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Nutrient Digestibility of Soybean Meal Products Based on In Vitro Procedures for Pigs

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Abstract: The present work aimed to assess the nutrient digestibility of soybean meal (SBM) products based on in vitro procedures. Two-step and three-step in vitro assays were performed to mimic the digestion and absorption of nutrients in the digestive tracts of growing swine. The two-step in vitro method was modified to reflect the digesta retention time and digestive enzymes of nursery piglets by decreasing incubation periods and digestive enzymes to half of those in the procedure for growing pigs and was used to determine the crude protein (CP) digestibility of nursery piglets. The seven ingredients included conventional SBM, thermo-mechanically processed SBM (TSBM), and five sources of fermented SBM (FSBM). The five sources of FSBM were produced using different microorganisms for fermentation, namely: (1) *Pediococcus pentosaceus* and *Bacillus subtilis*, (2) *Enterococcus faecium* (FSBM-EF), (3) *Aspergillus oryzae* and *Bacillus subtilis*, (4) *Aspergillus oryzae*, and (5) *Bacillus licheniformis*. Based on the conventional procedure, the in vitro ileal disappearance of CP in TSBM was greater ($p < 0.05$) compared with that in FSBM sources. Based on the in vitro assays for total tract digestibility, organic matter in TSBM was better digested ($p < 0.05$) compared with that in FSBM except for FSBM-EF. Based on the in vitro procedure for nursery piglets, the ileal disappearance of CP in TSBM was greater ($p < 0.05$) than that in the other SBM products. Taken together, thermo-mechanical processing rather than microbial fermentation of SBM improves the nutrient digestibility of SBM, particularly in nursery pigs.

Keywords: crude protein; in vitro procedures; soybean meal products; swine

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1. Introduction

Soybean products are widely used in swine feeds as a protein supplement [1]. However, these ingredients contain various antinutritional factors, including trypsin inhibitors, phytate, and oligosaccharides [2]. Unfortunately, nursery pigs have limited tolerance to these antinutritional factors, resulting in the restricted use of soybean products in nursery pig diets [3]. Therefore, various methods are employed to reduce these antinutritional factors in soybean products [4,5]. Soybean meal (SBM) is produced by extracting or expelling oils from soybeans and contains fewer trypsin inhibitors compared with raw soybeans due to the heat treatments used during the production procedure [6]. Fermentation can be used to enhance the nutritional quality of SBM by degrading antinutritional factors such as raffinose and stachyose and hydrolyzing proteins [4], resulting in fermented SBM (FSBM) which is often used in nursery pig diets [4,7]. Additionally, a thermo-mechanical processing method is also available to enhance the nutritional value of SBM [1]. To use processed SBM products as a feedstuff in pig diets, it is essential to determine their nutritional values. However, the effects of the fermentation or thermo-mechanical processing of SBM on its nutritional quality are inconsistent in the literature [1,7–9].

In vitro methods have been widely used to assess the nutrient digestibility of feedstuffs and have shown similar values compared with in vivo experiments [10]. However, the conventional in vitro method was developed to simulate the digestion and absorption of nutrients by growing pigs [11]. To more accurately determine the nutritional values of

processed SBM products, which are mostly used for the nursery stage of pigs, a modification of the conventional in vitro procedure is needed. To the best of our knowledge, there is a lack of information regarding the nutritional values of various processed SBM products based on the in vitro procedure for nursery pigs. Therefore, this work aimed to measure the nutrient digestibility of SBM products using conventional and modified in vitro procedures. The hypothesis was that the nutrient digestibility of SBM would be improved with thermo-mechanical processing or microbial fermentation.

2. Materials and Methods

2.1. Preparation of Test Ingredients

Seven SBM products were tested, including conventional SBM (CSBM), thermo-mechanically processed SBM (TSBM), and five sources of FSBM. The 5 sources of FSBM were produced using different microorganisms for fermentation: (1) SBM fermented by *Pediococcus pentosaceus* and *Bacillus subtilis* (FSBM-PB); (2) SBM fermented by *Enterococcus faecium* (FSBM-EF); (3) SBM fermented by *Aspergillus oryzae* and *Bacillus subtilis* (FSBM-AB); (4) SBM fermented by *Aspergillus oryzae* (FSBM-AO); and (5) SBM fermented by *Bacillus licheniformis* (FSBM-BL).

