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Effects of Fermentation on the Apparent Metabolizable Energy and Standardized Ileal Digestibility of Amino Acids in Soybean Meal Fed to Broiler Chickens

Yang Li [1,†], Shuzhen Li [1,†], Chong Li [1,2], Wenhuan Chang [1], Huiyi Cai [1] and Guohua Liu [1,*]

[1] The Key Laboratory of Feed Biotechnology of Ministry of Agriculture, National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2] Precision Livestock and Nutrition Laboratory, Teaching and Research Centre (TERRA), Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium
* Correspondence: liuguohua@caas.cn; Tel.: +86-10-8210-7727
† These authors contributed equally to this work.

Abstract: Two experiments were conducted to test the hypothesis that the apparent metabolizable energy (AME) and standardized ileal digestibility (SID) of amino acids (AA) in fermented soybean meal (FSBM) are greater than those in soybean meal (SBM). FSBM was produced by fermenting SBM with a mixture of *Bacillus amyloliquefaciens*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*. The fermentation process decreased trypsin inhibitor and crude fiber levels by 67.80% and 7.56%, while it increased the total amino acid content by 2.56%. In the first experiment, a substitution method was used to determine the AME and nitrogen-corrected AME (AMEn) of SBM and FSBM. A corn-SBM basal diet and two test diets consisting of 70% of the basal diet plus 30% SBM or FSBM were formulated. The results show that fermentation did not have an effect on the AME and AMEn concentrations of SBM ($p > 0.05$); the respective AME and AMEn values were 10.29 and 10.62 MJ/kg (DM basis) and 9.09 and 9.23 MJ/kg for SBM and FSBM. In the second experiment, a nitrogen-free diet was formulated to measure the endogenous AA flow, and the other two semi-purified diets containing SBM or FSBM as the sole source of AA were formulated. The results show that the AID and SID of isoleucine, leucine, phenylalanine, valine, cysteine, tyrosine, and aspartic acid were greater in FSBM than in SBM ($p < 0.05$). In conclusion, the fermentation of SBM by a mixture of *B. amyloliquefaciens*, *L. acidophilus*, and *S. cerevisiae* can improve its nutritional values and is a promising protein resource for broiler production.

Keywords: soybean meal; fermentation; metabolizable energy; standardized ileal digestibility of amino acids; broilers

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1. Introduction

Soybean meal (SBM), a remaining byproduct after oil extraction from soy seed, is the most widely used plant-derived protein source in the food and feed industries because of its relatively well-balanced amino acid profile, low price, and steady supply [1–3]. However, SBM contains a variety of anti-nutritional factors (ANFs), such as soybean antigenic proteins, non-starch polysaccharides, trypsin inhibitors, and other factors that can impede the digestion, absorption, and utilization of its nutrients and have detrimental effects on animal health, especially on young animals [4–6]. The antigenic proteins glycinin and β-conglycinin are known to directly affect intestinal epithelial permeability by inhibiting the proliferation and destroying the cytoskeleton of intestinal cells, and they elicit an inflammatory response via promoting the secretion of inflammatory cytokines [7,8]. Extensive studies have shown that solid-state microbial fermentation is an effective technique for improving the nutritional quality of SBM by decreasing the concentration of ANFs and increasing nutrient bioavailability [9–12]. According to Medeiros et al. [9], the soybean

macromolecular protein was broken down to less than 25 kDa, antigenicity against glycinin and β-conglycinin was completely eliminated, and raffinose and stachyose were totally hydrolyzed in SBM when SBM was fermented by *Bacillus amyloliquefaciens* for 48 h. Tsai et al. [12] found that the solid-state fermentation of SBM with *Bacillus velezensis* for 60 h degraded 97.54% of trypsin inhibitors and 63.76% of allergic protein and increased the concentration of trichloroacetic-acid-soluble protein (TCA-SP) from 4.21% to 15.17%.

