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NON RUMINANT NUTRITION

# Nutritional value of a new source of fermented soybean meal fed to growing pigs

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## Abstract

Three experiments were conducted to test the hypothesis that the standardized ileal digestibility (**SID**) of amino acids (**AA**), concentrations of digestible energy (**DE**) and metabolizable energy (**ME**), and the standardized total tract digestibility (**STTD**) of P in a new source of fermented soybean meal (Fermex 200) are greater than in conventional soybean meal (**SBM-CV**). In experiment 1, 9 barrows (initial body weight: 9.17 ± 1.03 kg) were surgically fitted with a T-cannula in the distal ileum and allotted to a triplicated 3 × 3 Latin square design. A nitrogen-free diet and 2 diets that contained cornstarch and SBM-CV or Fermex 200 as the sole source of crude protein (**CP**), and AA were formulated. Results indicated that there were no difference between SBM-CV and Fermex 200 for SID of CP and AA. In experiment 2, 24 growing pigs (initial body weight: 14.19 ± 1.18 kg) were housed individually in metabolism crates. Pigs were allotted to a corn-based diet or 2 diets that contained corn and SBM-CV or corn and Fermex 200. Feces and urine samples were collected using the marker-to-marker approach with 5-d adaptation and 4-d collection periods. Results indicated that the concentration of DE and ME in Fermex 200 were not different from DE and ME in SBM-CV. In experiment 3, 40 barrows (initial body weight: 11.01 ± 1.38 kg) were allotted to 1 of 4 diets with 10 replicate pigs per diet. Four diets were formulated to contain Fermex 200 or SBM-CV and either 0 or 1,000 units/kg of microbial phytase. Pigs were housed individually in metabolism crates. Fecal samples were collected as explained for experiment 2. Results indicated that the STTD of P in Fermex 200 was greater ($P < 0.01$) than in SBM-CV, but the addition of microbial phytase to the diets increased the ATTD and STTD of P in SBM-CV, but not in Fermex 200 (interaction; $P < 0.01$). In conclusion, the SID of AA and concentrations of DE and ME in Fermex 200 were not different from values determined for SBM-CV, but the STTD of P was greater in Fermex 200 than in SBM-CV if microbial phytase was not added to the diet.

**Key words:** amino acid digestibility, energy digestibility, fermented soybean meal, phosphorus digestibility, pigs, soybean meal

## Introduction

Soybean meal (**SBM**) is one of the most important protein sources in swine diets because of its wide availability, and its high concentration and digestibility of amino acids (**AA**; Stein et al., 2008). However, one negative aspect of using SBM in diets for young pigs is the presence of anti-nutritional factors such

**Abbreviations**

| | |
|---|---|
| AA | amino acids |
| AEE | acid hydrolyzed ether extract |
| AID | apparent ileal digestibility |
| ATTD | apparent total tract digestibility |
| CP | crude protein |
| DE | digestible energy |
| DM | dry matter |
| EPL | endogenous phosphorus loss |
| FTU | phytase units |
| GE | gross energy |
| IDF | insoluble dietary fiber |
| ME | metabolizable energy |
| SBM | soybean meal |
| SBM-CV | conventional soybean meal |
| SDF | soluble dietary fiber |
| SID | standardized ileal digestibility |
| STTD | standardized total tract digestibility |
| TDF | total dietary fiber |
| TIU | trypsin inhibitor units |

as trypsin inhibitors, lectins, antigens, and allergens, which negatively affect nutrient availability, feed efficiency, and health of the animals (Smiricky-Tjardes et al., 2003; Palacios et al., 2004; Stein et al., 2008). Soybean meal also contains nondigestible oligosaccharides, mainly stachyose, raffinose, and verbascose (Grieshop et al., 2003), which may increase diarrhea incidence (Liying et al., 2003) and reduce nursery growth performance (Hong et al., 2004). The concentration of oligosaccharides in SBM can be reduced via alcohol extraction (Lenehan et al., 2007), fermentation (Cervantes-Pahm and Stein, 2010; Rojas and Stein, 2013), or enzyme treatment (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011), or a combination of nonalcohol extraction and enzyme treatment (Oliveira and Stein, 2016). These technologies also result in increased concentrations of gross energy (**GE**), crude protein (**CP**), AA, and ash because of the removal of oligosaccharides and other soluble carbohydrates in SBM (NRC, 2012). It has, therefore, been demonstrated that processed SBM with reduced concentrations of oligosaccharides are better tolerated by weanling pigs than conventional SBM (**SBM-CV**; Jones et al., 2010; Kim et al., 2010).

Fermex 200 (Fermentation Experts USA LLC) is a new source of fermented SBM that may serve as an alternative to other protein sources in diets fed to pigs. There are, however, no data demonstrating the nutritional value of Fermex 200 in diets for pigs. Therefore, the objective of this research was to test the hypothesis that values for standardized ileal digestibility (**SID**) of AA, concentrations of digestible energy (**DE**) and metabolizable energy (**ME**), and the standardized total tract digestibility (**STTD**) of P are greater in Fermex 200 than in SBM-CV.