2.2. Conventional In Vitro Procedures for Growing Pigs

An in vitro procedure consisting of two steps was performed to determine the in vitro ileal disappearance (IVID) of dry matter (DM) and crude protein (CP) in the SBM products by simulating the digestion and absorption in the gastrointestinal tract of swine [12,13]. Briefly, each SBM product (1 g) was placed into a 100 mL conical flask in the first step. After adding 25 mL of a buffer solution (0.1 M and pH = 6.0) and 10 mL of HCl (0.2 M and pH = 0.7) to the conical flask, the pH was lowered to 2.0 using either a 1 M HCl or 1 M NaOH solution to replicate the acidic conditions of a growing pig's stomach. Additionally, 1 mL of pepsin solution at a concentration of 10 mg/mL made from a pepsin product consisting of >250 units/mg solid was introduced into the conical flask to simulate the action of gastric enzymes. After adding 0.5 mL of chloramphenicol solution, the flasks were placed into a shaking incubator at 39 °C for 6 h. The second step aimed to replicate the small intestine environment in growing pigs. Initially, 10 mL of a buffer solution (0.2 M and pH = 6.8) and 5 mL of NaOH solution at a concentration of 0.6 M were added to the conical flasks. The pH was then raised to 6.8. Next, 1 mL of pancreatin solution at a concentration of 50 mg/mL was added to simulate the action of intestinal enzymes. The flasks were once again incubated in the shaking incubator at 39 °C for 18 h. Following incubation, 5 mL of sulfosalicylic acid solution at a concentration of 20% was added to the flasks, and the flasks were kept under room ambient conditions for 30 min. Following precipitation, undigested samples underwent the process of filtration using filter crucibles. The flasks underwent two rinses with 1% sulfosalicylic acid. Then, ethanol and acetone were each added twice to the filter crucibles containing undigested samples followed by drying at 80 °C for 24 h. The filter crucibles containing residues and Celite were weighed to determine the ileal digestibility of DM in the SBM products. The residues in the filter crucibles were collected to assess CP concentrations and to compute the IVID of CP. To account for nutrients that did not originate from the SBM products, a blank flask was employed in the two-step in vitro procedure for the correction of the nutrient contents in the residues. The entire two-step in vitro procedure was conducted in triplicate.

To mimic the digestion and absorption of nutrients in the entire gut of growing pigs, the in vitro total tract disappearance (IVTTD) of nutrients in the SBM products was assessed using a three-step in vitro procedure [11,12]. The initial two steps of this procedure were very comparable to the IVID procedure but with some modifications. Briefly, the SBM products (0.5 g) were utilized, and the pepsin and pancreatic concentrations used were 25 and 100 mg/mL, respectively. The samples were incubated for 2 and 4 h in the first and second steps, respectively. In the third step of the IVTTD procedure, 10 mL of ethylenediaminetetraacetic acid solution (0.2 M) was added to the flasks. Additionally, a

multi-enzyme solution (0.5 mL) was added to the flasks followed by an 18 h incubation in a shaking incubator at 39 °C. After incubation, the SBM product samples were filtered and dried at 130 °C for 6 h. The ash contents in the residues were assessed to compute the IVTTD of organic matter (OM) in the SBM products. The 3-step in vitro procedure was performed in triplicate.

2.3. Modified Two-Step In Vitro Procedure for Nursery Pigs

The two-step in vitro procedure for the ileal digestibility of growing pigs described by Boisen and Fernández [13] was modified for nursery pigs. The first and second steps of this modified procedure were comparable to the IVID procedure for growing pigs except for the concentrations of the digestive enzymes and incubation periods. To account for the lower quantity of pepsin and pancreatic secretions in nursery pigs compared with growing pigs, the concentrations of the enzymes were reduced to 5 and 25 mg/mL, respectively. Additionally, the incubation periods in the first and second steps were shortened to 3 and 9 h, respectively. The other procedures were the same as those in the conventional method for growing pigs. These procedures were conducted in triplicate.

2.4. Chemical Analyses

After in vitro digestion, the remaining residues were subjected to DM analysis (method 930.15) [14]. Additionally, the CP (method 990.03) and OM (method 942.05) in the SBM products and the residues were determined following the procedures described in AOAC [14]. Briefly, 0.2 g of each sample was placed in a digestion tube to determine the CP concentration in the SBM product. Sulfuric acid (20 mL, 96% H_2SO_4; OCI Company Ltd., Seoul, Republic of Korea) and a tablet (a catalyst + potassium sulfate, 1000Kjeltabs S/3.5; FOSS Analytical AB, Höganäs, Sweden) were added to the sample. The tubes were located in the digestion block at 340 to 370 °C for 1 h 30 min using the digestion apparatus (Büchi Labortechnik AG, Flawil, Switzerland). Then, in a distillation–titration unit, 20 mL of 32% NaOH solution (OCI Company Ltd., Seoul, Republic of Korea) was added. The solutions were distilled for approximately 6 min. The ammonia collected was titrated using the standard 0.1 M HCl. For the determination of the CP in the residues after in vitro digestion, a total quantity of each residue including Celite was placed in a digestion tube before digestion with sulfuric acid.

