DETERMINATION OF REACTIVE LYSINE IN SOYBEAN PROTEINS VIA THE HOMOARGININE PROCEDURE OR ANALYSIS FOR REDUCING SUGARS TO DETERMINE NUTRITIONAL VALUE

BY

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DISSERTATION

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ABSTRACT

Seven experiments were conducted to validate the determination of reactive Lys by the homoarginine procedure and to evaluate the effects of processing and heat damage on the chemical composition, amino acid (**AA**) digestibility, nitrogen utilization, and growth performance of pigs fed soybean products. Experiments 1 and 2 were conducted to test the reproducibility of the homoarginine procedure for determining reactive and total Lys across independent laboratories at the University of Illinois. Results confirmed reproducibility ($R^2 > 0.98$) and analytical precision, validating the homoarginine procedure as a reliable method for quantifying reactive Lys in protein ingredients used in swine nutrition. Experiment 3 included the analysis of 86 samples of conventional soybean meal (**SBM**), enzyme-treated soybean meal (**ESBM**), fermented soybean meal (**FSBM**), and soy protein concentrate (**SPC**). Concentrations of crude protein (**CP**) and AA were greater ($P < 0.001$) in ESBM, FSBM, and SPC than in SBM, whereas the Lys to CP ratio was below 6.0% in ESBM and FSBM, indicating mild heat damage and partial loss of reactive Lys. Experiment 4 tested the hypothesis that heat exposure and reducing sugars contribute to Lys degradation. Samples of SBM were autoclaved at 120°C for 0, 15, 30, 45, 120, or 180 min. Increasing autoclaving time reduced reactive Lys, stachyose, raffinose, and sucrose, whereas glucose and fructose increased. Reactive Lys was negatively correlated with fructose ($r = –0.95$; $P < 0.001$), confirming the involvement of reducing sugars in the Maillard reaction and Lys degradation. Experiment 5 evaluated the impact of heat damage on AA digestibility using twelve weanling pigs (initial BW: 11.4 ± 1.0 kg). Pigs were fed diets containing SBM or ESBM autoclaved for 0, 30, 45, or 120 min. Autoclaving reduced reactive Lys from 3.07 to 2.29% and linearly decreased ($P < 0.05$) the standardized ileal digestibility of indispensable AA and the apparent ileal digestibility of dry matter and gross energy. Experiment

6 was conducted to test the hypotheses that growth performance of weanling pigs can be predicted from the concentration of reactive Lys in soybean ingredients and that supplementation with crystalline AA can compensate for reduced reactive Lys in heat-damaged SBM and ESBM. One hundred sixty weanling pigs (initial BW: 5.7 ± 0.6 kg) were used in a 28-day growth performance trial with SBM or ESBM included in four levels of heat damage and diets formulated without or with crystalline AA. Feeding heat-damaged ingredients reduced ($P <$ 0.001) average daily gain and final body weight, whereas crystalline AA supplementation partially restored growth performance. Reactive Lys intake was strongly correlated ($P < 0.001$) with average daily gain, demonstrating that reactive Lys is a reliable indicator of protein quality in heat-processed soybean ingredients. Experiment 7 tested the hypothesis that inclusion of ESBM in low-protein diets for weanling pigs increases nitrogen retention compared with conventional SBM. Results indicated that reducing dietary crude protein from 22% to 18% decreased ($P < 0.001$) nitrogen intake, urinary nitrogen excretion, daily nitrogen retention, and apparent total tract digestibility of nitrogen, but increased ($P < 0.001$) nitrogen retention relative to intake and the biological value of dietary nitrogen. Blood urea nitrogen was less ($P < 0.001$) in pigs fed low-CP diets compared with those fed 22% CP diets. However, no differences were observed between protein sources for nitrogen balance variables, and there were no interactions between CP level and protein source. Plasma total protein, albumin, and immunoglobulin G remained within normal physiological ranges, and peptide YY tended ($P < 0.10$) to be greater in pigs fed low-protein diets containing ESBM compared with those fed low-protein SBM diets, indicating a possible effect on satiety-related signaling. In conclusion, results from these seven experiments collectively demonstrated that the homoarginine procedure is a reproducible method for quantifying reactive Lys in protein ingredients used in swine nutrition. Heat processing of

soybean products reduces reactive Lys concentration, AA digestibility, and growth performance of pigs, confirming the susceptibility of Lys to Maillard reactions involving reducing sugars. Reactive Lys is an indicator of protein quality and a strong predictor of growth in weanling pigs fed heat-processed soybean ingredients. Furthermore, ESBM supported efficient nitrogen utilization and maintained normal physiological and metabolic responses when included in low-protein diets.

Keywords: amino acid digestibility, enzyme-treated soybean meal, growth performance, homoarginine method, Maillard reaction, reactive Lys.

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CHAPTER 1: INTRODUCTION

Soybeans are one of the most important agricultural crops in the world, and global production is around 389 mill metric tons per year (USDA, 2022). Most soybeans are crushed to provide soybean oil and soybean meal (**SBM**; Galkanda-Arachchige et al., 2021). The majority of SBM is used as a source of amino acids (**AA**) in diets for pigs and poultry. The three major producers of soybeans in the world are Brazil, the United States, and Argentina. In fact, in 2021, 33% of the agricultural crop area planted in the United States was for soybeans, and Illinois was the state with the most acres planted with soybeans (4,290,000 hectares; ASA, 2022). Soybean meal produced in the U.S. often has a greater concentration of AA compared with SBM from other countries (Lagos et al. 2017). Specifically, the concentration of Lys and Trp is greater in SBM than in other plant proteins.

Conventional toasted and dehulled SBM is produced by removing the fat from dehulled soybeans using a solvent. After this procedure, a heat treatment is necessary to reduce trypsin inhibitors, and lectins (Cervantes-Pahm et al., 2010), and to remove the residual solvent, which is the toasting step (Mukherjee et al., 2016). The resulting SBM is an excellent source of AA for growing-finishing pigs, but because of antigens and oligosaccharides (i.e., stachyose and raffinose) SBM cannot be used as the only source of AA in diets for weanling pigs (De Fatima et al., 2005; Cervantes-Pahm et al., 2010; Navarro et al., 2017). However, it is possible to remove oligosaccharides and reduce remaining antigens in SBM via fermentation or enzyme treatment. Fermented soybean meal (**FSBM**) is produced by inoculating conventional SBM with mold, yeast, or bacterium. The fermentation conditions and nutritional quality of FSBM can vary depending on the type of microorganism and production procedure (Mukherjee et al., 2016). Enzyme-treated soybean meal (**ESBM**) is produced by treating dehulled, solvent-extracted

soybean meal for several hours with a proprietary blend of enzymes (Goebel and Stein, 2011) based on a patented bioconversion process (Ma et al., 2019). Both fermentation and enzyme treatment of SBM results in removal of oligosaccharides, and FSBM and ESBM can therefore be used in diets for young pigs instead of animal proteins (Cervantes-Pahm et al., 2010).

The nutritional components in ESBM can be variable depending on the origin or the types of enzymes used (Navarro et al., 2017). As an example, if the enzyme mixture included phytase (Goebel and Stein, 2011), the concentration of non-phytate P is greater than if no phytase was used. Usage of steam during the process may also affect the nutritional value of the end product because the temperature of the sample is increased and it can generate overheating in the SBM (Ton-Nu et al., 2020).

As a consequence of overheating during the drying process, some sources of SBM have reduced SID of Lys due to Maillard reaction (Navarro et al., 2017; Ton-Nu et al., 2020). The Maillard reaction starts when the epsilon amino group in Lys binds to reducing sugars due to heat treatment, which results in Lys being turned into Amadori compounds and later on to melanoidins (Kim et al., 2012). This will result in lower values for analyzed Lys in the ingredient and the ileal digestibility of Lys will also be reduced (Kim et al., 2012). Some of the Amadori compounds may be analyzed as Lys and may also be absorbed, but because they are damaged by the Maillard reaction, they cannot be used in protein synthesis and are considered un-reactive. The amount of reducing sugars in the ingredient or diet, the water concentration, and the pH influence at which temperature the Maillard reaction will start. Because some of the Amadori compounds are analyzed as Lys in the normal AA analysis, the analyzed concentration of Lys includes both the Lys that can be used in protein synthesis by the animal (i.e., the reactive Lys) and the unreactive Lys that cannot be used. To separate the reactive Lys from un-reactive Lys, a

specific analysis for reactive Lys is therefore needed and several different procedures have been used for this purpose (Pahm et al., 2008). One of these procedures is the homoarginine procedure, which involves conversion of Lys to homoargine via a chemical inoculation step (Moughan and Rutherfurd, 1996; Pahm et al., 2008). The homoarginine procedure has been recommended as the best procedure to determine reactive Lys in food proteins (FAO, 2013), but this procedure has not been widely used in the animal feed industry. However, because all soybean products are heat treated, and FSBM and ESBM are heated twice, there is a risk of heat damage due to Maillard reactions in all sources of soy protein. As a consequence, being able to determine reactive Lys in soybean ingredients will enable the industry to identify sources of soy protein that are heat damaged and therefore have a lower feeding value due to lower digestibility of Lys and lower availability of the digestible Lys.

The objective of this dissertation is to apply the homoarginine procedure to ESBM and other soybean products to determine concentrations of reactive Lys. By correlating reactive Lys concentrations in soybean proteins with concentrations of reducing sugars, the hypothesis that reducing sugar analysis can be used to predict reactive Lys in these ingredients will be tested. Additionally, by feeding soybean proteins with varying degrees of heat damage, and consequently varying concentrations of reactive Lys, to growing pigs, the hypothesis that pig growth performance can be predicted based on the concentration of reactive Lys in the ingredient will be also tested. If confirmed, this will support the conclusion that formulating diets based on digestible reactive Lys, rather than digestible total Lys, may improve animal growth performance.

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CHAPTER 2: CONVENTIONAL AND PROCESSED SOYBEAN MEAL IN WEANLING PIG NUTRITION: COMPOSITION, HEAT DAMAGE, AND PROTEIN QUALITY EVALUATION: REVIEW OF LITERATURE

INTRODUCTION

Conventional toasted and dehulled soybean meal (**SBM**) is produced by removing the fat from dehulled soybeans using a solvent. After this procedure, a toasting step is necessary to reduce trypsin inhibitors and lectins (Cervantes-Pahm and Stein, 2010), and to remove the residual solvent (Mukherjee et al., 2016). The resulting SBM is an excellent source of amino acids (**AA**) for growing-finishing pigs.

Soybean meal produced in the U.S. often has a greater concentration of AA compared with SBM from other countries (Karr-Lilienthal et al., 2004; Lagos and Stein, 2017), but regardless of origin, SBM contains all the indispensable AA needed by pigs and poultry. Specifically, the concentration of Lys and Trp is greater in SBM than in most other plant proteins. However, because of antigens and oligosaccharides, SBM cannot be used as the only source of AA in diets for weanling pigs (Cervantes-Pahm and Stein, 2010; Navarro et al., 2017). The formulation of diets for weaning pigs is particularly complex due to the dramatic physiological, immunological, and digestive transitions that occur during this phase. During weaning, pigs experience a transition from milk to solid feed, which often leads to impaired gut function, reduced nutrient digestibility, and a higher incidence of diarrhea (Ma et al., 2019; Tang et al., 2023). Compounding this challenge is the immaturity of the digestive system, which limits the weaning pigs to utilize plant-based proteins rich in anti-nutritional factors such as trypsin inhibitors, glycinin, β-conglycinin, raffinose, and stachyose with efficiency (Li et al., 2021; Yang

et al., 2022; Deng et al., 2023). Therefore, diets for newly weaned pigs often contain animal proteins to supply sufficient AA to the diets. However, it is possible to remove oligosaccharides and reduce antigens in SBM via fermentation or enzyme treatment.

ENZYME-TREATED SOYBEAN MEAL AND FERMENTED SOYBEAN MEAL

The anti-nutritional factors (**ANF**) in SBM include trypsin inhibitors, oligosaccharides (Zhou et al., 2010; Ma et al., 2019ab), and the antigens glycinin and beta-conglycinin (Zhou et al., 2010). The presence of ANF in SBM reduces AA digestibility (Ma et al., 2019b), is detrimental to animal health because it can cause gastrointestinal disturbances, intestinal damage, and increased disease susceptibility, and negatively affects growth performance of pigs (Zhou et al., 2010; Long et al., 2021).

When conventional SBM is inoculated with a mold, yeast, or bacterium, the resulting meal is called fermented soybean meal (**FSBM**). The fermentation conditions and nutritional quality of FSBM vary depending on the type of microorganism used and other production procedures (Mukherjee et al., 2016). Enzyme-treated soybean meal (**ESBM**) is produced by fermenting dehulled, solvent-extracted soybean meal with yeast and then treating the fermented product for several hours with a proprietary blend of enzymes (Goebel and Stein, 2011).

Fermentation removes the oligosaccharides in SBM, and enzyme treatment reduces the concentration of glycinin and beta-conglycinin (Cervantes-Pahm and Stein, 2010; Li et al., 2021), and sucrose, raffinose, and stachyose are reduced by around 94% in ESBM compared with SBM (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Navarro et al., 2017; Ma et al., 2019b). Likewise, ESBM contains 93% less glycinin and 96% less β-conglycinin than

SBM (Cervantes-Pahm and Stein, 2010; Ma et al, 2019a; Li et al., 2021). The enzymatic treatment of SBM may also reduce trypsin inhibitors (Zhou et al., 2010).

Because soybeans may be grown in different geographies and different environments, the nutritional value of SBM can vary and the concentration of protein and AA may also vary (Lagos and Stein, 2017). The concentration of protein in SBM is usually between 44 and 48% (Sauvant et al., 2004; NRC, 2012; Stein et al., 2016; Rostagno et al., 2017). Because oligosaccharides and sucrose are removed during production of FSBM and ESBM the concentration of crude protein (**CP**) and other nutrients increase, and FSBM and ESBM, therefore, usually contain 52 to 58% CP (Table 2.1; Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012; Navarro et al., 2017).

Fermentation is a traditional way of food processing to preserve food and has been used to improve the nutritional value of a food or feed ingredients (Mukherjee et al., 2016). Because fermentation is preceded by soaking to initiate inoculation with microbes or yeast, it is necessary to dry the fermented product after fermentation. There is, therefore, a risk of overheating during drying, which may reduce the concentration of Lys and the digestibility of AA because of Maillard reaction (Navarro et al., 2017; Ton-Nu et al., 2020; Oliveira et al., 2020ab).

MAILLARD REACTION

Browning of foods occurs by enzymatic and non-enzymatic mechanisms. Non-enzymatic browning occurs during heating and involves several steps. The reaction is called the Maillard reaction and was named after the person who initially described it, the French physicist and chemist Louis Camille Maillard (Tamanna and Mahmood, 2015). The Maillard reaction occurs between the amino group of an AA and the carbonyl group of reducing sugars (e.g., glucose,

lactose, fructose; Koubaa et al., 2019). The reaction is divided into three stages: initial, intermediate, and final Maillard reaction (Rauh and Xiao, 2022). In the initial stage, the reducing sugar is condensed with the amino group from the AA, forming a Schiff base. The intermediate Maillard reaction is when the unstable Schiff base undergoes a rearrangement to form a more stable compound called an Amadori product. In the final stage, Amadori compounds are condensed and polymerized, which results in formation of glycation end products, heterocyclic nitrogen compounds, and melanoidins (Pahm, 2008; Koubaa et al., 2019; Rauh and Xiao, 2022).

The Maillard reaction occurs primarily in products subjected to high-temperature processing, but it can also be initiated at moderate temperatures when the exposure time is prolonged. Initial stages of the reaction can begin at 60-70 °C, but significant browning and loss of nutritional quality typically occur when temperatures exceed 120-140 °C, particularly under low-moisture or dry conditions, which are common in thermal processing of feed ingredients (Martins et al., 2000). The rate and extent of the reaction are also influenced by physicochemical factors, including moisture content (~30%), water activity (0.65–0.70), and pH around 6, which provide ideal conditions for the initial nucleophilic attack of the ε-amino group of amino acids on the carbonyl group of reducing sugars (Ajandouz and Puigserver, 1999; Rauh and Xiao, 2022). In dry systems, such as during autoclaving, drying, or extrusion, these conditions are easily met, accelerating the Maillard reaction even in a relatively short processing time.

Among AA, Lys is most vulnerable to the Maillard reaction due to its free ε-amino group on the side chain, which is highly reactive and not involved in peptide bonds. Once Lys reacts with reducing sugars to form Amadori compounds, it loses its bioavailability and cannot be utilized for protein synthesis, enzyme activity, or growth functions in animals (Pahm, 2008; Lund and Ray, 2017). The degradation of Lys through this reaction leads to an overestimation of

total Lys during acid hydrolysis-based AA analysis, as some unreactive forms are artificially regenerated during testing (Moughan and Rutherfurd, 1996; Tamanna and Mahmood, 2015; Koubaa et al., 2019; Rauh and Xiao, 2022).

Reducing sugar

Reducing sugars are monosaccharides and disaccharides that possess a free carbonyl group, either an aldehyde or a ketone, which allows them to act as electron donors in redox reactions. This chemical reactivity enables them to initiate Maillard reactions when they interact with amino groups, especially the ε-amino group of Lys, leading to the formation of Amadori compounds and other advanced glycation end-products (Moughan and Rutherfurd, 1996; Martins et al., 2000; Fontaine et al., 2007).

The most relevant reducing sugars in animal nutrition include glucose, fructose, maltose, and lactose. These sugars differ in structure and reactivity, with glucose and fructose being particularly reactive. Notably, fructose, due to its ketone group and its higher proportion in the open-chain form, tends to participate more rapidly in Maillard reactions than glucose under identical conditions (Ajandouz and Puigserver, 1999; Fontaine et al., 2007). Disaccharides like maltose, which yield glucose upon hydrolysis, also contribute to this reactivity.

In terms of stereoisomerism, D-isomers of reducing sugars are more abundant in nature and are the primary forms involved in Maillard reactions in biological systems. D-glucose and D-fructose are the predominant forms in plant-derived feed ingredients and are significantly more reactive in the Maillard reaction than their L-isomer counterparts, which are rarely found in natural feedstuffs and lack metabolic relevance (Whistler and BeMiller, 1997). This is due to the stereospecificity of enzymatic and non-enzymatic pathways in animal systems, which are

adapted to D-forms for both metabolism and unintended reactions like glycation (Martins et al., 2000).

The concentration of reducing sugars varies widely among feed ingredients due to origin, processing methods, and the extent of fermentation or enzymatic hydrolysis. For example, ingredients such as ESBM contain lower levels of reducing sugars because during the enzymatic treatment, it is utilized as a substrate (Navarro et al., 2018). In contrast, conventionally processed SBM or cassava products may retain higher levels of sucrose, glucose, or stachyose, which can hydrolyze into reducing sugars under thermal or enzymatic conditions (Fanelli et al., 2023).

PROCEDURES TO ESTIMATE HEAT DAMAGE

In normal undamaged SBM, Lys usually is between 6.0 and 6.3% of the protein. However, in heat-damaged SBM, the concentration of Lys is reduced, and if the Lys:CP ratio is less than 6.0, the SBM has usually been heat-damaged (Pedersen et al., 2016). The Lys:CP ratio should be the same in all soybean proteins including FSBM and ESBM and it is, therefore, believed that all soy proteins that are not heat damaged have a Lys to CP ratio that is greater than 6.0. Other proteins have other ratios between Lys and protein, but the ratios will remain constant if products are not heat-damaged. The Lys:CP ratio can, therefore, be used as an indicator of heat damage in SBM, 00-rapeseed meal, canola meal, sunflower meal, cottonseed meal, and other ingredients (Almeida et al., 2013; 2014; Oliveira et al., 2021). However, the Lys to CP ratio is not a quantitative measure of heat damage, and to accurately determine the amount of Lys that is available for usage by the animal, a more advanced procedure is needed. One of the challenges with determining the impact of overheating of protein is that some of the Lys that is included in the Amadory compounds that are generated during heating, and that cannot be used in protein

synthesis by the animal, are regenerated during the acid hydrolysis step that precedes AA analysis. The analyzed Lys, therefore, consists of both the Lys that can be used in protein synthesis, the reactive Lys, and some of the Lys that cannot be used (un-reactive Lys). To avoid an over-estimation of the Lys that is available for protein synthesis, it is necessary to be able to analyze only reactive Lys. Reactive Lys can be determined via a guanidination process (Moughan and Rutherfurd, 1996; Torbatinejad et al., 2005; Rutherfurd et al., 2006; Fontaine et al., 2007) using omethylisourea (**OMIU**; Rutherfurd and Moughan, 2008). The guanidination reaction results in an interaction between the ε-amino group of Lys and OMIU, which generates homoarginine (Rutherfurd and Moughan, 2005; Pahm et al., 2008). Only the reactive Lys, which has not been affected by the Maillard reaction and has an intact epsilon amino group will react with the OMIU and, there is, therefore, no influence of OMIU on unreactive Lys (Pahm et al., 2010; Almeida et al., 2013). Lysine and homoarginine are resistant to degradation under strong acidic conditions, which means they remain intact during acid hydrolysis. As a result, when proteins are hydrolyzed using hydrochloric acid, both amino acids are released from the protein structure without being destroyed. This stability is essential for accurate quantification of Lys or homoarginine in protein samples using chemical analysis (Moughan and Rutherfurd, 1996; Torbatinejad et al., 2005; Rutherfurd et al., 2006; Pahm et al, 2008). To achieve complete conversion of Lys to homoarginine, the guanidination reagent has to be adequately prepared and the incubation conditions need to be optimized. Optimum conditions for guanidination may be achieved by varying pH and reaction times (Almeida et al., 2013). Following incubation, the reactive Lys is analyzed as the homoarginine in the sample.

Reactive Lys can also be calculated using the Furosine method, which is based on the fact that some of the Lys bound Amadori compounds will be converted to furosine during acid

hydrolysis. Whereas it is assumed that 40% of the Lys bound in Amadori compounds will be analyzed as Lys, it is assumed that 32% is converted to furosine (Pahm et al., 2008; Kim et al., 2012). By analyzing furosine, the proportion of Lys bound in Amadori compounds but still measured as total Lys can be estimated. Using this proportion, the amount of regenerated Lys can be calculated, and consequently, the concentration of reactive Lys can be determined (Pahm et al., 2008; Kim et al., 2012; Almeida et al., 2013; Oliveira et al., 2021). The furosine procedure is faster and less tedious than the homoarginine procedure, but the accuracy of the procedure relies on the assumption that the regenerated Lys is always 40% and the amount of Amadori-bound Lys that is converted to furosine is always 32%.

Reactive Lys can also be determined via conversion of reactive Lys to dinitrophenyl or trinitrophenyl-Lys using fluor dinitrobenzene (**FDNB**; Rutherfurd and Gilani, 2009; Oliveira et al., 2021). This method can be divided into two different measurements. In the direct method reactive Lys is converted to dinitrophenyl or trinitrophenyl-Lys and analysis of this component is done using colorimetry or reverse-phase High-performance liquid chromatography (**HPLC**; Pahm, 2008; Rutherfurd and Gilani, 2009; Almeida et al., 2013; Oliveira et al., 2021). In the second procedure, the difference between total Lys and unreactive Lys is determined because the unreactive Lys may not react with FDNB and remains colorless after the reaction. However, a disadvantage of both methods is that FDNB may also react with carbohydrates during acid hydrolysis, leading to color deterioration of the dinitrophenyl-lysine, which may necessitate the use of correction factors (Moughan and Rutherfurd, 1996; Almeida et al., 2013; Oliveira et al., 2021).

High-Performance Liquid Chromatography

It is a physical separation technique widely used in analytical chemistry, biochemistry, and nutrition research to identify and quantify components in complex mixtures (Fallon et al., 1987; Dong, 2006). The separation is achieved through two phases: a mobile phase, which carries the sample through the system, and a stationary phase, which is packed inside a column. Compounds in the sample interact differently with the stationary phase based on their chemical properties, allowing separation based on polarity, size, or charge. The method uses high pressure that forces the mobile phase through the column at a constant flow rate, which significantly improves resolution and efficiency (Izydorczyk, 2017). After separation, an in-line detector, often UV or fluorescence-based, monitors the components as they elute from the column and generates a chromatogram, where each peak corresponds to a specific compound (Dong, 2006).

High-performance liquid chromatography is a non-destructive, highly sensitive, and reliable method for analyzing AA, sugars, and peptides in biological samples. In the context of animal nutrition, HPLC is particularly valuable for measuring Lys concentration and reactive Lys content in feed ingredients. Total Lys is typically quantified after acid hydrolysis using 6*N* hydrochloric acid, while reactive Lys is determined through a guanidination reaction with O-Methylisourea, which converts Lys to homoarginine. Both Lys and homoarginine can be separated and quantified using reversed-phase HPLC with appropriate solvent gradients and column chemistry (Moughan and Rutherfurd, 1996; Pahm et al., 2008).

Ultra High-Performance Liquid Chromatography (**UHPLC**) operates in a similar principes as the HPLC. However, UHPLC uses lower particle size column and higher pressures than the HPLC, allowing faster flow rate, decrease the time of each run, and optimizing chromatographic resolution (Serrano et al., 2013). This method ensures selective measurement of

bioavailable Lys, as unreactive Lys modified by the Maillard reaction does not undergo guanidination and is thus excluded from quantification. Due to its precision, flexibility, and sensitivity to low concentrations, HPLC remains the gold standard for analyzing AA and sugars in nutritional studies, especially when evaluating heat damage, digestibility, and protein quality in processed soybean meals and other protein-rich feed ingredients.

IMPACT OF HEAT DAMAGE ON AMINO ACID AND ENERGY DIGESTIBILITY

Heat processing is necessary to reduce ANF in some feed ingredients, including SBM, and digestibility of nutrients and energy may be improved by heat processing. However, overheating ingredients may decrease concentration, digestibility, and utilization of AA and energy due to the Maillard reaction, crosslinking of proteins within or between molecules, denaturation of protein, changing AA side chains, racemization, and other reactions (Hulshof, 2016). Reduced digestibility of AA and/or energy following heat processing has been demonstrated in sunflower meal and cottonseed meal (Almeida et al., 2014a), distillers dried grains with soluble (**DDGS**) and SBM (González-Vega et al., 2011; Almeida et al., 2014c), canola meal (Almeida et al., 2014b), soybean expellers (Oliveira et al., 2021), and 00-rapeseed meal (Oliveira et al., 2020a).

The Maillard reaction reduces digestibility and concentration of Lys (Fontaine et al., 2007; González-Vega et al., 2011; Almeida et al., 2014a). Advanced Maillard reaction products, such as pre-melanoidins and melanoidins, result in AA and protein being less accessible to digestive enzymes because of the obstruction of absorption sites through steric hindrance (González-Vega et al., 2011). Therefore, the overall digestibility of CP and AA is reduced (Almeida et al., 2014ac). Autoclaving can be used to induce the Maillard reaction in ingredients,

and reduced standardized ileal digestibility (**SID**) of AA is usually observed in autoclaved ingredients (González-Vega et al., 2011; Almeida et al., 2014abc). Sunflower meal that was autoclaved for 20 min at 130 °C did not have reduced SID of CP and AA compared with non-autoclaved sunflower meal, except for the SID of Lys, which was reduced from 83.2 to 81.2%. However, after 40 min of autoclaving the SID of all AA was linearly reduced. Likewise, if cottonseed meal is autoclaved for 15 min at 130 °C, the SID of all AA decreased by at least 4% (Almeida et al., 2014b). One of the ingredients that is highly affected by advanced Maillard reaction or other heat reactions is DDGS because of the presence of reducing sugars in the distilled grain that is off after fermentation. When DDGS was autoclaved at 130 °C for 10 min, the SID of all AA was reduced (Almeida et al., 2013). The reason DDGS is highly susceptible to heat damage is that it contains soluble sugars, which can easily be used in the Maillard reaction. When 00-rapeseed meal is autoclaved at 110 °C for 15 or 30 min, the SID of AA was not reduced, but if 00-rapeseed was autoclaved at 150 °C for 3 min, the SID of all AA was reduced, which demonstrates that both the time and the severity of heating is important for the reactions (Oliveira et al., 2020ab). Soybean meal autoclaved at 125 °C for 15 min resulted in a reduction of SID of Lys from 93.0 to 91.1%, indicating that Maillard reaction was initiated and after 30 min, the SID of all AA was reduced (González-Vega et al., 2011).

During advanced reactions following severe heating, some cross-linkages between Lys and other AA and polypeptide chains within the protein may occur, which may reduce the efficiency of proteolytic enzymes to access the proteins and hydrolyze peptide bonds (Moughan and Rutherfurd, 1996; González-Vega et al., 2011; Almeida et al., 2013; Almeida et al., 2014a). Digestibility may also be reduced due to reduced transport of AA and peptides in the small intestinal lumen (Oliveira et al., 2020ab).

High heat and pressure can cause AA to undergo racemization, turning L-AA into their D-forms. Because the D-form of most AA is less efficiently used, or not used at all by animals, this will reduce protein utilization (Almeida et al., 2013). Heat treatment may affect the digestibility of other nutrients, such as dry matter in SBM, 00-rapeseed meal, and soybean expellers, followed by a reduction in digestible and metabolizable energy (Oliveira et al., 2020ab; Espinosa et al., 2021). The binding of reducing sugars to Lys during the Maillard reaction may reduce the digestibility of energy (Oliveira et al., 2020a). Heat damage may also increase the concentration of acid detergent fiber and neutral detergent fiber because of formation of a lignin-like matrix (Almeida et al., 2013; 2014bc), which was observed in DDGS and 00-rapeseed meal (Almeida et al., 2013; Oliveira et al., 2020a). Lignin has the capacity to bind components to the cell wall, making them less digestible, reducing digestibility of dry matter, and therefore, the digestibility of energy. In addition, nitrogen-fiber complexes that may reduce fiber fermentation and fatty acids may be converted to cyclic polymers that resist digestibility (Oliveira et al., 2020ab; Jaksics et al., 2023).

TABLES AND FIGURE

Table 2.1. Concentrations of dry matter, fat, crude protein, energy, amino acids, ash, and minerals in soybean meal (SBM), enzyme-treated soybean meal (ESBM), and fermented soybean meal (FSBM), as-is basis[1]

Item	SBM	ESBM	FSBM
Dry matter, %	89.98	92.70	92.88
Ether extract, %	1.52	1.82	2.30
Acid ether extract, %	2.86	-	-
Gross energy, kcal/kg	4,256	4,451	4,533
Digestible energy, kcal/kg	3,619	3,914	3,975
Metabolizable energy, kcal/kg	3,294	3,536	3,607
Crude protein, %	47.73	55.62	54.07
Indispensable AA, %			
Arg	3.45	3.95	3.70
His	1.28	1.41	1.37
Ile	2.14	2.48	2.55
Leu	3.62	4.09	4.25
Lys	2.96	3.20	3.14
Met	0.66	0.71	0.75
Phe	2.40	2.78	2.87
Thr	1.86	2.13	2.09
Trp	0.66	0.72	0.69
Val	2.23	2.57	2.67

Table 2.1. (cont.)

Dispensable AA, %			
Ala	2.06	2.41	2.45
Asp	5.41	6.14	5.98
Cys	0.70	0.78	0.77
Glu	8.54	9.62	9.12
Gly	1.99	2.32	2.34
Pro	2.53	2.73	2.74
Ser	2.36	2.66	2.51
Tyr	1.59	2.03	2.08
Ash	6.27	7.05	6.98
Macro minerals, %			
Ca	0.33	0.31	0.29
P	0.71	0.75	0.80

[1] Values obtained from NRC, 2012.

Table 2.2. Lys terminology and definitions[1]

Recommended term	Definition	Terms that have been used
Reactive Lys	Unmodified Lys possesses a free side chain amino group and can be either free or protein-bound	Reactive Lys Chemically reactive Lys Chemically available Lys Available Lys Total available Lys Bioavailable Lys
Unreactive Lys	Lys for which the side chain amino group has reacted with another compound, e.g. Amadori compounds	Bound Lys Unreactive Lys Modified Lys
Reverted Lys	Lys that has reverted from unreactive Lys during the acid hydrolysis step of conventional amino acid analysis	Reverted Lys Regenerated Lys
Total Lys	Refers to the concentration of reactive Lys plus the concentration of unreactive Lys	Total Lys
Analyzed Lys	Analyzed Lys comprises reactive Lys and reverted Lys	Total Lys

[1] Adapted from Cervantes-Pahm and Stein, 2010; Almeida et al., 2013.

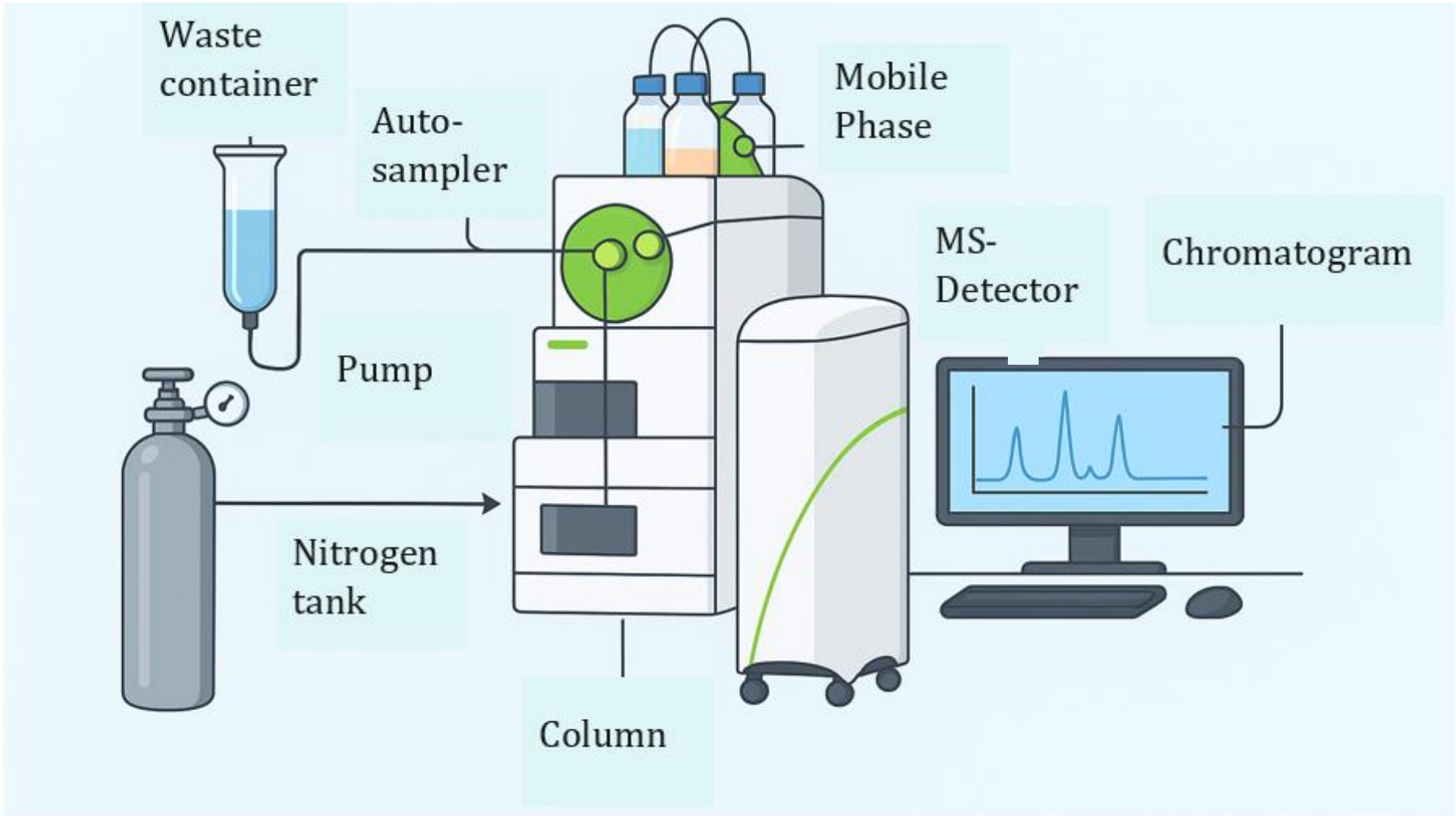

Figure 2.1. Ultra High-Performance Liquid Chromatograph.