## Materials and Methods

Protocols for 3 experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois and protocols were approved prior to initiation of the experiments. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used in all experiments. Conventional SBM was sourced from the same batch of SBM that was used to produce Fermex 200. The SBM-CV used in the production of Fermex 200 was produced by cracking and dehulling full-fat soybeans that were subsequently defatted using a hexane solvent, desolventized, toasted, and ground. Fermex 200 was produced via fermentation of SBM-CV in the presence of *Lactobacillus*. The SBM-CV and Fermex 200 used in the 3 experiments originated from the same batches (Table 1).

### Experiment 1: amino acid digestibility

#### *Animals and treatments*

Nine barrows (initial body weight: 9.17 ± 1.03 kg) were used. Pigs had a T-cannula installed in the distal ileum and were allotted to a triplicated 3 × 3 Latin square design with 3 diets and three 7-d periods in each square (Kim and Stein, 2009). There was, therefore, a total of 9 observations per treatment. Pigs were placed in individual pens (1.2 ×1.5 m) that were equipped with a

**Table 1.** Analyzed nutrient composition of ingredients (as-fed basis)

| Item | Fermex 200[1] | SBM-CV[1] |
|---|---|---|
| Dry matter, % | 87.26 | 88.48 |
| GE, kcal/kg | 4,166 | 4,091 |
| CP, % | 47.98 | 45.67 |
| AEE, % | 0.86 | 0.80 |
| IDF, % | 18.30 | 18.30 |
| SDF, % | 5.20 | 0.10 |
| TDF, % | 23.80 | 18.40 |
| Ash, % | 6.26 | 6.16 |
| Ca, % | 0.26 | 0.32 |
| Total P, % | 0.62 | 0.63 |
| Phytic acid, % | 0.79 | 1.62 |
| Phytate bound P[2], % | 0.22 | 0.46 |
| Non-phytate P[3], % | 0.40 | 0.17 |
| Trypsin inhibitor, TIU[4]/mg | <1.00 | 5.10 |
| Carbohydrates | | |
| Glucose, % | 0.94 | ND[5] |
| Sucrose, % | 0.70 | 7.65 |
| Maltose, % | 0.06 | ND |
| Fructose, % | 0.05 | ND |
| Stachyose, % | 0.48 | 5.50 |
| Raffinose, % | 0.34 | 0.97 |
| Indispensable AA, % | | |
| Arg | 3.71 | 3.51 |
| His | 1.33 | 1.26 |
| Ile | 2.54 | 2.45 |
| Leu | 3.97 | 3.77 |
| Lys | 3.18 | 3.13 |
| Met | 0.72 | 0.71 |
| Phe | 2.68 | 2.50 |
| Thr | 1.93 | 1.88 |
| Trp | 0.66 | 0.62 |
| Val | 2.29 | 2.31 |
| Dispensable AA, % | | |
| Ala | 2.21 | 2.10 |
| Asp | 5.83 | 5.57 |
| Cys | 0.75 | 0.76 |
| Glu | 9.23 | 8.86 |
| Gly | 2.17 | 2.06 |
| Pro | 2.24 | 2.13 |
| Tyr | 1.64 | 1.61 |
| Ser | 2.08 | 2.03 |
| Lys:CP | 6.63 | 6.85 |

[1]Fermex 200 = fermented SBM (Fermentation Experts USA LLC).
[2]Calculated as 28.2% of phytic acid (Tran and Sauvant, 2004).
[3]Calculated as total P – phytate P.
[4]TIU, trypsin inhibitor units.
[5]ND, not detected.

self-feeder, a nipple waterer, and a slatted tri-bar floor. Two diets were based on Fermex 200 or SBM-CV as the only AA-containing ingredient (Table 2). A nitrogen-free diet was included in the experiment to measure basal endogenous losses of CP and AA. Thus, a total of 3 diets were formulated. 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.

