NUTRITIONAL EVALUATION OF FERMENTED SOYBEAN MEAL AND FERMENTED FULL-FAT SOYBEANS FED TO CHICKENS AND PIGS

BY

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THESIS

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ABSTRACT

Eight experiments were conducted to determine the nutritional value of conventional solvent-extracted defatted soybean meal (SBM-CV), fermented conventional soybean meal (FSBM), full-fat soybeans (FFSB), and fermented full-fat soybeans (FFFSB) in chickens and pigs. In Experiments 1 and 2, two precision-fed rooster assays were performed to determine the nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid (AA) digestibility among the test ingredients using conventional and cecectomized roosters, respectively. Full-fat ingredients presented greater TME_n values than conventional ingredients ($P<0.05$). Fermentation had a positive effect on TME_n of SBM-CV and a negative effect on FFSB. There were no differences in standardized AA digestibility between SBM-CV and FFSB. The fermented ingredients had lower ($P<0.05$) standardized AA digestibility values compared with their unfermented counterparts. In Experiment 3, an ad libitum-fed broiler chicken assay was conducted to determine apparent ileal P digestibility and total tract P retention at two dietary Ca levels (0.2% and 0.75%) among the test ingredients. Diets contained a Ca:non-phytate P (NPP) ratio of either 2 or 7.5. Greater ($P<0.05$) apparent ileal P digestibility values were observed at the low Ca level than at the high Ca level. At the high Ca level, fermentation increased the ileal P digestibility and total tract P retention for both conventional and full-fat samples, while at the low Ca level, there was a reduction ($P<0.05$) in total tract P retention for FFFSB. In Experiments 4 and 5, two 17 d chick trials were conducted to determine the P bioavailability of the test ingredients relative to KH_2PO_4 using crossbred chicks (Experiment 4) and a similar trial but using only SBM-CV and FSBM in commercial broiler chicks (Experiment 5). Multiple regression of bone ash in mg/tibia and % on supplemental P intake yielded slope-ratio relative P bioavailabilities between 23% and 48%. Fermentation did not affect relative P

bioavailability in SBM-CV and increased the relative bioavailability values in full-fat samples in crossbred chicks. In commercial broiler chicks, there were no differences in relative P bioavailability between SBM-CV and FSBM. In Experiment 6, 40 growing barrows and gilts (initial BW: 13.9 ± 1.3 kg) were housed individually in metabolism crates and used in a complete randomized design. Pigs were fed a corn-based diet or four diets containing corn and each source of soybean product with 8 replicate pigs per diet. Fecal and urine samples were collected for 4 d after 5 d of adaptation. Results from Experiment 6 indicated that the concentration of ME in the test ingredients was not different between SBM and FSBM, but FFFSB had a lower ME concentration than FFSB ($P<0.05$). In Experiment 7, 10 growing barrows (initial BW: 11.3 ± 0.8 kg) with a T-cannula in the distal ileum were allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods for a total of 10 replicate pigs per diet. Four diets included either SBM-CV, FSBM, FFSB, or FFFSB as the sole source of crude protein (CP) and AA. A N-free diet was used to determine the basal endogenous losses of CP and AA. Ileal digesta were collected on days 6 and 7 of each period after 5 d of adaptation to the diets. Results from Experiment 7 indicated that fermentation reduced ($P<0.05$) the AID and SID of indispensable AA in SBM-CV and FFSB. In Experiment 8, 80 growing barrows and gilts (initial BW: 12.3 ± 1.6 kg) were placed in metabolism crates and allotted to four diets with 8 pigs per diet using a 2 × 2 × 2 factorial treatment arrangement. Each source of soybean product was included in a diet without microbial phytase and in a diet with microbial phytase (500 units/kg diet). Pigs were adapted to the diets for 5 d, and fecal samples were collected for 4 d. Results from Experiment 8 indicated that ATTD and STTD of P were greater ($P<0.05$) in fermented ingredients compared with non-fermented ingredients. The ATTD and STTD of P was also greater ($P<0.05$) in full-fat ingredients compared with conventional ingredients. The ATTD and STTD of P was greater

(P<0.05) in diets with phytase inclusion compared with diets without phytase inclusion. In summary, results from the poultry experiments indicated that fermentation increased TME_n in SBM-CV but had a negative effect on FFSB. Fermentation had no significant effect on indispensable AA with the exception of a decrease in Lys digestibility for both SBM-CV and FFSB, suggesting possible heat damage. Fermentation had a positive effect on apparent ileal P digestibility and total tract P retention in both SBM-CV and FFSB when diets contained 0.75% Ca and also increased relative P bioavailability of FFSB in crossbred chicks. Results from the swine experiments indicated that fermentation affected the ME concentration of FFSB negatively, had a positive effect on STTD of P but reduced SID of indispensable AA in SBM-CV and FFSB in growing pigs, supporting the possibility of heat damage of the fermented ingredients. Therefore, the fermentation technique used for SBM-CV and FFSB in the current study may improve ME concentration for poultry and the technique may be improved to avoid the negative effects of heat damage on digestibility of AA and possibly increase even more the availability of P for poultry and swine.

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Soybeans are used as the primary protein source in monogastric nutrition mainly due to their protein content and adequate amino acid profile. However, soybeans in the form of soybean meal (SBM) or full-fat soybeans (FFSB) contain antinutritional factors (ANF) that have a negative effect on the digestibility of the nutrients and growth performance of chickens and pigs, especially at an early age (Stein et al., 2008). The conventional processing of soybeans helps to reduce the ANF content, but some of the ANF are still present in the SBM.

Fermentation is proposed as a method to reduce the antinutritional factors even to undetectable values and improve the nutritional value of the substrate (Cervantes-Pahm and Stein, 2010). It has been reported that fermentation can reduce trypsin inhibitors, phytic acid, and oligosaccharides in soybeans (Hirabayashi et al., 1998; Rojas, 2012). Other benefits of fermented ingredients are the addition of organic acids and probiotics that remain from the fermentation process, increased small peptide concentration, inhibition of intestinal pathogenic microorganisms, enhanced intestinal enzymatic activity, increased immune and antioxidant capacity, and overall improved utilization of nutrients (Feng et al., 2007; Park and Kim, 2019a). Investigators have successfully included and replaced SBM with fermented ingredients in diets for chickens and pigs (Rojas, 2012; Park and Kim, 2019a).

There are different methods for fermentation that vary in the process and the microorganisms used, which produce fermented ingredients with different nutritional compositions (Liu et al., 2020). In addition, new methods are being developed to increase the efficiency of the process and the nutritive quality of the products. A new method for

fermentation has been developed in Costa Rica, taking advantage of the weather conditions to produce fermented soybeans, and fermented full-fat soybeans. Since data about feeding these ingredients to chickens and pigs is limited, more research about these products is required.

SOYBEANS IN ANIMAL FEEDING

Conventional soybean meal

Soybeans are one of the most used crops in the feed industry. The main products obtained from soy come from the oil and protein fractions. Most of the total value of the soybeans comes from the protein fraction, and that is why soybeans are known as a protein crop. Soy oil is mainly used for cooking and industrial processing of different food products (Johnson and Smith, 2017). After the oil extraction, the product left is the soybean meal (SBM), and due to its nutritional characteristics, SBM is one of the main protein ingredients in monogastric diets. Poultry consume nearly 54% of the total SBM production in the US, followed by swine with 26% (Stein et al., 2008).

The nutritional value of SBM is influenced by the method used for oil extraction. The most common methods are solvent extraction and mechanical extraction. There is also a third method that combines extrusion and expelling. The most used method is the solvent extraction of dehulled soybeans, and it is also the most efficient method for oil extraction with only 1.5% of oil left in the meal. The product obtained is the conventional SBM (SBM-CV) (Baker and Stein, 2009). The mechanical extraction or expelling method leaves approximately 5% oil in the SBM (Johnson and Smith, 2017), and it is also desired for animal feeding due to its greater energy content compared with SBM-CV.

The SBM-CV contains 48% CP (NRC, 2012), but protein content can vary from 45% to 48% depending on the area of origin (Ahasic, 2020). Regarding AA content, SBM-CV is known

to have greater concentrations and digestibility of Lys than corn, sorghum, and other vegetable meals, but is deficient in Met, Cys, Thr, and Val (Stein et al., 2008). The concentrations of Lys, Met, and Cys have been reported to be 3.14%, 0.68%, and 0.65%, respectively (Baker et al., 2011). The nitrogen-corrected true metabolizable energy (TME_n) concentration of SBM for poultry is reported to be 2,485 kcal/kg (NRC, 1994) and 3,294 kcal/kg as-is for pigs (NRC, 2012), and to be greater than in other oilseeds meals. This characteristic is attributed to the lower fiber content of FSBM compared with other vegetable ingredients (Stein et al., 2008).

Full-fat soybeans

The FFSB, as SBM-CV, is a good source of protein, but it has also been used in monogastric feeding due to its great fat content, which can enhance the energy content of the diets. The use of FFSB may also reduce the addition of fat when pelleting. Other situations where FFSB are used include small farms that do not have access to expelling or solvent extraction facilities due to their high price. The FFSB contain around 37% CP, 15% crude fat, and 11% carbohydrates (National Research Council, 2012). Since FFSB has not gone through the different heating processes used for SBM-CV, its content of ANF is relatively greater than in SBM-CV. Therefore, to use this ingredient, it usually requires a dehulling and extrusion process. Methods to process raw soybeans include extrusion, expansion, jet-sploding, flaking, cooking, roasting, micronizing, and microwaving (Waldroup, 1982). If done correctly, processing can increase the digestibility of amino acids, fat, and other nutrients in diets for chickens and pigs (Waldroup, 1982; Reese, 1990).

Antinutritional factors of soybeans

There are some nutrients in SBM that have a negative effect on the digestion of animals. These nutrients are known as ANF, and they may inhibit the action of some digestive enzymes,

block the digestion of nutrients, cause allergies, or be non-digestible (Chachaj et al., 2019a; Guo et al., 2020). Some of these ANF are trypsin inhibitors, non-starch polysaccharides, phytate, allergens, high fiber content, and others. These ANF limit the use of SBM in monogastrics.

Phytate, or phytic acid, is present in vegetable ingredients as the form of storage of P in the plants, and up to two-thirds of the total P of these ingredients is bound to phytate, known as phytate-P (Sens et al., 2021). Phytate-P is hardly digested and absorbed by poultry or swine, which means a low bioavailability of P in plant-based diets. Phytate can interact with proteins, carbohydrates, and other minerals such as zinc, iron, and calcium, and reduce their digestibility (Chen et al., 2014). As a result, phytate can negatively affect growth performance and feed efficiency. It is essential to mention that phytate is not destroyed by heat treatment (Stein et al., 2008).

Trypsin inhibitors in soybeans include the Kunitz factor and Bowman-Birk factor, and these factors inhibit the activity of trypsin, chymotrypsin, and other proteases. The resulting effect is poor digestion of proteins and reduced digestibility of all AA in SBM (Stein et al., 2008), and therefore a reduced growth performance in swine and poultry (Adeyemo and Onilude, 2013). Trypsin inhibitors in raw soybeans range from 70 to more than 100 mg/g, and their concentration is reduced up to 90% in SBM due to the heat treatment processing (Hoffmann et al., 2019; Wedekind et al., 2020). The reaction to trypsin inhibitors differs among species, with chickens being more sensitive than piglets (Yasothai, 2016). Hoffman et al., (2019) reports acceptable levels for chickens at $\leq$ 4 mg/g and $\leq$ 4.7 mg/g for piglets.

Non-starch polysaccharides in SBM include cellulose, b-glucan, arabinogalactan, galactomannan, xyloglucan and rhamnogalacturonans, and can be found up to 30% on DM basis. Some of these molecules can increase viscosity and water holding capacity of the digesta and

negatively affect the digestion of starch, protein and especially lipids. However, non-viscus NSPs may decrease the time that the intestinal contents are retained and provide structure for bacteria to attach, and thus be beneficial for the animal (Smits et al., 1997).

Oligosaccharides in SBM include stachyose, mannose, raffinose, verbascose and are present in SBM up to 11% on DM basis. Oligosaccharides present α-1,6 linkages in their structure, which make them indigestible by poultry due to the lack of α-1,6-galactosidase. The presence of oligosaccharides in the G.I. can also increase the digesta flow rate, which reduces fiber digestion and TME_n (Chen et al., 2013). It has been demonstrated that low oligosaccharide soybean varieties can have greater nutritional value than SBM-CV (Baker et al., 2011).

Other ANF include tannins, lectins, saponins, and allergens. Lectins are glycoproteins that bind to the intestinal epithelium, disrupting the brush border membrane and negatively affecting growth performance and increasing mortality (Ahasic, 2020). Saponins and tannins are secondary metabolites of plants. Saponins can form soap-like foams that can be beneficial at the proper levels, but otherwise can cause growth depression (Hanson et al., 1956). Tannins, similar to trypsin inhibitors, can inhibit digestive enzymes such as trypsin and α-amylase (El-Shemy et al., 2000). Allergens found in soybean include glycinin and β-conglycinin proteins that are poorly digested by gastric enzymes and can disrupt the intestinal wall, cause diarrhea, and reduce nutrient utilization of young animals (Park et al., 2020).

FERMENTED SOYBEAN MEAL

Fermented soybean products have been used for many years, especially in Asian food. There are reports of soldiers with gastrointestinal disorders, during World War II, who were able to consume and satisfactorily digest tempeh (fungus fermented soybeans) (Zamora and Veum,

1979). For animal feeding, soybeans are fermented with the purpose of reducing the ANF and increasing the nutritional value of the ingredient. For the fermentation process, it often uses a bacterium and a fungus that work together to increase the efficiency of the fermentation. Fermentation can reduce trypsin inhibitors to undetectable values, reduce oligosaccharides such as stachyose and raffinose (Cervantes-Pahm and Stein, 2010), increase the crude protein and AA concentration (Song et al., 2008; Cervantes-Pahm and Stein, 2010) and increase the non-phytate P concentration (Rojas, 2012). Young animals are more susceptible to the ANF in SBM therefore, there is a particular interest in including FSBM in starter or nursery diets for both chickens and pigs (Stein et al., 2008). Many studies have tested the FSBM inclusion in chicken and pig diets up to 30%, with beneficial effects on performance (Hirabayashi et al., 1998; Song et al., 2010).

Conventional fermentation method

The fermentation process differs depending on the desired product and the used microorganism. Most techniques involve sorting, dehulling, soaking, steaming, inoculation, heating, fermentation, or storage (Liu et al., 2020). The different methods of fermentation can be classified as submerged fermentation (SmF) and solid-state fermentation (SSF); the main difference is whether the fermentation takes place in a liquid medium (Mukherjee et al., 2015; Doriya et al., 2016).

The advantages of SmF are better diffusion of heat and microorganisms, and it is more suitable for large-scale operations. In contrast, the disadvantages are complexity, higher operational costs, and greater effluents. The SSF offers less water utilization, has more resistance to contamination, and has high yield and product activity. At the same time, the diasdvantages

are the risk of heat buildup, difficulty controlling process parameters, and challenges with scaling up (Doriya et al., 2016).

The most effective microbes for SBM fermentation are the *Aspergillus usamii* and *Aspergillus oryzaee* due to their capacity to produce hemicellulases, hydrolases, pectinases, protease, amylase, lipases, and tannases. Although, some authors suggest the addition of *Lactobacillus* and *Bacillus subtilis* to improve the action of the fungus (Mukherjee et al., 2015). These two types of microorganisms can work together with the fungus, creating tunnels where the bacteria can penetrate and produce enzymes to degrade the substrate (Sewell, 2015; Park and Kim, 2019b).

FSBM in poultry

Research about the nutritional value of fermented soybeans is limited in poultry compared with the studies done in pigs, especially for FFFSB. However, other types of fermented ingredients have been tested in chickens and other poultry species with positive results on nutritional value and growth performance. Park and Kim (2019a) replaced 10% of SBM-CV or guar meal with FSBM and fermented guar meal respectively in broiler diets. There was no adverse effect on growth performance or retention of dry matter, nitrogen, and energy in broilers fed FSBM compared with SBM-CV. For fermented guar meal, the results showed an improved growth performance and retention of nutrients compared with guar meal. Furthermore, the broilers fed the fermented ingredients presented improved caecal microflora with increased *Lactobacillus*, decreased coliforms, and less ammonia emission. These findings agree with (Zhang et al., 2021) who studied the effects of feeding fermented wheat bran (FBW) on growth performance and nutrient digestibility in broilers. The investigator successfully included wet FBW up to 5% in the diets without affecting the growth performance or the apparent DM,

energy, and nitrogen digestibility. These results also agree with Feng et al. (2007) who observed increased activity of trypsin, lipase, and protease in intestinal contents and improved intestinal morphology of starter broilers fed FSBM compared with the group fed SBM-CV.

Regarding digestibility of AA and metabolizable energy (ME), Wu et al. (2020) evaluated the effects of fermentation on standardized ileal digestibility (SID) of AA and apparent metabolizable energy (AME) in rapeseed meal fed to broilers. For this study, the AME and nitrogen-corrected AME (AME_n) were determined by substitution method, and the SID of AA was determined using the test ingredients as the sole source of AA and a nitrogen-free diet. The results showed an increase of 14% and 15% in AME and AME_n, respectively, in fermented rapeseed meal compared with rapeseed meal. Fermented rapeseed meal also showed greater apparent ileal digestibility (AID) and SID of most of the indispensable AA, including lysine, while no differences were found in AID and SID of AA such as methionine, cysteine, or threonine. The author attributes the improved nutrient digestibility and utilization to the reduced concentration of ANF such as glucosinolates, increased concentration of CP, gross energy, polypeptides, and lactic acid in the fermented rapeseed meal.

Investigators have reported increased bioavailability of P in FSBM compared with SBM-CV. Hirabayashi et al. (1998) investigated the effect of FSBM on P excretion in chicks. For this study, the investigator fed three experimental diets to 30 chicks. The diets consisted of a negative control P deficient SBM diet, the negative control SBM diet with inorganic P added, and a FSBM diet without supplemental inorganic P. The FSBM and P supplemented diets yielded greater BW gain, greater retained P, and greater femoral P than the control group. Also, the FSBM group had greater P retention than the P supplemented group. The author attributed the

increased bioavailability of P in FSBM to the capacity of *Aspergillus usamii* to almost degrade phytate P completely into inorganic P with fermentation.

Fermented full-fat soybeans have been successfully included in quail diets with positive results. In a study by Chah et al. (1976) full-fat soybeans were fermented with *Aspergillus oryzae*. The fermented ingredient, compared with the regular soybeans, contained greater concentrations of DM, CP, ether extract, and AA such as Lys, Arg, Phe, and Thr. Fermented full-fat soybeans were included in starter and layer quail diets at 50% and 30%, respectively, replacing regular full-fat soybeans in their totality. Quail fed the fermented soybeans showed a significantly superior weight gain and feed efficiency than the group fed the regular soybeans over a 4-week period. The authors attribute these results to the better AA balance in fermented diets. There were no differences among the diets on hen-day egg production and egg weight, and fertility and hatchability were not affected by the inclusion of the fermented ingredients.

