NOVEL SOYBEAN PRODUCTION FED TO WEANLING PIGS

BY

KURTIS P. GOEBEL

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, 2010

Urbana, Illinois

Advisor:

Professor Hans Henrik Stein

ABSTRACT

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Kurtis P. Goebel

2010

Several studies were conducted to measure the potential impact of feeding novel soybean products to young swine. The first experiment was conducted to determine the impact of heat treatment on AA digestibility with an emphasis on the effect that trypsin inhibitors have on protein digestion. The apparent (AID) and standardized (SID) ileal digestibility were determined using low temperature and high temperature-processed full-fat soybeans (FFSB) as well as a low temperature and high temperature-processed low-Kunitz variety of soybean. The protein source used in the control diet was conventional soybean meal (SBM). The 2 low temperature-processed FFSB had lower ($P < 0.05$) AID and SID values for all indispensable AA than the 2 high temperature-processed FFSB and SBM. The low temperature-processed low-Kunitz FFSB had greater ($P < 0.05$) SID values than the low temperature-processed conventional FFSB, however, the high temperature-processed soybeans had greater ($P < 0.05$) AID and SID values than both low temperature-processed soybeans. The next 2 experiments measured P and energy digestibility in different soybean products (HP-200, HP-310, and HP-340) that had been enzymatically treated. In the production of HP-340, phytase was included in the enzyme mixture while no phytase was involved in the enzymes used to produce HP-200 and HP-310. The enzyme treatment involves a proprietary blend of enzymes that help to remove the antinutritional factors in soybeans to lessen the sensitivity for young swine. Results indicated that the apparent total tract digestibility (ATTD) of P in HP-310 and SBM increased ($P < 0.05$) as phytase was included

in the diet (from 59.8 to 77.7% for HP-310 and from 65.5 to 79.5% for SBM), but the ATTD of P in HP-340 was not different (83.8 and 87.7%, respectively). These results indicate that treatment of SBM with an enzyme mixture containing phytase results in increased P-digestibility. The second experiment measured the energy digestibility of HP-200, HP-310, and SBM. Results showed that the concentration of DE in HP-200, HP-310, and SBM were not different (4,333, 4,316, and 4,347 kcal/kg DM, for HP-200, HP-300, and SBM respectively) and ME (3,926, 3,914, and 3,980 kcal/kg DM, respectively) were not different among the 3 sources of SBM. Therefore, it was concluded that enzyme treatment of SBM to remove antigen does not change the P or energy digestibility in the SBM. In the last experiment, HP-300 and HP-350 were used. As HP-300 is similar to HP-310, HP-350 is different because lecithin is added to this SBM. Lecithin is extracted from soybeans and may be used as a fat emulsifier. Four diets were formulated to contain HP-300 or HP-350 without and with choice white grease or soybean oil. Results indicated that the ATTD of DM and GE were not different regardless of the soybean or fat source used. In addition, the ATTD of acid-hydrolyzed ether extract in HP-300 and HP-350 was not different regardless of the fat source, suggesting that the added lecithin in HP-350 does not increase fat digestibility.

Key words: amino acid digestibility, enzyme treated soybean meal, lecithin, low-Kunitz soybeans, phytase, pigs

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude for those who have made this accomplishment such a great success as I complete another chapter in my life but open many more:

First and foremost, thank you Dr. Hans H. Stein for the patience and guidance through this amazing journey. As my degree began down another path, I was directed back to Dr. Stein as he accepted me into his program with open arms and I am truly thankful of that.

Secondly, I thank you, past and present members of the Stein lab during my time here. I am extremely appreciative to have worked with the best of the best including Dr. Beob Kim, Grant Petersen, Ferdinando Almeida, Dr. Ameer Pahm, Sarah Pahm, Dr. Pedro Urriola, Dr. Dong Yong Kil, Kate Baker, Scott Baker, Won Seok Ju, Jung Wook Lee, and Caroline Gonzalez. In addition, thanks to Dr. Carl Parson and Dr. Elvira de Mejia for serving on my M.S. defense committee.

Also, I am grateful to have such superb farm crews at both the Swine Research Center and the Imported Swine Research Laboratory, which helped out with my research projects and I thank everyone at both of these swine units. In addition, a sincere thanks to HiDee Ekstrom for her organization and patience throughout this whole process. She was the "go to person" when paperwork needed completed or when lab supplies needed ordered.

Last but definitely not least, thanks to all my friends and family for the encouragement and support. A special thanks to my wife, Shannon, for her love and the confidence she has in me. You all have made me into the person I am today.

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CHAPTER 1

Introduction

The widespread use of soybean meal (**SBM**) in swine diets as the main protein source is well justified. Not only does SBM provide a great AA composition, but pigs have relatively high digestibility of AA when fed SBM. Therefore, swine producers have benefited from using a corn-SBM based diet because SBM complements the high energy value of corn by providing a high quality protein (Dilger et al., 2004). In addition to the protein value of SBM, soybeans are grown in the Midwest, which is at the heart of pork production. Even though SBM provides a great source of protein, there are several limitations that affect pig performance.

The biggest limitation when feeding soybeans are the anti-nutritional factors. Trypsin inhibitors (**TI**) inhibit protease activity, and therefore, decrease AA digestibility (Rackis, 1972). Antigens are present in SBM in the form of β-conglycinin and glycinin, which may damage the villi in the small intestine and decrease protein digestion (Li et al., 1990; Friesen et al., 1993). Also, SBM contains lectins that are proteins which are resistant to the proteolytic digestive enzymes and lectins may also damage the intestinal villi. To inactivate the majority of the antinutritional factors, SBM is heat treated, which ameliorates some of the negative effects of unheated SBM by inhibiting the TI (Herkelman et al., 1992; Palacios et al., 2004). Despite the heat treatment, SBM still contains anti-nutritional compounds that negatively affect the young pig. Therefore, swine producers use less SBM in weanling pig diets (Friesen et al., 1993) whereas SBM may be used as the sole source of protein in diets fed to growing-finishing pigs and sows. However, technology advancements have made it possible to develop new varieties of

soybeans and new processing procedures have been implemented to produce soybean products that may be tolerated by young pigs.

By using such technologies, swine producers will potentially be able to feed unheated soybeans that have been commercially selected and bred to contain less TI than conventional soybeans. Swine producers will then be able to feed soybeans at a lower cost due to the savings from not having to heat treat the beans. Furthermore, the use of new processing methods, which removes the antigens in SBM, may provide pork producers with SBM that is tolerated by young swine. The advantages of this is that SBM can be used instead of higher priced animal proteins. There is, however, a need for research to evaluate the nutritional value of these new sources of SBM.

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CHAPTER 2

Challenges associated with feeding soybean products to weanling pigs: Literature review

Introduction

Soybean meal (**SBM**) is widely used in diet formulations throughout the swine industry. Use of SBM in corn-based diets was initiated in the 1950s. Soybean meal provides a quality protein source with an excellent AA profile (Dilger et al., 2004). Currently, SBM is produced from a process that includes de-hulling and oil extraction. Another important step is that the processed soybeans are heat treated to inactivate most antinutritional factors. Despite the advantages of using SBM in diets fed to pigs, there are many challenges as well.

One challenge is the pigs' sensitivity to trypsin inhibitors (TI), which is a common antinutritional factor in soybeans. Currently, SBM is heat treated to inactivate these trypsin inhibitors. However, a soybean variety that contains less trypsin inhibitors may provide pork producers with a SBM that does not need to be heat treated, thus resulting in reduced feed costs due to the energy saved from heat treatment.

As TI may be detrimental antinutritional factors, oligosaccharides (**OS**) are common antinutritional factors in SBM as well. The use of SBM in diets fed to nonruminants has been reported to cause gas production, diarrhea, and animal discomfort (Kuriyama and Mendel, 1917; Rackis, 1975). This is due to the inability of the small intestine to digest oligosaccharides because it lacks the enzyme alpha-galactosidase. Therefore, the use of supplemented α-galactosidase or the use of a low-oligosaccharide soybean variety may provide benefits to pork producers.

Conventional SBM also contains antigens that are harmful to young pigs and the inclusion of SBM is, therefore, often restricted in diets fed to weanling pigs (Friesen et al., 1993). Instead, higher priced animal proteins are usually used in these diets. However, technologies to remove antigens from SBM have recently been discovered and it is, therefore, possible that the concentration of SBM can be increased in diets fed to weanling pigs.

Trypsin Inhibitors

The excellent feeding value of soybeans when fed to pigs is due to the high protein quality. However, when feeding raw soybeans to pigs, protein digestibility is reduced because of the presence of TI in the beans. Kunitz and Bowman-Birk are the 2 TIs present in soybeans that reduce the activity of the proteolytic enzymes secreted by the pancreas (Rackis, 1972). Therefore, when feeding intact soybeans to pigs, a decrease in protein digestibility is observed (Yen et al., 1977). The Kunitz inhibitors make up 60% of the total TI in the seed (Losso, 2008) and Bowman-Birk makes up nearly 40% of the TI (Liener, 1981).

Heat treatment is used to inactivate the TI, because heat inactivates the Kunitz inhibitors, which results in an increase in AA digestibility and improved growth performance (Herkelman et al., 1992; Palacios et al., 2004). However, the Bowman-Birk inhibitor (**BBI**) is more resistant to heat treatment than the Kunitz inhibitor. At a pH of 2 and temperatures at 100°C, the BBI is only inactivated slightly. Therefore, to fully inactive the BBI inhibitors, temperatures need to exceed 100°C to break the disulfide bridges within the protein molecule (Losso, 2008). As a consequence, in the production of SBM, temperatures often reach 150°C to inactivate all the TI and this is the reason for the low concentration of TI in SBM (Webster et al., 2003).

Although heat treatment is important, new varieties of low-Kunitz soybeans may support optimal pig performance without using heat treatment. Reducing energy costs associated with heat treatment would be a cost effective way to reduce input costs, thus increasing the profit in swine production. Varieties of soybeans containing low levels of Kunitz TI have been selected and used in experiments with pigs. However, even if low-Kunitz soybeans are used, AA digestibilities may not be maximized because of the presence of BBI in the seed (Liener, 1981). Previous research has, therefore, demonstrated that performance of pigs fed low-Kunitz SBM is less than that of pigs fed heat treated SBM. However, new varieties of low-Kunitz soybeans are being developed.

Antigens

Post-weaning stress in pigs has provided many challenges for the swine industry, but also has allowed for many opportunities. The nursery stage is the most crucial period because the diet for the pig is changed from a milk-based diet to a dry-feed based diet. This transition is difficult and providing the newly weaned pigs with a palatable, balanced diet that they will eat and that supports the integrity of the small intestine is a challenge. High priced animal proteins are widely used during this phase. For example, fish meal, which is a very digestible protein, contains a high concentration of AA, vitamins, and minerals (Mason and Weidner, 1964). The major reason for using animal proteins such as fish meal in diets fed to nursery pigs is the poor immune responses that is a result of feeding antigens that are associated with feeding SBM to young pigs (Kim and Easter, 2001).

The 2 major antigens that cause severe allergic reactions in animals are glycinin and β–conglycinin (Sissons 1982; Miller et al., 1984). β-conglycinin activity is the best predictor for measuring the quality of protein from soybeans (Lallès et al., 1996) as it is the main storage

protein, but is has also been characterized as one of the major soybean allergens (Ogawa et al., 1995; Lallès et al., 1999). This is because β-conglycinin is more resistant to the proteolytic enzymes in the digestive processes than other proteins in soybeans (Zhao et al., 2008). The symptoms of young pigs consuming antigens are decreased performance and "disorders of immune function" (Hao et al., 2009). Young pigs fed antigens from SBM also often have damaged villi in the small intestine, which will result in poor protein absorption. The pigs transient hypersensitivity to glycinin and β-conglycinin is detrimental to the productivity of a swine herd as it will cause severe reduction in post-weaning performance (Li et al., 1990; Friesen et al., 1993). Not only is performance in the nursery negatively affected, but AA digestibility is greatly reduced because pigs fed SBM have lower digestibilities of both indispensable and dispensable AA compared with pigs fed dried skim milk or soybean protein concentrates in which the antinutritional factors have been removed (Sohn et al., 1994). Therefore, antigens in soy protein fed to pigs need to be removed if soy protein is fed to early weaned pigs.

