APPARENT AND STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY OF PROCESSED SOYBEAN PRODUCTS FED TO PIGS

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2007

Urbana, Illinois

Adviser:

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ABSTRACT. Three experiments were conducted to evaluate the feeding value of processed soybean (SB) products fed to pigs. The first objective of experiment 1 was to measure the apparent (AID) and standardized (SID) of CP and AA in a high protein SB fed to growing pigs. Values obtained were compared to the AID and SID of CP and AA in conventional SB, conventional soybean meal (SBM) and soy protein concentrate (SPC). Four cornstarch based diets were formulated using each soy product as the only source of CP and AA in the diet. The second objective was to measure the effect of adding oil on amino acid digestibility of SBM and SPC. Two additional diets were formulated by adding 7.55 and 7.35% oil to SBM and SPC, respectively. A N-free diet was used to measure the basal endogenous losses of CP and AA. The AID and SID of CP and AA in the high protein SB were not different from conventional SB and the SID of most AA in the high protein SB was similar to SBM with oil and SPC. The addition of oil improved the SID of most AA in SBM and SPC.

Experiment 2 was conducted to measure the AID and SID of CP and AA of 4 SB products, fishmeal (FM), and casein fed to weanling pigs. The 4 SB products were SBM, a soy protein product (SPP), soy protein isolate (SPI), and fermented SBM (FSBM). Each protein source was included as the only source of CP and AA in cornstarch based diets. An N-free based diet was also used to measure basal endogenous losses of CP and AA. The SID of most AA in casein was superior compared with the other protein sources. However, the SID of most AA in SPP and SPI were similar to casein. Except for Lys, the SID for all indispensable AA in FSBM was not different from FM, SPP, and SPI. No differences were also observed in the SID of AA between SBM and FSBM.

Experiment 3 was conducted to evaluate the impact of processing in conventional SBM and 3 SBM that had been treated to increase the amount of rumen undegraded protein (RUP). The objective of part 1 of this experiment was to measure the apparent (ADD) and standardized (SDD) duodenal digestibility, AID and SID of CP and AA and the ADD, AID, and the small intestinal disappearance of reactive Lys in the 4 SBM using the pig as a model. Four cornstarch based diets were formulated using each of the SBM as the only source of CP and AA in the diets. A N-free based diet was also used to measure basal endogenous losses of CP and AA. The objective of part 2 of this experiment was to test the hypothesis that reactive Lys procedures can be used to predict RUP in heated SBM. Rumen undegraded protein was calculated from CP fractions obtained from the N disappearance of each of the SBM incubated in the rumen of dairy cows. Regression equations were obtained correlating RUP with the concentration of reactive Lys in the 4 SBM. Conventional SBM had greater concentration of AA, greater ($P < 0.05$) ADD, and SID of AA, greater ($P < 0.05$) concentration of reactive Lys, and greater ($P < 0.05$) ADD of reactive Lys but lower ($P < 0.05$) RUP than treated SBM. The SID of Lys, the concentration of reactive Lys, and the AID of reactive Lys was not different among the treated SBM. The concentration of blocked Lys measured using the homoarginine procedure provided the best estimate (R^2=.77) for RUP in SBM.

Keywords: amino acids, digestibility, fermented soybean meal, high protein soybean, pigs, rumen undegraded protein

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CHAPTER 1

INTRODUCTION

Soybeans grown in the Orient where traditionally prepared as a source of protein for humans but in North America, the demand for soybeans is driven by the demand for soybean meal (**SBM**). A large proportion of SBM produced in the United States is fed to livestock (ASA, 2007). The popularity of SBM as a protein source for animals came from the fact that SBM provides the AA that are deficient in corn (Baker, 2000). The AA in SBM, therefore, complements the AA in corn better than other protein sources. The selective breeding of high protein soybeans, such as SSeed HP.290, is designed to optimize the potentials of soybeans as a protein source.

Although SBM is an excellent source of protein, it has limitations when fed to young pigs and dairy cows. Feeding SBM to young pigs often cause digestive disturbances resulting in poor growth (Liener, 2000). Pepsoygen, a fermented SBM product was recently introduced. The fermentation of SBM is effective in reducing the concentration of trypsin inhibitors and increasing the concentration of small peptides in SBM (Hong et al., 2004). The fermentation process also removes the oligossacharides in SBM (Nout and Kiers, 2005). Fermented SBM, therefore, has the potential to be used in diets fed to young pigs without the adverse effects of SBM. Amino acid digestibility may also be improved because of the pre-digested proteins in fermented SBM.

The high rumen degradability of SBM proteins limits its use in dairy cows (NRC, 2001). Heating has been employed to increase the concentration of rumen undegraded protein (RUP) in SBM. The addition of lignosulfonates and soyhulls to SBM prior to heating is also effective in increasing RUP (Castro et al., 2007). However, heat treatment has the risk of reducing the digestibility of AA in proteins but more information on the assessment of the consequences of heating in SBM is needed.

This thesis will primarily evaluate the AA digestibility of some soybeans products as affected by breeding, by the addition of oil, by fermentation and by heating. Specifically, the objectives of this thesis are:

1. To determine the apparent (AID) and standardized (SID) ileal AA digestibility of a new high protein soybean and to evaluate the effect of oil addition on the AA digestibility of SBM and soy protein concentrate fed to growing pigs
2. To determine the AID and SID of fermented SBM fed to weanling pigs
3. To determine the intestinal digestibility of treated SBM using the pig as a model

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CHAPTER 2

Soy Products in Animal Nutrition: Literature Review

INTRODUCTION

The soybean (*Glycine max*) is often referred to as a "miracle crop" because it has numerous applications not only in the food and animal industry, but in other industries as well (ASA, 2007). The recent discovery that bioactive peptides in soybean (**SB**) proteins may prevent chronic diseases adds another dimension to the usefulness of soy proteins in the field of health and medicine (Liu, 1997).

The use of SB as a rich source of CP and oil was long recognized in China (Snyder and Kwon, 1987). In Asia, SB is prepared in a variety of ways which results in different forms and textures of digestible SB products that complement rice-based diets (Snyder and Kwon, 1987). The use of SB as a healthy alternative protein source is also recognized in North America. From the traditional products of SBM for livestock, and oil for industrial use, advances in processing technology in combination with the functional versatility of SB proteins triggered the production of different types of products available for human and animal consumption (Liu, 1997).

SOYBEAN PRODUCTS USED IN THE FEED AND FOOD INDUSTRIES

Soybean Meal

Soybean meal **(SBM)** is produced by the extraction of oil from the SB seed (Liu, 1997). It is considered to be the "gold standard" for protein evaluation because of its excellent AA profile and high digestibility (Drakely, 2000). Several factors influence the concentration of CP and AA in SBM. The method of extraction can result in differences in the CP and AA concentration in SBM. The expeller method is the traditional way of removing oil from the seed. The SB flows through an auger with reducing diameter until it is crushed at the terminal end and pressed into a die to break the cell structure and let the oil flow (Liu, 1997). This method of oil removal retains approximately 6% oil in the meal. The most common current commercial procedure of oil extraction is the solvent extraction method, which is a more efficient way of removing oil from SB. Solvent extracted SBM contains less than 1% oil and has greater concentrations of CP and AA than expeller SBM (Opapeju, et al., 2006), but because of the lower oil concentration, they contain less energy than expeller SBM (Woodworth et al., 2001). Dry extrusion in combination with expeller method has shown promise because of increased oil recovery in a single processing run and because it produces a properly cooked SBM (Nelson et al., 1987).

Soybeans may be dehulled prior to crushing and the resulting SBM contains more CP and AA than non-dehulled SBM (Woodworth et al., 2001). Soybean meals processed from SB grown in the northern part of the United States also contain less AA than SBM processed from SB grown in the central and southern parts of the United States, which indicates that variations in the composition of SBM within the US exists (Grieshop et al., 2003; Karr-Lilienthal et al., 2005).

Soybean Oil

Soybean oil is the second most economically important component in SB. It is the most consumed oil in the world after palm oil (ASA, 2007). Most of the soybean oil produced is refined to cooking oil or salad oil. Soybean oil is mainly composed of unsaturated fatty acids and its high concentration of linolenic acid (9%) makes it susceptible to oxidation (Snyder and Kwon, 1987). Therefore, research has been focused towards the breeding of low linolenic acid SB (Liu, 1997).

Soy oil is included in swine diets primarily to add energy, but soy oil can also contribute essential fatty acids and fat soluble vitamins (Azain, 2001). An additional advantage of using oil in swine diets is that it also reduces the dustiness of the feeds (Barbi, 1996).

Full Fat Soybeans

Whole SB are referred as “full fat” SB (Barbi, 1996). The acceptance of the use of full fat SB was based on its economic advantages over the use of SBM and oil. But later research has shown that the use of full fat SB has other additional benefits (Hancock and Behnke, 2001).

Most of the full fat SB is processed by either roasting or extrusion. Roasting involves dry heating of the whole soybeans at 110º -170ºC for short periods (Barbi, 1996; Hanson, 1996). The whole SB is then ground, or flaked before mixing into the diet. Extrusion involves moving the whole soybeans in a screw conveyor with reducing configurations until the soybeans are pressed through a die to achieve tissue disruption (Barbi, 1996). Heat that is generated by friction and added pressure increases bean temperature from ambient temperature to 135ºC in the 30 seconds that the beans are inside the extruder (Nelson et al., 1987). A high quality SB is produced because the high temperature used during a short time prevents overheating that can reduce the nutritional quality of the full fat SB (Nelson et al., 1987; Parsons et al., 1992). The process of extrusion may be wet or dry depending on if steam is added or not (Hanson, 1996).

Full fat SB contain 35% CP, which is lower than SBM, but it contains 18% fat (NRC, 2001). Because of the high fat concentrations, FFSB contain more energy than SBM when fed to growing or finishing pigs (Kim et al., 2000).

Soy Protein Concentrate

The removal of soluble carbohydrates (i.e., sucrose, raffinose, stachyose, and verbascose) from SBM results in a product called soy protein concentrate (**SPC**; Liu, 1997). It contains approximately 70% CP in DM basis and is produced by 3 different methods (alcohol extraction, acid extraction, or moist heat and water leaching extraction). The composition and characteristics of SPC, therefore, vary according to its manufacturing process (Berk, 1992).

Soy protein concentrate is mainly used in diets fed to young pigs and calves because of their immature digestive tract and sensitivity to antigenic factors in SBM (Dawson et al., 1988; Sohn et al., 1994). Glycinin and β-conglycinin in SBM are the antigenic factors that cause the immune stimulation in pigs (Li et al., 1990). The absence of oligosaccharides, the reduced concentration of trypsin inhibitors, and the reduced concentration of antigenic factors in SPC make young pigs tolerate SPC better than SBM resulting in improved performance (Sohn et al., 1994).

Soy Protein Isolate

Soy protein isolate (**SPI**) contains approximately 90% CP and is the most concentrated form of SB protein (Liu, 1987). Its manufacture starts with the solubilization of the soy protein in SBM achieved at a slightly alkaline pH. After the proteins go into solution, the proteins are precipitated by acidification, neutralized and dried (Berk, 1992). Soy protein isolates are use as a substitute for milk products in newly-weaned pigs but N digestibility and performance are inferior in animals fed soy based diets than milk based diets (Dawson et al., 1988). Compared to SBM, SPI contains relatively less Lys, sulfur AA, and Thr because some of the AA are removed during the isolation and precipitation process (Liu, 1987; Emmert and Baker, 1995).

Fermented SBM

Methods for the fermentation of SB or SBM were developed in China and fermented soy products have been recognized as healthy sources of protein (Liu, 1997). The fermentation process is facilitated by the use of a mold or bacteria or both with *Aspergillus Oryzae* and *Bacillus subtilis* as the predominant strains of mold and bacteria, respectively (Hong et al, 2004, Yang et al., 2007). The fermentation process reduces the concentration of trypsin inhibitors in SBM, but increases the concentration of CP, AA, fat, and P. The AA profile of the fermented product was also different from conventional SBM because of the preferential utilization of some AA by the fungi (Hong et al., 2004). Fermented SBM also contain smaller peptides than SBM which indicates that protein has been digested by the proteases produced by *A. oryzae* (Sardjono et al., 1998; Hong et al., 2004).

Enzymatically digested SBM

This is a commercial product under the trade name of HP 300 (www.hamletprotein.com). It is an enzymatically treated SBM with reduced concentrations of trypsin inhibitors, oligosaccharides, and other anti-nutritional components that are present in SBM (Zhu et al., 1998). The AA composition of HP 300 is similar to SBM, but the apparent (**AID**) and standardized (**SID**) ileal digestibility of most AA in HP 300 is greater than SBM (Yang et al., 2007) and similar to value obtained in SPC and fermented SBM (Yang et al., 2007).

Heated SBM with increased Rumen By-pass Protein

Heating was shown to increase the ruminal by-pass value of the proteins in SBM (Faldet et al., 1992). A protein source with a high by-pass value is needed in the feed for high yielding dairy cows because these cows have a high AA requirement (Stern et al., 1994). Heating of SBM induces the Maillard reaction, which is believed to be the mechanism of protecting the SBM from rumen degradation (Faldet et al. 1992). Methods to increase the by-pass value of proteins include mixing SBM with lignosulfonates or soyhulls prior to heating (Can and Yilmaz, 2002, Heitritter et. al., 1998). Exposing SBM to severe heat using a mechanical expeller is also an effective way to increase by-pass SBM (Castro et al., 2007).

Soy Hulls

Soy hulls are by-products of soybean processing. They are removed during the cleaning process before oil extraction (Liu, 1997). The complex carbohydrate in soy hulls are composed of 30% pectin, 50% hemicellulose, and 20% cellulose (Kikuchi et al., 1971). Increasing levels of dietary soy hulls resulted in a linear reduction in the AID and SID of AA in SBM fed to pigs (Dilger et al., 2004). Therefore the use of soy hulls in diets fed to swine is limited.

NEW VARIETIES OF SOYBEANS.

High Protein Soybean

A major reason for breeding high protein SB is that CP concentration is positively correlated with the yield of soy milk and tofu (Liu, 1997). Conventional SB contains 35-

37 % CP whereas high protein SB contains at least 41% CP (Chohan et al., 1993; NRC, 2001; Yaklich, 2001). The increase in CP is associated with an increase in AA concentration, but the concentration of sulfur AA does not usually increase as much as the concentration of other AA (Krishnan, et al., 2005). The true ileal digestibility of AA in roosters fed 2 SBM processed from 2 high protein SB varieties, were not different to the conventional SBM however, more research is needed to evaluate the feeding value of these new varieties in swine diets.

Low Oligosaccharide Soybean

The feeding of soybeans to young animals, poultry and humans is constrained by the inherent presence of indigestible oligosaccharides in soybeans. The α-1,6 galactosidic linkage that connects the galactose in raffinose and stachyose can not be digested in the small intestines of monogastric animals because of the lack of endogenous α-galactosidase (Liener, 2000). This enzyme is necessary to digest the α-1,6 galactosidic bonds and break the oligosaccharide to its component sugars. Microbial fermentation in the distal ileum and colon digests these oligosaccharides producing gas which is the main reason for the flatulence observed in humans eating beans and other legumes containing α-galactosides (Suarez et al., 1999). The low ME in SBM fed to poultry is also attributed to these oligosaccharides. The development of a low-oligosaccharide SB has resulted in SBM that have 86% lower raffinose and stachyose concentration than conventional SBM (Parsons et al., 2000). The reduction in the oligosaccharide concentration of the modified SB result in a 7% improvement in true metabolizable energy compared with conventional SB (Parsons et al., 2000). However, in dogs, nutrient digestibility of low-oligosaccharide SB and conventional soybean were not different (Zuo et al., 1996).

Low Trypsin Inhibitor Soybean

The low-trypsin inhibitor SB contain 40-50% less trypsin inhibitors than conventional raw SB but the AA concentration is similar to conventional SB (Chohan et al., 1992; Herkelman et al., 1992). Birds fed low-trypsin inhibitor SB had a similar N digestibility and performance as birds fed raw SB, but birds fed roasted SB performed better than birds fed raw and low-trypsin inhibitor SB (Chohan et al., 1992). This indicates that the concentration of trypsin inhibitors in low-trypsin inhibitor SB has not been reduced enough to reduce the negative effects on growth performance. In pigs, apparent ileal digestibility of CP and AA in low-trypsin inhibitor SB is greater than in conventional raw SB but heating improved CP and AA digestibility in low-trypsin inhibitor SB to a greater extent than heated conventional SB (Herkelman et al., 1992). This further suggests that heating is necessary to optimize the feeding value of low-trypsin inhibitor SB and the use of these SB has no advantage over conventional SB. However, if the concentration of trypsin inhibitors can be further reduced, these varieties could be an alternative o conventional SB.

FACTORS AFFECTING AMINO ACID DIGESTIBILITY IN SOYBEAN PRODUCTS

Anti-nutritional Factors

Protease Inhibitors. Two types of protease inhibitors are inherent in soybeans. The Kunitz inhibitor that binds trypsin, and the Bowman-Birk inhibitor that binds both trypsin and chymotrypsin (Liener, 2000). These protease inhibitors are responsible for most of the reduction of CP and AA digestibility that is observed in raw SB compared with heated SB (Herkelman et al., 1992). The reason for this is that they reduce the digestion of proteins by inactivating trypsin and chymotrypsin. The compensatory response of the body to increase secretion of these 2 enzymes contributes to the reduction in CP and AA digestibility of feedstuffs (Liener, 2000).

Oligosaccharides. The major oligosaccharides in soybeans are raffinose and stachyose that are present at approximately 1% and 6% in SBM dry matter (Grieshop et al., 2003). The addition of 9% soy oligosaccharide reduced the AID of AA in SPC- fed pigs. However, pigs fed SBM that contained increasing levels of soy oligossacharides did not have a reduction in AID of AA (Smiricky, et al., 2002). Dogs fed low oligosaccharide SBM had similar nutrient digestibility as dogs fed conventional SBM (Zuo et al., 1996). But weanling pigs fed 1 and 2% added stachyose had lower weight gains than milk based diets. Diarrhea incidence was also greater in pigs fed SBM diets than milk based diets. The above studies indicate that the age of the animal influences the impact of oligossacharides on nutrient digestibility.

Processing

Heating is an effective way to reduce the anti-nutritional factors in SBM and the reduction in trypsin inhibitor concentration results in improved CP and AA digestibility (Herkelman et al., 1992). Different methods of heat application are used and the efficiency by which the anti-nutritional factors are reduced is influenced by the method of heating. The processing facilities, the temperature, and the duration of heating, therefore, influence the digestibility of amino acids (Woodworth et al., 2001; Opapeju et al. 2006).

The direct measure of trypsin inhibitor activity or concentration is a good indicator of underheated SBM, however, the simplicity of the urease activity assay makes it the method of choice in evaluating SBM quality (Snyder and Kwon, 1987). It is based on the principle that the urease enzyme in SBM is denatured at the same rate as protease inhibitors during heating (Parsons, 2000). The optimum range of urease pH change is 0.05-0.20 (AOCS, 1973) but SBM that have a zero urease pH change also showed Lys digestibility of not less than 90% using the cecectomized rooster assay (Parsons, 2000). This suggests that the urease activity assay is not a sensitive measure of overheating (Parsons, 2000).

Extrusion. The denaturation of SB native proteins and the disruption of SB fat globules during extrusion may contribute to the greater feeding value of extruded SB compared with roasted SB (Hancock and Behnke, 2001). Apparent ileal digestibility of most AA in extruded SB is greater than in roasted or jet-sploded SB, but lower than in conventional SBM (Marty et al., 1994; Marty and Chavez, 1995; Kim et al., 2000). The reduced AID of AA in roasted SB is associated with greater endogenous losses of Lys and greater ileal recovery of dietary Lys in the distal ileum (Marty and Chavez, 1995). Regardless of the equipment used to heat soybeans, overheating of soybeans reduces the true digestibility of some AA, particularly Lys (Parsons, et al., 1992). When fed to pigs, the AID of AA in roasted SBM that was heated to increase rumen by-pass was also lower than in conventional SBM (Marty and Chavez, 1995).