In a study conducted in 2020, researchers prepared a mixture of four parts of corn with three parts of cottonseed meal and three parts of rapeseed meal and fermented the mixture with *L. plantarum* and L. acidophilus for 72 h at 32 °C (Zhu et al., 2020). The fermented feed was included in White Leghorn chick diets at 7.5%, partially replacing corn, SBM, and wheat bran, and fed to the chicks for 21 d. The group fed the fermented feed had increased average BW, but reduced feed conversion ratio (FCR), compared with the control group. The fermented feed also significantly increased immune function and improved the antioxidant capacity of the chicks (Zhu et al., 2020).

FSBM in swine

Fermented soybean meal has been used in nursery pig diets due to the reduced ANF compared with SBM-CV, which helps the low digestion capacity of young pigs. Rojas (2012)

determined the digestibility of P in FSBM and SBM-CV with and without microbial phytase by weanling pigs. The investigator fed diets containing FSBM or SBM-CV with no phytase and other similar diets with phytase added, and a P-free diet was used as well to feed barrows of 14 kg initial BW. The results showed increased ATTD and STTD of P up to 61 and 66% in pigs fed the FSBM diets, and these were greater than the pigs fed the SBM-CV. In contrast, when phytase was added to the diets, there were no differences in ATTD or STTD of P between FSBM and SBM-CV. The author attributed the increased P digestibility in FSBM to the reduced phytate bound P.

In a subsequent study, Rojas (2012) determined digestibility of energy, concentration of digestible energy (DE) and ME, and digestibility of AA in FSBM, SBM-CV, and fish meal (FM). In Experiment 1, the investigators fed pigs of 22 kg with four diets consisting of a corn-based diet and three other diets containing FSBM, conventional SBM, and FM, respectively. The results were not different for ATTD of GE for corn, FSBM, and SBM-CV and all were greater than in FM. The concentrations of DE, and ME in FSBM were 4,296, and 3,781 kcal/kg DM, respectively, and these values were lower than in SBM-CV but greater than corn and FM. In Experiment 2, the investigator used eight cannulated barrows of 10 kg initial BW and fed them three diets containing a mixture of cornstarch and FSBM, SBM-CV, and FM, respectively, to measure SID of AA. A fourth N-free diet was used to determine the basal endogenous losses of CP and AA. The results showed that the SID of all indispensable AA were greater in FSBM than in FM except for Lys, Thr, and Trp. The SID of all indispensable AA were not different between FSBM and SBM-CV except for Met and Val, which were greater in FSBM. The author attributed the superiority of FSBM to FM to the low quality of the FM used in the experiment, since the values obtained for FM were inferior to the values reported by previous studies, and the

greater SID of indispensable AA in FSBM than FM is attributed to the capacity of the fermentation to increase the concentration of small peptides that have better digestibility in young pigs.

In another study, Jones et al. (2010) included FSBM at 3.75%, 6%, and 7.5% in nursery phase 2 diets and compared it with fish meal and dried porcine solubles. The FSBM at 6% inclusion had similar results to dried porcine solubles, and both were superior to FM or control diets on ADG and G:F from 0 to 14 d. Increasing FSBM levels from 3 to 7% improved the G:F. Feeding diets combining FSBM and dried porcine solubles yielded better ADG and G:F than diets containing FM, and better ADG and ADFI compared with diets containing FSBM. The authors suggest that combining specialty animal protein sources may have additive benefits. These results agreed with a study conducted by Rojas (2012) where FSBM was included in nursery diets replacing chicken meal and poultry by-product meal during the initial 28 d post-weaning in 2 or 3 phase programs. There were no differences in the final BW and G:F ratio for the overall experiments, and only FM diets yielded a superior G:F ratio to FSBM in one of the experiments.

In another study, Song et al. (2010) included 40% of SBM-CV in nursery diets and successfully replaced it with FSBM in 1/3, 2/3, and 3/3 of the total protein supplied by the SBM-CV. The results were not different for final BW, ADG, ADFI, and G:F ratio from days 1 to 14 between SBM-CV and FSBM diets; although, the author suggested that the optimum substitution level of SBM-CV by FSBM was at 2/3. The investigator also measured fecal scores per pen on a scale from 1 (severe diarrhea) to 5 (firm dry feces), and the results showed reduced diarrhea with the inclusion of FSBM in the diets from 1 to 14 d. The author attributed the reduction in diarrhea to the degradation of allergenic soybean proteins (glycinin and β-conglycinin) and reduced trypsin

inhibitors content, the partial degradation of proteins and carbohydrates that facilitated the digestion of nutrients, and the presence of microorganisms in FSBM that inhibited the intestinal colonization of pathogens.

New fermentation technique

The new fermentation technique developed by INOLASA (Industrial de Oleaginosas Americanas, Puntarenas, Costa Rica) is based on submerged fermentation and takes advantage of the warm weather which ranges from 25 °C to 36 °C. The fermentation can be applied to SBM-CV and FFSB. The fermentation process starts with the substrate, in this case, SBM-CV or FFSB, that goes through a steam treatment to add moisture up to 46% where it reaches temperatures near 100 °C. After that, the substrate is cooled, stored in bags, and transported to the fermentation room, where the microbes (*Lactobacillus subtillis*) are added, and it is left for 24 hours at 37 °C. In the final stage of the process, the fermented mass is air-dried and goes through a milling machine, and then is stored or packaged for distribution. (Kang et al., 2016; Olukomaiya et al., 2019).

The resulting products contain greater CP, greater GE, reduced carbohydrates including oligosaccharides, reduced trypsin inhibitors, and reduced fat content, especially in the FFFSB compared with the unfermented ingredients. The FSBM contains around 88.9% DM, 50% CP, 0.66% crude fat, and 6.28% ash, while the FFSB contains 90.12% DM, 38.42% CP, 18.73% AEE, and 5.22% ash.

METHODS FOR DETERMINATION OF DIGESTIBILITY AND CONCENTRATION OF NUTRIENTS IN POULTRY AND SWINE

Methods

Digestibility values are determined by measuring the intake and output of a nutrient by the animal. Two common methods used to determine the digestibility of nutrients in pigs and poultry include the total collection method and index method. The total collection method consists of feeding the animals for a period of time and collecting the feces or feces and urine depending on the objective of the study. The experimental diets are usually fed for approximately 5 d as an adaptation period to the diets and another 5 d as the collection period. Urine and fecal samples are collected separately for pigs, but for poultry, feces plus urine are collected. The samples are then dried, quantified, and ground. Urine samples are usually filtered prior to drying. Dried fecal and urine samples are then analyzed for the nutrient of study, such as gross energy, nitrogen content, mineral concentration, or others. The index method is an alternative to the quantification of feed intake or feces. In this method, indigestible markers are used as an index to measure digestibility. Index compounds should be nonabsorbable, nonessential, nontoxic, inert, and completely voided in the feces. Also, index compounds should be easy to mix with feed and to analyze for. The most used markers are chromic oxide, acid insoluble ash, and titanium dioxide, which are usually added at 0.1% to 0.5% (Zhang and Adeola, 2017). Since this method does not require the use of metabolism crates, the recycling of the index marker through coprophagy must be prevented. Samples are processed like those in the total collection method, but the index compound concentration also needs to be analyzed (Adeola, 2001).

Two approaches can be applied to the methods described above. The direct approach consists in feeding diets where the totality of the nutrient being evaluated comes from the test ingredient (Adeola, 2001). When the test ingredient presents limitations like formulation restrictions or reduced palatability (e.g., blood meal, feather meal, and full-fat oil seeds), the indirect approach is recommended (Stein, 1997). In this approach, the experimental diets are formulated to include the test ingredient and a basal diet. The digestibility of the test ingredient is determined by the difference of the digestibility of the basal diet plus the test ingredient and the digestibility of the basal diet fed alone. In this approach, it is assumed that the feedstuffs in the diet do not interact with each other to enhance or depress the digestibility of the component being determined (Adeola, 2001).

Metabolizable energy in poultry

The difference between energy intake and the energy voided in feces is known as apparent DE (ADE). The term apparent means that ADE does not account for the energy coming from the metabolic fraction of the feces, which includes abraded cells from the intestinal mucosa, bile, and digestive fluid. After subtracting the metabolic fraction energy from the total feces energy, true DE can be determined. When, additionally, subtracting the energy loss of urine from the total feces energy, AME can be determined. In this case, the TME is determined after subtracting the portion of the urinary energy loss that comes from the endogenous fraction from tissue catabolism (Sibbald, 1980). Since fasted birds are used to determine endogenous losses of energy associated with loss of nitrogen and fasted birds have been reported to have greater losses of nitrogen than fed birds, TME should be corrected to nitrogen equilibrium or zero nitrogen retention (TME_n) for more accurate measurement of ME (Parsons et al., 1982). By measuring and subtracting the heat increment of metabolism from the ME, net energy (NE) can be

calculated. The NE of a feedstuff is, therefore, the energy that is available for body maintenance and production.

The ME determination in poultry feedstuffs is the preferred system for to various reasons. First, it is very difficult to measure ADE or TDE of feedstuffs because urine and feces are voided together in the excreta. Furthermore, the improvement in accuracy of predicting bird growth performance and energy retention of NE compared with ME is small, and poultry feedstuffs tend to be less variable in digestible nutrient content compared with feedstuffs in pig diets. Therefore, TME_n is usually determined and applied in poultry feedstuffs (de Lange and Birkett, 2005).

A traditional determination of AME consists of feeding birds in metabolism batteries that allow for feed intake and excreta collection quantification. The bioassay can be addressed with the total collection or index method previously described. Special care needs to be taken when measuring feed consumption and excreta quantification since it influences the AME values. The total collection method is mostly used for the ME determination compared with the index method. In some cases, the index method has been reported not to be as accurate as the total collection method (Dourado et al., 2010). From the total collection methods, the precision-fed rooster assay is of common use due to its low cost, accuracy, quick results, and overall efficacy. This method allows the determination of TME_n. The process consists of fasting birds for 24 h and then intubating feed, usually 30 g, into the crop. The intubated feed includes the test ingredient alone or mixed with other ingredients such as corn. The roosters are fasted again for 48 h, and total excreta are collected during this period. The energy intake and energy voided in feces are measured, and the endogenous energy losses are also accounted for and corrected to zero nitrogen retention to obtain the TME_n value (Ahasic, 2020).

Amino acid digestibility in poultry

Between the two methods to determine AA availability, the AA digestibility assays based on the total collection and index methods are preferred. The most used assays are the ileal digesta collection of euthanized birds and the excreta collection of cecectomized roosters. Cannulated birds have also been successfully used for AA digestibility trials (Johns et al., 1986), but this method is rarely used due to its feasibility.

The first assay is based on the index method and the difference approach, and consists in collecting the digesta of the ileum part. By doing this, the effect of the ceca microbes is avoided. For this method, the birds are fed diets that include the test ingredients and an indigestible marker. The birds are then euthanized, and the ileum section is located from the Meckel's diverticulum to the ileo-cecal junction. The ileal digesta from birds of each replicate are pooled, freeze-dried, and analyzed for AA content (Kong and Adeola, 2014).

In the second assay, cecectomized birds are commonly used. In this assay, the total collection method and direct approach are used, and the calculation for AA digestibility is like that used in the determination of energy where the intake and output of AA are measured. For this assay, it is assumed that the AA concentration in urine is not significant (Ravindran and Bryden, 1999). The AA digestibilities can differ between intact and cecectomized birds because of the effect of the microbes of the ceca on AA digestibility. In intact birds, undigested protein and nitrogen sources from the intestine can be used by microorganisms in the ceca to synthesize other AA that will remain in the excreta, which will reduce the accuracy of the digestibility values. This is the reason why cecectomized birds are commonly used (Kong and Adeola, 2014).

A N-free diet can be fed to determine basal endogenous losses of AA and correct apparent digestibility and determine standardized ileal digestibility or standardized digestibility of AA, respectively, for the two methods mentioned above (Kong and Adeola, 2014).

Phosphorus digestibility and relative bioavailability in poultry

There are different methods to measure the bioavailability of P in poultry feedstuffs, and the results among these methods can show differences even when using the same source of P (Rodehutscord, 2009). A common qualitative method to determine the relative bioavailability of different P sources consists in using the values for bone strength, bone ash weight, or percentage bone ash of birds fed the test ingredient and comparing them with the same parameters of birds fed a standard P source. The relative bioavailability of the standard P source is established as 100%, and the biological availability of the test ingredient is relatively estimated from the standard using a slope-ratio method. The standard reference P source can be potassium phosphates, sodium phosphates, mono-, di-, and tricalcium phosphates, and others. The biological availability of the test ingredients obtained with this method can vary depending on the reference P standard used, the test ingredient, molecular formula, age, and species of poultry used in the assay (Coon et al., 2007). The experimental diets need to have a dietary P level below the requirement; otherwise, the excretion of P will increase regardless of the quality of the P source (Rodehutscord, 2009). When using the tibia to determine the bioavailability of P in poultry, the right or left tibia is collected from a euthanized chick and analyzed for its ash content. The legs are autoclaved to facilitate the removal of the tissue from the tibias. After the bones have been collected and cleaned, they are dried at 100 °C and then dry-ashed at 600 °C. The bones can be fat extracted, although it may not affect the accuracy of the relative bioavailability values (Garcia and Dale, 2006).

A preferred assay for a quantitative approach is the ileal digestible P determination. This assay is preferred over the retainable P determination since the latter involves P excreted in feces and urine, which is often not desired. When compared, the ileal digestible P values are usually greater than the retainable P values (Dilger and Adeola, 2006; Adeola and Applegate, 2010).

To determine total tract P retention in poultry, the P intake and the P excreted are measured. The difference between these two will be the P retention. A concern that arises would be the P excreted in the urine, but it may not be significant when the birds are fed a dietary P level well below the requirement (Li et al., 2016). Therefore, a total collection of excreta can be used to determine the P retention with an indigestible marker as an index. For this assay, birds are housed in cages and fed the experimental diets for 3 to 5 d, and then the total excreta are collected, dried, and analyzed for the concentration of the indigestible marker and P. An adaptation period to the experimental diets of not less than 5 d is recommended to allow the birds to adjust P excretion to the respective level of P intake with the experimental diets; however, this recommended period of 5 d by Rodehutscord (2009) is controversial and many labs use only 3 to 4 d or less.

Ileal P digestibility is measured following a similar method to the ileal AA determination where the section between the Meckel's diverticulum and the cecal junction is collected, avoiding the effect of post ileal microbial activity and urinary excreted P. In these assays, an indigestible marker is included in the diets to aid to the measurement of P concentration in the ileal samples. The collected ileal samples are pooled within each replicate and then freeze-dried, ground, and analyzed for concentration of the indigestible marker and P (Rodehutscord, 2009).

Digestible and metabolizable energy in swine

The DE is relatively easier to measure in pigs than in poultry since feces and urine can be collected separately. However, DE does not truly measure the energy of the nutrients absorbed by the digestive tract, and it also includes the energy of endogenous secretions and intestinal cell debris (Velayudhan et al., 2015). Factors that affect the DE content include dietary fiber level and the age of the animal. If measured for the same diets, DE will be greater in older pigs compared with young ones due to the greater capacity to digest dietary fiber. Thus, it is necessary to determine DE for the different physiological stages of growth (Velayudhan et al., 2015).

The ME is determined by subtracting the energy of feces, urine, and gas production from the gross energy of the feed. Gas produced by pigs is linked to the dietary fiber level and age, and can increase the ME values by up to 3%. Since the energy of gasses represents a small amount of the ME, it is usually overlooked when determining ME values of pig feedstuffs. In contrast, urinary energy may represent a greater and more variable percentage of the DE since it depends on the urinary N content, and this depends on the amount of digestible protein of the diet. Increased protein levels in the diet will likely cause greater urinary N excretion. Since urinary N excretion is not accounted for DE determination, DE values are usually higher and less precise than ME. Therefore, ME is preferred to be used to meet energy requirements for pigs.

The procedure to determine DE and ME involves feeding the pigs for a period of time and collecting the feces and urine. Fecal and urine samples are quantified, dried, and ground. Urine samples are usually filtered prior to drying. Dried fecal and urine samples are then analyzed in a calorimeter for gross energy content. The digestibility of energy can be determined using the methods and approaches previously described. As mentioned before, the gross energy

of the feces contains endogenous losses, but the determination of energy endogenous losses is not practicable in pigs. The correction for N equilibrium in growing pigs may not be necessary since they do not usually use the retained protein for energy purposes (Kong and Adeola, 2014).

Amino acid digestibility in swine

Like poultry, many studies have demonstrated that almost the total absorption of AA occurs in the small intestine of pigs (Stein, 1997). Therefore, the most accurate method to measure AA digestibilities of feedstuffs involves the collection of digesta from the end of the ileum (Adeola et al., 2016). In this way, the effect of the microbial activity from the hindgut is avoided. There are several methods to collect ileal digesta, such as the use of re-entrant cannulas, simple T-cannulas, post valve T-cannulas, ileorectal anastomy, and slaughter of the animal. Among these methods, the use of T-cannula is the most common due to its simple surgical procedure and minimum variation of the values obtained from trial to trial (Stein, 1997). For this matter, usually, cannulas of 10 to 15 mm inner diameter are surgically fitted in the distal ileum 10 to 20 cm before the ileo-colic valve. Smaller cannulas can be used for younger pigs. Since T-cannulas only allow for partial collection of digesta, an indigestible marker is used. The most commonly used markers are chromium oxide and titanium dioxide. As the determination of digestibility of energy, direct or difference approaches can be used to determine AA digestibility. For the difference approach, it needs to be assumed that there is no interaction in the digestibility coefficients between the basal and the test ingredients. The slaughter technique, as used in poultry, consists of feeding pigs for 5 to 7 d and then sacrificing the animals and removing the ileum. The use of electrical stunning should be avoided to minimize the shedding of epithelial cells into the intestinal lumen. Anesthesia or barbiturate intoxication are preferred for this technique. The ileal digesta are collected from the distal 20 to 150 cm of the ileum with the aid

of distilled water or saline solution. Due to the nature of the technique, the feeding and collection timing is crucial, and sampling at 9 h after the start of feeding is recommended. The values obtained by the slaughter method have been demonstrated to be reliable compared with the T-cannula method. However, the disadvantages of the slaughter method are that only one sample per animal can be collected, and in some cases, obtaining a representative sample may not be possible (Stein, 1997). Standardized ileal digestibility can be determined after correcting apparent ileal digestibility values for basal endogenous losses of AA. Basal endogenous losses are commonly determined by feeding N-free diets to pigs (Kong and Adeola, 2014).