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CHAPTER 3: ENZYME-TREATED SOYBEAN MEAL IN DIETS FOR WEANLING PIGS: A SYSTEMATIC REVIEW AND QUANTITATIVE DESCRIPTIVE SYNTHESIS.

ABSTRACT

Soybean meal (**SBM**) is an important source of amino acids (**AA**) in diets for pigs, poultry, dairy, and other animals. However, SBM contains anti-nutritional factors (**ANF)** such as trypsin inhibitors, phytate, lectins, saponins, oligosaccharides, glycinin, and β-conglycinin, which reduce nutrient digestibility, have negative impacts on animal health, and negatively affect growth performance of pigs. After treatment of SBM with yeast and a proprietary blend of enzymes, antigens, and oligosaccharides may be eliminated and enzyme-treated SBM (**ESBM**) may, therefore, be used in diets for weanling pigs. However, no comprehensive review of the use of ESBM in diets for pigs has been published, and summaries of nutrient composition and digestibility of energy and nutrients in ESBM are not available. Reviews of the impact of ESBM in diets fed to weanling pigs on pig growth performance and health have also not been published. Therefore, this literature review was conducted to identify experiments in which ESBM was used as a protein source in diets for weanling pigs. Only experiments conducted during the last 20 years are included in the review. Most experiments focused on the nutritional value of ESBM and reported possible beneficial effects of ESBM on growth performance and health of weanling pigs. Results of the review indicate that ESBM is an excellent source of AA and other nutrients and has a high concentration of small peptides, which may result in improved digestion of proteins. Additionally, ESBM may reduce gastrointestinal diseases, promote beneficial gut microbiota, and improve intestinal barrier functions. Results of most experiments also

demonstrated that pigs fed ESBM have improved growth performance, and reduced diarrhea compared with pigs fed a diet containing conventional SBM. In conclusion, ESBM has increased concentration of nutrients and contains fewer ANF than conventional SBM, which results in greater intestinal health and improved growth performance of weanling pigs.
Keywords: anti-nutritional factor, digestibility, enzyme-treated soybean meal, soybean meal, weanling pigs.

Abbreviations: AA, amino acids; ADF, acid detergent fiber; AEE, acid-hydrolyzed ether extract; AID, apparent ileal digestibility; ANF, anti-nutritional factor; ATTD, apparent total-tract digestibility; CP, crude protein; CV, coefficient of variation; DM, dry matter; EE, ether extract; ESBM, enzyme-treated soybean meal; GE, gross energy; IDF, insoluble dietary fiber; NDF, neutral detergent fiber; SBM, soybean meal; SD, standard deviation; SDF, soluble dietary fiber; SID, standardized ileal digestibility; TDF, total dietary fiber.

INTRODUCTION

Soybean is one of the major agricultural crops in the world, and global annual production is close to 400 million metric tons (USDA, 2023). The three major producers of soybeans in the world are Brazil, the United States, and Argentina, which produce 39, 29, and 13%, respectively, of global production (USDA, 2023). In 2023, soybean production in the United States was around 115 million metric tons, and Illinois was the state with the most hectares planted with soybeans (ASA, 2022; USDA, 2023). Most soybeans are crushed to provide soybean oil and soybean meal (**SBM**; Galkanda-Arachchige et al., 2021). Soybean oil is used in food

applications, biodiesel production, or production of other industrial products, but most SBM is used as a source of amino acids (**AA**) in diets for pigs, poultry, dairy, and other animals.

Soybean meal produced in the United States often has a greater concentration of AA compared with SBM from China or Argentina (Karr-Lilienthal et al., 2004; Lagos et al., 2017), but regardless of origin, SBM contains all the indispensable AA needed by pigs and poultry. Specifically, the concentration of Lys and Trp is greater in SBM than in most other plant proteins. Conventional dehulled and toasted SBM is produced by removing the fat from dehulled soybeans using a solvent. After this procedure, a toasting step is needed to reduce trypsin inhibitors and lectins (Cervantes-Pahm and Stein, 2010; Zhou et al., 2011; Tang et al., 2023) and to remove residual solvent (Mukherjee et al., 2016). The resulting SBM is an excellent source of AA for growing-finishing pigs and reproducing sows, but because of antigens and oligosaccharides (Cervantes-Pahm and Stein, 2010; NRC, 2012), SBM cannot be used as the only source of AA in diets for weanling pigs (Navarro et al., 2017). Therefore, diets for weanling pigs have traditionally contained animal proteins (i.e., fish meal, milk protein, blood protein) in addition to limited quantities of conventional SBM. However, due to increased demand for animal proteins from other industries, the supply of animal proteins for pig feed has been reduced over the last few decades.

If conventional SBM is inoculated with yeast and enzymes, both antigens and oligosaccharides can be eliminated (Mukherjee et al., 2016). The resulting product is called enzyme-treated soybean meal (**ESBM**) and is produced by treating dehulled, solvent-extracted SBM for several hours with yeast and a proprietary blend of enzymes (Goebel and Stein, 2011). Enzymes in the mixture include pectinase, alpha-galactosidase, cellulase, and sucrase (Islam and Ju, 2023), and the degree to which antigens and oligosaccharides are inactivated during the

process is influenced by the enzyme mixture, fermentation conditions, time, temperature, and pH. Due to the reduced concentrations of oligosaccharides and antigens, ESBM may be used in diets for weanling pigs.

The nutritional composition of ESBM including the concentration of anti-nutritional factors (**ANF**), has been reported (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Li et al., 2021; Islam and Ju, 2023). The impact of adding ESBM to diets for weanling pigs has also been determined (Min et al., 2004; Navarro et al., 2017; Li et al., 2019; Ma et al., 2019; Li et al., 2021), and effects of ESBM on intestinal health of weanling pigs have been investigated (Ma et al., 2019; Ruckman et al., 2020; Li et al., 2021; Song et al., 2023). However, no comprehensive reviews covering all aspects of ESBM fed to weanling pigs are available and the optimal inclusion rate of ESBM in diets for weanling pigs has not been reported. Likewise, there are no published feeding guidelines for ESBM based on published data in the scientific literature.

The objective of the present contribution is, therefore, to provide a summary of the nutritional value of ESBM, and review effects of ESBM on growth performance and immune status of weanling pigs. Guidelines for inclusion rates of ESBM in diets for weanling pigs will also be provided based on the published literature. However, it is outside the scope of this review to discuss management practices, housing conditions, and health status of pigs, and how these factors may influence growth performance and immune responses independently of ESBM inclusion.

METHODS

The literature review was conducted by searching the following databases: Google Scholar, Agricola, and PubMed. In Google Scholar, the search was conducted using “ESBM and

weanling pigs" and 189 publications were initially identified, but after manually tittle examination, only 23 publications were selected. In Agricola, the search was conducted using "enzyme treated soybean meal in pigs" and the search identified 25 publications, and after reviewing the titles and abstracts for relevance, 9 publications were selected that specifically examined ESBM in swine diets or related nutritional outcomes.

In PubMed (MEDLINE), queries targeted ESBM in weanling pigs and were designed to retrieve studies on composition, digestibility, and health/performance outcomes (White et al., 2024). To standardize and document reproducibility, we generated a set of complex Boolean queries using custom Python code with the Biopython Entrez utilities (Cock et al., 2009). The code assembled: (i) an ESBM term set (e.g., "enzyme-treated soybean meal", "enzymolytic soybean meal", "enzymatically treated soybean meal", "enzyme-modified soybean meal", ESBM, "HP300", "HP310", plus spacing variants), (ii) a porcine/weanling term set (e.g., "weanling pig*", "nursery pig*", piglet*, porcine, swine, *Sus scrofa*), and (iii) three thematic term sets (composition, digestibility, health/performance) that included common synonyms (e.g., "nutrient/chemical composition", "ileal/total tract/amino acid digestibility", "gut health", "intestinal morphology", "immune/oxidative/diarrhea/growth performance").

This final code was entered into PubMed as the search query: (("enzyme-treated soybean meal"[TIAB] OR "enzymolytic soybean meal"[TIAB] OR "enzymatically treated soybean meal"[TIAB] OR "enzyme-modified soybean meal"[TIAB] OR ESBM[TIAB] OR "HP300"[TIAB] OR "HP310"[TIAB]) AND ("weanling pig*"[TIAB] OR "nursery pig*"[TIAB] OR piglet*[TIAB] OR porcine[TIAB] OR swine[TIAB] OR "Sus scrofa"[MeSH Terms]) AND (composition[TIAB] OR "nutrient composition"[TIAB] OR "chemical composition"[TIAB] OR digestibility[TIAB] OR "amino acid digestibility"[TIAB] OR "ileal digestibility"[TIAB] OR "gut

health"[TIAB] OR "intestinal morphology"[TIAB] OR "immune response"[TIAB] OR diarrhea[TIAB] OR "growth performance"[TIAB])).

The selection of papers for the review followed a systematic process. Initially, papers were identified by their titles to determine relevance to the topic of ESBM in weanling pigs. Each final query required at least one ESBM term, one porcine/weanling term, and one theme term, with appropriate field tags used for PubMed. The code then searched each individual query on PubMed MEDLINE and returned an output file (i.e., a Microsoft Excel workbook) containing 22 results. Five of these papers were removed based on their titles; thus, a total of 49 publications were initially selected from the three databases. However, because many publications appeared in more than one database, 25 duplicate entries were identified by matching titles, authors, and publication years and were removed manually. Of these, 13 publications were duplicates present in two databases (Google Scholar and PubMed: 10; PubMed and Agricola: 1; Google Scholar and Agricola: 2), which were removed once. An additional 6 publications were found in all three databases and were removed twice to prevent double counting. After de-duplication, 24 unique publications were included in the final review.

These papers were manually reviewed by reading to verify their relevance to the specific topics of composition, digestibility, and health/performance outcomes. A table was created to document the key findings from each selected paper, including study outcomes, methodologies, and results. The information was then organized for clarity and consistency, with simple statistical analyses conducted to summarize and highlight patterns across the selected studies. The baseline characteristics of the publications that met the final criteria are summarized in Table 3.1 and Figure 3.1.

Statistical analysis

Basic descriptive statistics were calculated when multiple values were available for each parameter. The average was used to represent the overall trend, while the standard deviation (**SD**) and coefficient of variation (**CV**) were included to reflect the variability in the data. This approach allowed for a more concise summary of the literature and helped highlight both patterns and discrepancies among reported results.

RESULTS AND DISCUSSION

Composition of enzyme-treated soybean meal

Enzyme treatment of SBM results in an increase in the concentration of crude protein (**CP**), AA, ether extract (**EE**), ash, acid detergent fiber (**ADF**), neutral detergent fiber (**NDF**)**,** crude fiber, and minerals compared with conventional SBM (Goebel and Stein, 2011; NRC, 2012). The reason for this increase is that fermentation of oligosaccharides and sucrose during the production of ESBM reduced the concentration of these components, which concentrates the other nutrients in the final product (Tables 3.2 and 3.3).

Enzyme-treated soybean meal also has slightly greater concentration of dry matter (**DM**) than conventional SBM because of the drying step that is needed after inoculation of the SBM with enzymes. However, the CV of 2.0% indicates that the moisture content is relatively stable among samples (Engelsmann et al., 2022; Yang et al., 2022; Garavito-Duarte et al., 2023; Mallea et al., 2023; Tan et al., 2024). The concentration of acid-hydrolyzed ether extract (**AEE**) in ESBM is only 1.7% on average and as in the case for other defatted soybean products, ESBM has low concentration of AEE (NRC, 2012).

The concentration of CP in ESBM is 54.2% on average with a range from 48.3 to 57.7%. The high protein concentration is one of the main reasons ESBM is a valuable ingredient in animal diets. The variation in concentration of CP may be due to differences in CP among batches of SBM used for ESBM production (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Navarro et al., 2017). The concentration of AA in ESBM reflects the profile in SBM and generally is high in indispensable AA including Lys and Trp (Acosta et al., 2021; Long et al., 2021; Nørgaard et al., 2021; Engelsmann et al., 2022; Zhang and Piao, 2022). The CV for the concentration of the indispensable AA ranges from 19.1% to 31.8%, which indicates some differences among samples, which may be due to differences in the SBM used in the production of ESBM, However, the CV for most AA is greater in ESBM than in conventional SBM, which indicates that variation among sources of ESBM is partly a result of variability in processing conditions (Liao et al., 2015; Oliveira et al., 2020; Ton-Nu et al., 2020). Concentrations of calcium and phosphorus in ESBM are 0.32% and 0.72%, respectively. However, other macro and micro minerals such as chlorine, potassium, magnesium, sodium, sulfur, chromium, copper, iron, iodine, manganese, selenium, and zinc are not reported (Soetan et al., 2010; Hossain et al., 2016; Jones et al., 2018; Li et al., 2019; Ton-Nu et al., 2020).

Enzyme-treated SBM has a low concentration of glucose, maltose, fructose, sucrose, and starch compared with conventional SBM, which is likely because these compounds are fermented during the production of ESBM. The average concentration of crude fiber is 3.84%; however, the CV of 25.19% indicates that the fiber content can vary significantly among batches of ESBM, which may be due to differences among sources in raw material or fermentation methods.

Neutral detergent fiber represents the hemicellulose, cellulose, and lignin and ESBM contains 11.92% NDF, and the CV is less than 20%. Acid detergent fiber includes cellulose and lignin and ADF in ESBM is 5.74%. Both NDF and ADF are greater in ESBM than in conventional SBM (NRC, 2012; Mallea et al., 2023; Nørgaard et al., 2021), which indicates that fiber is not being fermented during the process to produce ESBM.

The total dietary fiber (**TDF**) combines both insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**). Insoluble dietary fiber in ESBM is 18.9%, whereas SDF is 2%. The TDF in ESBM is 21% on average, which is greater than in conventional SBM further indicating that fiber is not lost during enzymatic treatment. The CV of TDF and IDF in ESBM is less than 20%, but because of the low concentration of SDF, the CV for SDF is much greater than the CV for TDF or IDF. Soybean meal contains oligosaccharides (Ma et al., 2019), trypsin inhibitors, lectins, urease, and allergenic proteins (Zhou et al., 2011), which may reduce AA digestibility (Ma et al., 2019) and can cause gastrointestinal disturbances and negatively affect growth performance of young pigs (Zhou et al., 2011; Long et al., 2021). However, in ESBM, the concentration of sucrose, raffinose, and stachyose is reduced by approximately 94% compared with conventional SBM (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Navarro et al., 2017; Ma et al., 2019). Because oligosaccharides may have a negative impact on growth performance of weanling pigs and can cause increased diarrhea, the reduction in oligosaccharides in ESBM may positively impact growth of pigs.

On average, the concentration of trypsin inhibitors and lectins in ESBM is 1.7 TIU/mg and 29 mg/kg, respectively, which is less than in conventional SBM (Yang et al., 2022; Tang et al., 2023). This reduction may be due to fermentation and drying after fermentation. The reduction in trypsin inhibitors is also evidenced by a reduction in urease (Zhou et al., 2011). Due

to the use of enzymes in the production of ESBM, the concentration of glycinin and β-conglycinin is reduced compared with conventional SBM (Cervantes-Pahm and Stein, 2010; Ma et al., 2019b; Li et al., 2021). The low concentration of trypsin inhibitors, glycinin, β-conglycinin, and oligosaccharides confirms that fermentation and enzyme treatment is an effective method for reducing these ANF.

Small or short peptides are defined as peptides with at least two and not more than 45 AA (Cervantes-Pahm and Stein, 2010) and ESBM has a greater concentration of small peptides compared with conventional SBM (Table 3.4) because of the enzymatic reaction during production (Zhou et al., 2011; Ma et al., 2019a; 2019b; Tan et al., 2024). Small peptides are believed to be more easily absorbed from the small intestine, and they may also improve the beneficial gut microbiota community (Long et al., 2021; Zhang and Piao, 2022; Deng et al., 2023). Increasing the concentration of small peptides may increase digestibility of AA and may also increase the speed of absorption of AA (Hossain et al., 2016; Apostolopoulos et al., 2021; Nørgaard et al., 2021; Deng et al., 2023). As a consequence, pigs fed a diet containing ESBM had a greater plasma appearance of AA than pigs fed conventional SBM during the first two hours after feeding, but three hours after feeding, no differences between the two sources of SBM were observed (Nørgaard et al., 2021). These observations indicate that the smaller peptide sizes in ESBM compared with conventional SBM result in a faster uptake of AA, which enhances protein synthesis, improves nutrient utilization, supports gut health and immunity, and contributes to better growth performance and feed efficiency in weaned pigs (Nørgaard et al., 2021).

Digestibility of AA in ESBM

Apparent ileal digestibility (**AID**) of AA refers to the proportion of dietary AA absorbed at the end of the small intestine, calculated by subtracting the amount of each AA recovered in the ileal digesta from the amount ingested. This measure does not account for endogenous AA losses and may therefore slightly underestimate true digestibility. Nevertheless, AID is widely used to evaluate the bioavailability of amino acids in feed ingredients. In ESBM, the average AID of CP is 77.85%. The CV of AID of AA and CP in ESBM was low, accounting for less than 12%, except for Pro.

The average of standardized ileal digestibility (**SID)** of CP is 87.6% (Table 3.6). For indispensable AA, the averages indicate that Arg and Met have SID of 94.6 and 91.6%, respectively. The SID of Ile, Leu, and Trp is between 89 and 90%. However, Lys and Thr have a lower SID with 86.1 and 85.1%, respectively. Some sources of ESBM have reduced SID of Lys, which may be due to the Maillard reaction during the drying of ESBM (Navarro et al., 2017; Ton-Nu et al., 2020). The Maillard reaction causes the Lys to bind to reducing sugars, which makes it unavailable for use in protein synthesis by the animals. The Maillard reaction will always reduce both the concentration of analyzed Lys and the SID of Lys (Cervantes-Pahm and Stein, 2010).

For dispensable AA, the SID of Pro in ESBM is 97% on average, indicating a high digestibility compared with all other AA. The SID of Tyr and Glu is 91.3 and 89.2%, respectively. The lowest average SID of dispensable AA is Gly and Cys. The CV for the SID of AA and CP in ESBM is less than 9%, except for proline.

Total tract digestibility of energy and nutrients in ESBM

The apparent total-tract digestibility (**ATTD**) of DM is 87.8%, and for CP, it is approximately 80%, whereas, the ATTD of gross energy on average is 84.9% (NRC, 2012; Navarro et al., 2017; Li et al., 2019; Ma et al., 2019; Ma et al., 2019; Mallea et al., 2024). It appears that enzyme treatment of soybean may increase gross energy in the ingredient, but ATTD of gross energy is not different in ESBM compared with SBM (Goebel and Stein, 2011).

The ATTD of calcium in ESBM ranges between 53.6% and 63.8%, averaging 58.7%, and ATTD of phosphorus averages 61.8% with a low CV of 3.2%. The greater digestibility of phosphorus in ESBM than in conventional SBM is a consequence of the fermentation process that results in the release of phosphorus from the phytate molecule, which makes more phosphorus digestible for the pig (Goebel and Stein, 2011). A greater digestibility of phosphorus results in reduced phosphorus excretion from pigs, and less phosphorus from feed phosphates will be needed in diets containing ESBM, which will contribute to a reduction in cost of production (Ekpe et al., 2002; Lee et al., 2023).

Effect of ESBM on intestinal health parameters

The reduced ANF in ESBM reduces gastrointestinal diseases in pigs with a subsequent increase in the health status of weanling pigs (Zhou et al., 2011). The increased concentration of small peptides in ESBM may also improve the intestinal microbiota and promote the abundance of beneficial bacteria in nursery pigs (Long et al., 2021). Indeed, feeding a diet containing ESBM increased villus height and crypt depth in the jejunum compared with feeding a diet containing extruded full-fat soybeans, which likely improves absorption of AA and microbial protein synthesis (Ma et al., 2019). Replacing 50% of full-fat soybeans in the diet with ESBM improved

intestinal health and reduced diarrhea in pigs because it prevented protein fermentation in the hindgut (Ruckman et al., 2020; Long et al., 2021).

Feeding pigs with high levels of protein can cause an inflammatory response, which may damage the intestine, reduce the digestibility of nutrients, and increase diarrhea (Ma et al., 2019b; Ruckman et al., 2020; Long et al., 2021). Using ESBM in diets decreased the levels of the pro-inflammatory cytokines tumor necrosis factor (**TNF-α**), interferon-γ (**IFN-γ**), interleukin-1β (**IL-1β**), and IL-6, which is an indication that inflammation was reduced (Ma et al., 2019b; Long et al., 2021). Additionally, pigs fed a diet containing ESBM had a greater concentration of IgG and IL-10 in serum than pigs fed a diet with conventional SBM, which may improve immunity of the animal (Long et al., 2021).

Intestinal health may be influenced by the intestinal microbiota, which is highly dependent on the diet Li et al., 2019; Song et al., 2023). Using *Lactobacillus* in diets for pigs may improve growth performance, carcass quality, and intestinal health (Valeriano et al., 2016). In contrast, the presence of *Streptococcus* in pigs is associated with septicemia and other infections (Song et al., 2023). Pigs fed diets containing ESBM had increased *g_Blautia* (*f_Lachnospiraceae*) and *g_Lactobacillus* compared with pigs fed extruded full-fat soybeans, and the concentration of *Bifidobacterium,* which is beneficial to intestinal health, was greater in the cecum of pigs fed the diet containing ESBM than in pigs fed the diet containing extruded full-fat soybeans (Li et al., 2019). These changes in intestinal microbiota may be the reason pigs fed ESBM had improved intestinal health.

The gut epithelial barrier is a critical defense system in the intestine, and maintaining its integrity helps minimize the detrimental effects of pathogens, toxins, and antigens on the mucosal surface. Diamine oxidase is a marker that is used to determine negative effects on the

gut barrier. Pigs fed a diet with ESBM have reduced concentrations of serum diamine oxidase, which indicates that these pigs have an improved gut barrier compared with pigs fed conventional SBM (Ruckman et al., 2020; Long et al., 2021). The reduced concentration of the main antigens in ESBM compared with conventional SBM also contributes to reduced diarrhea, improved immunity, improved intestinal barrier function, increased villus height, and increased nutrient digestibility (Ma et al., 2019b; Long et al., 2021). The concentration of the volatile fatty acids acetate, propionate, and butyrate, and total volatile fatty acids in ileal contents increased as ESBM inclusion increased ($P < 0.05$), which may be a consequence of the reduced digesta transit rate in the ileum caused by ESBM, which may have resulted in increased fermentation of fiber in the small intestine (Ruckman et al., 2020).

Effect of ESBM on growth performance of pigs

Pigs fed diets with high levels of conventional SBM have low digestibility and absorption of nutrients, low growth rate, and increased incidence of diarrhea, which is believed to be due to the ANF in SBM (Min et al., 2004; Ma et al., 2019; Long et al., 2021). However, because of the reduced ANF in ESBM, growth rate of pigs may be increased if ESBM is used instead of conventional SBM (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Navarro et al., 2017; Long et al., 2021). Pigs fed diets containing ESBM also have reduced diarrhea compared with pigs fed diets with extruded full-fat SBM, conventional SBM, soy protein concentrate, fermented soybean meal, or fish meal (Zhou et al., 2011; Long et al., 2021; Jones et al., 2018; Li et al., 2019; Zhang and Piao, 2022).

Enzyme-treated SBM is inexpensive compared with milk proteins and blood proteins, which are sometimes used as AA-containing ingredients in diets for weanling pigs. The average daily feed intake of pigs fed a diet containing ESBM instead of extruded full-fat SBM was

reduced, but there was no change in average daily gain, which resulted in an increased gain-to-feed ratio for pigs fed the ESBM diet (Li et al., 2019; Long et al., 2021). Ruckman et al. (2020) included 7, 14, or 21% ESBM in diets for weanling pigs and pigs fed the diets with 14 or 21% ESBM had reduced average daily gain and final body weight compared with pigs fed the diet with 7% ESBM, but the gain-to-feed ratio was not affected. It is, however, possible that the source of ESBM used in the experiment may have been heat-damaged, but because no measures for heat damage were reported, this hypothesis cannot be verified. Regardless, as is the case for any other feed ingredient, it is important that ESBM is not heat-damaged during production or drying because that reduces growth performance (Navarro et al., 2017).

Substituting SBM for 6 to 8% ESBM improved growth performance in weanling pigs by enhancing nutrient digestibility, boosting antioxidant capacity, and strengthening intestinal barrier function (Tan et al., 2024). Similarly, dietary inclusion of 8% of ESBM reduced the diarrhea index and improved nutrient utilization, contributing to better growth outcomes (Tang et al., 2023). Enzyme-treated SBM, when included at 10 to 15% of the diet, along with soy protein concentrate and fermented soybean meal, enabled a reduction in inclusion of animal protein in diets for pigs without compromising growth or intestinal health. In comparison to fish meal, including 7 to 14% of ESBM, provided equal or improved growth performance and reduced the incidence of diarrhea, and also supporting better antioxidant capacity and intestinal integrity markers (Yang et al., 2022). Increased average daily gain, improved immune responses, and enhanced antioxidant status in pigs fed diets containing 9 to 12% ESBM have also been reported, which may have been associated with better nutrient absorption and reduced protein fermentation in the hindgut (Li et al., 2021, Zhang and Piao, 2022).

Improved growth performance due to enhanced immune response and improved intestinal morphology was observed when 10% ESBM was included in diets for weanling pigs (Long et al., 2021). Specifically, pigs fed the ESBM diet had an average daily gain of 445 g, whereas pigs fed a diet containing full-fat soybeans gained only 366 g per day (Long et al., 2021). In low-protein diets, inclusion of up to 15% ESBM maintained growth performance and reduced nitrogen excretion compared with pigs fed diets based on fish meal, indicating both nutritional and environmental advantages (Li et al., 2019). Additionally, partial replacement of SBM and soy protein isolate with ESBM improved feed intake, weight gain, and immune function in weanling piglets (Zhou et al., 2011).

Recommended inclusion rates and feeding guidelines

Enzyme-treated SBM is recommended for inclusion in weanling pig diets at levels of 6 to 10% during the early nursery phase (7–11 kg body weight). At this stage, ESBM improve growth performance, reduce post-weaning diarrhea, and enhance intestinal health by increasing villus height, promoting barrier function, and modulating inflammatory responses (Ma et al., 2019a; 2019b; Long et al., 2021; Tang et al., 2023; Tan et al., 2024). As pigs transition into the post-nursery phase (11–25 kg body weight), inclusion rates of 10 to 15% are appropriate, as ESBM can effectively replace animal protein supplements without compromising intestinal health or average daily gain (Yang et al., 2022; Deng et al., 2023). In low-protein, AA-supplemented diets, ESBM may be included by up to 15% without negatively impacting growth performance.

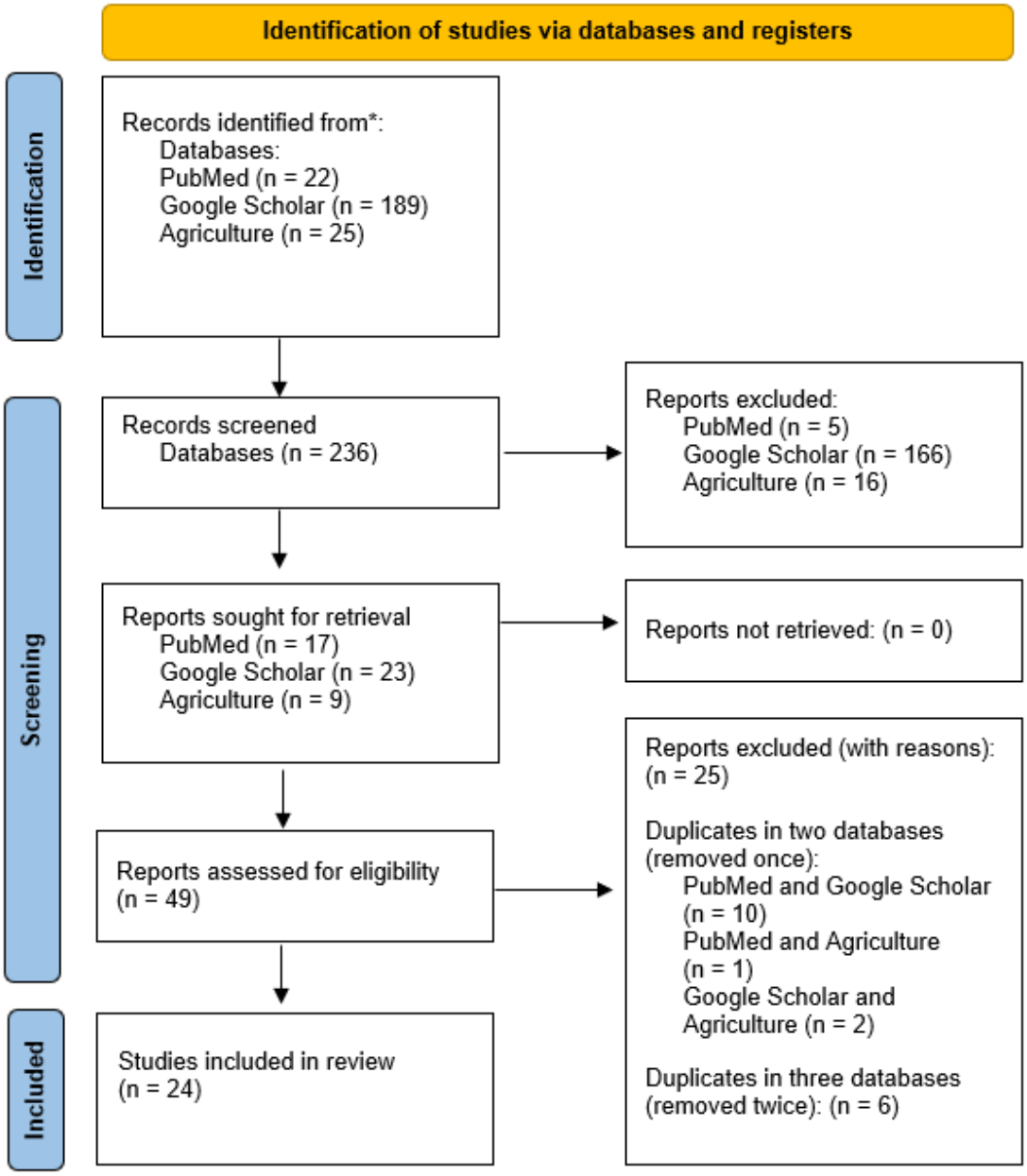
CONCLUSION

Enzyme-treated SBM has a greater concentration of nutrients and contains fewer ANF than conventional SBM, which results in improved intestinal health and growth performance of

weanling pigs. Therefore, ESBM can replace animal proteins in diets for young pigs. It is, however, important to make sure that ESBM is not heat-damaged during production because that will reduce the digestibility of other nutrients, including Lys. Inclusion of 6 to 10% ESBM in starter diets and 10 to 15% in diets fed during the post-weaning period appears to provide the best growth performance. In low-protein diets, it is possible that slightly greater inclusion rates may be used.

FIGURES AND TABLES

Figure 3.1. Flow diagram of study selection for enzyme-treated soybean meal (ESBM) in weanling pigs[1]



[1] Adapted from Page et al. (2020) and White et al. (2024).

Figure 3.2. Processes involved in producing soybean meal and enzyme-treated soybean meal.