Processing by alcohol, acid, or heat and water. Processing of SBM improves AA digestibility. The AID of AA in young pigs fed SPI and SPC containing diets were greater than the AID of AA in pigs fed SBM (Walker et al., 1986; Sohn et al., 1994). The improvement in the AID of AA in SPC and SPI was attributed to the removal of indigestible carbohydrates and antigenic proteins in these products (Walker et al., 1986; Sohn et al., 1994). However, the advantage of SPC over SBM may be limited to young pigs because the SID of AA in growing pigs fed SPC was not different from values obtained in pigs fed SBM (Smiricky, et al., 2002).

Fermentation. Fermentation of SBM using *A. oryzae* produces a product that contains more peptides than conventional SBM (Hong et al., 2004). Peptides are absorbed in the small intestines at a faster rate than free AA (Webb, 1990). As a result, the fermentation of SBM is expected to improve AA digestibility. However, the fermentation of SBM improved the AID and SID of some, but not all, AA in fermented SBM compared with conventional SBM (Yun et al., 2005; Yang et al., 2007). The AID of AA in fermented SBM fed to early-weaned pigs was not different from the AID of AA in fish meal, SPC, and HP 300 (Yun et al., 2005; Yang et al., 2007).

Oil addition

There is limited information about effect of dietary oil on AA digestibility. The addition of graded levels of oil improved the AID of AA (Albin et al., 2001; Imbeah and Sauer, 1991), which may be a result of a reduction in the rate of gastric emptying (Gentilcore et al., 2006) or a reduction in the rate of passage of the digesta through the small intestines (Valaja and Silijander, 2001). This would provide longer time for digestion and absorption of AA and peptides (Zhao et al., 2000). However, addition of oil to SBM in order to simulate the fat content in full-fat soybeans did not result in similar AA digestibilities between the two products (Fan et al., 1995; Marty and Chavez, 1995; Bruce et al., 2006). The lower values for AID of AA in FFSB than in SBM with added oil was attributed to a greater concentration of trypsin inhibitors and a greater concentration of dietary fiber from soy hulls in FFSB than in SBM with oil diet (Fan et al., 1995; Bruce et al., 2006). Greater endogenous loss of Lys was also observed at the distal ileum of pigs fed micronized, jet-sploded or roasted FFSB compared with SBM with oil (Marty and Chavez, 1994).

Fiber

The soy hulls contain most of the fiber in soybeans. It is removed before oil extraction and sometimes is added back to the meal to produce a meal with 42- 44% CP (Liu, 1997). Additions of soy hulls to SBM reduce the AID and SID of AA (Dilger, et al., 2004). This maybe a result of increasing concentration of neutral detergent fiber (NDF) in the diet because increasing NDF can reduce AA digestibility in 2 ways. First, it may increase pancreatic secretions of enzymes (Ikegami et al., 1990) and increase sloughing of intestinal epithelium (Shah et al., 1982), which may result in an increase in endogenous CP and AA losses. Second, NDF particularly pectin which is present in soy hulls, may increase digesta viscosity which may reduce the interaction between nutrients and enzymes resulting in a slow rate of nutrient absorption (Mosenthin et al., 1994; Buraczewska et al., 2007).

CONCLUSION AND PERSPECTIVES

As world population continues to increase, the demand for protein also increases. The demand for an inexpensive high quality protein as food for man and animals alike, is the driving force to explore the full potential of SB proteins and expand the use of SB in human food systems and animal production systems.

Selective breeding of SB to increase the concentration of CP may improve the feeding value of SB as a protein source for human and animal consumption. However, there is a lack of information on the AID and SID of AA in high protein SB compared with conventional SB and more research is needed to measure the AA digestibility in high protein SB.

Pepsoygen, a fermented SBM product, was recently introduced. The fermentation process was suggested to overcome the constraints of the use of conventional SBM in starter diets. Therefore, this product can potentially be a substitute for more expensive soy products such as SPC and SPI, FM, and dairy products in starter diets.

Different methods are used to produce rumen by-pass SBM. The process of inducing the Maillard reaction through heat and the addition of reducing sugars not only increases RUP but also decreases the concentration of reactive Lys. Measuring RUP using rumen-cannulated cows is tedious and expensive. *In-vitro* procedures for measuring the concentration of reactive Lys may be useful in predicting RUP of by-pass SBM.

Formulating diets to meet the CP and AA requirement of animals requires that reliable data on SID of AA in feed ingredients are available. Knowing the SID of CP and AA in high protein SB and fermented SBM will enable nutritionists to make sound decisions in choosing suitable feed ingredients relative to their price.

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Table 2.1 CP and AA composition (%) of soy products[1], as fed basis

Parameter	CSBM[2]	Extruded FFSB[3]	High CP FFSB[4]	By-pass SBM[5]	Extruded SPC[6]	SPI[7]	HP 300[8]	FSBM[9]
CP, %	47.50	35.71	41.87	50.11	66.39	90.23	56.00	50.46
Indispensable AA, %								
Arg	3.48	2.56	2.60	3.39	4.53	6.50	3.87	3.57
Hist	1.28	0.92	0.89	1.20	1.70	2.28	1.40	1.34
Ile	2.16	1.51	1.68	2.28	2.83	4.22	2.47	2.38
Leu	3.66	2.59	2.81	4.46	5.00	7.19	4.14	4.12
Lys	3.02	2.14	3.49	2.89	4.05	5.68	3.28	2.98
Met	0.67	0.51	0.93	0.66	0.38	1.30	0.70	0.75
Phe	2.39	1.68	1.88	2.80	3.21	4.66	2.69	2.64
Thr	1.85	1.24	1.36	2.16	2.45	3.37	2.10	2.04
Try	0.65	0.35	-	0.64	-	-	0.73	0.66
Val	2.27	2.55	1.80	2.73	2.92	4.37	2.58	2.63
Dispensable AA, %								
Ala	-	1.33	-	-	2.73	3.86	-	2.21
Asp	-	3.81	-	-	7.54	10.30	-	5.74
Cys	0.74	0.53	0.30	0.73	1.23	0.97	0.75	0.90
Glu	-	5.67	-	-	11.88	17.52	-	9.00
Gly	-	1.26	-	-	2.73	3.70	-	2.22
Pro	-	1.65	-	-	3.39	4.55	-	2.49

Ser	-	1.59	-	-	3.39	4.65	-	2.43
Tyr	-	1.29	-	-	2.17	3.37	1.99	1.87

[1] CSBM =conventional soybean meal; FFSB = full fat soybean; SPC = soy protein concentrate; SPI = soy protein isolate; HP 300 = Hamlet Protein 300; FSBM = fermented soybean meal

[2] NRC, 1998.

[3] Rudolph et al., 1983.

[4] Chohan et al., 1993.

[5] NRC, 2001..

[6] Clapper et al., 2001.

[7] Walker et al., 1986.

[8] Data from manufacturer; www.hamletprotein.com.

[9] Hong et al., 2004.

Table 2.2 Nutrient composition (%) of soy products[1], as fed basis

Parameter	Unit	CSBM	Extruded FFSB	High CP FFSB	Extruded SPC	SPI	HP 300	FSBM
DM	%	90.00[2]	91.25[3]	95.60[4]	94.30[5]	-	92.00[6]	91.21[6]
Crude fat	%	3.00[2]	19.87[3]	16.83[4]	0.75[5]	-	2.52[8]	5.23[6]
Fiber								
TDF	%	17.25[9]	-	-	16.50[5]	-	-	-
Crude fiber		-	4.44[3]	-	-	-	3.86[8]	-
ADF	%	5.40[2]	-	-	-	-	-	-
NDF	%	8.90[2]	-	-	-	-	-	-
Oligosaccharides								
Sucrose	%	5.81[9]	3.89[9]	-	-	0.10[10]	Trace[8]	-
Stachyose	%	4.40[9]	3.02[9]	-	-	0[10]	0.25[8]	-
Raffinose	%	1.04[9]	0.49[9]	-	-	0[10]	0.21[8]	-
Verbascose	%	0.19[9]	0.13[9]	-	-	-	-	-
Trypsin Inhibitor Activity								
	mg/g	2.50[11]	12.82[3]	-	0.38[12]	-	1.00[6]	0.42[6]
	TIU g^{-}CP	9.50[13]	12.90[13]	11.20[4]	-	-	-	-
Urease	pH units	0.02[9]	1.23[3]	-	-	-	-	-
Antigens								
Glycinin	Log$_2$	12[12]	-	-	0[12]	-	-	-
β-conglycinin	Log$_2$	11[12]	-	-	0[12]	-	-	-

ppm	-	-	-	-	-	2.00[6]	-

[1] CSBM = conventional soybean meal; FFSB = full fat soybean; SPC = soy protein concentrate; SPI = soy protein isolate; HP 300 = Hamlet Protein 300; FSBM = fermented soybean meal

[2] NRC, 1998

[3] Rudolph et al., 1983

[4] Chohan et al., 1993

[5] Clapper et al., 2001

[6] Data from manufacturer; www.hamletprotein.com

[7] Hong et al., 2004

[8] Jiang et al., 2006

[9] Grieshop et al., 2003

[10] Eldridge et al., 1979

[11] Herkelman et al., 1992

[12] Friesen et al., 1993; values of glycinin and *β*-conglycinin for conventional soybean meal is based on soy flakes

[13] Marty et al., 1994

CHAPTER 3

Ileal amino acid digestibility of high protein soybeans, conventional soybeans, and soybean meal and soy protein concentrate without and with added oil fed to growing pigs

ABSTRACT. A study was conducted to measure the apparent (AID) and standardized (SID) ileal digestibility of CP and AA in a new high protein variety of soybeans (SSeed.HP 290) fed to growing pigs and compare these values to the AID and SID of CP and AA in conventional full fat soybeans (FFSB), soybean meal (SBM), and soy protein concentrate (SPC). A second objective of the study was to evaluate the effect of oil addition on CP and AA digestibility in SBM and SPC. Four cornstarch-based diets were prepared using each soy product as the sole source of CP and AA. Two additional diets were formulated by adding soybean oil (7.55 and 7.35%, respectively) to SBM and SPC. A N-free diet was also used to measure basal endogenous losses of CP and AA. Before the experiment, SSeed.HP 290 and FFSB were extruded at 150^{o}C. Seven growing barrows (initial BW: 26.2 ± 2.24 kg) were fitted with a T-cannula in the distal ileum and allotted to a 7 x 7 Latin square design with 7 diets and 7 periods. Ileal digesta were collected from the pigs on d 6 and 7 of each period. All digesta samples were lyophilized and analyzed for DM, CP, AA, and chromium, and values for AID and SID of CP and AA were calculated. Results of the experiment showed that the SID for 6 of the indispensable AA in SSeed.HP290 were greater ($P < 0.05$) than in conventional FFSB and the SID for all indispensable AA except Met, were greater ($P < 0.05$) in SSeed.HP 290 than in SBM. However, the SID for most AA in SSeed.HP 290 were similar to SBM with oil and SPC, but these values were lower ($P < 0.05$) than in SPC with oil. Except for the SID of Lys, Phe, Trp, and Met, the SID of the indispensable AA were not different among conventional FFSB, SBM, and SBM with oil. The addition of oil improved ($P < 0.05$) the SID for most indispensable AA in SBM and SPC. In conclusion, the SID of AA in Seed.HP 290 were greater than in SBM and similar to conventional FFSB and SPC. The addition of oil improved the SID of most AA in SBM and SPC fed to growing pigs.

Keywords: amino acids, digestibility, high protein soybean, oil, pigs, soybean meal

INTRODUCTION

New varieties of soybeans are constantly being developed for agronomic or nutritional reasons. Soybeans that are free of lectin and kunitz trypsin inhibitors have been developed and evaluated (Palacios et al., 2004). Recently, a high CP variety of soybeans was developed (SSeed.HP 290; Shillinger Seed, Des Moines, IA). This variety provides greater concentrations of CP and AA than regular soybeans in diets fed to pigs, but the digestibility of AA in SSeed.HP 290 has not yet been measured.

Full fat soybeans (**FFSB**) have a high CP and lipid concentration that contribute significant amounts of energy and protein to compound feeds (Zarkadas and Wiseman, 2005), but soybean meal (**SBM**) is the most popular source of protein in diets fed to animals (Baker, 2000). Soy protein concentrate (**SPC**) is also used in animal feeding and the AA in SPC are considered more digestible than in SBM (NRC, 1998). However, it is not known if the AA in SSeed.HP 290 are more or less digestible than AA in SBM and SPC.

One of the differences between FFSB and SBM is the concentration of oil (Cromwell, 2000). Studies that evaluated the effect of oil on AA digestibility are few (Li and Sauer, 1994; Jørgensen et al., 1985; Albin et al., 2001) and these studies measured apparent ileal digestibility **(AID)** of AA**.** However, standardized ileal digestibility values (**SID**) for CP and AA take into account the AA contributions that are of endogenous origins and is, therefore, a more accurate estimate of the digestibility of AA in a feed ingredient (Stein et al., 2007). But limited information is available on the effect of fat addition on the SID of AA in SBM and SPC. The objective of this experiment was to measure the AID and SID of CP and AA in SSeed.HP 290 and compare these values to values obtained in commercial sources of FFSB, SBM, and SPC. A second objective was to evaluate the effect of oil addition to SBM and SPC on AA digestibility.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

Seven growing barrows (initial BW: 26.2 ± 2.2 kg) originating from the matings of SP-1 boars to line 13 sows (Ausgene Intl. Inc, Gridley, IL) were equipped with a T-cannula in the distal ileum using the method described by Stein et al. (1998). After surgery, pigs were transferred to individual pens (1.2 x 1.8 m) in a temperature controlled room (22^{o}C) where they were allowed to recover for 14 d. A standard corn-soybean meal diet (16% CP) was provided on an ad libitum basis during this time. Pigs were then allotted to a 7 X 7 Latin square design with pigs and periods comprising the rows and columns, respectively. The animal part of the study was conducted at South Dakota State University, and the experiment was approved by the Institutional Animal Care and Use Committee at South Dakota State University.

Ingredients, Diets, and Feeding

Four soybean products were used in this experiment (Table 1). The 4 sources were a high protein FFSB (SSeed.HP 290), a conventional source of FFSB, conventional SBM, and a commercial source of SPC (Profine E, Central Soya Co. Inc., Fort Wayne, IN). The conventional FFSB and SBM were obtained locally. Before the experiment, both sources of FFSB were ground and extruded at 150 to 160^{o}C using an Insta Pro extruder, Model 2500 (Insta Pro, Des Moines, IA). Immediately following extrusion, beans were cooled to 43^{o}C using a tumble drum cooler (Insta Pro, Des Moines, IA).

Seven diets were formulated (Tables 2 and 3). Four diets contained each of the soybean products as the only protein and AA source. Two additional diets were formulated by adding soybean oil to the diets containing SBM and SPC, respectively, to bring the total concentration of ether extract in those diets close to the concentrations in the 2 diets containing FFSB. A N-free diet was used to measure basal ileal endogenous losses of AA. Chromic oxide was included (0.40%) in all diets as an inert marker.

Feed was provided in quantities equal to 3 times the estimated daily maintenance energy requirement of the pigs (106 kcal ME per kg $^{0.75}$; NRC, 1998). The daily allotment of feed was divided into 2 equal meals that were provided at 0830 and 1600. Water was available at all times.

Data and Sample Collection

Pig BW were recorded at the beginning of the experiment and at the end of each period. Each period lasted 7 d and pigs were allowed to adapt to their diet during the initial 5 d. On d 6 and 7, ileal digesta were collected for 8 h. A 225-ml plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 min and replaced with a new one. Digesta were immediately stored at –20^{o}C to prevent bacterial degradation of the AA in the digesta. Ileal samples obtained over the 2-d collection period were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. A sample of each diet and of each of the protein sources was collected as well. Digesta samples were lyophilized and finely ground prior to chemical analysis.

Chemical Analysis

The soy products were analyzed for urease activity (procedure Ba9-58; AOCS, 1998) and NDF using the procedure of Holst (1973). Sucrose, raffinose, and stachyose were analyzed using the procedure of Janauer and Englmaier (1978). All soy products and diets were also analyzed for ether extract (procedure 4.5.01; AOAC, 2000), DM (procedure 4.1.06; AOAC, 2000), and CP (procedure 4.2.08; AOAC, 2000). Ileal digesta samples were also analyzed for DM and CP. Amino acids were analyzed in all samples on a Beckman 6300 Amino Acid Analyzer (Beckman Instruments Corp., Palo Alto, 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^{o}C (procedure 4.1.11, alternative 3; AOAC, 1998). Methionine and Cys were determined as Met sulfone and cysteic acid respectively, after cold performic acid oxidation overnight prior to hydrolysis (procedure 4.1.11, alternative 1; AOAC, 1998). Tryptophan was determined after NaOH hydrolysis for 22 h at 110^{o}C (procedure 45.4.04; AOAC, 2000). Chromium concentrations of diets and ileal digesta were determined after nitric acid-perchloric acid wet ash sample preparation (procedure 9.2.39; AOAC 2000).

Calculations

Apparent ileal digestibility values for AA in samples obtained from feeding the protein-containing diets were calculated using equation [1] (Stein et al., 2007):

$$\text{AID} = (1 - [(\text{AAd}/\text{AAf}) \times (\text{Crf}/\text{Crd})] \times 100\ \% \qquad \textbf{[1]}$$

where AID is the apparent ileal digestibility of an AA (%), AAd is the concentration of that AA in the ileal digesta DM, AAf is the AA concentration of that AA in the feed DM, Crf is the chromium concentration in the feed DM, and Crd is the chromium concentration in the ileal digesta DM. The AID for CP was also calculated using this equation.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 2007):

$$\text{IAA}_{end} = [\ \text{AAd} \times (\text{Crf}/\text{Crd})] \qquad \textbf{[2]}$$

where IAA_{end} is the basal endogenous loss of an AA (g per kg DMI). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each AA, standardized ileal AA digestibility values were calculated using equation [3] (Stein et al., 2007):

$$\text{SID} = \text{AID} + [(\text{IAA}_{end}/\text{AAf}) \times 100] \qquad \textbf{[3]}$$

where SID is the standardized ileal digestibility value (%).

Concentrations of standardized ileal digestible AA in each protein source were calculated using equation [4]:

$$\text{Digestible AA (g/kgDM)} = [\text{AA}_{ing} \times (\text{SID}/100)] \qquad \textbf{[4]}$$

where AA_{ing} is the AA concentration (g/kg DM) in the protein source. The concentration of standardized ileal digestible protein was also calculated using this equation.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to confirm the homogeneity of the variance. Outliers were determined as values that were more than 3 SD above or below the mean. No outliers were identified. An analysis of variance was conducted with diet as the fixed effect and pig and period as random effects. Whenever differences were detected, treatment means were separated using the Least Significant Difference test of PROC MIXED. Orthogonal contrasts were used to compare SBM and SPC vs. SBM with oil and SPC with oil to determine the effect of oil addition on AA digestibility. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among treatments.

RESULTS

The CP and AA concentrations were greater in SSeed.HP 290 compared with conventional FFSB and SBM (Table 3.1). However, the concentration of ether extract was lower in SSeed.HP 290 than in conventional FFSB. Soybean meal contained more CP and AA than conventional FFSB, but the concentration of CP and AA in SPC was greater than in the other soybean sources. The concentration of NDF was greater in SBM than in the other protein sources. The concentration of sucrose, raffinose, and stachyose were lower in SPC compared with the other soybean products, but the concentration of sucrose was similar between SBM and conventional FFSB. However, these values were greater than in SSeed.HP 290. The concentration of raffinose was lower in conventional FFSB than in SSeed.HP 290, but the stachyose concentration was not different for FFSB and SBM. The urease activity was within the range of 0.05 to 0.20 for all samples.