The SBM products were analyzed for ash (method 942.05) [14]. The concentrations of amylase-treated neutral detergent fiber (aNDF; method 2002.04) and acid detergent fiber (ADF; method 973.18) in the SBM products were assessed following the procedures described in AOAC [14]. Briefly, 0.5 g of each sample was placed in a filter crucible. To facilitate the filtration process, 0.5 g of Celite was added to the filter crucible before loading the sample. The crucible with the sample was placed in a hot extraction unit (Fibertec 1020 Hot Extraction, FOSS Ltd., Hillerød, Denmark) and 50 mL of a neutral detergent solution (containing sodium dodecyl sulfate at 30 g/L, ethylenediaminetetraacetic acid at 18.61 g/L, sodium tetraborate at 6.81 g/L, Na_2HPO_4 at 4.56 g/L, and alpha-amylase at 2 mL/L) was added. In the apparatus, the filter crucible with the sample was boiled for 1 h from the boiling point. After heating, the detergent solution was filtered with a vacuum using the pressure function of the apparatus. The fibrous residue in the filter crucible was rinsed three times with hot water and acetone, respectively. After the acetone vapors had dissipated, the filter crucible with the residue was placed in a drying oven and dried at 105 °C for 4 h. After analyzing the aNDF concentration, the concentration of ADF was determined. Most of the procedure of the ADF analysis was similar to that of the aNDF analysis except for the detergent solution. To determine the ADF concentration, an acid detergent solution (containing 5 M H_2SO_4 at 28 mL/L and cetrimonium bromide at 20 g/L) was used.

2.5. Calculations

The IVID and IVTTD of nutrients were computed using the equation suggested by Ha et al. [12], with minor modifications:

$$\text{In vitro disappearance of nutrient (\%)} = (\text{Nutr}_{\text{ingredient}} - \text{Nutr}_{\text{residue}} + \text{Nutr}_{\text{blank}}) \div \text{Nutr}_{\text{ingredient}} \times 100$$

where $\text{Nutr}_{\text{ingredient}}$ (g) is the quantity of nutrient in an SBM product, and $\text{Nutr}_{\text{residue}}$ (g) represents the quantity of the residual nutrient after in vitro digestion, while $\text{Nutr}_{\text{blank}}$ (g) represents the quantity of the residual nutrient after the in vitro digestion in the blank flask.

2.6. Statistical Analysis

The experimental data underwent statistical analysis using the generalized linear model procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The source of an SBM product was regarded as a fixed variable in the model. Least squares means were computed for the IVID of nutrients as well as the IVTTD of nutrients for each SBM product. To compare the means, the PDIFF option with Tukey's adjustment was employed. Each flask was regarded as an experimental unit, and statistical significance was determined at a p-value of less than 0.05.

2.7. Collection of References for Ileal Digestibility of FSBM

To overview the in vivo data for the effects of fermentation on the ileal digestibility of CP in SBM fed to pigs, peer-reviewed publications were obtained using a systematic search. Multiple searches were performed in August 2023 to find relevant publications related to FSBM. The databases used in the literature search included PubMed and Google Scholar, using the keywords CP, fermentation, FSBM, ileal digestibility, pigs, and SBM. The search included publications from 2000 onwards and excluded results from patents and publications not in English. After obtaining all relevant publications, strict selection criteria were applied to ensure the inclusion of only appropriate experiments. Studies that used CSBM and FSBM as the sole sources of CP and determined the ileal digestibility of CP in the ingredients were exclusively included in the dataset. Standardized ileal digestibility based on apparent ileal digestibility using a prediction equation for basal endogenous losses of CP [15] was used when standardized ileal digestibility values were not available in the papers.

3. Results

The CP concentrations in the seven sources of SBM products ranged from 45.7 to 53.6% (as-is basis; Table 1). The aNDF and ADF contents in the SBM products ranged from 7.5 to 16.8% and 4.5 to 7.7%, respectively (as-is basis).

Table 1. Analyzed nutrient concentrations in soybean meal (SBM) products (%, as-is basis).

Item	CSBM	TSBM	FSBM-PB	FSBM-EF	FSBM-AB	FSBM-AO	FSBM-BL
Dry matter	88.5	93.7	91.0	90.3	90.3	91.5	91.6
Ash	6.2	7.1	7.0	7.1	7.0	7.1	7.0
Crude protein	45.7	51.3	50.9	52.4	52.6	52.9	53.6
Amylase-treated neutral detergent fiber	11.0	16.8	7.7	7.6	7.5	8.3	7.9
Acid detergent fiber	5.9	6.4	4.5	4.6	4.6	7.7	4.9

CSBM, conventional SBM; TSBM, thermo-mechanically processed SBM; FSBM-PB, SBM fermented by *Pediococcus pentosaceus* and *Bacillus subtilis*; FSBM-EF, SBM fermented by *Enterococcus faecium*; FSBM-AB, SBM fermented by *Aspergillus oryzae* and *Bacillus subtilis*; FSBM-AO, SBM fermented by *Aspergillus oryzae*; FSBM-BL, SBM fermented by *Bacillus licheniformis*.