Oligosaccharides

The excellent AA profile in SBM is well recognized, but variation in the nutritional value among different varieties of soybeans exists (Smiricky et al., 2002). These variations may be due to many factors, but the presence of oligosaccharides (i.e., stachyose and raffinose) in SBM after processing will negatively affect performance of pigs (Liener, 1981; Anderson and Wolf, 1995). Even with processing, stachyose and raffinose are not removed (Leske et al., 1993). Stachyose and raffinose represent 4 to 6% of the DM in SBM and they may cause diarrhea when fed to rats (Kuriyama and Mendel, 1917). Steggerda (1968) reported that oligosaccharides caused diarrhea not only in rats, but also in dogs and humans. The cause of diarrhea may be a result of the detrimental effects oligosaccharides have on the intestinal morphology. Animals do not

synthesize α-1,6-galactosidase, which is the enzyme that is responsible for digesting raffinose and stachyose. Therefore, oligosaccharides are not digested in the small intestine (Gitzelman and Auricchio, 1965). Not only do oligosaccharidess cause diarrhea, but they also cause flatulence and extensive distress for nonruminants (Saini, 1989). These ill effects occur because oligosaccharides are fermented in the lower intestinal tract, which harbors the anaerobic bacteria that may produce hydrogen, carbon dioxide, and small amounts of methane gases (Rackis, 1975). To ameliorate the adverse effects of feeding oligosaccharides, a dietary α-galactosidase may be used to improve nutrient digestion (Smiricky et al., 2002). Feeding soybeans that have been selectively bred to contain less oligosaccharides may also be a way to reduce the negative effects of oligosaccharides (Baker and Stein, 2009). Use of a low-oligosaccharide variety of soybean may provide substantial benefits by improving AA digestibility without the pig experiencing diarrhea and flatulence. However, Baker and Stein (2009) did not observe any favorable effects of using a low-oligosaccharide variety compared with a conventional variety of soybeans on AA digestibility. In addition, no differences were observed in energy digestibilities when the same soybean sources were used. The lack of a positive response to a low-oligosaccharide SBM on AA and energy digestibility indicates that the negative effects of oligosaccharides may be limited. Smiricky et al. (2002) reported that AA digestibility was not improved in pigs fed diets that were supplemented with the α-galactosidase enzyme, which further indicates that pigs are not negatively affected by oligosaccharides to a great extent. Furthermore, the use of prebiotics have been introduced to the feed industry as a "non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the host health (Gibson and Roberfroid, 1995)." However, when prebiotics are fed in a corn-SBM diet to chicks, no positive response was

reported due in part from the high concentrations of soy oligosaccharides which may have masked the potential benefits of the prebiotics (Jacobs and Parsons, 2009).

Development of Enzyme Treated SBM

When feeding soy proteins to young pigs, it has been observed that the antinutritional factors in soy proteins cause transient hypersensitivity in the gut, which increases crypt cell production because of villi atrophy and reduced absorption of nutrients (Stokes et al., 1986; Li et al., 1990).

Hamlet Protein A/S (Horsens, Denmark) has developed a patented process to reduce the antinutritional factors in SBM, which supplies a novel soy product for the early weaned pig. The removal of the antinutritional factors is achieved by enzymatic treatment using a proprietary blend of enzymes. This creates enzymatically treated SBM, which is now available to the U.S. feed industry.

The production of Hamlet SBM is similar to the production of conventional SBM, but a few major differences exist after the soybeans are dehulled and defatted. In the case of the conventional soybean meal, the SBM is dehulled, defatted, and finally heated to produce conventional SBM. In the case of Hamlet SBM, the dehulled, defatted SBM is put through a special biotechnological processing step in which the proprietary blend of enzymes are added. After the completion of this step, the enzymes are inactivated and the product is dried and milled before the final Hamlet SBM is produced. The digestibility of AA in enzyme treated soybean meal (HP-300, Hamlet Protein, Horsens, Denmark) has been measured (Zhu et al., 1998; Min et. al., 2004; Pahm and Stein, 2007; Urbaityte et. al., 2009) and it was reported that there was an increase in DM, CP, and AA digestibilities as well as improved ADG in pigs fed diets with HP-

300. There are, however, no digestibility values available for energy, P, and acid-hydrolyzed ether extract in enzyme treated SBM and it is not known if enzyme treatment influences the digestibility of energy, P, or acid-hydrolyzed ether extract.

Fat Digestibility

The addition of 32% butterfat to a diet fed to neonatal pigs was beneficial in increasing growth performance (Wolfe et al., 1977). The inclusion level and source of fat used in this experiment was derived from previous research (Perrin, 1955; deMan, and Bowland, 1963) that analyzed the DM of milk from sows to contain nearly 30% fat. However, fat in sows milk contain medium chain fatty acids whereas fat in most common fat sources added to pig diets contain long chain fatty acids. To better utilize fat that is added to the diets of young pigs, it may be beneficial to use emulsifying agents that can improve the digestion and absorption of long chain fatty acids, because for an animal to utilize and digest fat, the fat has to be emulsified in the gastrointestinal tract.

Soybeans contain lecithin, also called phosphatidyl choline, which is a phospholipid that can be extracted from the soybean and used for fat emulsification. Because fat is insoluble in water, emulsification is required to digest and absorb fat in the gastrointestinal tract. Therefore, lecithin can be used as an exogenous emulsifier added to a weanling pig diet to increase fat digestibility instead of depending solely on bile acids (Jones et al., 1990a, b; Øverland et al., 1993a). Addition of lecithin to diets have increased fat digestibility in humans (Aldersberg and Sobotka, 1943), chicks (Polin, 1980), and calves (Hopkins et al., 1959). However, research to measure effects of exogenous emulsifiers in young pigs has resulted in inconsistent responses (Frobish et al., 1969; Jones et al., 1990a, b; Øverland et al., 1993a, b). These differences may be a result of different fat sources used or simply by the age of pig. For example, Allee et al. (1971)

suggested that the energy:AA ratio may affect fat digestibility and Cera et al. (1988) suggested that differences in pig age cause differences in fat digestibility. The source of fat also may influence the response to added fat because weaned pigs have greater fat digestibilities when the diets they consume contain corn oil rather than animal fat (Cera et al., 1988). Sewell and Miller (1965) reported similar results, which suggest that weanling pigs utilize diets supplemented with corn oil better than diets supplemented with animal fat. Similarly, Jin et al. (1998) discovered increased digestibility of nutrients when vegetable oil was added to diets compared with pigs fed diets with tallow. In fact, with added lecithin, growth rate and feed efficiency were improved in diets with vegetables compared with diets with tallow and lecithin. As demonstrated, the source of fat used in diets fed to young pigs is important because short-chained and medium-chained fatty acids are more digestible than long-chained fatty acids (Cera et al., 1989). In addition, adding lecithin to these diets may provide an even greater improvement in digestibility. However, research indicates that lecithin added to diets fed to pigs yields inconsistent responses. For example, pigs did not respond to lecithin in some experiments (Øverland et al., 1993a. b; Cho et al., 2008), but other studies reported increased digestibility of both fat and other nutrients (Jones et al., 1990a. b, 1992; Jin et al., 1998). There is, therefore, a need for more research to determine the effects of lecithin in SBM-based diets fed to weanling pigs.

Conclusion

The popularity of feeding pigs corn-SBM diets has been mainly due to the excellent AA profile of SBM. Feeding SBM to pigs has resulted in optimal growth performance but pork producers have limited the inclusion of SBM in diets for young pigs because of the young pig's inability to fully utilize SBM due to anti-nutritional factors. These factors include TI, antigens, and oligosaccharides. However, new soybean varieties and processing methods make it possible

to replace highly priced animal protein products with new soybean products in diets fed to young pigs.

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CHAPTER 3

Ileal digestibility of amino acids in conventional and low-Kunitz soybean products fed to weanling pigs

Abstract: An experiment was conducted to determine the standardized ileal digestibility (SID) of amino acids (AA) in 4 sources of full-fat soybeans (FFSB) and in 1 source of dehulled soybean meal (SBM). The FFSB had different concentrations of trypsin inhibitor units (TIU) and included 2 sources of conventional FFSB, and 2 sources of a soybean variety that was selected for a reduced concentration of the Kunitz trypsin inhibitor. The conventional FFSB was either low temperature-processed (LT-FFSB-CV; 37.7% CP, 35.4 TIU/mg) or high temperature-processed (HT-FFSB-CV; 40.5% CP, 4.4 TIU/mg). The low-Kunitz FFSB was also either low temperature-processed (LT-FFSB-LK; 36.2% CP, 23.5 TIU/mg) or high temperature-processed HT-FFSB-LK; (38.2% CP, 4.0 TIU/mg). The SBM contained 47.5% CP and 3.20 TIU/mg. Twelve weanling barrows (initial BW: 11.1±1.3 kg) were fitted with a T-cannula in the distal ileum. Pigs were allotted to a replicated 6 x 6 Latin square design with 6 diets and 6 periods per square. Five diets were prepared using each of the soybean sources as the only source of AA in the diet. An N-free diet was also included in the experiment to measure basal endogenous losses of AA. The AID and SID values for CP were greater ($P < 0.05$) in HT-FFSB-CV, HT-FFSB-LK, and SBM than in LT-FFSB-CV and LT-FFSB-LK. The 2 low temperature-processed FFSB had lower ($P < 0.05$) AID and SID values for all indispensable AA than the 2 high temperature-processed FFSB and SBM. The SID values for all indispensible AA except Trp were greater ($P < 0.05$) in LT-FFSB-LK than in LT-FFSB-CV, but the SID of AA in HT-FFSB-CV and HT-FFSB-LK were not different. The SID of AA in SBM were not different from the SID in HT-FFSB-CV

and in HT-FFSB-LK. Results of this experiment show that a reduction of the TIU from 35.4 to 23.5 TIU/mg will improve the SID of AA, but this reduction is not sufficient to completely ameliorate the negative impact of trypsin inhibitors. Results also show that the SID of AA in high temperature-processed FFSB is similar to that in de-hulled SBM.

Key Words: Amino Acid Digestibility, Low Kunitz Soybeans, Full-fat Soybeans, Trypsin Inhibitors

Introduction

Trypsin inhibitors are the most important antinutritional factors in raw soybeans. Kunitz and Bowman-Birk are the two major types of trypsin inhibitors in soybeans (Rackis, 1972), but the Kunitz trypsin inhibitor is of particular interest because it is heat labile, whereas the Bowman-Birk inhibitor exhibits a considerable resistance to heat treatment (Clemente et al., 2007). After isolation and characterization of the Kunitz trypsin inhibitor (Kunitz, 1947a, b), it was demonstrated that this inhibitor results in decreased protein digestibility in pigs due to a reduction in the activity of trypsin, chymotrypsin, and other pancreatic enzymes (Yen et al., 1977).

Heat treatment of soybean products inactivates the Kunitz inhibitor (Liener and Kakade, 1980) and heat treatment of soybean products is, therefore, routinely done before soybean products are used in diets fed to swine. The apparent ileal digestibility (**AID**) of amino acids (**AA**) in soybeans is also improved with heat treatment by approximately 15 percentage units (Herkelman et al., 1992), but there is no information about the effect of trypsin inhibitors on the standardized ileal digestibility (**SID**) of AA in soybean meal (**SBM**). Because of the negative impact of the Kunitz trypsin inhibitor on protein digestibility, plant breeders have tried to select

varieties of soybeans with a low concentration of the Kunitz inhibitor (Clark and Hymowitz, 1972). Previous research has demonstrated that growth performance in pigs is improved if unheated varieties of low-Kunitz soybeans instead of unheated conventional soybeans are fed to pigs (Yen et al., 1974; Cook et al., 1988; Palacios et al., 2004). However, in all of these experiments, pigs fed the low-Kunitz soybeans had performance that was lower than that of pigs fed heat-treated soybeans. Schillinger Genetics (Des Moines, IA, USA) has recently selected a new variety of low-Kunitz soybeans, but there are no data on the nutritional quality of this variety of soybeans.

The objective of this experiment was, therefore, to test the hypothesis that low-Kunitz soybeans from Schillinger Genetics Inc. have greater AID and SID of crude protein (**CP**) and AA than conventional soybeans with normal concentrations of trypsin inhibitors and that heat treatment of low-Kunitz soybeans is not necessary to maximize AA digestibility.

Materials and Methods

Animals, Housing, and Experimental Design

The experimental protocol for this experiment was reviewed and approved by the Animal Care and Use Committee at the University of Illinois. Twelve growing barrows (initial BW: 11.1±1.3 kg) were allotted to a replicated 6 x 6 Latin square design with 6 diets and 6 periods balanced for potential residual effects using the Balanced Latin Square Designer (Kim and Stein, 2009). Each pig was surgically equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Pigs were housed in individual pens (1.8 x 2.7 m) in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen.

Diets and Feeding

Five sources of soybean products were used in the experiment (Table 3.1). The control source was a conventional dehulled SBM containing 47.5% CP. Two sources of full-fat soybeans (**FFSB**) were also used. One of these FFSB was selected for a low concentration of Kunitz trypsin inhibitors and the other source was a conventional FFSB with normal activity of trypsin inhibitors. After isolation and characterization of the Kunitz trypsin inhibitor (Kunitz, 1947a, b), it was demonstrated that his inhibitor results in decreased protein digestion in pigs (Yen et al., 1977). Therefore, newly developed varieties with low Kunitz trypsin inhibitor were used. The 2 varieties of soybeans were grown in northeastern Indiana and were planted May 20, 2008, and harvested October 15, 2008. The growing season began very wet and cool and finished with very dry conditions. The conventional and low-Kunitz sources of soybeans were used as low temperature-processed FFSB (**LT-FFSB-CV** and **LT-FFSB-LK**) and high temperature-processed FFSB (**HT-FFSB-CV** and **HT-FFSB-LK**, respectively). The LT-FFSB-LK and LT-FFSB-CV were both dehulled and cracked through a Roskamp Double Roller Mill (Roskamp Champion, Waterloo, IA) at an ambient temperature of 21°C. The high temperature-processed FFSB were processed using the Insta-Pro model 600 autogenous extruder (Insta-Pro International, Des Moines, IA) with a 0.8 cm die operating at a rate of 182 kg per h. The extrusion temperature was 154°C for HT-FFSB-CV and 143°C for HT-FFSB-LK. The low temperature-processed soybeans and the extrudate from both high temperature-processed FFSB sources were ground to 1.18 mm through a Bauer mill in the Ag Bioprocess Laboratory at the University of Illinois.