The AID for CP in SSeed.HP 290 (81.0%) was similar to the AID in SPC with oil (81.5%) and both of these values were greater ($P < 0.05$) than the AID obtained for the other diets (Table 3.4). The AID for CP in SBM (68.1%) and SBM with oil (71.5%) were similar and these values were lower ($P < 0.05$) than the AID for CP in FFSB (76.4%). However, the AID for CP in FFSB and SBM with oil were similar to SPC (75.4%).

For most AA, no differences in the AID were observed among SSeed.HP 290, SPC, and SPC with oil. However, the AID for most AA in SBM, and SBM with oil was lower ($P < 0.05$) than in SSeed.HP 290, SPC, and SPC with oil. The AID for 6 of the 10 indispensable AA (Arg, His, Ile, Leu, Phe, and Val) was greater ($P < 0.05$) in SSeed.HP 290 than in conventional FFSB, but the AID for most AA in conventional FFSB was similar to SBM and SBM with oil. Diets containing SPC and SPC with oil had greater ($P < 0.05$) AID for most indispensable AA compared with conventional FFSB, but the AID for most dispensable AA in conventional FFSB were similar to the SPC-containing diets.

The addition of oil to SBM and SPC improved ($P < 0.05$) the AID of most indispensable AA. Among the dispensable AA, only the AID for Ala, Cys, and Glu were improved ($P < 0.05$) by oil addition.

The SID for CP in SBM (84.8%) was lower ($P < 0.05$) than in all other diets, and the SID for CP in SPC plus oil (97.6%) was greater ($P < 0.05$) than in all other diets except the value (94.1%) obtained for SSeed.HP 290 (Table 3.5). The SID for most AA in SSeed.HP 290 were similar to SBM with oil and SPC, but these values were lower ($P < 0.05$) than in SPC with added oil. The SID for Ile, Leu, Phe, Val, Ser, and Tyr in SSeed.HP 290 were greater ($P < 0.05$) than in conventional FFSB, but the SID for the remaining AA where not different between the 2 sources of FFSB. The SID for most of the indispensable AA in conventional FFSB were similar to SBM and SBM plus oil. The SID of Lys, Phe, and Trp were greater ($P < 0.05$) in conventional FFSB than in SBM, but the SID of Met was lower ($P < 0.05$) in conventional FFSB compared with SBM plus oil. However, for the remaining indispensable AA, no differences among these 3 diets were observed. The SID for most AA in SPC and SPC plus oil were greater ($P < 0.05$) than for conventional FFSB and SBM. The addition of oil improved ($P < 0.05$) the SID for most indispensable AA in SBM and SPC, but greater improvements were observed for SBM than for SPC.

Among all ingredients, conventional FFSB had the lowest ($P < 0.05$) concentration (352 g) of SID CP (Table 3.6). The concentration of SID CP in SSeed.HP 290 (472 g) was greater ($P < 0.05$) than in SBM (406 g) and SBM with oil (430 g). Soy protein concentrate had a greater ($P < 0.05$) concentration (652 g) of SID CP than SSeed.HP 290, but SPC with oil (691 g) had the greatest ($P < 0.05$) concentration of SID CP among all treatments.

Concentration of most of the digestible AA in SSeed.HP 290 were greater ($P < 0.01$) than in conventional FFSB, SBM, and SBM with oil, but the concentration of digestible Lys in SSeed.HP 290 was similar to SBM and SBM with oil. In contrast, the concentration of digestible Met and Trp were lower ($P < 0.05$) in SSeed.HP 290 than in SBM and SBM with oil. The concentration of digestible Thr in SSeed.HP 290, however, was similar to SBM, but lower ($P < 0.05$) than in SBM with oil. The concentration of digestible AA in SSeed.HP 290 were lower ($P < 0.01$) than in SPC and SPC with oil, but conventional FFSB had the lowest ($P < 0.05$) concentration of digestible AA among all diets. The concentration of digestible Ile, Leu, Met, Trp, and Val was greater ($P < 0.01$) in SBM with oil than in SBM, but for the remaining indispensable AA, no differences between these 2 diets were observed. Soy protein concentrate had a greater ($P < 0.01$) concentration of digestible AA than SBM with oil, and the addition of oil to SPC increased ($P < 0.01$) the concentrations of all indispensable AA except Met and Phe. Therefore, SPC with oil had a greater ($P < 0.05$) concentration of digestible AA than all other diets.

DISCUSSION

Composition of Ingredients

To increase the feeding value of soybeans, varieties with increased concentration of CP or reduced concentration of trypsin inhibitors and oligosaccharides have been selected. The SSeed.HP 290 variety was selectively bred for greater concentration of CP. The increase in CP was achieved partly at the expense of ether extract that was reduced in SSeed.HP 290 compared with conventional FFSB. The negative correlation of soybean protein to seed oil and yield are the major obstacles that hinder the development of high CP soybeans for commercial use because soybeans are traded on a weight basis and high CP lines often have a lower yield and contain less oil (Yaklich, 2001). The CP concentration of soybeans is also negatively correlated with the concentration of sucrose but positively correlated with the concentration of stachyose (Hartwig et al., 1997). This inverse relationship between protein and sucrose concentration explains the lower sucrose concentration in SSeed.HP 290 than in conventional FFSB, but the stachyose concentration was similar between the 2 soybean varieties. The stachyose concentration in high protein soybeans averaged 4.13% (Hartwig et al., 1997), which is close to the value obtained in this study for both SSeed.HP 290 and conventional FFSB.

Aqueous alcohol extraction removes the sucrose, raffinose, and stachyose from defatted soy flakes (Eldridge et al., 1979). The concentrations of oligosaccharides in SPC are, therefore, lower than in SBM. The concentration of sucrose, raffinose, and stachyose in SPC and SBM were within the range of values reported in other studies (Eldridge et al., 1979; Bach Knudsen, 1997, Grieshop et al., 2003).

The fiber in soybean is mainly present in the seed coat. The fiber in the seed coat contains approximately 80% polysaccharides, which can be separated into cellulose, hemicellulose, and pectin on the basis of solubility (Stombaugh et al., 2000). Seed coat contribution to the total seed weight is relatively constant. Although the increase in the concentration of CP results in a reduction in ether extract, the combined concentration of CP and ether extract increases, therefore, the fiber and carbohydrate components decrease when the soybeans are selected for high protein concentration (Hartwig et al., 1997). This may explain the lower concentration of NDF in SSeed.HP 290 than in conventional FFSB. On the other hand, the extraction of fat from the soybean seed results in a greater concentration of NDF in SBM than in FFSB and the values for NDF obtained in this study are similar to the values reported by Edwards et al. (2000), but lower than the values reported by Grieshop et al. (2003). Genotypic variation may account for the difference in the polysaccharides present in the seed coat (Stombaugh et al., 2000).

The CP and AA concentration in conventional FFSB and SBM used in this study were similar to the values reported by NRC (1998) and from other studies (Kim et al., 2000; Clapper et al., 2001; Grieshop et al., 2003). However, the AA concentration in

SPC was slightly lower than the values reported by NRC (1998) but similar to the values reported by Clapper et al. (2001). Differences in processing methods during the production of SPC may result in SPC with different characteristics (Berk, 1992).

Comparison of SSeed. HP290 and other Soy Products

The total concentration of AA in SSeed.HP 290 was 25% greater than in conventional FFSB, which indicates that this new variety of soybeans may have a greater feeding value than conventional FFSB. Among the indispensable AA, Arg and His concentrations had the greatest improvement (28%). The concentration of Arg usually increases with increasing CP concentration (Krishnan et al., 2007). In contrast, the concentration of Trp usually decreases with increasing CP concentration (Krishnan et al., 2007); however, in this study, no difference in Trp concentration was observed between SSeed.HP 290 and conventional FFSB. Among the dispensable AA, Glu followed by Asp had the greatest increase in concentration (30%) and this is consistent with the observation of Zarkadas et al., (1993) who reported that Glu and Asp are the most abundant AA in FFSB comprising 26-28% of the CP in soybeans regardless of cultivar. The concentration of Met and Cys in SSeed.HP 290 was only 8% greater than in conventional FFSB.

The lack of a difference in AID and SID of most AA between SSeed.HP 290 and conventional FFSB is consistent with the findings of Marty and Chavez (1994) who reported that the AID of AA in 3 soybean cultivars were similar. Although there is no difference in the SID of AA between SSeed.HP 290 and FFSB, the concentration of SID AA in SSeed.HP 290 was greater than in FFSB because of the greater concentration of

AA in SSeed.HP 290 compared with FFSB. Using SSeed.HP 290 instead of FFSB will, therefore, increase the contribution of digestible AA to the diet.

Effect of Oil Addition to SBM and SPC

The increased AID and SID of AA when oil was included in the diet may be due to slower gastric emptying, which increase the time that feed proteins are exposed to proteolytic enzymes (Gentilcore et al., 2006). The presence of fat in the small intestine may also reduce the passage rate of the ingested feed (Valaja and Silijander-Rasi, 2001). This may provide a longer time for AA and peptides to be absorbed (Zhao et al., 2000).

Addition of graded levels of canola oil to SBM resulted in a linear improvement in the AID of most AA (Li and Sauer, 1994), but both linear and quadratic improvements were observed in other studies upon oil addition (Imbeah and Sauer, 1991; Albin et al., 2001). Thus, the results of this experiment showing an increase in AID of AA in SBM when oil was added to the diet agree with previous reports. However, results from the present experiment also demonstrated that the SID for AA in SBM improved by the addition of oil. To our knowledge, this is the first study to report on the effect of oil on the SID of AA in SBM. The results of this study also showed that the AID and SID of AA in a highly digestible protein source such as SPC will increase when oil is added to the diet.

Previous studies have shown that the digestibility of AA in FFSB is lower than in SBM with added oil (Fan et al., 1995; Marty et al., 1994; Bruce et al., 2006). The reason for the reduced digestibilities has been attributed to greater concentration of trypsin inhibitors (Fan et al., 1995) and soy hulls (Bruce et al., 2006) in FFSB than in SBM. It has also been reported that the FFSB induces a greater endogenous Lys loss than SBM with added oil (Marty et al., 1994). In the present experiment, there were no differences in AA digestibility between FFSB and SBM with added oil. The SBM used in this experiment was not dehulled and the concentration of hulls was expected to be similar in SBM and FFSB. In addition, both FFSM and SBM were adequately heated as reflected by their urease activity which is within the range of 0.05 to 0.20, the generally acceptable index for adequate cooking of SBM (Parson, 2000).

Based on these observations, it is concluded that the major reason for the increased digestibility of AA in both sources of FFSB as compared with SBM was the presence of more oil in FFSB. When oil was added to SBM, the digestibility increased to a level that was similar to the digestibility in FFSB.

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Table 3.1. Analyzed nutrient composition of SSeed.HP 290, extruded full fat soybeans (FFSB), soybean meal (SBM), and soy protein concentrate (SPC), as-fed basis

Item Ingredient:	SSeed. HP290	FFSB	SBM	SPC
DM, %	94.91	93.43	89.60	90.55
CP, %	47.64	35.78	42.92	64.15
Ether extract, %	16.40	20.80	1.53	0.11
NDF, %	8.68	9.75	11.26	9.34
Sucrose, %	4.69	7.30	8.14	0.70
Raffinose, %	1.19	0.73	0.99	0.15
Stachyose, %	4.11	4.07	4.51	0.86
Urease Activity,[1] pH rise	0.12	0.10	0.18	0.12
Indispensable AA, %				
Arg	3.83	3.00	3.00	4.70
His	1.24	0.98	1.15	1.69
Ile	2.02	1.64	1.83	2.94
Leu	3.43	2.76	3.24	5.00
Lys	2.81	2.35	2.74	4.05
Met	0.64	0.59	0.67	0.94
Phe	2.31	1.84	2.14	3.23
Thr	1.66	1.39	1.69	2.47
Trp	0.33	0.33	0.56	0.81
Val	2.16	1.77	1.97	3.11

Dispensable AA, %				
Ala	1.89	1.57	1.86	2.77
Asp	5.25	4.08	4.8	7.29
Cys	0.66	0.61	0.68	0.92
Glu	8.31	6.39	7.48	11.36
Gly	1.89	1.54	1.8	2.72
Pro	2.17	1.7	2.0	3.04
Ser	2.21	1.74	2.18	3.17
Tyr	1.63	1.33	1.56	2.27
Total AA, %	44.43	35.17	41.35	62.49

[1] Urease activity was measured in the 2 sources of FFSB after extrusion.

Table 3.2. Ingredient composition of experimental diets, as-fed basis

Ingredient, % Diet:	SSeed. HP290	FFSB[1]	SBM[1]	SBM[1] + oil	SPC[1]	SPC[1] + oil	N-free
SSeed. HP 290	45.00	-	-	-	-	-	-
Commercial soybeans	-	45.00	-	-	-	-	-
Soybean meal, 44%	-	-	36.50	36.50	-	-	-
Soy protein concentrate	-	-	-	-	25.00	25.00	-
Soybean oil	-	-	-	7.55	-	7.35	4.00
Cornstarch	42.65	42.65	51.15	43.60	58.52	51.17	68.27
Sugar	10.00	10.00	10.00	10.00	10.00	10.00	20.00
Solka floc [2]	-	-	-	-	4.00	4.00	4.00
Limestone	0.75	0.75	0.75	0.75	0.80	0.80	0.60
Monocalcium phosphate	0.62	0.62	0.62	0.62	0.70	0.70	1.65
Magnesium oxide	-	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix [3]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Micromineral premix [4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] FFSB = conventional full fat soybean; SBM = soybean meal; SPC = soy protein concentrate.

[2] Fiber Sales and Development Corp., Urbana, OH.

[3] The vitamin premix provided the following quantities of vitamins per kilogram of complete diet: Vitamin A, 6,594 IU as vitamin A acetate; vitamin D_3, 989 IU as D-activated animal sterol; vitamin E, 33 IU as alpha tocopherol acetate; vitamin K_3, 2.6 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 2.0 mg as thiamine mononitrate; riboflavin, 5.9 mg; Pyridoxine, 2.0 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.026 mg; D-pantothenic acid, 20 mg as calcium pantothenate; niacin, 33 mg; folic acid, 0.66 mg; and biotin, 0.1 mg.

[4] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 3.3. Analyzed nutrient composition of experimental diets, as-fed basis

Item	Diet:	SSeed.HP290	FFSB[1]	SBM[1]	SBM[1] + oil	SPC[1]	SPC[1] + oil	N-free
DM, %		93.44	92.92	91.21	91.63	91.97	91.88	92.76
CP, %		20.72	17.32	15.92	14.60	16.14	16.59	1.00
Ether extract, %		7.35	9.29	0.69	7.50	0.24	6.70	-
Indispensable AA, %								
Arg		1.69	1.17	1.21	1.05	1.25	1.11	-
His		0.56	0.46	0.47	0.41	0.47	0.42	-
Ile		0.91	0.76	0.79	0.70	0.81	0.71	-
Leu		1.56	1.29	1.33	1.17	1.37	1.19	0.01
Lys		1.28	1.11	1.13	0.99	1.12	0.99	0.01
Met		0.31	0.28	0.30	0.26	0.28	0.25	-
Phe		1.04	0.86	0.88	0.77	0.89	0.78	-
Thr		0.75	0.65	0.67	0.58	0.67	0.58	-
Trp		0.27	0.22	0.23	0.23	0.25	0.22	<0.04
Val		0.99	0.82	0.85	0.75	0.86	0.76	0.01
Dispensable AA, %								
Ala		0.86	0.73	0.76	0.67	0.76	0.65	0.01
Asp		2.38	1.90	1.86	1.72	2.00	1.71	0.01
Cys		0.31	0.28	0.29	0.23	0.27	0.23	0.01
Glu		3.76	2.90	3.05	2.68	3.17	2.70	0.03
Gly		0.86	0.72	0.74	0.65	0.74	0.64	-

Pro	0.99	0.80	0.82	0.73	0.84	0.78	0.01
Ser	0.98	0.82	0.83	0.72	0.85	0.74	0.01
Tyr	0.57	0.49	0.52	0.45	0.48	0.41	-
Total AA, %	20.06	16.54	17.01	14.98	17.08	14.88	0.11

[1] FFSB = full fat soybean; SBM = soybean meal; SPC = soy protein concentrate.

Table 3.4. Apparent ileal digestibility (%) of CP and AA in experimental diets [1]

Item Diet	SSeed. HP290	FFSB[2]	SBM[2]	SBM[2] + oil	SPC[2]	SPC[2] + oil	SEM	*P*-Value[3]	Effect of oil[4] SEM	Effect of oil[4] *P*- value
CP, %	81.0^{u}	76.4^{z}	68.1^{x}	71.5^{xy}	75.4y^{z}	81.5^{u}	1.75	0.001	2.76	0.01
Indispensable AA, %										
Arg	93.1^{z}	88.1^{x}	86.5^{x}	87.1^{x}	89.5^{xy}	92.6^{yz}	1.22	0.001	2.19	0.12
His	88.5^{y}	85.8^{x}	84.0^{x}	85.0^{x}	88.5^{y}	90.8^{z}	1.00	0.001	1.51	0.04
Ile	87.6^{z}	83.7^{xy}	82.9^{x}	84.7^{y}	87.6^{z}	88.5^{z}	0.81	0.001	1.22	0.04
Leu	87.1^{z}	83.1^{xy}	81.9^{x}	83.9^{y}	86.9^{z}	87.9^{z}	0.86	0.001	1.33	0.04
Lys	88.2^{zu}	87.0^{yz}	82.4^{x}	84.5^{xy}	87.9^{yz}	91.8^{u}	1.62	0.001	2.54	0.03
Met	88.0^{xyz}	85.7^{x}	86.7^{xy}	88.7^{yz}	89.5^{z}	89.8^{z}	1.18	0.013	1.70	0.18
Phe	89.0^{z}	85.1^{xy}	83.5^{x}	85.2^{y}	89.0^{z}	89.8^{z}	0.77	0.001	1.19	0.04
Thr	76.7^{yz}	73.9^{xy}	71.7^{x}	72.3^{x}	77.5^{z}	77.3^{z}	1.43	0.002	2.23	0.87
Trp	83.9^{yz}	81.8^{xy}	79.4^{x}	83.0^{y}	86.5^{zu}	88.1^{u}	1.41	0.001	2.21	0.02
Val	84.0^{y}	79.8^{x}	78.6^{x}	80.6^{x}	84.1^{y}	85.3^{y}	1.10	0.001	1.67	0.06

Mean	87.5 ^{y}	83.8 ^{x}	81.9 ^{x}	83.6 ^{x}	86.8 ^{y}	88.5 ^{y}	0.98	0.001	1.53	0.04
Dispensable AA, %										
Ala	81.5 ^{z}	78.1 ^{yz}	73.7 ^{x}	76.5 ^{xy}	78.2 ^{yz}	81.60 ^{z}	1.43	0.001	2.64	0.03
Asp	86.0 ^{zu}	83.6 ^{yzu}	79.4 ^{x}	80.5 ^{xy}	84.4 ^{zu}	86.3 ^{u}	1.44	0.001	2.33	0.21
Cys	74.8 ^{xy}	74.1 ^{x}	70.3 ^{x}	73.5 ^{x}	73.9 ^{x}	79.8 ^{y}	2.76	0.048	3.87	0.03
Glu	87.3 ^{yz}	86.0 ^{yz}	80.4 ^{x}	82.6 ^{xy}	85.6 ^{y}	90.8 ^{z}	2.11	0.003	3.37	0.04
Gly	61.8 ^{z}	51.1 ^{xy}	41.6 ^{x}	48.0 ^{xy}	53.1 ^{yz}	57.4 ^{yz}	4.21	0.009	7.07	0.14
Pro	62.80	42.00	18.30	15.10	14.60	14.30	18.40	0.256	33.20	0.92
Ser	83.2 ^{y}	79.3 ^{x}	77.2 ^{x}	79.0 ^{x}	84.0 ^{y}	84.6 ^{y}	1.02	0.001	1.62	0.15
Tyr	88.4 ^{z}	85.0 ^{x}	85.6 ^{xy}	86.9 ^{yz}	90.9 ^{u}	90.7 ^{u}	0.81	0.001	1.24	0.36
Mean	81.4 ^{z}	76.6 ^{xyz}	70.2 ^{xy}	72.0 ^{xy}	75.0 ^{xyz}	77.6 ^{yz}	2.55	0.026	4.59	0.34
All AA, %	84.2 ^{z}	79.9 ^{yz}	75.6 ^{x}	77.4 ^{xy}	80.6 ^{yz}	82.7 ^{z}	1.67	0.004	2.98	0.20

$^{u-z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least square means of 7 observations per treatment.