**Table 2.** Composition of diets used in experiment 1

| | Diet | | |
|---|---|---|---|
| **Ingredient, %** | **Fermex 200[1]** | **SBM-CV[1]** | **Nitrogen free** |
| Fermex 200 | 35.00 | — | — |
| SBM-CV | — | 46.50 | — |
| Soybean oil | 3.00 | 3.00 | 4.00 |
| Solka floc | — | — | 4.00 |
| Dicalcium phosphate | 1.10 | 1.10 | 2.15 |
| Limestone | 0.85 | 0.75 | 0.45 |
| Lactose | 20.00 | 20.00 | 20.00 |
| Chromic oxide | 0.40 | 0.40 | 0.40 |
| Cornstarch | 39.10 | 27.70 | 67.95 |
| Magnesium oxide | — | — | 0.10 |
| Potassium carbonate | — | — | 0.40 |
| Salt | 0.40 | 0.40 | 0.40 |
| Vitamin–mineral premix[2] | 0.15 | 0.15 | 0.15 |
| Analyzed composition | | | |
| DM % | 92.98 | 92.87 | 96.29 |
| CP, % | 20.45 | 23.26 | 1.80 |
| Indispensable AA, % | | | |
| Arg | 1.39 | 1.65 | 0.01 |
| His | 0.51 | 0.59 | 0.01 |
| Ile | 0.95 | 1.12 | 0.02 |
| Leu | 1.52 | 1.78 | 0.03 |
| Lys | 1.23 | 1.48 | 0.01 |
| Met | 0.27 | 0.32 | 0.00 |
| Phe | 1.01 | 1.18 | 0.01 |
| Thr | 0.75 | 0.89 | 0.01 |
| Trp | 0.26 | 0.30 | 0.02 |
| Val | 0.98 | 1.16 | 0.01 |
| Dispensable AA, % | | | |
| Ala | 0.85 | 0.99 | 0.01 |
| Asp | 2.24 | 2.63 | 0.02 |
| Cys | 0.29 | 0.36 | 0.01 |
| Glu | 3.60 | 4.22 | 0.03 |
| Gly | 0.83 | 0.97 | 0.01 |
| Pro | 0.94 | 1.11 | 0.07 |
| Tyr | 0.67 | 0.80 | 0.01 |
| Ser | 0.83 | 0.98 | 0.01 |

[1]Fermex 200 = fermented SBM (Fermentation Experts USA LLC); SBM-CV = conventional SBM.
[2]Provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-α tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg (0.15 mg as sodium selenite and 0.15 mg as selenium yeast); and Zn, 125.1 mg as zinc hydroxychloride.

### *Experimental procedure*

Pigs were limit fed to 3 times their estimated ME requirement for maintenance (i.e., 197 kcal/kg body weight$^{0.60}$; NRC, 2012). Throughout the experiment, pigs had free access to water. The first 5 d of each period was considered the adaptation period to the diet, whereas ileal digesta were collected for 8 hr on days 6 and 7 of each period. A 225-mL plastic bag was attached to the cannula barrel using a cable tie, and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta—or at least once every 30 min—and immediately frozen at – 20 °C to prevent bacterial degradation of AA in the digesta. On the completion of 1 experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered.

### *Sample analyses*

After the experiment, ileal digesta samples were thawed and mixed within animal and diet, and a subsample was collected for analysis. Samples of all diets and AA-containing ingredients were also collected. Ileal digesta were lyophilized and finely ground before analysis. Concentrations of dry matter (**DM**) and all nutrients except AA were analyzed in duplicate. Samples of diets, digesta, and ingredients were analyzed for DM (Method 930.15; AOAC Int., 2007), and N was analyzed (Method 990.03; AOAC Int., 2007) on an FP628 protein analyzer (Leco Corporation, St. Joseph, MI) with CP calculated as N × 6.25. AA were analyzed on a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Diets and digesta samples were analyzed for chromium (Method 990.08; AOAC Int., 2007).

### *Calculation and statistical analyses*

The apparent ileal digestibility (**AID**) and the SID of CP and AA were calculated for the two diets containing Fermex 200 or SBM-CV (Stein et al., 2007). Values calculated for these 2 diets also represent the values for each ingredient. The basal endogenous losses of CP and AA were calculated from pigs fed the nitrogen-free diet.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure, and this procedure was also used to test for outliers. Means that deviated from the treatment mean by more than 3 times the interquartile range were considered outliers. One pig fed the Fermex 200 diet in period 3 was identified as an outlier and removed from the data set. Data for AID and SID of CP and AA were analyzed using a model that included diet as fixed effect and pig and period as random effects. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

## Experiment 2: energy measurements

### *Animals and treatments*

A corn-based diet and two diets containing corn and Fermex 200 or corn and SBM-CV were formulated (Table 3). Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Twenty-four growing pigs (initial body weight: 14.19 ± 1.18 kg) were allotted to a completely randomized design with 3 diets and 8 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials.

**Table 3.** Composition of diets used in experiment 2

| Ingredient, % | Diet | | |
|---|---|---|---|
| | Corn | Fermex 200[1] | SBM-CV[1] |
| Ground corn | 97.55 | 72.25 | 69.75 |
| Fermex 200 | — | 25.50 | —— |
| SBM-CV | — | — | 28.00 |
| Monocalcium phosphate | 0.80 | 0.70 | 0.70 |
| Ground limestone | 1.10 | 1.00 | 1.00 |
| Salt | 0.40 | 0.40 | 0.40 |
| Vitamin–mineral premix[2] | 0.15 | 0.15 | 0.15 |
| Analyzed composition | | | |
| DM, % | 87.32 | 87.95 | 87.90 |
| GE, kcal/kg | 3,687 | 3,816 | 3,837 |

[1]Fermex 200 = fermented SBM (Fermentation Experts USA LLC); SBM-CV = conventional SBM.
[2]Provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-α tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 (0.15 mg as sodium selenite and 0.15 mg as selenium yeast); and Zn, 125.1 mg as zinc hydroxychloride.