Six diets were prepared (Tables 3.2 and 3.3). Five of the diets contained one of the soybean sources and starch, sugar, and oil. The last diet was an N-free diet that was used to

calculate basal endogenous losses of CP and AA. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 1998). All diets were supplemented with 0.4% chromic oxide as an indigestible marker.

All pigs were fed at a daily level of three times the maintenance energy requirement (106 kcal of ME per $kg^{0.75}$; NRC, 1998). The daily allotment of feed was provided at 0700 h. Water was available at all times throughout the experiment.

Data Recording and Sample Collection

Pig body weights were recorded at the beginning and at the end of each period and the amount of feed supplied each day was recorded. The initial 4 d of each period was considered an adaptation period to the diet. Ileal digesta were collected for 8 h on d 5 and 6. The cannulas were opened and a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and stored at – 20^{o}C to prevent bacterial degradation of the AA in the digesta.

Chemical Analysis

At the conclusion of the experiment, ileal samples 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 soybean products was collected as well. Digesta samples were lyophilized and finely ground prior to chemical analysis. All samples were analyzed for DM (method 930.15; AOAC Int., 2007) and CP (method 990.03; AOAC Int., 2007). Chromium concentrations of diets and ileal digesta were determined using an inductive coupled plasma atomic emission spectrometric method (method 990.08; AOAC Int., 2007). Samples were prepared for analysis using nitric acid-perchloric acid (method 968.088D; AOAC Int., 2007). Amino acids were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.;

Pleasaton, CA, USA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (method 982.30; AOAC Int., 2006). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007). Sucrose, raffinose, and stachyose were analyzed using HPLC (method 982.14; AOAC Int., 2007). The concentration of total fat in these samples was measured after HCl hydrolysis, followed by petroleum ether extraction (method 954.02; AOAC Int., 2007), and the concentration of crude fiber was analyzed using the Ankom method (procedure Ba 6a-05; AOCS; 2006). Trypsin inhibitor concentrations were also analyzed in each source of SBM (method Ba 12-75; AOCS; 2006).

Calculations and Statistical Analysis

The AID for CP and AA in samples obtained from feeding the five diets containing FFSB or SBM were calculated. Because the soybean products were the only feed ingredients contributing CP and AA in each of the diets, these digestibility values also represented the digestibility values for each of the soybean products. Equation [1] (Stein et al., 2007) was used to calculate the AID:

$$AID_{AA}, \% = [1 - (AA_{digesta}/AA_{feed}) \times (Cr_{feed}/Cr_{digesta})] \times 100 \quad \textbf{[1]}$$

where AID_{AA} is the AID value (%) of an AA, $AA_{digesta}$ is the concentration of that AA in the ileal digesta, AA_{feed} is the AA concentration of that AA in the feed, Cr_{feed} is the Cr concentration in the feed, and $Cr_{digesta}$ is the Cr concentration in the ileal digesta. The mean Cr concentration of the 6 diets was used for all diets in order to overcome potential errors from feed sampling, Cr analytical calibration, or both. The AID for CP was also calculated using the same 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):

$$IAA_{end} = AA_{digesta} \times (Cr_{feed}/Cr_{digesta}) \quad \textbf{[2]}$$

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

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

$$SID_{AA} = [(AID + IAA_{end})/AA_{feed}] \quad \textbf{[3]}$$

where SID_{AA} is the SID value (%) of each AA. The SID for CP was also calculated using this equation.

The Proc UNIVARIATE procedure (SAS Institute Inc., Cary, NC) was used to identify outliers and one outlier was removed within the LT-FFSB-CV treatment. Data were analyzed using the Proc GLM procedure of SAS. The initial model included diet, period, and animal, but period and animal were not significant, and thus, removed from the final model. Orthogonal contrasts were used to determine the effects of the variety of FFSB, the thermal treatment, and the interaction between variety and thermal treatment. Treatment means were separated using the PDIFF option with Tukey's adjustment. The mean separation output was converted to letter groupings using a SAS macro program (Saxton, 1998). 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 SBM than in any of the FFSB sources (Table 3.1). However, the concentration of crude fat was lower in SBM than in the other soybean products, but the concentration of sucrose was not different among the soybean

products. The TIU concentration in LT-FFSB-CV (35.4 TIU/mg) was greater than in LT-FFSB-LK (23.5 TIU/mg), but HT-FFSB-CV and HT-FFSB-LK had TIU concentrations of only 4.4 and 4.0 TIU/mg, and SBM contained 3.2 TIU/mg.

The AID of CP and all AA except Pro in HT-FFSB-CV, HT-FFSB-LK, and SBM was greater ($P < 0.05$) than the AID of AA in LT-FFSB-CV and LT-FFSB-LK (Table 3.4). The AID of CP and all AA except Trp in LT-FFSB-CV was less ($P < 0.05$) than in LT-FFSB-LK, but no differences in the AID of CP and AA among HT-FFSB-CV, HT-FFSB-LK, and SBM were observed. The AID for CP and all AA was greater ($P < 0.05$) in HT-FFSB-LK and HT-FFSB-CV than in LT-FFSB-LK and in LT-FFSB-CV and the AID of all AA except Trp and Pro were greater ($P < 0.05$) in the 2 low-Kunitz meals than in the two conventional meals.

No differences in SID for CP and AA were observed among HT-FFSB-CV, HT-FFSB-LK, and SBM, but all of these soybean products had SID values for CP and all AA except Pro that were greater ($P < 0.05$) than the SID of CP and AA in LT-FFSB-LK and LT-FFSB-CV (Table 3.5). The SID of CP and all AA except Trp in LT-FFSB-LK was greater ($P < 0.05$) than in LT-FFSB-CV. The SID for CP and all AA except Trp and Pro in HT-FFSB-LK and LT-FFSB-LK was greater ($P < 0.05$) than in HT-FFSB-CV and LT-FFSB-CV, and the SID of CP and all AA the HT-FFSB-LK and HT-FFSB-CV were greater ($P < 0.05$) than in LT-FFSB-LK and LT-FFSB-CV.

Discussion

The two major types of trypsin inhibitors in legumes, particularly soybeans, are the Kunitz and Bowman-Birk trypsin inhibitors, which make up nearly 60% of the protein in soybeans (Losso, 2008). Bowman-Birk makes up approximately 40% of the trypsin inhibitors

and Kunitz the remaining 60% (Liener, 1981). Similar to Kunitz, Bowman-Birk inhibitors reduce the activity of the proteolytic enzymes secreted by the pancreas. Heat treatment inactivates the Kunitz trypsin inhibitors in soybeans, thus allowing for increased AA digestibilities and improved growth performance (Herkelman et al., 1992; Palacios et al., 2004). However, the Bowman-Birk inhibitor loses no activity if treated at a pH of two or heated to 100°C for ten minutes (Losso, 2008). The resistance of Bowman-Birk inhibitor to highly acidic conditions and to heat treatment up to 100°C is due to the presence of disulfide bridges within the protein molecules (Losso, 2008). This structure will tightly bind up pancreatic proteases and cause severe inhibitory responses (Clemente et al., 2008). To inactivate the Bowman-Birk inhibitors, the temperature needs to exceed 100°C. During soybean extrusion, the temperature at the end of the extruder may range from approximately 143 to 165°C, which is effective in inactivating both Kunitz and Bowman-Birk inhibitors (Webster et al., 2003). The low TIU levels in both of the high temperature-processed FFSB used in the present study is, therefore, a result of the heat treatment these products had undergone. However, if the beans are processed under low temperatures, the Bowman-Birk inhibitors are active.

The reason the SID of most AA was greater in LT-FFSB-LK than in LT-FFSB-CV is that the concentration of TIU is reduced in the low-Kunitz beans compared with the conventional soybeans. Heat treatment improves the AID of AA in conventional FFSB fed to pigs (Herkelman et al., 1992), but the results of this experiment document that heat treatment also improves the AID and the SID of AA in low-Kunitz beans. This observation concurs with Kim et al. (1999) who reported that weanling pig performance was improved when low-Kunitz soybeans were processed at high temperatures. The reason for this observation is most likely that although the concentration of trypsin inhibitors was reduced in the low-Kunitz soybeans, the

concentration was not nearly as low as it was in heat treated soybean products. Improved nutrient digestibility was also observed by Qin et al. (1996) who reported that by heat treating soybeans to reduce trypsin inhibitor concentrations, nutrient digestibility of soybeans was greatly improved. It, therefore, appears that additional efforts need to be placed on selecting soybean varieties that have lower concentrations of trypsin inhibitors than the low-Kunitz beans used in this experiment.

The AID and SID of AA were not different between the high temperature-processed FFSB and the conventional SBM. The differences in chemical composition between the 2 FFSB and SBM are due to processing. Unlike SBM, oil is not removed from FFSB, which is the reason that the conventional SBM has a lower concentration of fat than the FFSB. Previous research showed that the greater fat concentration in FFSB resulted in greater AA digestibility in FFSB than in non-dehulled SBM (Cervantes-Pahm and Stein, 2008). This may be due to a slower gastric and intestinal emptying, which gives the feed proteins increased exposure to proteolytic enzymes (Gentilcore et al., 2006). In contrast, AA digestibility is reduced if soy hulls are added to SBM (Dilger et al., 2004). In this experiment, a dehulled source of SBM and dehulled FFSB were used, and the AID and SID of AA in HT-FFSB-CV and HT-FFSB-LK were similar to the AID and SID in SBM. The AID values for AA in SBM that were measured in this experiment are similar to values previously reported (NRC, 1998). However, AID values for HT-FFSB-CV and HT-FFSB-LK were greater than those obtained by Herkelman et al. (1992). This may be due to processing differences between the heated soybean sources used in the different experiments.

Values for basal endogenous losses of AA were measured in the present experiment, which allowed for the calculation of SID for all AA. To our knowledge, SID values have not previously been measured in low-Kunitz soybeans. However, the values obtained for the SID of

AA in HT-FFSB-CV and in SBM concur with the values reported by Cervantes-Pahm and Stein (2008) but they are greater than the values published by NRC (1998).

In conclusion, if conventional or low-Kunitz de-hulled FFSB are processed at 140 to 160°C, values for the SID of AA are similar to those for de-hulled SBM. Even though it is advantageous to use a low-Kunitz variety of soybeans compared with conventional beans, the SID of AA in the non-heated beans is lower than the SID of AA in beans processed at 140 to 160°C. Processing at these temperatures increase SID of AA in both soybean varieties and the removal of the Kunitz trypsin inhibitor increases the SID of AA only in soybeans that have not been heat-treated. Therefore, even if low Kunitz varieties of soybeans are used, they still need to be heat treated to maximize AA digestibility.

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Table 3.1. Chemical composition of soybean meal (SBM) produced from low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and in conventional soybean meal (SBM), as-is basis

Item	Ingredient				
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM
Dry matter, %	90.43	88.33	95.77	94.02	87.50
Crude protein, %	37.70	36.17	40.45	38.19	47.47
Crude fat, %	19.86	20.72	21.22	20.60	1.48
Crude fiber, %	4.50	4.40	3.80	4.20	3.50
Ash, %	4.79	5.28	5.23	5.72	6.06
Trypsin inhibitor, (TIU/mg)[1]	35.40	23.50	4.40	4.00	3.20
Sucrose, %	5.91	6.09	6.02	5.91	7.05
Stachyose, %	4.74	4.46	4.56	4.84	4.61
Raffinose, %	0.62	0.96	1.00	0.64	0.93
Indispensable amino acids, %					

Table 3.1 (cont.)

Arg	2.98	2.87	3.21	2.91	3.56
His	1.08	1.07	1.17	1.09	1.25
Ile	1.90	1.80	2.13	1.90	2.25
Leu	3.10	3.02	3.47	3.14	3.76
Lys	2.74	2.63	2.90	2.67	3.14
Met	0.61	0.60	0.64	0.61	0.68
Phe	2.04	1.98	2.24	2.02	2.48
Thr	1.65	1.61	1.75	1.59	1.83
Trp	0.57	0.59	0.72	0.68	0.69
Val	1.99	1.89	2.23	2.02	2.36
Dispensable amino acids, %					
Ala	1.82	1.75	1.99	1.79	2.07
Asp	4.68	4.53	5.12	4.63	5.40
Cys	0.59	0.63	0.62	0.59	0.65
Glu	7.74	7.29	8.06	7.39	8.54

Table 3.1 (cont.)