Phosphorus digestibility in swine

In contrast to AA digestibility determination, Zhan and Adeola (2017) reported that there is no difference between ileal and total tract digestibility of P and Ca, which means that the absorption or excretion of P and Ca in the hindgut does not significantly affect the overall digestion of P. It is, therefore, recommended to determine the total tract digestibility of P and Ca using total collection along with the direct or index approach previously described (Zhang and Adeola, 2017).

Using these methods, it is relatively easy to determine ATTD of P or Ca by simply measuring total intake and subtracting the fecal output of the mineral. However, ATTD of P and Ca values may be affected by the dietary levels of organic sources of P and Ca. Another concern is that ATTD of P or Ca may be underestimated because the endogenous losses as a proportion of intake of P or Ca can be greater when pigs are fed low concentrations of P or Ca. In consequence, the additivity assumption for ATTD of P or Ca may not be right when applied to mixed diets (Zhang and Adeola, 2017). Therefore, the use of STTD and TTTD values are recommended instead of ATTD values (Fang et al., 2007).

The TTTD estimation involves the correction of ATTD for basal and specific ileal endogenous losses (Stein et al., 2007). The methods to determine specific ileal endogenous losses are expensive and complex, while the determination of basal endogenous losses is more practical. Since STTD determination only involves correcting basal endogenous losses, it is preferred over TTTD (Zhang and Adeola, 2017).

CONCLUSIONS

Soybean meal is still one of the main ingredients in monogastric diets, but it is limited by its ANF content. Several studies have shown how further processing can improve the nutritional value for monogastric animals. Fermentation is proposed as one of these methods with great potential to reduce ANF and increase the digestibility of nutrients by young animals. Although, due to the different soybean sources and various techniques of fermentation, the fermented products differ from each other in their nutritional content. Moreover, new methods for fermentation are being developed, and resulting products have not been tested in animals. Therefore, the nutritional value of new fermented products requires experimental evaluation using standardized procedures in poultry and swine to include them in commercial diets.

The objective of this study was to evaluate the effect of fermentation on the nutritional characteristics of SBM-CV and FFSB when fed to chickens and pigs. To achieve the objective, 8 experiments were conducted to determine TMEn concentration and standardized digestibility of AA in rooster, apparent ileal P digestibility and total tract P retention, and relative P bioavailability in crossbred and commercial broiler chicks, concentrations of DE and ME, SID of AA, and STTD of P in growing pigs.

LITERATURE CITED

Adeola, O. 2010. Digestion and balance techniques in pigs. Pages 895-908 in Swine Nutrition. A. J. Lewis and L. L. Southern. ed. CRC Press, Boca Raton, FL.

Adeola, O., and T. J. Applegate. 2010. Phosphorus and calcium equivalency of phytase. Proc. 1st International Phytase Summit. 1:28–30.

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274–283.

Adeyemo, S. M., and A. A. Onilude. 2013. Enzymatic reduction of anti-nutritional factors in fermenting soybeans by lactobacillus plantarum isolates from fermenting cereals. Niger. Food J. 31:84–90.

Ahasic, E. M. 2020. Effect of particle size on nutritional value of soybean meal for poultry. MS thesis. Univ. Illinois, Urbana, IL.

Baker, K. M., and H. H. Stein. 2009. Amino acid digestibility and concentration of digestible and metabolizable energy in soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to growing pigs. J. Anim. Sci. 87:2282–2290.

Baker, K. M., P. L. Utterback, C. M. Parsons, and H. H. Stein. 2011. Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. Poult. Sci. 90:390–395.

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674–2683.

Chachaj, R., I. Sembratowicz, M. Krauze, and K. Ognik. 2019. The effect of partial replacement of soybean meal with fermented soybean meal on chicken performance and immune status. J. Anim. Feed Sci. 28:263–271.

Chah, C. C., R. A. Nelson, C. W. Carlson, G. Semeniuk, I. S. Palmer, and C. W. Hesseltine. 1976. Fungus-Fermented Soybeans Benefit the Life Cycle of Japanese Quail (Coturnix coturnix japonica). Poult. Sci. 55:975–981.

Chen, X., C. M. Parsons, and N. Bajjalieh. 2013. Nutritional evaluation of new reduced oligosaccharide soybean meal in poultry. Poult. Sci. 92:1830–1836.

Chen, L., P. V. Vadlani, and R. L. Madl. 2014. High-efficiency removal of phytic acid in soy meal using two-stage temperature-induced Aspergillus oryzae solid-state fermentation. J. Sci. Food Agric. 94:113–118.

Coon, C. N., S. Seo, and M. K. Manangi. 2007. The determination of retainable phosphorus, relative biological availability, and relative biological value of phosphorus sources for broilers. Poult. Sci. 86:857–868.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

Doriya, K., N. Jose, M. Gowda, and D. S. Kumar. 2016. Solid-state fermentation vs submerged fermentation for the production of l-asparaginase. Adv. Food Nutr. Res. 78:115–135.

Dourado, L., J. Siqueira, N. Sakomura, S. Pinheiro, S. Marcato, J. Fernandes, and J. Silva. 2010. Poultry feed metabolizable energy determination using total or partial excreta collection methods. Braz. J. of Poult. Sci. 12:129–132.

El-Shemy, H., E. Abdel-Rahim, O. Shaban, A. Ragab, E. Carnovale, and K. Fujita. 2000. Comparison of nutritional and antinutritional factors in soybean and fababean seeds with or without cortex. Soil Sci. Plant Nutr. 46:515–524.

Fang, R. J., T. J. Li, F. G. Yin, Y. L. Yin, X. F. Kong, K. N. Wang, Z. Yuan, G. Y. Wu, J. H. He, Z. Y. Deng, and M. Z. Fan. 2007. The additivity of true or apparent phosphorus digestibility values in some feed ingredients for growing pigs. Asian-Australas. J. Anim. Sci. 20:1092–1099.

Feng, J., X. Liu, Z. R. Xu, Y. Z. Wang, and J. X. Liu. 2007. Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. Poult. Sci. 86:1149–1154.

Garcia, A. R., and N. M. Dale. 2006. Foot Ash as a Means of Quantifying Bone Mineralization in Chicks. J. Appl. Poult. Res. 15:103–109.

Guo, S., Y. Zhang, Q. Cheng, J. Xv, Y. Hou, X. Wu, E. Du, and B. Ding. 2020. Partial substitution of fermented soybean meal for soybean meal influences the carcass traits and meat quality of broiler chickens. Animals 10:225.

Hanson, L. J., D. E. Becker, S. W. Terrill, A. H. Jensen, and H. W. Norton. 1956. The inhibitory effect of dehydrated alfalfa meal in the diet of swine. J. Anim. Sci. 15:830–839

Hirabayashi, M., T. Matsui, H. Yano, and T. Nakajima. 1998. Fermentation of soybean meal with Aspergillus usamii reduces phosphorus excretion in chicks. Poult. Sci. 77:552–556.

Hoffmann, D., S. Thurner, D. Ankerst, K. Damme, W. Windisch, and D. Brugger. 2019. Chickens' growth performance and pancreas development exposed to soy cake varying in trypsin inhibitor activity, heat-degraded lysine concentration, and protein solubility in potassium hydroxide. Poult. Sci. 98:2489–2499.

Johns, D. C., C. K. Low, and K. A. C. James. 2007. Comparison of amino acid digestibility using the ileal digesta from growing chickens and cannulated adult cockerels. Br. Poult. Sci. 27:679-685.

Johnson, L., and K. Smith. 2017. Soybean Processing. Soybean meal info center. Accessed Nov. 2021. https://www.soymeal.org/soy-meal-articles/soybean-processing-2/

Jones, C. K., J. M. Derouchey, J. L. Nelssen, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2010. Effects of fermented soybean meal and specialty animal protein sources on nursery pig performance. J. Anim. Sci. 88:1725–1732

Kang, K. I., J. S. Heo, S. J. Cho, T. B. Kim, and S. W. Park. 2016. Process for preparing fermented soybean meal. CJ CHEILJEDANG Corp, Seoul, KR. US Pat. No. 0015055

Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917–925.

Lange, C. F. M. De, and H. Birkett. 2011. Characterization of useful energy content in swine and poultry feed ingredients. Can. J. Anim. Sci. 85:269-280.

Li, X., D. Zhang, T. Y. Yang, and W. L. Bryden. 2016. phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. Agriculture 6:25.

Liu, L., X. Chen, L. Hao, G. Zhang, Z. Jin, C. Li, Y. Yang, J. Rao, and B. Chen. 2020. Traditional fermented soybean products: processing, flavor formation, nutritional and biological activities. Crit. Rev. Food Sci. Nutr. 1-19.

Mukherjee, R., R. Chakraborty, and A. Dutta. 2015. Role of Fermentation in Improving Nutritional Quality of Soybean Meal — A Review. Asian-Australas. J. Anim. Sci. 29:1523–1529.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Olukomaiya, O., C. Fernando, R. Mereddy, X. Li, and Y. Sultanbawa. 2019. Solid-state fermented plant protein sources in the diets of broiler chickens: A review. Anim. Nutr. 5:319–330.

Park, J. H., and I. H. Kim. 2019. A comparative evaluation of fermented and unfermented soybean and guar meal on growth performance, nutrient retention, caecal microflora, and excreta gas emission of broiler chickens. Eur. Poult. Sci. 83:1–12.

Park, S., J. W. Lee, A. J. Cowieson, G. Pappenberger, and T. A. Woyengo. 2020. Soybean meal allergenic protein degradation and gut health of piglets fed protease-supplemented diets. J. Anim. Sci. 98:1–9.

Parsons, C. M., L. M. Potter, and B. A. Bliss. 1982. True metabolizable energy corrected to nitrogen equilibrium. Poult. Sci. 61:2241–2246.

Ravindran, V., and W. L. Bryden. 1999. Amino acid availability in poultry - in vitro and in vivo measurements. Aust. J. of Agric. Res. 50:889–908.

Reese, D. 1990. Full-Fat Soybeans for Pigs. University of Nebraska-Lincoln Extension. Accessed Nov. 2021. https://digitalcommons.unl.edu/extensionhist/1379.

Rodehutscord, M. 2009. Approaches and challenges for evaluating phosphorus sources for poultry. 17th Eur. Symp. Poult. Nutr. 17:2–6.

Rojas, O. J. 2012. Nutritional evaluation of fermented soybean meal fed to weanling pigs. MS thesis. Univ. Illinois, Urbana.

Sens, R. F., L. S. Bassi, L. M. Almeida, D. F. Rosso, L. V. Teixeira, and A. Maiorka. 2021. Effect of different doses of phytase and protein content of soybean meal on growth performance, nutrient digestibility, and bone characteristics of broilers. Poult. Sci. 100:100917.

Sewell, J. 2015. Soybean meal through solid-state fermentation. Feed Strategy. Accessed Nov. 2021. https://www.feedstrategy.com/pig-nutrition/soybean-meal-through-solid-state-fermentation.

Sibbald, I. R. 1980. Metabolizable energy in poultry nutrition. Bioscience 30:736–741.

Smits, C. H. M., A. Veldman, M. W. A. Verstegen, and A. C. Beynen. 1997. Nutrient requirements and interactions dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr 127:483–487.

Song, Y. S., J. Frias, C. Martinez-Villaluenga, C. Vidal-Valdeverde, and E. G. De Mejia. 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. Food Chem. 108:571–581.

Song, Y. S., V. G. Pérez, J. E. Pettigrew, C. Martinez-Villaluenga, and E. G. De Mejia. 2010. Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immunoreactivity in the plasma. Anim. Feed Sci. Technol. 159:41–49.

Stein, H. H. 1997. Comparative amino acid digestibilities in growing pigs and sows. PhD Dissertation. Univ. Illinois, Urbana.

Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey, D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their coproducts. Pages 613-

660 in Soybeans: Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White, and R. Galloway. Urbana, IL.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. De Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282–285.

Velayudhan, D. E., I. H. Kim, and C. M. Nyachoti. 2015. Characterization of dietary energy in swine feed and feed ingredients: A review of recent research results. Asian-Australas. J. Anim. Sci. 28:1-13.

Waldroup, P. W. 1982. Whole Soybeans for Poultry Feeds. Worlds Poult. Sci. J. 38:28–35.

Wedekind, K. J., J. Chen, F. Yan, J. Escobar, and M. Vazquez-Anon. 2020. Efficacy of a mono-component protease is affected by trypsin inhibitor concentration in soybean meal. Anim. Feed Sci. Technol. 265:114502.

Wu, Z., J. Liu, J. Chen, S. A. Pirzado, Y. Li, H. Cai, and G. Liu. 2020. Effects of fermentation on standardized ileal digestibility of amino acids and apparent metabolizable energy in rapeseed meal fed to broiler chickens. Animals. 10:1774.

Yasothai, R. 2016. Antinutritional factors in soybean meal and its deactivation. Int. J. Sci. Environ. Technol. 6:3793-3797.

Zamora, R. G., and T. L. Veum. 1979. Whole soybeans fermented with aspergillus oryzae and rhizopus oligosporus for growing pigs. J. Anim. Sci. 48:63–68.

Zhang, F., and O. Adeola. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. Anim. Nutr. 3:344–352.

Zhang, A. R., M. Wei, L. Yan, G. L. Zhou, Y. Li, H. M. Wang, Y. Y. Yang, W. Yin, J. Q. Guo, X. H. Cai, J. X. Li, H. Zhou, and Y. X. Liang. 2021. Effects of feeding solid-state

fermented wheat bran on growth performance and nutrient digestibility in broiler chickens. Poult. Sci. 101402.

Zhu, F., B. Zhang, J. Li, and L. Zhu. 2020. Effects of fermented feed on growth performance, immune response, and antioxidant capacity in laying hen chicks and the underlying molecular mechanism involving nuclear factor-κb. Poult. Sci. 99:2573–2580.

CHAPTER 2

DETERMINATION OF TME_n, STANDARDIZED AMINO ACID DIGESTIBILITY, PHOSPHORUS DIGESTIBILITY, AND PHOSPHORUS BIOAVAILABILITY OF FERMENTED SOYBEAN MEAL AND FERMENTED FULL-FAT SOYBEANS FED TO CHICKENS

ABSTRACT

Five experiments were conducted to evaluate the nutritional value of dehulled solvent-extracted soybean meal (SBM-CV), fermented SBM-CV (FSBM), dehulled extruded full-fat soybeans (FFSB), and fermented FFSB (FFFSB). In Experiments 1 and 2, two precision-fed rooster assays were performed to determine the nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid (AA) digestibility among the test ingredients using conventional and cecectomized roosters, respectively. Full-fat ingredients presented greater TME_n values than conventional ingredients (P<0.05). Fermentation had a positive effect on TME_n of SBM-CV and a negative effect on FFSB. There were no differences in standardized AA digestibility between conventional and full-fat ingredients. Fermented ingredients had lower (P<0.05) standardized AA digestibility values compared with their unfermented counterparts. In Experiment 3, an ad libitum-fed broiler chicken assay was conducted to determine apparent ileal P digestibility and total tract P retention at two Ca levels among the test ingredients. Diets contained a Ca:non-phytate P (NPP) ratio of either 2 or 7.5. Greater (P<0.05) apparent ileal P digestibility values were observed at the low Ca level than at high Ca levels. At the high Ca level, fermentation increased the ileal P digestibility and total tract P retention for both conventional and full-fat samples, while at the low Ca level, there was a reduction (P<0.05) in

total tract P retention for FFFSB. In Experiments 4 and 5, two 17 d chick trials were conducted to determine P bioavailability of the test ingredients relative to KH_2PO_4 using crossbred chicks (Experiment 4) and another similar trial using SBM-CV and FSBM in commercial broiler chicks (Experiment 5). Multiple regression of bone ash in mg/tibia and % on supplemental P intake yielded slope-ratio relative P bioavailabilities from 23% to 48%. Fermentation did not affect relative P bioavailability in SBM-CV and increased the relative bioavailability values in full-fat samples in crossbred chicks. In commercial broiler chicks, there were no differences in relative P bioavailability between SBM-CV and FSBM. In summary, fermentation increased TME_n in SBM-CV but had a negative effect on FFSB. Fermentation had no significant effect on indispensable AA with the exception of a decrease in Lys digestibility for both SBM-CV and FFSB, suggesting possible heat damage. Fermentation had a positive effect on apparent ileal P digestibility and total tract P retention in both SBM-CV and FFSB when diets contained 0.75% Ca and also increased relative P bioavailability of FFSB in crossbred chicks.

INTRODUCTION

Soybean meal (SBM) has excellent nutritional characteristics for monogastric nutrition. These include high crude protein content, good amino acid (AA) profile, moderate concentration of metabolizable energy, and a considerable amount of P and Ca. However, the bean also contains antinutritional factors (ANF) that can inhibit the action of some digestive enzymes, reduce the digestion of nutrients, cause allergies, or be non-digestible. Some of the ANF's present in SBM are trypsin inhibitors, non-starch polysaccharides, phytate, and elevated fiber content (Stein et al., 2008). Another factor affecting the nutritional value of SBM is the

production process itself, more specifically, overheating the ingredient (Fernandez and Parsons, 1996).

Overheating and trypsin inhibitor content have been demonstrated to reduce the AA digestibility in SBM (Hoffmann et al., 2019). The presence of NSPs, oligosaccharides and elevated fiber content can also reduce protein digestibility (Chen et al., 2013), negatively affect the digestibility of starch and fat, and reduce the concentration of metabolizable energy in the ingredient (Smits et al., 1997). Phytate directly affects the utilization of P, reducing the amount of P that is bioavailable for the animal, and can also have a negative effect on Ca metabolism (Karr-Lilienthal et al., 2005; Stein et al., 2008).

Further processing of SBM, such as fermentation, may provide multiple beneficial effects at once, but it requires the action of microbial agents on the ingredient (Mukherjee et al., 2015). Fermentation can reduce trypsin inhibitors, NSP's and phytate in the ingredient. In consequence, fermentation can improve AA, energy, and P digestibility (Rojas, 2012). Fermentation can also provide lactic acid, which, working as an acidifier, can improve the digestion of SBM nutrients (Chachaj et al., 2019b; Soumeh et al., 2019).

Although some authors reported the beneficial effects of fermented SBM, there is limited research about the effects of fermentation on full-fat soybeans and new techniques of fermentation. Therefore, the objective of this study was to determine nitrogen-corrected true metabolizable energy (TME_n), standardized AA digestibility, apparent ileal P digestibility, total tract P retention, and relative P bioavailability in fermented SBM (FSBM) and fermented full-fat soybeans (FFSB) fed to chickens, and to test the hypothesis that these values are greater than in conventional SBM (SBM-CV) and full-fat soybeans (FFSB).

MATERIALS AND METHODS

Five experiments were conducted, and the Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for these experiments.