### *Experimental procedure*

Pigs were fed at 3 times the ME requirement for maintenance (i.e., 197 kcal/kg × body weight$^{0.60}$), which were provided each day in 2 equal meals at 0800 and 1600 hours. Water was available at all times. The initial 5 d was considered an adaptation period to the diet. Fecal markers were fed on day 6 (chromic oxide) and on day 10 (ferric oxide). Fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at –20 °C immediately after collection. Urine collections were initiated on day 6 at 1600 hours and ceased on day 10 at 1600 hours. Urine buckets were placed under the metabolism crates to allow for the total collection of urine. Buckets were emptied every morning, and a preservative of 50 mL of 6N HCl was added to each bucket when they were emptied. The collected urine was weighed, and a 10% subsample was stored at –20 °C.

### *Sample analyses*

All samples were analyzed in duplicate. Fecal samples were thawed and mixed within pig and diet, and then dried at 50 °C using a forced-air drying oven. Fecal samples were finely ground through a 1-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) before analysis. Urine samples were thawed and mixed within animal and diet, and a sub-sample was lyophilized before analysis (Kim et al., 2009). Diets and fecal samples were analyzed for DM as explained for experiment 1. Ingredients, diets, fecal, and urine samples were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Ingredients were analyzed for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) according to method 991.43 (AOAC Int., 2007) using the Ankom$^{TDF}$ Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Total dietary fiber was calculated as the sum of IDF and SDF. The SBM-CV and Fermex 200 used in the experiment were also analyzed for sugars and oligosaccharides. Glucose, fructose, maltose, sucrose, stachyose, and raffinose were analyzed in SBM-CV and Fermex 200 by extracting and quantifying the sugars using high-performance liquid chromatography with an autosampler (Alcott, Norcross, GA), a pump (Waters 510, Milford, MA), a column (Dionex CarboPac PA1, Sunnyvale, CA), and a pulsed amperometric detector (Dionex) based on the procedure of the study by Rocklin et al. (1998). Results were compared with known standards for glucose, sucrose, maltose, and fructose (Chem Service, West Chester, PA) and known standards for stachyose and raffinose (Sigma-Aldrich, St. Louis, MO) to determine concentrations of monosaccharides, disaccharides, and oligosaccharides. The trypsin inhibitor activity in SBM-CV and Fermex 200 was also determined (Method Ba 12–75; AOCS, 2006).

### *Calculation and statistical analyses*

The apparent total tract digestibility (**ATTD**) of GE and DM was calculated for each diet. The DE and ME of corn were calculated by dividing the DE and ME of the corn diet by the inclusion rate of corn in the diet. The contribution of DE and ME from corn to the DE and ME in the diets containing Fermex 200 or SBM-CV was then calculated and subtracted from the DE and ME of these diets, and the DE and ME of Fermex 200 or SBM-CV were calculated by difference (Adeola, 2001). The ATTD of GE and DM in Fermex 200 and SBM-CV was calculated using the same procedure.

Data were analyzed using the MIXED procedure (SAS Inst. Inc.) with pig as the experimental unit. Homogeneity of the variances was confirmed as explained for experiment 1. The fixed effect was diet, and replicate was considered as the random effect. The LS means statement was used to calculate treatment means, and the PDIFF option was used to separate means if differences were detected. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

## Experiment 3: phosphorus digestibility

### *Animals and treatments*

Two diets were formulated to contain Fermex 200 and either 0 or 1,000 phytase units/kg of complete diet (Ronozyme HiPhos, DSM, Kaiseraugst, Switzerland; Table 4). Two additional diets were formulated to contain SBM-CV and either 0 or 1,000 phytase units/kg of complete diet. The only source of P in the diets was Fermex 200 or SBM-CV. Vitamins and minerals, except P, were included in all diets to meet or exceed the requirements for weanling pigs (NRC, 2012). Forty growing barrows (initial body weight: 11.01 ± 1.38 kg) were allotted to a completely randomized design with 4 diets and 10 replicate pigs per diet. Pigs were housed individually in metabolism crates that were 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 materials.

### *Experimental procedure*

Pigs were fed 3 times the daily ME requirement for maintenance (i.e., 197 kcal/kg × body weight$^{0.60}$), which were provided in 2 equal meals at 0800 and 1600 hours. Water was available at all times. The initial 5 d was considered an adaptation period to the diet. Feces were collected twice daily from days 6 to 10 as explained for experiment 2, and stored at –20 °C immediately after collection.