Gly	1.83	1.71	1.95	1.79	2.00
Pro	1.96	1.92	2.14	1.96	2.36
Ser	1.96	1.96	2.08	1.88	2.10
Tyr	1.50	1.47	1.63	1.49	1.70

[1]TIU = Trypsin inhibitor units.

Table 3.2. Ingredient composition of experimental diets containing low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and conventional soybean meal (SBM), as-is basis

	Diet					
Ingredient, %	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	N-Free
Soybean product	50.00	50.00	50.00	50.00	40.00	-
Cornstarch	43.05	33.05	33.05	33.05	33.05	67.85
Soybean oil	3.60	3.60	3.60	3.60	3.60	4.00
Sugar	10.00	10.00	10.00	10.00	10.00	20.00
Solka floc[a]	-	-	-	-	-	4.00
Ground limestone	0.75	0.75	0.75	0.75	0.75	0.85
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.70
Magnesium oxide	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40

Table 3.2 (cont.)

Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00

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

[2] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.3. Chemical composition of experimental diets containing low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and conventional soybean meal (SBM), as-is basis

Item	Diet					
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	N-Free
Dry matter, %	90.40	90.31	93.59	93.20	90.57	91.02
Crude protein, %	15.99	17.95	21.42	20.0	25.5	1.95
Indispensable Amino Acids, %						
Arg	0.94	1.27	1.44	1.30	1.87	0.11
His	0.36	0.49	0.53	0.51	0.68	0.04
Ile	0.64	0.86	0.96	0.89	1.23	0.09
Leu	1.08	1.42	1.59	1.47	2.01	0.14
Lys	0.87	1.18	1.30	1.21	1.63	0.10
Met	0.19	0.26	0.27	0.29	0.37	0.02
Phe	0.68	0.92	1.05	0.96	1.33	0.08

Table 3.3 (cont.)

Thr	0.54	0.70	0.80	0.73	0.97	0.06
Trp	0.18	0.22	0.30	0.29	0.34	0.03
Val	0.69	0.93	1.03	0.96	1.29	0.09
Dispensable Amino Acids, %						
Ala	0.63	0.81	0.91	0.85	1.13	0.08
Asp	1.52	2.05	2.32	2.14	2.94	0.19
Cys	0.19	0.25	0.29	0.26	0.34	0.02
Glu	2.41	3.21	3.59	3.39	4.53	0.32
Gly	0.60	0.78	0.88	0.82	1.09	0.07
Pro	0.69	0.97	1.00	0.94	1.30	0.08
Ser	0.64	0.76	0.90	0.82	1.09	0.07
Tyr	0.44	0.61	0.68	0.60	0.85	0.04

Table 3.4. Apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in low temperature-processed conventional soybeans (LT-FFSB-CV), low temperature-processed low-Kunitz soybeans (LT-FFSB-LK), high temperature-processed conventional soybeans (HT-FFSB-CV), high temperature-processed low-Kunitz soybeans (HT-FFSB-LK), and in soybean meal (SBM) [1]

	Diet						*P* - values[2,3]		
Item	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	SEM	Overall	LT vs. HT	CV vs. LK
CP	49.4[c]	59.6[b]	81.10 [a]	81.22[a]	81.90[a]	2.29	<0.001	<0.001	0.023
Indispensable AA, %									
Arg	57.9[c]	74.1[b]	91.4[a]	92.3[a]	93.7[a]	1.51	<0.001	<0.001	<0.001
His	56.6[c]	71.3[b]	86.9[a]	88.4[a]	89.3[a]	1.79	<0.001	<0.001	<0.001
Ile	48.2[c]	63.1[b]	84.9[a]	87.3[a]	88.7[a]	2.00	<0.001	<0.001	<0.001
Leu	47.1[c]	61.3[b]	84.2[a]	86.3[a]	88.0[a]	2.13	<0.001	<0.001	<0.001
Lys	49.3[c]	65.0[b]	85.1[a]	86.6[a]	86.3[a]	2.22	<0.001	<0.001	<0.001
Met	52.5[c]	67.4[b]	86.3[a]	89.8[a]	90.8[a]	1.94	<0.001	<0.001	<0.001

Table 3.4 (cont.)

Phe	50.1[c]	64.7[b]	86.5[a]	87.9[a]	88.8[a]	1.94	<0.001	<0.001	<0.001
Thr	45.2[c]	57.9[b]	78.6[a]	80.0[a]	82.1[a]	2.57	<0.001	<0.001	0.005
Trp	59.7[b]	65.9[b]	87.3[a]	88.5[a]	87.7[a]	2.23	<0.001	<0.001	0.079
Val	44.8[c]	60.1[b]	81.2[a]	83.2[a]	85.3[a]	2.36	<0.001	<0.001	<0.001
Mean	50.2[c]	64.8[b]	85.3[a]	87.0[a]	88.2[a]	1.95	<0.001	<0.001	<0.001
Dispensable AA, %									
Ala	44.3[c]	58.2[b]	80.8[a]	82.7[a]	82.9[a]	2.48	<0.001	<0.001	0.001
Asp	53.3[c]	66.4[b]	84.8[a]	86.1[a]	85.5[a]	1.84	<0.001	<0.001	<0.001
Cys	29.5[c]	44.0[b]	76.6[a]	77.3[a]	76.7[a]	2.95	<0.001	<0.001	0.009
Glu	61.6[c]	73.5[b]	88.2[a]	88.2[a]	84.0[a]	1.92	<0.001	<0.001	0.002
Gly	32.4[c]	51.2[b]	75.1[a]	74.2[a]	76.1[a]	3.08	<0.001	<0.001	0.003

Table 3.4 (cont.)

Pro	3.2[c]	38.7[bc]	72.4[ab]	70.0[ab]	79.8[a]	9.57	<0.001	<0.001	0.072
Ser	49.1[c]	58.8[b]	83.5[a]	85.0[a]	87.1[a]	2.12	<0.001	<0.001	0.007
Tyr	49.1[c]	65.1[b]	84.9[a]	85.7[a]	87.4[a]	2.14	<0.001	<0.001	<0.001
Mean	47.4[c]	62.7[b]	83.3[a]	83.7[a]	83.5[a]	2.12	<0.001	<0.001	<0.001

[a-c]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 12 observations.

[2]P-values: Overall = the p-value for the comparison of diets containing all 5 ingredients; LT vs. HT = comparison of the two low temperature-processed full-fat meals vs. the two high temperature-processed meals; CV vs. LK = comparison of the two conventional soybeans vs. the two low-Kunitz soybeans.

[3] The interaction between the effect of processing temperature and the variety of soybeans was significant (P <0.05) for CP and all AA except Trp.

Table 3.5. Standardized ileal digestibility (%) of crude protein (CP) and amino acids (AA) in low temperature processed conventional soybeans (LT-FFSB-CV), low temperature processed low-Kunitz soybeans (LT-FFSB-LK), high temperature processed conventional soybeans (HT-FFSB-CV), high temperature processed low-Kunitz soybeans (HT-FFSB-LK), and in soybean meal (SBM) [1, 2]

Item	Diet						*P* - values[3, 4]		
	LT-FFSB-CV	LT-FFSB-LK	HT-FFSB-CV	HT-FFSB-LK	SBM	SEM	Overall	LT vs. HT	CV vs. LK
CP	65.2 [b]	70.2 [b]	90.5 [a]	91.2 [a]	89.5 [a]	2.16	<0.001	<0.001	0.218
Indispensable AA, %									
Arg	64.6[c]	79.0[b]	95.9[a]	97.2[a]	97.1[a]	1.62	<0.001	<0.001	<0.001
His	63.5[c]	76.3[b]	91.8[a]	93.2[a]	92.9[a]	1.91	<0.001	<0.001	<0.001
Ile	55.3[c]	68.4[b]	89.8[a]	92.3[a]	92.4[a]	2.14	<0.001	<0.001	<0.001
Leu	54.3[c]	66.7[b]	89.2[a]	91.4[a]	91.8[a]	2.27	<0.001	<0.001	<0.001
Lys	57.5[c]	71.0[b]	90.7[a]	92.5[a]	90.6[a]	2.38	<0.001	<0.001	<0.001

Table 3.5 (cont.)

Met	58.7[c]	71.9[b]	90.8[a]	93.8[a]	94.0[a]	2.07	<0.001	<0.001	<0.001
Phe	56.6[c]	69.5[b]	90.9[a]	92.4[a]	92.1[a]	2.08	<0.001	<0.001	<0.001
Thr	56.4[c]	66.5[b]	86.4[a]	88.0[a]	88.3[a]	2.74	<0.001	<0.001	0.022
Trp	66.9[b]	71.9[b]	91.8[a]	92.8[a]	91.6[a]	2.38	<0.001	<0.001	0.176
Val	54.7[c]	67.5[b]	88.1[a]	90.0[a]	90.6[a]	2.52	<0.001	<0.001	0.002
Mean	58.0[c]	70.7[b]	90.6[a]	92.4[a]	92.3[a]	2.09	<0.001	<0.001	<0.001
Dispensable AA, %									
Ala	54.6[c]	66.3[b]	88.2[a]	90.3[a]	88.7[a]	2.66	<0.001	<0.001	0.006
Asp	60.2[c]	71.5[b]	89.5[a]	91.2[a]	89.1[a]	1.98	<0.001	<0.001	<0.001
Cys	40.8[c]	52.6[b]	84.3[a]	85.5[a]	83.1[a]	3.16	<0.001	<0.001	0.028
Glu	67.1[c]	77.7[b]	92.1[a]	92.8[a]	87.0[a]	2.05	<0.001	<0.001	0.004

Table 3.5 (cont.)

Gly	55.1[c]	68.6[b]	91.1[a]	91.7[a]	88.6[a]	3.29	<0.001	<0.001	0.022
Pro	51.3[c]	72.9[bc]	106.7[ab]	107.3[ab]	105.3[a]	10.25	<0.001	<0.001	0.237
Ser	58.1[c]	66.4[b]	90.2[a]	91.8[a]	92.4[a]	2.26	<0.001	<0.001	0.02
Tyr	56.9[c]	70.7[b]	90.2[a]	91.1[a]	91.4[a]	2.29	<0.001	<0.001	<0.001
Mean	59.9[c]	72.1[b]	92.0[a]	93.2[a]	90.2[a]	2.27	<0.001	<0.001	0.002

[a-c] Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 12 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 from pigs fed the N-free diet as (g/kg Dry Matter Intake): CP, 21.45; Arg, 0.70; His, 0.28; Ile, 0.51; Leu, 0.87; Lys, 0.74; Met, 0.13; Phe, 0.50; Thr, 0.68; Trp, 0.14; Val, 0.77; Ala, 0.72; Asp, 1.17; Cys, 0.24; Glu, 1.50; Gly, 1.47; Pro, 3.29; Ser, 0.64; Tyr, 0.39.

Table 3.5 (cont.)

[3] P-values: Overall = the p-value for the comparison of diets containing all 5 ingredients; LT vs. HT = comparison of the two low temperature-processed full-fat meals vs. the two high temperature-processed meals; CV vs. LK = comparison of the two conventional soybeans vs. the two low-Kunitz soybeans.

[4] The interaction between the effect of processing temperature and the variety of soybeans was significant (P <0.05) for all AA except for Trp, Ala, and Pro.

CHAPTER 4

Phosphorus and energy digestibility of conventional and enzyme treated soybean meal fed to weanling pigs

Abstract: Two experiments were conducted to measure P and energy digestibility in conventional soybean meal (SBM-CV) and in soybean meal (SBM) that had been enzyme treated to remove the antigens in the meals. Phosphorus digestibility in SBM-CV and in 2 enzyme treated SBM (HP-310 and HP-340) was measured using 36 barrows (initial BW: 21.9 ± 1.1 kg) that were placed in metabolism cages and randomly allotted to 6 diets with 6 replicate pigs per diet. During production, HP-310 had been treated with an enzyme mixture containing no phytase while HP-340 was treated with an enzyme mixture that contained exogenous phytase. Three diets containing HP-310, HP-340, or SBM-CV as the sole source of P were formulated. Three additional diets also contained HP-310, HP-340, and SBM-CV, but each of these diets were fortified with 500 units of microbial phytase (Optiphos 2000, Enzyvia LLC, Sheridan, IN). Pigs were fed their experimental diets for 14 d with the final 5 d used for collection of fecal materials on a quantitative basis. The apparent total tract digestibility (ATTD) of P in HP-310 and SBM-CV increased ($P < 0.05$) as phytase was included in the diet (from 59.8 to 77.7% for HP-310 and from 65.5 to 79.5% for SBM-CV) but the ATTD of P in HP-340 without and with phytase was not different (83.8 and 87.7%, respectively). There were no differences in ATTD of P between HP-310 and SBM-CV, but the ATTD of P in HP-340 was greater ($P < 0.05$) than in the other 2 meals, which demonstrates that treatment of SBM with an enzyme mixture that contains phytase will result in increased P-digestibility. The DE and ME in corn, SBM-CV and in 2 sources of enzyme treated SBM (HP-200 and HP-310) were measured using 28 barrows (initial BW: 16.8 ± 2.5 kg BW) that were placed in metabolism cages. Pigs were randomly

allotted to 4 diets with 7 replicate pigs per diet. A corn-diet consisting of 96.45% corn and vitamins and minerals was formulated. Three additional diets were formulated by mixing corn and each source of SBM with vitamins and minerals. The experiment lasted 14 d and urine and feces were quantitatively collected during the last 5 d. The concentration of DE in HP-200, HP-310, and SBM-CV was 4,333, 4,316, and 4,347 kcal/kg DM, respectively. These values were not different, but they were greater ($P < 0.05$) than the DE in corn (3,891 kcal/kg DM). The concentration of ME was 3,780, 3,926, 3,914, and 3,980 kcal/kg DM in corn, HP-200, HP-310, and SBM-CV, respectively. These values were not different. It is concluded that enzyme treatment of SBM to remove antigens does not change the digestibility of P or energy in the meals.