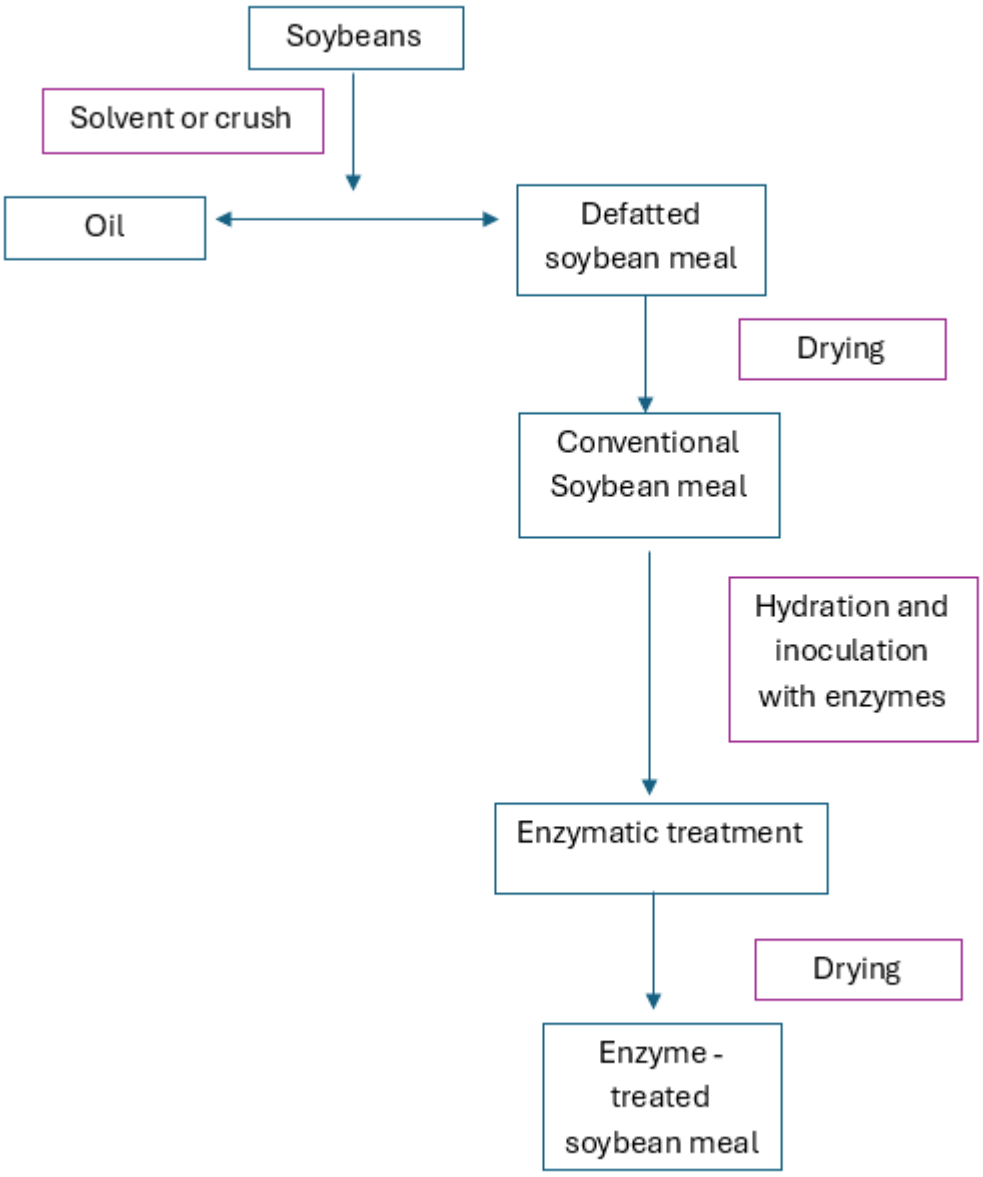


Table 3.1. Papers reporting results of experiments in which enzyme-treated soybean meal was included in diets for weanling pigs

Author	Publication	Exposure	Outcomes/conclusions	Search
Tan et al. (2024)	Enzymolytic soybean meal- impact on growth performance, nutrient digestibility, antioxidative capacity, and intestinal health of weaned piglets	ESBM composition	Amino acids, small peptides, maltose, sucrose, raffinose, stachyose, starch, glycinin, and β-conglycinin.	PubMed, Google Scholar
		Digestibility	Apparent ileal digestibility of dry matter, gross energy, crude protein, and ether extract in weaned piglets.	
		Growth performance/gut health	Substitution of SBM with ESBM resulted in growth improvement in weaned pig, attributed to enhanced nutrient digestibility, antioxidant capacity, and intestinal barrier function.	
Tang et al. (2023)	Effect of heating, microbial fermentation, and enzymatic hydrolysis of soybean meal on growth performance, nutrient digestibility, and intestinal microbiota of weaned piglets	ESBM composition	Lectins, glycinin, and β-conglycinin.	PubMed, Google Scholar
		Digestibility	Apparent total tract digestibility of energy and dry matter.	
		Growth performance/gut health	Dietary supplementation with ESBM reduced the diarrhea index and improved nutrient digestibility.	
Mallea et al. (2023)	Nutritional value of a new source of cheese coproduct fed to weanling pigs	ESBM composition	Dry matter, crude protein, acid-hydrolyzed ether extract, ash, amino acids, and total dietary fiber.	PubMed, Google Scholar
		Digestibility	Apparent ileal digestibility and standardized ileal digestibility of crude protein and amino acids. Apparent total tract digestibility of dry matter and energy.	
Garavito et al. (2023)	Nutritional value of high protein ingredients fed to growing pigs in comparison to commonly used protein sources in swine diets	ESBM composition	Dry matter, crude protein, crude fiber, ash, amino acids.	PubMed, Google Scholar
		Digestibility	Apparent ileal digestibility of amino acids. Standardized ileal digestibility of amino acids.	
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	

Table 3.1. (cont.)

Deng et al. (2023)	Comparative effects of soy protein concentrate, enzyme-treated soybean meal, and fermented soybean meal replacing animal protein supplements in feeds on growth performance and intestinal health of nursery pigs	ESBM composition	Glycinin and β-conglycinin.	PubMed, Agricola
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, and energy.	
		Growth performance/gut health	Soy protein concentrate, enzyme-treated soybean meal, and fermented soybean meal reduced the use of animal protein supplements up to 33% until 7 kg, up to 67% from 7 to 11, and entirely after 11 kg without affecting intestinal health or growth performance.	
Yang et al. (2022)	Comparative efficacy of fish meal replacement with enzymatically treated soybean meal on growth performance, immunity, oxidative capacity and fecal microbiota in weaned pigs	ESBM composition	Dry matter, crude protein, ash, amino acids, trypsin inhibitors, glycinin, and β-conglycinin.	PubMed, Google Scholar
		Growth performance/gut health	Replacing fish meal with enzymatically treated soybean protein shows equal or better growth performance, accompanied by a decreasing diarrhea rate. ESBM has a positive impact on serum concentrations of indicators for intestinal integrity, and antioxidant capacity.	
Engelsmann et al. (2022)	Age-dependent development in protein digestibility and intestinal morphology in weaned pigs fed different protein sources	Digestibility	Standardized ileal digestibility of crude protein and amino acids	PubMed, Google Scholar, Agricola
		Growth performance/gut health	No differences between wheat, SBM, ESBM, rapeseed meal, and casein were found in terms of morphological characteristics of the small intestine or incidence of diarrhea.	
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	

Table 3.1. (cont.)

Zhang et al. (2022)	Different dietary protein sources influence growth performance, antioxidant capacity, immunity, fecal microbiota, and metabolites in weaned piglets	ESBM composition	Dry matter, crude protein, crude fiber, Lys, Met, Thr, Trp, calcium, phosphorus, starch.	PubMed, Google Scholar, Agricola
		Digestibility	Apparent total tract digestibility of crude protein and gross energy	
		Growth performance/gut health	ESBM increased average daily gain and improved antioxidant status and immunity via increased serum levels and modulated fecal microbiota composition because reduced protein fermentation.	
Li et al., (2021)	Enzyme-treated soybean meal replacing extruded full-fat soybean affects nitrogen digestibility, cecal fermentation characteristics, and bacterial community of newly weaned piglets	ESBM composition	Dry matter, crude protein, ether extract, ash, gross energy, neutral detergent fiber, acid detergent fiber, trypsin inhibitors, glycinin and β-conglycinin	PubMed, Google Scholar
		Digestibility	Apparent ileal digestibility of amino acids	
		Growth performance/gut health	ESBM improved nitrogen utilization and inhibited protein fermentation in the hindgut of weaned piglets.	
Long et al. (2021)	Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets	Growth performance/gut health	ESBM enhances growth performance via improving antioxidant status, immune response, intestinal morphology, and barrier function of nursery pigs.	PubMed, Google Scholar, Agricola
Acosta et al. (2021)	Corn protein has greater concentrations of digestible amino acids and energy than low-oil corn distillers dried grains with soluble when fed to pigs but does not affect the growth performance of weanling pigs	ESBM composition	Dry matter, crude protein, acid-hydrolyzed ether extract, ash, amino acids.	PubMed, Google Scholar

Table 3.1. (cont.)

Nørgaard et al. (2021)	Amino acid absorption profiles in growing pigs fed different protein sources.	ESBM composition	Dry matter, crude protein, amino acids, and total dietary fiber.	Google Scholar, Agricola
Ruckman et al. (2020)	The effects of enzymatically treated soybean meal on growth performance and intestinal structure, barrier integrity, inflammation, oxidative status, and volatile fatty acid production of nursery pigs	Growth performance/gut health	ESBM improved oxidative status and intestinal barrier integrity, but impact on intestinal inflammation and morphology was minimal.	PubMed, Google Scholar
Ton-Nu et al. (2020)	Thermomechanical and enzyme-facilitated processing of soybean meal enhanced in vitro kinetics of protein digestion and protein and amino acid digestibility in weaned pigs	ESBM composition	Dry matter, crude protein, crude fiber, ash, amino acids, calcium, phosphorus, starch, neutral detergent fiber, acid detergent fiber, trypsin inhibitors, lectins, glycinin, and β-conglycinin	Google Scholar
		Digestibility	Apparent ileal digestibility of crude protein and amino acids	
Li et al. (2019)	Effects of different protein sources completely replacing fish meal in a low-protein diet on growth performance, intestinal digestive physiology, and nitrogen digestion and metabolism in nursery pigs	ESBM composition	Dry matter, crude protein, crude fiber, ether extract, ash, amino acids, calcium, phosphorus, starch, trypsin inhibitors, glycinin, and β-conglycinin	PubMed, Google Scholar, Agricola
		Digestibility	Apparent ileal digestibility of crude protein and amino acids.	
		Growth performance/gut health	ESBM replaced fish meal in low-protein, AA-supplemented diets without affecting growth performance and maintained intestinal digestive physiology, nitrogen digestion and metabolism in nursery pigs. Feeding pigs with HP300 decreased fecal nitrogen excretion.	
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	

Table 3.1. (cont.)

Ma et al. (2019)	Comparative effects of enzymolytic soybean meal and antibiotics in diets on growth performance, antioxidant capacity, immunity, and intestinal barrier function in weaned pigs	ESBM composition	Dry matter, crude protein, ether extract, ash, amino acids, gross energy, sucrose, raffinose, verbascose, neutral detergent fiber, acid detergent fiber, trypsin inhibitors, glycinin, and β-conglycinin	Google Scholar
		Digestibility	Apparent total tract digestibility of dry matter, crude protein, and gross energy.	
		Growth performance/gut health	ESBM reduced diarrhea incidence and improved growth performance by improving antioxidant capacity, immunity, and intestinal barrier function.	
Ma et al. (2019)	Effects of replacing soybean meal, soy protein concentrate, fermented soybean meal or fish meal with enzyme-treated soybean meal on growth performance, nutrient digestibility, antioxidant capacity, immunity, and intestinal morphology in weaned pigs.	ESBM composition	Dry matter, crude protein, ether extract, ash, amino acids, gross energy, sucrose, raffinose, verbascose, neutral detergent fiber, acid detergent fiber, trypsin inhibitors, glycinin, and β-conglycinin. Concentration of small peptides.	Google Scholar, Agricola
		Growth performance/gut health	ESBM improved growth performance, immune function, and antioxidant capacity as effectively as fish meal. ESBM could replace soybean meal, soy protein concentrate, fermented soybean meal or fish meal in weaned pig diets. ESBM could be used in weaned pig diets for at least 28 days.	
Jones et al. (2018)	Evaluating the effects of fish meal source and level on growth performance of nursery pigs	ESBM composition	Dry matter, crude protein, ether extract, ash, amino acids, calcium, and phosphorus	PubMed, Google Scholar
Navarro et al. (2017)	Amino acid digestibility by weanling pigs of processed ingredients originating from soybeans, 00-rapeseeds, or a fermented mixture of plant ingredients	ESBM composition	Dry matter, crude protein, acid-hydrolyzed ether extract, ash, amino acids, calcium, phosphorus, energy, sucrose, raffinose, stachyose, neutral detergent fiber, acid detergent fiber, and trypsin inhibitors.	PubMed, Google Scholar
		Digestibility	Apparent ileal digestibility of crude protein, ash, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	

Table 3.1. (cont.)

Hossain et al. (2016)	Apparent total tract digestibility and ileal digestibility of dry matter, nitrogen, energy, and amino acids in conventional, Bacillus subtilis-fermented, and enzyme-treated soybean meal fed to weanling pigs.	ESBM composition	Dry matter, crude protein, crude fiber, Lys, Met, Thr, Trp, calcium, phosphorus, starch, ether extract, ash, amino acids, calcium, phosphorus,	Google Scholar
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, energy, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	
Haghbayan et al. (2015)	The effect of replacing fish meal in the diet with enzyme-treated soybean meal (HP310) on growth and body composition of rainbow trout fry	ESBM composition	Crude protein, crude fiber, ether extract, ash, amino acids, trypsin inhibitors, and phytic acid.	Google Scholar
Goebel and Stein. (2011)	Phosphorus digestibility and energy concentration of enzyme-treated and conventional soybean meal fed to weanling pigs	ESBM composition	Dry matter, crude protein, ether extract, ash, amino acids, calcium, phosphorus, sucrose, raffinose, stachyose, neutral detergent fiber, acid detergent fiber, trypsin inhibitors, glycinin, and β-conglycinin	PubMed, Google Scholar, Agricola
		Digestibility	Apparent ileal digestibility of dry matter, ash, gross energy, crude protein, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	
Zhou et al. (2010)	Effect of feeding enzymolytic soybean meal on performance, digestion, and immunity of weaned pigs	ESBM composition	Dry matter, crude protein, ash, small peptide concentration, and trypsin inhibitors.	Google Scholar
		Growth performance/gut health	Enzymolytic soybean meal partially replacing common soybean meal and soy protein isolate improved feed intake, weight gain, and immune function of weaned piglets.	
Cervantes-Pahm et al. (2010)	Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs	ESBM composition	Dry matter, crude protein, crude fiber, ether extract, amino acids, calcium, phosphorus, glucose, fructose, raffinose stachyose, trypsin inhibitors, glycinin, and β-conglycinin	PubMed, Google Scholar, Agricola
		Digestibility	Apparent ileal digestibility of dry matter, crude protein, and amino acids. Standardized ileal digestibility of crude protein and amino acids.	

Table 3.2. Concentrations of dry matter, fat, crude protein, amino acids (AA), ash, and minerals in soybean meal (SBM) and enzyme-treated soybean meal (ESBM), as-is basis[1]

Item	n	SBM	SD[2]	CV	n	ESBM	SD	CV
Dry matter, %	9	89.00	1.32	1.49	22	92.11	1.84	2.00
Ether extract, %	4	1.31	0.55	42.18	11	1.68	0.59	35.34
Acid-hydrolyzed ether extract, %	2	2.40	1.65	68.79	4	1.70	0.64	37.70
Crude protein, %	11	46.50	1.72	3.69	23	54.25	2.27	4.18
Indispensable AA, %								
Arg	11	3.36	0.30	8.91	20	3.93	0.81	20.48
His	11	1.19	0.10	8.67	18	1.59	0.44	27.43
Ile	11	2.15	0.30	13.72	20	2.57	0.52	20.23
Leu	11	3.61	0.30	8.24	20	4.25	0.82	19.22
Lys	11	2.94	0.20	6.80	22	3.45	0.67	19.30
Met	11	0.63	0.07	11.74	21	0.69	0.22	31.80
Phe	11	2.53	0.66	26.15	20	3.04	0.89	29.41
Thr	11	1.81	0.13	7.23	21	2.19	0.42	19.11
Trp	8	1.35	1.92	141.58	17	0.70	0.17	23.54
Val	11	2.23	0.30	13.27	20	2.68	0.55	20.69
Dispensable AA, %								
Ala	9	1.96	0.22	11.27	18	2.31	0.23	9.79
Asp	10	5.34	0.43	8.02	18	5.91	0.48	8.08
Cys	11	0.76	0.23	30.04	18	0.79	0.21	26.52

Table 3.2. (cont.)

Glu	10	8.54	0.64	8.62	18	9.36	0.58	6.22
Gly	10	1.96	0.16	8.30	18	2.35	0.38	16.12
Pro	10	2.33	0.22	9.54	18	2.69	0.23	8.49
Ser	10	2.22	0.32	14.63	18	2.46	0.28	11.54
Tyr	9	1.59	0.23	14.18	18	1.87	0.25	13.49
Ash, %	8	6.33	0.47	7.47	19	6.79	0.54	7.90
Ca, %	5	0.28	0.05	17.00	13	0.32	0.04	13.79
P, %	5	0.64	0.03	4.89	13	0.72	0.03	3.92
Phytate P, %	1	0.48	-	-	1	0.11	-	-

[1]Values obtained from references included in this review.

[2] SD, standard deviation; CV, coefficient of variation.

Table 3.3. Concentrations of energy, carbohydrates, and anti-nutritional factors in conventional soybean meal (SBM) and enzyme-treated soybean meal (ESBM), as-is basis[1]

Item	n	SBM	SD[2]	CV	n	ESBM	SD	CV
Energy, kcal/kg								
Gross energy	4	4,203	72.58	1.73	6	4,493	54.15	1.21
Digestible energy	-	-	-	-	1	3,887	-	-
Metabolizable energy	-	-	-	-	1	3,634	-	-
Glucose, %	-	-	-	-	1	0.49	-	-
Maltose, %	1	0.45	-	-	1	0.01	-	-
Fructose, %	1	0.63	-	-	1	1.11	-	-
Sucrose, %	6	5.63	1.53	27.27	8	1.15	1.68	146.63
Starch, %	2	1.83	0.09	4.72	4	1.02	0.41	40.56
Crude fiber, %	5	3.93	1.79	45.48	10	3.84	0.97	25.19
Neutral detergent fiber, %	5	9.97	3.83	38.43	9	11.92	2.38	19.96
Acid detergent fiber, %	5	6.17	1.93	31.33	9	5.74	1.01	17.62
Total dietary fiber, %	1	20.30	-	-	2	21.02	3.82	18.17
Insoluble dietary fiber, %	1	17.50	-	-	2	18.97	2.37	12.47

Table 3.3. (cont.)

Soluble dietary fiber, %	1	2.80	-	-	2	2.06	1.46	70.80
Raffinose, %	5	1.08	0.10	9.42	9	0.49	0.60	122.28
Stachyose, %	5	4.29	1.31	30.51	9	0.81	0.80	99.54
Anti-Nutritional factors								
Trypsin inhibitors, TIU/mg	6	5.58	2.62	46.86	15	1.71	0.86	50.12
Lectins, mg/kg	1	300.00	-	-	1	29.00	-	-
β-Conglycinin, mg/g	6	40.04	57.54	143.74	13	1.22	2.11	172.64
Phytic Acid, g/100g	1	1.70	-	-	1	0.40	-	-

[1]Values obtained from references included in this review.

[2] SD, standard deviation; CV, coefficient of variation.

Table 3.4. Peptide size of protein in soybean meal (SBM) and enzyme-treated soybean meal (ESBM)

Peptide Size concentration g/100 g of CP	SBM	ESBM
> 60kDa	27.00	28.87
20-60 kDa	43.16	37.51
< 20kDa	29.84	33.62
Small peptide <500 Da[1]	3.66	18.30

[1] as-feed basis; Cervantes-Pahm et al., 2010; Ma et al., 2019.

Table 3.5. Apparent ileal digestibility of dry matter, ash, gross energy (GE), crude protein, and amino acids (AA) in enzyme-treated soybean meal

Item	NRC. 2012	Tan et al. 2024	Garavito-Duarte et al. 2023	Mallea et al. 2023	Li et al. 2021	Ton-Nu et al. 2020	Li et al. 2019	Navarro et al. 2017	Navarro et al. 2017	Hossain et al. 2016	Cervantes-Pahm and Stein. 2010	Average	SD[1]	CV
Dry matter, %	-	-	54.5	-	-	-	-	-	-	82.6	61.3	66.1	10.4	15.7
Crude protein, %	82.0	72.8	61.7	-	-	84.2	83.2	81.9	75.1	83.0	76.8	77.3	6.7	8.7
Ash, %	-	-	-	-	-	-	-	37.9	37.2	-	-	37.5	0.3	0.8
GE, %	-	-	55.6	-	-	-	-	-	-	81.7	-	68.6	10.6	15.5
Indispensable AA, %														
Arg	92.0	88.9	-	92.5	75.1	93.1	89.0	91.6	87.4	81.0	90.4	87.7	5.3	6.1
His	87.0	81.6	-	91.9	88.3	89.2	83.9	89.4	86.1	80.7	82.7	86.0	3.5	4.1
Ile	86.0	81.4	-	92.0	70.7	88.8	85.1	88.7	85.3	83.3	84.4	84.4	5.4	6.4
Leu	86.0	83.0	-	91.7	69.8	88.4	82.0	88.5	85.7	80.5	83.7	83.7	5.6	6.7
Lys	83.0	76.6	-	87.3	85.0	89.3	85.8	84.5	79.1	77.9	82.2	83.1	3.9	4.7

Table 3.5. (cont.)

Met	88.0	83.4	-	93.0	86.5	91.0	89.1	90.3	87.6	84.6	86.2	88.0	2.8	3.2
Phe	83.0	83.5	-	92.3	92.1	89.2	85.2	89.6	86.6	83.9	85.5	87.5	3.0	3.4
Thr	78.0	69.0	-	85.6	74.9	81.7	79.1	80.7	76.5	77.2	72.5	77.5	4.5	5.8
Trp	80.0	79.4	-	90.7	77.6	90.3	83.1	89.5	85.8	-	82.1	84.8	4.5	5.3
Val	84.0	76.9	-	88.8	73.8	85.5	83.2	85.3	80.7	84.6	78.9	82.0	4.3	5.2
Dispensable AA, %														
Ala	82.0	72.6	-	85.9	66.0	85.1	79.1	81.1	75.8	76.7	77.3	77.7	5.5	7.1
Asp	83.0	76.7	-	88.6	80.9	86.2	82.0	85.3	82.6	79.7	82.4	82.7	3.2	3.9
Cys	68.0	52.2	-	79.6	75.6	76.5	74.0	74.8	69.2	78.2	73.7	72.6	7.3	10.1
Glu	86.0	80.5	-	91.4	81.9	89.4	83.0	87.6	84.3	81.7	89.3	85.5	3.6	4.2
Gly	76.0	56	-	71.5	73.4	78.4	63.8	64.6	52.5	73.6	59.3	65.9	7.9	12.0
Pro	73.0	74.8	-	39.5	78.8	75.8	79.8	31.0	-0.2	80.4	43.9	63.0	18.5	29.4
Ser	83.0	76.7	-	89.6	77.3	86.4	84.3	86.7	83.6	82.0	78.9	82.8	4.0	4.8
Tyr	86.0	81.1	-	89.9	71.9	89.1	85.2	89.2	85.7	88.0	86.0	85.1	5.1	5.9

[1] SD, standard deviation; CV, coefficient of variation.

Table 3.6. Standardized ileal digestibility of crude protein and amino acids (AA) in enzyme-treated soybean meal

Item	Mallea et al. 2023	Garavito-Duarte et al. 2023	Ton-Nu et al. 2020	Navarro et al. 2017	Navarro et al. 2017	Hossain et al. 2016	NRC. 2012	Cervantes-Pahm and Stein. 2010	Average	SD[1]	CV
Crude protein, %	82.0	-	-	89.9	85.2	88.9	88.0	91.9	87.6	3.5	4.0
Indispensable AA, %											
Arg	92.8	99.6	96.0	96.9	93.5	84.1	96.0	98.1	94.4	4.8	5.1
His	86.5	95.5	92.6	93.4	90.6	84.5	90.0	88.8	90.3	3.6	4.0
Ile	86.3	95.1	91.9	91.7	88.9	86.3	89.0	89.7	90.0	3.0	3.3
Leu	86.6	94.9	91.6	91.7	89.2	83.9	89.0	89.2	89.6	3.4	3.7
Lys	81.1	89.4	92.3	87.3	82.5	82.0	86.0	88.1	86.1	3.9	4.6
Met	87.7	95.9	93.6	92.9	90.5	88.8	91.0	92.1	91.6	2.6	2.9
Phe	87.9	95.3	91.9	92.6	90.1	86.6	86.0	91.8	90.9	2.7	3.0
Thr	79.5	89.5	88.4	87.5	83.9	83.1	83.0	85.7	85.4	3.2	3.8
Trp	85.4	93.8	94.0	93.3	90.0	-	83.0	87.5	90.7	3.3	3.7
Val	83.4	92.6	90.3	90.0	86.2	89.3	89.0	89.4	88.7	2.8	3.2

Table 3.6. (cont.)

Dispensable AA, %											
Ala	80.1	90.6	90.1	88.3	83.9	85.7	86.0	88.5	86.7	3.5	4.0
Asp	80.8	91.1	89.2	88.5	86.3	83.5	86.0	88.1	86.8	3.3	3.8
Cys	63.6	82.1	84.5	82.3	77.3	85.1	73.0	85.1	80.0	7.2	8.9
Glu	83.8	93.7	91.6	90.1	87.1	85.9	88.0	93.5	89.4	3.6	4.0
Gly	71.9	78.0	88.4	86.6	77.7	87.4	89.0	94.4	83.5	7.2	8.7
Pro	96.1	53.1	97.8	101.2	80.3	87.3	112.0	148.2	94.8	26.5	27.9
Ser	84.3	94.1	91.4	92.0	89.2	87.9	87.0	89.2	89.7	2.9	3.3
Tyr	86.7	92.8	92.5	92.4	89.5	92.3	92.0	92.0	91.2	2.1	2.3

[1] SD, standard deviation; CV, coefficient of variation.

Table 3.7. Apparent total tract digestibility of dry matter, crude protein, ether extract, gross energy, Ca, and P in enzyme-treated soybean meal

Item	Tan et al. 2024	Tan et al. 2024	Tan et al. 2024	Mallea et al. 2023	Tang et al. 2023	Ma et al. 2019	Hossain et al. 2016	Goebel and Stein. 2011	Goebel and Stein. 2011	Goebel and Stein. 2011	Average	SD[1]	CV
Dry matter, %	82.7	85.7	82.0	85.6	86.8	81.5	88.9	-	96.4	96.3	87.3	5.3	6.1
Crude protein, %	78.9	79.6	81.8	-	72.5	76.0	88.8	-	-	-	79.6	5.1	6.4
Ether extract, %	64.1	71.0	61.2	-	-	-	-	-	-	-	65.4	4.1	6.3
Gross energy, %	82.2	84.9	80.9	86.4	82.3	81.9	87.7	87.6	88.0	-	84.6	2.7	3.2
Ca, %	-	-	-	-	-	-	-	-	53.6	63.8	58.7	5.1	8.7
P, %	-	-	-	-	-	-	-	-	59.8	63.8	61.8	2.0	3.2

[1] SD, standard deviation; CV, coefficient of variation.

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CHAPTER 4: ANALYSIS FOR TOTAL LYSINE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND REACTIVE LYSINE DETERMINATION BY THE HOMOARGININE PROCEDURE IN ANIMAL- AND PLANT-BASED PROTEIN INGREDIENTS

ABSTRACT

Two experiments were conducted to determine total and reactive Lys in feed ingredients, based on the hypothesis that their concentrations are consistent between laboratories and that the homoarginine procedure accurately determines reactive Lys in protein ingredients. In Experiment 1, sixteen soy protein samples, including enzyme-treated soybean meal (**ESBM**), soybean meal (**SBM**), soy protein concentrate (**SPC**), fermented soybean meal (**FSBM**), and extruded soybean meal (**EXSBM**), were analyzed for dry matter, crude protein, acid-hydrolyzed ether extract, fiber, sugars, minerals, and total Lys. Total Lys was determined by high-performance liquid chromatography after hydrolysis with 6 *N* HCl for 24 h at 110 °C. To confirm the reproducibility of the analytical procedure, samples were also analyzed at an external laboratory. The average total Lys among samples was analyzed at 3.10% at the external laboratory and 3.20% at the University of Illinois, and these values were not different. The coefficient of variation (**CV**) was less than 5% for most samples, indicating high reproducibility of results from the Lys analysis. In Experiment 2, reactive Lys was determined using the homoarginine method, which includes the guanidination of each ingredient using an O-Methylisourea solution for 72 h under alkaline conditions, followed by hydrolysis with 6 *N* hydrochloric acid for 24 h at 110 °C. Ten plant- or animal-derived protein ingredients were analyzed, and to verify the accuracy of this procedure, samples were also analyzed at an external

laboratory, and results from the external laboratory were compared with results from the University of Illinois. The average reactive Lys among samples was analyzed at 1.05% at the external laboratory and 1.03% at the University of Illinois, and these values were not different. The CV was less than 10% for most ingredients, indicating consistency for the method. In conclusion, the proposed procedure to analyze total Lys can be used to determine the concentration of Lys in soybean ingredients, and the proposed procedure to analyze plant- and animal-derived protein ingredients for reactive Lys generated results that were not different from values obtained at an external laboratory.

Keywords: animal protein, homoarginine, Lys, plant protein, reactive Lys.

Abbreviations: AA, amino acids; AEE, acid-hydrolyzed ether extract; CP, crude protein; CV, coefficient of variation; DM, dry matter; ESBM, enzyme-treated soybean meal; EXSBM, extruded soybean meal; FSBM, fermented soybean meal; HPLC, high-performance liquid chromatography; SBM, soybean meal; SD, standard deviation; SPC, soy protein concentrate; UHPLC, ultra high-performance liquid chromatography.

INTRODUCTION

Feed ingredients from soybeans are important agriculture crops around the world. Soybeans are crushed to provide soybean oil and soybean meal (**SBM**; Galkanda-Arachchige et al., 2021). Most SBM is used as a source of amino acids (**AA**) in diets for pigs, poultry, dairy, and other animals. Lysine is often a limiting AA when pigs are fed diets based on cereal grains. Because of the trypsin inhibitors in raw soybeans, heat treatment is necessary before ingredients

from soybeans can be used in diets for pigs and poultry. However, the structure of Lys may be modified during heat processing, which may reduce the concentration and digestibility of Lys in heat-processed ingredients (Fontaine et al., 2007) due to the Maillard reaction and other reactions related to heat damage (Navarro et al., 2017; Ton et al., 2020). The Maillard reaction causes the Ɛ-amino group of Lys to bind to reducing sugars, which makes the Lys unavailable for use in protein synthesis by animals. This has been demonstrated in SBM and soybean expellers (González-Vega et al., 2011; Oliveira et al., 2020b; Espinosa et al., 2021), and in other ingredients (Almeida et al., 2013; 2014a; 2014b; 2014c; Oliveira et al., 2020a; Sung et al., 2025). Total Lys in a feed ingredient or diet can be determined after hydrolysis of the sample with 6 *N* hydrochloric acid [method 982.30 E(a); AOAC Int., 2019], but in heat-damaged soybean products, some of the modified or unreactive Lys can revert back to Lys during acid hydrolysis, leading to an overestimation of the Lys that is available for protein synthesis (Kim et al., 2012). Therefore, to determine the amount of Lys that can be used for protein synthesis (the reactive Lys), it is necessary to be able to analyze only the Lys that has not been heat damaged and not the damaged Lys that reverted back to Lys during acid hydrolysis. Several methods have been developed to determine only the reactive Lys in feed and food ingredients. One such method is the homoarginine procedure, which involves guanidination of an ingredient with O-Methylisourea (Moughan and Rutherfurd, 1996). This will change the reactive Lys to homoarginine, which can then be analyzed. The homoarginine procedure has been recommended as the method of choice to determine reactive Lys in food proteins (FAO, 2013). However, there is no laboratory in the United States that analyzes reactive Lys using the homoarginine procedure, and it is not known if values for reactive Lys obtained in the other parts of the world are reproducible in the United States. Therefore, two experiments were conducted to test the

hypothesis that total Lys can be accurately analyzed, and that reactive Lys can be accurately determined using the homoarginine procedure in plant- and animal-based protein ingredients.

MATERIALS AND METHODS

Experiment 1: Sixteen soy protein samples from Hamlet Protein A/S (Horsens, Denmark) were analyzed for Lys using high-performance liquid chromatography (**HPLC**). There were seven sources of enzyme-treated soybean meal (**ESBM**), four sources of conventional SBM, two sources of extruded soybean meal (**EXSBM**), two sources of soy protein concentrate (**SPC**), and one source of fermented soybean meal (**FSBM**). The ESBM samples were lab-scale experimental batches, not commercial lots, and reflected production-trial, pilot, or simulated processing conditions. All samples were ground and sieved using a fine test sieve pore size 500 μm (Millipore, Sigma, St. Louis, MO, USA). Each ingredient was divided into two batches. One batch was analyzed for Lys at an external laboratory (University of Missouri, Columbia, MO, USA), and the other batch was analyzed at the University of Illinois (Urbana-Champaign, IL, USA). At the external laboratory, Lys was analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for post-column derivatization and norleucine as the internal standard. At the University of Illinois, all ingredients were analyzed for total Lys on an ultra high-performance liquid chromatography (**UHPLC**), Model QSight LX50 (PerkinElmer; Shelton, CT, USA) using an Agilent InfinityLab Poroshell 120 PFP, 2.1 × 100 mm, 2.7 μm, narrow bore LC column (Luque-Cordoba et al., 2022). The strong mobile phase was 0.1% formic acid in acetonitrile, and the soft mobile phase was 0.1% formic acid in HPLC water. Prior to analysis, samples were hydrolyzed with 6 *N* hydrochloric acid for 24 h at 110 °C [method 982.30 E (a); AOAC Int., 2019]. An L-Lysine

standard was purchased from Sigma-Aldrich, St. Louis, MO, USA, and was diluted to 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 g/L, and a calibration curve was created. The determination coefficient was 0.9989. Prior to analysis, at both laboratories, samples were hydrolyzed with 6 *N* hydrochloric acid for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019].

Experiment 2: Ten ingredient samples which included navy beans, boiled carrots, roasted turkey breast, boiled cassava, boiled plantain, whole meal bread, wheat bagel, pea protein isolate, chia seeds, and sunflower seeds were analyzed for reactive Lys by the homoarginine procedure at an external laboratory (Nutrition Laboratory, Massey University, Palmerston North, New Zealand), as described by Moughan and Rutherfurd (1996). The same 10 ingredients were analyzed for reactive Lys at the University of Illinois, where samples were analyzed on an UHPLC, Model QSight LX50 (PerkinElmer; Shelton, CT, USA) using an Agilent InfinityLab Poroshell 120 PFP, 2.1 × 100 mm, 2.7 µm, narrow bore LC column (Luque-Cordoba et al., 2022). The QSight 210 mass spectrometer was operated in positive electrospray at 5,000 V with a 200 °C source, nebulizer gas at 200 µL/min, drying gas at 100 µL/min, and hot surface induced desolvation temperature of 320 °C. Chromatographic separation on the QSight LX50 UHPLC utilized a 7.0 µL needle and a 20.2 µL loop in partial-loop-fill mode with a 2.0 mm needle height. The autosampler was maintained at 10 °C, and the column oven was set at 40 °C. The needle wash used 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The method used a 2 min equilibration, a flow rate of 0.15 mL/min, and a total run time of 3 min (Martens-Lobenhoffer et al., 2013). To convert reactive Lys to homoarginine 200 mg of each sample were incubated for 72 h at room temperature with 8 mL of O-Methylisourea solution at pH 10.6 to initiate the guanidination reaction (Moughan and Rutherfurd, 1996; Torbatinejad et al., 2005;

Rutherfurd et al., 2006; Pahm et al, 2008). Following guanidination, samples were hydrolyzed using 6 *N* hydrochloric acid at 110 °C for 24 h [method 982.30 E (a); AOAC Int., 2019].