Previous studies have indicated that the fermentation of SBM using fungi or bacteria changes the native composition and improves the nutritional value of SBM [13–16]. Yang et al. [17] indicated that the crude protein, TCA-SP, and total amino acid (AA) contents significantly increased by 12%, 452%, and 5% in fermented SBM (FSBM) produced by a combination of *B. subtilis*, *Lactobacillus casei*, and yeast, as compared with those in SBM. In addition, the contents of lysine, phenylalanine, valine, and methionine were increased by 9–42% after fermentation. Wu et al. [18] documented that the fermentation of SBM with *B. stearothermophilus* for 48 h significantly improved the crude protein, peptide, and ash contents, while it reduced the trypsin inhibitor and crude fat contents. The nutrient composition changes after fermentation, such as the increased crude protein and small peptide levels and the reduced oligosaccharide and trypsin inhibitor levels, were expected to contribute to an increased apparent metabolizable energy (AME) and ileal digestibility of AA and nitrogen in FSBM when fed to animals. It has been shown that the standardized ileal digestibility (SID) of the dry matter, nitrogen, and energy in FSBM is similar to that of fish meal [19]. The SID of crude protein (CP), arginine, methionine, alanine, and glycine was higher in FSBM fermented by *Aureobasidium pullulans* than in fish meal fed to 10 kg pigs, and no differences were observed in the SID of the other AA tested in this experiment [20]. Feeding trials in piglets revealed that 50% of fish meal in the diet can be replaced by FSBM without adverse effects on the growth performance and total tract digestibility of dry matter, nitrogen, and energy in weaned pigs [21]. It has, however, also been reported that there were no differences in the metabolizable energy and SID of CP and AA between SBM and FSBM when fed to pigs [22,23]. To date, few groups have studied the effects of FSBM on growth performance and nutrient digestibility in broilers. The partial or complete replacement of fish meal with FSBM produced by *Saccharomyces cerevisiae* did not impair the growth performance of broiler chickens and increased the calcium and phosphorus digestibility and absorption in broilers [24]. In contrast, feeding broilers with 5% FSBM manufactured by probiotics in combination with protease did not improve the utilization of dry matter, crude protein, and gross energy (GE) [4]. Thus, to gain more insight into how broilers respond to dietary FSBM fermented by various kinds of microorganisms and processing parameters, there is a need to further study the nutritional value of FSBM.

Currently, to the best of our knowledge, no research has been reported on the effects of fermentation on the AME, AID, and SID of FSBM in broiler chickens. We hypothesized that fermentation could improve the AME and SID of AA in SBM. Therefore, the objective of this study was to determine the nutritional value of FSBM and to provide data for formulating feed for broiler chickens.

2. Materials and Methods

2.1. Ethics Statement

All animal experiments were licensed by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences and conducted in accordance with the guidelines for animal experiments set out by the National Institute of Animal Health (statement no. AEC-CAAS-20191208).

2.2. Preparation of FSBM

Commercial SBM was obtained from Bunge Chia Tai Grain & Oil Co., Ltd. (Tianjin, China). FSBM was prepared by fermenting SBM with a mixture of *B. amyloliquefaciens*, *L. acidophilus*, and *S. cerevisiae*. The *B. amyloliquefaciens* strain was isolated from a healthy cow's rumen with a special screening plate, upon which the antigenic protein extracted

from SBM was the sole nitrogen source. The *L. acidophilus* strain was previously selected from silage. The above two strains have been deposited in the China General Microbiological Culture Collection Center (CGMCC) under accession numbers 18,230 and 14,437, respectively. The *S. cerevisiae* strain was preserved in our laboratory. The fermentation process was performed in accordance with the method reported by Shi et al. [25], with minor modifications. Briefly, each kilogram of SBM as the fermentation substrate was mixed and inoculated with 1 L of distilled water containing 6% (*v*/*v*) *B. amyloliquefaciens*, 2% (*v*/*v*) *L. acidophilus*, and 2% (*v*/*v*) *S. cerevisiae*, and aerobic fermentation was carried out at 37 °C for 24 h. After the first stage, the aerobically fermented mixture was transferred to a plastic bag equipped with a gas-pressure opening valve (Rou Duoduo Biotechnology Co., Beijing, China) and then fermented under anaerobic conditions at 37 °C for 24 h. After fermentation, the obtained FSBM was dried for 10 h at 60 °C.