Key words: energy, enzyme treated soybean meal, pigs, phosphorus, phytase, soybean meal

Introduction

Inclusion of soy proteins in diets fed to weanling pigs may negatively affect the pig by causing transient hypersensitivity, villi death, and mal-absorption of nutrients (Stokes et al., 1986; Li et al., 1990). Early weaned pigs fed soybean meal (**SBM**) based diets also have reduced growth performance compared with pigs fed diets containing no SBM because of the presence of antigens in SBM (Friesen et al., 1993). Therefore, animal proteins are usually included in diets fed to newly weaned pigs despite their higher costs. However, it is possible that the high priced animal proteins may be replaced by soy proteins if the antigens in the soy protein are removed. Antigens in SBM may be removed by enzymatic treatment using a proprietary blend of enzymes and enzymatically treated SBM is now available to the U.S. feed industry. The digestibility of AA in enzyme treated SBM has been measured (Min et al., 2004; Pahm and Stein, 2007;

Urbaityte et al., 2009), but there is no information on the digestibility of P and energy in enzyme treated SBM. The objective of this research, therefore, was to measure P digestibility and the concentration of DE and ME in enzyme treated SBM and in conventional SBM (**SBM-CV**) and to test the hypothesis that enzyme treatment of SBM does not compromise P and energy digestibility.

Materials and Methods

The experimental protocols were reviewed and approved by the Animal Care and Use Committee at the University of Illinois. Two experiments were conducted and SBM used in both experiments was sourced from Hamlet Protein A/S, Horsens, Denmark. No antibiotic growth promoters were used. Pigs used in the experiments were the offspring of Landrace boars that were mated to Yorkshire x Duroc females (Pig Improvement Company, Hendersonville, TN).

Exp. 1, Phosphorus Digestibility

Experiment 1 was designed to measure the digestibility of P in SBM-CV and in 2 sources of enzyme treated SBM (HP-310 and HP-340; Table 4.1). The 2 enzyme treated SBM and the SBM-CV were produced from the same batch of non-genetically modified soybeans. The enzymatic treatment takes place in a continuous-flow system where SBM is exposed to the enzyme preparation for several hours. The difference between HP-310 and HP-340 is that HP-340 was treated with an enzyme mixture that included phytase (Rhonozyme, DSM Nutritional Products AG, Basel, Switzerland) while no phytase was included in the enzymes used to produce HP-310.

Six diets were formulated (Tables 4.2 and 4.3). Three diets were formulated by mixing cornstarch, sugar, and each of the 3 sources of SBM. Three additional diets that were similar to

the initial 3 diets with the exception that 500 units of microbial phytase (Optiphos 2000, Enzyvia LLC, Sheridan, IN) was added to these diets were also formulated. Vitamins and all minerals except P were added to the diets to meet or exceed current requirement estimates (NRC, 1998). No inorganic P was added to the diets and the only source of P in the diets, therefore, was the P contributed by the SBM.

Thirty-six weanling barrows (initial BW: 21.9 ± 1.1 kg) were randomly allotted to the 6 diets with 6 replicate pigs per diet. Pigs were housed in metabolism cages that allowed for total collection of fecal materials. The daily feed allowance was calculated as 3 times the maintenance energy requirement (i.e., 106 kcal ME per kg $^{0.75}$; NRC, 1998) and divided into 2 equal meals. Water was available at all times. Pigs were fed their experimental diets for 14 d. The initial 7 d was an adaptation period to the diet. A marker, chromic oxide, was added to the morning meals on d 8 and d 13. Fecal collections were initiated upon first appearance of the marker in the feces after d 8, while fecal collections ceased when the marker first appeared in the feces after d 13 (Petersen and Stein, 2006).

Fecal samples were stored at -20^{o}C immediately after collection. At the conclusion of the experiment, fecal samples were dried in a forced air oven and finely ground. All samples of SBM, diets, and feces, were analyzed for DM by oven drying duplicate samples at 135°C for 2 h (method 930.15; AOAC Int., 2007). Phosphorus and Ca concentrations were analyzed in these samples by the inductively coupled plasma (ICP) spectroscopy procedure (method 985.01; AOAC Int., 2007) after wet ash sample preparation (method 975.03; AOAC Int., 2007). Ingredients and diets were also analyzed for CP (method 990.03; AOAC Int., 2007) and AA were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800. (Hitachi High Technologies America, Inc; Pleasaton, CA) using ninhydrin for postcolum derivatization and

norleucine as the internal standard. Before analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C (method 982.30 E (a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007). All diets were also analyzed for phytase activity (Phytex Method, version 1; Eurofins, Des Moines, IA), and ingredients were analyzed for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL). Benzoic acid was used as the internal standard for calibration. Additional analyses on ingredients included analysis for ether extract (method 2003.06; AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), sucrose, stachyose, and raffinose (method 982.14; AOAC Int., 2007) and trypsin inhibitor activity (method Ba 12-75; AOCS; 2006). The concentration of β-conglycinin and glycinin was also measured in the 3 sources of SBM (Van Biert and Hessing, 1993).

Following chemical analysis, the apparent total tract digestibility (**ATTD**) of P was calculated for each diet (Petersen and Stein, 2006). Because soybean meal was the only P contributing ingredient in the diets, the calculated ATTD for each diet also represents the digestibility of P in each source of SBM.

Data were analyzed using the Proc GLM procedure in SAS (SAS Institute Inc., Cary, NC). The Proc UNIVARIATE procedure was used to identify outliers. Values greater than 3 SD above or below the mean were classified as outliers and 1 outlier was removed from the HP-310 diet containing no added phytase. Treatment means were separated using the LSD test in Proc GLM. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among means.

Exp. 2, Energy Concentration

Experiment 2 was designed to measure the ATTD of energy and the DE and ME in SBM-CV and in 2 sources of enzyme treated SBM (HP-200 and HP-310). The difference between HP-200 and HP-310 is that HP-200 has a lower concentration of CP and AA than HP-310. The reason for a decrease in CP is because HP-200 goes through the same process as HP-300 but faster, leaving more sugars and anti-nutritional factors. Also, some fibers are added to HP-200 before enzyme treatment which explains the increase in CF content. A corn diet that contained 96.45% corn and vitamins and minerals was formulated. Three additional diets were formulated by mixing corn, each source of SBM, and vitamins and minerals (Tables 4.4 and 4.5).

A total of 28 weanling barrows (initial BW: 16.8 ± 2.5 kg) were randomly allotted to the 4 diets with 7 replicate pigs per diet. Pigs were placed in metabolism cages that were equipped with a feeder and a nipple drinker, slatted floors, a screen floor, and urine trays, which allowed for the total, but separate, collection of urine and fecal materials from each pig.

The quantity of feed provided per pig daily was calculated as 3 times the estimated requirement for maintenance energy for the smallest pig in each replicate and divided into 2 equal meals. Water was available at all times. The experiment lasted 14 d. The initial 7 d was considered an adaptation period to the diet, and fecal materials were collected during the following 5 d according to standard procedures using the marker to marker approach as explained for the P-digestibility experiment. Urine was collected from d 8 to d 13 in urine buckets over a preservative of 50 mL of HCl. Fecal samples and 20% of the collected urine were stored at -20^{o}C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a sub-sample was collected for analysis.

Fecal samples were dried in a forced air oven and finely ground. HP 200 was analyzed as described for the ingredients used in Exp. 1. Diets were analyzed for DM, GE, CP, Ca, P, NDF, ADF, and AA, and fecal samples were analyzed in duplicate for GE. The GE in urine was determined in triplicate samples. Approximately 10 mL of urine were added to a small cotton ball (0.2 to 0.3 g) that was placed in a plastic bag (approximately 0.2 g). The weights of the plastic bag, the cotton ball, and of the plastic bag containing the cotton ball and the urine were recorded. The bag was then lyophilized, the weight was recorded again, and the GE of the bag containing the cotton and the lyophilized urine was measured. The weight and the GE of 6 empty plastic bags and of 6 virgin cotton balls were also recorded, and the average GE of the 6 bags and of the 6 cotton balls on a per gram basis was assumed to represent the GE of the bags and the cotton, respectively. These values were then multiplied by the weight of the bag and the cotton ball, respectively, that had been bombed together with the urine, and the GE contributed by the plastic bag and the cotton ball was subtracted from the total GE that was measured in the bag containing the cotton ball and the urine to calculate the GE of the urine in the sample. Values for ATTD were calculated for energy in each diet (Widmer et al., 2007). The amount of energy lost in the feces and in the urine, respectively, was calculated and the quantities of DE and ME in each of the 4 diets were calculated (Widmer et al., 2007). The DE and ME in corn was calculated by dividing the DE and ME values for the corn diet by the inclusion rate of corn in the diet. These values were then used to calculate the contribution from corn to the DE and ME in the corn-SBM diets, and the DE and ME in each source of soybean meal was calculated by difference as previously described (Widmer et al., 2007).

Data were analyzed using the Proc GLM procedure and outliers were analyzed using Proc Univariate as described for Exp. 1. One outlier was removed from the HP-310 treatment.

An analysis of variance was conducted with pigs and diets as main effects. Treatment means were calculated using the LS Means statement in Proc GLM and if significant, means were separated using a LSD test. The pig was the experimental unit for all analyses and an alpha value of 0.05 was used to assess significance among means.

Results

The CP and AA concentrations were greater in HP-200, HP-310 and HP-340 than in SBM-CV (Table 1), but the concentration of CP and AA was lower in HP-200 than in HP-310 and HP-340. The concentration of ADF and NDF were also greater in HP-200, HP-310, and HP-340 than in SBM-CV, but the concentrations of sucrose and alpha-galactosides were much greater in SBM-CV than in the enzyme treated SBM. The trypsin inhibitor activity was low in all sources of SBM, but the concentration of β-conglycinin in SBM-CV was much greater than in the 3 enzyme treated SBM. The concentration of glycinin was also much greater in SBM-CV than in the other 3 SBM, but HP-200 contained more glycinin than HP-310 and HP-340.

Exp. 1, Phosphorus Digestibility

Feed intake and fecal output were not different among diets (Table 4.6). The percentage of DM digestibility and percentage of Ca in feces was also not different among individual treatments resulting in no differences in the ATTD of DM and Ca. However, for all sources of SBM, the percentage of P in feces was less ($P < 0.05$) when phytase was added to the diet than if no phytase was added to the diet. Pigs fed the HP-340 diets also had a lower ($P < 0.05$) concentration of P in the feces than pigs fed the HP-310 or the SBM-CV diets regardless of whether or not phytase was added to the diet. The total fecal output of P was reduced ($P < 0.01$) when phytase was added to the diets (1.26 vs. 0.74, 0.48 vs. 0.38, and 1.16 vs. 0.70 g/d for HP-

310, HP-340, and SBM-CV). The output of P from pigs fed the HP-340 diet without phytase was less ($P < 0.05$) than the P-output from pigs fed the HP-310 and the SBM-CV diets without phytase. The output of P from pigs fed the HP-340 diet with phytase was also less ($P < 0.05$) than for pigs fed the HP-310 diet with phytase. Pigs fed diets containing phytase also had greater ($P < 0.01$) ATTD of P than pigs fed diets without phytase (77.7 vs. 59.8% and 79.5 vs. 65.0% for HP-310 and SBM-CV) except for HP-340 (87.7 vs. 83.8%). The ATTD of P was also greater ($P < 0.05$) for HP-340 than for HP-310 and SBM-CV if no phytase was used, and if phytase was used, the ATTD of P in HP-340 was greater than in HP-310.

Exp. 2, Energy Digestibility

No differences were observed in GE intake or in GE excreted in the feces among pigs fed the 4 experimental diets (Table 4.7). Pigs fed the corn diet, however, excreted less ($P < 0.05$) GE in the urine than pigs fed the 3 SBM-containing diets. The ATTD of GE was not different among diets, but the DE in the HP-200, HP-310, and the SBM-CV diets were greater ($P < 0.001$) than the DE of the corn diet (3,454, 3,469, and 3,448 vs. 3,300 kcal/kg). The ME in the 3 SBM-containing diets was also greater ($P < 0.05$) than the ME in the corn diet (3,282, 3,297, and 3,285 vs. 3,207 kcal/kg), but no differences among the SBM diets were observed.