[2] FFSB = full fat soybean; SBM = soybean meal; SPC = soy protein concentrate.

[3] *P*-value for the ANOVA comparing all 6 diets.

[4] *P*-value for the effect of adding oil to SBM and SPC.

Table 3.5. Standardized ileal digestibility (%) of CP and AA in experimental diets[1,2]

Item Diet	SSeed. HP290	FFSB[2]	SBM[2]	SBM[2] + oil	SPC[2]	SPC[2] + oil	SEM	*P*-Value[3]	Effect of oil[4]	
									SEM	*P*- value
CP, %	94.1 ^{yz}	92.1 ^{y}	84.8 ^{x}	89.8 ^{y}	92.0 ^{y}	97.6 ^{z}	1.75	0.001	2.76	0.006
Indispensable AA, %										
Arg	99.1 ^{yz}	96.7 ^{xy}	94.7 ^{x}	96.6 ^{xy}	97.4 ^{xy}	101.4 ^{z}	1.23	0.005	2.21	0.014
His	93.3 ^{yz}	91.6 ^{xy}	89.5 ^{x}	91.5 ^{xy}	94.1 ^{z}	97.1 ^{u}	1.00	0.001	1.52	0.004
Ile	93.1 ^{zu}	90.2 ^{xy}	89.1 ^{x}	91.7 ^{yz}	93.6 ^{uv}	95.4 ^{v}	0.81	0.001	1.22	0.002
Leu	92.5 ^{z}	89.7 ^{xy}	88.2 ^{x}	91.0 ^{yz}	93.0 ^{zu}	94.9 ^{u}	0.86	0.001	1.33	0.002
Lys	93.0 ^{y}	92.5 ^{y}	87.7 ^{x}	90.6 ^{xy}	93.2 ^{y}	97.9 ^{z}	1.63	0.001	2.55	0.008
Met	94.0 ^{xy}	92.2 ^{x}	92.6 ^{x}	95.5 ^{yz}	95.9 ^{yz}	97.0 ^{z}	1.18	0.028	1.70	0.028
Phe	93.7 ^{z}	90.7 ^{y}	88.9 ^{x}	91.5 ^{y}	94.4 ^{zu}	96.0 ^{u}	0.77	0.002	1.19	0.002
Thr	87.6 ^{y}	86.4 ^{xy}	83.6 ^{x}	86.1 ^{xy}	89.4 ^{yz}	91.2 ^{z}	1.44	0.071	2.23	0.071
Trp	90.1 ^{yz}	89.4 ^{y}	86.1 ^{x}	90.1 ^{yz}	93.0 ^{zu}	95.6 ^{u}	1.41	0.007	2.21	0.007
Val	91.7 ^{z}	89.0 ^{xy}	87.3 ^{x}	90.6 ^{yz}	92.7 ^{zu}	95.2 ^{u}	1.10	0.002	1.67	0.002

Mean	92.3^{z}	89.7^{xy}	87.5^{x}	90.0^{yv}	92.3^{zv}	94.8^{u}	0.98	0.001	1.53	0.004
Dispensable AA, %										
Ala	92.7^{yz}	91.1^{y}	86.0^{x}	90.5^{y}	90.5^{y}	96.1^{z}	1.43	0.001	2.64	0.001
Asp	91.0^{z}	89.7^{yz}	85.3^{x}	87.3^{xy}	90.2^{yz}	93.1^{z}	1.44	0.001	2.33	0.046
Cys	84.9^{x}	85.0^{x}	80.6^{x}	85.0^{x}	85.1^{x}	92.8^{y}	2.76	0.006	3.87	0.005
Glu	91.0^{y}	90.7^{y}	84.9^{x}	87.7^{xy}	89.9^{xy}	95.9^{z}	2.11	0.003	3.37	0.015
Gly	93.9^{yz}	89.2^{y}	77.9^{x}	89.6^{yz}	89.6^{yz}	99.7^{z}	4.50	0.008	7.08	0.006
Pro	153.6	153.7	125.2	135.8	119.8	127.6	18.55	0.621	33.26	0.588
Ser	91.1^{zu}	88.6^{y}	86.3^{x}	89.5^{yz}	92.9^{uv}	94.9^{v}	1.02	0.001	1.62	0.004
Tyr	93.6^{y}	90.9^{x}	91.1^{x}	93.3^{y}	96.9^{z}	97.7^{z}	0.81	0.001	1.24	0.021
Mean	93.8^{y}	91.7^{y}	84.6^{x}	88.5^{xy}	89.1^{xy}	94.2^{y}	2.56	0.057	4.60	0.064
All AA, %	94.8^{yz}	92.7^{yz}	87.9^{x}	91.4^{xy}	92.6^{yz}	96.7^{z}	1.68	0.008	2.99	0.019

[u-z] Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least square means of 7 observations per treatment.

[2] Standardized ileal digestibility values were calculated by correcting the AID values for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg DMI): CP, 29.11; Arg, 1.09; His, 0.29; Ile, 0.53; Leu, 0.91;

Lys, 0.66; Met, 0.20; Phe, 0.53; Thr, 0.88; Trp, 0.18; Val, 0.81; Ala, 1.02; Asp, 1.27; Cys, 0.33; Glu, 1.50; Gly, 3.95; Pro, 9.62; Ser, 0.82, Tyr, 0.31.

[3] FFSB = full fat soybean; SBM = soybean meal; SPC = soy protein concentrate.

[4] *P*-value for the ANOVA comparing all 6 diets.

[5] *P*-value for the effect of adding oil to SBM and SPC.

Table 3.6. Concentration of standardized ileal digestible CP and AA (g/kg DM) in each protein source.[1]

Item	Ingredient:	SSeed. HP290	FFSB[2]	SBM[2]	SBM[2] + oil	SPC[2]	SPC[2] + oil	SEM	*P*-value
CP, %		472.3^{z}	352.7^{x}	406.2^{y}	430.2^{y}	651.8^{u}	691.4^{v}	9.73	0.001
Indispensable AA, %									
Arg		40.0^{z}	26.6^{x}	31.7^{y}	32.3^{y}	50.6^{u}	52.6^{v}	0.48	0.001
His		12.2^{z}	9.9^{x}	11.5^{y}	11.7^{y}	17.6^{u}	18.1^{v}	0.14	0.001
Ile		19.8^{u}	15.9^{x}	18.2^{y}	18.7^{z}	30.4^{v}	30.9^{w}	0.18	0.001
Leu		33.4^{z}	26.5^{x}	31.9^{y}	32.9^{z}	51.5^{u}	52.4^{v}	0.33	0.001
Lys		27.6^{y}	23.3^{x}	26.8^{y}	27.7^{y}	41.7^{z}	43.7^{u}	0.54	0.001
Met		6.3^{y}	5.8^{x}	7.0^{z}	7.2^{u}	10.0^{v}	10.1^{v}	0.10	0.001
Phe		22.8^{u}	17.9^{x}	21.2^{yz}	21.9^{z}	33.7^{v}	34.2^{w}	0.19	0.001
Thr		15.3^{y}	12.9^{x}	15.8^{yz}	16.2^{z}	24.5^{u}	24.9^{u}	0.28	0.001
Trp		3.1^{x}	3.20^{x}	5.4^{y}	5.6^{z}	8.3^{u}	8.5^{v}	0.08	0.001
Val		20.9^{u}	16.9^{x}	19.2^{y}	19.9^{z}	31.9^{v}	32.6^{w}	0.26	0.001
Dispensable AA, %									
Ala		18.4^{yz}	15.3^{x}	17.8^{y}	18.8^{z}	27.7^{u}	29.4^{v}	0.32	0.001
Asp		50.3^{z}	39.2^{x}	45.7^{y}	47.0^{y}	72.7^{u}	75.9^{u}	0.90	0.001
Cys		5.9^{xy}	5.5^{x}	6.1^{yz}	6.4^{z}	8.7^{u}	9.4^{v}	0.22	0.001
Glu		79.9^{z}	62.0^{x}	70.9^{y}	73.3^{y}	112.9^{u}	120.2^{v}	2.12	0.001
Gly		18.7^{z}	14.7^{x}	15.6^{xy}	18.0^{yz}	26.9^{u}	30.0^{v}	21.43	0.001
Pro		35.1	28.0	27.8	30.2	39.3	42.5	5.08	0.161

Ser	21.2 ^{yz}	16.5 ^{x}	21.0 ^{y}	21.8 ^{z}	32.6 ^{u}	33.2 ^{u}	0.26	0.001
Tyr	16.1 ^{yz}	12.9 ^{x}	15.8 ^{y}	16.2 ^{z}	24.3 ^{u}	24.5 ^{u}	0.14	0.001
All AA, %	443.7 ^{z}	349.9 ^{x}	405.2 ^{y}	421.7 ^{yz}	639.6 ^{u}	667.6 ^{v}	8.99	0.001

$^{u-z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data were calculated by multiplying the AA concentration in each ingredient by the measured value for the standardized ileal digestibility. N=7.

[2] FFSB = conventional full fat soybean; SBM = soybean meal; SPC = soy protein concentrate.

CHAPTER 4

Ileal amino acid digestibility of soybean and animal protein sources fed to weanling pigs

ABSTRACT. An experiment was conducted to determine the apparent (AID) and standardized (SID) ileal digestibility of CP and AA by weanling pigs of 4 soybean products, fishmeal (FM), and casein. The 4 soybean products were soybean meal (SBM), a soy protein product (SPP), soy protein isolate (SPI), and fermented soybean meal (FSBM). Seven weanling barrows (initial BW: 10.9 kg) were fitted with a T-cannula in the distal ileum. The barrows were arranged in a 7 x 7 Latin square design with 7 diets and 7 periods. Six cornstarch-based diets were prepared using each of the protein sources as the only source of CP and AA in one diet. A N-Free diet was also used to measure basal endogenous losses of CP and AA. Results of the experiment showed that casein had the greatest ($P < 0.05$) SID for most AA, but the SID for Arg, Lys, Thr, and Trp in SPP and SPI were similar to casein. There were no differences in SID for any AA among FM, SPP, and SPI. Except for Lys, the SID for all indispensable AA in FSBM was not different from FM, SPP, and SPI, and there were no differences in SID between FSBM and SBM. In conclusion, although the SID of AA in FSBM is similar to SBM, FSBM contains more digestible AA than SBM because it has a greater AA concentration than SBM. With the exception of Lys, the SID of AA in FSBM is also similar to FM, SPP, and SPI, but the SID of most AA in casein is greater than any of the other protein sources.

Keywords: amino acids, casein, digestibility, fermented soybean meal, fish meal, soy protein isolate

INTRODUCTION

Soybeans are commonly used as a source of dietary AA for humans and animals. Proper heat treatment of raw soybeans, however, is necessary to inactivate anti-nutritional factors such as trypsin inhibitors and lectins to make it suitable for consumption (Baker, 2000). Soybean meal (**SBM**) usually has low concentrations of trypsin inhibitors and lectins (Lallès, 2000) because it has undergone heating during the process of fat extraction (Baker, 2000). However, when SBM is fed to young pigs, digestive disturbances are sometimes observed. The presence of indigestible carbohydrate complexes (Sohn et al., 1994), antigenic soy proteins (Li et al., 1990), and residual trypsin inhibitors (Lallès, 2000) may contribute to this problem. Therefore, fish meal (**FM**) or milk proteins are usually used as protein sources in pig starter diets and only limited quantities of SBM are included in these diets. Soy protein products such as soy protein isolate (**SPI**) and HP 300 (Hamlet Protein, Horsens, Denmark) are defatted soy flakes that are further processed to remove the heat stable oligosaccharides and antigens (Cromwell, 2000; Zhu et al., 1998). These products, therefore, are better tolerated by young pigs than regular SBM (Zhu et al., 1998; Li et al., 1991).

Recently, fermented soybean meal (**FSBM**) was introduced. It has a lower trypsin inhibitor concentration and a greater CP concentration than SBM and it is reported to contain higher amounts of smaller peptides (<20 kDa) compared with unfermented SBM (Hong, et al., 2004). Therefore, FSBM is thought to be more digestible to young animals than other soybean products. However, there is limited information on the digestibility of AA in FSBM it is not known how SID for AA values in FSBM compare with animal proteins. Therefore, the objective of this experiment was to measure AID and SID of CP and AA in SBM, HP

300, SPI, and FSBM and compare these values to the AID and SID of CP and AA in FM and casein.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

Seven growing barrows (initial BW: 10.9 ± 2.3 kg) originating from the matings of SP-1 boars to line 13 sows (Ausgene Intl. Inc., Gridley, IL) were equipped with a T-cannula in the distal ileum for digesta collection using the method described by Stein et al. (1998). After surgery, pigs were transferred to individual pens (1.2 x 1.8 m) in a temperature controlled room (22^{o} C) where they were allowed to recover for 10 d. A standard corn-soybean meal nursery diet (20% CP) was provided on an ad libitum basis during this time. Pigs were allotted to a 7 x 7 Latin square design with pigs and periods comprising the rows and columns, respectively. The animal part of the study was conducted at South Dakota State University, Brookings, and the experiment was approved by the Institutional Animal Care and Use Committee at South Dakota State University (# 06-A022).

Ingredients, Diets, and Feeding

The test proteins consisted of 4 soybean products, FM, and casein (Table 1). The soybean products were conventional SBM, a soy protein product (HP 300, Hamlet Protein, Horsens, Denmark), SPI (Ardex AF, Archer Daniel Midland, Decatur, IL), and FSBM (Pepsoygen, Genebiotech Co., Ltd, South Korea). Fish meal was mainly menhaden (Menhaden Select) from Omega Protein, Houston, TX and casein was obtained from Erie Foods Intl., Erie, IL.

The conventional SBM was a commercial source of dehulled SBM whereas the soy protein product (SPP) is HP 300, an enzymatically treated soy protein product that has decreased concentrations of oligosaccharides and anti-nutritional factors compared with SBM (Jiang et al., 2006). Soy protein isolate is the proteinacious fraction of the soybean meal (Baker, 2000). It is produced by solubilizing the protein at neutral or slightly alkaline pH and precipitating the extract by acidification to obtain the protein isolate (Berk, 1992). Pepsoygen is a product of the fermentation of soybean meal with *Aspergillus oryzae* GB-107 (Hong et al., 2004). It is believed to have a low trypsin inhibitor concentration and the partial digestion of large peptides by proteases secreted by *A. Oryzae* during fermentation may increase the concentrations of small peptides in the product (Hong et al., 2004). Menhaden select FM is a protein source that is commonly used in diets fed to young pigs (Maxwell and Carter, 2001). Menhaden fish is steamed, strained, and pressed to remove oil and other liquids and the remaining cake is then dried to produce the FM (FAO, 1986). Casein is the solid residue obtained by acid coagulation of defatted milk (Kellems et al., 2002). It is a milk protein and has been shown to have superior nutrient digestibility compared with soy proteins (Veum and Odle, 2001) when fed to young pigs.

Six diets were formulated to contain each protein ingredient as the only protein and AA source (Tables 2 and 3). A N- Free diet was also included to determine the basal endogenous losses of CP and AA. Chromic oxide (0.40%) was included in all diets as an inert marker. Pigs were allowed ad libitum access to water and their respective diets throughout the experiment.

Data and Sample Collection

Pig BW were recorded at the beginning of the experiment and at the end of each period. Each period lasted 7 d and pigs were allowed to adapt to their diet during the initial 5 d. On d 6 and 7, ileal digesta were collected for 8 h as described by Stein et al. (1999).

Briefly, a 225-ml plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 min and replaced with a new one. Digesta were immediately stored at – 20^{o}C to prevent bacterial degradation of the AA in the digesta. At the completion of 1 experimental period, all the remaining feeds in the feeder was removed and pigs were given access to a new diet.

Ileal samples obtained over the 2-d collection period were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. A sample of each diet and of each of the protein sources was taken as well. Digesta samples were lyophilized and finely ground prior to chemical analysis.

Chemical Analysis

All protein sources were analyzed for ether extract (procedure 4.5.01; AOAC, 2000), crude fiber (procedure 4.6.02; AOAC, 2000), Ca (procedure 4.8.02; AOAC, 2000), and P (procedure 4.8.13; AOAC, 2000). Protein separation in these ingredients was performed by SDS-PAGE according to the method of Laemmli (1970) using a Bio-Rad Criterion Electrophoresis system (Bio-Rad, Hercules, CA). Proteins (40 µg) were applied to a 12% Criterion XT precast gel (Bio-Rad, Hercules, CA) and separated at 150 V for 60 minutes. After electrophoresis, the gel was stained with Coomassie Blue (Sigma-Aldrich, St. Louis, MO). Molecular weights were estimated by comparing the bands with known standards (Mark 12, Invitrogen; Carlsbad, CA) and protein quantification was performed on a Typhoon 9400 using Image Quant (R) software (GE Healthcare, Fairfield, CT). The bands were classified as large peptides (60 kDa and greater), medium (20-60 kDa), and smaller peptides (less than 20 kDa). The amount of each peptide in each class was expressed as percent of total protein in a sample.

Concentrations of glycinin and β-conglycinin in the soybean proteins were measured by SDS-PAGE using the method of Wu et al. (1999). Densitometry was accomplished by using the Kodak 1D mage analysis (Kodak, Rochester, NY) on scanned images produced by a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ) Trypsin inhibitor concentration was also determined (procedure Ba 12-75, AOCS). The sugar profile of soybean products was determined using a high-performance liquid chromatography with an Alcott autosampler (Norcross, GA), a Waters 510 pump (Milford, MA), a Dionex CarboPac PA1 column (Sunnyvale, Ca) and a Dionex pulsed amperometric detector (Sunnyvale, Ca) based on the procedure of Rocklin et. al (1998). Results from samples were compared with known standards for stachyose and raffinose (Sigma-Aldrich, St. Louise, MO) and known standards for glucose, sucrose, maltose, and fructose (Chem Service, West Chester, PA) to determine concentrations of monosaccharides and oligosaccharides.

All samples were analyzed for DM (procedure 4.1.06; AOAC, 2000) and for CP (procedure 4.2.08, AOAC, 2000). Amino acids were analyzed in ingredients, diets, and ileal samples on a Beckman 6300 Amino Acid Analyzer (Beckman Instruments Corp., Palo Alto, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Before analysis, samples were hydrolyzed with 6 *N* HCL for 24 h at 110^{o}C (procedure 4.1.11, alternative 3; AOAC, 1998). Methionine and Cys were determined as Met sulfone and cysteic acid, respectively, after cold performic acid oxidation overnight prior to hydrolysis (procedure 4.1.11, alternative 1; AOAC, 1998). Tryptophan was determined after NaOH hydrolysis for 22 h at 110^{o}C (procedure 45.4.04; AOAC, 2000). Chromium concentrations of diets and ileal digesta were determined after nitric acid- perchloric acid wet ash sample preparation (procedure 9.2.39; AOAC, 2000).