2.3. Experiment 1: AME Measurements

For the first experiment, newly hatched 1-day-old Cobb 500 male broilers with an initial body weight of 43.2 ± 2.4 g were obtained from a local commercial hatchery (Beijing Dafa Chia Tai Co., Ltd., Beijing, China) to determine the AME values of SBM and FSBM. The broilers were reared together in the Nankou experimental base of the Chinese Academy of Agricultural Science (CAAS, Beijing, China) and fed a corn-SBM starter basal diet until day 20. All birds were housed in wire cages and raised in an environmentally controlled room with 16 h of light and 8 h of darkness, with free access to purified water via nipple drinkers and feed in pellet form. The room temperature was initially set at 33 °C for the first 3 days and then gradually brought down according to the age of the birds until 23 °C at 21 days. On day 21, after 6 h of fasting, all birds were weighed, and 72 healthy broilers of uniform body weight were selected and randomly assigned to three experimental groups consisting of six replicate cages with four birds per cage. The AME values of SBM and FSBM were determined using a substitution method, as described by Ravindran et al. [2] and Azam et al. [26]. According to this method, a corn-SBM basal diet was formulated as the reference diet based on the nutritional requirement for broilers determined by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (NY/T33—2004). The two test diets were prepared to contain 30% (*w*/*w*) SBM or FSBM at the expense of energy-yielding ingredients in the reference diet. The composition and nutrient levels of the basal and test diets are presented in Table 1.

Table 1. Ingredient composition and nutrient levels of the basal diet and test diets (air-dry basis, %).

Items	Basal Diet	Test Diets	
		SBM	FSBM
Ingredient			
Corn	59.21	40.68	40.68
Soybean meal	28.99	19.91	19.91
Fermented soybean meal	0	30.00	30.00
Corn gluten meal	3.35	2.30	2.30
Soybean oil	4.28	2.94	2.94
Dicalcium phosphate	1.85	1.85	1.85
DL-Methionine	0.14	0.14	0.14
L-Lysine	0.13	0.13	0.13
Limestone	1.05	1.05	1.05
Salt	0.28	0.28	0.28
50% Choline Chloride	0.10	0.10	0.10
Premix [1]	0.22	0.22	0.22
TiO_2	0.40	0.40	0.40
Total	100.00	100.00	100.00

Table 1. *Cont.*

Items	Basal Diet	Test Diets	
		SBM	FSBM
Nutrient level [2]			
AME (MJ/kg)	12.96	11.95	11.95
Crude protein, %	20.00	27.73	29.86
Calcium, %	0.90	0.87	0.87
Available phosphorous, %	0.40	0.38	0.38
Lysine, %	1.00	1.53	1.53
Methionine, %	0.40	0.51	0.51

[1] The premix provided the following quantities of vitamins and microminerals per kg of complete feed: vitamin A, 12,000 IU; vitamin D_3, 2000 IU; vitamin E, 20 IU; vitamin K_3, 2.5 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_3, 2 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.025 mg; D-pantothenic acid, 12 mg; biotin, 0.12 mg; folic acid, 1.25 mg; nicotinic acid, 50 mg; 80 mg Fe ($FeSO_4 \cdot H_2O$); 8 mg Cu ($CuSO_4 \cdot 5H_2O$); 0.15 mg Se (Na_2SeO_3); 100 mg Mn ($MnSO_4 \cdot H_2O$); 78 mg Zn ($ZnSO_4$); 0.34 mg (KI). [2] Crude protein is the analyzed value, and Ca, AP, Lys, and Met are the calculated values.

The AME was measured using the classical total excreta collection method. The birds were provided experimental diets at 22 days, followed by a 4-day adaptation period. From 26 to 30 days, the total excreta were collected daily (free from feed and feather contamination), weighed, pooled within a cage, and stored at −20 °C. The collected excreta were pooled and thoroughly mixed for each cage over 4 days and then oven-dried. The dried excreta were ground, passed through a 60-mesh screen, and stored at 4 °C before laboratory analysis. The average daily feed intake was monitored for each treatment.

2.4. Experiment 2: Ileal Digestibility Measurements

For the second experiment, 120 1-day-old Cobb 500 male broilers were purchased from a local commercial hatchery (Beijing Dafa Chia Tai Co., Ltd., Beijing, China) to measure the ileal digestibility of the AA in SBM and FSBM. All broilers were raised together and received a corn-SBM basal diet from 1 to 22 days. On day 22, after 6 h of fasting, all birds were weighed, and 108 healthy broilers with a similar average body weight were selected and randomly allocated to three experimental groups consisting of six replicate cages with six birds per cage. A nitrogen-free diet (NFD) was formulated to measure the endogenous AA flow, and the other two semi-purified diets were formulated to contain 20% crude protein, resulting in inclusion levels of 42.62% and 36.86% for SBM and FSBM, respectively. All experimental diets were balanced in terms of phosphorus and calcium and supplemented with equal contents of mineral and vitamin premix. Each diet contained 0.4% titanium dioxide (TiO_2) as an exogenous digestible marker. The composition and nutrient levels of the NFD and experimental diets are shown in Table 2. The broilers' feeding conditions were consistent with those in Experiment 1.