The guanidination conversion in samples analyzed at the external laboratory was performed as described by Moughan and Rutherfurd (1996). At the University of Illinois, four grams of barium hydroxide octahydrate were mixed with 16 mL of boiling distilled deionized water (preboiled for 30 min) in a 50 mL centrifuge tube (Moughan and Rutherfurd, 1996; Rutherfurd et al., 1997a; 1997b). The solution was placed in a hot-water bath at 100°C for approximately 30 minutes (Pahm et al., 2008). Two g of O-Methylisourea was then added to the tube (Moughan and Rutherfurd, 1996). The new solution was mixed and cooled to room temperature, centrifuged at 7,000 g for 10 min, and the supernatant was transferred to a new tube and centrifuged a second time at 7,000 g for 10 min. The precipitate was washed with 2 mL of boiling distilled deionized water and centrifuged at 7,000 g for 10 min. The liquid was transferred to a new tube, and the pH was determined. If the pH was less than 12, a new solution was prepared (Moughan and Rutherfurd, 1996; Torbatinejad et al., 2005), and the pH was adjusted to 10.6 using 2 *N* hydrochloric acid and filled to 20 mL with boiling distilled and deionized water (Moughan and Rutherfurd, 1996; Torbatinejad et al., 2005; Rutherfurd et al., 2006; Rutherfurd and Moughan, 2008; Pahm et al., 2008).

Ingredients used in experiment 1 and in experiment 2 were also analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019). Crude protein (**CP**) in each sample was calculated as nitrogen × 6.25, and nitrogen was measured using the combustion procedure (method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI). Ingredients from experiment 1 were analyzed for ash (method 942.05; AOAC Int., 2019). Acid-hydrolyzed ether extract (**AEE**) was analyzed using *3 N* HCl (AnkomHCl, Ankom Technology,

Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY). These ingredients were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int., 2019) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Stachyose, raffinose, galactose, glucose, maltose, and sucrose in feed ingredients were analyzed by HPLC using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA; Navarro et al., 2018). Minerals were also analyzed (method 985.01 a, b, and c; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200; PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600°C for 2 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). For all ingredients used in experiment 1, all analyzed components were added and subtracted from the concentration of DM to calculate the rest fraction using the following equation (Fanelli et al., 2023):

Rest fraction = [dry matter - (crude protein + acid-hydrolyzed ether extract + ash + total dietary fiber + glucose + galactose + maltose + sucrose + stachyose + raffinose + fructose)].

Statistical analysis

In Experiment 1, a paired t-test was used to compare total Lys concentrations in samples analyzed at the University of Illinois with values analyzed at the external laboratory. In Experiment 2, a paired t-test was also used to compare reactive Lys concentrations in samples analyzed at the external laboratory and reactive Lys in samples analyzed at the University of Illinois. For both experiments, the experimental unit was the individual feed ingredient. The standard deviation (**SD**) were calculated between laboratories to assess consistency and

reproducibility in both experiments. All statistical analyses were completed using SAS software (SAS Institute Inc., Cary, NC, USA). Statistical significance was declared at an α-level of 0.05.

RESULTS

Experiment 1: Dry matter in samples ranged from 91.16% to 92.87% (Table 4.1). Soy protein concentrate contained 62.48% CP, followed by ESBM with 54.73%, FSBM with 50.21%, EXSBM with 48.50%, and SBM with 48.19%. The Lys/CP ratio in SPC was 6.14, and SBM and EXSBM 6.40% had a Lys/CP ratio of 6.19 and 6.40, respectively, whereas ESBM and FSBM had Lys/CP ratios of 5.90% and 4.68%, respectively.

The concentration of Lys in all samples varied from 2.86 to 3.33% if analyzed at the external laboratory and from 3.03 to 3.45% if analyzed at the University of Illinois (Table 4.2). Soybean meal samples analyzed from 2.98 to 3.10% at the external laboratory and from 2.84 to 3.33% at the University of Illinois. For EXSBM, Lys varied from 3.00 to 3.02% at the external laboratory and from 3.03 to 3.17% at the University of Illinois. The concentration of Lys in SPC ranged from 3.74 to 4.14% at the external laboratory and from 3.73 to 3.91% at the University of Illinois. In contrast, FSBM analyzed 2.38% at the external laboratory and 2.35% Lys at the University of Illinois. Statistical analysis demonstrated that there were no differences in Lys concentrations between the two laboratories ($P = 0.175$) and the standard error of the mean was 0.023 indicating low variability among samples.

Experiment 2: Ingredients were nutritionally diverse with DM ranging from 12.80% to 96.09% (Table 4.3). Crude protein ranged from 0.86% to 79.69% and Lys between 0.03% to 6.01%. Boiled cassava, plantain, and carrots contained less than 0.90% CP and Lys in these samples was between 0.03% and 0.06%. Whole grain bread and wheat bagels contained 10.55%

and 12.23% CP, respectively, and 0.24% and 0.31% Lys. Navy beans contained 7.72% CP and 0.65% Lys, whereas chia and sunflower contained 19.59% and 24.82% CP and 0.97% and 0.98% Lys. Crude protein and Lys were 28.34% and 2.41%, respectively, in roasted turkey, whereas, pea protein isolate contained 79.69% CP and 6.01% Lys. Reactive Lys values determined by the external laboratory and the University of Illinois were consistent for all samples (Table 4.4).

DISCUSSION

Experiment 1: The chemical compositions of SBM, ESBM, FSBM, EXSBM, and SPC agreed with reported values (González-Vega et al., 2011; NRC, 2012; Almeida et al., 2014; Deng et al., 2023). Processing methods applied to SBM may influence the concentration of Lys, which is susceptible to heat damage. Conventional SBM production involves dehulling, oil extraction, and toasting, which effectively reduce anti-nutritional factors, but also promote Maillard reactions that may impair Lys availability (Cervantes-Pahm and Stein, 2010; Mukherjee et al., 2016). In EXSBM, a second thermal-mechanical treatment may further disrupt fiber and starch structures, potentially enhancing digestibility, but the additional heat may reduce Lys and reactive Lys (Mukherjee et al., 2016). Enzymatic treatment of SBM degrades oligosaccharides and antigenic proteins, increasing digestibility and preserving both total and reactive Lys (Goebel and Stein, 2011; Ma et al., 2019), although the ingredient does need to be dried after inoculation with enzymes, which includes the risk for overheating, and therefore, heat damage. Fermentation can also improve the quality of SBM by reducing anti-nutritional compounds such as trypsin inhibitors and phytates, and by modifying protein structure; however, some microbes may utilize Lys during fermentation, reducing its final concentration and resulting in a reduced Lys:CP ratio (Thomas and Ingledew, 1992; Cervantes-Pahm and Stein, 2010; Kim et al., 2012).

Like ESBM, FSBM needs an additional drying step following fermentation, which increases the risk of heat damage. Soy protein concentrate is produced by alcohol or acid extraction, which removes soluble carbohydrates and non-protein components, resulting in a greater concentration of protein and Lys (Deng et al., 2023).

Conventional SBM contained less Lys than ESBM and SPC, reflecting the influence of processing on nutrient concentration. In contrast, the second thermomechanical treatment used for EXSBM did not appear to change the total Lys compared with conventional SBM. However, the total Lys values observed for SBM, ESBM, EXSBM, and SPC are within the range of published values for these ingredients (Almeida et al., 2014; González-Vega et al., 2011; Hossain et al., 2016), whereas FSBM contained less Lys than previously published ranges (Cervantes-Pahm and Stein, 2010; Hossain et al., 2016). The Lys:CP ratio in SBM, SPC, and EXSBM was greater than 6.0, indicating that these ingredients likely were not affected by the heat used in processing (Oliveira et al., 2021). In contrast, ESBM and FSBM had Lys:CP ratios below 6, indicating heat-damage during processing, which resulted in destruction of Lys (González-Vega et al., 2011), although some Lys could also have been used during fermentation.

Because these ingredients differed in their susceptibility to heat damage and Lys destruction, it was important to verify that total Lys was measured consistently between laboratories. Despite differences in procedure between the two laboratories, both procedures quantified total Lys using the same initial step of acid hydrolysis to release AA from the protein. The distinction between laboratories was primarily in the analytical platforms used to detect the released Lys; the external laboratory used an ion-exchange AA analyzer with post-column ninhydrin derivatization, whereas the University of Illinois used reversed-phase UHPLC with mass-spectrometric detection, which provides greater selectivity and improved separation of AA

in complex matrices (Serrano et al., 2013). Despite these methodological differences, both procedures measure the same hydrolyzed Lys, and the close agreement in results demonstrates that both analytical approaches may be used to quantify total Lys in soybean ingredients.

Experiment 2: The concentrations of DM, CP, and Lys in all analyzed ingredients were within the ranges previously reported (NRC, 2012; Woyengo et al., 2015; USDA, 2019). These ingredients represented a broad spectrum of protein sources, differing in origin, processing intensity, and structural composition, allowing evaluation of the homoarginine procedure across a wide range of nutritional profiles.

The reactive Lys procedure has been widely used to assess the degree of heat damage in protein sources (Fontaine et al., 2007; Pahm et al., 2008). The homoarginine method quantifies only the Lys residues with unmodified ε-amino groups, excluding those blocked through Maillard or other nonenzymatic reactions. This selectivity makes the procedure a valuable tool for distinguishing nutritionally available Lys from chemically modified Lys that cannot be utilized for protein synthesis.

Several analytical methods have been proposed for determining reactive Lys in protein sources (Moughan and Rutherfurd, 1996; Fontaine et al., 2007; Pahm et al., 2008). The furosine method estimates reactive Lys indirectly by measuring Amadori compounds formed during the Maillard reaction, but its accuracy depends on the conversion factors used in the calculations, which may vary among ingredients (Pahm et al., 2008; Kim et al., 2012; Oliveira et al., 2021). The dinitrophenyl derivatization method measures free ε-amino groups through dinitrophenyl- or trinitrophenyl-Lys formation, but side reactions with carbohydrates during hydrolysis can affect color development and require correction factors (Moughan and Rutherfurd, 1996; Rutherfurd and Gilani, 2009; Oliveira et al., 2021). In contrast, the homoarginine procedure directly converts

unmodified ε-amino groups of Lys into homoarginine, allowing quantification of nutritionally available Lys (Moughan and Rutherfurd, 1996; Rutherfurd and Moughan, 2005; Pahm et al., 2008; Oliveira et al., 2021). The reproducibility of results between two laboratories observed in the present experiment support the conclusion that the homoarginine method provides a robust approach for assessing reactive Lys in feed and food proteins.

The efficiency of Lys conversion to homoarginine varies among ingredients and depends on pH, incubation time, and the Lys-to-O-Methylisourea ratio (Pahm et al., 2008). Previous studies have reported conversion rates above 80% in casein and milk-based products, 60 to 80% in soybean and oilseed meals, and less than 60% in cereal ingredients (Rutherfurd and Moughan, 2005; 2006; Fontaine et al., 2007). These differences are attributed to the structural characteristics of proteins and the degree of heat treatment, which can reduce the availability of free ε-amino groups for guanidination (Pahm et al., 2009). The consistency of reactive Lys results between laboratories in the present study indicates that the reaction conditions used were adequate for achieving reliable conversion among diverse protein matrices.

A key difference between laboratories was the amount of sample used for the guanidination reaction. The external laboratory followed the original Moughan and Rutherfurd (1996) method, which uses small subsamples of 5-10 mg, as it was developed for ingredients with relatively high protein and Lys concentrations. In contrast, the University of Illinois used 200 mg of sample to ensure adequate recovery of homoarginine after hydrolysis, particularly for ingredients with low protein or low Lys content (Pahm et al., 2008). The volume of O-Methylisourea solution added was also adjusted according to the expected Lys concentration of each ingredient to maintain an adequate reagent-to-Lys ratio and ensure complete guanidination.

The approaches used to quantify reactive Lys differed between the external laboratory

and at the University of Illinois. The external laboratory used the classical homoarginine procedure of Moughan and Rutherfurd (1996), which relies on ion-exchange HPLC with post-column ninhydrin derivatization to quantify homoarginine formed during guanidination. In contrast, the University of Illinois applied a procedure in which homoarginine was quantified using reversed-phase UHPLC coupled to tandem mass spectrometry. Despite differences in instrumentation, chromatographic separation, and detection technology, both methods rely on the same underlying chemical principle: the conversion of the ε-amino group of reactive Lys into homoarginine using O-Methylisourea under alkaline conditions. The close agreement in values between laboratories indicates that both methods accurately quantified reactive Lys in ingredients.

CONCLUSION

Results from experiment 1 demonstrated that total Lys was measured consistently between laboratories using different analytical platforms, confirming that both ion-exchange AA analysis and UHPLC provided accurate and comparable values. Results of experiment 2 confirmed the hypothesis that reactive Lys can be accurately analyzed in plant- and animal-based protein ingredients using the homoarginine procedure. Despite the absence of laboratories in the United States routinely using this method and uncertainty regarding the reproducibility of results generated in other countries, the present data demonstrate that the homoarginine procedure provides consistent values for reactive Lys.

TABLES

Table 4.1. Analyzed nutrient composition of seven samples of enzyme-treated soybean meal (ESBM), four samples of soybean meal (SBM), two samples of extruded SBM (EXSBM), two samples of soy protein concentrate (SPC), and one sample of fermented by lactic acid bacteria soybean meal (FSBM), as-fed basis[1], experiment 1

Item	ESBM	SD	CV	SBM	SD	CV	EXSBM	SD	SPC	SD	FSBM
Dry matter, %	92.06	0.63	0.68	91.80	0.55	0.60	92.65	1.24	92.87	0.63	91.16
Crude protein, %	54.73	0.89	1.63	48.19	0.31	0.65	48.50	0.58	62.48	6.83	50.21
Lys/CP	5.90	0.33	5.58	6.19	0.51	8.25	6.40	0.13	6.14	0.47	4.68
AEE, %	1.15	0.72	62.17	2.92	0.59	20.34	1.02	0.49	1.12	0.45	1.52
Ash, %	7.07	0.54	7.64	5.66	1.07	18.89	7.12	0.41	4.90	2.66	7.31
Glucose, %	-	-	-	0.05	0.00	0.00	0.06	0.01	-	-	-
Galactose, %	1.22	0.93	76.83	-	-	-	-	-	-	-	0.40
Sucrose, %	0.36	0.84	237.14	6.31	0.57	9.05	5.45	0.13	-	-	-
Maltose, %	-	-	-	0.42	0.09	22.07	0.46	0.13	-	-	-
Fructose, %	-	-	-	0.07	0.01	14.18	0.10	0.00	-	-	-
Stachyose, raffinose, %	0.72	0.51	70.82	6.81	0.43	6.32	6.29	0.07	2.70	2.97	2.50

Table 4.1. (cont.)

Insoluble dietary fiber, %	18.17	1.88	10.32	16.63	1.08	6.49	15.35	0.49	17.80	2.26	18.45
Soluble dietary fiber, %	1.57	0.63	40.37	0.63	0.10	15.32	1.05	0.21	1.20	1.70	1.60
Total dietary fiber, %	19.74	2.39	12.11	17.25	1.03	6.00	16.40	0.71	19.00	3.96	20.05
Ca, %	0.35	0.04	11.23	0.29	0.09	30.37	0.34	0.03	0.26	0.09	0.33
P, %	0.91	0.04	4.07	0.71	0.15	21.37	0.85	0.07	0.80	0.02	0.89
K, %	2.29	0.17	7.40	1.82	0.47	25.95	2.33	0.21	1.25	1.20	2.37
Mg, %	0.33	0.04	12.42	0.26	0.06	23.17	0.32	0.04	0.19	0.19	0.35
Na, %	0.02	<0.01	16.40	0.02	0.01	38.49	0.02	0.01	0.13	0.17	0.05
Cu, mg/kg	44.75	2.97	6.64	35.57	7.94	22.31	40.96	1.78	34.98	0.84	45.92
Fe, mg/kg	206.12	92.64	44.94	164.13	80.57	49.09	215.45	46.24	189.37	33.43	362.17
Mn, mg/kg	89.25	7.72	8.65	77.72	19.32	24.86	98.51	3.39	75.06	19.94	106.04
Zn, mg/kg	59.66	2.75	4.61	48.05	10.38	21.59	58.20	3.08	38.24	12.12	67.15
Rest fraction[2], %	7.53	2.79	37.03	4.13	0.73	17.80	7.25	0.04	2.66	2.58	9.17

[1]SD, standard deviation; CV, coefficient of variation; Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract.

[2]Rest fraction = calculated using the following equation [dry matter - (crude protein + acid-hydrolyzed ether extract + ash + total dietary fiber + glucose + galactose + maltose + sucrose + stachyose + raffinose + fructose)].

Table 4.2. Lys concentration in percentage in enzyme-treated soybean meal (ESBM), soybean meal (SBM), soy protein concentrate (SPC), SBM fermented by lactic acid bacteria (FSBM), and extruded soybean meal (EXSBM), as-fed basis[1] experiment 1

	ESBM							SBM				EXSBM		SPC		FSBM		*P*-
Item	1	2	3	4	5	6	7	1	2	3	4	1	2	1	2	1	SEM	value
MO	3.08	2.86	2.95	3.33	3.12	2.87	2.81	2.98	3.03	3.02	3.10	3.02	3.00	3.74	4.14	2.38		
IL	3.11	3.03	3.33	3.33	3.12	3.45	3.22	2.88	2.88	2.84	3.33	3.17	3.03	3.73	3.91	2.35	0.023	0.175

[1] MO, University of Missouri, Columbia, MO, USA; IL, University of Illinois at Urbana-Champaign, USA.

Table 4.3. Analyzed nutrient composition of plant and animal-derived protein ingredients, as-fed basis, experiment 2

Item	Dry matter, %	Crude protein, %	Lys, %
Navy beans	38.68	7.72	0.65
Boiled carrots	12.80	0.90	0.06
Roasted turkey breast	31.72	28.34	2.41
Boiled cassava	34.82	0.87	0.03
Boiled plantain	33.90	0.86	0.06
Whole grain bread	61.14	12.23	0.31
Wheat bagel	67.29	10.55	0.24
Pea protein isolate	93.33	79.69	6.01
Chia seeds	92.17	19.59	0.97
Sunflower seeds	96.09	24.82	0.98

Table 4.4. Reactive Lys of ingredients analyzed at an external laboratory and at the University of Illinois, experiment 2

Item, %	Reactive Lys		SEM	P-value
	External laboratory	University of Illinois		
Navy beans	0.632	0.623	0.023	0.514
Boiled carrots	0.051	0.052		
Roasted turkey breast	2.511	2.313		
Boiled cassava	0.024	0.029		
Boiled plantain	0.048	0.055		
Whole meal bread	0.210	0.192		
Wheat bagel	0.167	0.136		
Pea protein isolate	5.388	5.341		
Chia seeds	0.706	0.757		
Sunflower seeds	0.752	0.832		

[1] CV, coefficient of variation.

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CHAPTER 5: NUTRITIONAL COMPOSITION AND REACTIVE LYS IN ENZYME-TREATED SOYBEAN MEAL, CONVENTIONAL SOYBEAN MEAL, FERMENTED SOYBEAN MEAL, AND SOY PROTEIN CONCENTRATE

ABSTRACT

An experiment was conducted to test the hypothesis that the chemical composition and reactive Lys concentration differ among enzyme-treated soybean meal (**ESBM**), fermented soybean meal (**FSBM**), soy protein concentrate (**SPC**), and conventional soybean meal (**SBM**). A total of 86 samples, including 74 samples of ESBM, seven samples of SBM, three samples of FSBM, and two samples of SPC, were used. During production, some of the ingredients were intentionally over-heated to create a range of total Lys and reactive Lys in the ingredients. All samples were analyzed for dry matter, crude protein, acid hydrolyzed ether extract, insoluble dietary fiber, soluble dietary fiber, amino acids, and minerals. Results indicated that SPC, FSBM, and ESBM had greater ($P < 0.001$) concentrations of crude protein compared with conventional SBM, and ESBM had the greatest ($P < 0.001$) concentration of total dietary fiber. Lys and reactive Lys were greater ($P < 0.001$) in SPC, ESBM, and FSBM compared with SBM. The Lys:CP ratio in SBM and SPC was above 6.0%, indicating that there was no heat damage in these ingredients, whereas ESBM and FSBM had Lys:CP ratios below 6.0%, indicating slight heat damage. Mineral concentrations, particularly phosphorus, potassium, magnesium, and copper, were greater in ESBM than in other ingredients, indicating that, because soluble carbohydrates were fermented during the production of ESBM, minerals were concentrated. In conclusion, soybean ingredients differ in nutrient composition depending on the processing method used to generate

the ingredients, and reactive Lys may be used as an indicator for assessing protein quality in heat-processed ingredients.

Keywords: chemical composition, enzyme-treated soybean meal, fermentation, Lys, reactive Lys, soybean products.

Abbreviations: AA, amino acids; AEE, acid hydrolyzed ether extract; CP, crude protein; CV, coefficient of variation; DM, dry matter; ESBM, enzyme-treated soybean meal; FSBM, fermented soybean meal; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; SBM soybean meal; SD, standard deviation; SPC, soy protein concentrate; TDF, total dietary fiber.

INTRODUCTION

Soybean meal (**SBM**) is used as a source of amino acids (**AA**) in diets for pigs, poultry, dairy, and other animals. However, because of antigens and oligosaccharides, it cannot be used as the only source of AA in diets for young animals (Cervantes-Pahm and Stein, 2010; Ma et al., 2019). To reduce anti-nutritional factors in SBM, processing including heat treatment, fermentation, or enzyme treatment may be used. Enzyme-treated soybean meal (**ESBM**) is produced by treating dehulled, solvent-extracted soybean meal for several hours with a proprietary blend of enzymes (Goebel and Stein, 2011). When conventional SBM is inoculated with a mold, yeast, or bacterium, the resulting meal is called fermented soybean meal (**FSBM**). The fermentation conditions and nutritional quality of ESBM and FSBM vary depending on the type of microorganism or enzymes used, and other production procedures (Mukherjee et al., 2016). Soy protein concentrate (**SPC**) is produced by acid or ethanol extraction of soluble

carbohydrates from SBM, which results in an ingredient with reduced soy allergens and greater crude protein (**CP**) than conventional SBM (Deng et al., 2023). Fermentation removes the oligosaccharides in SBM, and enzyme treatment reduces the concentration of glycinin and B-conglycinin (Cervantes-Pahm and Stein, 2010; Li et al., 2021). However, after fermentation or enzymatic hydrolysis, there may be residual compounds that should not be included in the animal diets such as lactic acid or ethanol (Mukherjee et al., 2016). These compounds are, therefore, evaporated by drying, and there is a risk of overheating during this process, which may reduce AA digestibility (Navarro et al., 2017; Ton-Nu et al., 2020; Oliveira et al., 2020). As a consequence of overheating during the drying process, some sources of SBM or ESBM have reduced standardized ileal digestibility of Lys (Navarro et al., 2017; Ton-Nu et al., 2020) which may be a result of Maillard reaction that causes the Lys to bind to reducing sugars, and thereby makes the Lys unavailable for use in protein synthesis by the animals. Therefore, a portion of the analyzed Lys in heated feed ingredients is "unreactive" because it cannot be used for protein synthesis, whereas another portion of the analyzed Lys is called "reactive Lys" because it was not heat-damaged during production. Reactive Lys is the Lys that can be used for protein synthesis (Kim et al., 2012). There is, therefore, a need to characterize soybean products and provide information about their chemical composition because all soybean products have gone through a heating process. However, there is limited information about reactive Lys in soybean products and it is therefore not known how much of the analyzed Lys can be used for protein synthesis. Therefore, the objectives of this work were to determine the chemical composition, including total and reactive Lys, of SBM, ESBM, FSBM, and SPC and to test the hypothesis that there is variation in chemical composition among soybean products.

MATERIALS AND METHODS

A total of 86 soybean samples were provided by Hamlet Protein (Findlay, OH, USA) and shipped to the University of Illinois, Urbana, IL, USA, where samples were labeled, cataloged, and analyzed. There were 74 ESBM samples, 7 SBM samples, 3 FSBM samples, and 2 SPC samples. The ESBM samples were lab-scale experimental batches, not commercial lots, and reflected production-trial, pilot, or simulated processing conditions.

All samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Samples were also analyzed for nitrogen by combustion using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI; method 990.03; AOAC Int., 2019). Crude protein was calculated as nitrogen × 6.25. Ingredients were analyzed for acid-hydrolyzed ether extract (**AEE**) using 3 *N* hydrochloric acid (AnkomHCl, Ankom Technology, Macedon, NY), followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY). Soy protein samples were analyzed for gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Glucose, sucrose, maltose, fructose, stachyose, raffinose, and galactose in feed ingredients were analyzed by high-performance liquid chromatography using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA; Navarro et al., 2018). Ingredients were analyzed also for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) according to method 991.43 (AOAC Int., 2019) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber (**TDF**) was calculated as the sum of IDF and SDF. Minerals were also analyzed (method 985.01 a, b, and c; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200; PerkinElmer, Waltham,

MA, USA). After dry ashing at 600°C for 2 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000).

Lysine was analyzed using Ultra High-Performance Liquid Chromatography (Model QSight LX50; PerkinElmer, Waltham, MA, USA). Prior to analysis, samples were hydrolyzed with 6 *N* hydrochloric acid for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2019). Reactive Lys was analyzed in ingredients using Ultra High-Performance Liquid Chromatography (Model QSight LX50; PerkinElmer, Waltham, MA, USA). Samples were incubated with an O-Methylisourea solution at pH 10.6 to initiate the guanidination reaction for 72 h at room temperature (Pahm et al., 2008), and after incubation, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019]. All other AA were analyzed on a Hitachi Amino 30 Acid Analyzer, Model No. L8800. (Hitachi High Technologies America, Inc; Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(c); AOAC Int., 2019].

Calculations and statistical analysis

For each analysis of each source of ingredients, all analyzed components were added and subtracted from the concentration of dry matter in each ingredient to calculate the rest fraction using the following equation (Fanelli et al., 2023ab):

Rest fraction= [dry matter - (crude protein + acid hydrolyzed ether extract + ash + total dietary fiber + sucrose + stachyose + raffinose + glucose + fructose + maltose + galactose)]

The coefficient of variation (**CV**) was calculated if there were more than 3 samples in each source, and the standard deviation (**SD**) and the average of all analyzed components from each group of ingredients were calculated. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). The replicate was the experimental unit for all analyses. The feed ingredient was the fixed effect, and the replicate sample was the random effect. Mean values were calculated using the LSMeans statement. An alpha value of 0.05 was used to determine significance among ingredients.

RESULTS

The analyzed nutrient composition of SBM, ESBM, SPC, and FSBM is summarized in Tables 5.1, 5.2, 5.3, and 5.4. Results indicated that the chemical composition of conventional SBM varied across samples. The average DM was 90.57%, and CP was 47.36%. The Lys/CP ratio averaged 6.42%, with a CV of 7.91%. The concentration of ash averaged 6.04%, and AEE was 2.96%, with a CV of 17.00%, indicating some variability in fat concentration among SBM samples. Total dietary fiber was 18.01% on average, with IDF as the main component. The Lys and reactive Lys were 3.04% and 2.95%, respectively. Mineral concentrations also varied considerably, especially for copper, iron, and manganese.

Enzyme-treated SBM had an average DM of 92.24% and a CP of 54.50%. The Lys/CP ratio averaged 5.88%, and Lys and reactive Lys were 3.20% and 3.11%, respectively. The total concentration of oligosaccharides and sucrose was 2.25%, with a high coefficient of variation (63.80%), indicating variability among samples. Enzyme-treated SBM contained 1.32% AEE and 6.99% ash. Total dietary fiber averaged 20.80%, composed mostly of IDF (18.39%) and some SDF (2.41%). Mineral concentrations showed moderate variation, with average values of

0.32% calcium, 0.91% phosphorus, 2.76% potassium, and 0.33% magnesium. Trace mineral concentrations were 49.44 mg/kg for copper, 195.63 mg/kg for iron, 110.20 mg/kg for manganese, and 57.79 mg/kg for zinc.

Fermented SBM varied across samples; the average DM and CP were 91.35% and 57.21%, respectively, and the CV of CP was 21.70%, indicating substantial variability. The Lys/CP ratio averaged 5.53%. Ash was 5.02%, and total dietary fiber averaged 20.08%, composed mainly of insoluble fiber (18.28%) and a smaller amount of soluble fiber (2.70%). Concentrations of Lys and reactive Lys averaged 3.17% and 3.08%, respectively, with CV values exceeding 26%. Mineral concentrations were also variable, with average values of 0.27% calcium, 0.68% phosphorus, 1.71% potassium, and 0.23% magnesium. Trace mineral concentrations were variable, particularly iron, which ranged from 127.35 to 362.17 mg/kg.

Soy protein concentrate differed depending on the extraction method. The average DM was 92.87% and CP was 57.65% in alcohol-extracted and 67.32% in water and acid-extracted SPC, with an average of 62.48%. The Lys/CP ratio averaged 6.14%. Acid-hydrolyzed ether extract averaged 1.12%. The average of ash was 4.90%. Sugars were higher in alcohol-extracted SPC (4.80%) than in acid-extracted SPC (0.60%), averaging 2.70%. TDF was 19.00%, primarily composed of IDF (17.80%). Concentrations of Lys and reactive Lys averaged 3.82% and 3.74%, respectively. Mineral concentrations were variable, with potassium, magnesium, sodium, and calcium showing notable differences between extraction methods. Potassium averaged 1.25%, but ranged from 0.40 to 2.10%, and sodium ranged from 0.01 to 0.25%. Trace mineral values included 34.98 mg/kg for copper, 189.37 mg/kg for iron, 75.06 mg/kg for manganese, and 38.24 mg/kg for zinc.

Dry matter was greater ($P < 0.05$) in ESBM and SPC compared with SBM or FSBM

(Table 5.5). Crude protein was greater ($P < 0.001$) in SPC, ESBM, and FSBM compared with conventional SBM. The concentration of AEE was greater ($P < 0.001$) in conventional SBM than in ESBM, SPC, and FSBM. Ash was greater ($P < 0.05$) in ESBM compared with FSBM and SPC. Fermented SBM had a greater gross energy ($P < 0.001$) than conventional SBM, with ESBM and SPC as intermediate values.

The concentration of TDF was greater ($P < 0.05$) in ESBM than in SBM, but no differences were observed between SPC and FSBM. Soluble dietary fiber was greater ($P < 0.05$) in ESBM compared with SBM, whereas values for SPC and FSBM were intermediate. Phosphorus, potassium, magnesium, and sodium differed among ingredients ($P < 0.05$), with SBM and FSBM generally having lower values. Copper was greater ($P < 0.05$) in ESBM compared with SBM. Manganese was greater ($P < 0.05$) in ESBM compared with SPC. Zinc was greater ($P < 0.001$) in ESBM and SBM than in SPC. The rest fraction was less ($P < 0.05$) in SBM and SPC, compared with FSBM or ESBM. Enzyme-treated SBM, SPC, and FSBM had greater concentrations of AA than conventional SBM ($P < 0.05$ or $P < 0.001$; Table5.6). Lysine and reactive Lys were both greater ($P < 0.05$) in SPC compared with ESBM, FSBM, and SBM. All other AA were greater ($P < 0.05$ or $P < 0.001$) in ESBM, FSBM, and SPC than conventional SBM.

DISCUSSION

The chemical compositions of SBM, ESBM, FSBM, and SPC were consistent with reported values (González-Vega et al., 2011; NRC, 2012; Almeida et al., 2014; Deng et al., 2023). The production of SBM includes dehulling, oil extraction, and toasting. The toasting step inactivates most anti-nutritional factors, but can also trigger Maillard reactions that reduce the

availability of certain AA, particularly Lys (Cervantes-Pahm and Stein, 2010; Mukherjee et al., 2016). After the production of SBM, oligosaccharides are present, which can negatively affect the health and performance of young pigs. Therefore, enzymatic treatment, fermentation, or protein concentration are used to reduce anti-nutritional factors and enhance the nutritional value of SBM. Results confirm that these processing methods increase the concentration of several nutrients compared with conventional SBM. Enzyme treatment of SBM degrades oligosaccharides and antigens, and increases the concentration of AA (González-Vega et al., 2011; Almeida et al., 2014). Fermentation can also improve Lys digestibility by reducing trypsin inhibitors and phytates, and by modifying protein structure; however, its effectiveness depends on the type of microbial strain used and the fermentation conditions (Cervantes-Pahm and Stein, 2010; Kim et al., 2012). In contrast, SPC production involves alcohol or acid extraction, which removes soluble carbohydrates and other non-protein components, which increases the concentration of protein, resulting in a higher concentration of Lys and other AA (Deng et al., 2023).

Soybean ingredients are a source of AA due to their high concentration and digestibility of indispensable AA. Enzymatic treatment, fermentation, or protein concentration can increase gross energy and may also enhance digestible and metabolizable energy by reducing oligosaccharides (Rojas and Stein, 2012; 2013; Cristobal et al., 2025). However, corn usually provides more metabolizable energy than soybean products, mainly due to its lower fiber content and highly digestible starch, which makes it a more efficient energy source overall (Stein et al., 2008).

The Lys:CP ratio is often used as an indicator of heat damage in protein ingredients. The observation that Lys:CP ratio in SBM and SPC was greater than 6 indicates that these ingredients

were not heat-damaged (Oliveira et al., 2021). However, the fact that Lys:CP ratio in FSBM was less than 6 indicates heat-damage may have occurred during production (González-Vega et al., 2011). It is also possible that microbial fermentation contributed to the reduction in Lys content, as Lys may be utilized by microorganisms during fermentation (Thomas and Ingledew, 1992). The Lys:CP ratio obtained for ESBM was also less than 6 on average, but this does not reflect the Lys:CP ratio in commercial ESBM because some of the ESBM used in this work were intentionally over-heated to create products with a range of Lys:CP ratio. Nevertheless, the values for Lys and Lys:CP ratio, for all ingredients, are within the range of previous data (Hossain et al., 2016; Nørgaard et al., 2021; Yang et al., 2022; Zhang and Piao, 2022; Garavito-Duarte et al., 2023).