Calculations

Apparent ileal digestibility values for AA in samples obtained from feeding the diets containing a protein source were calculated using equation [1] (Stein et al., 2007):

$$AID = (1 – [(AAd/AAf) \times (Crf/Crd)] \times 100\ \% \qquad \textbf{[1]}$$

where AID is the apparent ileal digestibility of an AA (%), AAd is the concentration of that AA in the ileal digesta DM, AAf is the AA concentration of that AA in the feed DM, Crf is the chromium concentration in the feed DM, and Crd is the chromium concentration in the ileal digesta DM. The AID for CP was also calculated using this equation.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 2007):

$$IAA_{end} = [\ AAd \times (Crf/Crd)] \qquad \textbf{[2]}$$

where IAA_{end} is the basal endogenous loss of an AA (g per kg DMI). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each AA, standardized ileal AA digestibility values were calculated using equation [3] (Stein et al., 2007):

$$SID = AID + [(IAA_{end}/AAf) \times 100] \qquad \textbf{[3]}$$

where SID is the standardized ileal digestibility value (%).

Concentration of digestible AA in the ingredients was calculated using equation [4]:

$$\text{Digestible AA} = AA_{ing} \times (SID/100) \qquad \textbf{[4]}$$

where digestible AA is the concentration of AA in the ingredients that was absorbed (g/kg DM) and AA_{ing} is the AA concentration in the protein source DM (g/kg)..

Statistical Analysis

Data were analyzed using the Proc Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure of SAS. Outliers were determined as values that were more than 3 SD above or below the mean. No outliers were identified. An analysis of variance was conducted with diet as the fixed effect and pig and period as random effects. Whenever differences were detected, treatment means were separated using the Least Significant Difference test of Proc Mixed. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among treatments.

RESULTS

Fermented soybean meal contained more DM, CP, Ca, and P but less ether extract than SBM (Table 4.1). Casein contained more CP and AA than the other ingredients, but the concentration of ether extract, Ca, and P were greater in FM than in the other ingredients. Fermented soybean meal also contained more AA than SBM, but the distribution of peptides did not differ between the 2 soybean products (Table 4.2). Fermented soybean meal did not contain sucrose, stachyose, and raffinose whereas these carbohydrates were present in SBM. Glycinin and β-conglycinin concentrations were greater in FSBM than in SBM, but the concentration of trypsin inhibitors was lower in FSBM than in the other soybean products.

The AID for CP in casein (81.7%) was greater ($P < 0.05$) than in SBM (66%), FSBM (70.4%), and FM (71.4%), but not different from the values of 77.2% and 79.3% obtained for SPP and SPI, respectively (Table 4.5). The AID for all indispensable AA, except Lys, in FSBM was similar to the AID for indispensable AA in SBM, SPP, SPI, and FM. The AID for Lys in FSBM was similar to SBM, but lower ($P < 0.05$) than in the other protein sources.

Soy protein concentrate and SPI had similar AID for all indispensable AA. With the exception of Arg, His, and Lys, the AID for all AA in casein was greater ($P < 0.05$) than in the other ingredients.

The AID for all dispensable AA was similar in FSBM, SBM, and FM. Soy protein concentrate and SPI had a greater ($P < 0.05$) AID for Asp, Glu, and Gly than SBM, but with a few exceptions, no differences between SPP, SPI, FSBM, and FM were observed. The AID for most dispensable AA in casein was also similar to the AID in SPP and SPI.

The SID for CP (97.0%) in casein was greater ($P < 0.05$) than in SBM (80.3%), FSBM (82.2%), and FM (85.0%), but not different from SPP and SPI that had an SID of 92.2% and 92.1%, respectively (Table 4.6). The SID for Lys in FSBM was lower than in FM ($P < 0.05$), but the SID for all other indispensable AA in FSBM was similar to SBM and FM. The SID for all AA except Arg, His, and Lys in FSBM were also similar to the SID in SPP and SPI. Soy protein concentrate and SPI had similar values for SID of all indispensable AA. Casein had greater ($P < 0.05$) values for SID of Ile, Leu, Met, Phe, and Val than all other protein sources, but for the remaining indispensable AA, the values obtained for casein were similar to SPI.

The SID for the dispensable AA in FSBM was similar to SBM and FM. Soy protein concentrate and SPI had similar values for SID of all dispensable AA, and with a few exceptions, these values were also similar to the SID in FSBM and casein.

Fermented soybean meal contained 480 g of SID CP, which is more ($P < 0.01$) than in SBM (Table 4.7). The concentration of SID CP in SPP (545 g) and FM (586 g) were similar but these values were lower ($P < 0.01$) than the concentration in SPP (757 g) and casein (924 g).

Concentrations of SID Lys and Trp in SBM were similar to FSBM but for the remaining AA, FSBM had a greater ($P < 0.01$) concentration of digestible AA than SBM. The concentration of most of the digestible AA in FSBM was also similar to SPP, but these values were lower ($P < 0.01$) than in SPI. Although digestible Trp concentration was low in FM, concentrations of most digestible AA in FM were greater ($P < 0.01$) than in all soybean products, but the concentrations of most digestible AA in casein were greater ($P < 0.01$) compared with all other protein sources.

Concentrations of SID dispensable AA in FM were similar to SBM for most AA and with the exception of Cys and Pro, concentrations of SID dispensable AA in FSBM were greater ($P < 0.01$) than in SBM. Soy protein isolate contained more ($P < 0.05$) digestible dispensable AA compared with SPP and FSBM, whereas casein contained more ($P < 0.01$) SID AA than all other protein sources.

DISCUSSION

Composition of Ingredients

The AA composition of FSBM used in this study was similar to the values obtained by Hong et al. (2004) and Yun et al. (2005). Fermented SBM also contained more DM, CP, Ca, and P than SBM which is consistent with the findings from other studies (Hong et al., 2004; Feng et al., 2007a,b). The AA composition of the SBM used in this study was similar to the values reported by NRC (1998) and by other studies (Rudolph, et al., 1983; Kim et al., 2000; Opapeju et al., 2006). Likewise, the concentration of raffinose and stachyose in SBM obtained in this study was also similar to the values reported by Grieshop (2003). The concentrations of most AA in FM and in casein used in this study were also similar to previously reported values (NRC, 1998; Kim and Easter, 2001). The CP and AA composition of SPP used in this study was similar to the values reported by Zhu et al. (1998) who used the same protein product in their study. The CP and AA concentration in the SPI used in this study were greater than NRC (1998) values. The use of new processing technology results in more efficient protein recovery (Koseoglu and Rhee, 1993), which may account for the higher AA concentration in the SPI used in the study.

The concentration of crude fiber was greater and the concentration of ether extract was lower in FSBM than in SBM. These results are not consistent with previous studies where a concentration of crude fiber was reduced but a concentration of crude fat was increased after fermentation (Zamora and Veum, 1979, 1988; Feng et al., 2006a,b). The SBM used in this study was obtained from a commercial source and the variation in the chemical characteristics of SBM may be the cause of the difference in the ether extract and crude fiber between FSBM and SBM (Grieshop et al., 2003). The absence of stachyose and raffinose in fermented soybean meal may be due to the production of α-galactosidase by *Aspergillus oryzae* during the fermentation process (Shankar and Mulimani, 2007) and the removal of the oligosaccharides in FSBM and the low concentrations of oligosaccharides in SPP and SPI may be an advantage because the diarrhea associated with feeding SBM to weanling pigs was suggested to be attributed to the oligosacharrides present in SBM (Liener, 2000). The fractionation of soy proteins during the production of soy protein isolates (Wolf, 1970) and the enzymatic cleavage of SBM to produce SPP may have contributed to more small peptides in SPP and SPI whereas the processing of SBM, SPP, and SPI may have contributed to their low trypsin inhibitor activity.

Ileal Amino Acid digestibility

Fermented soybean meal contained fewer anti-nutritional factors (trypsin inhibitors, stachyose, and raffinose) than regular SBM which was shown to be a result of the fermentation process (Hong et al., 2004). This has the potential to enhance the digestibility of AA in FSBM when fed to young animals. Hong et al. (2004) showed that FSBM contained greater amount of small peptides than SBM. The rate and extent of absorption for small peptides was reported to be greater than for free AA (Webb, 1990) therefore, having more small peptides could improve the digestibility of FSBM. However, CP and AA digestibility were not greater in FSBM than in SBM. Fermented SBM and SBM contained similar amounts of small peptides which may explain the lack of difference in the AID and SID of AA between FSBM and SBM. The concentration of glycinin and β-conglycinin in FSBM is two fold greater than in SBM. The reason for this is unknown however the differences in the concentration of glycinin and β-conglycinin among the 4 soy protein products may be partially attributed to the variations in the growing conditions of the soybean, soybean genotype and the type of processing the soybean product was subjected to (Murphy and Resurrecion, 1984; Wu et al., 2004).

The presence of trypsin inhibitors and soybean oligosaccharides reduced AA digestibility (Smiricky et al., 2002; Święch et al., 2004) but despite the absence of oligosaccharides and a low trypsin inhibitor concentration in FSBM, no improvement in the SID of AA in FSBM was observed compared with SBM. However, the concentration of raffinose and stachyose in SPC and SPI were closer to the values obtained for FSBM. This may explain the lack of difference of the AID and SID of most AA among FSBM, SPP, and SPI. The difference in the concentration of trypsin inhibitors in the 4 soybean products is unlikely to greatly influence the SID of AA. Defatted soy flakes containing trypsin inhibitor activity that vary from 1.4 to 6.2 mg had similar AID of CP and AA when fed to pigs (Vandergrift, et. al., 1983). Similarly, true digestibility of N was not different among soybean products that contained trypsin inhibitor concentrations in the range of 3.4 to 16.85 mg when fed to rats (Taciak et al., 2004). Although the SID of AA between FSBM and SBM were similar, the feeding value of FSBM is greater than SBM because it has more concentration of AA than SBM.

Previous studies did not concur with the results of this study. Previous studies have shown that the AID of most AA in FSBM is greater than SBM (Yun et al., 2005; Yang et al., 2007). The difference in the results between this study and previous studies may be due to the use of 17 to 23 day old pigs in the previous study as compared to the 11 kg pigs used in this study. Older pigs are less sensitive to antigenic components in soy proteins and oligosaccharides than early weaned pigs (Li et al., 1990; Smiricky et al., 2002), and this may be the reason for the lack of difference in the AID of AA between FSBM and SBM in this study. Further, the type of microorganism used in the fermentation process may influence the degree of proteolysis of the substrate protein (Baumann and Bisping, 1995; Wilson et al., 2005) and these may account for the differences in the AID of AA observed among FSBM. However, the SID of AA is a better indicator of the digestibility of AA in a feedstuff because it accounts for the basal endogenous losses (Stein et al., 2007). In this study, no difference was observed in the SID of AA between FSBM and SBM. Although the SID of Arg, Ile, Lys, Gly, and Pro was greater in FSBM than SBM, the study of Yang et al. (2007) observed that the SID of the remaining AA was not different between FSBM and SBM.

The nutrient digestibility of plant proteins was shown to be inferior compared with the nutrient digestibility of animal proteins (Yun et al., 2005). In this study, the AID and SID of most AA in the 4 soybean products were not lower than the AID and SID of AA in FM. Among the soy proteins, most AA in SPP and SPI were absorbed at the same extent as casein. This suggests that digestive capacity of the pigs at this age were sufficient to break the dietary proteins in the forms by which it can be absorbed. However, the combination of a low concentration of raffinose and stachyose and the presence of more small peptides in SPP and SPI may have contributed to the greater AID and SID of AA in SPC and SPI compared with SBM and FSBM (Sohn et al., 1994; Webb, 1990).

Most of the studies that evaluated the effect of soy proteins on AA digestibility used early weaned pigs (Sohn et al., 1994b; Walker et al., 1996) and at this age, aside from being sensitive to anti-nutritional factors that reduces AA digestibility, the digestive capacity of the gastro-intestinal tract is limited (Veum and Oodle, 2000). Thus, the soy proteins were reported to be not as digestible as milk proteins (Walker et al., 1986; Viljoen et al., 1998). However, since older pigs are used in this study, no difference was observed in the AID and SID of most AA in SPP, SPI, and casein which concurs with the study of Sohn, et al., (1994a) that the AID of most AA in processed soybean products are not different from dried skim milk.

The AID of AA in casein obtained in this study was lower compared with the values reported by NRC (1998) but similar to the values reported by Walker et al. (1986). Both of these studies used young pigs and ileal AA digestibility was shown to increase over time. Milk proteins are still the ideal protein source for young pigs and this is shown by its superior SID of AA when fed to young pigs and it contributes more SID AA (g/kg) than SPI.

In conclusion, the SID of FSBM is similar to SBM but FSBM has a greater feeding value than SBM because it contains more AA than SBM. The SID of AA in SBM, FSBM, SPP and SPI was not different from FM but the SID for most of the AA in SPP and SPI were similar to casein. Casein is still superior source of SID AA among the other protein sources tested.

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Table 4.1. Analyzed nutrient composition (%) of soybean meal, soy protein product, soy protein isolate, fermented soybean meal, fishmeal, and casein, as-fed basis

Item Ingredient:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein
DM, %	89.32	91.48	94.10	91.33	91.71	91.12
CP, %	45.07	54.40	77.89	53.74	63.52	87.33
Ether extract, %	1.07	1.13	1.24	0.80	8.73	0.09
Crude fiber, %	2.78	3.75	0.28	3.31	0.07	0.00
Ca, %	0.26	0.35	0.17	0.29	5.21	0.00
P, %	0.67	0.74	0.72	0.82	2.88	0.69
Indispensable AA, %						
Arg	3.06	3.75	5.55	3.50	3.56	3.24
His	1.13	1.35	1.96	1.30	1.39	2.64
Ile	1.89	2.31	3.64	2.48	2.33	4.61
Leu	3.37	3.98	5.94	4.09	4.10	8.30
Lys	2.77	3.06	4.78	3.11	4.50	7.02
Met	0.63	0.71	1.03	0.76	1.59	2.54
Phe	2.23	2.74	3.91	2.71	2.41	4.61
Thr	1.71	2.02	2.76	1.98	2.32	3.51
Trp	0.62	0.69	1.03	0.67	0.54	1.07
Val	1.96	2.40	3.84	2.69	2.83	5.98
Dispensable AA, %						

Ala	1.86	2.25	3.13	2.29	3.70	2.58
Asp	4.80	5.71	8.49	5.67	5.12	6.01
Cys	0.67	0.76	0.97	0.77	0.50	0.32
Glu	7.48	8.75	13.55	8.56	7.22	18.33
Gly	1.77	2.26	3.10	2.23	4.50	1.58
Pro	2.08	2.46	3.70	2.45	2.75	9.15
Ser	1.97	2.35	3.23	2.24	1.99	4.03
Tyr	1.67	2.03	2.81	1.97	1.88	5.11
Total AA	41.67	49.58	73.42	49.47	53.23	90.63

Table 4.2. Biochemical characteristics of soybean meal, soy protein product, soy protein isolate, and fermented soybean meal

Item Ingredient:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal
Peptide size distribution (% of total protein)				
60 kDa and higher	27.00	28.87	23.59	29.21
20-60 kDa	43.16	37.51	41.55	42.68
20 kDa and lower	29.84	33.62	34.86	28.11
Carbohydrate[1], %				
Glucose	0.0	0.54	0.0	0.39
Sucrose	8.74	0	0	0
Maltose	0	0	0	0
Fructose	0.70	1.21	0	0.77
Stachyose	5.79	0.78	0.15	0.00
Raffinose	1.21	0.18	0.05	0.00
Storage proteins[2]				
Glycinin	16.24	64.27	55.30	33.40
βνινιχψλγνοχ−	4.83	19.03	19.00	8.23
Trypsin inhibitor, TIU/mg	4.00	2.10	7.20	<1.00

[1] DM basis.

[2] Expressed as % of protein.

Table 4.3. Ingredient composition of experimental diets, as-fed basis

Ingredient, % Diet:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein	N-free
Soybean meal, 47%	30.00	-	-	-	-	-	-
Soy protein concentrate	-	21.50	-	-	-	-	-
Soy protein isolate	-	-	17.20	-	-	-	-
Fermented soybean meal	-	-	-	30.00	-	-	-
Fish meal	-	-	-	-	20.00	-	-
Casein	-	-	-	-	-	13.00	-
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	4.00
Cornstarch	55.32	63.77	67.82	55.32	67.02	72.42	68.42
Sugar	10.00	10.00	10.00	10.00	10.00	10.00	20.00
Solka floc [1]	-	-	-	-	-	-	4.00
Limestone	0.75	0.80	0.90	0.75	-	0.90	0.90
Monocalcium phosphate	0.95	0.95	1.10	0.95	-	0.70	1.20
Magnesium oxide	-	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Vitamin premix [2]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Micro mineral premix [3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

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

[2] Provided the following quantities of vitamins per kilogram of complete diet: Vitamin A, 6,594 IU as vitamin A acetate; vitamin D_3, 989 IU as D-activated animal sterol; vitamin E, 33 IU as alpha tocopherol acetate; vitamin K_3, 2.6 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 2.0 mg as thiamine mononitrate; riboflavin, 5.9 mg; Pyridoxine, 2.0 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.026 mg; D-pantothenic acid, 20 mg as calcium pantothenate; niacin, 33 mg; folic acid, 0.66 mg; and biotin, 0.1 mg.

[3] Provided the following quantities of minerals per kilogram of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

Table 4.4. Analyzed nutrient composition of experimental diets, as-fed basis

Item Diet:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein	N-free
DM, %	91.22	92.01	91.55	92.60	94.67	95.25	91.94
CP, %	12.20	11.67	13.62	15.01	13.26	11.80	0.35
Indispensable AA, %							
Arg	0.83	0.79	0.96	1.05	0.70	0.41	0
His	0.34	0.29	0.34	0.42	0.30	0.34	0
Ile	0.57	0.55	0.66	0.77	0.49	0.62	0
Leu	0.94	0.88	1.08	1.26	0.85	1.10	0.01
Lys	0.79	0.69	0.87	0.94	0.93	0.94	0
Met	0.15	0.18	0.20	0.24	0.34	0.34	0
Phe	0.63	0.60	0.72	0.82	0.49	0.62	0.01
Thr	0.45	0.42	0.49	0.59	0.47	0.46	0
Trp	0.19	0.17	0.19	0.21	0.14	0.16	<0.04
Val	0.63	0.59	0.71	0.84	0.59	0.80	0.01
Dispensable AA, %							
Ala	0.53	0.51	0.58	0.72	0.78	0.36	0.01
Asp	1.35	1.27	1.55	1.75	1.06	0.81	0.01
Cys	0.20	0.17	0.18	0.24	0.11	0.07	0
Glu	2.21	2.07	2.62	2.83	1.59	2.61	0.01
Gly	0.51	0.47	0.57	0.70	0.94	0.22	0

Pro	0.57	0.54	0.65	0.75	0.57	1.17	0.01
Ser	0.51	0.48	0.60	0.67	0.41	0.56	0
Tyr	0.31	0.30	0.35	0.46	0.25	0.51	0.01
Total AA, %	11.71	10.97	13.32	15.26	11.01	12.10	0.08

Table 4.5. Apparent ileal digestibility (%) of DM, CP, and AA in experimental diets [1]

Item Diet:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein	SEM	*P*-value
DM, %	57.1^{x}	61.4^{y}	70.9^{z}	61.1^{y}	76.3^{u}	83.3^{v}	1.61	0.001
CP, %	66.0^{x}	77.2^{yzu}	79.3^{zu}	70.4^{xy}	71.4^{xyz}	81.7^{u}	3.63	0.009
Indispensable AA, %								
Arg	83.6^{xy}	90.5^{z}	92.2^{z}	87.6^{yz}	85.2^{xy}	81.8^{x}	2.21	0.001
His	78.9^{x}	82.8^{xy}	85.5^{yz}	80.2^{xy}	81.0^{xy}	90.3^{z}	2.16	0.003
Ile	77.8^{x}	84.5^{y}	85.2^{y}	82.0^{xy}	81.4^{xy}	90.9^{z}	2.20	0.002
Leu	76.9^{xy}	83.8^{z}	84.4^{z}	81.5^{xyz}	82.5^{xyz}	92.0^{u}	2.24	0.001
Lys	74.2^{xy}	82.4^{yz}	86.2^{zu}	72.9^{x}	83.2^{yzu}	92.8^{u}	3.80	0.001
Met	78.6^{x}	86.2^{y}	86.4^{y}	83.8^{xy}	86.2^{y}	95.2^{z}	2.04	0.001
Phe	78.1^{x}	85.6^{y}	86.5^{y}	82.6^{xy}	79.5^{x}	91.1^{z}	2.09	0.001
Thr	65.2	72.6	73.8	69.0	74.8	78.5	3.30	0.062
Trp	80.0	82.1	83.1	79.1	81.9	86.0	2.12	0.128
Val	72.2^{x}	79.0^{y}	80.7^{y}	76.9^{xy}	78.3^{xy}	88.9^{z}	2.41	0.001
Mean	76.5^{x}	83.5^{y}	85.0^{uz}	79.8^{xy}	81.6^{xy}	89.6^{z}	2.38	0.003
Dispensable AA, %								
Ala	66.4	77.5	76.8	73.1	80.9	76.6	3.8	0.054
Asp	74.1^{x}	82.5^{yz}	83.23^{yz}	77.5^{xy}	74.0^{x}	84.6^{z}	2.9	0.014
Cys	63.7	73.9	71.4	61.6	57.8	54.8	5.9	0.073
Glu	77.1^{x}	89.5^{zu}	88.3^{yzu}	81.1^{xy}	81.1^{xyz}	92.6^{u}	3.3	0.004

Gly	33.0^{y}	59.8^{z}	59.4^{z}	51.0^{yz}	67.2^{z}	-1.8^{x}	8.11	0.001
Pro	22.7	45.0	60.5	56.9	15.0	62.4	17.87	0.145
Ser	72.9^{x}	79.1^{xy}	82.3^{y}	74.8^{x}	74.2^{x}	83.9^{y}	2.53	0.005
Tyr	80.3^{xy}	86.1^{yz}	86.0^{y}	83.8^{xy}	78.3^{x}	92.1^{z}	2.26	0.002
Mean	66.4^{x}	79.1^{yz}	80.6^{z}	74.0^{xyz}	70.0^{xy}	80.6^{z}	4.14	0.025
All AA	71.2^{x}	81.2^{yzu}	82.7^{zu}	76.8^{xyz}	75.6^{xyz}	85.0^{u}	3.18	0.009

^{x,y,z,u} Means within a row lacking a common superscript letter differ (P <0.05).