Ingredients and analysis

The test ingredients were SBM-CV, FSBM, FFSB and FFFSB, processed from the same batch of U.S. soybeans. The fermented ingredients were produced by submerged fermentation in presence of *Lactobacillus subtilis*. The test ingredients were processed and provided by Inolasa (San Jose, Costa Rica). Most of the analyses were performed at the Department of Animal Sciences of the University of Illinois at Urbana-Champaign. Dry matter (DM) was measured using a drying oven for 2 h at 135 °C (method 930.15; AOAC International, 2007), gross energy (GE) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), crude protein (CP) by multiplying N × 6.25 and N determined via the combustion procedure (method 990.03; AOAC International, 2007) on a LECO FP628 (LECO Corp., Saint Joseph, MI), crude fat via acid hydrolysis using 3 N HCl ($Ankom^{HCl}$, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether ($Ankom^{XT15}$, Ankom Technology, Macedon, NY), insoluble and soluble dietary fiber using the $Ankom^{TDF}$ Dietary Fiber Analyzer (method 991.43, AOAC International, 2007), ash (method 942.05; AOAC International, 2007), and Ca and P by inductively coupled plasma spectroscopy (method 985.01 A, B, and C; AOAC International, 2007) after wet ash sample preparation (method 975.03 B, AOAC International, 2007). Amino acid concentrations were determined at the University of Missouri Analytical Laboratories on a Hitachi Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110 °C (method 982.30 E(a);

AOAC International, 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 International, 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C (method 982.30 E(c); AOAC International, 2007). Carbohydrate content were determined at Illinois Crop Improvement Association Laboratories as described by Cervantes-Pahm and Stein (2010). Phytic acid (Ellis et al., 1977) and trypsin inhibitor units (method Ba 12-75; AOCS, 2006) were determined by Eurofins Scientific Inc. Protein dispersibility index (method BA 10-65; AOCS, 2006), KOH protein solubility (Araba and Dale, 1990), and urease activity (method BA 9-58; AOCS, 2006) were determined by Dairyland laboratories. Titanium concentrations in experimental diets, ileal digesta, and excreta were measured using UV spectroscopy (Myers et al., 2004).

Experiments 1 and 2: TME_n and Standardized AA Digestibility

In Experiment 1, conventional Single Comb White Leghorn roosters were used to determine TME_n of the four test ingredients. There were 6 replicate roosters per treatment and each treatment consisted of each test ingredient. The roosters were fasted for 26 h to empty their gastrointestinal tracts. After the fasting period, each rooster was tube fed 25 g of a test ingredient. After the tube feeding, each rooster was placed in an individual wire cage with an excreta collection tray underneath. Excreta were collected during the following 48 h after the tube feeding. Excreta samples were freeze dried, weighed, and ground prior being analyzed. For Experiment 1, excreta samples were analyzed for GE and N, and TME_n was calculated as described by Parsons et al. (1982). Endogenous losses of GE were determined from roosters that were fasted for 48 h.

For Experiment 2, Single Comb cecectomized White Leghorn roosters were used, and the procedures was identical to Experiment 1. Excreta samples were analyzed for AA concentration. Standardized AA digestibility values were calculated following the method described by Engster et al. (1985). Basal endogenous AA losses were determined using cecectomized birds that were fasted for 48 h.

Experiment 3: Apparent ileal P digestibility

This experiment was conducted using commercial broiler chicks to determine apparent ileal P digestibility and total tract P retention of the four test ingredients at two dietary Ca inclusion levels. Commercial Ross 308 males were placed in Petersime batteries with raised wire floors in an environmentally controlled room. The chicks were fed a standard, nutritionally complete corn-SBM diet for 16 d and had ad libitum access to water and feed. On day 16, chicks were fasted overnight. On d 17, the chicks were weighed, wingbanded, and allotted to one of eight dietary treatments, ensuring consistency in average body weight across treatments in a completely randomized design with a 2 × 2 × 2 factorial treatment arrangement where SBM type (SBM-CV vs FFSB), fermentation (fermented vs non-fermented), and Ca inclusion level (0.2% vs 0.75%) were considered the factors. The average initial body weight at the start of the experimental period was 514 g and there were five replicate pens of five chicks for each dietary treatment, resulting in a total of 200 chicks. The experimental diets were provided for ad libitum consumption from d 17 to 21. Diets 1, 3, 5, and 7 were formulated to contain 0.2% Ca and included SBM-CV, FSBM, FFSB, and FFFSB, respectively. Diets 2, 4, 6, and 8 were formulated to contain 0.75% Ca achieved by adding limestone at the expense of dextrose and included SBM-CV, FSBM, FFSB, and FFFSB, respectively. All diets contained approximately 0.1% NPP with the test ingredients serving as the sole source of P. Titanium dioxide was added at 0.5% of

the diet as an indigestible marker. The composition of the experimental diets is presented in Table 2.1. Chicks were euthanized on the last day of the experimental period (d 21) via asphyxiation with carbon dioxide gas. Ileal digesta content (from Meckel's diverticulum to ileal-cecal junction) and excreta were collected and analyzed for P and Ti as previously described.

Experiments 4 and 5: Relative P Bioavailability

For Experiment 4, crossbred (New Hampshire × Columbian) male chicks, hatched at the University of Illinois at Urbana-Champaign poultry research field laboratory, were used to determine the bioavailability of P in the test ingredients relative to the P in KH_2PO_4. Chicks were housed in heated Petersime batteries and fed a standard, nutritionally complete corn-SBM pretest diet until 7 d of age. At 7 d of age, chicks were weighed, wingbanded, and allotted to one of the eleven dietary treatments, maintaining a consistent average initial body weight across treatments. The experiment was a completely randomized design with five replicate pens of five chicks for each dietary treatment, resulting in a total number of 275 chicks. The average initial body weight at the start of the experimental period was 80.8 g/chick. Chicks were fed the experimental diets from 7-21 d of age. Composition of the experimental diets are shown in Table 2.2. Diet 1 was a P-deficient cornstarch-dextrose-SBM diet containing 0.18% non-phytate P, Diets 2-3 contained 0.05% and 0.10% supplemental P from KH_2PO_4, respectively, Diets 4-5 contained added 12.5 and 25% test SBM-CV, respectively, Diets 6-7 contained added 12.5 and 25% test FSBM, respectively, Diets 8-9 contained added 12.5 and 25% test FFSB, respectively, Diets 10-11 contained added 12.5 and 25% test FFFSB, respectively. The KH_2PO_4 and the test ingredients were added in place of cornstarch and dextrose. At the end of the experimental period, all chicks and feeders were weighed and recorded for analysis. Weight gain, feed consumption, and feed efficiency were calculated for each replicate. Chicks were euthanized on the last day of the

experimental period via asphyxiation with carbon dioxide gas. The right leg was collected from each chick for subsequent tibia ash analysis. The right tibia was autoclaved, cleaned of any adhering tissue, oven-dried at 100 degrees Celsius for 24 h, and ashed at 600 degrees Celsius in a muffle furnace for 24 h.

Experiment 5 was conducted similarly to Experiment 4 except that commercial Ross 308 broiler males were used, and the test ingredients were only SBM-CV and FSBM. This experiment was conducted to determine if relative P bioavailability is not different for crossbred and commercial broiler chicks. The average initial body weight was 82.8 g. The experimental design was completely randomized with five replicate pens of five chicks for each of the seven treatments, resulting in a total of 175 chicks. Diet 1 was a P deficient cornstarch-dextrose-SBM diet containing 0.18% non-phytate P, Diets 2-3 contained 0.05% and 0.10% supplemental P from KH_2PO_4, respectively, Diets 4-5 contained added 12.5 and 25% test SBM-CV, respectively, and Diets 6-7 contained added 12.5 and 25% test FSBM, respectively. The compositions of the seven experimental diets used in Experiment 5 were identical to the first seven diets used in Experiment 4 and are shown in Table 2.4.

Statistical Analysis

Data from all five experiments were analyzed using the GLM procedure in SAS (SAS Institute. INC., 2010). An ANOVA procedure was utilized for each experiment and their respective design, and the Fisher's least significant difference test was used to determine if differences among treatments were significant at $P<0.05$. For Experiments 1 and 2, each individual rooster was considered the experimental unit, and a factorial analysis was used to determine the main effects of SBM type (SBM-CV vs FFSB), fermentation, and the interaction. For Experiments 3, 4, and 5, each pen containing 5 chicks was considered the experimental unit.

For Experiment 3, a 2 × 2 × 2 factorial analysis was used to determine the main effects of SBM type, fermentation, and Ca inclusion level and the interaction. For Experiments 4 and 5, a multiple linear regression (GLM procedure of SAS) was computed by regressing either tibia ash content (mg/tibia) or tibia ash concentration (%) on supplemental P intake (g/chick) from KH_2PO_4 or the test ingredients. The slope ratio method was then used to calculate the bioavailability of P in the test ingredients relative to KH_2PO_4 (Finney, 1964). Phosphorus bioavailability values for potassium phosphate were set at 100%.

RESULTS AND DISCUSSIONS

Nutritional Composition

The nutrient composition of the test ingredients is presented in Table 2.3. The nutrient composition of SBM-CV and FFSB was not different than the values reported by the NRC (2012), except that FFSB contained 20.48% of AEE and 16.1% of TDF, and these values were greater for AEE and lower for TDF than the values reported by NRC (2012). In contrast, the values for FSBM were different than the values reported by NRC (2012). The FSBM contained lower DM, CP, and P, similar GE, and greater Ca percentages compared with the values reported by NRC (2012). The FFFSB contained similar values for GE, AEE, and ash compared with the values for heated full-fat soybeans fermented with fungus reported by Zamora and Veum (1987). The exception was for CP and essential AA, which were slightly lower in the FFFSB than the values reported by Zamora and Veum (1987). In general, values reported for FFFSB in the literature are limited.

Due to the fermentation process, there were observed clear differences when comparing the fermented ingredients with their unfermented counterparts. The first difference was the reduction of carbohydrates such as sucrose and stachyose and the reduction of trypsin inhibitors

in FSBM and FFFSB, which was also observed in a previous study (Mukherjee et al., 2015). Fermentation had a greater negative effect on AEE content in FFSB compared with the effect on SBM-CV, where it barely decreased the AEE, and this may be due to the low content of AEE in SBM-CV. The AEE reduction with fermentation is not common or expected, but it was also reported by by Drazbo et al. (2018) in yeast enzyme fermented rapeseed cake where ether extract was reduced by 0.7 percentage points while the concentration of other nutrients increased or did not change after fermentation. According to Ketharpaul and Chauhan (1989), pure culture fermentation with either *Saccharomyces* or *Lactobacillus* species can significantly reduce the crude fat content in pearl millet flour, and the reduction is greater with *Lactobacillus* due that some yeasts strains are able to produce fat which will be accounted for in the crude fat analysis. Since there was no variation in the DM content of the fermented ingredients, and because of the reduction of the nutrients previously mentioned, the concentration of the other nutrients increased. It was observed that GE and CP increased in the fermented ingredients and TDF content slightly increased in FSBM but decreased in FFFSB, compared with SBM-CV and FFSB, respectively, although the differences were only 1 – 2 percentage points.

The concentration of total Ca and P slightly increased with fermentation while phytic acid concentration barely changed. These changes caused a slight increase in nonphytate-P, measured as a percentage of total P in the fermented ingredients compared with their unfermented counterparts.

Trypsin inhibitor activity (TIA, mg/g) content in FFSB was not different than the values reported by Van Eys et al. (2012) for full-fat soybeans extruded at 126 °C. The value for TIA content for SBM-CV was in the range of the values reported by the same author in the same study for SBM. The TIA values were reduced in the fermented ingredients. Phytate bound P for

SBM-CV and FFSB were in within the range, but stachyose and raffinose levels were slightly above the range reported by Van Eys (2012).

Experiment 1: True Metabolizable Energy

The TME_n value obtained for SBM-CV was 2,897 kcal/kg of DM (Table 2.3), and it was within the range of 2,761 and 2,963 kcal/kg of DM as reported by NRC (1994) and Baker et al. (2011), respectively. The FSBM had a TME_n value of 3,004 kcal/kg DM, which was greater than the value for SBM-CV ($P<0.05$). In contrast, FFFSB presented a TME_n of 4,090 kcal/kg DM, which was lower compared with 4,189 kcal/kg DM for FFSB ($P<0.05$), although both values were greater than the TME_n values of 3,322 kcal/kg of DM reported by NRC (1994) and 3960 kcal/kg of DM for apparent ME reported by Van Eys (2012) for roasted full-fat soybeans. There was a significant interaction ($P<0.05$) between fermentation and SBM type. When fermentation was applied to SBM-CV, the TME_n content increased, while when fermentation was applied to FFSB, the TME_n content decreased. The positive effect of fermentation on TME_n for SBM-CV may be explained by the increased CP and reduction of oligosaccharide content which has been demonstrated to increase TME_n (Parsons et al., 2000). Even though fermentation had similar effects on FFSB, increasing the CP and reducing oligosaccharides, it also reduced the fat content to a greater extent compared with SBM-CV. The reduction of fat content may have been enough to also reduce the TME_n concentration since fat supplies more ME than proteins or carbohydrates.

Experiment 2: Standardized AA Digestibility

Total AA concentration, standardized AA digestibility values, and digestible AA concentrations for the four test ingredients are presented in Table 2.4. Total concentrations of AA were greater in the conventional ingredients compared with the full-fat ones. When

comparing the fermented ingredients with their respective unfermented counterparts, there was variation in the AA content, where Lys and Arg were reduced by fermentation, while other AA maintained or increased in concentration, causing a reduction in the Lys:CP ratio in the fermented ingredients. The total concentration of AA in SBM-CV, FSBM, and FFSB was in accordance with the values reported by NRC (2012) for the respective ingredient. The total AA concentration of FFFSB was lower than the values reported by Zamora and Veum (1987), but the difference was likely due to the higher CP content in the ingredient used in that study compared with the ingredient from this study. When compared based on total AA:CP ratio, the ratio values were not different between FFFSB in the current study and that in Zamora (1987). The standardized AA digestibility values observed for indispensable AA in SBM-CV agreed with those reported by Ahasic (2020) for SBM. The values obtained for FFSB agreed with the values reported by Thanabalan et al. (2021) for the same ingredient in 21-d broiler chickens. No differences (P>0.05) were observed for standardized AA digestibility values of each AA among the four ingredients, except for Lys and Glu, which were lower (P>0.05) in the fermented ingredients than in the unfermented ones. The digestibility reduction in Lys due to fermentation was numerically greater in SBM-CV than in FFSB, and this effect may be explained by the fat possibly alleviating the negative effect on AA digestibility of the fermentation. There was no interaction between fermentation and fat. The digestible concentration of essential AA increased in the fermented ingredients compared with the unfermented ones, except for Lys and Arg, that which were reduced due to the reduction in total concentration and digestibility coefficients of these AA with fermentation.

The increased CP in the fermented ingredients is in accordance with several authors (Cervantes-Pahm and Stein, 2010; Mukherjee et al., 2015; Chachaj et al., 2019), although the

reduction in Lys content disagrees with these reports. Usually, a reduction in Lys to CP ratio, a darkened color of the ingredient, and reduction of ileal digestibility are characteristics associated with heat-damaged SBM (González-Vega et al., 2011). According to the quality check analyses of the test ingredients (Table 2.3), the PDI and urease activity results may not be conclusive on whether heat damage occurred in the fermented ingredients. According to Van Eys (2012), KOH solubility is a better indicator of overcooked SBM, and the lower values for the fermented ingredients may indicate some heat damage due to the fermentation process. However, a reduction in total Lys and changes in sulfur amino acids due to fermentation have been reported (Osman, 2011; Çabuk et al., 2018). Changes in the color of the ingredient have also been reported as an effect of fermentation attributed to increased phenolic compounds and the drying step during the process (Cui et al., 2012). Therefore, the reduction in total Lys concentration and digestibility of Lys may be partially due to both heat damage and fermentation. The reason for the effect of fermentation on digestibility values for Glu is unknown.

Experiment 3: Apparent Ileal P digestibility and total tract P retention

Apparent ileal P digestibility values for the four test ingredients are presented in Table 2.5. The apparent ileal P digestibility values ranged from 41.3 to 79%. At 0.2% dietary Ca or a Ca:NPP ratio of 2.0, the test ingredients presented greater apparent ileal P digestibility values compared with the values obtained at 0.75% Ca or Ca:NPP ratio of 7.5 (significant main effect of dietary Ca level, $P<0.05$). The values for SBM-CV and FFSB at 0.2% Ca were lower than the values obtained by Ahasic (2020) for the same level of Ca in SBM using ad-libitum fed chicks, and this may be due to the lower Ca:NPP ratio of the diets in that study based on the analyzed Ca values reported. The values for SBM-CV and FFSB were greater than the values obtained by Munoz (2020) for SBM using precision-fed chicks. At 0.75% Ca, the values obtained were close

to the values reported by Ahasic (2020) for the same level of Ca. There was an interaction between dietary Ca level and fermentation ($P<0.01$). At 0.75% Ca, the fermented ingredients presented greater apparent ileal P digestibility than the unfermented ones, while a different effect was observed at 0.2% Ca, where no significant differences were observed among the four test ingredients. There were no significant differences in P digestibility when comparing SBM-CV with FFSBM or FSBM with FFFSBM at both Ca levels.

Total tract P retention values ranged from 33.9 to 61.2%. In contrast to apparent ileal P digestibility, there was no significant main effect of dietary Ca level on total tract P retention ($P>0.05$). At 0.2% Ca, the total tract P retention values for SBM-CV and FFSB were lower than the values reported by Ahasic (2020), but the values were not different at 0.75% C. There was an interaction between SBM type and fermentation ($P<0.001$) and between diet Ca level and fermentation ($P<0.001$). For the diet Ca level and fermentation interaction, fermentation had a positive effect at 0.75% Ca but had no effect or a negative effect at 0.2% Ca. For the SBM type and fermentation interaction, when averaged over both diet Ca levels, fermentation produced an increase in total tract P retention for SBM but had little or no effect for FFSB.

The positive effect of fermentation on apparent ileal digestibility and apparent total tract retention of P may be due to reduced phytate-P relative to total P in the fermented ingredients compared with unfermented ingredients (Table 2.3). The capacity of fermentation to increase the availability of P has been reported in several studies (Hirabayashi et al., 1998; Chen et al., 2014; Mukherjee et al., 2015). These results agree with Hirabayashi et al. (1998), who reported greater P retention as a percentage of P intake in 1-wk-old White Leghorn chicks fed SBM and FSBM diets. The negative effect of wider Ca:NPP ratios on ileal digestibility and retention of P observed herein has been reported to be due to reduced P absorption due to an increase in the

intestinal pH and formation of Ca-phytate complexes (Applegate et al., 2003; Liu et al., 2013). This negative effect of increased diet Ca on digestibility of P has also been reported in several studies in broilers, hens, and pigs (Applegate et al., 2003; Stein et al., 2011; Rama Rao et al., 2014).