Table 2. Ingredient composition and nutrient levels of the nitrogen-free, soybean meal, and fermented soybean meal diets (air-dry basis, %).

Items	NFD	SBM	FSBM
Soybean meal	0	42.62	0
Fermented soybean meal	0	0	36.86
Corn starch	68.10	40.18	45.94
Sucrose	19.98	10.00	10.00
Crystallitic cellulose	5.00	0	0
Soybean oil	3.00	3.00	3.00
Dicalcium phosphate	1.90	1.90	1.90
Limestone	1.00	1.00	1.00
Salt	0.30	0.30	0.30
50% Choline	0.10	0.10	0.10
TiO_2	0.40	0.40	0.40

Table 2. *Cont.*

Items	NFD	SBM	FSBM
Vitamin Premix [1]	0.02	0.02	0.02
Mineral Premix	0.20	0.20	0.20
Zeolite Powder	0	0.28	0.28
Total	100.00	100.00	100.00
Nutrients levels [2]			
ME (MJ/kg)	13.31	12.32	12.50
Crude protein, %	0.20	19.89	19.92
Calcium, %	0.80	0.89	0.89
Available phosphorous, %	0.35	0.39	0.38
Lysine, %	0.01	1.30	1.19
Methionine, %	0.01	0.23	0.25

[1] The premix provided the following quantities of vitamins and microminerals per kg of complete feed: vitamin A, 12,000 IU; vitamin D_3, 2000 IU; vitamin E, 20 IU; vitamin K_3, 2.5 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_3, 2 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.025 mg; D-pantothenic acid, 12 mg; biotin, 0.12 mg; folic acid, 1.25 mg; nicotinic acid, 50 mg; 80 mg Fe ($FeSO_4 \cdot H_2O$); 8 mg Cu ($CuSO_4 \cdot 5H_2O$); 0.15 mg Se (Na_2SeO_3); 100 mg Mn ($MnSO_4 \cdot H_2O$); 78 mg Zn ($ZnSO_4$); 0.34 mg (KI). [2] Crude protein, Lys, and Met are analyzed values, and Ca and AP are calculated values.

The birds received experimental diets at day 22. Following a 3-day acclimation period, on day 26, all birds were euthanized using sodium pentobarbitone and were immediately dissected. The ileum contents from the Meckel's diverticulum to approximately 50 mm to a point to the ileo-cecal junction were collected by gently flushing with distilled water into a plastic culture plate. The ileal samples from the birds in a replicate cage were pooled, immediately frozen at −20 °C, and subsequently placed in a lyophilizer (LGL-10D, Beijing Sihuan Scientific Instrument Factory Co., Ltd., Beijing, China). After freeze-drying, the digesta samples were ground through a 60-mesh sieve and stored in plastic tubes at −4 °C until chemical analysis.

2.5. Chemical Analysis

The dry matter contents of SBM, FSBM, excreta, and diets were determined according to AOAC International (2006) [27]. The crude fiber contents in SBM and FSBM were analyzed using method 978.10 of AOAC International (2006) [27]. The total nitrogen content was measured by a combustion analyzer (Dumatherm, Gerhardt, Germany), using ethylenediaminetetraacetic acid as the calibration standard, with the crude protein being calculated as N × 6.25. The GE of the feed and excreta was determined in a bomb calorimeter (C2000, IKA, Guangzhou, China) standardized with benzoic acid. The AA profile of the samples was determined by an automatic amino acid analyzer (Hitachi L-8900, Tokyo, Japan) after hydrolyzing the samples with 6 M HCl for 24 h at 110 °C, and methionine and cysteine were oxidized with cold performic acid before acid hydrolysis. The TCA-SP content in SBM or FSBM was measured as described by our previous research. The concentrations of two allergic proteins, glycinin and β-conglycinin, and trypsin inhibitors in SBM and FSBM were measured using commercial competitive enzyme-linked immunosorbent assay (ELISA) kits (Longkefangzhou Bio-Engineering Technology Company, Beijing, China). The TiO_2 concentrations were estimated according to the method of Tigemeyer et al. [28]. All analyses were conducted in triplicate, and the average values were used for further statistical analysis.