In the conventional in vitro procedure, the IVID of CP in TSBM was the greatest ($p < 0.05$) among the SBM products, followed by CSBM and FSBM-EF (Table 2). The IVTTD of OM in TSBM was greater ($p < 0.05$) than that in most FSBM sources but did not differ

from that in CSBM. In the in vitro method for nursery pigs, the IVID of CP in TSBM was the greatest ($p < 0.05$) among the SBM products, followed by FSBM-EF and FSBM-AO.

Table 2. In vitro ileal disappearance (IVID, %) of nutrients and in vitro total tract disappearance (IVTTD, %) of nutrients in soybean meal (SBM) products for growing pigs and nursery pigs [1].

Item	CSBM	TSBM	FSBM-PB	FSBM-EF	FSBM-AB	FSBM-AO	FSBM-BL	SEM [2]	*p*-Value
Growing pigs									
IVID of dry matter	74.7 ^{bc}	79.1 ^{a}	73.2 ^{c}	72.7 ^{c}	77.1 ^{ab}	75.4 ^{bc}	74.3 ^{bc}	0.8	0.001
IVID of crude protein	91.2 ^{b}	94.5 ^{a}	89.2 ^{bcd}	90.8 ^{b}	88.5 ^{cd}	87.4 ^{d}	90.3 ^{bc}	0.5	<0.001
IVTTD of dry matter	92.1 ^{c}	96.0 ^{a}	93.9 ^{b}	94.8 ^{ab}	92.3 ^{c}	92.0 ^{c}	94.0 ^{b}	0.3	<0.001
IVTTD of organic matter	92.7 ^{ab}	93.5 ^{a}	91.4 ^{bc}	92.1 ^{ab}	89.8 ^{cd}	89.4 ^{d}	91.4 ^{bc}	0.4	<0.001
Nursery pigs									
IVID of dry matter	65.1 ^{c}	77.1 ^{a}	67.0 ^{c}	66.1 ^{c}	72.5 ^{b}	71.7 ^{b}	66.8 ^{c}	0.6	<0.001
IVID of crude protein	80.2 ^{c}	92.8 ^{a}	81.0 ^{c}	83.5 ^{b}	81.4 ^{bc}	81.7 ^{bc}	80.4 ^{c}	0.5	<0.001

CSBM, conventional SBM; TSBM, thermo-mechanically processed SBM; FSBM-PB, SBM fermented by *Pediococcus pentosaceus* and *Bacillus subtilis*; FSBM-EF, SBM fermented by *Enterococcus faecium*; FSBM-AB, SBM fermented by *Aspergillus oryzae* and *Bacillus subtilis*; FSBM-AO, SBM fermented by *Aspergillus oryzae*; FSBM-BL, SBM fermented by *Bacillus licheniformis*. [1] Each least squares mean represents 3 observations.[2] SEM, standard error of the means. $^{a–d}$ Least squares of means within a row without a common superscript letter are different ($p < 0.05$).

The standardized ileal digestibility of CP in SBM was increased by 3.1 percentage units on average based on data from 20 FSBM in 13 studies (Table 3). When the SBM was fermented using *Bacillus*, the standardized ileal digestibility of CP was increased by 3.3 percentage units based on data from 11 FSBM in eight studies.

Table 3. Effects of fermentation on ileal digestibility of crude protein (CP) in soybean meal (SBM) fed to pigs.

Reference	Body Weight, kg	Type of Microorganism	Response Criterium	CSBM	FSBM	Δ% [1]
[7]	6.5	*Aspergillus oryzae*	AID of CP	90.0	91.3	1.3
			SID of CP	93.5	94.6	1.1
[16]	9.2	*Lactobacillus*	AID of CP	78.0	77.8	−0.2
			SID of CP	86.9	87.8	0.9
[4]	10.0	*Enterococcus faecium*	AID of CP	78.3	83.2	4.9
			SID of CP [2]	86.9	91.8	4.9
[17]	10.4	*Bacillus subtilis* and *Aspergillus oryzae*	AID of CP	60.0	65.0	5.0
			SID of CP	80.0	80.0	0
[9]	10.9	*Aspergillus oryzae*	AID of CP	70.0	70.1	0.1
			SID of CP	84.3	81.8	−2.5
[18]	14.1	*Bacillus subtilis*, *Lactobacillus*, and *yeast*	AID of CP	40.6	54.7	14.1
			SID of CP	78.4	90.4	12.0
			AID of CP	40.6	50.4	9.8
			SID of CP	78.4	84.8	6.4
[19]	14.2	*Bacillus subtilis*	SID of CP	84.0	87.7	3.7
[20]	15.6	*Enterococcus faecium*	AID of CP	75.3	76.0	0.7
			SID of CP [2]	90.0	90.7	0.7