The DE in HP-310 (3,940 kcal/kg) was greater ($P < 0.05$) than in SBM-CV (3,779 kcal/kg), but the DE in HP-200 (3,887 kcal/kg) was not different from the DE in the other 2 sources of SBM. The DE in all 3 SBM was, however, greater ($P < 0.001$) than the DE in corn (3,422 kcal/kg). The ME in HP-200 and HP-310 (3,522 and 3,573 kcal/kg) were also greater ($P < 0.05$) than the ME in corn (3,325 kcal/kg), but the ME in SBM-CV (3,460 kcal/kg) was not different from that of any of the other ingredients. When calculated on a DM basis, no differences in the DE among HP-200, HP-310, and SBM-CV (4,333, 4,316, and 4,347 kcal/kg

DM) were observed, but all those values were greater ($P < 0.001$) than the DE of corn (3,891 kcal/kg DM). However, the ME was not different among ingredients (3,780, 3,926, 3,914, and 3980 kcal/kg DM for corn, HP-200, HP-310, and SBM-CV, respectively).

Discussion

Composition of Ingredients

The composition of the conventional SBM agrees with published values (NRC, 1998; Sauvant et al., 2004; Cervantes-Pahm and Stein, 2008). The enzyme treated SBM contains much lower concentrations of sucrose and alpha galactosides than SBM-CV. The reason for this difference is most likely that sucrose and oligosaccharides are either fermented during the enzyme treatment or they are separated from the SBM after enzyme treatment. The total concentration of sucrose and alpha galactosides in SBM-CV is approximately 10.6% and because of the disappearance of these carbohydrates in the enzyme treated SBM, the concentration of the remaining nutrients increase. Therefore, the concentrations of CP, ether extract, ADF, and NDF were greater in the enzyme treated SBM than in SBM-CV. The concentration of glycinin and β-conglycinin was also much less in the enzyme treated SBM than in SBM-CV, which indicates that enzyme treatment is effective in removing the antigens from SBM. The enzyme treated SBM may, therefore, be better tolerated by weanling pigs than SBM-CV, and it may be possible to include greater concentrations of enzyme treated SBM in diets fed to weanling pigs than if SBM-CV is used. The concentration of glycinin was less in HP-340 than in HP-200 and HP-310, but β-conglycinin concentration is the best predictor for the quantity of antigens in soybeans (Lalles et al., 1996) because β-conglycinin is more resistant to the proteolytic enzymes in the digestive processes than glycinin and other proteins (Zhao et al., 2008). As a consequence, β-conglycin contributes a relatively greater proportion of antigens in

SBM than glycinin does and there was no differences among the enzyme treated SBM in the concentration of β-conglycinin.

Phosphorus and Ca Digestibility

The digestibility of P in SBM-CV without phytase that was measured in this experiment is greater than most published values that have been in the range of 32 to 59% (Ajakaiye et al., 2003; Sauvant et al., 2004; Bohlke et al., 2005, Almeida et al., 2009). It is unknown why the ATTD of P in this particular source of SBM was greater than previous values, but the fact that the response obtained by adding phytase to the diet containing SBM-CV is similar to what has been reported in other experiments makes it unlikely that the value is erroneous. Relatively large variability in the ATTD of P among different sources of distillers dried grains with solubles has been reported (Pedersen et al., 2007). There are, however, very few published values for the ATTD of P in SBM and to our knowledge, there are no data on the variability in the ATTD of P among sources of SBM. Nevertheless, the ATTD of P in SBM-CV that was measured in this experiment and in other experiments during the last decade indicates that the digestibility of P in SBM produced from current varieties of soybeans is much greater than values for the relative bioavailability of P that have been published (NRC, 1998).

The ATTD of P in HP-310 was not different from the ATTD of P in SBM-CV, which indicates that enzyme treatment of this SBM does not increase the ATTD of P. However, the response to exogenous phytase in HP-310 is similar to that obtained for SBM-CV. We are unaware of any other data for the ATTD of P in enzyme treated SBM, but results of this experiment indicate that the ATTD of P in enzyme treated SBM is similar to that of SBM-CV and the response to phytase is similar for enzyme treated SBM and SBM-CV.

The ATTD of P in HP 340 was much greater than the ATTD of P in HP-310 and in SBM-CV, which is most likely a result of the fact that microbial phytase was included in the enzyme mixture that was used to treat this source of SBM. It appears, therefore, that treatment of SBM with phytase is very effective in improving the ATTD of P. The present results also demonstrate that addition of microbial phytase to a SBM that has already been treated with phytase has a much smaller effect in terms of increasing the ATTD of P than if phytase is added to a source of SBM that has not been treated with phytase. We are not aware of any previous experiments in which the effects of treating SBM with exogenous phytase have been measured.

All diets contained 0.70% limestone and assuming that limestone contains 36% Ca (NRC, 1998), this amount of limestone contributed approximately 0.25% Ca to the diets, which is equivalent to about 70% of all the Ca in the diets with the remaining 30% coming from the SBM. The values for ATTD of Ca in the diets are, therefore, a combination of the ATTD of Ca in limestone and the ATTD of Ca in SBM. If the ATTD of Ca in SBM is assumed to be 46.7% (Bohlke et al., 2005) and the ATTD of Ca in limestone is between 75 and 80% (Stein et al., unpublished) then the ATTD of the diets used in the present experiment should be approximately 65%, which is reasonably close to the values that were measured in the experiment. The ATTD of Ca in a diet containing 54% corn, 20% SBM and 0.69% limestone is 62.3% (Stein et al., 2008), and the values for the ATTD of Ca that were measured in this experiment also agree with this value.

There was no effect of phytase on the ATTD of Ca, which is most likely because most of the Ca in the diets originated from limestone. The fact that the ATTD of Ca in the HP-340 diet was not greater than the ATTD of Ca in the HP-310 diet indicates that treatment of SBM with phytase does not influence the digestibility of Ca in SBM.

The lack of any treatment differences on the output and ATTD of DM shows that enzyme treatment of SBM does not affect DM digestibility even though the gross composition of the meals were changed. This observation is in agreement with data showing that addition of exogenous phytase to SBM does not affect DM digestibility (Nitrayova et al., 2009).

Energy Digestibility

The values for DE and ME in corn that were measured in this experiment are in close agreement with previous data (NRC, 1998; Sauvant et al., 2004; Baker and Stein, 2009). The DE and ME values for SBM-CV are slightly greater than the values reported by Sauvant et al. (2004), but close to other published values (NRC, 1998). The lack of a difference in ME between corn and SBM-CV is also in agreement with the data reported by Baker and Stein (2009). Results from this experiment demonstrate that there are no differences in the DE and ME between SBM-CV and enzyme treated SBM despite the fact that nearly all the sucrose and oligosaccharides in the enzyme treated SBM were removed during processing. The concentration of ADF and NDF was, therefore, greater in the enzyme treated SBM than in SBM-CV, but HP-200 and HP-310 also contained more CP than SBM-CV, which is likely the reason that no differences in DE and ME among the SBM were observed. We are not aware of any other published data on the DE and ME in enzyme treated SBM, but results from the present experiment demonstrate that enzymatic treatment of SBM does not reduce the DE and ME. As a consequence, values for DE and ME in SBM-CV can also be used for enzyme treated SBM.

Conclusions

Enzymatic treatment of SBM changes the composition of the meals because sucrose, stachyose, and raffinose disappear during or after enzyme treatment. The concentration of CP,

ether extract, ADF, and NDF is, therefore, greater in enzyme treated SBM than in SBM-CV. However, these changes do not influence the digestibility of P and Ca, but a greatly improved ATTD of P is obtained if SBM is treated in the presence of microbial phytase. The concentration of DE and ME in the enzyme treated SBM is not different from that of SBM-CV and corn.

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Table 4.1. Chemical composition of enzymatically treated soybean meal and conventional soybean meal, as fed basis

Item	Ingredient			
	HP-200[1]	HP-310[1]	HP-340[1]	SBM-CV[1]
DM, %	89.70	91.28	90.98	87.32
GE, kcal/kg	4,435	4,473	4,446	4,159
CP, %	52.62	57.71	56.50	48.31
Ca, %	0.25	0.31	0.27	0.23
P, %	0.70	0.78	0.74	0.65
Ether extract, %	1.39	1.27	0.98	0.95
ADF, %	5.54	5.31	5.27	4.55
NDF, %	11.79	12.60	9.90	7.63
Ash, %	5.93	6.33	6.26	5.74
Trypsin inhibitor, (TIU^2/mg)	3.10	2.40	1.80	5.70
β-conglycinin, ppm	3	4	5	130,000
Glycinin, ppm	17,000	3,300	90	420,000

Table 4.1 (cont.)

Sucrose, %	0.20	0.20	0.20	5.78
Stachyose, %	0.26	0.27	0.20	3.78
Raffinose, %	0.42	0.43	0.21	1.05
Indispensable AA, %				
Arg	3.88	4.05	3.96	3.63
His	1.41	1.44	1.43	1.27
Ile	2.62	2.70	2.65	2.36
Leu	4.25	4.42	4.32	3.88
Lys	3.24	3.54	3.59	3.17
Met	0.72	0.73	0.73	0.70
Phe	2.76	2.89	2.80	2.51
Thr	2.03	2.11	2.09	1.89
Trp	0.76	0.81	0.79	0.72
Val	2.69	2.74	2.74	2.42
Dispensable AA, %				

Table 4.1 (cont.)

Ala	2.39	2.43	2.43	2.14
Asp	6.07	6.34	6.14	5.65
Cys	0.68	0.69	0.69	0.69
Glu	9.35	9.84	9.53	8.85
Gly	2.28	2.36	2.32	2.06
Pro	2.55	2.71	2.69	2.39
Ser	2.27	2.46	2.39	2.16
Tyr	1.90	2.01	1.95	1.78

[1]HP-200= low protein enzyme treated soybean meal; HP-310= enzyme treated soybean meal; HP-340 = enzyme treated soybean meal with phytase; SBM-CV= conventional soybean meal that has not been enzyme treated.

[2]TIU= trypsin inhibitor units.

Table 4.2. Ingredient composition of experimental diets (as-fed basis), Exp. 1

	No Phytase			Phytase		
Ingredient, %	HP-310[1]	HP-340[1]	SBM-CV[1]	HP-310[1]	HP-340[1]	SBM-CV[1]
HP-310[1]	38.00	-	-	38.00	-	-
HP-340[1]	-	38.00	-	-	38.00	-
SBM-CV[1]	-	-	44.00	-	-	44.00
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0
Ground limestone	0.70	0.70	0.70	0.70	0.70	0.70
Sugar	15.00	15.00	15.00	15.00	15.00	15.00
Cornstarch	42.60	42.60	36.60	42.58	42.58	36.58
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase premix [2]	-	-	-	0.03	0.03	0.03
Vit. mineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30

[1]HP-310= enzyme treated soybean meal; HP-340 = enzyme treated soybean meal with phytase; SBM-CV= conventional soybean meal that has not been enzyme treated.

[2]Optiphos 2000 (Enzyvia LLC, Sheridan, IN) included at 0.025% provides 500 units of phytase per kilogram of complete diet.

Table 4.2 (cont.)

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.3. Analyzed composition of experimental diets (as-fed basis), Exp 1

Item	No Phytase			Phytase		
	HP-310[1]	HP-340[1]	SBM-CV[1]	HP-310[1]	HP-340[1]	SBM[1]
DM, %	91.60	91.53	90.12	91.64	91.50	90.27
CP, %	22.19	21.37	21.48	21.48	21.74	20.46
Ca, %	0.37	0.38	0.44	0.44	0.37	0.46
P, %	0.31	0.29	0.32	0.32	0.30	0.33
Phytase, units/kg	85	130	<70	820	680	640
Indispensable AA, %						
Arg	1.63	1.55	1.52	1.57	1.54	1.51
His	0.57	0.57	0.56	0.55	0.56	0.54
Ile	1.09	1.04	0.99	1.07	1.03	1.00
Leu	1.82	1.73	1.66	1.76	1.71	1.66
Lys	1.42	1.41	1.34	1.37	1.40	1.33
Met	0.29	0.28	0.29	0.28	0.28	0.29
Phe	1.18	1.12	1.08	1.14	1.11	1.07
Thr	0.86	0.83	0.81	0.81	0.82	0.79

Table 4.3 (cont.)

Trp	0.31	0.31	0.31	0.31	0.30	0.30
Val	1.11	1.08	1.02	1.10	1.07	1.04
Dispensable AA, %						
Ala	1.00	0.98	0.92	0.97	0.97	0.91
Asp	2.61	2.47	2.40	2.52	2.44	2.38
Cys	0.28	0.27	0.29	0.26	0.27	0.30
Glu	4.12	3.89	3.78	3.96	3.84	3.75
Gly	0.96	0.93	0.89	0.93	0.92	0.88
Pro	1.12	1.07	1.03	1.06	1.04	1.02
Ser	1.00	0.94	0.91	0.92	0.92	0.87
Tyr	0.66	0.65	0.67	0.65	0.67	0.68

[1]HP-310= enzyme treated soybean meal; HP-340 = enzyme treated soybean meal with phytase; SBM-CV= conventional soybean meal that has not been enzyme treated.