Reactive Lys is considered a more accurate indicator of protein quality in feed ingredients than CP or total Lys concentrations (Pahm et al., 2008), as it represents the fraction of Lys that can be utilized for protein synthesis by pigs (Kim et al., 2012). However, excessive heat processing can reduce reactive Lys and Lys digestibility due to the Maillard reaction and other heat-induced modifications (Cervantes-Pahm and Stein, 2010). A reduction in reactive Lys limits the bioavailability of Lys for protein synthesis, thereby impairing the ability of the pigs to effectively utilize dietary AA for tissue deposition. It may negatively affect the average daily gain and feed conversion and the overall nutritional value of the diet, particularly in young pigs with high AA requirements. Soybean ingredients with high CP may fail to support optimal growth performance if a significant portion of Lys is unavailable due to Maillard reaction or fermentation by-products. (Pahm et al., 2008; González-Vega et al., 2011; Almeida et al., 2014; Oliveira et al., 2021).

The rest fraction represents unmeasured or unknown components. The analyzed

components of SBM and SPC closely matched the total DM, indicating that all nutrients in these ingredients were accounted for. However, despite extensive nutrient analysis, a small portion of ESBM and FSBM remained unaccounted for, and the composition of these remaining fractions is unclear. It is possible that in ESBM and FSBM, compounds such as lactic acid or ethanol may be present, as they can be produced during fermentation or enzymatic processing (Mukherjee et al., 2016).

CONCLUSION

Results of this work confirm that SBM, ESBM, FSBM, and SPC differ in nutrient composition, including Lys and reactive Lys concentrations. Results highlight the importance of characterizing soybean ingredients to ensure accurate diet formulation. Soybean co-products have a high concentration of Lys compared with other plant proteins; however, ESBM also contains high levels of TDF, ash, and several minerals compared with other plant proteins, which may contribute additional nutrients to the diet of pigs.

TABLES

Table 5.1. Analyzed nutrient composition in 7 sources of conventional soybean meal (SBM), as-fed basis

Item	SBM 1	SBM 2	SBM 3	SBM 4	SBM 5	SBM 6	SBM 7	Average	SD[1]	CV
Dry matter, %	91.75	91.31	91.55	92.58	89.89	90.88	91.39	91.34	0.82	0.90
Ash, %	5.03	4.52	6.24	6.84	6.04	6.20	7.41	6.04	0.99	16.44
Crude protein, %	48.61	48.24	47.98	47.93	45.54	45.57	47.64	47.36	1.27	2.67
Lys/CP	5.92	5.96	5.91	6.95	6.90	6.98	6.33	6.42	0.51	7.91
AEE, %	3.40	3.20	3.02	2.06	3.34	2.67	3.02	2.96	0.46	15.71
Glucose, %	0.05	0.05	0.05	0.05	0.05	0.11	0.05	0.06	0.02	38.72
Sucrose, %	5.49	6.40	6.79	6.57	8.37	7.93	5.76	6.76	1.06	15.68
Maltose, %	0.55	0.41	0.35	0.36	0.29	0.26	0.60	0.40	0.13	31.78
Fructose, %	0.07	0.08	0.06	0.06	0.05	0.07	0.10	0.07	0.02	23.33
Stachyose, %	4.69	5.29	5.52	5.15	5.48	5.13	4.76	5.15	0.32	6.29
Raffinose, %	1.83	2.05	1.44	1.25	0.96	1.03	1.72	1.47	0.41	28.23
Insoluble dietary fiber, %	17.60	15.30	16.20	17.40	16.20	17.90	20.90	17.36	1.82	10.47
Soluble dietary fiber, %	0.50	0.70	0.60	0.70	NA	2.10	NA	0.66	0.70	107.19

Table 5.1. (cont.)

Total dietary fiber, %	18.10	16.00	16.80	18.10	16.20	20.00	20.90	18.01	1.88	10.41
Lys, %	2.88	2.88	2.84	3.33	3.14	3.18	3.02	3.04	0.19	6.16
Reactive Lys, %	2.76	2.76	2.76	3.26	3.07	3.08	2.93	2.95	0.20	6.66
Ca, %	0.29	0.17	0.31	0.38	0.23	0.27	0.40	0.29	0.08	28.06
P, %	0.77	0.48	0.80	0.77	0.78	0.75	1.04	0.77	0.16	21.11
K, %	1.75	1.18	2.14	2.21	2.37	2.32	3.31	2.18	0.65	29.69
Mg, %	0.30	0.17	0.27	0.29	0.25	0.24	0.39	0.27	0.07	24.05
Na, %	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	40.51
Cu, mg/kg	40.01	23.68	39.60	38.98	34.59	32.82	67.02	39.53	13.40	33.90
Fe, mg/kg	189.92	87.09	113.72	265.78	104.16	104.84	245.90	158.77	74.24	46.76
Mn, mg/kg	81.57	51.70	79.25	98.35	70.45	66.49	158.74	86.65	34.89	40.27
Zn, mg/kg	51.67	32.80	51.68	56.06	49.19	45.61	66.05	50.44	10.12	20.06
Rest fraction, %	3.93	5.07	3.30	4.21	3.57	1.91	-0.57	3.06	1.87	61.05

[1] SD, standard deviation; CV, coefficient of variation; Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract; NA, not analyzable.

Table 5.2. Analyzed nutrient composition in 74 sources of enzyme-treated soybean meal (ESBM), as-fed basis

Item	n	Average	SD[1]	CV
Dry matter, %	74	92.24	0.87	0.95
Ash, %	74	6.99	0.78	11.13
Crude protein, %	74	54.50	2.00	3.67
Lys/CP, %	74	5.88	0.41	6.98
AEE, %	74	1.32	0.63	47.26
Stachyose + Raffinose, %	74	0.72	0.51	70.82
Galactose, %	74	1.22	0.93	76.83
Sucrose, %	74	0.36	0.84	237.14
Insoluble dietary fiber, %	74	18.39	2.24	12.20
Soluble dietary fiber, %	74	2.41	1.38	57.38
Total dietary fiber, %	74	20.80	2.36	11.35
Lys, %	74	3.20	0.22	6.85
Reactive Lys, %	74	3.11	0.22	7.05
Ca, %	74	0.32	0.05	14.81
P, %	74	0.91	0.10	11.06
K, %	74	2.76	0.36	13.05
Mg, %	74	0.33	0.05	15.52
Na, %	74	0.02	0.01	37.57
Cu, mg/kg	74	49.44	8.60	17.40
Fe, mg/kg	74	195.63	73.20	37.42

Table 5.2. (cont.)

Mn, mg/kg	74	110.20	24.56	22.28
Zn, mg/kg	74	57.79	6.78	11.73
Rest fraction, %	74	6.46	3.38	52.24

[1] SD, standard deviation; CV, coefficient of variation; Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract.

Table 5.3. Analyzed nutrient composition in 3 sources of fermented soybean meal (FSBM), as-fed basis

Item	FSBM 1	FSBM 2	FSBM 3	Average	SD[1]	CV
Dry matter, %	92.54	90.60	90.91	91.35	1.04	1.14
Ash, %	1.31	7.31	6.43	5.02	3.24	64.59
Crude protein, %	71.54	50.21	49.87	57.21	12.42	21.70
Lys/CP	5.66	4.68	6.24	5.53	0.79	14.23
AEE, %	0.37	1.52	1.14	1.01	0.59	58.01
Stachyose + Raffinose, %	NA	0.40	2.40	1.40	1.41	101.02
Galactose, %	NA	NA	0.40	0.40	-	-
Sucrose, %	NA	NA	NA	-	-	-
Insoluble dietary fiber, %	15.80	18.45	20.60	18.28	2.40	13.15
Soluble dietary fiber, %	3.80	1.60	NA	2.70	1.56	57.62
Total dietary fiber, %	19.60	20.05	20.60	20.08	0.50	2.49
Lys, %	4.05	2.35	3.11	3.17	0.85	26.86
Reactive Lys, %	3.94	2.28	3.03	3.08	0.83	26.96
Ca, %	0.20	0.33	0.28	0.27	0.07	24.29
P, %	0.33	0.89	0.81	0.68	0.30	44.76
K, %	0.24	2.37	2.52	1.71	1.28	74.58
Mg, %	0.04	0.35	0.29	0.23	0.16	72.54
Na, %	0.01	0.05	0.03	0.03	0.02	66.67
Cu, mg/kg	30.31	45.92	40.56	38.93	7.93	20.37
Fe, mg/kg	127.35	362.17	179.29	222.94	123.34	55.33

Table 5.3. (cont.)

Mn, mg/kg	43.03	106.04	96.18	81.75	33.89	41.46
Zn, mg/kg	43.03	67.15	54.04	54.74	12.08	22.06
Rest fraction, %	-0.28	11.12	10.07	6.97	6.30	90.39

[1] SD, standard deviation; CV, coefficient of variation; Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract; NA, not analyzable.

Table 5.4. Analyzed nutrient composition in 2 sources of soy protein concentrate (SPC), as-fed basis

Item	Alcohol extracted SPC	Water and acid extracted SPC	Average	SD[1]	CV
Dry matter, %	93.31	92.42	92.87	0.63	0.67
Ash, %	6.79	3.02	4.90	2.66	54.23
Crude protein, %	57.65	67.32	62.48	6.83	10.94
Lys/CP	6.47	5.81	6.14	0.47	7.66
AEE, %	1.44	0.80	1.12	0.45	40.50
Stachyose + Raffinose, %	4.80	0.60	2.70	2.97	109.99
Galactose, %	NA	NA	-	-	-
Sucrose, %	NA	NA	-	-	-
Insoluble dietary fiber, %	19.40	16.20	17.80	2.26	12.71
Soluble dietary fiber, %	2.40	NA	1.20	1.70	141.42
Total dietary fiber, %	21.80	16.20	19.00	3.96	20.84
Lys, %	3.73	3.91	3.82	0.13	3.29
Reactive Lys, %	3.66	3.83	3.74	0.13	3.36
Ca, %	0.32	0.19	0.26	0.09	34.75
P, %	0.81	0.79	0.80	0.02	2.02
K, %	2.10	0.40	1.25	1.20	95.83
Mg, %	0.32	0.06	0.19	0.19	99.65
Na, %	0.01	0.25	0.13	0.17	129.61
Cu, mg/kg	34.39	35.57	34.98	0.84	2.39

Table 5.4. (cont.)

Fe, mg/kg	165.73	213.01	189.37	33.43	17.65
Mn, mg/kg	89.16	60.96	75.06	19.94	26.57
Zn, mg/kg	46.81	29.67	38.24	12.12	31.70
Rest fraction, %	0.83	4.49	2.66	2.58	97.13

[1] SD, standard deviation; CV, coefficient of variation; Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract; NA, not analyzable.

Table 5.5. Comparison of chemical composition of enzyme-treated soybean meal (ESBM), soybean meal (SBM), soy protein concentrate (SPC), and fermented by lactic acid bacteria soybean meal (FSBM), as-fed basis

Item	ESBM	SBM	SPC	FSBM	SEM	*P*-value
Dry matter, %	92.24[a]	91.34[b]	92.87[a]	91.35[b]	0.387	0.017
Ash, %	6.99[a]	6.04[ab]	4.90[b]	5.02[b]	0.434	<0.001
Crude protein, %	54.50[b]	47.36[c]	62.48[a]	57.21[ab]	1.230	<0.001
Lys/CP[1]	5.88[b]	6.42[a]	6.14[ab]	5.53[b]	0.192	0.008
AEE, %	1.32[b]	2.96[a]	1.12[b]	1.01[b]	0.272	<0.001
Gross energy, kcal/kg	4465[b]	4382[c]	4569[ab]	4659[a]	36.306	<0.001
Sugars[2], %	2.25[b]	13.90[a]	2.70[b]	1.07[b]	0.637	<0.001
Insoluble dietary fiber, %	18.39	17.36	17.80	18.28	0.996	0.529
Soluble dietary fiber, %	2.41[a]	0.66[b]	1.20[ab]	2.70[ab]	0.607	0.010
Total dietary fiber, %	20.80[a]	18.01[b]	19.00[ab]	20.08[ab]	1.045	0.015
Ca, %	0.32	0.29	0.26	0.27	0.023	0.061
P, %	0.91[a]	0.77[b]	0.80[ab]	0.68[b]	0.051	<0.001
K, %	2.76[a]	2.18[b]	1.25[b]	1.71[b]	0.201	<0.001
Mg, %	0.33[a]	0.27[ab]	0.19[bc]	0.23[c]	0.024	<0.001
Na, %	0.02[b]	0.01[b]	0.13[a]	0.03[b]	0.009	<0.001
Cu, mg/kg	49.44[a]	39.53[b]	34.98[ab]	38.93[ab]	3.995	0.003
Fe, mg/kg	195.63	158.77	189.37	222.94	33.173	0.565
Mn, mg/kg	110.20[a]	86.65[ab]	75.06[b]	81.75[ab]	11.424	0.013
Zn, mg/kg	57.79[a]	50.44[ab]	38.24[b]	54.74[ab]	2.265	<0.001

Table 5.5. (cont.)

Rest fraction, %	6.46$_a$	3.06^b	2.66^b	6.97^a	1.669	0.040

[1] Lys/CP, Lys to crude protein ratio; AEE, acid-hydrolyzed ether extract.

[2] Glucose, fructose, maltose, stachyose, raffinose, galactose, and sucrose were included if detected.

Table 5.6. Comparison of amino acid (AA) composition of enzyme-treated soybean meal (ESBM), soybean meal (SBM), soy protein concentrate (SPC), and fermented by lactic acid bacteria soybean meal (FSBM), as-fed basis

Item, %	ESBM	SBM	SPC	FSBM	SEM	*P*-value
Indispensable AA						
Arg	3.89^{ab}	3.69^{b}	4.09^{a}	4.15^{a}	0.087	0.005
His	1.50^{a}	1.40^{b}	1.55^{a}	1.58^{a}	0.035	0.006
Ile	2.73^{a}	2.52^{b}	2.90^{a}	2.96^{a}	0.076	0.001
Leu	4.34^{a}	4.03^{b}	4.63^{a}	4.78^{a}	0.114	<0.001
Lys	3.20^{b}	3.03^{b}	3.82^{a}	3.17^{b}	0.112	0.003
Reactive Lys	3.11^{b}	2.95^{b}	3.74^{a}	3.08^{b}	0.112	0.002
Met	0.76^{a}	0.71^{b}	0.81^{a}	0.82^{a}	0.018	<0.001
Phe	2.94^{b}	2.72^{c}	3.13^{ab}	3.28^{a}	0.076	<0.001
Thr	2.19^{a}	2.03^{b}	2.28^{a}	2.33^{a}	0.052	0.001
Trp	0.69^{a}	0.64^{b}	0.74^{a}	0.74^{a}	0.016	<0.001
Val	2.79^{a}	2.57^{b}	2.95^{a}	2.98^{a}	0.076	0.002
Dispensable AA						
Ala	2.43^{a}	2.25^{b}	2.54^{a}	2.62^{a}	0.061	0.001
Asp	6.31^{a}	5.94^{b}	6.73^{a}	6.80^{a}	0.150	0.001
Cys	0.81^{b}	0.76^{c}	0.85^{ab}	0.91^{a}	0.021	<0.001
Glu	10.10^{ab}	9.65^{b}	10.80^{a}	10.94^{a}	0.242	0.004
Gly	2.33^{a}	2.17^{b}	2.47^{a}	2.48^{a}	0.056	0.002

Table 5.6. (cont.)

Pro	2.86^{bc}	2.71^{c}	3.12^{a}	3.15^{ab}	0.072	<0.001
Ser	2.45^{ab}	2.30^{b}	2.56^{ab}	2.70^{a}	0.068	0.007
Tyr	2.09^{a}	1.88^{b}	2.14^{a}	2.18^{a}	0.048	<0.001

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CHAPTER 6: CORRELATION BETWEEN SUGARS AND REACTIVE LYS IN SOYBEAN MEAL AND AUTOCLAVED SOYBEAN MEAL

ABSTRACT

An experiment was conducted to test the hypothesis that reactive Lys in soybean meal (**SBM**) is correlated with the concentrations of sugars and that thermal processing reduces both reactive Lys and sugar concentrations. One source of conventional SBM was divided into four batches: one batch was used without further processing, and the other three batches were autoclaved at 120 ± 1 ℃ for 15, 30, or 45 min. A second source of SBM was divided into three batches: one batch was used without further processing, and the other two batches were autoclaved at 120 ± 1 ℃ for 30 or 45 min. Two additional sources of SBM were each divided into four batches, for a total of 8 batches: two batches were used without further processing, and the other six batches were autoclaved at 120 ± 1 ℃ for 60, 120, or 180 min. Samples were analyzed for dry matter, crude protein, Lys, reactive Lys, and concentrations of stachyose, raffinose, galactose, sucrose, glucose, maltose, fructose, and total carbohydrates. Pearson correlation coefficients (**r**) and simple linear regression analyses were conducted to determine associations between reactive Lys and individual sugar and oligosaccharide concentrations. Multiple linear regression was also performed using all sugars and oligosaccharides as independent variables. Results indicated that autoclaving reduced reactive Lys, stachyose, raffinose, maltose, and sucrose, whereas glucose and fructose increased with longer autoclaving times. Reactive Lys was associated with reducing sugars, especially fructose ($R^2 = 0.91$), and a multivariable model including all sugars explained 94% of the variation in reactive Lys ($R^2 = 0.94$). In conclusion, heat processing reduces

concentrations of reactive Lys, oligosaccharides, glucose, and fructose in SBM, and strong correlations were observed between reactive Lys and some reducing sugars.

Keywords: autoclaving, Maillard reaction, soybean meal, reactive Lys, reducing sugars.

Abbreviations: AA, amino acids; CP, crude protein; DM, dry matter; SBM, soybean meal.

INTRODUCTION

Soybean meal (**SBM**) is among the most widely used plant protein sources in animal nutrition, primarily due to its high concentration of amino acids (**AA**), particularly Lys (Cervantes-Pahm and Stein, 2010). Conventional toasted and dehulled SBM is produced by removing the fat from dehulled soybeans using a solvent extraction process, followed by a toasting step to reduce concentrations of anti-nutritional factors such as trypsin inhibitors and lectins (Mukherjee et al., 2016). However, heat processing conditions can initiate chemical reactions that negatively impact the nutritional quality of SBM, particularly through heat-damage of Lys. Among all AA, Lys is especially susceptible to degradation because of the high reactivity of its free ε-amino group, which is not involved in peptide bonds (Pahm, 2008; Lund and Ray, 2017). The reduction of Lys and Lys availability may affect protein synthesis and animal growth, and one of the main chemical reactions responsible for this reduction is known as the Maillard reaction, which involves the binding between the amino group of AA and the carbonyl group of a reducing sugar. The Maillard reaction involves three stages, starting with the early stage, which consists of initial condensation to form Schiff bases. In the intermediate phase, rearrangement to produce Amadori products occurs, and in the final phase, pre-melanoid and melanoidins are

formed (Pahm, 2008; Koubaa et al., 2019; Rauh and Xiao, 2022). Maillard reactions can be initiated at low temperatures, but damage may increase at higher temperatures, especially under low-moisture conditions typical of feed ingredient processing (Martins et al., 2000). Reducing sugars are key initiators of the Maillard reaction (Moughan and Rutherfurd, 1996; Martins et al., 2000; Fontaine et al., 2007). Fructose is more reactive than glucose due to its chemical structure in the initial stage of Maillard reaction (Dills, 1993; Yang and Hiramatsu, 2024). The predominant reducing sugars in feed ingredients are usually present as D-enantiomers, which are more reactive and metabolically relevant than L-isomers (Whistler and BeMiller, 1997; Martins et al., 2000). The concentration of reducing sugars varies among feed ingredients depending on origin, processing, and enzymatic treatment. Enzyme-treated SBM typically contains reduced levels of sugars due to reduction during the enzymatic process (Navarro et al., 2018). However, conventionally processed SBM or cassava-derived ingredients may contain more sucrose, glucose, or stachyose, which can be hydrolyzed into reactive reducing sugars during thermal or enzymatic processing (Fanelli et al., 2023). Standard acid hydrolysis procedures used for AA analysis can regenerate some modified forms of Lys, which results in an overestimation of total available Lys (Moughan and Rutherfurd, 1996; Tamanna and Mahmood, 2015; Koubaa et al., 2019; Rauh and Xiao, 2022). This complicates an accurate assessment of reactive Lys concentrations in processed feed ingredients. Therefore, the objective of this work was to test the hypothesis that the concentration of reactive Lys in intentionally heat-damaged SBM is correlated with the concentration of reducing sugars.

MATERIALS AND METHODS

Sample preparation

One source of conventional SBM was divided into four batches: one batch was used without further processing, and the other three batches were autoclaved at 120 ± 1 ℃ for 15, 30, or 45 min. A second source of SBM was divided into three batches: one batch was used without further processing, and the other two batches were autoclaved at 120 ± 1 ℃ for 30 or 45 min. A third source of SBM was divided into four batches, and they were autoclaved at 120 ± 1 ℃ for 0, 60, 120, or 180 min. The fourth source was divided into four batches, and they were also autoclaved at 120 ± 1 ℃ for 60, 120, or 180 min (Table 6.1). It was expected that, following autoclaving, all samples would contain different quantities of reactive Lys and sugars.

Chemical analysis

Feed ingredients were analyzed for dry matter (**DM;** method 930.15; AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Acid-hydrolyzed ether extract was analyzed using 3 *N* HCl (AnkomHCl, Ankom Technology, Macedon, NY), followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY). Nitrogen was analyzed by combustion using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI; method 990.03; AOAC Int., 2019). Crude protein (**CP**) was calculated as nitrogen × 6.25. Lysine was analyzed using Ultra High-Performance Liquid Chromatography (Model QSight LX50; PerkinElmer, Waltham, MA, USA). Prior to analysis, samples were hydrolyzed with 6 *N* hydrochloric acid for 24 h at 110 ± 1 ℃ (method 982.30 E(a); AOAC Int., 2019). Reactive Lys was analyzed in ingredients using Ultra High-Performance Liquid Chromatography (Model QSight LX50; PerkinElmer, Waltham, MA, USA). Samples were incubated with an O-Methylisourea solution at pH 10.6 to initiate the guanidination reaction for 72 h at room

temperature (Moughan and Rutherfurd, 1996; Pahm et al., 2008), and after incubation, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 ± 1 ℃ [method 982.30 E(a); AOAC Int., 2019]. Stachyose, raffinose, galactose, sucrose, glucose, maltose, and fructose in SBM and autoclaved SBM were analyzed by high-performance liquid chromatography using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA; Navarro et al., 2018).

Instrumental color was measured in each sample, determined by measuring L* (lightness), a* (redness), and b* (yellowness) using a HunterLab Miniscan EZ Plus apparatus, Model 45/0-L, spectrophotometer. The instrument was standardized using black and white tiles and operated with a D65 light source and a 10° observer angle (González- Vega et al., 2011).

Statistical analysis

Pearson correlation coefficients (**r**) were calculated using the PROC CORR procedure of SAS (SAS Institute Inc., Cary, NC) to determine the relationship between reactive Lys and individual sugars and oligosaccharides (stachyose, raffinose, galactose, sucrose, glucose, maltose, and fructose) as well as total carbohydrates, and color parameters.

Simple linear regression analyses were conducted using the PROC REG procedure of SAS (SAS Institute Inc., Cary, NC) to determine associations between reactive Lys and the concentration of each carbohydrate and color parameters. In each regression model, reactive Lys was used as the dependent variable, and concentration of each carbohydrate was used as the independent variable.

A multiple linear regression model was also conducted using the PROC REG procedure of SAS to determine the association between reactive Lys and stachyose, raffinose, galactose, sucrose, glucose, maltose, and fructose. In this model, reactive Lys was used as the dependent

variable. For all analyses, statistical significance was considered at $P \leq 0.05$, and tendencies were considered if $P > 0.05$ and ≤ 0.10.

RESULTS

The DM, CP, Lys, and reactive Lys of SBM are reported in Table 6.1 for increasing autoclaving times. In the first SBM, DM varied from 89.83% in non-autoclaved SBM to 84.32% after 45 min, whereas DM in the fourth SBM ranged from 92.65% to 90.64% after 120 min of autoclaving. Crude protein decreased from 45.61% to 43.25% in the first SBM and from 46.49% to 45.51% in the fourth SBM when autoclaving time increased. Total Lys decreased when autoclaving time increased in all sources of SBM. Reactive Lys showed a similar pattern, decreasing from 2.71% to 2.34% in the first source of SBM and from 2.92% to 1.42% in the fourth source of SBM as autoclaving time increased. The proportion of reactive Lys relative to total Lys varied among SBM sources and autoclaving durations (Table 6.1). In the first SBM source, reactive Lys/Lys values were 97.48% in non-autoclaved SBM and ranged from 97.43% to 96.69% after 15 to 45 min of autoclaving. In the second SBM source, reactive Lys/Lys was 95.00% in non-autoclaved SBM and decreased to 93.33% and 90.58% after 30 and 45 min of autoclaving, respectively. In the third SBM source, reactive Lys/Lys values were 95.81% in non-autoclaved SBM and were 90.53%, 78.97%, and 66.83% after 60, 120, and 180 min of autoclaving, respectively. Similarly, in the fourth SBM source, reactive Lys/Lys decreased from 93.89% in non-autoclaved SBM to 88.53%, 76.42%, and 68.60% after 60, 120, and 180 min of autoclaving.

The concentrations of stachyose, raffinose, sucrose, and total carbohydrates in SBM decreased as autoclaving time increased, although the extent of reduction varied among batches.

In the first source of SBM, stachyose concentration remained relatively stable, ranging from 5.80% in non-autoclaved SBM to 5.90%, 6.00%, and 5.90% after 15, 30, and 45 min, respectively. In contrast, greater reductions in stachyose were observed in the third and fourth SBM, where the reduction was from 4.75 in non-autoclaved SBM to 4.42%, 3.81%, and 3.19% after 60, 120, or 180 min for the third source of SBM and from 6.09% to 5.59%, 4.95%, and 3.90% for the fourth source of SBM. Raffinose decreased progressively in all SBM with increasing autoclaving time. In the third source of SBM, raffinose decreased from 0.96% to 0.91%, 0.79%, and 0.63% after 60, 120, and 180 min, respectively. A similar pattern was observed for raffinose in the fourth source of SBM.

Sucrose was reduced when the autoclaving time increased. In the third source of SBM, sucrose decreased from 5.98% to 5.55%, 4.67%, and 3.98% after 60, 120, and 180 min, respectively. In the fourth source, sucrose decreased from 7.95% in non-autoclaved SBM to 7.41%, 6.28%, and 4.92% after 60, 120, or 180 min. Concentrations of glucose, maltose, and fructose were low in all samples and generally did not change markedly except for slight increase in fructose and glucose with longer autoclaving times.

The L* values decreased from 80.37 to 73.91 after 30 min of autoclaving and to 69.15 after 45 min. For the third and fourth sources of soybean meal, L* values were reduced from 83.28 and 82.92 at 0 min to 69.90 and 68.85 at 60 min, 58.35 and 57.28 at 120 min, and 56.84 and 54.36 at 180 min. The a* values increased from 4.37 at 0 min to 6.55 and 8.20 at 30 and 45 min, respectively, and from 2.93 to 3.56 at 0 min to 8.23 and 9.37 at 60 min, 12.35 and 13.05 at 120 min, and 12.76 and 13.22 at 180 min. Similarly, b* values increased from 25.85 at 0 min to 27.28 and 28.64 at 30 and 45 min, and from 19.94 and 23.88 at 0 min to 26.68 and 29.55 at 60 min, 32.27 and 33.63 at 120 min, and 32.65 and 32.63 at 180 min.

The Pearson correlation analysis demonstrated negative correlations between reactive Lys and fructose (r = -0.953; $P < 0.001$), glucose (r = -0.841, $P < 0.001$), a* (r = -0.910; $P < 0.001$), and b* (r = -0.805; $P < 0.001$) in Table 6.2. In contrast, positive correlations were observed between reactive Lys and stachyose (r = 0.767; $P < 0.001$), raffinose (r = 0.759; $P < 0.001$), sucrose (r = 0.737; $P < 0.05$), total carbohydrates (r = 0.811, $P < 0.001$), and L* (r = 0.939; $P < 0.001$). However, no correlation between reactive Lys and galactose or maltose was observed.

The linear regression analysis demonstrated that fructose and glucose had the greatest R^2 values ($R^2 = 0.91$; $P < 0.001$, and $R^2 = 0.71$; $P < 0.001$), confirming their strong negative association with reactive Lys (Table 6.3). Total carbohydrates ($R^2 = 0.66$; $P < 0.001$), stachyose ($R^2 = 0.59$; $P < 0.001$), raffinose ($R^2 = 0.58$; $P < 0.001$), and sucrose ($R^2 = 0.54$; $P < 0.05$) had a positive correlation with reactive Lys. Maltose ($R^2 = 0.16$; $P = 0.228$) and galactose ($R^2 = 0.08$; $P = 0.319$) had no correlation with reactive Lys. The color parameters L*, a*, and b* also showed a strong R^2 values ($R^2 = 0.94$; $P < 0.001$) and both a* ($R^2 = 0.91$; $P < 0.001$) and b* ($R^2 = 0.80$; $P < 0.001$).

Based on the simple linear regression analysis, three prediction equations were developed to estimate reactive Lys in SBM as a function of individual sugar concentrations. The first equation was based on fructose, which had the strongest correlation with reactive Lys ($R^2 = 0.91$), and was represented as: Reactive Lys = $3.226 - 4.301 \times$ Fructose.

The second equation used total carbohydrate concentration as the predictor ($R^2 = 0.66$) and was described as: Reactive Lys = $- 0.005 + 0.154 \times$ Total carbohydrates.

The third equation was based on stachyose concentration ($R^2 = 0.59$) and was represented as: Reactive Lys = $0.188 + 0.421 \times$ Stachyose.

These models indicate that reactive Lys concentration can be predicted using concentrations of fructose, total carbohydrates, or stachyose, with fructose providing the most accurate prediction due to its very strong inverse relationship with reactive Lys.

The multiple linear regression model explained 94.30% of the variability in reactive Lys ($R^2 = 0.94$) and included the following parameters:

Reactive Lys = 3.540 + 0.936 (Stachyose) + 1.279 (Raffinose) + 1.672 (Galactose) − 1.081 (Sucrose) + 12.201 (Glucose) + 2.446 (Maltose) − 7.554 (Fructose).

DISCUSSION

The reason DM and CP decreased slightly with increasing autoclaving time, whereas moisture increased, is that heat treatment promotes partial degradation of nutrients and structural breakdown of the feed matrix, leading to loss of nutrients (Fontaine et al., 2007; González-Vega et al., 2011). The high humidity during the autoclave also contributes to water absorption or retention in SBM, due to the effects of moist heat on ingredient structure and hydration (González-Vega et al., 2011).

The progressive reduction in concentrations of Lys and reactive Lys as autoclaving time increased is consistent with reported values (Pahm et al., 2008; González-Vega et al., 2011; Almeida et al., 2014; Oliveira et al., 2021) and demonstrates the sensitivity of Lys to heat damage, the contribution of prolonged heating to AA degradation, and the value of reactive Lys as an indicator of protein quality loss during processing. This reduction in Lys and reactive Lys observed in autoclaved samples is mainly a result of the Maillard reaction, which involves the condensation of reducing sugars with the ε-amino group of Lys (Martins et al., 2000). The reaction proceeds through the formation of Schiff bases and Amadori compounds, resulting in a

loss of Lys bioavailability (Ajandouz and Puigserver, 1999). Lysine is particularly susceptible to heat damage because its free ε-amino group is highly reactive. When Lys is bound in Maillard products, it can no longer be used for protein synthesis (Pahm et al., 2008; Kim et al., 2012).

The pronounced decline in reactive Lys/Lys observed in SBM exposed to longer autoclaving durations indicates an increasing degree of heat damage and supports the use of reactive Lys/Lys as a sensitive indicator of protein quality loss. Differences among SBM sources suggest that initial composition and processing history influence the susceptibility of Lys to heat-induced modification. These findings further explain the reductions in ileal digestibility of Lys and other AA observed in heat-damaged SBM, as chemically bound Lys may limit protein hydrolysis and reduce overall AA availability to the pig (Pahm et al., 2008; González-Vega et al., 2011; Almeida et al., 2014).

The increased concentration of glucose and fructose following autoclaving is consistent with partial hydrolysis of sucrose and possibly more complex sugars, and heating, therefore, provides more substrates for the Maillard reaction (Fontaine et al., 2007; Murai et al., 2024). The reduction in stachyose, raffinose, sucrose, and maltose as autoclaving time was increased indicates degradation of oligosaccharides or disaccharides during autoclaving. In contrast, the increased glucose and fructose that were observed as autoclaving time increased indicate partial hydrolysis of sucrose and possibly also some complex carbohydrates (Cervantes-Pahm and Stein, 2010; Murai et al., 2024). The decrease in total carbohydrates that was observed with increased time of autoclaving reflects sugar loss during autoclaving (Almeida et al., 2014; Navarro et al., 2018).

Because soybean genotype and origin alter the oligosaccharide profile of SBM, the relationship between sugars and reactive Lys is unlikely to be universal across sources.

Experiment with broilers demonstrated that SBM from different origins, including U.S. origin beans processed in Spain, differ in sucrose, raffinose, and stachyose profiles, and these differences impact digestibility of AA (de Coca-Sinova et al., 2008). Likewise, low-oligosaccharide soybean lines yield higher metabolizable energy than conventional meals due to a high concentration of sucrose compared with conventional SBM (Parsons et al., 2000). During thermal processing, sucrose, stachyose, and raffinose are hydrolyzed, which results in increased concentrations of reducing sugars that can be used in Maillard reactions (Cervantes-Pahm and Stein, 2010; Almeida et al., 2014; Navarro et al., 2018; Murai et al., 2024). As a consequence, genetic effects on the starting sugar profile can modify reactive Lys loss under a given amount of heat and moisture. However, because SBM contains more sucrose than other oilseed meals and cereal grains, it is likely that SBM is more susceptible to the Maillard reaction than other ingredients. Conventional SBM typically contains 6% to 8% sucrose, which may be hydrolyzed during heat processing to yield glucose and fructose (Martins et al., 2000; Cervantes-Pahm and Stein, 2010; Navarro et al., 2018). Likewise, milk-based ingredients such as whey protein concentrates and skim milk powder naturally contain high levels of lactose, with a concentration around 4% to 5%, which hydrolyzes into glucose and galactose during thermal treatment, which may contribute to the Maillard reaction (Wu et al., 2021). Additionally, the type and concentration of reducing sugars can also be influenced by the processing methods applied. For example, enzyme-treated SBM and fermented SBM contain less reducing sugars because these sugars are consumed as substrates during fermentation (Goebel and Stein, 2011; Navarro et al., 2017; Navarro et al., 2018; Torres-Mendoza et al., 2025). Each ingredient has a different sugar profile, and specific analyses are essential for accurately assessing the nutritional consequences of heat damage across various ingredients.