2.6. Scanning Electron Microscopy of SBM and FSBM

The surface morphology of SBM and FSBM was observed with a field-emission scanning electron microscope (ZEISS Merlin, Oberkochen, Germany) at an acceleration voltage of 15 kV. Before the scanning electron microscope was used, SBM and FSBM power were coated with gold using an ion coater.

2.7. Calculations

The AME values of SBM and FSBM were calculated using the following formulas:

$$AME_{diet}\ (MJ/kg) = ((\text{feed intake} \times GE_{diet}) - (\text{excreta output} \times GE_{excreta}))/\text{feed intake} \quad (1)$$

$$AME_{SBM\ or\ FSBM}\ (MJ/kg) = ((\text{AME of test diet} - (\text{AME of basal diet} \times 0.70))/0.3 \quad (2)$$

$$AMEn\ (MJ/kg) = AME_{SBM\ or\ FSBM} - 8.22 \times ((\text{feed intake} \times N_{diet}) - (\text{excreta output} \times N_{excreta}))/\text{feed intake} \quad (3)$$

where GE is the gross energy and N is the nitrogen content. AMEn is the apparent metabolizable energy of diets corrected by N, and 8.22 is the nitrogen correction factor for broilers.

The AID values of SBM and FSBM were calculated as follows:

$$AID\ (\%) = 1 - (TiO_2 \text{ in diet}/TiO_2 \text{ in ileal digesta}) \times (\text{amino acid in ileal digesta}/\text{amino acid in diet}) \quad (4)$$

The AID values were converted to SID values using the basal endogenous ileal amino acid loss values obtained from the birds fed the NFD diet:

$$\text{ileal amino acid content (mg/kg, DM intake)} = (\text{amino acid in ileal digesta}) \times (TiO_2 \text{ in diet}/TiO_2 \text{ in ileal digesta}) \quad (5)$$

$$SID\ (\%) = AID\ (\%) + (\text{ileal amino acid content}/\text{amino acid in raw material}) \times 100\% \quad (6)$$

2.8. Statistical Analysis

All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The data for the chemical composition of SBM and FSBM, the AME measurements, and the ileal digestibility measurements were analyzed using Student's *t*-test. Differences between the means were considered significant at $p < 0.05$.

3. Results

3.1. Effects of Fermentation on the Chemical Composition and Total Amino Acids of SBM

Table 3 shows the chemical composition and total amino acids of SBM and FSBM. The trypsin inhibitor and crude fiber contents in FSBM were significantly decreased by 67.80% and 7.56%, respectively, compared with SBM. An increase of 2.56% in the total amino acid content was found in FSBM compared with SBM. Among the essential amino acids, the methionine, isoleucine, leucine, phenylalanine, and valine contents in FSBM were significantly increased by 17.47%, 5.04%, 5.42%, 11.03%, and 9.05%, respectively. Among non-essential amino acids, the glycine, alanine, tyrosine, proline, and glutamic acid contents were increased by 5.01%, 13.64%, 5.52%, 11.47%, and 12.98%, respectively, as compared to those in SBM. However, the arginine, threonine, cysteine, and serine contents were lower in FSBM.

Table 3. The crude protein, TCA-SP, antigenic protein, and amino acid contents of SBM and FSBM (air-dry basis, %).

Items	SBM	FSBM	Change, %
Trypsion inhibitors, mg/g	25.87	8.33	−67.80
Crude fiber, %	6.22	5.75	−7.56
	Essential amino acid		
Arg	3.65	2.85	−21.73
His	1.31	1.33	1.73
Lys	3.06	3.12	1.80
Met	0.47	0.55	17.47
Thr	2.08	1.94	−6.79
Ile	2.38	2.50	5.04
Leu	4.03	4.25	5.42
Phe	2.55	2.83	11.03
Val	2.68	2.92	9.05

Table 3. *Cont.*

Items	SBM	FSBM	Change, %
Non-essential amino acids			
Cys	0.89	0.56	−37.76
Gly	2.21	2.32	5.01
Ala	2.26	2.57	13.64
Tyr	1.63	1.72	5.52
Asp	5.99	6.05	0.97
Ser	2.75	2.14	−22.04
Pro	2.54	2.84	11.47
Glu	9.92	11.21	12.98
EAA	22.20	22.28	0.39
NEAA	28.20	29.40	4.27
Total	50.39	51.68	2.56

3.2. Microscopic Observation

Scanning electron microscopy (SEM) was carried out to assess the physical structures of SBM and FSBM. Figure 1 shows the morphological features of SBM and FSBM at magnification factors of 400× and 3000×. It can be seen that FSBM had fragmental, cracked, and plurilocular structures, whereas SBM had relatively large, compact, and smooth structures.