Experiment 4 and 5: Relative P Bioavailability

Growth performance and tibia ash values from Experiments 4 and 5 are presented in Table 2.6 and Table 2.7. In Experiments 4 and 5, weight gain and feed efficiency were increased (P<0.05) with increasing inclusion of KH_2PO_4 and the different types of SBM compared with the P deficient diet. Feed intake and tibia ash (content and concentration) linearly increased (P<0.05) with increasing KH_2PO_4 and increasing test SBM inclusion from 12.5 to 25%.

For Experiment 4, the bioavailability values of P in the test ingredients relative KH_2PO_4 from the multiple linear regression analysis for both tibia ash content (mg/tibia) and tibia ash concentration (%) on supplemental P intakes are presented in Table 2.8. The values ranged from 23.6 to 35.3% based on tibia ash content (mg/tibia) and 23.1 to 47.9% based on tibia ash concentration (%). The relative bioavailability of P in the test ingredients determined from regression of tibia ash content (mg/tibia) was much lower than the apparent P digestibility values determined at 0.2% dietary Ca in Experiment 3. The relative P bioavailability values were in better agreement with the apparent ileal P digestibility and total tract P retention values determined at 0.75% dietary Ca but they were still numerically greater in several instances. This may be possibly explained by bioavailability including post-absorptive P metabolism and deposition in the bone that occurs after P digestion. Therefore, it is likely that not all the P digested is deposited or retained in the bone (Ahasic, 2020). The relative bioavailability values determined using tibia ash content (mg/tibia) were generally numerically lower than the values

determined using tibia ash concentration (%). There were no significant differences of the bioavailability values among the test ingredients except for FFSB which presented the lowest bioavailability value compared with the other three test ingredients (significant on tibia ash concentration, $P<0.05$). The bioavailable content of P in each test ingredient was calculated by multiplying the total P of the sample with its corresponding bioavailable P value. The relative bioavailability of P based on tibia ash content (mg/tibia) and the respective calculated bioavailable content of SBM-CV agreed with the values obtained in a similar study by Ahasic (2020), but the values were below the values reported by Munoz et al. (2018). The greater values obtained by Munoz et al. (2018) may be explained by the greater NPP relative to the total P of the SBM used in that study compared with the same ingredient of this study. Another possible reason for the difference in relative P bioavailability values may be that the rooster assay used by Munoz et al. (2018) was determined to be highly variable for determining P digestibility and retention.

For Experiment 5, the bioavailability values of P in the test ingredients relative to KH_2PO_4 determined in commercial broiler chicks are presented in Table 2.9. The values ranged from 26.6% to 32.1% based on tibia ash content (mg/tibia) and from 20.9% to 23.7% based on tibia ash concentration (%). The relative bioavailability values based on tibia ash content (mg/tibia) were numerically greater than the values based on tibia ash concentration (%). The relative bioavailability values based on tibia ash content (mg/tibia) were not different to the values from Experiment 4, whereas relative bioavailability values based on tibia ash concentration (%) were numerically higher for commercial broiler chicks than the crossbred chicks in Experiment 4. There were no significant differences in relative bioavailability of P between SBM-CV and FSBM for commercial broiler chicks in Experiment 5. Thus, there were

no consistent differences between crossbred and commercial broiler chicks for relative P bioavailability values in Experiments 4 and 5.

In summary, fermentation increased TME_n in SBM-CV but had a negative effect on FFSB. Fermentation generally had no significant effect on digestibility of indispensable AA with the exception of a decrease in Lys digestibility for both SBM and FFSB, suggesting possible heat damage. Fermentation had a positive effect on apparent ileal P digestibility and total tract P retention in both SBM and FFSB when diets contained 0.75% Ca and also increased relative P bioavailability of FFSB in crossbred chicks.

TABLES

Table 2.1. Ingredient composition of experimental diets in Experiment 3 for determination of apparent ileal P digestibility and total tract P retention.

	Dietary treatment							
Ingredient, %	1	2	3	4	5	6	7	8
Dextrose	34.58	33.13	34.61	33.16	29.49	28.04	29.53	28.08
Test SBM-CV[1]	45.00	45.00	-	-	-	-	-	-
Test FSBM[1]	-	-	45.00	45.00	-	-	-	-
Test FFSB[1]	-	-	-	-	50.00	50.00	-	-
Test FFFSB[1]	-	-	-	-	-	-	50.00	50.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Cornstarch	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	0.07	1.52	0.04	1.49	0.16	1.61	0.12	1.57
Solka floc[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline Cl 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Analyzed, %:								
Ca	0.23	0.73	0.26	0.85	0.24	0.75	0.31	0.93
P	0.28	0.30	0.31	0.31	0.27	0.27	0.28	0.30

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[2]Powdered cellulose; International fiber Corporation, Urbana, OH 43078.

[3]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

Table 2.1. (Cont.)

[4]Provided as milligrams per kilogram of diet: manganese, 75 mg from $MnSO_4 \cdot H_2O$; iron, 75 mg from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 mg from ethylene diamine dihydroiodide; selenium, 0.1 mg from Na_2SeO_3.

Table 2.2. Ingredient composition of experimental diets in Experiment 4 for determination of relative P bioavailability in crossbred chickens[1].

	Dietary treatment										
Ingredient, %	1	2	3	4	5	6	7	8	9	10	11
Dextrose	8.25	8.25	8.25	4.17	-	4.17	-	4.17	-	4.17	-
Cornstarch	16.75	16.52	16.29	8.33	-	8.33	-	8.33	-	8.33	-
Corn	24.85	24.85	24.85	24.85	2.11	24.85	24.85	24.85	24.85	24.85	24.85
KH_2PO_4	-	0.23	0.46	-	-	-	-	-	-	-	-
Test SBM-CV[2]	-	-	-	12.50	25.00	-	-	-	-	-	-
Test FSBM[2]	-	-	-	-	-	12.50	25.00	-	-	-	-
Test FFSB[2]	-	-	-	-	-	-	-	12.50	25.00	-	-
Test FFFSB[2]	-	-	-	-	-	-	-	-	-	12.50	25.00
Non-test SBM	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59
Dicalcium phosphate	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
L-Thr	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choline Cl (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Table 2.2. (Cont.)

Calculated nutrients:											
Ca	0.80	0.80	0.80	0.85	0.90	0.86	0.91	0.84	0.87	0.84	0.88
Available P	0.18	0.23	0.28	0.21	0.24	0.21	0.24	0.21	0.24	0.21	0.24

[1]Diets used in Experiment 5 were the same as diets 1-7 used in Experiment 4 but fed to commercial broiler chicks.

[2]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[3]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

[4]Provided as milligrams per kilogram of diet: manganese, 75 mg from $MnSO_4 \cdot H_2O$; iron, 75 mg from $FeSO_4 \cdot H2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 mg from ethylene diamine dihydroiodide; selenium, 0.1 mg from Na_2SeO_3.

Table 2.3. Analyzed composition and TME_n of the test ingredients, as-fed basis.

Item, %	SBM-CV[1]	FSBM[1]	FFSB[1]	FFFSB[1]
DM[1]	88.82	88.92	91.15	90.12
GE[1], kcal/kg	4213	4316	5226	5242
CP[1]	48.81	50.44	36.42	38.48
Lys:CP	0.063	0.057	0.063	0.058
Total dietary fiber	17.80	19.20	24.10	21.70
Soluble dietary fiber	2.3	3.5	3.2	3.5
Insoluble dietary fiber	15.5	15.7	20.9	18.2
Neutral detergent fiber	7.49	10.07	8.94	6.66
Acid detergent fiber	4.08	4.83	5.47	4.35
Acid hydrolyzed ether extract	0.93	0.66	20.48	18.73
Ash	6.28	6.71	4.91	5.22
Ca	0.39	0.41	0.28	0.31
Total P	0.64	0.69	0.52	0.53
Phytate-P[3]	0.47	0.49	0.36	0.36
Phytate-P, % of total P	72.59	70.91	69.54	67.55
Nonphytate-P[4]	0.18	0.20	0.16	0.17
Indispensable amino acids				
Arg	3.51	3.34	2.61	2.38
His	1.26	1.27	0.95	0.95
Ile	2.39	2.46	1.80	1.91
Leu	3.73	3.87	2.77	2.90
Lys	3.06	2.88	2.28	2.22
Met	0.62	0.64	0.46	0.49
Phe	2.50	2.55	1.86	1.96
Thr	1.81	1.87	1.34	1.38
Trp	0.65	0.68	0.49	0.50
Val	2.46	2.56	1.87	1.98
Total	21.99	22.12	16.43	16.67
Dispensable amino acids				

Table 2.3. (Cont.)

Ala	2.08	2.22	1.55	1.69
Asp	5.44	5.66	4.00	4.26
Cys	0.69	0.73	0.53	0.54
Glu	8.64	8.99	6.18	6.45
Gly	2.03	2.15	1.53	1.61
Pro	2.40	2.49	1.80	1.89
Ser	2.07	2.24	1.49	1.50
Tyr	1.80	1.78	1.29	1.33
Total	25.15	26.26	18.37	19.27
Other amino acids				
Hydroxylysine	0.08	0.05	0.06	0.07
Hydroxyproline	0.06	0.07	0.06	0.07
Lanthionine	0.06	ND[1]	ND	ND
Ornithine	0.04	0.21	0.03	0.26
Taurine	0.08	0.06	0.06	0.06
Total amino acids	47.46	48.77	35.01	36.40
Trypsin inhibitor, TIU/mg	3.60	1.20	2.20	<1.00
Trypsin inhibitor[5], TIA (mg/g)	1.89	0.63	1.16	<1.00
Sugar profile				
Glucose	ND	0.07	ND	0.07
Sucrose	6.61	0.06	5.18	0.08
Maltose	ND	ND	ND	ND
Fructose	0.06	0.13	0.07	0.08
Stachyose	5.93	0.13	4.39	0.17
Raffinose	1.86	ND	1.33	ND
TME_n (kcal/kg DM)	2897[d]	3004[c]	4189[a]	4090[b]
Quality check analysis:				
Protein dispersibility index	10.75	23.82	8.28	28.96
Urease activity, pH units	0.01	0.02	0.01	0.01
KOH solubility	79.28	78.23	69.76	67.77

Table 2.3. (Cont.)

[a-d]TME_n values within a row with no common superscript are significantly different ($P<0.05$). Values are means of 6 individually-caged conventional roosters. Pooled SEM = 31.9.

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. DM= dry matter. GE = gross energy. CP = crude protein. ND = not detectable.

[2]TME_n values are means of six individually-caged conventional roosters.

[3]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).

[4]Nonphytate-P was calculated as the difference between total P and phytate-P.

[5]Calculated using a conversion factor: TIU/mg = 1.9 × TI, mg/g (Hamerstrand et al. 1981).

Table 2.4. Total amino acids, standardized amino acid digestibility values, and digestible amino acid concentrations for the test ingredients from the precision-fed rooster assay in Experiment 2 (DM basis).

	SBM-CV[1]			FSBM[1]			FFSB[1]			FFFSB[1]			
	Total	Digest. value	Digest. conc.[2]	Total	Digest. value	Digest. conc.	Total	Digest. value	Digest. conc.	Total	Digest. value	Digest. conc.	Pooled SEM[3]
Indispensable AA													
Arg	3.95	92.0	3.63	3.76	91.8	3.45	2.86	93.1	2.66	2.64	92.6	2.44	0.54
His	1.42	89.9	1.28	1.43	87.1	1.25	1.04	88.7	0.92	1.05	88.2	0.93	0.78
Ile	2.69	88.4	2.38	2.77	88.5	2.45	1.97	88.9	1.75	2.12	89.4	1.89	0.73
Leu	4.20	88.4	3.71	4.35	89.1	3.88	3.04	89.3	2.71	3.22	90.2	2.90	0.78
Lys	3.45	88.5[a]	3.05	3.24	82.0[b]	2.66	2.50	87.5[a]	2.19	2.46	83.6[b]	2.06	0.71
Met	0.70	87.3	0.61	0.72	88.9	0.64	0.50	87.0	0.44	0.54	87.2	0.47	0.90
Phe	2.81	88.9	2.50	2.87	89.1	2.56	2.04	89.0	1.81	2.17	89.9	1.95	0.77
Thr	2.04	86.7	1.77	2.10	86.4	1.81	1.47	86.3	1.27	1.53	85.7	1.31	0.95
Trp	0.73	96.7	0.71	0.76	97.0	0.74	0.54	97.4	0.53	0.55	97.4	0.54	0.34
Val	2.77	86.7	2.40	2.88	87.7	2.53	2.05	87.1	1.79	2.20	88.1	1.94	0.93
Dispensable AA													
Ala	2.34	84.1	1.97	2.22	84.8	1.88	1.55	85.0	1.32	1.69	85.8	1.45	0.86
Asp	6.12	89.3	5.46	5.66	87.7	4.96	4.00	87.7	3.51	4.26	86.6	3.69	0.76
Cys	0.78	85.4	0.67	0.73	81.5	0.59	0.53	83.8	0.44	0.54	81.6	0.44	1.44
Gly	2.29	-	-	2.15	-	-	1.53	-	-	1.61	-	-	-
Glu	9.73	92.0[a]	8.96	8.99	89.5[b]	8.05	6.18	91.3[a]	5.64	6.45	88.9[b]	5.74	0.59
Pro	2.70	90.9	2.45	2.49	89.2	2.22	1.80	90.6	1.63	1.89	89.3	1.69	0.82

Table 2.4. (Cont.)

Ser	2.33	89.3	2.08	2.24	90.8	2.03	1.49	88.7	1.32	1.50	87.8	1.32	0.75
Tyr	2.03	89.6	1.82	1.78	89.1	1.59	1.29	88.8	1.14	1.33	88.5	1.18	0.69

[a-b]Standardized digestibility values within a row with no common superscript are significantly different (P<0.05). Values are means of 6 individually-caged cecectomized roosters.

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[2]Digestible concentration = (total × standardized digestibility values)/100.

[3]Pooled SEM for standardized digestibility values. There was a significant main effect of fermentation on digestibility values for Glu and Lys (P<0.05). There were no significant interactions between SBM type and fermentation for any AA (P>0.05).

Table 2.5. Apparent ileal P digestibility and total tract P retention values for chicks in Experiment 3[1].

SBM type	Diet Ca level[3] (%)	Ileal P digestibility (%)[4]	Total tract P retention (%)[5]
SBM-CV[2]	0.2	76.3[a]	51.1[bc]
FSBM[2]	0.2	79.0[a]	49.6[c]
FFSB[2]	0.2	75.2[a]	61.0[a]
FFFSB[2]	0.2	73.9[a]	52.5[bc]
SBM-CV	0.75	41.3[c]	33.9[d]
FSBM	0.75	61.7[b]	61.2[a]
FFSB	0.75	44.8[c]	47.5[c]
FFFSB	0.75	57.7[b]	56.9[ab]
Pooled SEM	-	3.12	2.22

[a-d] Means within a column with no common superscript differ (P<0.05).

[1]Values are means of five pens of five chicks at 18 days of age for ileal P digestibility and total tract P retention.

[2]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[3]Diet Ca levels are calculated values.

[4]Significant main effect of diet Ca level and a significant interaction between SBM type and diet Ca level (P<0.05).

[5]Significant interaction between SBM type and fermentation and between diet Ca level and fermentation (P<0.05).

Table 2.6. Growth performance and tibia ash for crossbred chicks in Experiment 4[1].

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Tibia ash[2] (mg/tibia)	Tibia ash[3] (%)
1. P deficient cornstarch-dextrose	248.2^{e}	359.0^{de}	688.4^{ab}	279.9^{e}	32.6^{fg}
2. As 1 + 0.05% P[4]	283.6^{b}	399.9^{b}	711.1^{a}	342.4^{c}	35.7^{bc}
3. As 1 + 0.1% P[4]	312.9^{a}	430.6^{a}	727.1^{a}	444.4^{a}	39.2^{a}
4. As 1 + 12.5% SBM-CV[5]	259.6^{de}	358.0^{e}	726.5^{a}	299.8^{de}	34.1^{de}
5. As 1 + 25% SBM-CV	265.4^{bcde}	401.2^{b}	661.4^{b}	341.0^{c}	36.6^{b}
6. As 1 + 12.5% FSBM[5]	263.7^{bcde}	383.2^{bcd}	688.1^{ab}	298.4^{de}	33.8^{ef}
7. As 1 + 25% FSBM	282.8^{bc}	429.9^{a}	657.2^{b}	367.1^{b}	38.3^{a}
8. As 1 + 12.5% FFSB[5]	263.2^{bcde}	359.6^{cde}	732.8^{a}	275.9^{e}	31.8^{g}
9. As 1 + 25% FFSB	277.8^{bcd}	388.0^{b}	715.9^{a}	319.4^{cd}	34.4^{cde}
10. As 1 + 12.5% FFFSB[5]	261.6^{cde}	360.2^{cde}	726.6^{a}	300.0^{de}	33.8^{ef}
11. As 1 + 25% FFFSB	277.4^{bcd}	383.6^{bc}	723.2^{a}	338.5^{c}	35.5^{bcd}
Pooled SEM	7.64	8.57	16.27	8.56	0.50

$^{a-g}$ Means within a column with no common superscript differ ($P<0.05$).

[1]Values are means of five pens of five chicks; average initial BW was 80.8 g. Diets were fed from 8 to 21 days of age.

[2]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), SBM-CV (X_2), FSBM (X_3), FFSB (X_4), FFFSB (X_5) yielded the equation: $Y = 265.3 + 409.2 \pm 24.8X_1 + 118.3 \pm 16.7X_2 + 131.9 \pm 14.4X_3 + 96.6 \pm 21.1X_4 + 144.3 \pm 20.9X_5$ ($R^2 = 0.857$) The (±) values are standard errors of the regression coefficients.

[3]Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), SBM-CV (X_2), FSBM (X_3), FFSB (X_4), FFFSB (X_5) yielded the equation: $Y = 32 + 17 \pm 1.55X_1 + 7.25 \pm 1.04X_2 + 8.14 \pm 0.90X_3 + 3.92 \pm 1.32X_4 + 7.19 \pm 1.31X_5$ ($R^2 = 0.765$) The (±) values are standard errors of the regression coefficients.

[4]From KH_2PO_4.

[5]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

Table 2.7. Growth performance and tibia ash for commercial chicks in Experiment 5[1].