### *Sample analyses*

At the conclusion of the experiment, fecal samples were thawed and mixed within pig and diet and dried and ground

**Table 4.** Composition of diets used in experiment 3

| | Fermex 200[1] | | SBM-CV[1] | |
|---|---|---|---|---|
| Ingredient, % | No phytase | 1,000 FTU[2]/kg | No phytase | 1,000 FTU/kg |
| Fermex 200 | 35.00 | 35.00 | — | — |
| SBM-CV | — | — | 40.00 | 40.00 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 |
| Sucrose | 10.00 | 10.00 | 10.00 | 10.00 |
| Cornstarch | 50.55 | 50.54 | 45.55 | 45.54 |
| Ground limestone | 0.90 | 0.90 | 0.90 | 0.90 |
| Salt | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin–mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |
| Phytase concentrate | — | 0.01 | — | 0.01 |
| Analyzed composition | | | | |
| Dry matter, % | 89.72 | 90.38 | 90.25 | 90.79 |
| Ash, % | 4.52 | 4.61 | 4.17 | 4.28 |
| Ca, % | 0.49 | 0.48 | 0.46 | 0.45 |
| P, % | 0.30 | 0.28 | 0.31 | 0.31 |
| Phytase, FTU/kg | <70 | 650 | <70 | 850 |

[1]Fermex 200 = fermented SBM (Fermentation Experts USA LLC); SBM-CV = conventional SBM.
[2]Ronozyme HiPhos (10,000 phytase units/g; DSM, Kaiseraugst, Switzerland); FTU = phytase units.
[3]Provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin $D_3$ as cholecalciferol, 2,210 IU; vitamin E as DL-α tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin $B_{12}$, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg (0.15 mg as sodium selenite and 0.15 mg as selenium yeast); and Zn, 125.1 mg as zinc hydroxychloride.

as explained for experiment 2. All samples were analyzed in duplicate. Fecal, ingredient, and diet samples were analyzed for DM as explained for experiment 1, and Ca and P were analyzed by inductively coupled plasma optical emission spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC, 2007]. Diets and ingredients were also analyzed for ash (Method 942.05; AOAC Int., 2007). Ingredients were analyzed for phytic acid (Ellis et al., 1977), and diets were analyzed for phytase activity (Method 2000.012; AOAC Int., 2007).

### *Calculation and statistical analyses*

The ATTD of P in Fermex 200 and in SBM-CV was calculated. By correcting these values for the basal endogenous loss of P (i.e., 190 mg per kg DM intake), values for the STTD of P in Fermex 200 and in SBM-CV were calculated (Almeida and Stein, 2010; NRC, 2012). The ATTD of Ca was also calculated for all 4 diets (NRC, 2012).

Data were analyzed in a 2 × 2 factorial arrangement using the MIXED procedure (SAS Inst. Inc.) with pig as the experimental unit. Homogeneity of the variances was confirmed, and outliers were identified as explained for experiment 1. One pig fed the Fermex 200 with phytase diet was identified as an outlier and removed from the data set. The model included the source of SBM, phytase, and the interaction between the source of SBM and phytase as the main effects. Pig was the random effect. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

## Results

### Experiment 1: AA digestibility

Pigs readily consumed their assigned diets and remained healthy throughout the experiment. No differences were observed between Fermex 200 and SBM-CV for the AID of CP and all AA, total indispensable AA, and total dispensable AA (Table 5). Likewise, SID values for CP and AA were not different between Fermex 200 and SBM-CV.

### Experiment 2: energy measurements

Pigs fed the Fermex 200 diet or the SBM-CV diet had greater ($P < 0.01$) GE intake compared with pigs fed the corn diet (Table 6). Pigs fed the corn diet had reduced ($P < 0.01$) fecal and urine excretion of GE compared with pigs fed the Fermex 200 or the SBM-CV diet. On an as-fed basis, the ATTD of GE and DM were not different among diets. However, concentration of DE in the SBM-CV diet tended to be greater ($P < 0.10$) than in the corn diet, but ME values were not different among diets.

The ATTD of GE and DM were not different among ingredients. However, the DE on an as-fed basis was greater ($P < 0.05$) in SBM-CV than in corn, but not different from Fermex 200. On a DM basis, the concentration of DE in SBM-CV tended to be greater ($P < 0.10$) than in corn, but ME values were not different among ingredients.

### Experiment 3: phosphorus digestibility

Neither the source of SBM nor phytase influenced daily feed intake or basal endogenous P loss (**EPL**; Table 7). However, daily P intake tended to be greater ($P < 0.10$) for pigs fed diets containing SBM-CV than for pigs fed diets containing Fermex 200. Pigs fed the SBM-CV diets had reduced P ($P < 0.05$) in feces when phytase was added, but that was not the case for pigs fed the Fermex 200 diets (interaction; $P < 0.01$). However, pigs fed the Fermex 200 diets had reduced ($P < 0.01$) excretion of P in feces compared with pigs fed the SBM-CV diets. When phytase was not included in diets, the ATTD and STTD of P were greater ($P < 0.01$) in Fermex 200 than in SBM-CV. Addition of microbial phytase to the diets increased ($P < 0.05$) the ATTD and STTD of P in diets containing SBM-CV, but not in Fermex 200 diets (interaction; $P < 0.01$). As a result, the ATTD and STTD of P in the SBM-CV diet with phytase