Table 3. *Cont.*

Reference	Body Weight, kg	Type of Microorganism	Response Criterium	CSBM	FSBM	Δ% [1]
[21]	17.0	*Bacillus subtilis*	AID of CP	74.3	77.3	3.0
			SID of CP	84.8	87.7	2.9
		Saccharomyces carlbergensis	AID of CP	74.3	75.8	1.5
			SID of CP	84.8	86.3	1.5
		Saccharomyces carlbergensis and *Bacillus amyloliquefaciens*	AID of CP	74.3	77.2	2.9
			SID of CP	84.8	87.8	3.0
[22]	26.8	*Bacillus subtilis*, *Streptococcus thermophilus*, and *Saccharomyces cerevisiae*	AID of CP	75.5	73.7	−1.8
			SID of CP	82.8	80.5	−2.3
		Not provided	AID of CP	75.5	81.6	6.1
			SID of CP	82.8	88.6	5.8
[23]	27.1	*Bacillus subtilis* (low protein solubility)	AID of CP	71.1	71.8	0.7
			SID of CP	80.7	82.4	1.7
		Bacillus subtilis (medium protein solubility)	AID of CP	71.1	73.7	2.6
			SID of CP	80.7	83.1	2.4
		Bacillus subtilis (high protein solubility)	AID of CP	71.1	71.3	0.2
			SID of CP	80.7	85.0	4.3
[1]	30.4	Not provided	AID of CP	80.8	89.0	8.2
			SID of CP	89.4	95.9	6.5
		Bacillus subtilis and *Aspergillus oryzae*	AID of CP	80.8	82.1	1.3
			SID of CP	89.4	91.7	2.3
[24]	32.0	*Bacillus subtilis* and *Enterococcus faecium*	AID of CP	78.3	84.2	5.9
			SID of CP [2]	87.6	94.0	6.4
n [3]	-	Genus	Response criterium	Mean		
				CSBM	FSBM	Δ% [1]
20		Total	SID of CP	84.5	87.6	3.1
11		*Bacillus*		76.3	79.6	3.3

CSBM, conventional SBM; FSBM, fermented SBM; AID, apparent ileal digestibility; SID, standardized ileal digestibility. [1] The increase or decrease as percentage unit of ileal digestibility for FSBM relative to CSBM. [2] SID of CP value was calculated using a prediction equation for basal endogenous losses of CP suggested by Park et al. [15]. [3] The number of FSBM sources that were used for the calculation.

4. Discussion

Soybean meal contains various antinutritional factors that can decrease the nutritional value of SBM. In the present study, thermo-mechanical processing and fermentation by microorganisms were used to improve the nutrient availability of SBM. For the fermentation process, various species of microorganisms were employed. These microorganisms have been reported to have beneficial effects when used for the fermentation of SBM. *Pediococcus pentosaceus* can degrade the raffinose and stachyose in SBM [25]. In addition, *Bacillus subtilis* and *Enterococcus faecium* hydrolyze antinutritional factors in SBM and decrease the molecular weights of proteins in SBM [26]. *Aspergillus oryzae* and *Bacillus licheniformis* can also increase the ratio of small peptides and reduce trypsin inhibitors [27,28]. Additionally, these microorganisms can break down fiber fractions in SBM. In contrast, thermo-mechanical processing can reduce trypsin inhibitors without reducing fiber fractions, which can serve as a prebiotic in the intestinal tracts of pigs [29]. These processes potentially affect the nutrient availability of SBM, which can be assessed by employing in vitro assays. However, information is lacking regarding the nutritional values of various processed SBM products based on the in vitro procedure for nursery pigs. Thus, the nutrient digestibility of SBM products was determined in the present work using conventional and modified in vitro procedures.

The analyzed CP concentration in CSBM in the present study was within the range of those in previous studies [1,7,9,12]. However, the aNDF concentration in CSBM in the present study was slightly greater than those reported in the literature [12,30], which is likely due to the inclusion of hulls in the CSBM used in this work. In the production of SBM, oils are extracted from soybeans after removing the hulls from soybeans. Soybean hulls removed before oil extraction are often added back into the SBM. The CP concentration in SBM is less than 51.6% (DM basis) if soybean hulls are added to the SBM [31]. As the CP concentration in the CSBM used in this study was 51.6% (DM basis), it is unclear if soybean hulls were included in the CSBM or not.

The CP concentration in TSBM in the present study was within the range of previous data [1]. Although the CP concentrations in TSBM and FSBM were comparable, the aNDF concentration in TSBM in the present study was much higher than that in FSBM. The reason for the high aNDF concentration in TSBM remains unclear. Regardless of the species of microorganisms used for fermentation, all FSBM had lower aNDF concentrations than CSBM in this study. This observation indicates that the microorganisms may have utilized the fiber fraction of SBM as a substrate during the fermentation process [9]. In contrast with CP and aNDF concentrations, the amount of ash was similar across the SBM products and fell within the range of previous studies [1,10,12]. These observations indicate that the processing method does not affect the ash concentration in SBM products.