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Tibia ash[2] (mg/tibia)	Tibia ash[3] (%)
1. P deficient cornstarch-dextrose	407.2[c]	468.8[c]	871.5	357.8[c]	32.7[bc]
2. As 1 + 0.05% P[4]	500.5[b]	597.3[b]	838.3	409.5[b]	34.8[b]
3. As 1 + 0.1% P[4]	586.9[a]	689.6[a]	852	556.4[a]	40.4[a]
4. As 1 + 12.5% SBM-CV[5]	392.0[c]	466.1[c]	842.1	360.9[c]	31.3[c]
5. As 1 + 25% SBM-CV	473.4[b]	548.4[b]	862	424.9[b]	33.8[b]
6. As 1 + 12.5% FSBM[5]	407.4[c]	497.1[c]	818.3	356.2[c]	30.8[c]
7. As 1 + 25% FSBM	491.9[b]	583.9[b]	842.6	420.7[b]	34.9[b]
Pooled SEM	19.74	17.55	29.08	14.26	0.85

[a-c] Means within a column with no common superscript differ ($P<0.05$).

[1] Values are means of five pens of five chicks; average initial BW was 82.8 g. Diets were fed from 8 to 21 days of age.

[2] Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), SBM-CV (X_2), FSBM (X_3) yielded the equation: $Y = 333 + 313.5 \pm 26.2X_1 + 100.6 \pm 20.6X_2 + 83.4 \pm 18X_3$ ($R^2 = 0.824$) The (±) values are standard errors of the regression coefficients.

[2] Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), SBM-CV (X_2), FSBM (X_3) yielded the equation: $Y = 31.1 + 13.28 \pm 1.69X_1 + 2.77 \pm 1.33X_2 + 3.15 \pm 1.16X_3$ ($R^2 = 0.681$) The (±) values are standard errors of the regression coefficients.

[4] From KH_2PO_4.

[2] SBM-CV = conventional soybean meal. FSBM = fermented soybean meal.

Table 2.8. Relative P bioavailability in the test ingredients in crossbred chicks in Experiment 4.

SBM[1] type	Total P (%)	Bioavailability values[2] (%)		Bioavailable content[3] (%)	
		Tibia ash (mg/tibia)	Tibia ash (%)	Tibia ash (mg/tibia)	Tibia ash (%)
SBM-CV[1]	0.64	28.9[ab]	42.6[a]	0.18	0.27
FSBM[1]	0.69	32.2[ab]	47.9[a]	0.22	0.33
FFSB[1]	0.52	23.6[b]	23.1[b]	0.12	0.12
FFFSB[1]	0.53	35.3[a]	42.3[a]	0.19	0.22

[a-b]Values within a column with no common superscript are different ($P<0.05$) as determined using the regression coefficients and standard errors in the multiple regression equations in footnotes 2 and 3 of Table 2.6.

[1]SBM = soybean meal. SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[2]Calculated by the slope-ratio method using the regression equation in footnotes 2 and 3 in Table 2.6. Bioavailability values are relative to the P in KH_2PO_4 which was set at 100%.

[3]Bioavailable content = (Total P × bioavailability value)/100. Values are presented on as-fed basis.

Table 2.9. Relative P bioavailability in the test ingredients[1] in commercial broilers in Experiment 5.

SBM type	Total P (%)	Bioavailability values[2] (%)		Bioavailability content[3] (%)	
		Tibia ash (mg/tibia)	Tibia ash (%)	Tibia ash (mg/tibia)	Tibia ash (%)
SBM-CV[1]	0.64	32.1	20.9	0.21	0.13
FSBM[1]	0.69	26.6	23.7	0.18	0.16

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal.

[2]Calculated by the slope-ratio method using the regression equation in footnotes 2 and 3 in Table 2.7. Bioavailability values are relative to the P in KH_2PO_4 which was set at 100%.

[3]Bioavailable content = (Total P × bioavailability value)/100. Values are presented on as-fed basis.

LITERATURE CITED

Ahasic, E. M. 2020. Effect of particle size on nutritional value of soybean meal for poultry. MS Diss. Univ. Illinois, Urbana, IL.

AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int, Gaithersburg, MD.

AOCS. 2006. Official Methods and Recommended Practices. 5th ed. Association of Oil Chemists Society, Urbana, IL.

Applegate, T., R. Angel, and H. Classen. 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci. 82:1140–1148.

Araba, M., and N. M. Dale. 1990. Evaluation of protein solubility as an indicator of overprocessing soybean meal. Poult. Sci. 69:76–83.

Baker, K. M., P. L. Utterback, C. M. Parsons, and H. H. Stein. 2011. Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. Poult. Sci. 90:390–395.

Çabuk, B., M. G. Nosworthy, A. K. Stone, D. R. Korber, T. Tanaka, J. D. House, and M. T. Nickerson. 2018. Effect of Fermentation on the Protein Digestibility and Levels of Non-Nutritive Compounds of Pea Protein Concentrate. Food Technol. Biotechnol. 56:257-264.

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674–2683.

Chachaj, R., I. Sembratowicz, M. Krauze, and K. Ognik. 2019. The effect of partial replacement of soybean meal with fermented soybean meal on chicken performance and immune status. J. Anim. Feed Sci. 28:263–271.

Chen, X., C. M. Parsons, and N. Bajjalieh. 2013. Nutritional evaluation of new reduced oligosaccharide soybean meal in poultry. Poult. Sci. 92:1830–1836.

Chen, L., P. v. Vadlani, and R. L. Madl. 2014. High-efficiency removal of phytic acid in soy meal using two-stage temperature-induced Aspergillus oryzae solid-state fermentation. J. Sci. Food Agric. 94:113–118.

Cui, L., D. Li, and C. Liu. 2012. Effect of fermentation on the nutritive value of maize. Int. J. Food Sci. Technol. 47:755–760.

Drażbo, A., K. Ognik, A. Zaworska, K. Ferenc, and J. Jankowski. 2018. The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys. Poult. Sci. 97:3910–3920.

Ellis, R., E. R. Morris, and C. Philpot. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77:536–539.

Engster, H. M., N. A. Cave, H. Likuski, J. M. McNab, C. A. Parsons, and F. E. Pfaff. 1985. A collaborative study to evaluate a precision-fed rooster assay for true amino acid availability in feed ingredients. Poult. Sci. 64:487–498.

Van Eys, J. E. 2012. Manual of quality analyses for soybean products in the feed industry. 2nd rev. ed. USSEC, Chesterfield, MO.

Fernandez, S. R., and C. M. Parsons. 1996. Bioavailability of digestible lysine in heat-damaged soybean meal for chick growth. Poult. Sci. 75:224–231.

Finney, D. J. 1964. Statistical method in biological assay. 2nd ed. ed. Charles Griffin & Co., London, UK.

González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci. 89:3617–3625.

Hamerstrand, G. E., L. T. Black, and J. D. Black. 1981. Trypsin inhibitors in soy products: modification of the standard analytical procedure. Cereal chem. 58:42-45.

Hirabayashi, M., T. Matsui, H. Yano, and T. Nakajima. 1998. Fermentation of soybean meal with Aspergillus usamii reduces phosphorus excretion in chicks. Poult. Sci. 77:552–556.

Hoffman, D., S. Thurner, D. Ankerst, K. Damme, W. Windisch, and D. Brugger. 2019. Chickens growth performance and pancreas development exposed to soycake varying in trypsin inhibitor activity, heat-degraded lysine concentration, and protein solubility in potassium hydroxide. Poult. Sci. 98:2489-2499.

Karr-Lilienthal, L. K., P. L. Utterback, C. Martinez Amezcua, C. M. Parsons, N. R. Merchen, and G. C. Fahey. 2005. Relative bioavailability of phosphorus and true amino acid digestibility by poultry as affected by soybean extraction time and use of low-phytate soybeans. Poult. Sci. 84:1555–1561.

Khetarpaul, N., and B. M. Chauhan. 1989. Effect of fermentation on protein, fat, minerals, and thiamine content of pearl millet. Plant Foods Hum. Nutr. 39:169-177.

Liu, J. B., D. W. Chen, and O. Adeola. 2013. Phosphorus digestibility response of broiler chickens to dietary calcium-to-phosphorus ratios. Poult. Sci. 92:1572–1578.

Mukherjee, R., R. Chakraborty, and A. Dutta. 2015. Role of Fermentation in Improving Nutritional Quality of Soybean Meal — A Review. Asian-Australas. J. Anim. Sci. 29:1523–1529.

Munoz, J. A., C. D. Hanna, P. L. Utterback, and C. M. Parsons. 2018. Phosphorus retention in corn, spray dried plasma protein, soybean meal, meat and bone meal, and canola meal using a precision-fed rooster assay. Poult. Sci. 97:4324–4329.

Munoz, J. A., P. L. Utterback, and C. M. Parsons. 2020. Phosphorus digestibility and bioavailability in soybean meal, spray-dried plasma protein, and meat and bone meal determined using different methods. Poult. Sci. 99:4998–5006.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179–183.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Osman, M. A. 2011. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. J. Saudi Soc. Agric. Sci. 10:1–6.

Parsons, C. M., L. M. Potter, and B. A. Bliss. 1982. True metabolizable energy corrected to nitrogen equilibrium. Poult. Sci. 61:2241–2246.

Parsons, C. M., Y. Zhang, and M. Araba. 2000. Nutritional evaluation of soybean meals varying in oligosaccharide content. Poult. Sci. 79:1127–1131.

Rama Rao, S. V., M. V. L. N. Raju, A. K. Panda, and O. K. Murthi. 2014. Effect of supplementing microbial phytase in diets containing graded concentrations of calcium on performance, shell quality and bone mineral parameters in wl layers. Anim. Feed Sci. Technol. 193:102–110.

Rojas, O. J. 2012. Nutritional evaluation of fermented soybean meal fed to weanling pigs. MS Diss. Univ. Illinois, Urbana.

Smits, C. H. M., A. Veldman, M. W. A. Verstegen, and A. C. Beynen. 1997. Nutrient requirements and interactions dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr 127:483–487.

Soumeh, E. A., H. Mohebodini, M. Toghyani, A. Shabani, A. Ashayerizadeh, and V. Jazi. 2019. Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. Poult. Sci. 98:6797–6807.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139–2144.

Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey, D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their coproducts. Pages 613-660 in Soybeans: Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White, and R. Galloway. Urbana, IL.

Thanabalan, A., M. Mohammadigheisar, and E. G. Kiarie. 2021. Amino acids and energy digestibility in extruded or roasted full fat soybean fed to broiler chickens without or with multienzyme supplement containing protease, phytase, and fiber degrading enzymes. Poult. Sci. 100:101511.

Zamora, R. G., and T. L. Veum. 1987. Nutritive value of whole soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by neonatal pigs. J. Nutr. 118:438–444.

CHAPTER 3

METABOLIZABLE ENERGY, AMINO ACID DIGESTIBILITY, AND PHOSPHORUS DIGESTIBILITY IN FERMENTED SOYBEAN MEAL AND FERMENTED FULL-FAT SOYBEANS FED TO PIGS

ABSTRACT

Three experiments were conducted to determine the concentration of metabolizable energy (ME), apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids (AA), and standardized total tract digestibility (STTD) of P in conventional soybean meal (SBM-CV), fermented conventional soybean meal (FSBM), full-fat soybeans (FFSB), and fermented full-fat soybeans (FFFSB) fed to pigs. In Experiment 1, 40 growing barrows and gilts (initial BW: 13.9 ± 1.3 kg) were housed individually in metabolism crates and used in a complete randomized design. Pigs were fed a corn-based diet or four diets containing corn and each source of soybean product with 8 replicate pigs per diet. Fecal and urine samples were collected for 4 d after 5 d of adaptation. Results from Experiment 1 indicated that the concentration of ME in the test ingredients was not different between SBM-CV and FSBM, but FFFSB had a lower ME concentration than FFSB ($P<0.05$). In Experiment 2, 10 growing barrows (initial BW: 11.3 ± 0.8 kg) with a T-cannula in the distal ileum were allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods for a total of 10 replicate pigs per diet. Four diets included SBM-CV, FSBM, FFSB, and FFFSB as the sole source of crude protein (CP) and AA. A N-free diet was used to determine the basal endogenous losses of CP and AA. Ileal digesta were collected on days 6 and 7 of each period after 5 d of adaptation to the diets. Results from Experiment 2 indicated that fermentation reduced ($P<0.05$) the AID and SID of indispensable AA in SBM-CV

and in FFSB when compared with non-fermented ingredients. In Experiment 3, 80 growing barrows and gilts (initial BW: 12.3 ± 1.6 kg) were placed in metabolism crates and allotted to four diets with eight pigs per diet using a 2 × 2 × 2 factorial treatment arrangement. The factors were SBM type, fermentation and phytase inclusion (500 units/kg). Pigs were adapted to the diets for 5 d, and fecal samples were collected for 4 d. Results from Experiment 3 indicated that ATTD and STTD of P were greater (P<0.05) in fermented ingredients compared with non-fermented ingredients. The ATTD and STTD of P was also greater (P<0.05) in full-fat ingredients compared with conventional ingredients. The ATTD and STTD of P was greater (P<0.05) in diets with phytase inclusion compared with diets without phytase inclusion. In conclusion, fermentation did not affect the ME concentration of SBM-CV but negatively affect ME concentration of FFSB. Further, fermentation had a positive effect on STTD of P but reduced SID of indispensable AA in SBM-CV and FFSB in growing pigs, supporting the possibility of heat damage of the fermented ingredients.

INTRODUCTION

Protein from conventional soybean meal (SBM-CV) contains antinutritional factors (ANF) such as trypsin inhibitors, oligosaccharides, lectins, and antigens that negatively affect the availability of nutrients and reduce growth performance, especially in weaning pigs. For this reason, the inclusion of SBM-CV in weaning pig diets is limited and increases as the pigs grow older. To improve nutrient availability of the diets for weaning pigs, animal protein sources are often used, but they may increase the cost of the diets.

Further processing of SBM, such as fermentation, may improve the availability of nutrients, but it requires the action of microbial agents on the ingredient (Mukherjee et al., 2015).

Fermentation can reduce trypsin inhibitors, non-starch polysaccharides (NSP) and phytate in the ingredient. As consequence, fermentation can improve amino acid, energy, and P digestibility (Rojas, 2012). Fermentation can also provide lactic acid, which, working as an acidifier, can improve the digestion of nutrients (Chachaj et al., 2019b; Soumeh et al., 2019).

Although some authors reported the beneficial effects of fermented soybean meal (FSBM), there is limited research about the effects of fermentation on full-fat soybeans (FFSB) and new techniques of fermentation. Therefore, the objective of this study was to determine the digestibility of gross energy (GE) and concentrations of DE and ME, apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids (AA), and standardized total tract digestibility (STTD) of P in FSBM and FFFSB fed to pigs, and to test the hypothesis that these values are greater than in SBM-CV and FFSB.

MATERIALS AND METHODS

The protocols for the three experiments were reviewed and approved by the Institutional Animal Acre and Use Committee at the University of Illinois. Pigs used in these experiments were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN). The 4 test ingredients used in the 3 experiments were SBM-CV, FSBM, FFSB, and FFFSB and were the same as used for the experiments in Chapter 2.

Experiment 1. Digestibility of GE and concentrations of DE and ME

Forty barrows and gilts (initial BW: 13.9 ± 1.3 kg) were allotted to a completely randomized design with 5 diets and 8 replicate pigs per diet. Pigs were individually placed in metabolism crates equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen and

urine pan was placed under the slatted floor to allow for the total, but separate, collection of urine and fecal samples.

A basal diet containing corn as the sole source of energy and four diets containing corn and each test ingredient were formulated; thus, a total of five diets were used (Table 3.1). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Pigs were limit fed at 3.2 times the ME requirement for maintenance; feed was provided each day in 2 equal meals at 0800 and 1600 hours. The ME concentration in the diets was calculated based on the ME concentration in the test ingredients (NRC, 2012). Water was available at all times. The initial 5 d were considered the adaptation period to the diets. Indigestible markers were fed on d 6 (chromic oxide) and 10 (ferric oxide). Fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared according to standard procedures using the marker-to-marker approach (Adeola, 2001). Feces were collected twice daily and stored at –20 °C immediately after collection. Urine collections were initiated on d 6 at 0900 hours and ceased on d 10 at 0900 hours. Urine was collected in buckets placed under the crates. The collected urine was weighed daily, and a 10% subsample was stored at -20 °C. Urine buckets were emptied every morning, and a preservative of 50 mL of 6N HCL was added to the urine buckets before the beginning of urine collection each day. A sample of each diet was collected at the time of diet mixing.

Experiment 2. AA digestibility

Ten barrows (initial BW: 11.3 ± 0.8 kg) that had a T-cannula installed in the distal ileum were used. Pigs were placed in 1.2 × 1.5 m individual pens equipped with a self-feeder, a nipple waterer, and fully slatted tri-bar floors. Pigs were allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods of 7 d each. There were two pigs per diet in each period for a

total of 10 observations per treatment. Each test ingredient was included in the respective diet as the sole source of AA (Table 3.2). A nitrogen-free diet was used to measure basal endogenous losses of AA. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets contained 0.40% chromic oxide as an indigestible marker. A sample of each diet was collected at the time of diet mixing.

Pigs were fed their respective diets at 3 times the maintenance requirement for ME (i.e., 197 kcal ME per kg $BW^{0.60}$; NRC, 2012) and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period. Ileal digesta were collected on d 6 and 7 for 9 h using standard procedures (Stein et al., 1998). Pigs were fed experimental diets at 0700 hours and ileal digesta samples were collected from 0700 to 1600 hours. Cannulas were opened at the beginning of collection and a 225-mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were collected and bags were replaced whenever they were full or at least once every 30 min. All samples were stored at -20 °C after collection. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for analysis.

Experiment 3. Digestibility of P and effects of microbial phytase

Eighty barrows and gilts (initial BW: 12.3 ± 1.6 kg) were allotted to a completely randomized design with 8 diets, and 10 replicate pigs per diet. Pigs were housed individually in metabolism crates equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen floor was placed under the slatted floor to allow for the total collection of fecal material. Eight diets were formulated. The composition and analyzed composition of the 8 diets are shown in Table 3.3. The 8 diets were formulated and arranged in a 2 × 2 × 2 factorial with 2 SBM types

(conventional vs full-fat), 2 types of processing (fermented vs non-fermented), and 2 levels of microbial phytase (0 vs 500 phytase units, FTU per kg; Quantum Blue, AB Vista, Marlborough, UK). Cornstarch and sucrose were included in the diets, and the test ingredients were the only source of P. Vitamins and minerals except P were included in all diets to meet or exceed the requirements for weanling pigs (NRC, 2012).

The feed and water were provided as in Experiment 1. The indigestible marker used was indigo blue and was supplied in the morning meals on d 6 and 10. Fecal collection started when the blue marker appeared in the feces after the first time the marker was fed and ceased after the marker appeared for the second time (Adeola, 2001). A sample of each diet was collected at the time of diet mixing.