**Table 5.** AID and SID of CP and AA in Fermex 200 and in SBM-CV, experiment 1[1,2]

| | AID | | | | SID | | | |
|---|---|---|---|---|---|---|---|---|
| Item, % | Fermex 200[3] | SBM-CV | SEM | P-value | Fermex 200 | SBM-CV | SEM | P-value |
| CP | 77.8 | 78.0 | 2.37 | 0.945 | 87.8 | 86.9 | 2.09 | 0.763 |
| Indispensable AA | | | | | | | | |
| Arg | 90.1 | 91.4 | 1.23 | 0.474 | 94.0 | 95.0 | 1.02 | 0.524 |
| His | 84.9 | 83.9 | 2.00 | 0.735 | 89.4 | 88.0 | 1.77 | 0.583 |
| Ile | 85.6 | 84.0 | 1.65 | 0.505 | 90.4 | 87.9 | 1.45 | 0.255 |
| Leu | 85.0 | 83.4 | 1.67 | 0.514 | 90.0 | 87.7 | 1.46 | 0.271 |
| Lys | 80.0 | 84.1 | 2.20 | 0.207 | 84.9 | 87.9 | 1.96 | 0.298 |
| Met | 87.3 | 86.8 | 1.55 | 0.801 | 91.5 | 90.0 | 1.36 | 0.452 |
| Phe | 85.8 | 83.8 | 1.63 | 0.399 | 90.7 | 88.0 | 1.43 | 0.212 |
| Thr | 76.0 | 76.8 | 2.39 | 0.822 | 87.0 | 86.1 | 2.11 | 0.751 |
| Trp | 85.3 | 85.6 | 1.85 | 0.934 | 90.3 | 89.8 | 1.64 | 0.820 |
| Val | 82.7 | 81.7 | 1.87 | 0.710 | 89.3 | 87.2 | 1.65 | 0.368 |
| Total indispensable AA | 84.2 | 84.3 | 1.71 | 0.951 | 89.6 | 88.9 | 1.51 | 0.746 |
| Dispensable AA | | | | | | | | |
| Ala | 77.7 | 78.8 | 2.47 | 0.766 | 85.5 | 85.2 | 2.17 | 0.938 |
| Asp | 81.8 | 82.9 | 2.08 | 0.729 | 86.7 | 86.8 | 1.83 | 0.974 |
| Cys | 69.3 | 71.9 | 3.42 | 0.603 | 79.6 | 79.4 | 3.08 | 0.954 |
| Glu | 82.5 | 80.0 | 2.52 | 0.496 | 85.6 | 83.0 | 2.23 | 0.415 |
| Gly | 69.1 | 67.5 | 3.92 | 0.781 | 91.4 | 86.9 | 3.46 | 0.382 |
| Tyr | 86.0 | 85.6 | 1.39 | 0.832 | 91.5 | 90.2 | 1.39 | 0.514 |
| Ser | 83.4 | 83.4 | 2.03 | 0.986 | 91.1 | 89.4 | 1.80 | 0.528 |
| Total dispensable AA | 80.3 | 79.3 | 2.18 | 0.764 | 89.4 | 87.0 | 2.24 | 0.462 |
| All AA | 80.7 | 84.1 | 2.81 | 0.413 | 89.8 | 88.5 | 2.26 | 0.638 |

[1]Data are least squares means of 8 and 9 observations for Fermex 200 treatment and SBM-CV treatment, respectively.
[2]Values for SID were calculated by correcting the values for AID for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DM intake) as CP, 22.16; Arg, 0.58; His, 0.25; Ile, 0.49; Leu, 0.83; Lys, 0.65; Met, 0.12; Phe, 0.53; Thr, 0.89; Trp, 0.14; Val 0.69; Ala, 0.71; Asp, 1.19; Cys, 0.32; Glu, 1.21; Gly, 1.99; Tyr, 0.40; and Ser, 0.69.
[3]Fermex 200 = fermented SBM (Fermentation Experts USA LLC).

**Table 6.** ATTD of GE and DM, and concentrations of DE and ME in experimental diets and in corn, Fermex 200, or SBM-CV, experiment 2[1]

| Item | Corn | Fermex 200[2] | SBM-CV | SEM | P-value |
|---|---|---|---|---|---|
| GE intake, diet, kcal/d | 1,975[b] | 2,925[a] | 3,203[a] | 160 | <0.001 |
| GE in feces, kcal/d | 236[b] | 403[a] | 415[a] | 33 | 0.001 |
| GE in urine, kcal/d | 37[b] | 114[a] | 91[a] | 11 | <0.001 |
| ATTD of GE, diet, % | 88.0 | 86.4 | 87.1 | 0.8 | 0.412 |
| ATTD of DM, diet, % | 90.0 | 88.5 | 88.7 | 0.7 | 0.259 |
| DE, diet, kcal/kg | 3,246[y] | 3,299[xy] | 3,343[x] | 31 | 0.091 |
| ME, diet, kcal/kg | 3,173 | 3,153 | 3,234 | 33 | 0.215 |
| ATTD of GE, ingredient, % | 88.0 | 82.2 | 84.9 | 2.2 | 0.199 |
| ATTD of DM, ingredient, % | 90.0 | 84.6 | 85.6 | 1.9 | 0.110 |
| As-fed basis | | | | | |
| DE, ingredient, kcal/kg | 3,328[b] | 3,507[ab] | 3,650[a] | 89 | 0.046 |
| ME, ingredient, kcal/kg | 3,253 | 3,149 | 3,446 | 102 | 0.143 |
| Dry matter basis | | | | | |
| DE, ingredient, kcal/kg | 3,798[y] | 4,040[xy] | 4,131[x] | 102 | 0.067 |
| ME, ingredient, kcal/kg | 3,712 | 3,627 | 3,900 | 117 | 0.266 |