Various reducing sugars, including glucose, galactose, ribose, lactose, maltose, and others, have been studied for their ability to conjugate with whey proteins or caseins via the Maillard reaction. Generally, monosaccharides are more reactive than disaccharides or oligosaccharides due to their smaller molecular size and greater accessibility to protein amino groups. The type of sugar affects not only the Maillard reaction products, may also impact functional properties of the resulting conjugates, such as heat stability, antioxidant activity, and emulsification. Differences in sugar structure (e.g., glycosidic linkages, molecular weight, charge, and chirality) influence protein conjugation outcomes. The type of sugar impacts Maillard reaction products, protein structure modifications, the biological activity of the glycated proteins, and the color of the ingredient (Cardoso et al., 2018; Wu et al., 2021; Sun et al., 2023).

The reduction in L* values and the increases in a* and b* with longer autoclaving times observed in the present study have been previously reported (González-Vega et al., 2011). This is a consequence of the Maillard reaction, which is induced by heat treatment. In the early stage of the Maillard reaction, initial color development can already be detected at the molecular level before visible browning occurs. The formation of Schiff bases and Amadori compounds was associated with an increase in absorbance at 294 nm, indicating that the early intermediates of the Maillard reaction contribute to the development of yellow coloration. Although intense brown pigments are formed only at more advanced stages of the reaction, these early intermediates subsequently undergo dehydration, fragmentation, and polymerization into melanoidins, leading to the pronounced decreases in L* and increases in a* and b* values observed in heat-damaged soybean meal (Sun et al., 2023).

The negative association between reactive Lys and glucose and fructose highlights the role that these monosaccharides play in heat-induced Lys loss in SBM. Fructose is especially

reactive due to its greater proportion of open-chain form compared with glucose, which increases its ability to participate in the early steps of the Maillard reaction, forming intermediates that progress to browning compounds and result in the nutritional loss of Lys (Ajandouz and Puigserver, 1999; Fontaine et al., 2007; Wu et al., 2021; Murai et al., 2024; Yang and Hiramatsu, 2024). Although glucose is less reactive than fructose, under heat processing conditions, glucose also contributes to the Maillard reaction, forming Amadori products and other intermediates that ultimately reduce Lys availability (Ajandouz and Puigserver, 1999; Lund and Ray, 2017). By contrast, the positive association between reactive Lys and the concentrations of stachyose, raffinose, and sucrose indicates that these sugars are less involved in Maillard reactions or may indicate samples with lower degrees of heat damage; however, they may also indirectly participate by hydrolyzing into single sugars during heat processing (Murai et al., 2024; Yang and Hiramatsu, 2024).

CONCLUSION

Results confirmed the hypothesis that the concentration of reactive Lys in intentionally heat-damaged SBM is strongly correlated with the concentration of reducing sugars, especially fructose. Autoclaving changed the chemical composition of SBM by reducing reactive Lys and oligosaccharides while increasing reducing sugars that contribute to Maillard reactions and the formation of dark brown pigments in SBM. The strong associations observed highlight the potential of using sugar profiles as indicators of heat damage in a specific ingredient. These observations provide a basis for integrating carbohydrate analysis with reactive Lys determination as a complementary approach to evaluate protein quality in processed soybean products.

TABLES

Table 6.1. Concentrations of dry matter, ash, fat, crude protein, Lys, reactive Lys, carbohydrates, and color parameters (L*, a*, b*) in 4 sources of soybean meal (SBM) autoclaved at different durations, as-is basis

Item, %	Autoclaved SBM, min				Autoclaved SBM, min			Autoclaved SBM, min				Autoclaved SBM, min			
	0	15	30	45	0	30	45	0	60	120	180	0	60	120	180
Dry matter, %	89.83	84.68	86.90	84.32	89.01	87.36	86.98	91.32	90.29	89.31	92.31	92.65	91.19	90.64	92.27
Ash, %	-	-	-	-	6.45	6.37	6.31	6.30	6.23	6.23	6.51	6.58	6.40	6.43	6.64
AEE[1], %	-	-	-	-	1.50	1.67	1.69	2.18	1.95	1.83	1.76	2.08	1.89	1.69	1.53
Crude protein, %	45.61	43.23	44.68	43.25	45.41	45.61	43.73	45.74	45.75	45.28	47.60	46.49	45.74	45.51	46.03
Moisture, %	10.17	15.32	13.10	15.68	10.99	12.64	13.02	8.68	9.71	10.69	7.69	7.35	8.81	9.36	7.73
Lys/CP	6.09	6.29	5.83	5.60	6.61	6.25	6.31	6.78	6.23	5.15	4.31	6.69	6.10	5.03	4.50
Lys, %	2.78	2.72	2.61	2.42	3.00	2.85	2.76	3.10	2.85	2.33	2.05	3.11	2.79	2.29	2.07
Reactive, % Lys	2.71	2.65	2.53	2.34	2.85	2.66	2.50	2.97	2.58	1.84	1.37	2.92	2.47	1.75	1.42

Table 6.1. (cont.)

Reactive Lys/Lys, %	97.48	97.43	96.93	96.69	95.00	93.33	90.58	95.81	90.53	78.97	66.83	93.89	88.53	76.42	68.60
Stachyose, %	5.80	5.90	6.00	5.90	6.28	5.84	5.92	4.75	4.42	3.81	3.19	6.09	5.59	4.95	3.90
Raffinose, %	1.20	1.20	1.20	1.10	1.10	1.20	1.20	0.96	0.91	0.79	0.63	1.13	1.08	0.97	0.78
Galactose, %	NA	NA	NA	NA	0.20	0.20	0.20	NA	NA	NA	NA	NA	NA	NA	NA
Sucrose, %	6.90	6.30	6.30	6.30	8.44	7.71	8.04	5.98	5.55	4.67	3.98	7.95	7.41	6.28	4.92
Glucose, %	NA	NA	NA	NA	0.05	0.05	0.05	0.05	0.05	0.06	0.12	0.06	0.05	0.08	0.12
Maltose, %	NA	NA	NA	NA	0.40	0.29	0.28	0.05	0.05	0.05	0.05	0.37	0.36	0.24	0.22
Fructose, %	NA	NA	NA	NA	0.10	0.16	0.14	0.06	0.15	0.28	0.42	0.11	0.14	0.28	0.48
Carbohydrates[2], %	13.90	13.40	13.50	13.30	16.57	15.45	15.83	11.85	11.13	9.66	8.39	15.71	14.63	12.8	10.42

Table 6.1. (cont.)

Lightness, L*[3]	-	-	-	-	80.37	73.91	69.15	83.28	69.90	58.35	56.84	82.92	68.85	57.28	54.36
Redness, a*[4]	-	-	-	-	4.37	6.55	8.20	2.93	8.23	12.35	12.76	3.56	9.37	13.05	13.22
Yellownes s, b*[5]	-	-	-	-	25.85	27.28	28.64	19.94	26.68	32.27	32.65	23.88	29.55	33.63	32.63

[1] AEE, acid-hydrolyzed ether extract; NA, not analyzable.

[2] Stachyose, raffinose, galactose, sucrose, glucose, maltose, fructose.

[3] Measures darkness (0) to lightness (100; greater L* indicates a lighter color).

[4] Measures redness (greater a* indicates a redder color).

[5] Measures yellowness (greater b* indicates a more yellow color).

Table 6.2. Pearson correlation between reactive Lys and individual or total carbohydrates and color parameters in non-autoclaved and autoclaved soybean meal (SBM)[1]

Item[2]	Pearson r	*P* -value	R^2	Strength	Direction
Fructose	-0.953	<0.001	0.91	Very strong	Negative
Glucose	-0.841	0.001	0.71	Strong	Negative
Total carbohydrates	0.811	<0.001	0.66	Strong	Positive
Stachyose	0.767	<0.001	0.59	Strong	Positive
Raffinose	0.759	0.001	0.58	Strong	Positive
Sucrose	0.737	0.002	0.54	Strong	Positive
Maltose	0.396	0.228	0.16	Weak	Positive
Galactose	0.276	0.319	0.08	Weak	Positive
Multiple carbohydrates model	-	0.067	0.94	Very strong	Mixed
Lightness, L*	0.969	<0.001	0.94	Very strong	Positive
Redness, a*	-0.954	<0.001	0.90	Very strong	Negative
Yellowness, b*	-0.897	<0.001	0.78	Strong	Negative

[1] Each correlation represents 11 to 15 observations.

[2] R^2, coefficient of determination.

Table 6.3. Parameter estimates and coefficient of determination (R^2) from simple linear regression models predicting reactive Lys from individual or total carbohydrates concentrations and color parameters[1]

Item	Intercept	Slope (Estimate)	R^2
Fructose	3.226	-4.301	0.91
Glucose	3.585	-18.827	0.71
Total carbohydrates	-0.005	0.154	0.66
Stachyose	0.188	0.421	0.59
Raffinose	0.085	2.231	0.58
Sucrose	0.434	0.302	0.54
Maltose	1.946	1.736	0.16
Galactose	2.311	1.796	0.08
Lightness, L*	-1.492	0.056	0.94
Redness, a*	3.614	-0.151	0.90
Yellowness, b*	6.013	-0.130	0.78

[1] Each coefficient represents 11 to 15 observations.

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CHAPTER 7: OVERHEATING REDUCES STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACIDS AND APPARENT ILEAL DIGESTIBILITY OF GROSS ENERGY IN ENZYME-TREATED SOYBEAN MEAL FED TO WEANLING PIGS

ABSTRACT

An experiment was conducted to test the hypothesis that increasing duration time of heat treatment of enzyme-treated soybean meal (**ESBM**) reduces the concentration of reactive Lys, the standardized ileal digestibility (**SID**) of Lys and other amino acids (**AA**) and apparent ileal digestibility (**AID**) of dry matter and gross energy when fed to weanling pigs. Twelve pigs with an average initial body weight of 11.38 ± 1.02 kg had a T-cannula installed in the distal ileum. Pigs were allotted to a replicated 6 × 6 Latin square design with six diets and six periods in each square. There were, therefore, 12 replicate pigs for each diet. One batch of ESBM was divided into four batches, which were autoclaved at 120 ℃ for 0, 30, 45, or 120 minutes. Conventional soybean meal (**SBM**) was also used, and each source of ESBM or SBM was included in one diet in which ESBM or SBM was the only AA-containing ingredient. A nitrogen-free diet was also used. Each diet was fed to pigs for 7 days, with 5 days of adaptation followed by two days of ileal digesta collection. Ileal digesta samples were thawed and mixed at the conclusion of the animal part of the experiment, and a subsample was lyophilized, ground, and analyzed for dry matter, gross energy, crude protein, and AA. The AID of dry matter, gross energy, crude protein, and AA, as well as the SID of crude protein and AA were calculated. The statistical model included diet as fixed variable and animal, period, and square as random variables. Linear and quadratic effects of increasing autoclaving time were analyzed using contrast coefficients.

Results indicated that analyzed reactive Lys in ESBM was reduced from 3.07 to 2.29% when duration time of autoclaving increased from 0 to 120 min. The AID of dry matter and gross energy was reduced (linear; $P < 0.05$) or tended to be reduced (quadratic; $P < 0.10$), respectively, as autoclaving time increased. The SID of Lys was greater in SBM than in non-autoclaved ESBM ($P < 0.05$), but the SID of other indispensable AA did not differ between SBM and non-autoclaved ESBM. The SID of all indispensable AA was reduced (quadratic; $P < 0.05$) by increasing duration time of autoclaving. In conclusion, autoclaving of ESBM reduced the concentration of all AA and reactive Lys as well as the SID of AA, and the AID of dry matter and gross energy when fed to weanling pigs.

Keywords: amino acids, digestibility, enzyme-treated soybean meal.

Abbreviations

AA, amino acids; AID, apparent ileal digestibility; CP, crude protein; DM, dry matter; ESBM, enzyme-treated soybean meal; GE, gross energy; SBM, soybean meal; SID, standardized ileal digestibility.

INTRODUCTION

Soybean meal (**SBM**) is an excellent source of amino acids (**AA**) for growing-finishing pigs, but because of antigens and oligosaccharides, SBM cannot be used as the only source of AA in diets for weanling pigs (Cervantes-Pahm et al., 2010; Navarro et al., 2017). Therefore, animal proteins are often included in diets for weanling pigs, which increases diet costs. Enzyme-treated soybean meal (**ESBM**) is produced by treating dehulled, solvent-extracted SBM for several hours with a

proprietary blend of enzymes, which results in a reduction in antigens and removal of oligosaccharides (Goebel and Stein, 2011; Ma et al., 2019). Therefore, ESBM can be used in diets for young pigs as an alternative to animal proteins (Cervantes-Pahm et al., 2010). However, if an ingredient is overheated during the drying process after fermentation, concentrations of AA and the standardized ileal digestibility (**SID**) of Lys and other AA may be reduced due to the Maillard reaction and other chemical reactions (Navarro et al., 2017; Ton-Nu et al., 2020). This reaction also leads to the development of characteristic color changes in the ingredient, which serve as visual indicators of the extent of heat damage (González-Vega et al., 2011; Sun et al., 2023). The Maillard reaction causes Lys to bind to reducing sugars, which makes it unavailable for protein synthesis. This has been demonstrated for SBM (González-Vega et al., 2011) and other ingredients (Kim et al., 2012; Almeida et al., 2013; 2014a; 2014b; 2014c). However, most reducing sugars in SBM are eliminated during production of ESBM (Cervantes-Pahm and Stein, 2010), and it is, therefore, possible that ESBM is less susceptible to heat damage than other ingredients. However, data to confirm this hypothesis have not been reported.

The Lys that becomes bound to reducing sugars during the Maillard reaction cannot be used for protein synthesis by the animal and is, therefore, called unreactive Lys. In contrast, the Lys that is not bound to a reducing sugar is called reactive Lys. Therefore, the reactive Lys is the Lys in a feed ingredient that is available for digestion, absorption, and utilization for protein synthesis, because it is Lys that has not undergone chemical reactions (Oliveira et al., 2021). However, some of the unreactive Lys will be analyzed as Lys because it converts back to Lys during the acid hydrolysis that precedes analysis for AA. Based on results from the AA analysis, it is, therefore, not possible to estimate how much Lys is available for protein synthesis. However, the concentration of reactive Lys in a feed ingredient relative to the concentration of

total analyzed Lys can be used as an indicator of heat damage in the ingredient because the amount of Lys that is available for protein synthesis is estimated. Analysis of reactive Lys has been used to estimate Lys in feed ingredients commonly used in diets for pigs (Fontaine et al., 2007; Pahm et al, 2008; 2010; Kim et al., 2012). However, to our knowledge, there is no information about reactive Lys in ESBM, and it is, therefore, not known if heat damage takes place during production of ESBM. In addition to causing reduced digestibility of Lys and other AA, heating may also result in reduced digestibility of dry matter (**DM**) and gross energy (**GE**) because binding of reducing sugars to Lys will prevent these sugars from being used as a source of energy by the pig (Oliveira et al., 2020a; 2020b). There are, however, no data for the impact of heating on the digestibility of DM and GE in ESBM. Therefore, the objective of this work was to test the hypothesis that overheating reduces the concentration of reactive Lys, total Lys, the SID of Lys, and apparent ileal digestibility (**AID**) of DM and GE when fed to weanling pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs were castrated male pigs that were the offspring of Line 800 males mated to Camborough females (Pig Improvement Company, Henderson, TN, USA).

One source of conventional SBM was divided into two batches; one batch was used without further processing, and the other batch was used to produce ESBM. The SBM and ESBM were provided by Hamlet Protein (Findlay, OH, USA). The ESBM was divided into four batches, that were not autoclaved or autoclaved at 120 °C for 30, 45, or 120 minutes. Concentration of AA and reactive Lys in all ingredients was analyzed before the experiment

started. Five experimental diets were formulated based on each source of ESBM or conventional SBM. These ingredients were the only AA-containing ingredients in each diet (Tables 7.2 and 7.3). A nitrogen-free diet was also used. There were, therefore, six experimental diets. Vitamins and minerals were included in all diets to meet current requirement estimates (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

Twelve weanling pigs with an average initial body weight of 11.38 ± 1.02 kg had a T-cannula installed in the distal ileum (Stein et al., 1998). Pigs were allotted to a replicated 6 × 6 Latin square design (Kim and Stein, 2009) with six diets and six periods in each square. There were, therefore, 12 replicate pigs for each diet. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sidings and fully slatted tribar floors, and a feeder and a nipple drinker were installed in each pen.

Experimental periods were seven days. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The initial five days of each period were considered an adaptation period to the diet, but ileal digesta were collected for nine hours on day 6 and also on day 7 using standard procedures (Stein et al., 1998). On the completion of one experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered.

Chemical analysis

At the conclusion of the experiment, ileal digesta samples were thawed and mixed within animal, and subsamples were collected for analysis. Ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Samples of diets, feed ingredients, and ileal digesta were analyzed for DM (method 930.15; AOAC Int., 2019) and nitrogen was analyzed by combustion using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI; method 990.03;

AOAC Int., 2019). Crude protein (**CP**) was calculated as nitrogen × 6.25. Ash in ingredients and diets was also analyzed (method 942.05; AOAC Int., 2019). Diet, ingredient, and ileal digesta samples were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Amino acids in feed ingredients, diets, and ileal digesta samples were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* hydrochloric acid for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E (b)]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(b); AOAC Int., 2019]. Reactive Lys was analyzed in SBM and in the four batches of ESBM using Ultra High-Performance Liquid Chromatography (Model QSight LX50; PerkinElmer, Waltham, MA, USA). Samples were incubated with an O-methylisourea solution at pH 10.6 to initiate the guanidination reaction for 72 h at room temperature (Pahm et al., 2008) and after incubation, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Chromium was analyzed in diets and ileal digesta using Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2019). Samples were prepared using nitric acid-perchloric acid [method 968.08D (b); AOAC Int., 2019]. Stachyose, raffinose, galactose, and sucrose in SBM, ESBM, autoclaved ESBM, and diets were analyzed by high-performance liquid chromatography using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA; Navarro et al., 2018).

Instrumental color was measured in SBM, ESBM, and autoclaved ESBM, determined by measuring L* (lightness), a* (redness), and b* (yellowness) using a HunterLab Miniscan EZ

Plus apparatus, Model 45/0-L, spectrophotometer. The instrument was standardized using black and white tiles and operated with a D65 light source and a 10° observer angle (González- Vega et al., 2011).

Calculations and statistical analysis

Basal endogenous losses of CP and AA were calculated from pigs fed the nitrogen-free diet (Stein et al., 2007). Apparent ileal digestibility of DM, GE, CP, and all AA was calculated using the analyzed DM, GE, CP, AA, and Cr concentrations in the diets and ileal digesta samples. The SID values were calculated by correcting AID values for the basal endogenous losses of CP and AA (Stein et al., 2007).

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified as values with internally studentized residuals outside the range of -3 to 3 (Tukey, 1977). However, no outliers were identified. The pig was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC). The statistical model included diet as fixed effect, and animal, period, and square as random effects. Mean values were calculated using the LSMeans statement. Linear and quadratic effects of increasing autoclaving time were also analyzed using contrast statements. Statistical significance was considered at $P \leq 0.05$, and tendencies were considered if $0.05 \leq P < 0.10$.

RESULTS

Pigs were healthy throughout the experiment, and no feed refusal was observed. Results indicated that the process used to produce ESBM reduced the concentrations of stachyose from 3.80% to 0.30%, raffinose from 0.90% to 0.10%, and sucrose from 6.40% to 0.10% (Table 7.1). The concentration of ash increased from 5.40 to 7.32%, and CP increased from 44.83 to 53.26%.

The concentration of AA was also greater in ESBM compared with SBM. However, reactive Lys was reduced from 3.07% in the non-autoclaved ESBM to 2.64, 2.53, and 2.29% in ESBM that was autoclaved for 30, 45, or 120 min, and regardless of the time of autoclaving, the concentration of all AA in autoclaved ESBM was reduced compared with conventional SBM. The L* value for SBM was 82.32, the a* value was 2.08, and the b* value was 25.59. For ESBM, the L* value decreased from 77.20 in the non-autoclaved ESBM to 65.87, 53.92, and 49.95 as autoclaving time increased to 30, 45, and 120 min, respectively. The corresponding a* values increased from 5.30 to 11.39, 16.01, and 16.72, and the b* values increased from 30.35 to 34.29, 38.52, and 38.93.

No differences in AID of DM and GE between SBM and non-autoclaved ESBM were observed, but AID of DM and GE in autoclaved ESBM was reduced (linear, $P < 0.01$) as autoclaving time increased (Table 7.4). The AID and SID of Lys and Cys were greater ($P < 0.05$) in SBM than in non-autoclaved ESBM (Tables 7.4 and 7.5), but the AID of Phe and Tyr tended to be less in SBM than in ESBM ($P < 0.10$), whereas the SID of Gly and Pro tended ($P < 0.10$) to be greater in SBM than in ESBM. However, for all other AA, no difference in the SID was observed between SBM and non-autoclaved ESBM. The AID of CP and all AA decreased (linear or linear and quadratic, $P < 0.05$) as autoclaving time for ESBM increased. Likewise, the SID of CP and all AA decreased (linear or quadratic, $P < 0.05$) as the time of autoclaving increased.

DISCUSSION

The reduced concentration of stachyose, raffinose, and sucrose in ESBM compared with SBM may be attributed to the fermentation process and agreed with reported values (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; Navarro et al., 2017; Ma et al., 2019). Because of the

removal of oligosaccharides and sucrose, ESBM has an increased concentration of ash, CP, and AA compared with SBM. However, the concentration of Lys in ESBM increased less than the concentration of other AA compared with conventional SBM, which is likely a result of the fact that Lys is consumed during fermentation (Thomas and Ingledew, 1992).

The increased concentration of CP and most AA in non-autoclaved ESBM compared with SBM was in agreement with reported data (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012). The Lys:CP ratio in SBM, which was 6.42 %, indicated that this source of SBM was not heat-damaged (Oliveira et al., 2021). However, the Lys:CP ratio for non-autoclaved ESBM (5.95 %) indicates slight heat-damage during production, because a Lys:CP ratio less than 6.0 % indicates heat damage (González-Vega et al., 2011). However, because Lys may also be fermented during the production of ESBM, fermentation may have contributed to the reduced Lys:CP ratio. Nevertheless, the Lys:CP ratio that was calculated for ESBM used in this experiment was within the range of previous data (Hossain et al., 2016; Nørgaard et al., 2021; Yang et al., 2022; Zhang and Piao, 2022; Garavito-Duarte et al., 2023).

The decrease in AA in the three sources of autoclaved ESBM that was observed as the time of autoclaving increased is due to the Maillard reaction or others forms of AA destruction by heat. However, after 45 min there were no reducing sugars in the autoclaved ESBM. Therefore, the reduction of Lys and other AA in ESBM autoclaved for 45 or 120 min is likely a result of heat-damage reactions other than Maillard reactions, which may include deamination, peptide bond cleavage, oxidation reactions, or interactions with dietary lipids (Kutzli et al., 2021). Reduction in the concentration of AA and reduced Lys:CP are common indicators of heat-damage in a feed ingredient (Almeida et al., 2013; 2014c; Oliveira et al., 2021).

Reactive Lys is a better indicator of the quality of protein in protein ingredients than analyzed CP or total Lys (Pahm et al., 2008). Reactive Lys is the Lys that can be absorbed from the small intestine and used for protein synthesis by the animal (Kim et al., 2012). However, if an ingredient is heat-processed and the ingredient or diet is overheated, reactive Lys is reduced, and the digestibility of Lys is also reduced due to Maillard reaction or other forms of heat-damage (Cervantes-Pahm and Stein, 2010). Because of this reduction, use of feed ingredients or diets that have been over-heated will result in reduced protein synthesis, reduced growth, and reduced feed efficiency (Pahm et al., 2008; González-Vega et al., 2011; Almeida et al., 2014c; Oliveira et al., 2021). If a heat-damaged feed ingredient is included in a diet for pigs, it is, therefore, necessary to add additional crystalline AA to the diet to avoid the negative effects of feeding pigs with insufficient AA compared with the requirements of pigs (Almeida et al., 2014c; Oliveira et al., 2021).

The L*, a*, and b* values for SBM were in agreement with reported values (González-Vega et al., 2011; Yang et al., 2020). The reduction in L* values and the increases in a* and b* values with longer autoclaving times observed in autoclaved ESBM have been previously reported in autoclaved SBM (González-Vega et al., 2011). This is a consequence of heat damage due to the Maillard reaction. The formation of Schiff bases and Amadori compounds in the early stage of the Maillard reaction was associated with the development of yellow coloration, whereas brown pigments are observed at the intermediates and advanced stage because of the synthesis of deoxyketosyl compounds and melanoidins, respectively (González-Vega et al., 2011; Sun et al., 2023).

The AID and SID of Lys in SBM and the ESBM that was not autoclaved were in agreement with reported values (NRC, 2012; Navarro et al., 2017; Li et al., 2019a; 2019b). The

reduced SID in the autoclaved ESBM compared with non-autoclaved ESBM may be attributed to Maillard reaction where sugars are bound to amino groups in AA by a covalent linkage between free amino groups of AA (usually Lys or Arg) and the carbonyl groups of a reducing sugar (Teodorowicz et al., 2017), which affects protein functionality and reduces absorption (Lund and Ray, 2017). The presence of Maillard reaction products in the intestinal tract of animals may also induce a reduction in the efficiency of digestive enzymes, which results in more undigested peptides in the ileal digesta (Rutherfurd et al., 2006). Thus, autoclaving of ESBM reduced the concentration of all AA and reactive Lys as well as the SID of AA in ESBM, and the longer ESBM was autoclaved, the greater was the reduction in both concentration and SID of ESBM. Reactive Lys represents the fraction of Lys with an unmodified ε-amino group that is available for enzymatic digestion and absorption. During autoclaving, the ε-amino group of Lys participates in the Maillard reaction, forming Schiff bases and Amadori compounds that are not digestible by endogenous proteases. As a result, a greater proportion of Lys becomes chemically bound and unavailable, which directly reduces the apparent and SID of Lys. Because Lys is often the first-limiting AA in soybean-based diets, reductions in reactive Lys may also impair the digestibility of other AA by limiting protein hydrolysis and absorption efficiency. Consequently, longer autoclaving times exacerbate the loss of reactive Lys and contribute to the observed reductions in AID and SID of AA in ESBM (Cervantes-Pahm and Stein, 2010).

The reduction in the AID of DM that was observed with increased time of autoclaving is in agreement with data for SBM, rapeseed meal, and soybean expellers that were heat-treated (Oliveira et al., 2020a; 2020b; Espinosa et al., 2021). This observation indicates that the digestibility of not only AA, but also other nutrients is negatively affected by heat treatment. It is possible that heating may result in formation of nitrogen-fiber complexes that may reduce fiber

fermentation and fatty acids may be converted to cycling polymers that resist digestibility (Oliveira et al., 2020a; 2020b; Jaksics et al., 2023). Additionally, Amadori compounds and melanoidins that are generated in the Maillard reaction are not bioavailable for pigs, and therefore, also contribute to the reduced AID of DM. As a consequence of the reduced AID of DM, the AID of GE was also reduced as ESBM was autoclaved. Concentration of digestible and metabolizable energy in 00-rapeseed meal, SBM, and soybean expellers were also reduced by autoclaving these ingredients (Oliveira et al., 2020a; 2020b; Espinosa et al., 2021). Therefore, it appears that an important impact of heat damage of feed ingredients is a reduction in the digestibility of GE.

CONCLUSION

Results indicate that enzyme treatment of SBM results in a reduced concentration of oligosaccharides and sucrose, but an increased concentration of other nutrients including ash, CP, and AA. The reduction in L* and the increases in a* and b* values confirmed the color darkening associated with heat damage. The SID of most AA and the AID of GE was not different in ESBM compared with SBM. However, autoclaving ESBM at 120 °C for 30, 45, or 120 min reduced the concentrations and digestibility of AA, DM, and GE due to heat damage and overheating during processing, thereby reducing the economic value of the ingredient.

TABLES

Table 7.1. Analyzed nutrient composition and color parameters in soybean meal (SBM), enzyme-treated soybean meal (ESBM), and autoclaved ESBM, as-fed basis

Item, %	Not autoclaved		ESBM autoclaved, min		
	SBM	ESBM	30	45	120
Dry matter	88.71	93.06	90.89	91.40	90.02
Ash	5.40	7.32	6.39	6.34	6.13
Gross energy, kcal/kg	4352	4605	4546	4546	4579
Stachyose	3.80	0.30	0.20	0.10	0.10
Raffinose	0.90	0.10	NA	NA	NA
Galactose	NA[1]	0.40	0.10	NA	NA
Sucrose	6.40	0.10	0.10	0.10	0.10
Crude protein	44.83	53.26	51.57	51.32	51.68
Lys:CP	6.42	5.95	5.47	5.12	4.62
Indispensable amino acids					
Arg	3.20	3.73	3.52	3.39	3.27
His	1.16	1.38	1.34	1.30	1.25
Ile	2.14	2.56	2.54	2.53	2.52
Leu	3.44	4.10	4.04	4.03	3.96
Lys	2.88	3.17	2.82	2.63	2.39
Reactive Lys	2.85	3.07	2.64	2.53	2.29
Met	0.60	0.71	0.70	0.70	0.69
Phe	2.26	2.73	2.67	2.63	2.58

Table 7.1. (cont.)

Thr	1.70	2.06	2.01	2.01	1.96
Trp	0.54	0.67	0.62	0.62	0.59
Val	2.24	2.70	2.67	2.67	2.67
Dispensable amino acids					
Ala	1.92	2.32	2.27	2.27	2.24
Asp	4.90	5.73	5.62	5.61	5.51
Cys	0.64	0.74	0.71	0.69	0.62
Glu	8.20	9.50	9.25	9.28	9.18
Gly	1.87	2.24	2.20	2.19	2.17
Pro	2.29	2.72	2.61	2.63	2.60
Ser	1.89	2.27	2.18	2.18	2.08
Tyr	1.64	1.92	1.90	1.86	1.80
Lightness, L*[2]	82.32	77.20	65.87	53.92	49.95
Redness, a*[3]	2.08	5.30	11.39	16.01	16.72
Yellowness, b*[4]	25.59	30.35	34.29	38.52	38.93

[1] NA: not analyzable.

[2] Measures darkness (0) to lightness (100; greater L* indicates a lighter color).

[3] Measures redness (greater a* indicates a redder color).

[4] Measures yellowness (greater b* indicates a more yellow color).

Table 7.2. Ingredient composition of experimental diets containing soybean meal (SBM) or enzyme-treated soybean meal (ESBM), as-fed basis

	Not autoclaved		ESBM autoclaved, min			
Ingredient, %	SBM	ESBM	30	45	120	N-free
SBM	40.00	-	-	-	-	-
ESBM	-	35.00	35.00	35.00	35.00	-
Cornstarch	30.75	35.75	35.75	35.75	35.75	70.25
Lactose	15.00	15.00	15.00	15.00	15.00	-
Sucrose	10.00	10.00	10.00	10.00	10.00	20.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	2.00
Solka floc[1]	-	-	-	-	-	4.00
Ground limestone	0.70	0.95	0.95	0.95	0.95	0.40
Dicalcium phosphate	1.25	1.00	1.00	1.00	1.00	1.55
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Potassium carbonate	-	-	-	-	-	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	-	-	-	-	-	0.10

[1]Fiber Sales and Development Corp., Urbana, OH, USA.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as

Table 7.2. (cont.)

menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 7.3. Analyzed nutrient composition in experimental diets containing soybean meal (SBM) or enzyme-treated soybean meal (ESBM), as fed basis

Item, %	Not autoclaved		Autoclaved ESBM, duration (min)			
	SBM	ESBM	30	45	120	N-free
Dry matter	92.59	93.63	93.34	93.40	93.13	93.60
Ash	4.60	4.77	4.47	4.73	4.64	3.24
Gross energy, kcal/kg	3927	3994	3979	3993	3995	3745
Crude protein	16.48	18.17	17.70	17.74	17.23	0.60
Stachyose	2.80	0.20	0.10	NA[1]	NA	NA
Raffinose	0.50	0.10	NA	NA	NA	NA
Galactose	NA	NA	NA	NA	NA	NA
Sucrose	15.20	12.40	12.40	12.00	12.00	14.80
Indispensable AA						
Arg	1.04	1.16	1.10	1.05	1.04	0.01
His	0.38	0.43	0.41	0.41	0.39	NA
Ile	0.72	0.81	0.79	0.79	0.78	0.01
Leu	1.16	1.31	1.28	1.28	1.26	0.01
Lys	0.96	1.00	0.90	0.83	0.77	0.01
Met	0.19	0.22	0.20	0.19	0.19	NA
Phe	0.73	0.83	0.81	0.81	0.80	0.01
Thr	0.56	0.65	0.63	0.63	0.62	0.01
Trp	0.21	0.23	0.21	0.21	0.20	< 0.02
Val	0.77	0.87	0.85	0.85	0.83	0.01

Table 7.3. (cont.)

Dispensable AA						
Ala	0.65	0.76	0.74	0.73	0.73	0.01
Asp	1.60	1.82	1.74	1.74	1.70	0.02
Cys	0.21	0.23	0.22	0.21	0.20	NA
Glu	2.83	3.19	3.10	3.08	3.05	0.02
Gly	0.64	0.73	0.71	0.71	0.70	0.01
Pro	0.81	0.92	0.91	0.88	0.86	0.03
Ser	0.64	0.73	0.71	0.71	0.70	0.01
Tyr	0.48	0.56	0.53	0.54	0.54	NA

[1] NA: not analyzable.