Table 4.4. Ingredient composition of experimental diets (as-fed basis), Exp 2

	Diet			
Ingredient, %	Corn	HP-200	HP-310	SBM-CV
Ground corn	96.45	68.00	68.00	62.00
HP-200[1]	-	29.00	-	-
HP-310[1]	-	-	29.00	-
SBM-CV[1]	-	-	-	35.00
Ground limestone	1.35	0.9	0.9	0.9
Monocalcium phosphate	1.50	1.40	1.40	1.40
Salt	0.40	0.40	0.40	0.40
Vitamin micromineral premix [2]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[1] HP-200 = low protein enzyme treated soybean meal; HP-310 = enzyme treated soybean meal; SBM-CV = conventional soybean meal that has not been enzyme treated.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24

Table 4.4 (cont.)

mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.5. Analyzed energy and nutrient composition of experimental diets (as-fed basis), Exp. 2

	Diet			
Item	Corn	HP-200[1]	HP-310[1]	SBM-CV[1]
DM, %	87.82	88.14	88.68	87.99
GE, kcal/kg	3,734	3,942	3,943	3,898
CP, %	8.22	21.23	21.52	20.87
Ca, %	0.76	0.66	0.67	0.72
P, %	0.54	0.62	0.65	0.67
NDF, %	8.99	9.65	12.69	9.62
ADF, %	2.03	3.12	3.09	2.94
Indispensable AA, %				
Arg	0.42	1.36	1.39	1.45
His	0.22	0.55	0.55	0.58
Ile	0.27	0.91	0.91	0.94
Leu	0.90	1.81	1.86	1.86
Lys	0.31	1.10	1.15	1.23

Table 4.5 (cont.)

Met	0.15	0.31	0.30	0.33
Phe	0.38	1.03	1.05	1.07
Thr	0.28	0.76	0.78	0.80
Trp	0.10	0.27	0.27	0.29
Val	0.37	1.00	0.99	1.03
Dispensable AA, %				
Ala	0.55	1.05	1.06	1.07
Asp	0.58	2.10	2.14	2.22
Cys	0.16	0.31	0.29	0.33
Glu	1.47	3.64	3.75	3.80
Gly	0.33	0.86	0.86	0.89
Pro	0.61	1.12	1.17	1.19
Ser	0.35	0.87	0.91	0.90
Tyr	0.28	0.68	0.69	0.71

[1] HP-200 = low protein enzyme treated soybean meal; HP-310 = enzyme treated soybean meal; SBM-CV = conventional soybean meal that has not been enzyme treated.

Table 4.6. Digestibility of DM, Ca, and P by pigs fed 3 sources of soybean meal without or with supplemental phytase[1,2]

	Soybean meal[3]									
	HP-310	HP-340	SBM-CV	HP-310	HP-340	SBM-CV		*P*-value		
Phytase, FTU/kg:	0	0	0	500	500	500	SEM	SBM	Phytase	SBM × Phytase
Feed intake										
Total feed intake, g/d	1,003	1,006	1,054	1,032	1,016	1,032	84.51	0.16	0.69	0.33
DM intake, g/d	919.2	921.4	950	946	930.2	931.8	77.20	0.62	0.65	0.36
Ca intake, g/d	3.7[b]	3.8[b]	4.6[a]	4.5[a]	3.8[b]	4.7[a]	0.34	<0.001	<0.001	<0.001
P intake, g/d	3.14	2.96	3.36	3.34	3.06	3.4	0.27	<0.001	0.02	0.37
Fecal output										
Total dried feces, g/d	36.4	36.6	33.8	37.4	36.0	34.8	10.21	0.46	0.79	0.92
DM in feces, %	91.0	93.4	92.1	93.0	93.9	91.7	0.73	0.04	0.22	0.25

Table 4.6 (cont.)

Ca in feces, %	4.8	3.7	3.3	4.8	3.1	2.4	0.39	<0.001	0.11	0.45
P in feces, %	3.5[a]	1.3[cd]	3.4[a]	2.0[bc]	1.1[d]	2.0[b]	0.13	<0.001	<0.001	<0.001
DM output, g/d	33	34.2	31.2	34.8	33.8	32	10.08	0.40	0.65	0.87
Ca output, g/d	1.7	1.4	1.1	1.8	1.1	0.8	0.73	<0.001	0.20	0.50
P output, g/d	1.3[a]	0.5[bc]	1.2[a]	0.7[b]	0.4[c]	0.7[bc]	0.32	<0.001	<0.001	0.01
Digestibility, %										
ATTD[4] of DM	96.4	96.3	96.7	96.3	96.4	96.6	0.19	0.19	0.78	0.85
ATTD of Ca	53.6	63.8	75.6	60.9	70.0	82.2	3.59	<0.001	0.03	0.99
ATTD of P	59.8[c]	83.8[ab]	65.5[c]	77.7[b]	87.7[a]	79.5[ab]	1.87	<0.001	<0.001	0.002

[a,b,c,d] Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 6 observations.

[2]Feed intake and fecal output were based on daily amounts collected.

Table 4.6 (cont.)

[3]HP-310 = enzyme treated soybean meal; HP-340 = enzyme treated soybean meal with phytase; SBM-CV = conventional SBM.

[4]ATTD = Apparent total tract digestibility.

Table 4.7. Energy digestibility and DE and ME in diets and ingredients, Exp. 2[1]

Item	Diet				SEM	*P*-value
	Corn	HP-200[2] 29%	HP-310[2] 29%	SBM-CV[2] 35%		
Diet intake, g/d	762	784	796	788	0.26	0.97
GE intake, kcal/d	2,843	3,092	3,141	3,074	995	0.74
GE fecal output, kcal/d	332	379	374	354	112	0.46
GE urine output, kcal/d	73[a]	135[b]	139[b]	130[b]	87.7	0.03
ATTD[3], %	88.4	87.6	88.0	88.5	0.5	0.55
DE in diets, kcal/kg	3,300[a]	3,454[b]	3,469[b]	3,448[b]	17.4	0.01
ME in diets, kcal/kg	3,207[a]	3,282[b]	3,297[b]	3,285[b]	23.7	0.03
DE in ingredients, kcal/kg	3,422[c]	3,887[ab]	3,940[a]	3,779[b]	56.7	0.01
ME in ingredients, kcal/kg	3,325[b]	3,522 [a]	3,573[a]	3,460[ab]	63.8	0.05
DE in ingredients, kcal/kg DM	3,891[b]	4,333[a]	4,316[a]	4,347[a]	63.4	0.01
ME in ingredients, kcal/kg DM	3,780	3,926	3,914	3,980	71.0	0.22

[a,b] Values within a row lacking a common superscript letter are different ($P < 0.05$).

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

[2]HP-200 = enzyme treated low protein soybean meal; HP310 = enzyme treated high protein soybean meal; SBM-CV= conventional soybean meal.

[3]ATTD = apparent total tract digestibility of energy.

CHAPTER 5

Fat digestibility in enzymatically treated soybean meal without and with choice white grease and vegetable oil

Abstract: An experiment was conducted to measure the digestibility of fat by weanling pigs fed enzymatically treated soybean meal and either soybean oil or choice white grease. Two sources of enzymatically treated soybean meals were used (HP-300 and HP-350). These meals are similar with the exception that an emulsifier, lecithin, is included in HP-350, but not in HP-300. The HP-300 meal contained 57.07% CP, 1.44% acid-hydrolyzed ether extract (AEE), and 2.30 trypsin inhibitor units (TIU) per mg, and HP-350 contained 53.60% CP, 3.73% AEE, and 1.50 TIU per mg. Two diets were formulated by mixing cornstarch, sugar, 6% soy oil, and each source of soybean meal. Two additional diets that were similar to the initial 2 diets with the exception that 6% choice white grease was substituted for the 6% soybean oil was added to these diets and were also formulated. Thirty-two weanling barrows (initial BW: 13.3 ± 0.8 kg) were randomly allotted to the 4 diets with 8 replicate pigs per diet in a 2 x 2 factorial design. Pigs were housed in metabolism cages. Pigs were fed experimental diets for 14 d with total collections of feces during the final 5 d. Feed intake and DM output were not different among treatments. The apparent total tract digestibility (ATTD) of DM and GE were not different among treatments regardless of soybean meal and fat source. The ATTD of AEE in HP-300 and HP-350 mixed with soybean oil was not different (80.4 and 75.7%, respectively). The ATTD of AEE in HP-300 and HP-350 mixed with choice white grease was also not different (80.2% and 79.3%, respectively). Results indicated that the added

lecithin in HP-350 did not increase fat digestibility in pigs fed diets supplemented with soybean oil or choice white grease.

Key words: fat digestibility, lecithin, pigs, soybean meal

Introduction

Fat is often added to diets fed to weanling pigs to increase the energy concentration of the diets. However, weanling pigs have a limited capacity to digest fat (Cera et al., 1988) and it is essential to feed newly weaned pigs with a fat source that is highly digestible (Cera et al., 1989). For an animal to utilize and digest fat, the fat has to emulsify in the gastrointestinal tract (Øverland et al., 1993a, b; Jensen et al., 1997). The main emulsifying agent is bile acid that breaks down the dietary fat globule and keeps it in smaller emulsions (Wieland et al., 1993). Bile acid also helps keep the digested fatty acids and monoglycerides in micelles that can be absorbed into the intestinal villi and transported via the lymphatic system to the tissues (Wilson et al., 1971; Rombeau and Caldwell, 1990; Wieland et al., 1993).

It has been suggested that weanling pigs are not capable of synthesizing enough bile acid to fully emulsify dietary fat, and addition of emulsifying agents has been suggested to improve fat digestibility in weanling pigs (Øverland et al., 1993a). An emulsifying agent that may be used to possibly help young pigs better digest lipids is lecithin, a phospholipid derived from phosphatidyl choline, which is extracted from soybeans (Øverland et al., 1993a). With added dietary lecithin, fat utilization by young pigs may be greater than if fat is added to diets containing no lecithin. Usually, lecithin is added directly to the diet, but a new source of enzyme treated soybean meal (HP-350)

contains added lecithin. It is possible, therefore, that inclusion of HP-350 to diets fed to weanling pigs will result in an improved digestibility of fat, but this hypothesis has not been experimentally verified. It was the objective of this experiment to measure the digestibility of fat by weanling pigs fed HP-350 and compare that to pigs fed a source of enzyme treated soybean meal that did not contain an emulsifier.

Materials and methods

Animals, Housing, and Experimental Design

The experimental protocol was reviewed and approved by the Animal Care and Use Committee at the University of Illinois. Thirty-two weanling barrows (initial BW: 13.3 ± 0.8 kg) were randomly allotted to 4 diets with 8 replicate pigs per diet in a 2 x 2 factorial design. Pigs were placed in metabolism cages that allow for total collection of fecal materials. Feed was provided in the amount of 3 times the maintenance energy requirement (106 kcal of ME/$kg^{0.75}$; NRC, 1998) and divided into 2 daily meals. Water was available at all times. Pigs were fed their experimental diets for 12 d. The initial 5 d was considered an adaptation period to the diet, while fecal materials were collected during the following 5 d using the marker to marker approach.

Ingredients, Diets, Feeding, and Sample Collection

Two enzyme treated SBM (HP-300 and HP-350) that were produced by Hamlet Protein A/S, Horsens, Denmark were used (Table 5.1). The difference between HP-300 and HP-350 is that HP-350 is treated with an enzyme mixture and lecithin while no lecithin was used in the production of HP-300. Two diets were formulated by mixing cornstarch, sugar, 6% soybean oil, and each source of soybean meal (Tables 5.2 and 5.3).

Two additional diets that were similar to the initial 2 diets with the exception that 6% choice white grease was used instead of soybean oil were also formulated. Vitamins and all minerals were added to all diets according to current requirement estimates (NRC, 1998). Pigs were fed their experimental diets for 14 d. The initial 7 d was an adaptation period to the diet. A marker was added to the morning meals on d 8 and d 13. Fecal collections were initiated upon first appearance of the marker in the feces after d 8, while fecal collections ceased when the marker first appeared in the feces after d 13 (Petersen and Stein, 2006).

Chemical Analysis

Fecal samples were stored at -20^{o}C immediately after collection. At the conclusion of the experiment, fecal samples were dried in a forced air oven and finely ground. Samples of HP-300 and HP-350, diets, and feces, were analyzed for DM by oven drying duplicate samples at 135°C for 2 h (method 930.15; AOAC Int., 2007). Phosphorus and Ca concentrations were analyzed in ingredients and diets by the inductively coupled plasma (ICP) spectroscopy procedure (method 985.01; AOAC Int., 2007) after wet ash sample preparation (method 975.03; AOAC Int., 2007). Ingredients and diets were also analyzed for CP (procedure 990.03; AOAC Int., 2007) and AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasaton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after

NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007). Ingredients, diets, and fecal samples were also analyzed for GE using a bomb calorimeter (Model 6300, Parr Instruments, Moline, IL). Benzoic acid was used as the internal standard for calibration. Additional analyses on ingredients and diets included analysis for ADF (method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Ingredients were also analyzed for ash (method 7.009, AOAC Int., 2007), sucrose, stachyose, and raffinose (method 982.14; AOAC Int., 2007), and trypsin inhibitor activity (method Ba 12-75; AOCS; 2006). The concentration of total fat was measured in diets, ingredients, and fecal samples after 3*N* HCl hydrolysis, followed by ether extraction (Sanderson, 1986).