[1]Data are least squares means of 8 observations per treatment.
[2]Fermex 200 = fermented SBM (Fermentation Experts USA LLC).
[a,b]Means within a row lacking a common letter are different ($P < 0.05$).
[x,y]Means within a row lacking a common letter tend to be different ($P < 0.10$).

were not different from values obtained for the Fermex 200 diet without phytase.

Intake of Ca was greater ($P < 0.05$) for pigs fed the Fermex 200 diets compared with pigs fed the SBM-CV diets. The concentration of Ca in feces was reduced ($P < 0.05$) if phytase was added to diets containing SBM-CV, but that was not the case if phytase was added to Fermex 200 diets (interaction; $P < 0.01$). When phytase was not included in diets, the ATTD of Ca was greater ($P < 0.01$) in Fermex 200 than in SBM-CV. However, when phytase was included in the diets, the ATTD of Ca increased in SBM-CV, but not in Fermex 200 (interaction; $P < 0.01$), and therefore, the ATTD of Ca in SBM-CV with phytase was not

**Table 7.** Effects of microbial phytase on ATTD of P and Ca and STTD of P in Fermex 200 and SBM-CV, experiment 3[1]

| | Fermex 200[2] | | SBM-CV | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | No phytase | 1,000 FTU[3]/kg | No phytase | 1,000 FTU/kg | SEM | Source | Phytase | Source × phytase |
| Feed intake, g/d | 737 | 738 | 730 | 733 | 21 | 0.796 | 0.908 | 0.964 |
| P intake, g/d | 2.2 | 2.1 | 2.3 | 2.3 | 0.1 | 0.054 | 0.321 | 0.216 |
| P in feces, % | 1.2[b] | 1.0[b] | 2.2[a] | 1.3[b] | 0.1 | <0.001 | <0.001 | 0.001 |
| ATTD of P, % | 78.6[ab] | 80.8[a] | 49.9[c] | 73.7[b] | 2.0 | <0.001 | <0.001 | <0.001 |
| Basal EPL[4], mg/d | 125 | 127 | 125 | 126 | 3.6 | 0.939 | 0.728 | 0.983 |
| STTD of P[5], % | 84.3[ab] | 87.1[a] | 55.5[c] | 79.3[b] | 2.1 | <0.001 | <0.001 | <0.001 |
| Ca intake, g/d | 3.6 | 3.5 | 3.4 | 3.3 | 0.1 | 0.036 | 0.317 | 0.667 |
| Ca in feces, % | 1.5[bc] | 1.8[b] | 2.5[a] | 1.4[c] | 0.1 | 0.095 | 0.011 | <0.001 |
| ATTD of Ca, % | 83.2[a] | 80.3[a] | 62.5[b] | 81.5[a] | 2.0 | <0.001 | 0.001 | <0.001 |

[1]Data are least squares means of 10 observations per treatment, except for Fermex 200 with phytase treatment, which represents 9 observations.
[2]Fermex 200 = fermented SBM (Fermentation Experts USA LLC).
[3]Ronozyme HiPhos (10,000 phytase units/g; DSM, Kaiseraugst, Switzerland); FTU = phytase units.
[4]This value was assumed to be 190 mg/kg DM intake (NRC, 2012). The daily basal EPL (mg/d) for each diet was calculated by multiplying the EPL (mg/kg dry matter intake) by the daily DM intake of each diet (Almeida and Stein, 2010).
[5]Values for STTD were calculated by correcting values for ATTD for the basal endogenous loss of P (NRC, 2012).
[a–c]Means within a row lacking a common letter are different ($P < 0.05$).

different from the ATTD of Ca in Fermex 200 without or with phytase.

## Discussion

The SBM-CV contained <46% CP, which is likely a result of the soybeans being grown in the north-western part of the corn belt where CP usually is less than in beans grown further to the east and south (Sotak-Peper et al., 2015). As a consequence, the analyzed concentration of CP in Fermex 200 was less compared with other fermented SBM (Yang et al., 2007; NRC, 2012; Rojas and Stein, 2013; 2015). The greater concentrations of CP and AA in Fermex 200 compared with SBM-CV is likely a result of the fermentation process, which ferments most of the low molecular weight carbohydrates (sucrose, rafinose, and stachyose) in SBM, and therefore results in an increased concentration of other nutrients (Hong et al., 2004; Cervantes-Pahm and Stein, 2010). The increased total dietary fiber (**TDF**) in fermented SBM is a result of the concentration of nutrients after fermentation of the low molecular weight carbohydrates, but the observation that IDF was not increased, whereas SDF increased, indicates that some of the IDF in SBM-CV may have been solubilized during fermentation, which has also been previously described (Tu et al., 2014).