Table 7.4. Apparent ileal digestibility (AID) of dry matter, crude protein, gross energy, and amino acids in soybean meal (SBM), enzyme-treated soybean meal (ESBM), and autoclaved ESBM fed to weanling pigs[1]

Item, %							Contrast *P*-value		
	Not autoclaved		Autoclaved ESBM, duration (min)				Not autoclaved	Autoclaved	
	SBM	ESBM	30	45	120	SEM	SBM vs. ESBM	Linear	Quadratic
Dry matter	78.99	79.87	79.29	76.82	77.16	0.77	0.335	0.005	0.068
Crude protein	77.05	76.25	71.98	60.41	56.35	1.93	0.680	< 0.001	0.001
Gross energy	78.64	80.31	79.70	76.67	76.97	0.89	0.110	0.002	0.064
Indispensable amino acids									
Arg	89.96	90.18	88.41	80.20	76.99	1.35	0.872	< 0.001	0.010
His	85.51	85.48	83.12	72.14	67.81	1.36	0.988	< 0.001	0.002
Ile	84.62	85.17	83.06	74.84	70.68	1.23	0.679	< 0.001	0.011
Leu	83.66	85.25	83.57	76.05	72.10	1.20	0.219	< 0.001	0.028
Lys	85.66	80.00	76.49	66.11	58.18	1.46	0.002	< 0.001	0.025

Table 7.4. (cont.)

Met	86.10	86.62	84.48	75.05	70.32	1.23	0.700	< 0.001	0.007
Phe	82.87	85.28	83.54	76.16	72.68	1.18	0.056	< 0.001	0.015
Thr	76.06	75.44	72.68	63.22	57.90	1.51	0.739	< 0.001	0.033
Trp	84.94	84.81	81.86	74.86	70.22	1.26	0.930	< 0.001	0.030
Val	82.11	82.17	81.68	72.83	68.62	1.30	0.965	< 0.001	0.029
Dispensable amino acids									
Ala	78.16	77.66	75.98	65.72	62.41	1.77	0.789	< 0.001	0.024
Asp	81.41	79.73	75.73	62.67	52.44	1.41	0.316	< 0.001	0.010
Cys	70.14	60.42	57.02	41.87	38.92	2.71	0.005	< 0.001	0.018
Glu	83.42	82.13	79.95	71.74	68.35	1.79	0.506	< 0.001	0.049
Gly	65.31	61.50	60.47	46.63	44.09	4.17	0.255	< 0.001	0.098
Pro	58.05	47.35	49.17	24.44	27.69	13.83	0.226	0.017	0.250
Ser	81.25	82.16	80.24	72.59	68.98	1.44	0.548	< 0.001	0.033
Tyr	81.70	84.09	82.03	75.54	72.52	1.18	0.072	< 0.001	0.017

[1]Each least squares mean for each treatment represents 12 observations.

Table 7.5. Standardized ileal digestibility (SID) of crude protein, and amino acids in soybean meal (SBM), enzyme-treated soybean meal (ESBM), and autoclaved ESBM to feed weanling pigs[1, 2]

Item, %							Contrast *P*-value		
	Not autoclaved		Autoclaved ESBM, duration (min)				Not autoclaved	Autoclaved	
	SBM	ESBM	30	45	120	SEM	SBM vs. ESBM	Linear	Quadratic
Crude protein	86.67	84.89	80.91	69.49	65.56	1.93	0.363	<0.001	0.001
Indispensable amino acids									
Arg	94.78	94.55	93.00	85.01	81.84	1.35	0.862	< 0.001	0.017
His	89.89	89.40	85.93	76.23	71.36	1.36	0.751	< 0.001	0.002
Ile	88.83	88.95	86.92	78.71	74.58	1.23	0.927	< 0.001	0.012
Leu	87.94	89.09	87.48	79.97	76.07	1.20	0.375	< 0.001	0.031
Lys	89.34	83.56	80.44	70.40	62.78	1.46	0.002	< 0.001	0.040
Met	89.84	89.89	88.07	78.82	74.09	1.23	0.974	< 0.001	0.014
Phe	87.23	89.16	87.51	80.12	76.68	1.18	0.123	< 0.001	0.017
Thr	85.44	83.61	81.08	71.63	66.42	1.51	0.329	< 0.001	0.040
Trp	90.14	89.63	87.10	80.02	75.67	1.26	0.747	< 0.001	0.041

Table 7.5. (cont.)

Val	87.70	87.17	85.48	77.01	72.84	1.30	0.719	< 0.001	0.031
Total	89.21	88.55	86.50	78.12	73.76	1.21	0.629	< 0.001	0.016
Dispensable amino acids									
Ala	86.88	85.20	83.71	73.55	70.22	1.77	0.370	< 0.001	0.032
Asp	85.49	83.36	79.52	66.46	56.31	1.41	0.205	< 0.001	0.011
Cys	79.42	68.99	65.95	51.24	48.73	2.71	0.003	< 0.001	0.023
Glu	86.37	84.78	82.67	74.47	71.10	1.78	0.411	< 0.001	0.052
Gly	85.58	79.47	78.88	65.06	62.73	4.17	0.072	< 0.001	0.118
Pro	104.59	88.79	90.93	67.65	71.78	13.83	0.077	0.037	0.274
Ser	88.28	88.40	86.63	78.98	75.44	1.44	0.938	< 0.001	0.039
Tyr	86.61	88.34	86.51	79.94	76.90	1.18	0.188	< 0.001	0.023
Total	88.03	84.62	82.82	71.45	67.64	2.55	0.123	< 0.001	0.037
Total amino acids	88.38	86.25	84.49	74.45	70.48	1.80	0.224	< 0.001	0.025

[1]Each least squares mean for each treatment represents 12 observations.

[2]Values for SID were calculated by correcting values for apparent ileal digestibility for basal ileal endogenous losses. The basal ileal endogenous losses were determined (g/kg dry matter intake) as CP, 13.18; Arg, 0.61; His, 0.18; Ile, 0.32; Leu, 0.53; Lys, 0.39; Met, 0.08; Phe, 0.33; Thr, 0.56; Trp, 0.12; Val, 0.46; Ala, 0.66; Asp, 0.70; Cys, 0.21; Glu, 0.91; Gly, 1.50; Pro, 4.57; Ser, 0.48; Tyr, 0.25; and total AA, 12.84.

LITERATURE CITED

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CHAPTER 8: EFFECTS OF HEAT DAMAGE AND SYNTHETIC AMINO ACID SUPPLEMENTATION ON GROWTH PERFORMANCE OF WEANLING PIGS FED DIETS BASED ON SOYBEAN MEAL AND ENZYME-TREATED SOYBEAN MEAL

ABSTRACT

An experiment was conducted to test the hypothesis that growth performance of weanling pigs can be predicted from the concentration of reactive Lys in heat-damaged soybean ingredients and that supplementation with crystalline amino acids (**AA**) can compensate for reduced reactive Lys. One source of conventional soybean meal (**SBM**) and one source of enzyme-treated soybean meal (**ESBM**) were each divided into three batches. One batch of SBM and one batch of ESBM were used without further processing, one batch of SBM and one batch of ESBM were autoclaved at 120 ± 1°C to moderately heat-damage the ingredients and reduce reactive Lys to 85% of total Lys in each ingredient, and the last batch of SBM and ESBM were autoclaved at 120 ± 1°C to severely heat-damage the ingredients and reduce reactive Lys to approximately 70% of total Lys. Four diets were formulated for phase 1 (day 0 to 14 post-weaning) and four additional diets were formulated for phase 2 (day 15 to 28 post-weaning). The four diets in each phase included a control diet containing the non-autoclaved SBM and ESBM, a diet containing moderately heat-damaged SBM and ESBM, a diet containing severely heat-damaged SBM and ESBM, and a diet containing severely heat-damaged SBM and ESBM supplemented with crystalline AA to match digestible AA levels in the control diet. A total of 160 weanling pigs with an initial body weight (**BW**) of 5.71 ± 0.55 kg were allotted to the 4 treatments in a randomized complete block design with 10 replicate pens per treatment. Growth performance

and feed intake were recorded over 28 days. Results indicated that autoclaving reduced reactive Lys, Lys to crude protein ratio, sucrose, stachyose, and raffinose, whereas glucose and fructose increased as autoclaving time increased. Pigs fed severely heat-damaged diets had reduced final BW and average daily gain (**ADG**) compared with pigs fed the control diet ($P < 0.001$). Supplementation with crystalline AA partially restored growth performance of pigs, but pigs fed the AA supplemented diet had reduced ($P < 0.05$) ADG and final BW compared with pigs fed the control diet. Reactive Lys intake was strongly correlated with final BW and ADG. In conclusion, heat-damage of soybean ingredients reduces reactive Lys and growth performance of pigs, but supplementation with crystalline AA can partially compensate for the loss of nutritional value.

Keywords: enzyme-treated soybean meal, growth performance, Maillard reaction, reactive Lys, soybean meal.

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; AEE, acid-hydrolyzed ether extract; BW, body weight; CP, crude protein; DM, dry matter; ESBM, enzyme-treated soybean meal; G:F, gain to feed ratio; SBM, soybean meal; SID, standardized ileal digestibility.

INTRODUCTION

Soybean meal (**SBM**) and enzyme-treated soybean meal (**ESBM**) are excellent sources of amino acids (**AA**) and may be combined to provide the majority of AA in diets for weanling pigs. However, if ingredients are overheated during processing, concentrations and digestibility of Lys

and other AA are reduced due to heat-damage and Maillard reactions (Navarro et al., 2017; Ton et al., 2020). This has been demonstrated in SBM (González-Vega et al., 2011) and other ingredients (Kim et al., 2012; Almeida et al., 2013; 2014a; 2014b). The Maillard reaction causes reducing sugars to bind to Lys, which makes the Lys unavailable for use in protein synthesis. Reactive Lys is the Lys that has not been used in the Maillard reaction, and therefore, can be absorbed from the small intestine and used for protein synthesis (Kim et al., 2012; Kutzli et al., 2021). However, if SBM or ESBM that is heat-damaged are used in diets, growth performance of pigs may be reduced (Almeida et al., 2014c). There are, however, no data to demonstrate the extent to which heat-damage of soybean ingredients will reduce growth performance, and it is not known if growth performance of pigs can be predicted from the concentration of reactive Lys in a diet. Therefore, the objective of this work was to test the hypothesis that growth performance of weanling pigs can be predicted from the concentration of reactive Lys in soybean ingredients and that the reduced growth performance caused by heat-damage can be corrected by adding crystalline AA to the diets to compensate for the reduced absorption and utilization of AA from heat-damaged ingredients.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs that were the offspring of Line 337 males mated to Camborough females (Pig Improvement Company, Henderson, TN) were used in the experiment.

One source of conventional SBM and one source of ESBM (HP 300, Hamlet Protein, Findlay, OH, USA) were obtained, and each source was divided into three batches. The reactive

Lys in both ingredients was analyzed, and it was confirmed that reactive Lys was greater than 95% of total Lys in both ingredients. One batch of each ingredient was used without further processing. The second batch was moderately heat-treated in an autoclave at 120 ± 1ºC to reduce reactive Lys to 85% of total Lys. The last batch was severely heat-treated in an autoclave at 120 ± 1ºC until reactive Lys was reduced to 70% of total Lys. There were, therefore, six batches in total (three batches of conventional SBM and three batches of ESBM). The standardized ileal digestibility (**SID**) of all AA in ESBM had been determined previously in a separate digestibility experiment, and the concentration of SID of AA in each batch of autoclaved ESBM was calculated.

A two-phase feeding program was used, with days 1 to 14 as phase 1 and days 15 to 28 as phase 2. Four diets were formulated. The control diet in each phase contained the two batches of non-heat-damaged SBM and ESBM, and this diet was formulated to contain 90% of the requirement for digestible Lys for 5 to 7 kg and 7 to 11 kg pigs, respectively (NRC, 2012). A second diet, which was identical to the control diet with the exception that this diet contained SBM and ESBM that were moderately heat-damaged, was also formulated within each phase. This diet, therefore, was expected to have reactive Lys that was 85% of the total Lys. A third diet for each phase was formulated by using SBM and ESBM with severe heat-damage and this diet was expected to have reactive Lys that was 70% of the total Lys. The fourth diet was identical to the third diet with the exception that crystalline AA were added to bring the concentration of digestible AA and reactive Lys to the same level as that used in the control diet. All diets were analyzed for Lys and reactive Lys before the animal part of the experiment was initiated to confirm correct diet mixing.

A total of 160 weaned pigs with an initial body weight (**BW**) of 5.71 ± 0.55 kg were allotted to the four dietary treatments. The experiment was conducted using a randomized complete block design with room as the blocking factor. Two blocks were used, each representing a separate room and containing 80 pigs. Within each room (block), pigs were randomly allotted to 20 pens, with five replicate pens per dietary treatment and four pigs per pen (two barrows and two gilts). Therefore, a total of 40 pens were used, resulting in 10 replicate pens per treatment. Pigs were weighed at the beginning of the experiment, on day 14, and on day 28, and feed allotments were recorded daily. Quantities of feed left in the feeders were also recorded on the days when pig weights were recorded. Data were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) for each treatment group. If a pig died or was removed from the experiment, the pig was weighed at the time of removal, and the feeder in that pen was also weighed to determine feed intake up to the time of removal (Laskoski et al., 2021). Feed intake was corrected based on the number of pigs that remained in the pen. Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea).

Chemical analyses

At the end of the experiment, diets and feed ingredients were analyzed for dry matter (**DM**; method 930.15). Ash in ingredients and diets was also analyzed (method 942.05; AOAC Int., 2019). Diets and ingredients were analyzed for gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Ingredients were analyzed for acid-hydrolyzed ether extract (**AEE**) using *3N* HCl (AnkomHCl, Ankom Technology, Macedon, NY), followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY).

Glucose, maltose, sucrose, fructose, stachyose, and raffinose in ingredients and diets were analyzed by High-Performance Liquid Chromatography using a pulsed amperometric detector (Dionex Tech. Notes 21 & 92, Sunnyvale, CA; Navarro et al., 2018). These samples were also analyzed for nitrogen by combustion using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI; method 990.03). Crude protein (**CP**) was calculated as nitrogen × 6.25. Trypsin inhibitor concentrations were analyzed in ingredients (method Ba 12-75; AOCS, 2006). Amino acids in feed ingredients and diets were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc: Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* hydrochloric acid for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E (b)]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(b); AOAC Int., 2019]. Reactive Lys was analyzed using Ultra High-Performance Liquid Chromatography, Model QSight LX50 (PerkinElmer, Waltham, MA). Samples were incubated with O-methylisourea to initiate the guanidination reaction (Moughan and Rutherfurd, 1996; Pahm et al., 2008). After incubation, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019].

Calcium, P, K, Mg, Na, Cu, Fe, Mn, and Zn in ingredients were analyzed (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000).

Calculations

Total reactive Lys intake (kg) was calculated by multiplying the analyzed concentration of reactive Lys in the diet (%) by the total feed intake (kg) in each period. Reactive Lys intake (g/day) was then determined by dividing the total reactive Lys intake by the number of days in the feeding period. These calculations allowed for quantifying the daily supply of reactive Lys provided from each diet.

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified as values with internally studentized residuals outside the range of -3 to 3 (Tukey, 1977). The pen was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS. The statistical model included diet as fixed effect, and block, and replicate within block as random effects. Mean values were calculated using the LSMeans statement. Contrast coefficients were used to compare the control diet versus the diet containing crystalline AA. Linear effects of decreasing reactive Lys in diets were also analyzed using contrast statements. Statistical significance was considered at $P \leq 0.05$, and tendencies will be considered if $0.05 \leq P < 0.10$.

Pearson correlation coefficients were calculated using the PROC CORR of SAS to evaluate associations between total reactive Lys intake (kg) and final BW, and between reactive Lys intake (g/d) and ADG. Prediction equations for final BW and ADG were developed using the PROC REG of SAS. In the initial models, final BW and ADG were regressed on total reactive Lys intake (kg) or daily intake (g/d), respectively. Separate models were constructed for each experimental phase and for the overall period. Final prediction equations were generated

using least squares regression estimates, and model fit was evaluated based on the coefficient of determination ($\mathbf{R^2}$).

RESULTS

During phase 1, two pigs fed the positive control diet died for unknown reasons. For phase 2, three pigs fed the severely heat-treated diet, and one pig fed the diet supplemented with crystalline AA were removed due to poor health. All other pigs completed the experiment.

Ingredients and diet composition

Concentrations of reducing sugars (glucose and fructose) increased in SBM as autoclaving time increased, whereas sucrose, stachyose, and raffinose decreased as the severity of heat treatment increased (Table 8.1). In ESBM, concentrations of reducing sugars, stachyose, and raffinose were less than 0.05% regardless of heat treatment. In SBM, the Lys:CP ratio decreased from 6.63 in the non-autoclaved SBM to 6.07 in moderate autoclaved SBM and 4.70 in the severely autoclaved SBM. Similarly, in ESBM, the Lys:CP ratio was reduced from 6.24 to 5.62 and 4.99 with increasing heat treatment. Lysine in SBM decreased from 3.00% in the non-autoclaved sample to 2.77% and 2.15% in the moderately and severely autoclaved SBM, respectively. In ESBM, Lys was reduced from 3.33% to 2.96% and 2.64% as heat treatment severity increased. Reactive Lys was reduced from 2.84% to 2.54% and 1.59% in SBM, whereas in ESBM it was reduced from 3.11% to 2.72% and 2.38% when the time in the autoclave increased. All indispensable AA showed slight reductions with heat-treatment in both SBM and ESBM. In SBM, trypsin inhibitors also decreased from 4.94 TIU/mg in the non-autoclaved sample to < 0.45 TIU/mg in the severely autoclaved product. In ESBM, trypsin inhibitor activity was also reduced from 2.16 TIU/mg to < 0.45 TIU/mg with severe autoclaving.

Reactive Lys in phase 1 diets were reduced from 1.43% to 1.27% and 1.03% and increased to 1.56% in the supplemented diet. In phase 2, reactive Lys was reduced from 1.26% in the positive control diet to 1.19% and 0.93% in the severely autoclaved diet and increased to 1.39% with AA supplementation.

Growth performance

Initial BW did not differ among treatments. However, on day 28, pigs fed the severely autoclaved diet had reduced final BW compared with pigs fed the positive control diet ($P < 0.001$). From day 1 to 14, ADG tended to be less by pigs fed the severely autoclaved diet compared with pigs fed the other diets. From day 15 to 28 and overall, pigs fed the positive control diet had greater ADG than pigs fed the moderate or severely autoclaved diets ($P < 0.001$). Average daily feed intake was also reduced by pigs fed the severely autoclaved diet compared with the positive control from day 15 to 28 ($P < 0.001$) and overall ($P < 0.05$). Gain to feed was greater in pigs fed the positive control diet during phase 2 and overall compared with pigs fed a severely autoclaved diet ($P < 0.001$ Supplementation with crystalline AA improved final BW, ADG, ADFI, and G:F in pigs fed the severely autoclaved diet; however, values were less compared with pigs fed the positive control diet ($P < 0.05$). Fecal scores were generally low for all treatments, although pigs fed the severely autoclaved diet had firmer feces overall compared with the positive control ($P < 0.05$).

Linear effects of decreasing reactive Lys concentrations across the positive control, moderate, and severe diets were observed for final BW on day 28 ($P < 0.05$). Similarly, ADG decreased linearly from day 15 to 28 and over the entire 28-day period as reactive Lys in the diets decreased ($P < 0.05$). A linear reduction was also observed in G:F during phase 2 and for the overall period as reactive Lys decreased ($P < 0.05$). In contrast, there were no linear effects

of dietary reactive Lys on ADFI. Reactive Lys intake was positively correlated with final BW during both phases of the experiment ($P < 0.001$). Similarly, a strong positive association was observed between reactive Lys intake and ADG ($P < 0.001$).

DISCUSSION

Ingredients

Soybean meal and ESBM are widely used protein sources in diets for weanling pigs, but because heat-damage can occur during processing, particularly during toasting of SBM and during drying after enzymatic treatment of ESBM, variation in nutritional value, may occur (Stein et al., 2008; González-Vega et al., 2011). The concentrations of CP and most indispensable AA in the non-autoclaved ESBM and conventional SBM used in this experiment were consistent with reported values (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012). These differences reflect the processing steps involved in the production of ESBM, in which some carbohydrates are removed which results in increased concentrations of CP and other nutrients.

The reduction of CP, carbohydrates, Lys, and reactive Lys in the autoclaved ingredients is due to the combined effects of thermal degradation, Maillard reactions, and formation of indigestible complexes. Prolonged time in the autoclave may result in loss of volatiles and structural changes in the sample (Oliveira et al., 2020b; Espinosa et al., 2021). The reduction in CP and Lys is primarily attributed to heat-damage, especially the Maillard reaction, in which the ε-amino group of Lys binds with reducing sugars to form Amadori products and melanoidins, making Lys unavailable for protein synthesis. However, non-enzymatic protein denaturation and crosslinking may also take place during heating (González-Vega et al., 2011; Almeida et al.,

2014c; Oliveira et al., 2020b). The reduction in carbohydrates observed in autoclaved SBM is likely due to the consumption of reducing sugars during Maillard reactions, which results in formation of glycation end products (Fontaine et al., 2007; Oliveira et al., 2020a) and the reduction in stachyose and raffinose indicates degradation of oligosaccharides and partial hydrolysis of complex carbohydrates (Navarro et al., 2018). The increased concentrations of glucose and fructose after autoclaving are likely a result of hydrolysis of sucrose and other complex sugars, and release of these simple sugars provides additional substrates that promote Maillard reactions during heat processing (Fontaine et al., 2007; Murai et al., 2024).

In undamaged soybean protein, Lys:CP is typically greater than 6%, which is indicative of minimal heat-damage, whereas a Lys:CP < 6 indicates heat-damage (González-Vega et al., 2011; Almeida et al., 2014c; Pedersen et al., 2016; Oliveira et al., 2021). Therefore, the non-autoclaved SBM and ESBM that were used in this experiment likely were not heat-damaged, whereas most of the autoclaved ingredients had Lys:CP below 6.0 indicating they were indeed heat-damaged.

Diets

The diets containing autoclaved SBM and ESBM without crystalline AA supplementation were formulated using the analyzed concentrations of AA from non-autoclaved SBM and ESBM, simulating a scenario where overestimation of AA concentration and digestibility occurs in the moderate and severely heat-damaged diets. The reduced concentrations of indispensable AA, especially Lys and reactive Lys in these diets indicate that autoclaving reduces the concentration of not only Lys but also other AA. However, no corrections were made for potential reductions in energy digestibility, although decreased concentration of digestible and metabolizable energy in 00-rapeseed meal, SBM, and soybean expellers have been

demonstrated in diets containing over-heated ingredients (Oliveira et al., 2020a; 2020b; Espinosa et al., 2021).

Growth performance

The reduction in ADG and final BW observed for pigs fed the severely autoclaved diet is in agreement with reported data and reflects the detrimental effects of heat-damage on AA and energy digestibility (Almeida et al., 2014c). This reduction is likely due to the decreased concentration of reactive Lys caused by Maillard reactions, and possibly also the reduction in the concentration of other indispensable AA caused by non-enzymatic heat damage during autoclaving (Fontaine et al., 2007; González-Vega et al., 2011; Almeida et al., 2014c). The reduction in reactive Lys and other AA compromises the ability of pigs to synthesize proteins efficiently, which likely is the reason for the impaired growth observed in this experiment that was observed for pigs fed diets with autoclaved SBM and ESBM (Pahm et al., 2008; Lund and Ray, 2017). The reduced ADFI of pigs fed the diet with severely autoclaved SBM and ESBM further contributed to the reduced ADG by limiting overall nutrient and energy intake (Pahm et al., 2008; Almeida et al., 2014c; Oliveira et al., 2020a; 2020b). These results confirm that excessive heat treatment negatively affects not only AA digestibility but also overall growth performance.

Supplementation with crystalline AA to the diet containing severely autoclaved SBM and ESBM was done in an attempt to compensate for AA deficiencies in the heat-damaged ingredients. However, the fact that growth performance was not fully restored indicates that heat damage may affect more than only AA concentration and digestibility. Other factors such as reduced energy digestibility, altered palatability, or impaired availability of digested AA may also contribute to the negative effects of heat damage (Almeida et al., 2014c; Oliveira et al.,

2021). These observations align with results of experiments that indicated that nutrient availability was reduced if heat-damaged ingredients were used, which ultimately had negative effects on growth performance (Zhang and Piao, 2022).

The correlation between reactive Lys intake and final BW and ADG demonstrates the importance of reactive Lys as a predictor of growth performance in weanling pigs, which is in agreement with results of research demonstrating that bioavailable Lys is important for protein accretion and growth because reactive Lys is a marker for available Lys in heat-processed ingredients (Pahm et al., 2008; Almeida et al., 2014c). It was also demonstrated that increasing dietary Lys may improve growth performance of pigs fed diets containing heat-damaged ingredients (Aymerich et al., 2020).

CONCLUSION

Heat treatment of SBM and ESBM at 120 ± 1 °C reduced Lys, reactive Lys, and other AA. Growth performance and G:F declined when heat-damaged ingredients were used without adjusting for AA losses, but adding crystalline AA to diets containing heat damaged SBM and ESBM can partially mitigate some of the negative effects of heat damage. The strong correlation between reactive Lys intake and ADG and final BW confirms that reactive Lys can be used as a predictor for growth of weanling g pigs.

TABLES

Table 8.1. Analyzed nutrient composition in soybean meal (SBM), enzyme-treated soybean meal (ESBM), as-fed basis

Item	SBM			ESBM		
	No autoclaving[1]	Moderate autoclaving	Severe autoclaving	No autoclaving	Moderate autoclaving	Severe autoclaving
Dry matter, %	89.08	89.13	89.21	92.96	92.28	92.14
Ash, %	6.14	6.39	6.36	7.03	7.12	7.20
Gross energy, kcal/kg	4237	4251	4295	4537	4500	4510
AEE[2], %	2.34	2.48	2.60	2.10	1.27	1.29
Glucose, %	<0.05	0.05	0.09	0.81	0.18	<0.05
Maltose, %	0.33	0.26	0.21	<0.05	<0.05	<0.05
Sucrose, %	7.10	6.86	5.35	<0.05	<0.05	<0.05
Fructose, %	0.06	0.15	0.32	<0.05	<0.05	<0.05
Stachyose, %	5.30	5.25	4.21	<0.05	<0.05	<0.05
Raffinose, %	1.15	1.15	0.94	<0.05	<0.05	<0.05
Crude protein, %	45.26	45.66	45.79	53.33	52.63	52.89
Lys:CP ratio	6.63	6.07	4.70	6.24	5.62	4.99
Trypsin inhibitors, TIU/mg	4.94	1.69	<0.45	2.16	0.706	<0.45
Indispensable amino acids, %						
Arg	3.40	3.21	2.76	3.92	3.69	3.49
His	1.23	1.20	1.16	1.49	1.43	1.38

Table 8.1. (cont.)

Ile	2.21	2.20	2.19	2.68	2.65	2.61
Leu	3.58	3.58	3.59	4.38	4.33	4.23
Lys	3.00	2.77	2.15	3.33	2.96	2.64
Reactive Lys	2.84	2.54	1.59	3.11	2.72	2.38
Met	0.63	0.61	0.65	0.78	0.76	0.76
Phe	2.40	2.41	2.45	2.96	2.93	2.83
Thr	1.79	1.79	1.82	2.27	2.24	2.21
Trp	0.56	0.60	0.57	0.70	0.66	0.65
Val	2.32	2.30	2.32	2.86	2.83	2.82
Dispensable amino acids, %						
Ala	2.01	2.00	2.02	2.46	2.42	2.40
Asp	5.19	5.16	5.14	6.32	6.20	6.15
Cys	0.65	0.60	0.56	0.82	0.79	0.75
Glu	8.56	8.57	8.55	10.10	9.87	9.83
Gly	1.98	1.96	1.96	2.43	2.38	2.34
Pro	2.36	2.36	2.32	2.87	2.81	2.79
Ser	1.99	2.00	2.00	2.53	2.47	2.42
Tyr	1.67	1.64	1.58	2.03	1.93	1.86

[1]No autoclaving (95% reactive Lys out of total Lys); Moderate autoclaving (85% reactive Lys out of total Lys); Severe autoclaving (<70% reactive Lys out of total Lys).

[2]AEE: acid-hydrolyzed ether extract.

Table 8.2. Analyzed mineral composition in soybean meal (SBM), enzyme-treated soybean meal (ESBM), and autoclaved products on, as-fed basis

Item	SBM			ESBM		
	No autoclaving	Moderate autoclaving	Severe autoclaving	No autoclaving	Moderate autoclaving	Severe autoclaving
Ca, %	0.27	0.27	0.29	0.33	0.32	0.33
P, %	0.68	0.69	0.71	0.76	0.78	0.78
Mg, %	0.24	0.23	0.25	0.23	0.23	0.23
K, %	1.86	1.76	1.93	1.94	1.89	1.89
Na, %	0.01	0.01	0.01	0.01	0.01	0.01
Cu, ug/g	8.71	8.59	9.64	8.30	7.84	7.48
Fe, ug/g	109.53	100.83	111.98	84.21	80.38	79.73
Mn, ug/g	26.58	25.64	28.14	24.67	23.92	23.30
Zn, ug/g	64.35	68.53	76.92	41.51	41.57	40.22

Table 8.3. Ingredient composition of experimental diets for phases 1 and 2 containing soybean meal (SBM) and enzyme-treated soybean meal (ESBM)

	Phase 1				Phase 2			
Ingredient, %	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA[1]	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA
Corn	44.05	44.05	44.05	43.34	45.56	45.56	45.56	44.77
SBM	21.00	21.00	21.00	21.00	25.00	25.00	25.00	25.00
ESBM	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Whey, dried	4.00	4.00	4.00	4.00	1.50	1.50	1.50	1.50
Blood Plasma	3.00	3.00	3.00	3.00	-	-	-	-
Soybean oil	2.40	2.40	2.40	2.40	2.70	2.70	2.70	2.70
L-Lys-HCl	0.18	0.18	0.18	0.71	0.12	0.12	0.12	0.72
DL-Met	0.13	0.13	0.13	0.22	0.11	0.11	0.11	0.21
L-Thr	0.01	0.01	0.01	0.10	-	-	-	0.09

Table 8.3. (cont.)

Dicalcium phosphate	1.43	1.43	1.43	1.43	1.27	1.27	1.27	1.27
Limestone	0.90	0.90	0.90	0.90	0.84	0.84	0.84	0.84
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1] AA, amino acid; ESBM, enzyme-treated soybean meal; SBM, soybean meal.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 8.4. Analyzed nutrient composition in experimental diets, as fed basis

Item	Phase 1				Phase 2			
	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA[1]	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA[1]
Dry matter, %	89.78	89.78	89.92	90.08	89.65	89.65	89.86	89.96
Ash, %	5.77	5.62	5.72	6.02	5.51	5.35	5.49	5.59
Gross energy, kcal/kg	4065	4100	4088	4101	4088	4087	4111	4133
Glucose, %	0.25	0.18	0.17	0.18	0.22	0.16	0.16	0.14
Maltose, %	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Sucrose, %	2.08	2.22	1.69	1.94	2.52	2.53	1.95	2.04
Fructose, %	0.15	0.16	0.18	0.14	0.16	0.18	0.16	0.18
Stachyose, %	1.27	1.23	0.84	1.07	1.42	1.39	1.03	1.10
Raffinose, %	0.32	0.32	0.23	0.29	0.36	0.35	0.28	0.31

Table 8.4. (cont.)

Crude protein, %	22.74	22.35	23.01	23.41	22.26	22.14	21.80	22.50
Indispensable AA, %								
Arg	1.45	1.39	1.22	1.33	1.47	1.42	1.28	1.26
His	0.61	0.59	0.56	0.59	0.59	0.58	0.56	0.56
Ile	1.00	0.97	0.93	1.01	1.03	1.01	1.02	0.99
Leu	1.97	1.93	1.92	1.96	1.90	1.88	1.90	1.85
Lys	1.51	1.42	1.20	1.73	1.39	1.32	1.09	1.56
Reactive Lys	1.43	1.27	1.03	1.56	1.26	1.19	0.93	1.39
Met	0.45	0.41	0.38	0.48	0.43	0.38	0.36	0.45
Phe	1.17	1.14	1.14	1.16	1.15	1.14	1.15	1.13
Thr	0.95	0.93	0.97	1.03	0.84	0.84	0.84	0.94
Trp	0.28	0.27	0.25	0.27	0.26	0.24	0.22	0.23
Val	1.17	1.12	1.11	1.19	1.12	1.13	1.11	1.10

Table 8.4. (cont.)

Dispensable								
AA, %								
Ala	1.11	1.10	1.09	1.11	1.08	1.08	1.09	1.08
Asp	2.34	2.28	2.28	2.34	2.31	2.31	2.28	2.31
Cys	0.40	0.39	0.36	0.38	0.34	0.34	0.31	0.31
Glu	4.09	4.01	3.97	4.08	4.13	4.13	4.09	4.07
Gly	0.93	0.91	0.89	0.91	0.93	0.92	0.92	0.92
Pro	1.28	1.27	1.26	1.28	1.26	1.25	1.26	1.24
Ser	1.03	1.03	1.03	0.99	0.96	0.96	0.94	0.94
Tyr	0.80	0.78	0.75	0.76	0.75	0.74	0.73	0.70

[1] AA, amino acid.

Table 8.5. Growth performance and fecal score of pigs fed experimental diets

Item	Diets				SEM	*P* -value	No autoclaving vs. Severe autoclaving + AA	Linear[2]
	No autoclaving	Moderate autoclaving	Severe autoclaving	Severe autoclaving + AA[1]				
Body weight, kg								
Initial body weight	5.75	5.72	5.73	5.70	0.463	0.433	0.116	0.294
d 14	6.62	6.51	6.12	6.33	0.738	0.111	0.183	0.384
d 28	11.10[a]	10.44[ab]	8.13[c]	9.89[b]	1.268	<0.001	0.006	0.005
ADG, g								
d 1 to 14	62	57	28	45	20.750	0.099	0.236	0.397
d 15 to 28	320[a]	280[ab]	143[c]	254[b]	39.440	<0.001	0.006	0.003
d 1 to 28	191[a]	169[ab]	86[c]	149[b]	29.140	<0.001	0.004	0.003
ADFI, g								
d 1 to 14	122	117	103	114	25.331	0.494	0.550	0.500
d 15 to 28	432[a]	440[a]	326[b]	397[ab]	56.821	0.001	0.200	0.548

Table 8.5. (cont.)

d 1 to 28	268[a]	278[a]	205[b]	253[ab]	44.720	0.009	0.490	0.846
G:F								
d 1 to 14	0.32	0.47	0.19	0.32	0.164	0.548	1.000	0.589
d 15 to 28	0.77[a]	0.63[a]	0.45[b]	0.64[a]	0.046	<0.001	0.042	0.005
d 1 to 28	0.77[a]	0.60[ab]	0.43[b]	0.59[ab]	0.050	<0.001	0.011	0.002
Fecal score								
d 1 to 14	1.55	1.58	1.37	1.43	0.058	0.039	0.123	0.795
d 15 to 28	1.55	1.61	1.45	1.36	0.078	0.084	0.070	0.744
d 1 to 28	1.53[ab]	1.58[a]	1.39[b]	1.38[b]	0.050	0.009	0.029	0.896

[1] AA, amino acid; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

[2] Linear effects of decreasing reactive Lys in the positive control, moderate, and severe diets.