Sample analysis

Fecal samples from Experiments 6 and 8 were dried in a 55 °C forced air drying oven for 7 d reaching <10% moisture in the samples. Urine samples from Experiment 1 were thawed, and a sub-sample was lyophilized before analysis using a standard procedure (Kim et al., 2009). For this procedure, 10 mL of urine was dripped on a cotton ball that was placed in a plastic bag, the bag with the urine and cotton ball was lyophilized, and GE was analyzed in the bag and in empty bags and cotton balls to calculate the GE in the 10 mL of urine. Ileal digesta samples from Experiment 2 were lyophilized and finely ground.

Dry matter in diets, freeze-dried ileal digesta, and oven-dried fecal samples was measured using a drying oven for 2 h at 135 °C (method 930.15; AOAC International, 2007). Ash in corn and diet samples from Experiment 3 was also analyzed (method 942.05; AOAC International, 2007). The CP in diets from Experiments 6 and 7 and ileal digesta samples was calculated as N × 6.25, and N was measured using the combustion procedure (method 990.03; AOAC

International, 2007) on a LECO FP628 (LECO Corp., Saint Joseph, MI). The GE in diet, fecal, and urine samples from Experiment 1 was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Amino acids in the diet and ileal digesta samples from Experiment 2 were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at 110 °C [method 982.30 E(a); AOAC International, 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 International, 2007]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC International, 2007]. Chromium in diet and ileal digesta samples from Experiment 2 was analyzed using Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC International, 2007). Calcium and P in diet and fecal samples from Experiment 3 were analyzed by inductively coupled plasma spectroscopy (AOAC International, 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC International, 2007; method 975.03 B(b)]. Phytase activity in the test ingredients and in diet samples from Experiment 3 was also measured (Phytex Method, Version 1; Eurofins, Des Moines, IA).

Calculations and Statistical analysis

In Experiment 1, The ATTD of GE and DM was calculated for each diet, and the DE and ME in each diet were calculated as well (NRC, 2012). The DE and ME in corn were calculated by dividing the DE and ME of the basal diet by the inclusion rate of corn in that diet. The contribution of DE and ME from corn to the DE and ME in the diets containing both corn and 1

of the 4 test ingredients were subtracted from the DE and ME of each diet, and the DE and ME in each test ingredient were calculated by difference (Adeola, 2001).

Data were analyzed using the PROC MIXED in SAS (SAS Institute Inc., Cary, NC). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure, and this procedure was also used to identify outliers, but no outliers were observed. Diet was the fixed effect and replicate was the random effect. Least squares means were calculated and separated using the PDIFF statement with Tukey's adjustment. Contrast statements were used to determine the effects of SBM type, fermentation, and inclusion of phytase. The pig was the experimental unit for all analyses, and an alpha level of 0.05 was used to assess significance among means.

In Experiment 2, AID and SID of CP and AA were calculated using the analyzed CP, AA, and Cr concentrations in the diets (Stein et al., 2007). Basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet as previously described (Stein et al., 2007).

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The model included diet as the fixed effect and square, period, and animal as the random effects. Mean values were calculated using the LSMeans statement. The pig was the experimental unit for all analyses, and an alpha level of 0.05 was used to assess significance among means.

In Experiment 3, the concentration of phytate-bound P in the test ingredients was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004). The ATTD of P and Ca in each diet was calculated (NRC, 2012), and the ATTD of P in the diets also represented the ATTD of P in each test ingredient because the test ingredient was the only source of P in the diets. Values for ATTD of P were determined based on calculated P in the diets. By correcting

these values for the basal endogenous losses of P (i.e., 190 mg per kg DM intake; NRC, 2012), the STTD of P in each test ingredient without and with phytase was calculated.

Data were analyzed using the PROC MIXED in SAS (SAS Institute Inc., Cary, NC). SBM type, fermentation, phytase, and the interaction between SBM type and fermentation, SBM type and phytase, fermentation and phytase, and SBM type, fermentation, and phytase were the fixed effects and replicate was the random effect. Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure, and this procedure was also used to test for outliers and 3 observations were removed. Least squares means were calculated and separated using the PDIFF statement with Tukey's adjustment. The pig was the experimental unit for all analyses, and an alpha level of 0.05 was used to assess significance among means.

RESULTS AND DISCUSSION

Experiment 1: ATTD of GE and Concentration of DE and ME

Daily GE intake in Experiment 1 was not different among diets (Table 3.5). Daily GE intake was lower (P<0.05) by pigs fed the corn diet compared with the non-fermented ingredient diets, but not different compared with the fermented ingredient diets. Fecal excretion of GE was not different among pigs fed the diets with the test ingredients but was greater (P<0.05) than in pigs fed the corn diet. Excretion of GE in urine was greater (P<0.05) from pigs fed the conventional ingredient diets than from pigs fed the full-fat ingredient diets, and pigs fed the corn diet had the least (P<0.05) urine excretion of GE. The ATTD of GE in the corn diet was greater (P<0.05) than in the other diets. The ATTD of GE among the test ingredient diets was not different among diets except that the SBM-CV diet had a greater (P<0.05) value than the FFFSB diet. Concentrations of DE and ME in the full-fat ingredient diets were greater (P<0.05)

compared with the conventional ingredient and corn diets. The DE in the conventional ingredient diets was not different than in the corn diet, but ME was lower (P<0.05) in the conventional ingredient diets than in the corn diet. The DE and ME in the full-fat ingredients were greater (P<0.05) compared with the conventional ingredients and corn. The ME was greater (P<0.05) in FFSB than in FFFSB. The DE was not different between the conventional ingredients and corn, but the conventional ingredients had a lower (P<0.05) concentration of ME than corn. There was an interaction (P<0.05) between SBM type and fermentation for DE and ME in the test diets and for the ME in the test ingredients. Fermentation reduced DE and ME more in FFSB than it did in SBM-CV.

The ATTD of GE of the corn diet obtained agreed with the values obtained in previous studies (Rojas and Stein, 2013; Espinosa et al., 2020). The values for DE and ME concentration in corn, SBM-CV, and FSBM were close to the values obtained by Espinosa et al. (2020) and NRC (2012) but were lower than the values obtained by Rojas and Stein (2013). The DE concentration values in FFSB were greater than the values reported in previous studies for FFSB (Woyengo et al., 2014; Kiarie et al., 2020). Reported values for the concentration of energy in FFFSB are limited.

The results from Table 2.3 demonstrated that fermentation increased the TMEn concentration in SBM-CV when fed to roosters. The fact that the concentrations of DE and ME did not differ between SBM-CV and FSBM in growing pigs may be due to the high quality of SBM-CV, which had a concentration of trypsin inhibitors of 1.89 mg/g within the ideal range for a properly processed SBM (van Eys, 2012). Another explanation may be that, although fermentation reduced the concentration of oligosaccharides in SBM-CV, removing oligosaccharides in soybeans may not affect the DE concentration when fed to pigs (Woyengo et

al., 2014). Therefore, reduction of oligosaccharides may have a greater effect on energy concentration in poultry than in pigs.

In the case of the FFSB, fermentation had a similar effect on ME concentration as observed in Table 2.3. The reduction in ME in FFFSB can be attributed to the reduced fat content compared with the FFSB, and this may be true for both poultry and pigs since fat supplies more ME/g than protein or carbohydrates.

Experiment 2: AID and SID of AA

Results from Experiment 2 indicated that the AID (Table 3.7) and SID (Table 3.8) of all AA in non-fermented ingredients were greater ($P<0.05$) than in the fermented ingredients, with the exception that the SID of Pro was not different among the ingredients (Table 3.6 and Table 3.7). Most values for AID and SID were not different between SBM and FFSB. An interaction ($P<0.05$) between SBM type and fermentation on the AID and SID of Leu, Lys, and Val was observed. The interaction may be explained due that fermentation reduced the AID and SID of Lys, Leu, and Val, more in SBM-CV than it did in FFSB.

The AID and SID of indispensable AA values obtained for SBM-CV and FFSB agreed with reported values (NRC, 2012; Espinosa et al., 2020). However, the AID and SID of indispensable AA for FSBM were lower than values obtained by Espinosa et al. (2020) but agreed with values reported by Cervantes-Pahm and Stein (2010).

Fermentation of SBM-CV may increase the digestibility of AA due to a greater concentration of small peptides in the fermented products with a greater AA absorption rate in the small intestine than free AA (Rojas, 2013). Another reason to expect an improved digestibility of AA with fermentation is the reduction in TI and oligosaccharides (Cervantes-Pahm and Stein, 2010). In other studies, fermentation did not affect the digestibility of AA

(Espinosa et al., 2020). However, the fact that fermentation reduced the digestibility of indispensable AA in both SBM-CV and FFSB in the current study may be due to a reduction in the quality of the protein. The Lys:CP ratio in the fermented ingredients was below 6.0, which is the minimum ratio recommended by Stein et al. (2008), and this may be caused by heat damage during the fermentation process. As discussed in Chapter 2, another possible cause for the reduced Lys:CP may be the type of microbe used and the fermentation itself (Osman, 2011; Çabuk et al., 2018).

The fact that the SID of indispensable AA in the fermented ingredients was lower in pigs than in poultry may be due to the animals and method used. In this study, young pigs were used, whereas adult roosters were used in the poultry experiment. Young pigs have lower digestibility of AA than older pigs (Pedersen et al., 2016). The effect of age on the digestibility of AA has also been reported in poultry (Barua et al., 2021). It is therefore possible, that adult animals may better tolerate ingredients which protein digestibility has been negatively affected by processing.

Experiment 3: STTD of P

Neither fermentation, SBM type or phytase influenced daily feed intake or basal endogenous P loss (EPL; Table 3.8). However, daily P intake was lower in full-fat ingredients compared with conventional ingredients ($P<0.05$). Fermentation reduced concentration of P in feces in SBM-CV but did not affect the FFSB (interaction; $P<0.05$). Fermentation reduced concentration of P in feces when phytase was not included in diets but did not affect the concentration of P in feces when phytase was included in diets (interaction; $P<0.05$). When there was no phytase included in diets, fermentation reduced the concentration of P in feces in SBM-CV but did not affect FFSB, and when phytase was included in diets, fermentation did not affect the concentration of P in feces in SBM-CV or FFSB (interaction; $P<0.05$). Full-fat soybeans

presented reduced (P<0.05) P excretion in feces compared with SBM-CV. When phytase was included to the diets, P excretion was reduced (P<0.05) in ingredients compared with diets when phytase was not included. The ATTD and STTD of P were greater (P<0.05) in fermented ingredients compared with non-fermented ingredients. The ATTD and STTD of P was also greater (P<0.05) in full-fat ingredients compared with conventional ingredients. The ATTD and STTD of P was greater (P<0.05) in diets with phytase inclusion compared with diets without phytase inclusion.

Fermentation increased daily Ca intake in SBM-CV but not in FFSB (interaction; P<0.05). Fermentation increased concentration of Ca in feces in SBM-CV but not in FFSB (interaction; P<0.05). Fermentation increased concentration of Ca in feces more when phytase was added to the diets than it did when phytase was not included in diets (interaction; P<0.05). Fermentation increased Ca excretion in feces when phytase was added to the diets but not when phytase was not included in diets (interaction; P<0.05). The ATTD of Ca was greater (P<0.05) in diets containing phytase compared with diets without phytase supplementation.

The ATTD and STTD of P in SBM-CV and FFSB without phytase supplementation agree with reported values (Rojas and Stein, 2012; NRC, 2012). In contrast, the ATTD and STTD of P for FSBM without phytase were lower than the values reported in previous studies (Rojas and Stein, 2012; NRC, 2012; Espinosa et al., 2020). Researchers that reported an improvement of ATTD and STTD of P in FSBM compared with SBM-CV, attributed that effect to the reduced phytate content in FSBM due to hydrolysis of phytate-bound P during the fermentation process; thereby increasing the non-phytate P of the ingredient (Rojas and Stein, 2012; Espinosa et al., 2020). In the study conducted by Rojas (2012), the microbe used was the *Aspergillus oryzae*, which degrade phytate in SBM (Chen et al., 2014). In the study conducted by

Espinosa et al. (2020), a *Bacillus subtillis* was used for fermentation, and even if it is the same microbe as the one used in the current study, details about the fermentation process were not published, and temperatures and length of fermentation may affect degradation of phytate in the substrate (Chen et al., 2014). In the current study, phytate P relative to total P was slightly reduced with fermentation, but the effect was not as great as it was in the earlier mentioned studies. However, although the reduction in phytate content was not as great as in the reported studies, it may had been enough to have a positive effect on ATTD and STTD of P. This may also explain the response that was observed when phytase was added to the diets meaning that there was still a substantial amount of P bound to phytate in the fermented ingredients that was released by the action of the phytase. The fact that the ATTD and STTD of P were greater in full-fat ingredients compared with conventional ingredients may be due to the fat reducing the passage rate of digesta allowing for more digestive enzymatic activity and nutrient absorption.

Calcium intake was greater ($P<0.05$) for pigs fed the diets containing FSBM than pigs fed the other diets, and this was likely because of the greater Ca content in the FSBM diet. The greater Ca intake with the FSBM diet likely led to the greater Ca output compared with other diets. Addition of phytase to the diets reduced Ca excretion in feces ($P<0.05$) and increased ATTD of Ca compared with diets without phytase. Effects of phytase on ATTD of Ca in pigs has been attributed to release of Ca from calcium carbonate that was chelated to phytate (Ca-phytate complex) in the intestine of pigs (Lee et al., 2019).

In summary, fermentation had no effect on DE and ME in SBM-CV but reduced the ME concentration in FFSB. Fermentation reduced the AID and SID of indispensable AA in SBM-CV and FFSB, with the SID of Lys being one of the lowest values, indicating possible heat damage. Fermentation had a positive effect on the digestibility of P in SBM-CV and FFSB.

TABLES

Table 3.1. Ingredient composition of experimental diets, as-fed basis, Experiment 1.

	Dietary treatments				
Ingredient, %	Corn	SBM-CV[1]	FSBM[1]	FFSB[1]	FFFSB[1]
Ground corn	96.70	67.20	69.25	57.15	59.15
SBM-CV	-	30.00	-	-	-
FSBM	-	-	28.00	-	-
FFSB	-	-	-	40.00	-
FFFSB	-	-	-	-	38.00
Calcium carbonate	0.80	0.75	0.95	0.80	0.75
Dicalcium phosphate	1.60	1.15	0.90	1.15	1.20
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50
Analyzed, %					
DM	87.57	88.95	88.37	89.77	88.85
GE[1], kcal/kg	3664	3825	3844	4286	4255
ME[3], kcal/kg	3283	3270	3361	3515	3622
CP	6.97	20.49	19.37	19.63	18.38

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. GE = gross energy.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg;

Table 3.1. (Cont.)

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

[3]Calculated from previous data (NRC, 2012).

Table 3.2. Ingredient composition of experimental diets, as-fed basis, Experiment 2.

	Dietary treatments				
Ingredient, %	SBM-CV[1]	FSBM[1]	FFSB[1]	FFFSB[1]	N-free
SBM-CV	40.00	-	-	-	-
FSBM	-	40.00	-	-	-
FFSB	-	-	50.00	-	-
FFFSB	-	-	-	50.00	-
Soybean oil	2.00	2.00	2.00	2.00	4.00
Calcium carbonate	0.60	0.80	0.65	0.60	0.45
Dicalcium phosphate	1.35	0.95	1.30	1.30	2.10
Sucrose	10.00	10.00	10.00	10.00	20.00
Cornstarch	44.75	44.95	34.75	34.80	67.65
Solka floc[2]					
Magnesium oxide	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.50	0.50	0.50	0.50	0.50

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

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

[3]The vitamin-micromineral premix will provide the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-

Table 3.2. (Cont.)

calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

[3]Calculated from previous values (NRC, 2012).

Table 3.3. Composition of experimental diets, as-fed basis, Experiment 3.

Phytase, unit/kg	0				500			
Ingredient, %	SBM-CV[1]	FSBM	FFSB	FFFSB	SBM-CV	FSBM	FFSB	FFFSB
SBM-CV	40.00	-	-	-	40.00	-	-	-
FSBM	-	40.00	-	-	-	40.00	-	-
FFSB	-	-	50.00	-	-	-	50.00	-
FFFSB	-	-	-	50.00	-	-	-	50.00
Phytase concentrate[2]	-	-	-	-	0.01	0.01	0.01	0.01
Cornstarch	46.82	46.5	36.76	36.79	46.81	46.49	36.75	36.78
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Calcium carbonate	0.28	0.60	0.34	0.31	0.28	0.60	0.34	0.31
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Analyzed, %								
ME[3], kcal/kg	3,704	3,816	3,954	4,111	3,704	3,816	3,954	4,111

Table 3.3. (Cont.)

DM	90.3	90.0	91.4	90.7	90.6	90.2	91.4	91.1
Ash	3.1	3.5	3.3	3.3	3.1	3.3	3.0	3.0
P	0.31	0.29	0.29	0.30	0.29	0.29	0.28	0.28
Ca	0.26	0.41	0.30	0.29	0.28	0.36	0.24	0.25
Phytase, unit/kg	<70	<70	<70	<70	460	340	310	370

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[2]Phytase concentrate was added to provide 500 units of phytase (Quantum Blue®, AB Vista, Marlborough, UK) per kilogram of diet.

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

[3]ME in all diets was calculated (NRC, 2012).

Table 3.4. Analyzed nutrient composition of experimental diets, as-fed basis, Experiment 2.

	Dietary treatments				
Item, %	SBM-CV[1]	FSBM	FFSB	FFFSB	N-free
ME[1], kcal/kg	3,621	3,755	3,874	4,031	3,737
DM	90.24	89.98	91.39	90.54	91.79
CP	20.32	21.80	19.72	18.55	0.02
Indispensable AA					
Arg	1.55	1.24	1.40	1.18	0.01
His	0.56	0.48	0.51	0.48	-
Ile	1.04	0.91	0.95	0.93	0.02
Leu	1.67	1.48	1.52	1.46	0.02
Lys	1.39	1.09	1.25	1.14	0.01
Met	0.28	0.24	0.26	0.24	0.01
Thr	1.13	0.99	0.99	0.95	0.01
Trp	0.83	0.73	0.76	0.72	0.01
Val	0.31	0.29	0.27	0.25	<0.02
Dispensable AA					
Ala	0.94	0.86	0.86	0.88	0.01
Asp	2.47	2.20	2.28	2.18	0.02
Cys	0.31	0.28	0.29	0.27	0.00
Glu	3.99	3.48	3.55	3.40	0.02
Gly	0.92	0.82	0.85	0.84	0.01
Pro	1.04	0.93	0.94	0.93	0.01

Table 3.4 (Cont.)

Ser	0.99	0.88	0.87	0.80	0.01
Tyr	0.73	0.63	0.64	0.58	0.01

[1]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans.

[2]Calculated from previous values (NRC, 2012).

Table 3.5. Concentration of digestible energy (DE) and metabolizable energy (ME) and apparent total tract digestibility (ATTD) of gross energy in experimental diets and SBM-CV, FSBM, FFSB, and FFFSB, as-fed basis, Experiment 1[1].