The lack of differences in AID and SID of CP and AA between Fermex 200 and SBM-CV is in contrast with previous data indicating that fermentation of SBM-CV resulted in a reduction in the SID of some AA (Cervantes-Pahm and Stein, 2010; Pedersen et al., 2016) due to heat damage in the fermented SBM. The Lys:CP ratio for Fermex 200 was 6.63%, which indicates that the fermented SBM was not heat damaged (Gonzalez-Vega et al., 2011). Lysine is the most susceptible AA to destruction via the Maillard reaction due to its ε-amino group, which reacts with reducing sugars in the presence of heat and moisture (Erbersdobler and Hupe, 1991). Therefore, it appears that the fermentation and drying procedures used to produce Fermex 200 were effective in maintaining AA digestibility in the ingredient. As a result, greater concentrations of digestible AA are provided to the diet by Fermex 200 compared with SBM-CV.

A corn-based diet was used in the energy balance experiment because corn is well tolerated by pigs, and therefore, corn is often used in experiments where DE and ME values are determined using the difference procedure. The lower GE intake for pigs fed the corn diet than for pigs fed the 2 SBM diets is the result of the fact that the ME:GE ratio in corn is greater than in SBM and because feed was provided to the same daily ME, less GE per day was fed of the corn diet than of the 2 SBM diets. In addition, there were more orts from pigs fed the corn diet than from pigs fed the diets containing Fermex 200 or SBM-CV, which also contributed to the reduced GE intake of the corn diet. The ATTD of GE in corn obtained in this experiment is in close agreement with reported data for young pigs (Rojas and Stein, 2013). However, values for DE and ME in corn from this experiment are less than previously reported values (Baker and Stein, 2009; Rojas and Stein, 2013; Espinosa and Stein, 2018), which is likely a result of the reduced concentration of GE and acid hydrolyzed ether extract (**AEE**), as well as increased concentration of IDF in the corn used in this experiment compared with published data (NRC, 2012; Stein et al., 2016; Rostagno et al., 2017; Espinosa and Stein, 2018). Values for DE and ME in SBM-CV are in agreement with reported data (NRC, 2012; Stein et al., 2016) and the observation that SBM-CV had a greater concentration of DE than corn also concurs with previous data (Rojas and Stein, 2013; Zhang et al., 2013). Because of the reduced concentrations of sucrose and oligosaccharides, Fermex 200 had increased CP and AEE compared with SBM-CV, which is likely the reason for the lack of differences between SBM-CV and Fermex 200 for DE and ME. This observation is in agreement with data by Goebel and Stein (2011), indicating that fermentation of SBM does not affect DE or ME despite compositional differences caused by fermentation.

The analyzed concentrations of ash, Ca, and P in SBM-CV used in the experiment agree with published values (NRC, 2012; Sotak-Peper et al., 2016; She et al., 2017). The analyzed values for ash, Ca, and P in Fermex 200 are also in agreement with previous data for fermented SBM (Rojas and Stein, 2012). The observation that the analyzed concentration of phytic acid in Fermex 200 is less than in SBM-CV also concurs with previous data (Rojas and Stein, 2012). Values for STTD of P are more additive in mixed diets compared with values for ATTD of P (She et al., 2018) and are determined by correcting ATTD values for the basal endogenous loss of P (Almeida and Stein, 2010; NRC, 2012). The

ATTD and STTD of P in SBM-CV without phytase concur with previous values (Bohlke et al., 2005; She et al., 2017), and the ATTD and STTD of P obtained for SBM-CV with phytase are in agreement with values reported by Almeida and Stein (2010). The observation that the STTD of P and ATTD of Ca were greater in Fermex 200 compared with SBM-CV if no microbial phytase is included in diets is likely a result of the partial degradation of phytate during fermentation, which results in increased Ca and P digestibility due to increased concentrations of non-phytate bound Ca and P in Fermex 200 (Ilyas et al., 1995). In contrast, the majority of the Ca and P in SBM-CV is bound to phytate, which results in reduced digestibility of both Ca and P. Therefore, when phytase was included in the diets, increased digestibility of Ca and P in SBM-CV was observed, but that was not the case for Fermex 200. These observations are in agreement with data indicating that the effect of microbial phytase is much greater in SBM-CV than in fermented SBM (Rojas and Stein, 2012).

## Conclusion

The DE and ME values, as well as SID of AA in Fermex 200 were not different from SBM-CV. The ATTD and STTD of P were greater in Fermex 200 than in SBM-CV if microbial phytase was not added to diets, which reduces the need for inclusion of inorganic P in diets containing Fermex 200. These results indicate that Fermex 200 can likely be used as a source of AA, energy, and P in diets for weanling pigs.

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## Conflict of Interest Statement

TLW and AJM are employees of Purina Animal Nutrition who distributes Fermex 200 in the United States. CDE, MSFO, LVL, and HHS declare no real or perceived conflicts of interest..

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