The IVID of DM and CP in CSBM based on the conventional in vitro procedure observed in the present study was comparable to those reported in previous studies [12,32]. In the present study, thermo-mechanical processing improved the IVID of CP of SBM in the conventional in vitro procedure. This indicates that amino acids in TSBM are more digestible compared with CSBM at the ileal level. These results are in agreement with a previous animal study [1] that reported a 7.4 percentage unit greater standardized ileal CP digestibility in TSBM compared with CSBM. Thermo-mechanical processing has been observed to improve nutrient utilization, but the reasons for this effect are not fully understood. Thermo-mechanical processing may shorten peptide bonds or increase enzyme accessibility in SBM. Increased physical flexibility with thermo-mechanical processing may also positively affect the nutrient digestibility of SBM.

In the case of FSBM, however, the effect of fermentation on the in vitro disappearance of SBM varied in the present study. In a previous in vivo study conducted with nursery pigs [4], FSBM-EF showed greater ileal digestibility of CP compared with CSBM, which is consistent with the present results based on the in vitro procedures modified for nursery pigs. Jeong et al. [4] suggested that antinutritional factors including trypsin inhibitors, raffinose, and stachyose in SBM were reduced via microbial fermentation. Another study [33] reported that the addition of *Enterococcus faecium* to the diet resulted in an increase in CP digestibility, suggesting that *Enterococcus faecium* may secrete a substantial quantity of microbial proteases. However, in the conventional in vitro procedure, the fermentation of SBM using *Enterococcus faecium* did not improve the IVID of CP in the SBM. These observations are likely attributed to differences in the digestive capacities of nursery pigs and growing pigs. Due to the longer incubation time and a greater digestive enzyme concentration in the in vitro procedure for growing pigs compared with nursery pigs, the beneficial effects of fermentation might be diluted by pepsin and pancreatin in the in vitro procedure for growing pigs.

In this work, FSBM-AB and FSBM-AO had lower IVID of CP values compared with CSBM when using the conventional in vitro method. Although the difference was not statistically significant, a previous study by Cervantes-Pahm and Stein [9] also reported a numerically less standardized ileal digestibility of CP (2.5 percentage unit) in FSBM-AO compared with CSBM, which is consistent with our findings. However, Yáñez et al. [1] reported that the standardized ileal digestibility of CP in FSBM-AB was 2.3 percentage units greater than that in CSBM. Generally, microbial fermentation causes increased amounts of small peptides in FSBM. As small peptides are more digestible than proteins in SBM, microbial fermentation is expected to increase CP digestibility. In the study by Cervantes-

Pahm and Stein [9], however, the amounts of small peptides did not differ between CSBM and FSBM-AO. Therefore, *Aspergillus oryzae* in the fermentation process may not have a positive impact on the utilization of CP in SBM, likely due to the lack of microbial effects on proteins in SBM.

Similar to FSBM-AB and FSBM-AO, *Bacillus licheniformis* did not have a beneficial impact on the IVID of CP in SBM in both conventional and modified in vitro procedures. Cheng et al. [34] reported that the addition of protease derived from *Bacillus licheniformis* did not improve CP digestibility in a cereal-SBM-based diet fed to pigs, which partially supports the present results.

The effects of fermentation on the ileal digestibility of SBM are controversial in the literature. In the present work, the results from the in vivo studies that investigated the influence of fermentation on the ileal digestibility of CP in SBM fed to pigs were summarized (Table 3). The positive effects of fermentation on the standardized ileal digestibility of CP were observed. However, some previous studies [9,16,17,22] reported that fermentation did not positively affect the ileal digestibility of SBM. The potential reasons for this inconsistency could include the fermentation conditions, types of microorganisms, and sources of SBM.

The value of the IVTTD of DM in CSBM observed in the present study is similar to those reported in previous studies [10,32]. The greater IVTTD of DM in TSBM compared with CSBM can be partially explained by the greater IVID of DM and IVID of CP in TSBM, indicating that thermo-mechanical processing may have made proteins more digestible in the upper gut of pigs. In contrast with the IVID of DM, the IVTTD of DM of FSBM-PB and FSBM-EF were greater compared with CSBM. The reason for the positive effects of fermentation by *Pediococcus pentosaceus*, *Bacillus subtilis*, and *Enterococcus faecium* only on the IVTTD of DM but not on the IVID of DM remains unclear. However, it is likely that the microbial enzymes secreted during the fermentation process may have greater activity in step 3, leading to an improved IVTTD of DM. Although FSBM-AB and FSBM-AO showed no difference compared with CSBM in terms of the IVTTD of DM, FSBM-AB and FSBM-AO had a lower IVTTD of OM compared with CSBM. Organic matter is calculated by subtracting the ash concentration from the DM. Therefore, the reason for this inconsistency can be explained by the decreased IVID of CP having a greater impact on the IVTTD of OM compared with the IVTTD of DM.