Item	Corn	SBM-CV[2]	FSBM[2]	FFSB[2]	FFFSB[2]	SEM	ST[2]	Ferm.	ST * Ferm.
Diets									
Intake									
Feed, g/d, DM basis	713[b]	820[a]	775[ab]	721[b]	689[b]	23	<0.001	0.102	0.787
GE[2], kcal/d	2984[b]	3526[a]	3371[ab]	3444[a]	3299[ab]	107	0.477	0.169	0.960
Fecal excretion									
Dry feces output, g/d	51[b]	93[a]	90[a]	92[a]	94[a]	5	0.755	0.951	0.581
GE, kcal/d	237[b]	425[a]	420[a]	446[a]	452[a]	22	0.236	0.967	0.800
Urinary excretion									
Urine output, g/d	1274[b]	3176[a]	2285[ab]	1740[ab]	2234[ab]	381	0.059	0.606	0.077
GE, kcal/d	47[c]	111[a]	107[a]	74[bc]	99[ab]	7	0.003	0.140	0.055
ATTD of GE, %	92.1[a]	88.0[b]	87.6[bc]	87.1[bc]	86.3[c]	0.4	0.012	0.134	0.610
Energy in diets, kcal/kg									
DE	3372[b]	3366[b]	3367[b]	3735[a]	3674[a]	15	<0.001	0.055	0.048
ME	3314[c]	3246[d]	3245[d]	3641[a]	3546[b]	16	<0.01	0.005	0.006
Energy in feed ingredients, kcal/kg									
As-fed basis									
DE	3488[b]	3407[b]	3398[b]	4355[a]	4239[a]	38	<0.001	0.108	0.164

Table 3.5. (Cont.)

ME	3427[c]	3143[d]	3113[d]	4207[a]	3998[b]	42	<0.001	0.008	0.042
DM basis									
DE	3988[b]	3836[b]	3822[b]	4778[a]	4704[a]	42	<0.001	0.305	0.484
ME	3919[b]	3539[c]	3501[c]	4615[a]	4436[a]	48	<0.001	0.029	0.146

[1]Data are least square means of 8 observations for all treatments.

[2]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. GE = gross energy. ATTD = Apparent total tract digestibility. ST = SBM type.

[a-d] Means within a row that do not have a common superscript differ, $P<0.05$.

Table 3.6. Apparent ileal digestibility (AID) of CP and AA in SBM-CV, FSBM, FFSB, and FFFSB, by growing pigs, Experiment 2[1].

	AID							
Item, %	SBM-CV[2]	FSBM[2]	FFSB[2]	FFFSB[2]	SEM	ST[2]	Ferm	ST*Ferm.
CP	80.3[a]	69.6[b]	77.8[a]	64.9[b]	2.0	0.009	<0.001	0.399
Indispensable AA								
Arg	90.9[a]	81.7[b]	89.6[a]	82.0[b]	1.6	0.553	<0.001	0.414
His	88.3[a]	76.0[b]	86.4[a]	76.0[b]	1.3	0.329	<0.001	0.335
Ile	88.3[a]	79.0[b]	85.1[a]	79.8[b]	1.1	0.221	<0.001	0.038
Leu	88.4[a]	80.1[b]	85.6[a]	81.4[b]	1.1	0.436	<0.001	0.036
Lys	87.7[a]	70.5[c]	83.6[b]	73.8[c]	1.2	0.704	<0.001	0.002
Met	90.6[a]	83.4[c]	86.6[b]	81.2[c]	1.0	0.001	<0.001	0.255
Phe	88.8[a]	80.6[b]	85.6[a]	81.1[b]	1.2	0.167	<0.001	0.067
Thr	81.3[a]	67.5[b]	77.3[a]	67.8[b]	1.6	0.153	<0.001	0.095
Trp	89.3[a]	83.7[b]	85.1[b]	82.8[b]	1.2	0.005	<0.001	0.068
Val	86.2[a]	76.0[b]	83.4[a]	78.6[b]	1.3	0.885	<0.001	0.020
Total Indisp.	88.0[a]	77.3[b]	85.0[a]	78.3[b]	1.2	0.320	<0.001	0.043
Dispensable AA								
Ala	84.3[a]	72.4[c]	80.1[ab]	75.3[bc]	1.6	0.627	<0.001	0.010
Asp	85.2[a]	70.3[c]	80.8[b]	70.6[c]	1.6	0.071	<0.001	0.041
Cys	73.6[a]	53.4[b]	73.0[a]	56.0[b]	2.4	0.604	<0.001	0.414
Glu	87.8[a]	71.3[b]	85.9[a]	71.8[b]	1.8	0.540	<0.001	0.295
Gly	68.7[a]	52.4[b]	66.3[a]	52.8[b]	3.5	0.625	<0.001	0.494
Pro	65.7[a]	45.8[b]	59.9[ab]	42.9[b]	6.5	0.241	<0.001	0.714

Table 3.6. (Cont.)

Ser	86.2[a]	76.3[b]	82.4[a]	74.1[b]	1.7	0.009	<0.001	0.460
Tyr	86.6[a]	77.3[b]	83.1[a]	75.3[b]	1.4	0.017	<0.001	0.485
Total Disp.	79.9[a]	63.3[b]	77.2[a]	64.5[b]	2.9	0.662	<0.001	0.241
Total AA	83.5[a]	69.5[b]	80.6[a]	70.7[b]	2.0	0.500	<0.001	0.116

[1]Each least squares mean for experimental diets from growing pigs represents 10 observations, respectively.

[2]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. ST = SBM type.

[a-c] Means within a row that do not have a common superscript differ, $P<0.05$.

Table 3.7. Standardized ileal digestibility (SID) of CP and AA in SBM-CV, FSBM, FFSB, and FFFSB, by growing pigs, Experiment 2[1,2].

	SID							
Item, %	SBM-CV[3]	FSBM[3]	FFSB[3]	FFFSB[3]	SEM	ST[3]	Ferm	ST*Ferm.
CP	87.8[a]	76.5[b]	85.6[a]	73.1[b]	2.0	0.036	<0.001	0.631
Indispensable AA								
Arg	94.8[a]	86.6[b]	94.0[a]	87.1[b]	1.6	0.846	<0.001	0.474
His	91.0[a]	79.2[b]	89.4[a]	79.2[b]	1.3	0.415	<0.001	0.410
Ile	91.0[a]	82.0[b]	88.0[a]	82.8[b]	1.1	0.271	<0.001	0.055
Leu	90.7[a]	82.6[b]	88.1[a]	84.0[b]	1.1	0.537	<0.001	0.045
Lys	90.4[a]	74.1[b]	86.7[a]	77.2[b]	1.2	0.782	<0.001	0.003
Met	92.6[a]	85.6[bc]	88.7[b]	83.5[c]	1.0	0.001	<0.001	0.298
Phe	90.9[a]	82.9[b]	88.0[a]	83.5[b]	1.2	0.243	<0.001	0.083
Thr	87.3[a]	74.4[b]	84.0[a]	74.8[b]	1.6	0.258	<0.001	0.138
Trp	92.5[a]	87.1[b]	88.8[b]	86.7[b]	1.2	0.022	<0.001	0.064
Val	89.4[a]	79.6[b]	86.7[a]	81.9[b]	1.3	0.888	<0.001	0.030
Total Indisp.	91.1[a]	80.9[b]	88.4[a]	82.0[b]	1.2	0.427	<0.001	0.061
Dispensable AA								
Ala	89.5[a]	78.1[b]	85.9[a]	81.0[b]	1.6	0.761	<0.001	0.018
Asp	87.7[a]	73.0 [b]	83.5[a]	73.3[b]	1.6	0.091	<0.001	0.049
Cys	79.7[a]	60.1 [b]	79.6[a]	63.0[b]	2.4	0.473	<0.001	0.445
Glu	89.3[a]	73.1 [b]	87.6[a]	73.6[b]	1.8	0.618	<0.001	0.327
Gly	87.3[a]	73.2 [b]	86.7[a]	73.2[b]	3.6	0.887	<0.001	0.872

Table 3.7. (Cont.)

Pro	119.6[a]	106.0 [a]	109.2[a]	104.1[a]	9.1	0.265	0.189	0.454
Ser	90.7[a]	81.3[b]	87.7[a]	79.7[b]	1.7	0.035	<0.001	0.503
Tyr	89.8[a]	81.0[b]	86.7[a]	79.3[b]	1.4	0.038	<0.001	0.528
Total Disp.	88.5[a]	73.0[b]	86.8[a]	74.5[b]	2.9	0.966	<0.001	0.347
Total AA	89.6[a]	76.5[b]	87.4[a]	77.8[b]	2.0	0.737	<0.001	0.176

[1]Each least squares mean for experimental diets from growing pigs represents 10 observations, respectively.

[2]Values for SID were calculated by correcting the values for AID for the basal ileal endogenous losses. The basal ileal endogenous losses were determined (g/kg DMI) as CP, 11.91; Arg, 0.46; His, 0.12; Ile, 0.21; Leu, 0.34; Lys, 0.26; Met, 0.06; Phe, 0.20; Thr, 0.34; Trp, 0.34; Trp, 0.07; Val, 0.36; Ala, 0.45; Asp, 0.51; Cys, 0.12; Glu, 0.62; Gly, 1.26; Pro, 4.35; Ser, 0.32; and Tyr, 0.17.

[3]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. ST =SBM type.

[a-d] Means within a row that do not have a common superscript differ, $P<0.05$.

Table 3.8. Effects of phytase on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of P in SBM-CV, FSBM, FFSB, and FFFSB, and ATTD of Ca in diets fed to growing pigs, as-fed basis, Experiment 3[1].

Phytase, unit/kg	0				500					*P*-value			*Interaction*			
Item, %	SBM-CV[2]	FSBM[2]	FFSB[2]	FFFSB[2]	SBM-CV	FSBM	FFSB	FFFSB	SEM	Fr[2]	T[2]	P[2]	Fr*T	Fr*P	T*P	Fr*T*P
Feed intake, g/d in DM basis	644	613	624	597	635	618	602	588	22.2	0.152	0.105	0.565	0.905	0.667	0.662	0.975
Dry feces output, g/d	35.0	39.2	46.8	47.2	33.6	33.1	45.9	46.6	2.2	0.484	0.425	<0.001	0.134	0.670	0.475	0.317
P digestibility																
P intake, g/d	1.8	1.9	1.8	1.8	1.8	1.9	1.7	1.7	0.1	0.381	0.008	0.519	0.204	0.668	0.658	0.925
P in feces, %	3.3[a]	2.7[b]	2.1[c]	2.0[c]	2.1[c]	1.9[c]	1.1[d]	0.9[d]	0.1	<0.001	<0.001	<0.001	0.003	0.035	0.585	0.001
P output, g/d	1.2	1.0	1.0	0.9	0.7	0.6	0.5	0.4	0.1	0.096	<0.001	<0.001	0.498	0.874	0.368	0.366
ATTD of P, %	37.7	46.3	46.5	46.1	61.7	67.0	71.3	74.6	2.4	0.012	<0.001	<0.001	0.096	0.930	0.182	0.276
BEL[3], mg/d	122	117	119	114	121	118	114	112	4.2	0.152	0.105	0.566	0.905	0.667	0.662	0.975
STTD[4] of P, %	44.4	52.3	53.1	52.5	68.3	73.0	77.9	81.1	2.4	0.021	<0.001	<0.001	0.124	0.928	0.183	0.274
Ca digestibility																
Ca intake, g/d	1.9[b]	2.7[a]	1.9[b]	1.8[b]	1.9[b]	2.7[a]	1.8[b]	1.8[b]	0.1	<0.001	<0.001	0.564	<0.001	0.704	0.700	0.918
Ca in feces, %	2.1[ab]	2.4[a]	1.5[c]	1.4[c]	1.3[c]	2.0[b]	0.9[d]	0.9[d]	0.1	<0.001	<0.001	<0.001	<0.001	0.006	0.834	0.194

Table 3.8. (Cont.)

Ca output, g/d	0.8[ab]	0.9[a]	0.7[b]	0.7[b]	0.4[c]	0.7[b]	0.4[c]	0.4[c]	0.1	0.003	<0.001	<0.001	0.003	0.281	0.850	0.859
ATTD of Ca, %	60.2	65.7	61.7	62.8	76.7	75.8	78.4	75.8	1.9	0.566	0.942	<0.001	0.252	0.062	0.580	0.625

[1]Data are least square means of 10 observations for all treatments, except for SBM-CV with 0 FTU and FSBM and FFFSB with 500 FTU, which represent 9 observations.

[2]SBM-CV = conventional soybean meal. FSBM = fermented soybean meal. FFSB = full-fat soybeans. FFFSB = fermented full-fat soybeans. Fr = fermentation, T = SBM type, P = phytase.

[3]The basal endogenous loss (BEL) of P expressed as milligram per day was calculated by multiplying the basal endogenous loss (mg/kg DMI) by the daily DM feed intake (kg/d) of each diet.

[4]Values for the STTD of P were calculated by correcting values for the ATTD of P with the basal endogenous loss (i.e., 190 mg/kg DMI, NRC, 2012).

[a-d]Means within a row that do not have a common superscript differ, P<0.05.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 895-908 in Swine Nutrition. A. J. Lewis and L. L. Southern. ed. CRC Press, Boca Raton, FL.

AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int, Gaithersburg, MD.

Barua, M., M.R. Abdollahi, F. Zaefarian, T.J. Wester, C.K. Girish, P.V. Chrystal, and V. Ravindran, 2021. An investigation into the influence of age on the standardized amino acid digestibility of wheat and sorghum in broilers. Poult. Sci. 100:101466.

Çabuk, B., M. G. Nosworthy, A. K. Stone, D. R. Korber, T. Tanaka, J. D. House, and M. T. Nickerson. 2018. Effect of fermentation on the protein digestibility and levels of non-nutritive compounds of pea protein concentrate. Food Technol. And Biotechnol. 56:257-264.

Cervantes-Pahm, S. K., and H. H. Stein. 2010. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674–2683.

Chachaj, R., I. Sembratowicz, M. Krauze, and K. Ognik. 2019. The effect of partial replacement of soybean meal with fermented soybean meal on chicken performance and immune status. J. Anim. Feed Sci. 28:263–271.

Chen, L., P. v. Vadlani, and R. L. Madl. 2014. High-efficiency removal of phytic acid in soy meal using two-stage temperature-induced Aspergillus oryzae solid-state fermentation. J. Sci. Food Agric. 94:113–118.

Espinosa, C. D., M. S. F. Oliveira, L. V. Lagos, T. L. Weeden, A. J. Mercado, and H. H. Stein. 2020. Nutritional value of a new source of fermented soybean meal fed to growing pigs. J. Anim. Sci. 98:1–9.

Van Eys, J. E. 2012. Manual of quality analyses for soybean products in the feed industry. 2nd rev. ed. USSEC, Chesterfield, MO.

Kiarie, E. G., I. A. Parenteau, C. Zhu, N. E. Ward, and A. J. Cowieson. 2020. Digestibility of amino acids, energy, and minerals in roasted full-fat soybean and expelled-extruded soybean meal fed to growing pigs without or with multienzyme supplement containing fiber-degrading enzymes, protease, and phytase. J. Anim. Sci. 98:1–10.

Kim, B. G., G. I. Petersen, R. B. Hinson, G. L. Allee, and H. H. Stein. 2009. Amino acid digestibility and energy concentration in a novel source of high-protein distillers dried grains and their effects on growth performance of pigs. J. Anim. Sci. 87:4013–4021.

Lee, S. A., L. V. Lagos, C. L. Walk, and H. H. Stein. 2019. Standardized total tract digestibility of calcium varies among sources of calcium carbonate, but not among sources of dicalcium phosphate, but microbial phytase increases calcium digestibility in calcium carbonate. J. Anim. Sci. 97:3440–3450.

Mukherjee, R., R. Chakraborty, and A. Dutta. 2015. Role of Fermentation in Improving Nutritional Quality of Soybean Meal — A Review. Asian-Australas. J. Anim. Sci. 29:1523–1529.

National Research Council. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Osman, M. A. 2011. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. J. Saudi Soc. Agric. Sci. 10:1–6.

Pedersen, C., J. S. Almeida, and H. H. Stein. 2016. Analysis of published data for standardized ileal digestibility of protein and amino acids in soy proteins fed to pigs. J. Anim. Sci. 94:340–343.

Rojas, O. J. 2012. Nutritional evaluation of fermented soybean meal fed to weanling pigs. MS Thesis Univ. Illinois, Urbana.

Rojas, O. J., and H. H. Stein. 2012. Digestibility of phosphorus by growing pigs of fermented and conventional soybean meal without and with microbial phytase. J. Anim. Sci. 90:1506–1512.

Rojas, O. J., and H. H. Stein. 2013. Concentration of digestible, metabolizable, and net energy and digestibility of energy and nutrients in fermented soybean meal, conventional soybean meal, and fish meal fed to weanling pigs. J. Anim. Sci. 91:4397–4405.

Soumeh, E. A., H. Mohebodini, M. Toghyani, A. Shabani, A. Ashayerizadeh, and V. Jazi. 2019. Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. Poult. Sci. 98:6797–6807.

Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey, D. C. Hernot, and C. M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their coproducts. Pages 613-660 in Soybeans: Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White, and R. Galloway. Urbana, IL.

Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. Pages 17–24 in Tables of composition and nutritional value of feed materials. Sauvant, D., Perez, J.-M., Tran, G. Paris, France.

Woyengo, T. A., R. Jha, E. Beltranena, A. Pharazyn, and R. T. Zijlstra. 2014. Nutrient digestibility of lentil and regular- and low-oligosaccharide, micronized full-fat soybean fed to grower pigs. J. Anim. Sci. 92:229–237.

CHAPTER 4

GENERAL CONCLUSIONS

The fermentation process evaluated herein demonstrated that it can affect the nutritional composition of SBM and FFSB. For example, it reduced the oligosaccharides concentration and trypsin inhibitors units. Fermentation also reduced the crude fat but increased the CP concentration.

Fermentation may have a positive effect on ME of SBM-CV for chickens, but the effect may not be as large for pigs. However, the fermentation technique used in the ingredients of the current study may negatively affect the ME in FFSB for both chickens and pigs.

Fermentation negatively affected the standardized AA digestibility of Lys of SBM-CV and FFSB in chickens. A similar effect was observed for swine for all indispensable AA. Considering the reduced digestibility of AA and the reduced Lys:CP ratio, it is possible that the negative effect may be due largely to heat damage caused during the fermentation and subsequent drying process.

Fermentation had a positive effect on apparent ileal digestibility of P and STTD of P in SBM-CV and FFSB in chickens and pigs.

It is possible that the fermentation technique used for the SBM-CV and FFSB in the current study could be improved to avoid the negative effects of heat damage on digestibility of AA and possibly increase even more the availability of P for poultry and swine.