[1] Data are least square means of 7 observations per treatment except for fish meal that has only 6 observations.

Table 4.6. Standardized ileal digestibility (%) of CP and AA in experimental diets[1,2]

Item Diet:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein	SEM	*P*-value
CP, %	80.3^{x}	92.2^{yz}	92.1^{yz}	82.2^{x}	85.0^{xy}	97.0^{z}	3.63	0.003
Indispensable AA, %								
Arg	90.9^{x}	98.2^{yz}	98.5^{z}	93.5^{xy}	94.2^{xyz}	97.2^{yz}	2.21	0.025
His	84.0^{x}	88.9^{xy}	90.7^{yz}	84.4^{x}	87.0^{xy}	95.7^{z}	2.16	0.002
Ile	82.9^{x}	89.8^{y}	89.6^{y}	85.8^{xy}	87.5^{xy}	95.7^{z}	2.20	0.002
Leu	82.0^{x}	89.3^{y}	88.9^{y}	85.4^{xy}	88.3^{y}	96.5^{z}	2.24	0.001
Lys	79.2^{xy}	88.3^{yz}	90.8^{z}	77.2^{x}	87.7^{yz}	97.3^{z}	3.80	0.002
Met	85.5^{x}	92.2^{y}	91.7^{y}	88.3^{xy}	89.5^{xy}	98.5^{z}	2.04	0.001
Phe	84.1^{x}	91.9^{y}	91.7^{y}	87.2^{xy}	87.7^{xy}	97.4^{z}	2.09	0.001
Thr	77.4^{x}	85.8^{xyz}	85.0^{xyz}	78.5^{xy}	86.9^{yz}	90.9^{z}	3.30	0.026
Trp	84.8^{x}	87.5^{xy}	87.9^{xy}	83.5^{xy}	88.7^{xy}	92.0^{y}	2.12	0.034
Val	81.9^{x}	89.5^{y}	89.3^{y}	84.3^{xy}	89.0^{y}	96.8^{z}	2.41	0.001
Mean	83.2^{x}	90.6^{yzu}	90.9^{zu}	85.1^{xy}	88.8^{xyz}	96.2^{u}	2.38	0.002
Dispensable AA, %								
Ala	77.0^{x}	88.7^{yz}	86.6^{yz}	81.0^{xy}	88.4^{yz}	93.0^{z}	3.77	0.014
Asp	79.5^{x}	88.3^{yz}	88.0^{yz}	81.7^{xy}	81.1^{xy}	94.0^{z}	2.91	0.002
Cys	73.4	85.2	82.0	69.7	76.0	84.6	5.87	0.171
Glu	81.1^{x}	93.7^{zu}	91.6^{yzu}	84.2^{xy}	86.8^{xyz}	96.1^{u}	3.27	0.005
Gly	65.0	94.9	88.2	74.6	85.2	75.7	8.11	0.071

Pro	120.7	149.4	146.7	132.5	116.7	112.3	17.87	0.352
Ser	82.5 ^{x}	89.4 ^{y}	90.8 ^{y}	82.2 ^{x}	86.6 ^{xy}	93.0 ^{y}	2.53	0.007
Tyr	86.1 ^{xy}	92.1 ^{yz}	91.2 ^{xyz}	87.7 ^{xy}	85.7 ^{x}	95.8 ^{z}	2.26	0.017
Mean	82.9 ^{x}	96.9 ^{z}	95.0 ^{yz}	86.8 ^{xy}	88.6 ^{xyz}	97.6 ^{z}	4.14	0.020
All AA	83.0 ^{x}	94.0 ^{zu}	93.2 ^{yzu}	86.0 ^{xy}	88.8 ^{xyz}	97.0 ^{u}	3.18	0.006

$^{x, y, z, u}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least square means of 7 observations per treatment except for fish meal that has only 6 observations.

[2] Standardized ileal digestibility values were calculated by correcting the values for apparent ileal digestibility for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg DMI): CP, 19.06; Arg, 0.67; His, 0.19; Ile, 0.32; Leu, 0.52; Lys, 0.44; Met, 0.12; Phe, 0.41; Thr, 0.60; Trp, 0.10; Val, 0.67; Ala, 0.62; Asp, 0.80; Cys, 0.21; Glu, 0.96; Gly, 1.79; Pro, 6.12; Ser, 0.54, and Tyr, 0.20.

Table 4.7. Concentration of standardized ileal digestible CP and AA (g/kg DM) in soybean meal, soy protein product, soy protein isolate, fermented soybean meal, fishmeal, and casein

Item	Ingredient:	Soybean meal	Soy protein product	Soy protein isolate	Fermented soybean meal	Fish meal	Casein	SEM	*P*-value
CP,%		403^{x}	545^{z}	757^{u}	480^{y}	586^{z}	924^{v}	23.99	0.001
Indispensable AA									
Arg		31^{x}	40^{u}	58^{v}	36^{yz}	36^{z}	34^{y}	0.86	0.001
His		11^{x}	13^{z}	19^{u}	12^{y}	13^{z}	28^{v}	0.37	0.001
Ile		18^{x}	23^{y}	35^{z}	23^{y}	22^{y}	48^{u}	0.67	0.001
Leu		31^{x}	39^{y}	56^{z}	38^{y}	40^{y}	88^{u}	1.15	0.001
Lys		24^{x}	29^{y}	46^{z}	26^{xy}	43^{z}	75^{u}	1.52	0.001
Met		6^{x}	7^{y}	10^{z}	7^{y}	15^{u}	27^{v}	0.21	0.001
Phe		21^{x}	28^{z}	38^{u}	25^{z}	23^{y}	49^{v}	0.69	0.001
Thr		15^{x}	19^{y}	25^{u}	17^{y}	22^{z}	35^{v}	0.84	0.001
Trp		6^{y}	7^{z}	10^{u}	6^{y}	5^{x}	11^{v}	0.16	0.001
Val		18^{x}	23^{y}	36^{u}	25^{y}	27^{z}	63^{v}	0.56	0.001
Dispensable AA									
Ala		16^{x}	22^{y}	29^{z}	20^{y}	35^{u}	26^{z}	1.06	0.001
Asp		43^{x}	55^{y}	79^{z}	50^{y}	45^{x}	62^{u}	1.93	0.001
Cys		6^{z}	7^{v}	8^{u}	6^{z}	4^{y}	3^{x}	0.42	0.001
Glu		68^{x}	89^{z}	131^{u}	79^{y}	68^{x}	192^{v}	3.5	0.001

Gly	13^{x}	23^{z}	29^{u}	18^{y}	41^{v}	13^{x}	1.84	0.001
Pro	28^{x}	40^{x}	57^{y}	35^{x}	34^{x}	110^{z}	7.01	0.001
Ser	18^{x}	23^{z}	31^{u}	20^{y}	19^{xy}	41^{v}	0.69	0.001
Tyr	16^{x}	20^{z}	27^{u}	19^{yz}	18^{xy}	54^{v}	0.57	0.001
All AA	386^{x}	506^{yz}	722^{u}	463^{y}	513^{z}	959^{v}	19.86	0.001

CHAPTER 5

Duodenal and ileal amino acid digestibility and reactive Lys digestibility in pigs, and rumen undegraded protein in dairy cows of conventional and treated soybean meals

ABSTRACT: Two experiments were conducted to evaluate the digestibility of CP and AA in a conventional soybean meal (SBM) and in 3 SBM that had been treated to increase the amount of rumen undegradable protein (RUP). Experiment 1 was conducted to measure the apparent (ADD) and standardized (SDD) duodenal and apparent (AID) and standardized (SID) ileal digestibility of CP and AA in each of the 4 SBM in pigs. Four cornstarch-based diets were prepared with each of the 4 sources of SBM as the only source of CP and AA in the diets. A nitrogen-free diet was also used to measure endogenous CP and AA losses. Five growing barrows were fitted with duodenal and ileal cannulas and were arranged in a 5 × 5 Latin square design with 5 diets and five 9-d periods. Ileal digesta were collected on d 6 and 7 of each period and duodenal digesta were collected on d 8 and 9. Collected samples were lyophilized and analyzed for DM, chromium, CP, and AA. Reactive Lys was measured in each source of SBM using 3 different procedures (homoarginine, furosine, and sodium borohydride). The ADD, SDD, AID, and SID of CP and AA and the ADD, AID, and small intestinal disappearance of reactive Lys were calculated. Experiment 2 was conducted to measure the RUP of each SBM using an in-situ procedure. Four lactating Holstein dairy cows that were fitted with a rumen cannula were used. Samples for each SBM were weighed into N-free bags that were either not incubated or incubated in the rumen for 8, 16, and 24 h. All bags, including the non-incubated bags, were rinsed in cold water and *in-situ* residues were

analyzed for DM and CP. Rumen undegraded protein was calculated from CP fractions obtained from the N disappearance. Results of the experiment showed that conventional SBM had greater concentrations of AA, greater SDD ($P < 0.05$) and SID of AA, greater ($P < 0.05$) concentration of reactive Lys, and greater ($P < 0.05$) ADD of reactive Lys, but lower ($P < 0.05$) RUP than treated SBM. The SDD and SID of most AA in 1 of the treated SBM was similar to the SDD and SID in conventional SBM, but the other 2 treated SBM had digestibility values for AA that were lower ($P < 0.05$) than conventional SBM. The SID of Lys, the concentration of reactive Lys, the AID of reactive Lys and the small intestinal disappearance of reactive Lys were not different among the treated SBM. Among the reactive Lys procedures, the homoarginine procedure provided the best estimate (R^2=0.77) for RUP in SBM.

Keywords: amino acids, dairy cows, digestibility, reactive Lys, rumen undegraded protein, soybean meal

INTRODUCTION

Heat treatment of soybean meal **(SBM)** protects the proteins from ruminal proteolysis and increases the supply of dietary proteins to the small intestine for absorption (Firkins and Fluharty, 2000). Heating SBM in the presence of reducing sugars promotes the Maillard reaction, which is believed to be the mechanism that makes proteins unavailable for microbial digestion in the rumen (Faldet et al., 1992). Among all AA, the availability of Lys is most affected during the Maillard reaction because the reducing sugar binds to the epsilon amino group of Lys, which makes the Lys biologically unavailable (Finot, 2005). However, upon reaching the acidic environment in the stomach, the Lys that is bound in the very early Maillard reaction products is believed to be released from the reducing sugar, making it available for absorption in the small intestine (Finot, 1982). The proper control of heating of the SBM is critical, because overheating of the SBM produces Amadori compounds and late Maillard reaction products that cannot be converted back to Lys. These compounds are unavailable to the animal, and their production results in a loss of nutritive value of the SBM.

Dietary Lys that has been damaged during heating may be analyzed as normal Lys although it is not bio-available to the animal. The dietary Lys that is bio-available is called reactive Lys. Several methods such as the homoarginine procedure, the furosine procedure, and the sodium borohydride procedure may be used to estimate the concentration of reactive Lys in a feed ingredient (Mauron and Bujard, 1964; Thomas, 1972). However, only the homoarginine procedure has been used to evaluate the effect of heating on the nutritional value of SBM (Rutherfurd et al., 1997).

The objective of this experiment was to estimate small intestinal disappearance of treated SBM using the pig as a model and to test the hypothesis that reactive Lys procedures can be used to predict the ruminal by-pass value in heated SBM.

MATERIALS AND METHODS

Soybean Meal samples

Three SBM that had been treated to increase the ruminal by-pass value, and a conventional non-treated SBM were used (Table 1). The conventional SBM was obtained from South Dakota Soybean Processors, Volga, SD. The 3 by-pass SBM was a SBM that was heated after being sprayed with lignosulfonate (**LS-SBM**; Surepro, Land O Lakes, Shoreview, MN), a SBM that was heated and mixed with soybean hulls via a patented process (**SBM-SH**; Amino Plus, AGP, Omaha, NE), and an expeller SBM (**ME-SBM**; Soy Best, Grain States Soya, Inc., West Point, NE).

Industrial processes in the manufacture of SBM with high by-pass value commonly involve heating to initiate the Maillard reaction (Firkins and Fluharty, 2000). Lignosulfonate SBM is manufactured by spraying lignosulfonate to the SBM prior to heating at 95°C (Castro, 2007). Lignosulfonate is a source of xylose, a highly reactive reducing sugar that easily participates in the Maillard reaction (Can and Yilmaz, 2002, Castro, 2007). The heating of SBM at 100°C after mixing SBM and soybean hulls at 10:1 ratio, also involves Maillard reaction because soy hulls is a source of reducing sugars (Karr-Lilienthal et al., 2005). However, the procedure used to produce SBM-SH is patented (Heitritter et al., 1998). The expeller process is the traditional way of removing oil from the soybean. The heat generated in the press as the soybean is crushed may induce the Maillard reaction because the temperature reaches 163°C (Liu, 1997).

Pig Experiment

Animals, Housing, and Experimental Design. Five growing barrows (initial BW:57.3 ± 3.46) originating from the matings of SP-1 boars to line 13 sows (Ausgene Intl. Inc., Gridley, IL) were equipped with a T-cannula in the anterior duodenum and another T-cannula in the distal ileum. After surgery, pigs were transferred to individual pens (1.2 x 1.8 m) in a temperature controlled room (22ºC) where they were allowed to recover for 14 d. A standard corn-soybean meal diet (16% CP) was provided on an ad libitum basis during this time. Pigs were then allotted to a 5 × 5 Latin square design with pigs and periods comprising the rows and columns, respectively. The animal part of the study was conducted at South Dakota State University, and the experiment was approved by the Institutional Animal Care and Use Committee at South Dakota State University.

Diets and Feeding. Five cornstarch based diets were formulated (Tables 2 and 3). Four diets contained each of the SBM as the only protein and AA source. The fifth diet was a N-Free diet that was used to measure basal ileal endogenous losses of AA. Chromic oxide was included (0.50%) in all diets as an inert marker.

Feed was provided in quantities equal to 3 times the estimated daily requirement for maintenance (106 kcal ME per kg $^{0.75}$; NRC, 1998). The daily allotment of feed was divided into 2 equal meals that were provided at 0800 and 2000. Water was available at all times.

Data and Sample Collections. Pig BW were recorded at the beginning of the experiment and at the end of each period. Each period lasted 9 d and pigs were allowed to adapt to their diet for the initial 5 d of each period. On d 6 and 7, ileal digesta were collected for 12 h as described by Stein et al. (1999). On d 8, duodenal samples were collected from 0800 to 1000, from 1200 to 1400, and from 1600 to 1800. On d 9, duodenal samples were collected from 1000 to 1200, from 1400 to 1600, and from 1800 to 2000. During the ileal and duodenal collections, a 225-ml plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 minutes or when full and replaced with a new bag. Digesta were immediately stored at -20ºC to prevent bacterial degradation of the AA in the digesta. At the completion of 1 experimental period, pigs were given access to a new diet.

Ileal and duodenal samples were thawed at the conclusion of the experiment, mixed within animal and diet, and a subsample was collected for chemical analysis.

Digesta samples were lyophilized and finely ground prior to analysis. A sample of each diet and each protein source was also collected.

Chemical Analysis. The SBM was analyzed for crude fat (Thiex et al. 2003) using a Soxtec 2050 automatic system (Foss analytical AB, Höganäs, Sweden) and NDF using the procedure of Holst (1973). All samples were analyzed for DM (procedure 4.1.06; AOAC, 2000), and CP (procedure 4.2.08; AOAC, 2000). Chromium concentrations in diets and ileal digesta were determined after nitric acid-perchloric acid wet ash sample preparation (procedure 9.2.39; AOAC 2000) and AA were determined as described by Stein et al. (1999).

Reactive Lysine Analysis. Three different reactive Lys procedures (i.e., the homoarginine procedure, the furosine procedure, and the sodium borohydride procedure) were used to estimate the concentration of reactive Lys in the SBM and in duodenal and ileal samples. The homoarginine procedure was used as described by Rutherford and Moughan (1990) and modified by Pahm (2008). Briefly, guanidinated samples were hydrolyzed with 6 N HCl and refluxed for 24 h (procedure 4.1.11; AOAC, 2000). The solution obtained was evaporated to dryness using a Centrifuge evaporator (Labconco, Kansas City, MO). Homoarginine was analyzed using cation exhange chromatography with norleucine (Sigma-Aldrich Inc., St. Louis, MO) as the internal standard and ninhydrin (Trione, Pickerings Laboratories, Mt. View, CA) for color development. Homoarginine was quantified using homoarginine hydrochloride (Fisher Scientific International Inc., Pittsburgh, PA) as the reference standard.

The furosine concentration in all samples was measured by adding 30 mL of 6 *N* HCl to approximately 0.2 g sample in a 250 mL flask. Hydrolysis of the samples and quantification by HPLC were performed as for the homoarginine analysis except that a furosine standard (ε-N-2-furoymethy-lysine, Neosystems Laboratory, Strasbourg, France) was used.

Reactive Lys was also analyzed using the sodium borohydride procedure (Thomas, 1972) but 12 *N* HCl was used to hydrolyze the samples. Ground samples weighing approximately 0.2 g were mixed with 13 mL deionized water in a 125-mL flask and stirred slowly for 1 min using a Multimagnestir 1278 (Labline Instruments, Melrose Park, IL) magnetic stirrer. The 125-mL flasks were packed in crushed ice for 15 min after which sodium borohydride was added at a rate of 5 mg per 5 min until 25 mg had been added. The samples were stirred with a magnetic stirrer after every sodium borohydride addition for 1 min. After 1 h, the flasks were removed from the ice bath and allowed to equilibriate at room temperature for 30 min. Two mL of deionized water was used to rinse the magnetic bar upon removal. Fifteen mL of 12 *N* HCl was added to the flasks and the samples were refluxed for 24 h and analyzed for total Lys using a procedure similar to the one used for homoarginine analysis.