Although the present in vitro procedure mimics the nutrient digestion and absorption in the gastrointestinal tract of pigs, the action of some antinutritional factors such as raffinose and stachyose is not fully simulated in the in vitro system. This may also partially explain the inconsistent effects of fermentation on the IVID or IVTTD of nutrients in SBM. To overcome this limitation, further experiments using nursery pigs are needed to validate the present results.

5. Conclusions

Thermo-mechanical processing rather than microbial fermentation of SBM improves the nutrient digestibility of SBM, particularly in nursery pigs, based on the present in vitro experiments. In vivo studies using nursery pigs are warranted to confirm the present in vitro results.

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References

1. Yáñez, J.L.; Woyengo, T.A.; Jha, R.; Van Kempen, T.A.; Zijlstra, R.T. Nutrient digestibility of soybean products in grower-finisher pigs. *J. Anim. Sci.* **2019**, *97*, 4598–4607. [CrossRef] [PubMed]
2. Cho, J.; Min, B.; Chen, Y.; Yoo, J.; Wang, Q.; Kim, J.; Kim, I. Evaluation of FSP (fermented soy protein) to replace soybean meal in weaned pigs: Growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. *Asian-Australas. J. Anim. Sci.* **2007**, *20*, 1874–1879. [CrossRef]
3. Opazo, R.; Ortuzar, F.; Navarrete, P.; Espejo, R.; Romero, J. Reduction of soybean meal non-starch polysaccharides and α-galactosides by solid-state fermentation using cellulolytic bacteria obtained from different environments. *PLoS ONE* **2012**, *7*, e44783. [CrossRef] [PubMed]
4. Jeong, J.S.; Park, J.W.; Lee, S.I.; Kim, I.H. Apparent ileal digestibility of nutrients and amino acids in soybean meal, fish meal, spray-dried plasma protein and fermented soybean meal to weaned pigs. *Anim. Sci. J.* **2016**, *87*, 697–702. [CrossRef]
5. Roh, S.G.; Carroll, J.A.; Kim, S.W. Effects of fermented soybean meal on innate immunity-related gene expressions in nursery pigs acutely challenged with lipopolysaccharides. *Anim. Sci. J.* **2015**, *86*, 508–516. [CrossRef]
6. Perez-Maldonado, R.; Mannion, P.; Farrell, D. Effects of heat treatment on the nutritional value of raw soybean selected for low trypsin inhibitor activity. *Br. Poult. Sci.* **2003**, *44*, 299–308. [CrossRef]
7. Kim, D.H.; Heo, P.S.; Jang, J.C.; Jin, S.S.; Hong, J.S.; Kim, Y.Y. Effect of different soybean meal type on ileal digestibility of amino acid in weaning pigs. *J. Anim. Sci. Technol.* **2015**, *57*, 11. [CrossRef]
8. Wang, Y.; Liu, X.; Wang, H.; Li, D.; Piao, X.; Lu, W. Optimization of processing conditions for solid-state fermented soybean meal and its effects on growth performance and nutrient digestibility of weanling pigs. *Livest. Sci.* **2014**, *170*, 91–99. [CrossRef]
9. Cervantes-Pahm, S.K.; Stein, H.H. 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.* **2010**, *88*, 2674–2683. [CrossRef]
10. Son, A.R.; Park, C.S.; Kim, B.G. Determination and prediction of digestible and metabolizable energy concentrations in byproduct feed ingredients fed to growing pigs. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 546–553. [CrossRef]
11. Boisen, S.; Fernández, J.A. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Anim. Feed Sci. Technol.* **1997**, *68*, 277–286. [CrossRef]
12. Ha, D.U.; Choi, H.; Kim, B.G. Supplemental protease improves in vitro disappearance of dry matter and crude protein in feather meal and copra meal for pigs. *Rev. Bras. Zootec.* **2020**, *49*. [CrossRef]
13. Boisen, S.; Fernández, J. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. *Anim. Feed Sci. Technol.* **1995**, *51*, 29–43. [CrossRef]
14. AOAC. *Official Methods of Analysis of AOAC International*, 21st ed.; AOAC International: Gaithersburg, MD, USA, 2019.
15. Park, C.S.; Oh, S.I.; Kim, B.G. Prediction of basal endogenous losses of amino acids based on body weight and feed intake in pigs fed nitrogen-free diets. *Rev. Colomb. Cienc. Pecu.* **2013**, *26*, 186–192.
16. Espinosa, C.D.; Oliveira, M.S.; Lagos, L.V.; Weeden, T.L.; Mercado, A.J.; Stein, H.H. Nutritional value of a new source of fermented soybean meal fed to growing pigs. *J. Anim. Sci.* **2020**, *98*, skaa357. [CrossRef]
17. Rojas, O.J.; Stein, H.H. 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.* **2013**, *91*, 4397–4405. [CrossRef]
18. Yan, H.; Jin, J.Q.; Yang, P.; Yu, B.; He, J.; Mao, X.B.; Yu, J.; Chen, D.W. Fermented soybean meal increases nutrient digestibility via the improvement of intestinal function, anti-oxidative capacity and immune function of weaned pigs. *Animal* **2022**, *16*, 100557. [CrossRef]
19. Zhang, H.Y.; Yi, J.Q.; Piao, X.S.; Li, P.F.; Zeng, Z.K.; Wang, D.; Liu, L.; Wang, G.Q.; Han, X. The metabolizable energy value, standardized ileal digestibility of amino acids in soybean meal, soy protein concentrate and fermented soybean meal, and the application of these products in early-weaned piglets. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 691. [CrossRef]
20. Muniyappan, M.; Lee, Y.; Kim, I.H. Comparative efficacy of soybean meal vs fermented soybean meal on ileal digestibility and urine contents in weaned pigs. *Livest. Sci.* **2022**, *263*, 105016. [CrossRef]
21. Upadhaya, S.D.; Kim, I.H. Ileal digestibility of nutrients and amino acids in unfermented, fermented soybean meal and canola meal for weaning pigs. *Anim. Sci. J.* **2015**, *86*, 408–414. [CrossRef]
22. Wang, Y.; Lu, W.Q.; Li, D.F.; Liu, X.T.; Wang, H.L.; Niu, S.; Piao, X.S. Energy and ileal digestible amino acid concentrations for growing pigs and performance of weanling pigs fed fermented or conventional soybean meal. *Asian-Australas. J. Anim. Sci.* **2014**, *27*, 706. [CrossRef]
23. Upadhaya, S.D.; Ryu, J.-H.; Kang, K.-I.; Cho, S.-J.; Kim, I.H. Effect of fermentation of soybean meal with varying protein solubility on ileal digestibility of nutrients in growing pigs. *Anim. Proc. Sci.* **2015**, *56*, 2023–2028. [CrossRef]
24. Akhtar, N.; Cai, H.Y.; Kiarie, E.G.; Li, J. A novel *Bacillus* sp. with rapid growth property and high enzyme activity that allows efficient fermentation of soybean meal for improving digestibility in growing pigs. *J. Appl. Microbiol.* **2022**, *133*, 3–17. [CrossRef] [PubMed]
25. Jiang, S.; Cai, L.; Lv, L.; Li, L. *Pediococcus pentosaceus*, a future additive or probiotic candidate. *Microb. Cell Factories* **2021**, *20*, 45. [CrossRef] [PubMed]