Table 8.6. Pearson correlation coefficients (r), coefficients of determination (R^2), and prediction equations for final body weight (BW) and average daily gain (ADG) based on reactive Lys intake in weanling pigs

Item	Pearson r	*P*-value	R^2	Strength	Direction	Prediction Equation
Final BW						
Phase 1	0.841	<0.001	0.71	Strong	Positive	Y = 4.33 + (26.50 × total reactive Lys intake, kg)
Phase 2	0.881	<0.001	0.78	Strong	Positive	Y = 4.92 + (19.97 × total reactive Lys intake, kg)
ADG						
Phase 1	0.910	<0.001	0.83	Very strong	Positive	Y = - 45.43 + (67.33 × reactive Lys intake, g/d)
Phase 2	0.790	<0.001	0.62	Strong	Positive	Y = 15.54 + (51.64 × reactive Lys intake, g/d)
Overall	0.880	<0.001	0.77	Very strong	Positive	Y = - 1.58 + (51.37 × reactive Lys intake, g/d)

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CHAPTER 9: EFFECTS OF ENZYME-TREATED SOYBEAN MEAL ON NITROGEN BALANCE OF WEANLING PIGS FED DIETS WITH NORMAL OR REDUCED LEVELS OF CRUDE PROTEIN

ABSTRACT

An experiment was conducted to test the hypothesis that low-protein diets for weanling pigs support greater nitrogen retention if enzyme-treated soybean meal (**ESBM**) is included than if only conventional soybean meal (**SBM**) is used. Two diets were formulated to include corn and SBM, or corn, SBM, and ESBM, and to contain 22% crude protein (**CP**). Two additional diets that also contained SBM or ESBM were formulated to contain 18% CP. All diets included synthetic amino acids (**AA**), vitamins, and minerals to meet current requirements for pigs, but the 18% CP diets contained greater quantities of synthetic AA than 22% CP diets. Diets were arranged in a 2 × 2 factorial with one factor being protein level (22% vs. 18% CP), and the other factor being protein source (SBM vs. SBM with the inclusion of ESBM). Forty barrows (average initial body weight: 10.58 ± 0.88 kg) were allotted to the four diets, with 10 replicate pigs per diet. Pigs were housed individually in metabolism crates and fed experimental diets for 13 days. Feces and urine were collected for five days to determine apparent total tract digestibility (**ATTD**) of dry matter and nitrogen, nitrogen balance, nitrogen retention, and biological value. Blood urea nitrogen, total protein, albumin, peptide YY, and immunoglobulin G were measured in blood samples collected on day 13. Results indicated that reducing CP reduced ($P < 0.001$) nitrogen intake and urine nitrogen output, decreased ATTD of nitrogen and daily nitrogen absorption and retention, but nitrogen retention as a percentage of intake and the

biological value of dietary nitrogen were increased in pigs fed diets with 18% CP compared with pigs fed diets with 22% CP. Blood urea nitrogen was less ($P < 0.001$) in pigs fed 18% CP diets compared with pigs fed diets with 22% CP. In conclusion, feeding 18% CP diets with synthetic AA to weanling pigs reduces ATTD of nitrogen and daily nitrogen retention, although nitrogen efficiency is increased. Inclusion of ESBM in low-CP diets did not affect nitrogen retention compared with SBM.

Keywords: amino acids, enzyme-treated soybean meal, nitrogen balance, nitrogen retention, soybean meal, weanling pigs.

Abbreviations: AA, amino acids; ATTD, apparent total tract digestibility; BUN, blood urea nitrogen; CP, crude protein; DM, dry matter; EDTA, ethylenediaminetetraacetic acid; ESBM, enzyme-treated soybean meal; SBM, soybean meal.

INTRODUCTION

Over the last few decades, there has been much interest in formulating diets for weanling pigs with reduced levels of crude protein (**CP**) and inclusion of synthetic amino acids (**AA**), but pigs fed low-protein diets often have reduced nitrogen retention compared with pigs fed diets containing normal levels of CP (Kerr and Easter, 1995; Le Bellego et al., 2001; Cristobal et al., 2025). It has been speculated that one reason for these results may be that synthetic AA are absorbed faster than AA provided from intact protein in soybean meal (**SBM**) and as a result, when these AA arrive at the cells where they are needed for protein synthesis, the cells are lacking the AA from the intact proteins, and therefore, deaminate the synthetic AA before other

AA arrive at the cell (Eugenio et al., 2022; Zhang et al., 2022). However, AA from enzyme-treated soybean meal (**ESBM**) are absorbed faster than AA from conventional SBM (Nørgaard et al., 2021), and it is, therefore, possible that inclusion of ESBM in low-protein diets will result in AA from the intact protein arriving at the cells at the same time as the synthetic AA, which may result in a greater protein synthesis, and therefore, greater nitrogen retention than if only conventional SBM is used. However, this speculation has not been experimentally verified. Therefore, an experiment was conducted to test the hypothesis that low-protein diets for weanling pigs will support greater nitrogen retention if ESBM is included in the diets than if no ESBM is used.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol prior to initiation of the experiment. Pigs that were the offspring of Line 800 males and Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used in the experiment.

Animals and housing

A total of 40 castrated male pigs (average initial body weight: 10.58 ± 0.88 kg) were randomly allotted to 4 dietary treatments with 10 pigs per treatment using a completely randomized design. Pigs were housed in individual metabolism crates equipped with a slatted floor, a nipple waterer, a feeder, and a mesh screen installed below the slatted floor of the crates. A urine pan was installed below the mesh screen.

Diets and feeding

Four diets were formulated (Table 9.1) and used in a 2 × 2 factorial arrangement with two levels of CP (i.e., 22 and 18%) and two sources of intact protein (i.e., SBM and SBM with the inclusion of ESBM). The diets containing 22% CP contained moderate levels of synthetic Lys, Met, and Thr, but the diets containing 18% CP contained greater levels of synthetic Lys, Met, and Thr, and also synthetic Trp and Val. All diets were formulated to meet minimum requirements for all standardized ileal digestible indispensable AA (NRC, 2012).

Pigs were fed experimental diets for 13 days. Daily feed allowance was 3.4 times the maintenance requirement for growing pigs (i.e., 197 kcal ME/kg $BW^{0.60}$; NRC, 2012). Daily feed allotments were divided into two equal meals, provided at 0800 and 1600 h every day. Water was available at all times.

Sample collection

The initial 5 days of the feeding period were considered the adaptation period to the diets followed by 5 days of collection using the marker-to-marker procedure (Adeola, 2001). Fecal collection commenced when the first marker (i.e., indigo carmine), which was added to the morning meal on day 6, appeared in the feces, and fecal collections ceased when the second marker (i.e., ferric oxide), which was added to the morning meal on day 11, appeared in the feces (Adeola, 2001). Collected feces were stored at -20 °C immediately after collection.

Urine was collected for 5 days, starting on day 6, using urine buckets containing 50 mL of 6 N HCl as a preservative, and placed under the metabolism crates. Buckets were emptied daily, and the weight of the collected urine was recorded. Each day, 20% of the collected urine was stored at -20 °C. Orts were collected daily prior to feeding the morning meal, pooled for the

duration of the collection period, dried in a 50 °C forced-air drying oven, and weighed at the conclusion of the experiment to determine feed intake.

On the last day of the experiment (i.e., on day 13), pigs were provided the morning meal at 0700 h, and 30 min later, all feeders were emptied if they still contained feed. Four hours after feeding, two blood samples were collected from each pig via venipuncture. One sample was collected in heparinized vacutainers, and the other sample was collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA), a chelating agent that binds calcium and also prevents coagulation. Blood samples were placed on ice immediately after collection and then centrifuged at 4,000 × g at 4 °C for 13 min, and plasma was collected and stored at -20 ºC until analysis.

Chemical Analyses

At the conclusion of the experiment, fecal samples were thawed, dried at 65 °C in a forced air oven (Thermo Fisher Scientific Inc.; model Heratherm OMH750, Waltham, MA, USA) and then finely ground using a 500 G stainless steel mill grinder (RRH, Zhejiang, China). Main ingredients (i.e., corn, SBM, and ESBM), diets, and dried fecal samples were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2019), and diets and ingredients were also analyzed for ash (method 942.05; AOAC Int., 2019). Diets and ingredients were analyzed for macrominerals and microminerals (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Diets and ingredients were also analyzed for acid hydrolyzed ether extract by acid-hydrolysis using 3 *N* HCl (AnkomHCl; Ankom Technology, Macedon, NY,

USA) followed by crude fat extraction using petroleum ether (method 2003.06; AOAC Int., 2019) on an Ankom fat analyzer (AnkomXT15; Ankom Technology, Macedon, NY, USA). Diets and ingredients were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int., 2019) using the Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Starch was analyzed in corn using the glucoamylase procedure (method 979.10; AOAC Int., 2019). Soybean meal and ESBM were analyzed for sugars including glucose, fructose, maltose, sucrose, stachyose, raffinose, and verbascose using high-performance liquid chromatography (Dionex App Notes 21 and 92). Diets and ingredients were also analyzed for AA [method 982.30 E (a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, USA). All ingredients, diets, fecal samples, and urine samples were analyzed for nitrogen using the Kjeldahl procedure (method 984.13; AOAC Int., 2019) on a Kjeltec 8400 (FOSS Inc., Eden Prairie, MN, USA) with subsequent calculation of CP using a conversion factor of 6.25. Blood samples collected in vacutainers containing heparin were analyzed for blood urea nitrogen (**BUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer at the University of Illinois Veterinary Diagnostic Laboratory (Urbana IL, USA). Samples collected in vacutainers containing EDTA were analyzed for peptide YY and immunoglobulin G using enzyme-linked immunosorbent assay, a colorimetric immunoassay for protein quantification kits, according to the recommendations from the manufacturer (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA; and Bethyl Laboratories Inc., Montgomery, TX, USA, respectively).

Calculations

For each analysis of each source of ingredients, all analyzed components were added and subtracted from the concentration of DM in each ingredient to calculate the rest fraction using the following equation (Fanelli et al., 2023ab):

Rest fraction of corn = [dry matter - (crude protein + acid hydrolyzed ether extract + ash + total dietary fiber + starch)]

Rest fraction of SBM and ESBM = [dry matter - (crude protein + acid hydrolyzed ether extract + ash + total dietary fiber + sucrose + stachyose + raffinose + glucose + fructose + maltose + galactose)]

The apparent total tract digestibility (**ATTD**) of DM and nitrogen in each experimental diet was calculated (NRC, 2012). Nitrogen retention in grams per day and as a percentage of intake was calculated for each pig and summarized within diet (Pedersen et al., 2007). The biological value of nitrogen in diets was calculated as described by Rojas and Stein (2013).

Statistical Analysis

Normality and homogeneity of the variance were verified (SAS Inst. Inc., Cary, NC, USA), and outliers were identified using Internally Studentized Residuals (Tukey, 1977). However, no outliers were identified. The pig was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included the level of CP, protein source, and the interaction between the level of CP and protein source as fixed effects, and replicate pig was the random effect. Least squares means were calculated using the LSMeans statement in SAS, and means were separated using the PDIFF statement with Tukey's adjustment if the model was significant. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All pigs were healthy throughout the experiment; no mortality or feed refusals were observed, and no veterinary interventions were required. There were no effects of treatments on feed intake, fecal output, fecal nitrogen, and urine output, but nitrogen (g/d) was less ($P < 0.001$) in urine from pigs fed 18% CP diets compared with pigs fed 22% CP diets (Table 9.6). Total nitrogen intake was greater ($P < 0.001$) by pigs fed 22% CP diets than by pigs fed 18% CP diets. The ATTD of DM did not differ among treatments, whereas ATTD of nitrogen, nitrogen absorbed, and nitrogen retained daily were greater by pigs fed 22% CP diets than by pigs fed 18% CP diets ($P < 0.05$). In contrast, expressed relative to intake, nitrogen retention and the biological value of dietary nitrogen were greater for pigs fed 18% CP diets than pigs fed 22% CP diets ($P < 0.001$).

Pigs fed the 18% CP diets had less BUN, total protein, and peptide YY than pigs fed 22% CP ($P < 0.05$; Table 9.7), and albumin tended to be reduced in pigs fed 18% CP diets compared with pigs fed 22% CP diets ($P < 0.10$). Peptide YY was not affected by protein sources in 22% CP diets, but pigs fed 18% CP diets tended to have greater peptide YY if fed the ESBM diet compared with the SBM (interaction, $P < 0.10$).

DISCUSSION

The analyzed nutrient composition of experimental diets was in close agreement with formulated values, confirming correct diet mixing. Nutrients in corn, SBM, and ESBM aligned with previous data (NRC, 2012; Torres-Mendoza and Stein, 2025). Almost all sucrose, stachyose, and raffinose were eliminated in ESBM, which is in agreement with reported data (Cervantes-Pahm and Stein, 2010). This is a result of these components being fermented in the

production of ESBM. The rest fraction represents unmeasured or unknown components. The analyzed components of corn and SBM closely matched the total DM, indicating that all nutrients in these ingredients were accounted for. However, a small portion of ESBM remained unaccounted for, and the composition of these remaining fractions is unclear. It is possible that in ESBM, compounds such as lactic acid or ethanol may be present, as they can be produced during enzymatic processing and fermentation (Mukherjee et al., 2016).

The lack of an effect of diet CP on daily feed intake and the reduced urine nitrogen excretion by pigs fed diets with 18% CP have been reported previously (Cristobal et al., 2025) and reflect the inclusion of greater quantities of synthetic AA in the 18% CP diets than in the 22% CP diets. By improving the AA balance, synthetic AA reduce excess of dietary AA, thereby limiting deamination and urea-cycle nitrogen excretion (Wang et al., 2018; Cristobal et al., 2025). The lack of an effect of protein source on ATTD of DM is in agreement with data reporting minimal differences in DM digestibility between SBM and ESBM (Rojas and Stein, 2013). The reduction in ATTD of nitrogen in 18% CP diets compared with diets containing 22% CP diets is in agreement with published data and may be a result of the increased amount of corn in the 18% CP diets (Cristobal et al., 2025) because corn has reduced ATTD of CP compared with SBM or ESBM.

Although ESBM contains greater concentrations of small peptides due to enzymatic hydrolysis (Cervantes-Pahm and Stein, 2010; Ma et al., 2019a; 2019b), and these peptides generally accelerate postprandial plasma AA appearance compared with conventional SBM (Nørgaard et al., 2021), no differences in nitrogen retention were observed between protein sources in the present experiment. This observation aligns with the understanding that plasma AA kinetics, and ultimately the AA available for protein synthesis, reflect not only digestion and

absorption rates, but also first-pass intestinal and hepatic metabolism, the balance of indispensable AA in the diet, and the synchrony between absorbed AA and dietary energy supply (Eugenio et al., 2023). Because synthetic AA were added to both SBM and ESBM-based diets to balance the standardized ileal digestible indispensable AA supply, the rapid early appearance of AA from ESBM was likely moderated by the balanced supply of AA appearing in the blood. As a result, despite ESBM providing a faster protein absorption, the coordinated AA availability required to maximize protein synthesis was not different among diets. Rapidly absorbed AA, such as those in synthetic AA supplements or hydrolyzed protein, can cause an early increase in plasma AA concentrations that exceed the capacity for anabolic utilization, leading to increased AA oxidation and reduced efficiency of AA use for growth (He et al., 2016). Faster AA absorption also results in a more rapid elevation and subsequent decrease in postprandial plasma AA concentrations, however, these rapidly absorbed forms undergo less first-pass metabolism, induce higher postprandial insulin, and do not necessarily enhance net protein accretion (Eugenio et al., 2023).

The reduced daily nitrogen absorbed by pigs fed 18% CP diets compared with pigs fed 22% CP diets primarily reflects reduced nitrogen intake as CP decreased (Kerr and Easter, 1995; Cristobal et al., 2025) and the reduction in daily nitrogen retention in pigs fed 18% CP diets may be a result of asynchrony in postprandial AA supply, because synthetic AA may be absorbed more rapidly than AA released from corn and SBM; as a result, some free AA may be oxidized before complementary AA from intact proteins arrive at the cell, thereby limiting protein synthesis (Hu et al., 2021; Wang et al., 2021; Eugenio et al., 2022; Cristobal et al., 2025).

The increased nitrogen retention as a percentage of feed intake and increased biological value of nitrogen that was observed as CP in diets was reduced may be a result of the improved

balance of indispensable AA in low-CP diets supplemented with synthetic AA (Wang et al., 2018; Cristobal et al., 2025). A reduction in excess nitrogen minimizes AA deamination and thereby enhances the efficiency with which absorbed nitrogen is retained (Hu et al., 2021; Bailey et al., 2023; Nisley et al., 2025). In growing pigs, replacing SBM with corn and synthetic AA reduced nitrogen retention, but increased nitrogen efficiency, as indicated by higher retention relative to intake and biological value, due to reduced AA catabolism and urea excretion (Cristobal et al., 2025). Likewise, increasing dietary CP elevated urinary nitrogen and BUN, confirming that excess protein reduced efficiency (Niyonsaba et al., 2023). Collectively, these data support the hypothesis that AA-balanced low-CP diets enhance efficiency of nitrogen utilization, even if daily nitrogen retention declines.

The reduction in BUN in pigs fed 18% CP diets compared with pigs fed 22% CP diets indicates that dietary protein level contributes to circulating urea in weanling pigs. Low dietary CP balanced with synthetic AA reduced excess AA catabolism, thereby decreasing transamination and deamination reactions and limiting ammonia entry into the urea cycle. As a result, hepatic urea synthesis and subsequent BUN concentration were reduced, which is consistent with the decrease in urinary nitrogen excretion and the greater nitrogen retention as a percentage of intake observed in pigs fed low-CP diets (Niyonsaba et al., 2023; Cristobal et al., 2025). Results of experiments in which multiple protein ingredients were compared indicated that ingredients do not effect BUN (Li et al., 2019; Tang et al., 2023), which may be the reason no differences in BUN between pigs fed SBM and ESBM were observed.

The concentration of peptide YY was in agreement with published data and with data showing no main effect of ingredient on peptide YY (Espinosa et al., 2020; Mallea et al., 2023). Peptide YY generally reflects postprandial energy/AA supply and the observation that pigs fed

18% CP diet with ESBM had greater peptide YY than pigs fed the SBM diet indicates that ESBM tended to sustain satiety signaling better than SBM, which may improve intake regulation (Casas and Stein, 2016). For immunoglobulin G, the absence of a protein-source effect is in agreement with results demonstrating no change in serum immunoglobulin G between pigs fed ESBM and SBM under non-challenged conditions (Zhang and Piao, 2022; Tang et al., 2023).

CONCLUSION

The hypothesis that low-protein diets for weanling pigs will support greater nitrogen retention if ESBM is included in the diets than if no ESBM is used was rejected. However, inclusion of ESBM in the diet tended to attenuate the reduction in peptide YY observed with SBM. Reducing dietary CP while balancing with synthetic AA reduced the ATTD of nitrogen as well as daily nitrogen absorption and retention, and decreased urinary nitrogen and BUN, but improved nitrogen retention relative to intake and the biological value of dietary nitrogen in 18% CP diets compared with 22% CP diets.

TABLES

Table 9.1. Ingredient composition of experimental diets containing 22 or 18% crude protein (CP) and conventional soybean meal (SBM) or enzyme-treated soybean meal (ESBM)

Item	22 % crude protein		18% crude protein	
Protein source:	SBM	ESBM	SBM	ESBM
Ground corn	54.15	59.85	66.02	71.80
SBM, 46% CP	38.85	18.76	26.10	5.82
ESBM[1]	-	15.00	-	15.00
Soybean oil	4.00	3.22	3.92	3.16
Ground limestone	0.80	0.92	0.78	0.86
Dicalcium phosphate	1.00	0.90	1.20	1.15
$_{L}$-Lys·HCl, 78% Lys	0.17	0.28	0.56	0.68
$_{DL}$-Met	0.10	0.11	0.20	0.22
$_{L}$-Thr	0.03	0.06	0.20	0.22
$_{L}$-Trp	-	-	0.04	0.05
$_{L}$-Val	-	-	0.07	0.10
$_{L}$-His	-	-	0.01	0.02
$_{L}$-Ile	-	-	-	0.02
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50

[1]HP 300 (Hamlet Protein; Findlay, OH, USA).

[2]The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as

Table 9.1. (cont.)

cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

Table 9.2. Analyzed nutrient composition in corn, soybean meal (SBM), and enzyme-treated soybean meal (ESBM), as-fed basis

Item	Corn[1]	SBM[2]	ESBM
Dry matter, %	86.94	88.11	93.15
Gross energy, kcal/kg	3,866	4,114	4,464
Ash, %	1.26	6.41	7.21
Crude protein, %	7.51	44.97	53.54
Acid hydrolyzed ether extract, %	3.90	1.96	2.58
Insoluble dietary fiber, %	10.20	17.00	19.50
Soluble dietary fiber, %	0.70	1.80	3.70
Total dietary fiber, %	10.90	18.80	23.20
Starch, %	64.40	-	-
Sugars, %			
Glucose	-	<0.05	0.84
Sucrose	-	6.66	0.16
Maltose	-	<0.05	<0.05
Fructose	-	0.08	<0.05
Stachyose	-	5.52	0.13
Raffinose	-	1.04	<0.05
Indispensable AA, %			
Arg	0.34	3.06	3.92
His	0.22	1.19	1.47
Ile	0.27	2.12	2.63

Table 9.2. (cont.)

Leu	0.86	3.39	4.22
Lys	0.28	2.81	3.29
Met	0.14	0.59	0.73
Phe	0.36	2.24	2.82
Thr	0.26	1.73	2.15
Trp	0.05	0.56	0.64
Val	0.36	2.23	2.79
Dispensable AA, %			
Ala	0.53	1.93	2.38
Asp	0.51	4.89	6.08
Cys	0.16	0.62	0.73
Glu	1.38	8.04	9.91
Gly	0.29	1.87	2.35
Pro	0.64	2.21	2.74
Ser	0.33	1.88	2.41
Tyr	0.21	1.55	1.94
Rest fraction, %	-1.03	2.57	5.34

[1]Rest fraction = calculated using the following equation [dry matter - (crude protein + acid hydrolyzed ether extract + ash + total dietary fiber + starch)].

[2]Rest fraction = calculated using the following equation [dry matter - (crude protein + acid hydrolyzed ether extract + ash + total dietary fiber + sucrose + stachyose + raffinose + glucose + fructose + maltose + galactose)].

Table 9.3. Analyzed mineral composition in corn, soybean meal (SBM), and enzyme-treated soybean meal (ESBM), as-fed basis

Item	Corn	SBM	ESBM
Ca, %	0.04	0.45	0.49
P, %	0.26	0.67	0.78
Mg, %	0.30	1.94	2.26
K, %	0.08	0.29	0.27
Na, %	0.01	0.09	0.02
Cu, µg/g	7.11	17.18	13.03
Fe, µg/g	26.97	113.57	100.23
Mn, µg/g	6.08	42.84	29.46
Zn, µg/g	49.20	59.10	53.54

Table 9.4. Analyzed composition of experimental diets containing 22 or 18% crude protein and conventional soybean meal (SBM) or enzyme-treated soybean meal (ESBM)

Item	22 % crude protein		18% crude protein	
Protein source:	SBM	ESBM	SBM	ESBM
Dry matter, %	88.44	89.21	88.28	88.69
Ash, %	4.97	4.60	4.34	4.38
Acid hydrolyzed ether extract, %	6.71	6.52	7.35	6.50
Insoluble dietary fiber, %	15.60	14.90	11.17	11.24
Soluble dietary fiber, %	0.20	2.40	0.93	1.16
Total dietary fiber, %	15.80	17.30	12.10	12.40
Gross energy, kcal/kg	4,126	4,146	4,076	4,054
Metabolizable energy, kcal/kg[1]	3,470	3,470	3,470	3,470
Net energy, kcal/kg[1]	2,525	2,525	2,530	2,530
Crude protein, %	22.35	22.19	17.85	18.03
Indispensable AA, %				
Arg	1.42	1.47	1.02	1.02
His	0.59	0.61	0.46	0.47
Ile	0.96	0.98	0.69	0.77
Leu	1.82	1.89	1.46	1.49
Lys	1.41	1.46	1.34	1.36
Met	0.39	0.42	0.40	0.46
Phe	1.09	1.13	0.82	0.84
Thr	0.87	0.91	0.90	0.78

Table 9.4. (cont.)

Trp	0.25	0.25	0.22	0.20
Val	1.04	1.07	0.82	0.93
Dispensable AA, %				
Ala	1.06	1.09	0.86	0.88
Asp	2.24	2.30	1.61	1.62
Cys	0.32	0.34	0.27	0.28
Glu	3.99	4.12	3.04	3.08
Gly	0.90	0.93	0.68	0.69
Pro	1.21	1.27	1.02	1.05
Ser	0.98	1.01	0.76	0.73
Tyr	0.76	0.78	0.57	0.58

[1]Calculated from NRC (2012).

Table 9.5. Analyzed mineral composition of diets containing 22 or 18% crude protein and conventional soybean meal (SBM) or enzyme-treated soybean meal (ESBM)

Item	22 % crude protein		18% crude protein	
Protein source:	SBM	ESBM	SBM	ESBM
Ca, %	0.76	0.70	0.73	0.67
P, %	0.64	0.58	0.61	0.56
Mg, %	0.92	0.87	0.64	0.65
K, %	0.17	0.15	0.13	0.13
Na, %	0.18	0.15	0.17	0.21
Cu, µg/g	25.85	25.08	32.95	19.88
Fe, µg/g	234.68	219.18	246.24	200.41
Mn, µg/g	81.75	77.54	84.26	66.20
Zn, µg/g	176.76	158.25	179.45	147.74

Table 9.6. Apparent total tract digestibility (ATTD) of dry matter and nitrogen balance in experimental diets fed to weanling pigs, as-fed basis[1]

Item	22% crude protein		18% crude protein		SEM	*P*-value	*P*-value	*P*-value
	SBM	ESBM	SBM	ESBM		Protein level	Protein source	Interaction
Intake								
Feed, g/d	716	708	741	726	20.42	0.152	0.429	0.807
Total nitrogen, g	126.00	124.27	106.30	101.86	3.348	<0.001	0.229	0.593
Nitrogen, g/day	25.20	24.85	21.26	20.37	0.670	<0.001	0.229	0.593
Fecal excretion								
Dry feces output, g/day	76.78	73.66	76.39	75.93	2.730	0.708	0.476	0.596
Total nitrogen, g	16.05	15.31	14.88	14.83	0.664	0.199	0.534	0.578
Nitrogen g/day	3.21	3.06	2.98	2.97	0.133	0.199	0.534	0.578
Urine excretion								
Urine output, kg/day	4.70	5.50	5.98	5.46	0.489	0.213	0.782	0.185

Table 9.6. (cont.)

Nitrogen, g/day	3.76	4.02	1.74	1.51	0.277	<0.001	0.952	0.379
ATTD of dry matter, %	88.66	89.10	88.83	88.86	0.405	0.933	0.565	0.611
Nitrogen balance								
Nitrogen absorbed, g/day	21.99	21.79	18.28	17.40	0.652	<0.001	0.314	0.521
Nitrogen retained, g/day	18.23	17.77	16.54	15.89	0.554	<0.001	0.324	0.865
ATTD of nitrogen, %	87.27	87.45	85.97	85.43	0.623	0.011	0.776	0.568
Nitrogen retention, % of intake	72.36	71.35	77.80	78.18	1.115	<0.001	0.778	0.537
Biological value of dietary nitrogen, %	82.92	81.61	90.51	91.52	1.178	<0.001	0.899	0.332

[1]Each least square mean for experimental diets represents 10 observations.

[2]Calculated by dividing retained nitrogen by absorbed nitrogen and multiplying by 100 (Rojas and Stein, 2013).

Table 9.7. Blood urea nitrogen, total protein, albumin, peptide YY, and immunoglobulin G in plasma of pigs fed the experimental diets containing 22 or 18% crude protein and conventional soybean meal (SBM) or enzyme-treated soybean meal (ESBM)[1]

Item,	22% crude protein		18% crude protein		SEM	*P*-value Protein level	*P*-value Protein source	*P*-value Interaction
	SBM	ESBM	SBM	ESBM				
Blood urea nitrogen, mg/dL	13.40	13.70	7.80	7.30	0.764	<0.001	0.878	0.541
Total protein, g/dL	5.44	5.31	5.20	5.04	0.078	0.002	0.070	0.848
Albumin, g/dL	3.11	3.13	3.04	2.90	0.092	0.099	0.500	0.370
Peptide YY, ng/mL	2.25	2.22	1.76	2.09	0.104	0.003	0.130	0.060
Immunoglobulin G, mg/mL	2.92	2.74	3.11	2.52	0.295	0.950	0.160	0.455

[1]Each least square mean for experimental diets represents 10 observations.

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CHAPTER 10: CONCLUSIONS

The overall objective of this dissertation is to apply the homoarginine procedure to soybean products to determine concentrations of reactive Lys, to assess the correlation between reactive Lys and reducing sugars, and to evaluate whether reducing sugars can be used to predict reactive Lys and whether reactive Lys can be used to predict growth performance. Because all soybean products are exposed to heat during processing, and enzyme-treated soybean meal (**ESBM**) and fermented soybean meal (**FSBM**) undergo additional thermal steps, there is a risk of heat damage that reduces amino acid (**AA**) digestibility, particularly that of Lys. Therefore, this research aimed to establish reliable analytical procedures to measure reactive Lys and to understand how heat damage affects the nutritional value of soybean products and pig performance.

A comprehensive literature review is essential because it provides the scientific context needed to understand the nutritional value, processing characteristics, and limitations of ESBM. Enzyme-treated soybean meal has a greater concentration of digestible nutrients and less concentration of anti-nutritional factors than conventional soybean meal (**SBM**), resulting in improved intestinal health and growth performance of weanling pigs. There are also reports of the optimal inclusion levels of 6 to 10% in starter feeds and 10 to 15% during the post-weaning period. However, the literature also emphasizes that ESBM must be protected from heat damage during production because overheating reduces the digestibility of Lys and other nutrients through Maillard reactions. The literature review demonstrated that examining previous research is critical for identifying both the nutritional advantages of ESBM and the risks associated with improper processing, thereby ensuring appropriate inclusion rates and optimal utilization of this ingredient in diets for young pigs.

Recognizing the risks associated with improper processing, the results of this dissertation confirmed that total and reactive Lys concentrations were reproducible across laboratories and that the homoarginine procedure accurately quantified reactive Lys in soybean-based ingredients. This demonstrates that these analytical methods are reliable tools for evaluating protein quality and detecting heat damage in feed ingredients.

It was essential to characterize the feed ingredients to determine the differences in nutrient composition among SBM, ESBM, FSBM, and soy protein concentrate, with particular emphasis on total and reactive Lys. Characterizing these ingredients provides valuable information for accurate diet formulation, as soybean co-products contain higher concentrations of Lys than most other plant proteins. However, ESBM also provides additional nutritional contributions, including greater concentrations of total dietary fiber, ash, and several minerals. In addition, soybean ingredients differ in their sugar profiles, which is particularly important because reducing sugars serve as substrates for the Maillard reaction and may influence the extent of heat damage that occurs during processing. This comprehensive characterization ensures that both the AA profile and the broader nutrient and carbohydrate matrix of soybean ingredients are understood when formulating diets for pigs.

A correlation between reactive Lys in intentionally heat-damaged SBM and the concentration of reducing sugars, especially fructose, was observed. Autoclaving changed the chemical composition of SBM by reducing reactive Lys and oligosaccharides while increasing reducing sugars that contribute to Maillard reactions and the formation of dark brown pigments in SBM. The strong associations observed highlight the potential of using sugar profiles as indicators of heat damage in a specific ingredient. These observations provide a basis for

integrating carbohydrate analysis with reactive Lys determination as a complementary approach to evaluate protein quality in processed soybean products.

These relationships between reducing sugars, reactive Lys, and heat-induced color changes in SBM provide important context for understanding how processing also affects ESBM. Results indicate that enzyme treatment of SBM results in a reduced concentration of oligosaccharides and sucrose, but an increased concentration of other nutrients, including ash, crude protein, and AA. The reduction in L* and the increases in a* and b* values confirmed the color darkening associated with heat damage. The SID of most AA and the apparent ileal digestibility of GE were not different in ESBM compared with SBM. However, autoclaving ESBM at 120 °C for 30, 45, or 120 min reduced the concentration and digestibility of AA, dry matter, and gross energy due to heat damage and overheating of ESBM during processing, which will reduce the economic value of the ingredient.

It is also confirmed that heat treatment of SBM and ESBM at 120 ± 1 °C reduced concentrations of Lys, reactive Lys, and other AA. As a result, growth performance and gain to feed ratio declined when heat-damaged ingredients were used without accounting for AA losses. Although supplementing diets with crystalline AA partially alleviated these negative effects, performance did not fully return to levels observed in pigs fed unheated ingredients. The strong correlation between reactive Lys intake and average daily gain and final body weight further confirms that reactive Lys is a reliable predictor of growth in weanling pigs and a sensitive indicator of heat-induced reductions in protein quality.

Despite notable compositional differences between SBM and ESBM, the nitrogen-balance results indicated that these differences were not sufficient to improve nitrogen utilization in young pigs. Lowering dietary CP while supplementing crystalline AA decreased the ATTD of

nitrogen, daily nitrogen absorption, and retention, and reduced urinary nitrogen and BUN, although nitrogen retention relative to intake and the biological value of dietary nitrogen improved. The ESBM did not enhance daily nitrogen retention or counteract the reduction associated with the low-CP diet, but its inclusion tended to lessen the decline in peptide YY observed in pigs fed SBM.