Calculations. Apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) values for AA in samples obtained from feeding the protein-containing diets were calculated using equations previously published (Stein et al., 2007). The basal endogenous flow to the distal ileum of each AA was determined from the flow obtained after feeding the N-free diet using the equation described by Stein et al. (2007).

Apparent duodenal digestibility **(ADD**) and standardized duodenal digestibility **(SDD)** values were also calculated using these equations but the values used in these calculations were the concentrations of AA in the duodenal samples instead of the ileal samples. Small intestinal disappearance of reactive Lys was calculated similarly except that the AA in the feed was replaced by the concentration of reactive Lys in the duodenum and the AA in the digesta was the concentration of the reactive Lys in the ileal samples.

The guanidination of the SBM samples resulted in the conversion of reactive Lys to homoarginine. The concentration of reactive Lys from homoarginine were calculated using Equation [1] :

Reactive Lys (g/kg) = [homoarginine (g/kg)/MW homoarginine] x MW Lys **[1]**

where the molecular weight (**MW**) of homoarginine and Lys were 188 g/mole and 146.14 g/mole, respectively.

The concentration of Lys that was not converted to homoarginine is eluted as the analytical Lys in the chromatograph. This Lys represents the Lys whose ε-amino group participated in Maillard reactions and is unavailable to react with O-methylisourea. This is referred to as blocked Lys.

The Amadori compound is an early Maillard reaction product that represents the major form of blocked Lys in heated proteins (Hurrel and Carpenter, 1981). Upon acid hydrolysis, the Amadori compound from heated proteins produce 32% furosine and 40% regenerated Lys (Finot and Mauron, 1972). Therefore, blocked Lys can be calculated using equation [2] (Finot et al., 1981):

$$\text{Blocked Lys} = 1/(32/100) \times \text{Furosine} \qquad \textbf{[2]}$$

The concentration of reactive Lys in the SBM can be calculated from the relative concentration of Lys and Furosine in the samples (equation 3).

$$\text{Reactive Lys}_{\text{SBM}}\ (\text{g/kg}) = [\text{total Lys} - ((40/32) \times \text{Furosine})] \qquad \textbf{[3]}$$

However, for digested proteins, i.e. proteins in duodenal and ileal samples, Amadori compounds produce 20.33% furosine and 49.5% regenerated Lys (Finot and Mauron, 1972). Therefore, a different equation is used to calculate the reactive Lys in these samples (equation 4).

$$\text{Reactive Lys}_{\text{digesta}}\ (\text{g/kg}) = [\text{total Lys} - ((49.5/20.33) \times \text{Furosine}] \qquad \textbf{[4]}$$

Sodium borohydride reduces the Amadori compounds and makes it acid stable, which prevents the Lys that is bound in the Amadori compound from being regenerated as Lys (Couch and Thomas, 1974; Hurrell and Carpenter, 1974). Therefore, the total Lys that is analyzed after the sample has been treated with sodium borohydride is the Lys that has not undergone Maillard reaction (Hurrell and Carpenter, 1981). Thus, the reactive Lys is measured directly if this procedure is used.

Statistical Analysis. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to confirm the homogeneity of the variance. Outliers were determined as values that were more than 3 SD above or below the mean. No outliers were identified. An analysis of variance was conducted to compare the effects of diet, site of digestion, and the interaction between diet and site of digestion on the concentration of reactive Lys. Analysis of variance was also used to compare the digestibility of reactive Lys in the SBM with diet as the fixed effect and pig and period as the random effects. Whenever differences were detected, treatment means were separated using the PDIFF option of SAS. The pig was the experimental unit for all analyses and differences among treatments were considered significant at an alpha level of 0.05.

In Situ Experiment

Animals, Housing, and Diets. Four lactating Holstein cows that were fitted with a rumen cannula (Bar Diamond, Parma, ID) were used to determine the extent of ruminal N disappearance in the 4 sources of SBM. The animals were housed in a tie-stall barn and fed a totally mixed ration at 1000 and 1700 h. The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Data and Sample Collection. Approximately 750 mg of each SBM was weighed into 5 × 10 cm N-free polyester bags (Ankom Technology, Macedon, NY) to obtain a sample size: surface area ratio of 15 mg/cm^2. All bags were sealed by an electric sealer. Duplicate samples were prepared for each SBM and each duplicate consisted of 4 bags with a total of 8 bags per SBM. The samples were grouped into sets. Each set was composed of all SBM in duplicates. Each set of samples was incubated in the rumen for 8, 16, and 24h. An additional set of samples was not incubated (0 h). After incubation, all bags, including time 0 bags, were rinsed in cold water and placed in a washing machine at cold cycle for the final rinse.

Chemical Analysis. All *in-situ* bags were dried at 55ºC to a constant weight in a Thelco oven (Precision Scientific, Chicago, IL). Nitrogen determination was conducted on the entire bag using the Kjeldahl method (procedure 978.02; AOAC, 2000).

Calculations. *In-situ* rumen degradability of CP was estimated according to the model of Mertens and Loften (1980) using nonlinear regression (Sherrod, 2000).

Estimates of the different N fractions were obtained from the model and rumen undegraded protein (**RUP**) was calculated based on equation [5] (NRC, 2001):

$$RUP = B[Kp/(Kd+Kp)] + C \qquad \textbf{[5]}$$

where B is the degradable CP fraction, Kp is the fractional rate of passage from the rumen (assumed to be $6.25\%\cdot h^{-1}$), Kd is the fractional rate of degradation of the B fraction, and C is the insoluble CP fraction.

Statistical Analysis. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). An analysis of variance was conducted to compare the different CP fractions and RUP with diet as the fixed effect and cow as random effect. The cow was the experimental unit. Whenever differences were detected, treatment means were separated using the PDIFF option of SAS. Differences among experimental treatments were considered significant at an alpha level of 0.05 and a tendency to be significant at an alpha level between 0.05 and 0.10. Correlation analysis between RUP and the concentration of reactive Lys, and between RUP and the concentration of blocked Lys was conducted using Proc REG of SAS.

RESULTS

Pig experiment

The concentration of CP and crude fat in LS-SBM and SBM-SH were similar to conventional SBM (Table 5.1). However, the concentration of crude fat in ME-SBM was greater (6.16%), and the concentration of CP was lower in ME-SBM than in the other SBM. The concentration of NDF was greater in all the treated SBM than in conventional SBM.

The ADD of CP was negative and ranged from -30.73% in ME-SBM to -3.01% in conventional SBM (Table 5.4). The ADD of CP and most AA in LS-SBM was similar to values obtained in conventional SBM, but greater ($P < 0.05$) than in SBM-SH and ME-SBM. With a few exceptions, the ADD of CP and AA in SBM-SH and ME-SBM were not different from each other, but they were lower ($P < 0.05$) than in conventional SBM and LS-SBM.

The SDD of CP ranged from -13.51 in ME-SBM to 10.44 in conventional SBM (Table 5.5). The SDD of CP and AA in LS-SBM was not different from conventional SBM. With the exception of Met, Trp, and Cys, the SDD of AA in SBM-SH were similar to ME-SBM, but these values were lower ($P < 0.05$) than in conventional SBM and LS-SBM.

The AID of CP ranged from 62.58% to 82.23%, but these values were not different among the SBM (Table 5.6). The AID of Lys and Cys were lower ($P < 0.05$) in all the treated SBM than in conventional SBM. The AID of Met was also lower ($P < 0.05$) in SBM-SH (69.18%) compared with conventional SBM (93.91%), but this value was not different from LS-SBM (87.93%) and ME-SBM (86.18%). The AID of Thr (58.02%) and Asp (61.15%) in SBM-SH were lower ($P < 0.05$) than in conventional SBM (80.94 and 86.88%, respectively) and in LS-SBM (72.82 and 76.96%, respectively), but they were not different from the AID in ME-SBM (66.75 and 73.32%, respectively).

The SID of CP (Table 5.7) ranged from 73.17% to 91.33% and was not different among the SBM, but the SID of Lys was lower ($P < 0.05$) in all treated SBM compared with conventional SBM (93.85%). The SID of Met (72.93%) and Asp (66.34%) in SBM-SH were lower ($P < 0.05$) than in conventional SBM (96.16 and 90.86%, respectively), but not different from ME-SBM (88.84 and 78.20%, respectively). In contrast, the SID of Met (90.45%) and Asp (81.14%) in LS-SBM, was similar to conventional SBM and to ME-SBM. Likewise, the AID of Cys was not different between LS-SBM (72.87%) and conventional SBM (88.64%), but this value was greater ($P < 0.05$) than in SBM-SH (60.88%) and ME-SBM (64.47%).

The concentration of reactive Lys in diets containing conventional SBM was greater ($P < 0.05$) than in diets containing treated SBM, but among the treated SBM, the concentration of reactive Lys in the diets were not different (Table 5.8). This was true regardless of the procedure used to measure reactive Lys. When estimated using the homoarginine or the furosine procedures, the concentration of reactive Lys in the duodenum of pigs fed SBM-SH (1.11%) and ME-SBM (1.08%) was greater ($P < 0.05$) than the concentration of reactive Lys in the diets (0.93 and 0.83, respectively) that contained these SBM. However, the concentration of reactive Lys in the duodenum of pigs fed conventional SBM and LS-SBM were not different from values in the diets. When the concentration of reactive Lys was measured using the sodium borohydride procedure, no differences between duodenal and diet samples were observed. Regardless of the method of estimation, the concentration of reactive Lys in the ileum was lower ($P < 0.05$) than the concentration of reactive Lys in the diet and in the duodenum.

The ADD of reactive Lys (Table 5.9) in ME-SBM (-33.61%) was not different from the values for SBM-SH (-19.18%), but was lower ($P < 0.05$) than for conventional SBM and LS-SBM (-3.14% and -5.85% respectively). The ADD of reactive Lys in LS-SBM was similar to the ADD in conventional SBM and SBM-HS except when measured using the sodium borohydride procedure where the value for SBM-SH was lower ($P <$

0.05) than the value for conventional SBM (4.67%) and LS-SBM (6.75%). For the AID and the apparent small intestinal disappearance of reactive Lys, no differences among SBM were observed, regardless of the procedure used to measure reactive Lys.

In Situ Experiment

The soluble fraction of the CP (Table 5.10) in ME-SBM was 33.5% and was greater ($P < 0.05$) than in the other SBM. The degradable fraction of CP in LS-SBM (85.2%) was not different from conventional SBM (81.3%), but was greater ($P < 0.05$) than in SBM-SH (65.0%) and ME-SBM (50.0%). The degradable fraction of CP in ME-SBM was not different from the degradable fraction of CP in SBM-SH, but lower ($P < 0.05$) than in conventional SBM. There was a tendency ($P = 0.06$) for a lower fractional rate of degradation of CP in LS-SBM than in conventional SBM, but no differences were observed for the fractional rate of degradation of CP in SBM-SH and ME-SBM compared with conventional SBM. There was also a tendency ($P = 0.07$) for a greater indigestible CP fraction in SBM-SH than in LS-SBM. The RUP ranged from 32.9% in conventional SBM to 64.4% in LS-SBM with values for all the treated SBM being greater ($P < 0.05$) than the value for conventional SBM.

The concentration of reactive Lys in the samples of SBM could not be used to predict the RUP value of SBM (Table 5.11). However, blocked Lys measured by the homoarginine or the furosine method provided estimates of RUP with an R^2 of 77% and 66% respectively.

DISCUSSION

The concentration of CP and AA in conventional SBM was similar to the values reported by NRC (1998; 2001), but the CP and AA values in LS-SBM, SBM-SH, and

ME-SBM were lower than previously reported (Awadeh et al., 2007; Castro et al., 2007). The reduced concentration of all AA, especially Lys, in treated SBM suggests that some AA have been destroyed during the heat treatment used to increase the RUP value. Destroyed AA are not recovered during acid hydrolysis (Hurrell and Carpenter, 1977) and can be detected as a reduction in AA concentration (Mauron, 1981). Advanced Maillard reactions can also destroy Arg, His, and Cys, which explains the low concentration of these 3 AA in the treated SBM (Hurrell and Carpenter, 1977). Low concentrations of Lys, Arg, and His were also observed by Castro, et al. (2007) in ME-SBM and LS-SBM.

The negative ADD for CP and most AA in the SBM suggest a contribution of endogenous CP prior to the anterior duodenum. This is consistent with the observation of Zebrowska et al. (1982) who reported that N and total AA contained in the duodenal digesta of pigs was 17% and 8% greater than the N and AA ingested, respectively. The increased N in the duodenal digesta can be attributed to the N in saliva, gastric juice, bile, and pancreatic juice secretions that flowed to the duodenum (Low, 1979). The relatively low values for the ADD of Gly and Pro suggest a high concentration of endogenous secretions in the duodenal digesta because Gly is a major constituent of glychocholic acid, which is the main bile salt in pigs (de Lange et al., 1989), and Pro is abundant in gastric mucus (van Klinken et al., 1998).

The negative value for ADD is in contrast to the observations of Wilfart et al. (2007) who obtained a low, but positive, ADD for CP. The difference between results obtained in the 2 studies may be due to the big variation in duodenal digestibility values that are usually observed (Low, 1979; Wilfart et al., 2007).

The very low ADD and SDD of CP and AA of SBM-SH and ME-SBM may be caused by the type of fiber in SBM-SH or the presence of advanced Maillard reaction products in ME-SBM. The carbohydrate fraction in soy hulls is composed of 30% pectin, 50% hemicellulose, and 20% cellulose (Kikuchi et al., 1971). Pectin is a soluble fiber that forms gel complexes in the small intestines. The reduced AA digestibility observed with the addition of pectin in the diet was attributed to increased endogenous secretions of AA (de Lange et al., 1989), impaired protein digestion (Mosenthin et al., 1994), and increased digesta viscosity (Buraczewska et al., 2007). The presence of advanced Maillard reaction products, such as premelanoidins, may also inhibit the activity of pancreatic carboxypeptidases (Hansen and Millington, 1979; Öste, et al., 1987), which may increase endogenous AA losses and reduce values for AID. This hypothesis is supported by the observation of Marty and Chavez (1995) who reported that the endogenous flow of Lys increased in pigs fed roasted SBM compared with pigs fed non-roasted SBM.

The reduced AID and SID of Lys in treated SBM compared with conventional SBM reflects the effect of heat treatment on Lys. Aside from Lys blockage through early Maillard reactions, cross-linkages established by Lys and other AA between polypeptide chains within a protein during heating are enzyme resistant (Hurrell and Finot, 1985; Gerrard, 2002). These modified proteins are not digested resulting in decreased digestibility of AA (Hurrell and Finot, 1985; Finot, 1982). The presence of premelanoidins may also block absorption sites due to steric hindrances, which reduces the digestibility of AA and peptides (Ford, 1973). This is consistent with the results of Marty and Chavez (1995) who reported greater endogenous losses and greater ileal recovery of exogenous Lys in pigs fed by-pass SBM compared with pigs fed conventional SBM.

The lower concentration of reactive Lys in the treated SBM than in conventional SBM supports the hypothesis that the Lys in the treated SBM was blocked or destroyed in Maillard reactions. Endogenous Lys contributed to the increase in the concentration of reactive Lys in the duodenum, but for pigs fed SBM-SH and ME-SBM, the release of Lys from Schiff bases, an intermediate formed after the condensation of the ε-amino group of Lys to a sugar, may be the reason for the increase in the concentration of reactive Lys in the duodenum. Schiff bases are unstable compounds but they are biologically available (Finot, 1982). In the low pH of the stomach, the Schiff bases disassociates to Lys and its corresponding reducing sugar (Finot, 1982). Thus, the concentration of the Schiff bases in SBM-SH and ME-SBM may have contributed to the increase in the concentration of reactive Lys in the duodenum to such an extent that the concentration was greater than the concentration of reactive Lys in the diet. To our knowledge, this is the first time that the 3 reactive Lys procedures have been used to evaluate treated SBM and the first time that the presence of Schiff bases has been suggested to contribute to the increase in the concentration of reactive Lys in the duodenum of pigs fed treated SBM. A similar observation was reported by Awadeh et al. (2007) who observed that the concentration of FDNB-reactive Lys in rumen residues where greater than in their respective treated SBM.

The concentration of RUP in conventional SBM that was measured in this study is similar to values reported previously (Can and Yilmaz, 2002; NRC, 2001). The low RUP of the conventional SBM is attributed to a high degradable CP fraction and a high fractional rate of degradation. The high RUP of the treated SBM confirms that controlled non-enzymatic browning is an effective method of increasing RUP (Can and Yilmax, 2002; Castro et al., 2007). However the RUP was different among the treated SBM and this is consistent with the results of Awawdeh et al. (2007) who reported that the RUP of ME-SBM is lower than that of LS-SBM. The low RUP in ME-SBM was a function of the low concentration of degradable CP, a higher fractional rate of degradation, and a greater fraction of soluble CP compared with LS-SBM. A greater fraction of soluble CP in ME-SBM was also observed by Awawdeh et al. (2007). In contrast, Castro et al. (2007) did not observe differences in the effective degradability among LS-SBM, SBM-SH, and ME-SBM.

The concentration of reactive Lys in the SBM did not correlate well with the RUP of the SBM as reflected by the low R^2 values. However, the calculated concentration of blocked Lys measured by the homoarginine procedure was a better predictor of RUP. The reason for this observation is that blocked Lys is a direct measure of the Lys that is bound in Amadori compounds (Hurrell and Carpenter, 1981). As more heat is applied, the concentration of blocked Lys increases, resulting in an increased RUP value but also a reduced digestibility of Lys.

The low AID of AA in pigs fed treated SBM suggests that the feeding value of treated SBM is lower than conventional SBM, however, treated SBM increases milk yield in dairy cows (Sahlu, et al., 1984). This indicates that the degree of damage attributed to heating, is less than the degree of damage caused by rumen microbial action in conventional SBM. More AA therefore, reaches the small intestines from heated SBM. This may also indicate that the pig is not a suitable model to evaluate treated SBM because the rumen microbes may reverse the negative effects of heating by producing enzymes that can hydrolyze the Amadori compounds. Micro-organisms from the hind gut can degrade Amadori compounds (Finot and Magnenat, 1981) and Amadoriases, enzymes that are capable of breaking the bond between the AA and the sugar in the Amadori compound, was isolated from several fungi and bacteria (Takahashi, et al. 1997; Capuano et al., 2007). The hypothesis that micro-organisms in the rumen may produce such enzymes is possible.

In conclusion, heat processing of SBM affected the AA concentration, SDD and SID of AA, the concentration of reactive Lysine, and the concentration of RUP in SBM. Conventional SBM contained a greater concentration of AA, had greater SDD and SID of AA, had a greater concentration of reactive Lys, but a lower RUP value compared with the treated SBM. Among the treated SBM, LS-SBM provided the greatest RUP and SDD and SID of most AA, except Lys. However, the SID of Lys, the concentration of reactive Lys, and the AID of reactive Lys was not different among the treated SBM. The concentration of blocked Lys estimated by the Homoarginine or the furosine procedure may be used to predict RUP values in treated SBM.