26. Shi, C.; Zhang, Y.; Yin, Y.; Wang, C.; Lu, Z.; Wang, F.; Feng, J.; Wang, Y. Amino acid and phosphorus digestibility of fermented corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* fed to pigs. *J. Anim. Sci.* **2017**, *95*, 3996–4004. [CrossRef] [PubMed]
27. Hong, K.J.; Lee, C.H.; Kim, S.W. *Aspergillus oryzae GB-107* fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food* **2004**, *7*, 430–435. [CrossRef]
28. Dai, C.; Hou, Y.; Xu, H.; Huang, L.; Dabbour, M.; Mintah, B.K.; He, R.; Ma, H. Effect of solid-state fermentation by three different *Bacillus* species on composition and protein structure of soybean meal. *J. Sci. Food Agric.* **2022**, *102*, 557–566. [CrossRef]
29. de Carvalho, A.W.; Natal, D.I.G.; da Silva, C.O.; de Dantas, M.I.d.S.; de Barros, E.G.; Ribeiro, S.M.R.; Costa, N.M.B.; Martino, H.S.D. Heat-treatment reduces anti-nutritional phytochemicals and maintains protein quality in genetically improved hulled soybean flour. *Food Sci. Technol.* **2013**, *33*, 310–315. [CrossRef]
30. NRC. *Nutrient Requirements of Swine*, 11th ed.; National Academy Press: Washington, DC, USA, 2012.
31. Lee, S.A.; Park, C.S.; Kim, B.G. Novel two-slope equations to predict amino acid concentrations using crude protein concentration in soybean meal. *Agriculture* **2021**, *11*, 280. [CrossRef]
32. Park, C.S.; Son, J.; Kim, B.G. Effects of supplemental β-mannanase on in vitro disappearance of dry matter in feed ingredients for swine. *Anim. Ind. Technol.* **2022**, *9*, 35–40. [CrossRef]
33. Yan, L.; Kim, I. Effect of probiotics supplementation in diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, faecal microbial population and faecal noxious gas content in growing pigs. *J. Appl. Anim. Res.* **2013**, *41*, 23–28. [CrossRef]
34. Cheng, L.; Jin, Y.H.; Kim, I.H. Effects of *Bacillus licheniformis* derived-protease supplementation, alone or in combination, with valine to low protein diet on growth performance and carcass quality grade in growing-finishing pigs. *J. Appl. Anim. Res.* **2021**, *49*, 180–184. [CrossRef]