Calculations and Data Analysis

Following chemical analysis, the daily balance of energy, DM, and AEE was calculated according to Adeola (2001). The apparent total tract digestibility (**ATTD**) values were calculated for acid-hydrolyzed ether extract (**AEE**) in each diet using the following equation (Adeola, 2001):

> ATTD of AEE, % = 100 x [(amount of AEE consumed – amount of AEE in feces)/amount of AEE consumed]

The ATTD of energy and DM were also calculated using this equation. Data were analyzed using the Proc GLM Procedure in SAS (SAS Inst. Inc., Cary, NC). The Proc UNIVARIATE procedure was used to identify outliers. Values greater than 3 SD above or below the mean were classified as outliers, but no outliers were identified. Least squares means were calculated using an LSD test and means were separated using the pdiff statement in Proc GLM. Diet and ingredient were the fixed effects and pig and

replicate were the random effects. Replicate and interaction were included in the initial model, but were excluded from the final model due to insignificance. The pig was the experimental unit for all calculations and an alpha level of 0.05 was used to assess significance among means.

Results

The concentration of CP was greater in HP-300 than in HP 350; 57.07% vs. 53.60% CP (Table 5.1). The concentration of all AA was also greater in HP-300 than in HP-350. However, the concentration of AEE in HP-350 (3.72%) was greater than in HP-300 (1.44%). The concentrations of sucrose, stachyose, and raffinose were low in both soybean meals. The TIU activity was also low in both sources of soybean meal (2.30 and 1.50 TIU/mg in HP-300 and HP-350, respectively).

Feed intake and DM intake were not different among treatments (Table 5.4). Pigs fed the diets containing HP-350 consumed slightly more ($P < 0.01$) GE than pigs fed the diets containing HP-300, and the intake of AEE was also greater ($P < 0.01$) for pigs fed HP-350 than for pigs fed HP-300. There was also a trend ($P = 0.096$) for pigs fed the soybean oil containing diets to have a greater AEE intake than pigs fed the choice white grease containing diets. There were no differences in the ATTD of DM, GE, or AEE between the 2 soybean meal sources, but there was a tendency ($P = 0.078$) for a greater ATTD of AEE from soybean oil than from choice white grease.

Discussion

In early weaned pigs, greater ATTD values of fat have been measured in pigs fed diets containing corn oil compared with diets containing animal fat (Cera et al., 1988) and

Sewell and Miller (1965) suggested that newly weaned pigs utilize diets supplemented with corn oil better than diets supplemented with animal fat. However, these differences in digestibility between fat sources are not observed in older pigs (Cera et al., 1988).

The phospholipid lecithin or phosphatidyl choline, is obtained by extraction from soybean oil and used for fat emulsification. Because fat is insoluble in water, emulsification is required for digestion and absorption of fat in the gastrointestinal tract. Therefore, lecithin can be used as an exogenous emulsifier added to a weanling pig diet to increase fat digestibility (Jones et al., 1990a, b; Øverland et al., 1993a). Lecithin has also been used to increase fat digestibility in humans (Aldersberg and Sobotka, 1943), chicks (Polin, 1980), and calves (Hopkins et al., 1959). However, in weanling pigs, inconsistent results with lecithin have been reported. These differences may be explained by the different fat sources used or simply by the age of the pig. For example, Allee et al. (1971) suggested that the energy:AA ratio may affect fat digestibility and Cera et al. (1988) suggested that differences in pig age would cause differences in fat digestibility.

In the present experiment, a source of enzymatically treated soybean meal that contains an emulsifier was used. To our knowledge, effects of adding such a feed ingredient to young pig diets have never before been investigated. It was expected that the pigs fed the diets containing HP-350 had greater ATTD of AEE than pigs fed HP-300, but that was not the case. This observation indicates that the type of emulsifier that was added to HP-350 was not effective in increasing the digestibility of fat. This response is in agreement with previous data suggesting that lecithin does not affect the ATTD of DM, GE, and fat (Cho et al., 2008). The ATTD of DM and energy were also not different among the soybean meal and fat sources although a tendency for a greater

ATTD of AEE in soybean oil than in choice white grease was observed. Previously, Cera et al. (1988) reported that the ATTD of fat in corn oil is greater than in tallow.

The small increases in GE intake and in AEE intake for pigs fed the diets containing HP-350 compared with pigs fed the diets containing HP-300 is probably a result of the greater concentration of fat in HP-350 than in HP-300. These small differences are not believed to have influenced the outcome of the experiment.

The concentration of emulsifier in HP-350 was not disclosed, but it is possible that the reason for the lack of a response to HP-350 on the ATTD of AEE is that the inclusion rate of the emulsifier to the final diet was too small to have an effect. With an inclusion rate of 10% of HP-350 to the diets, the inclusion of the emulsifier in the final diet is only 10% of that included in HP-350.

In conclusion, pigs fed diets containing a source of soybean meal that has an emulsifier added to it did not have a greater digestibility of DM, energy, and AEE than pigs fed diets containing soybean meal without the emulsifier.

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Table 5.1. Chemical composition of enzymatically treated soybean meal, as fed basis

	Ingredient	
Ingredients, %	HP-300[1]	HP-350[1]
DM, %	92.84	92.38
GE, kcal/kg	4,699	4,751
CP, %	57.07	53.6
Ca, %	0.33	0.31
P, %	0.75	0.62
AEE[2], %	1.44	3.72
ADF, %	4.85	4.6
NDF, %	8.46	20.18
Ash, %	7.36	8.73
Trypsin inhibitor, (TIU/mg)	2.30	1.50
Sucrose, %	<0.20	<0.20
Stachyose, %	0.32	0.43
Raffinose, %	0.58	0.48
Indispensable AA, %		

Table 5.1 (cont.)

Arg	4.14	3.24
His	1.51	1.33
Ile	2.9	2.62
Leu	4.68	4.17
Lys	3.45	3.19
Met	0.75	0.66
Phe	3.1	2.76
Thr	2.08	1.88
Trp	0.79	0.7
Val	3.04	2.71
Dispensable AA, %		
Ala	2.49	2.27
Asp	6.45	5.83
Cys	0.77	0.61
Glu	9.57	8.76
Gly	2.37	2.19

Table 5.1 (cont.)

Pro	2.72	2.39
Ser	2.11	1.99
Tyr	2.29	1.98

[1]HP-300= enzyme treated soybean meal; HP-350= enzyme treated soybean meal with lecithin.

[2]AEE= acid-hydrolyzed ether extract.

Table 5.2. Composition of experimental diets containing HP-300 or HP-350[1], as-fed basis

Ingredients, %	HP-300		HP-350	
	Soybean oil	Choice white grease	Soybean oil	Choice white grease
Ground corn	50.95	50.95	50.95	50.95
Soybean meal, 48%	20.00	20.00	20.00	20.00
HP 300	10.00	10.00	-	-
HP 350	-	-	10.00	10.00
Soybean oil	6.00	-	6.00	-
Choice white grease	-	6.00	-	6.00
Lactose	10.00	10.00	10.00	10.00
Ground limestone	1.05	1.05	1.05	1.05
Monocalcium phosphate	1.10	1.10	1.10	1.10
L-lysine HCL	0.15	0.15	0.15	0.15
DL-methionine	0.05	0.05	0.05	0.05
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30

Table 5.2 (cont.)

[1]HP-300= enzyme treated soybean meal; HP-350= enzyme treated soybean meal with lecithin.

[2] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 5.3. Analyzed composition of experimental diets, as fed basis

	HP-300[1]		HP-350[1]	
Item	Soybean oil	Choice white grease	Soybean oil	Choice white grease
DM, %	89.04	89.33	89.33	89.31
GE, kcal/kg	4,172	4,213	4,217	4,482
CP, %	18.84	19.03	18.62	18.58
Ca, %	0.83	0.81	0.79	0.84
P, %	0.55	0.55	0.58	0.59
ADF, %	3.08	2.74	2.99	2.91
NDF, %	8.13	7.51	7.87	8.92
AEE[2], %	8.11	7.88	8.37	8.49
Indispensable AA, %				
Arg	1.21	1.23	1.18	1.09
His	0.46	0.47	0.48	0.43
Ile	0.81	0.82	0.84	0.75
Leu	1.45	1.51	1.54	1.34
Lys	1.11	1.17	1.17	1.08
Met	0.31	0.31	0.31	0.29

Table 5.3 (cont.)

Phe	0.89	0.91	0.93	0.84
Thr	0.65	0.67	0.66	0.62
Trp	0.23	0.24	0.22	0.22
Val	0.9	0.91	0.92	0.85
Dispensable AA, %				
Ala	0.86	0.87	0.88	0.82
Asp	1.86	1.91	1.91	1.76
Cys	0.28	0.29	0.29	0.27
Glu	3.00	3.07	3.10	2.87
Gly	0.75	0.77	0.77	0.71
Pro	0.91	0.97	0.98	0.85
Ser	0.69	0.71	0.70	0.68
Tyr	0.58	0.59	0.60	0.53

[1]HP-300= enzyme treated soybean meal; HP-350= enzyme treated soybean meal with lecithin.

[2] AEE= acid-hydrolyzed ether extract.

Table 5.4. Daily balance and apparent total tract digestibility (ATTD) of acid-hydrolyzed ether extract (AEE) in diets containing 2 sources of enzyme treated soybean meal with addition of either soybean oil or choice white grease, as fed basis[1]

	HP-300[2]		HP-350[2]			*P*-values	
Item	Soybean oil	Choice white grease	Soybean oil	Choice white grease	RMSE	Soybean meal	Fat source
Feed intake, g/d	615	594	622	611	164	0.310	0.179
DMI, g/d	548	530	555	546	146	0.275	0.205
GE intake, kcal/d	2,567	2,512	2,622	2,740	699	0.006	0.595
AEE intake, g/d	49.8	46.8	52.0	51.9	13.2	0.001	0.096
Feces, g/d	63.1	64.8	63.2	64.7	54.5	0.998	0.678
Feces AEE, %	15.7	17.7	16.3	16.6	2.35	0.774	0.170
Feces output, kcal/d	304	318	308	315	275	0.984	0.589
AEE output, g/d	9.8	11.6	10.3	10.8	11.85	0.894	0.191

Table 5.4 (cont.)

ATTD of DM, %	89.3	88.7	89.3	88.9	1.63	0.876	0.357
ATTD of GE, %	88.2	87.4	88.3	88.5	1.84	0.361	0.713
ATTD of AEE[2], %	80.4	75.7	80.2	79.3	4.38	0.281	0.078

[1] The interaction between fat source and source of soybean meal was analyzed but not significant. The interaction term was, therefore, removed from the final model.

[2] HP-300= enzyme treated soybean meal; HP-350= enzyme treated soybean meal with lecithin.

CHAPTER 6

Conclusion

Results of the 4 experiments in this thesis have demonstrated that swine producers may benefit from using new soybean products rather than conventional soybean meal (SBM). The biggest limiting factor in feeding SBM is the high concentrations of anti-nutritional factors, which include trypsin inhibitors, antigens, lectins, and oligosaccharides. However, with new technology, scientists have been able to develop new hybrids and processing methods. For example, the first experiment measured AA digestibility of a new variety of soybean with a low concentration of Kunitz trypsin inhibitors, one of the 2 trypsin inhibitors present in soybeans. It was demonstrated that it would be advantageous to use a low-Kunitz variety of soybean compared with using a conventional bean; however, heat treated soybeans, regardless of variety, maximize AA digestibility. It is, therefore, necessary to use heat treatment of conventional as well as low-Kunitz soybeans.

The new processing methods to treat soybeans with a proprietary blend of enzymes produced several new soy products including HP-200, HP-300, HP-310, HP-340, and HP-350. All of these soy products have been enzymatically processed to remove most of the antinutritional factors. Therefore, 3 experiments were conducted to measure P, energy, and fat digestibility in these new sources of SBM.

Results showed that enzyme treatment does not influence the digestibility of P, however the ATTD of P is greatly improved if SBM is treated in the presence of microbial phytase. Furthermore, energy digestibility of enzyme treated SBM was not different from that of

conventional SBM. Lastly, fat digestibility was not improved when emulsifiers, such as lecithins, were added to the enzyme treatment as was the case for HP-350.

In conclusion, it is critical to feed young pigs only soybeans that have been heat treated. Currently, hybrids with low-Kunitz concentrations are available, but if they are not heated they do not have AA digestibility similar to that of conventional SBM. Enzyme treated SBM can be fed to young pigs and have similar P and energy digestibilities as conventional SBM despite containing less sugars and oligosaccharides. Therefore, these new soybean products can be used successfully in diets fed to weanling pigs.