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Table 5.1. Analyzed nutrient composition (%) of conventional soybean meal and 3 treated soybean meals, as-fed basis

	Soybean meal treatment			
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction
CP, %	48.02	47.88	46.36	43.04
Crude fat, %	1.13	1.45	1.64	6.16
NDF, %	8.92	29.34	24.57	18.53
Indispensable AA, %				
Arg	3.38	3.06	3.09	2.81
His	1.23	1.13	1.15	1.04
Ile	2.10	2.04	2.00	1.85
Leu	3.62	3.47	3.45	3.13
Lys	2.96	2.56	2.64	2.44
Met	0.71	0.67	0.68	0.64
Phe	2.35	2.26	2.25	2.02
Thr	1.83	1.74	1.74	1.58
Trp	0.68	0.67	0.65	0.57
Val	2.18	2.13	2.09	1.95
Dispensable AA, %				
Ala	2.03	1.91	1.92	1.75
Asp	5.24	5.07	5.03	4.51
Cys	0.65	0.60	0.58	0.59

Glu	8.20	7.88	7.79	6.95
Gly	1.98	1.89	1.88	1.73
Pro	2.35	2.33	2.19	2.00
Ser	2.20	2.06	2.05	1.88
Tyr	1.72	1.64	1.63	1.49
Total, %	45.41	43.11	42.81	38.93

Table 5.2. Ingredient composition of experimental diets, as-fed basis, pig experiment

Item Diet:	Soybean meal diets	N-free diet
Ingredient, %		
Soybean meal	37.50	-
Cornstarch	46.50	69.50
Sugar	10.00	20.00
Solka floc[1]	-	3.00
Soybean oil	3.00	3.00
Limestone	0.75	0.50
Monocalcium phosphate	1.15	2.40
Chromic oxide	0.50	0.50
Salt	0.40	0.40
Vitamin premix[2]	0.05	0.05
Micromineral premix[3]	0.15	0.15
Magnesium oxide	-	0.10
Potassium carbonate	-	0.40
Total, %	100.00	100.00

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

[2] Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 10,990 IU; vitamin D_3, 1,648 IU; vitamin E, 55 IU; vitamin K, 4.4 mg; thiamin, 3.3 mg; riboflavin, 9.9 mg; pyridoxine, 3.3 mg; vitamin B_{12}, 0.044 mg; D-pantothenic acid, 33 mg; niacin, 55 mg; folic acid, 1.1 mg; and biotin, 0.17 mg.

Table 5.3. Analyzed nutrient composition (%) of diets, as-fed basis, pig experiment

[3] Provided the following quantities of minerals per kg of complete diet: Cu, 16 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

	Soybean meal treatment			
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction
CP, %	18.34	16.5	15.61	14.33
Indispensable AA, %				
Arg	1.30	1.19	0.97	1.06
His	0.48	0.45	0.37	0.4
Ile	0.82	0.81	0.64	0.7
Leu	1.42	1.38	1.11	1.2
Lys	1.19	0.99	0.87	0.92
Met	0.30	0.27	0.18	0.26
Phe	0.93	0.89	0.71	0.77
Thr	0.73	0.69	0.56	0.60
Try	0.26	0.25	0.24	0.19
Val	0.87	0.83	0.68	0.74
Dispensable AA, %				
Ala	0.81	0.77	0.63	0.68
Asp	2.12	2.00	1.61	1.73
Cys	0.28	0.26	0.18	0.24
Glu	3.27	3.18	2.55	2.73
Gly	0.79	0.76	0.62	0.67
Pro	0.89	0.91	0.71	0.77
Ser	0.84	0.81	0.66	0.71

Tyr	0.55	0.50	0.45	0.51
Total AA, %	17.85	16.93	13.74	14.87

Table 5.4. Apparent duodenal digestibilities (%) of CP and AA in conventional soybean meal and 3 treated soybean meals, pig experiment[1]

	Soybean meal treatment					
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
CP	-3.01^{y}	-4.52^{y}	-22.23^{x}	-30.73^{x}	5.75	0.006
Indispensable AA, %						
Arg	4.56^{y}	8.35^{y}	-25.88^{x}	-14.82^{x}	5.50	0.001
His	2.99^{y}	7.95^{y}	-24.54^{x}	-14.38^{x}	4.79	0.001
Ile	-2.14^{y}	5.47^{y}	-28.30^{x}	-21.53^{x}	5.18	0.001
Leu	1.38^{y}	8.18^{y}	-26.86^{x}	-18.72^{x}	5.19	0.001
Lys	3.50^{y}	3.77^{y}	-24.50^{x}	-17.99^{x}	4.61	0.001
Met	20.60^{y}	24.34^{z}	-35.11^{x}	-4.30^{y}	10.93	0.001
Phe	3.12^{y}	9.36^{y}	-26.60^{x}	-17.17^{x}	5.17	0.001
Thr	-1.10^{y}	3.09^{y}	-31.51^{x}	-26.82^{x}	5.06	0.001
Trp	-13.12^{y}	-13.60^{y}	-25.35^{y}	-50.27^{x}	6.96	0.005
Val	-2.49^{y}	0.52^{y}	-32.27^{x}	-24.02^{x}	5.46	0.002
Mean	1.80^{y}	6.13^{y}	-27.48^{x}	-19.53^{x}	5.12	0.001
Dispensable AA, %						
Ala	-2.71^{y}	-1.20^{y}	-35.44^{x}	-23.26^{x}	4.91	0.001
Asp	1.76^{y}	5.37^{y}	-27.26^{x}	-21.03^{x}	4.91	0.001
Cys	-10.02^{z}	-9.17^{z}	-66.28^{x}	-35.13^{y}	5.70	0.001

Glu	1.54 ^{y}	7.57 ^{y}	-23.89 ^{x}	-17.21 ^{x}	4.63	0.001
Gly	-35.79 ^{y}	-39.38 ^{y}	-72.19 ^{x}	-59.08 ^{y}	8.90	0.032
Pro	-41.39 ^{y}	-47.94 ^{y}	-84.94 ^{x}	-53.08 ^{y}	13.99	0.022
Ser	-5.20 ^{y}	0.92 ^{y}	-33.71 ^{x}	-30.29 ^{x}	5.40	0.001
Tyr	1.15 ^{y}	2.79 ^{y}	-34.50 ^{x}	-26.69 ^{x}	6.02	0.001
Mean	-5.89 ^{y}	10.37 ^{y}	-46.88 ^{x}	-34.52 ^{x}	5.56	0.001
All AA	-2.26 ^{y}	0.39 ^{y}	-33.19 ^{x}	-23.09 ^{x}	5.08	0.001

$^{x\text{-}z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least squares means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

Table 5.5. Standardized duodenal digestibilities (%) of CP and AA in conventional soybean meal and 3 treated soybean meals, pig experiment[1]

	Soybean meal treatment					
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
CP, %	10.44 [y]	10.28 [y]	-6.58 [x]	-13.51 [x]	5.75	0.012
Indispensable AA, %						
Arg	15.88 [y]	20.6 [y]	-10.85 [x]	-0.93 [x]	5.5	0.001
His	9.05 [y]	14.35 [y]	-16.76 [x]	-7.11 [x]	4.79	0.001
Ile	3.43 [y]	11.05 [y]	-21.24 [x]	-15.00 [x]	5.18	0.002
Leu	7.96 [y]	14.89 [y]	-18.52 [x]	-10.94 [x]	5.19	0.001
Lys	12.05 [y]	13.94 [y]	-12.93 [x]	-6.94 [x]	4.61	0.002
Met	29.49 [z]	34.33 [z]	-20.26 [x]	6.20 [y]	10.93	0.001
Phe	9.58 [y]	16.06 [y]	-18.21 [x]	-9.36 [x]	5.17	0.001
Thr	8.01 [y]	12. 64 [y]	-19.74 [x]	-15.73 [x]	5.06	0.001
Trp	-2.40 [y]	-2.87 [y]	-14.17 [y]	-36.00 [x]	6.96	0.008
Val	6.58 [y]	9.93 [y]	-20.78 [x]	-13.37 [x]	5.46	0.003
Mean	9.95 [y]	14.78 [y]	-16.89 [x]	-9.63 [x]	5.12	0.001
Dispensable AA, %						
Ala	15.72 [y]	18.01 [y]	-11.97 [x]	-1.30 [x]	4.90	0.002
Asp	9.21 [y]	13.19 [y]	-17.54 [x]	-11.90 [x]	4.91	0.001
Cys	10.17 [z]	12.87 [z]	-34.83 [x]	-11.09 [y]	5.70	0.001

Glu	8.21 [y]	14.37 [y]	-15.42 [x]	-9.22 [x]	4.63	0.001
Gly	12.52	10.35	-11.23	-2.11	8.91	0.216
Pro	68.66	58.66	51.68	74.14	13.99	0.348
Ser	5.69 [y]	12.10 [y]	-19.98 [x]	-17.40 [x]	5.40	0.001
Tyr	7.32 [y]	10.60 [y]	-25.83 [x]	-18.96 [x]	6.02	0.001
Mean	15.83 [y]	13.28 [y]	-17.36 [x]	-6.96 [x]	5.56	0.002
All AA, %	13.15 [y]	16.48 [y]	-13.36 [x]	-4.59 [x]	5.08	0.002

[x-z] Means within a row lacking a common superscript letter differ (*P* < 0.05).

[1] Data are least squares means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

[2] Standardized duodenal digestibility values were calculated by correcting the ADD values for the basal duodenal endogenous losses. Basal duodenal endogenous losses were determined as (g/kg DMI): CP, 25.89; Arg, 1.55; His, 0.31; Ile, 0.48; Leu, 0.98; Lys, 1.07; Met, 0.28; Phe, 0.33; Thr, 0.70; Trp, 0.28; Val, 0.83; Ala, 1.57; Asp, 1.66; Cys, 0.60; Glu, 2.29; Gly, 4.01; Pro, 10.28; Ser, 0.96, Tyr, 0.41.

Table 5.6. Apparent ileal digestibilities (%) of CP and AA in conventional soybean meal and 3 treated soybean meals, pig experiment [1]

	Soybean meal treatment					
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
CP, %	82.23	75.93	62.58	66.02	6.06	0.084
Indispensable AA,%						
Arg	93.33	86.57	79.06	85.82	4.38	0.116
His	89.91	80.95	68.71	77.62	5.51	0.051
Ile	87.76	80.29	67.2	76.97	6.13	0.103
Leu	87.52	80.82	68.15	77.79	5.73	0.098
Lys	89.82[y]	77.60[x]	68.89[x]	76.85[x]	4.53	0.018
Met	93.91[y]	87.93[xy]	69.18[x]	86.18[xy]	5.79	0.027
Phe	88.62	81.95	70.02	79.30	5.61	0.106
Thr	80.94[y]	72.82[y]	58.02[x]	66.75[xy]	5.73	0.039
Trp	87.63	80.43	76.11	72.45	5.02	0.140
Val	84.80	76.21	62.99	72.95	6.00	0.073
Mean	88.42	80.41	68.96	77.66	5.34	0.070
Dispensable AA, %						
Ala	81.44	72.59	57.27	68.96	6.73	0.078
Asp	86.88[z]	76.96[yz]	61.15[x]	73.32[xy]	4.90	0.009
Cys	78.55[y]	61.85[x]	45.15[x]	52.45[x]	6.64	0.013

Glu	89.04	82.33	69.51	78.57	5.36	0.064
Gly	72.52	58.92	43.85	50.97	7.57	0.056
Pro	66.15	41.38	40.11	54.96	12.63	0.219
Ser	84.63	76.71	65.72	73.75	5.00	0.061
Tyr	91.57	85.66	70.79	78.15	5.60	0.051
Mean	83.98 ^{y}	71.76 ^{xy}	58.39 ^{x}	69.27 ^{xy}	6.04	0.036
All AA, %	86.00	76.52	64.62	74.13	5.47	0.054

$^{x\text{-}z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least squares means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

Table 5.7. Standardized ileal digestibilities (%) of CP and AA in conventional soybean meal and 3 treated soybean meals, pig experiment[1,2]

	Soybean meal treatment					
Item	Conventional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
CP, %	91.33	85.96	73.17	77.67	6.06	0.129
Indispensable AA, %						
Arg	97.36	90.93	84.41	90.77	4.38	0.169
His	93.56	84.81	73.40	82.01	5.51	0.067
Ile	92.39	84.93	73.07	82.39	6.13	0.136
Leu	91.71	85.10	73.47	82.75	5.73	0.128
Lys	93.85^{y}	82.41^{x}	74.36^{x}	82.06^{x}	4.53	0.028
Met	96.16^{y}	90.45^{y}	72.93^{x}	88.84^{xy}	5.79	0.039
Phe	92.70	86.17	75.32	84.23	5.61	0.142
Thr	88.48	80.72	67.75	75.92	5.73	0.068
Trp	92.50	85.44	81.33	79.11	5.02	0.198
Val	91.31	82.97	71.24	80.61	6.00	0.107
Mean	93.04	85.31	74.95	83.27	5.34	0.099
Dispensable AA, %						
Ala	89.78	81.29	67.90	78.90	6.73	0.125
Asp	90.86^{y}	81.14^{y}	66.34^{x}	78.20^{xy}	4.89	0.012
Cys	88.64^{y}	72.87^{xy}	60.88^{x}	64.47^{x}	6.64	0.031

Glu	92.49	85.85	73.90	82.71	5.36	0.082
Gly	88.77	75.65	64.36	70.13	7.57	0.118
Pro	117.75	91.36	104.17	114.61	107.47	0.228
Ser	90.75	83.00	73.44	80.99	5.00	0.094
Tyr	95.21	89.62	75.19	82.07	5.59	0.060
Mean	93.95	82.62	71.95	81.93	6.04	0.079
All AA, %	93.48	84.33	74.25	83.11	5.47	0.091

[x-z] Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least squares means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

[2] Standardized ileal digestibility values were calculated by correcting the AID values for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg DMI): CP, 17.53; Arg, 0.55; His, 0.18; Ile, 0.40; Leu, 0.63; Lys, 0.50; Met, 0.07; Phe, 0.40; Thr, 0.58; Trp, 0.13; Val, 0.59; Ala, 0.71; Asp, 0.89; Cys, 0.30; Glu, 1.18; Gly, 1.35; Pro, 4.82; Ser, 0.54, Tyr, 0.21.

Table 5.8. Concentration of reactive Lys (%) estimated by homoarginine, furosine, or sodium borohydride procedures in conventional soybean meal and 3 treated soybean meals in diets, duodenal, and ileal samples, pig experiment [1]

	Soybean meal treatment					*P*-value		
	Conven-tional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	Diet	Site	Diet* Site
Homoarginine								
Diet	1.19^{y}	0.87^{x}	0.93^{x}	0.83^{x}	0.05	0.001	0.001	0.001
Duodenum[2]	1.23^{z}	0.93^{x}	1.11^{y}	1.08^{y}				
Ileum[3]	0.12	0.18	0.22	0.17				
Furosine								
Diet	1.06^{y}	0.84^{x}	0.88^{x}	0.82^{x}	0.05	0.021	0.001	0.003
Duodenum[2]	1.20^{z}	0.94^{x}	1.07^{y}	1.07^{y}				
Ileum[3]	0.11^{x}	0.20^{xy}	0.23^{y}	0.21^{xy}				
Sodium borohydride								
Diet	1.14^{y}	0.90^{x}	0.87^{x}	0.89^{x}	0.05	0.014	0.001	0.003
Duodenum	1.04^{z}	0.85^{x}	1.02^{yz}	0.95^{xy}				
Ileum[3]	0.10	0.18	0.20	0.18				

$^{x-z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least squares means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

[2] Values in the duodenum for soybean meal with soyhulls and mechanically-extracted soybean meal are different ($P < 0.05$) from values in the diet, but for the other soybean meals, no differences between diet and duodenum values was observed.

[3] Ileal values are different ($P < 0.05$) from values in the duodenum and diet for all soybean meals.

Table 5.9. Apparent digestibility and small intestinal disappearance of reactive Lys (%) measured by homoarginine, furosine, and sodium borohydride procedures in regular soybean meal (SBM) and different treated soybean meals, pig experiment [1]

	Soybean meal treatment					
Procedure and Site	Conventional	Lignosulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
Homoarginine						
Duodenal digestibility, %	-3.14[z]	-5.85[yz]	-19.18[xy]	-33.61[x]	5.01	0.003
Ileal digestibility %	90.28	79.64	76.20	78.36	4.35	0.133
Small intestinal disappearance, %	90.41	80.71	79.88	83.90	3.49	0.158
Furosine						
Duodenal digestibility, %	-12.88[y]	-10.50[y]	-21.39[xy]	-34.82[x]	5.52	0.036
Ileal digestibility, %	90.28	76.64	73.57	74.19	4.45	0.052
Small intestinal disappearance, %	90.56	78.79	77.92	80.51	3.76	0.094
Sodium borohydride						
Duodenal digestibility, %	4.67[yz]	6.75[z]	-16.72[x]	-9.82[xy]	4.70	0.007
Ileal digestibility, %	90.90	80.75	76.58	78.89	4.03	0.093
Small intestinal disappearance, %	90.26	79.31	79.56	80.48	3.80	0.155

[x-z] Means within a row lacking a common superscript letter differ (*P* < 0.05).

[1] Data are least square means of 5 observations per treatment except for mechanically extracted SBM that had 4 observations.

Table 5.10. CP fractions, fractional rate of degradation, and rumen undegraded CP of conventional soybean meal and 3 treated soybean meals, *in situ* experiment [1]

	Soybean meal treatment					
Components	Conven-tional	Ligno-sulfonate	Soy hulls	Mechanical extraction	SEM	*P*-value
Soluble fraction, % of CP	15.7 ^{x}	15.0 ^{x}	16.5 ^{x}	33.5 ^{y}	2.8	0.001
Degradable fraction, % of CP	81.3 ^{yz}	85.2 ^{z}	65.0 ^{xy}	50.0 ^{x}	8.9	0.006
Fractional rate of degradation, %/hour	11.0 ^{y}	2.0 ^{x}	7.2 ^{xy}	9.2 ^{y}	2.5	0.060
Indigestible fraction, % of CP	3.0 ^{xy}	0.0 ^{x}	18.8 ^{y}	16.8 ^{xy}	7.1	0.074
Rumen undegraded CP, % of CP	32.9 ^{x}	64.4 ^{u}	52.8 ^{z}	42.4 ^{y}	2.0	0.001

$^{x-z}$ Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1] Data are least squares means of 4 observations per treatment except for conventional SBM that had 3 observations.

Table 5.11. Correlation between values for reactive Lys and blocked Lys and rumen undegraded CP in 4 sources of soybean meal.

Reactive Lys measured by:	Regression equation	R^2
Homoarginine procedure	Y= 3.520 - 0.016x	0.42
Furosine procedure	Y= 3.245 - 0.010x	0.23
Sodium borohydride procedure	Y= 3.022 - 0.013x	0.37
Blocked Lys calculated from:		
Homoarginine procedure	Y= 0.038 + 0.004x	0.77
Furosine procedure	Y= -0.072 + 0.002x	0.66

CHAPTER 6

CONCLUSION

Advances in breeding and processing technology have expanded the application of soybeans (SB) and SB products in food and feed. Advances in breeding, such as the genetic selection of SB with high concentrations of CP and AA resulted to a product that has AA concentrations more than conventional SB. Although CP and AA digestibility of conventional and high protein SB is not different, the feeding value of high protein SB is greater because it contributes greater digestible AA per kg/DM.

The addition of oil increases the AA digestibility. This is demonstrated in this thesis where the addition of soybean oil to soybean meal (SBM) improve AA digestibility to the level of SB. The main difference between SB and SBM is the oil content therefore the increase AA digestibility of SB compared with SBM is because of the oil content.

Amino acid digestibility of fermented SBM is not different from conventional SBM but because the AA concentrations in fermented SBM is more than conventional SBM, the feeding value of fermented SBM is greater than conventional SBM. The absence of the non-digestible oligosaccharides, stachyose and raffinose, in fermented SBM may also contribute to the greater feeding value of this product compared to SBM when fed to weanling pigs.

Treatment of SBM is effective in increasing the concentration of rumen undegraded protein (RUP) but the feeding value of the treated SBM, as assessed by measuring AA digestibility in pigs, is low. However, feeding treated SBM to dairy cows resulted to increased milk yield which indicates that the pig is not a good model to estimate digestibility of treated SBM because microbes in the rumen may reverse the negative effects of heating. The concentration of blocked Lys using the homoarginine or the furosine procedure may be used to predict RUP values in treated SBM.