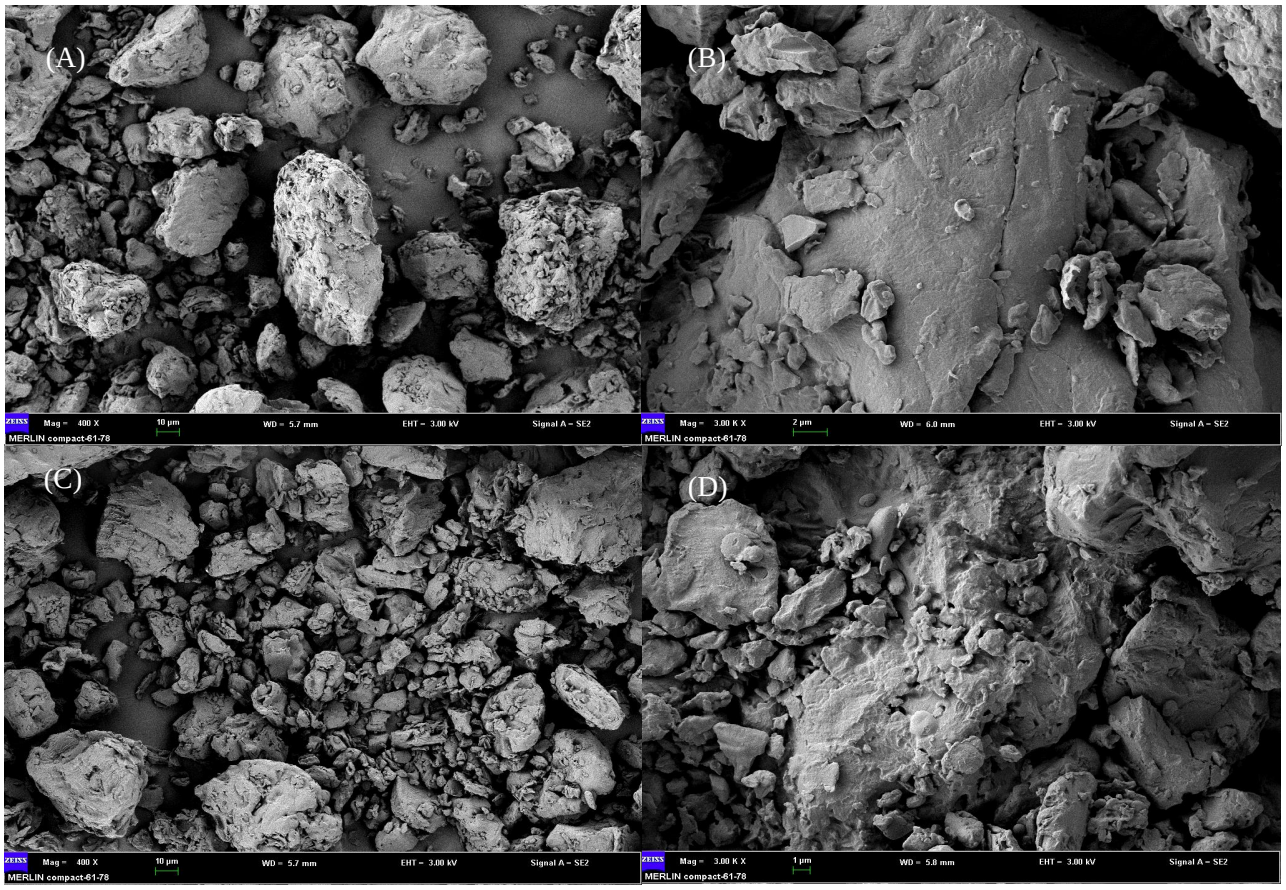

Figure 1. Scanning electron microscopy images of SBM and FSBM: (**A**) unfermented SBM (400×), (**B**) unfermented SBM (3000×), (**C**) FSBM (400×), (**D**) FSBM (3000×).

3.3. Apparent Metabolizable Energy of SBM and FSBM

The data for the AME and AMEn of SBM and FSBM are presented in Table 4. No significant differences in the AME and AMEn values were observed between SBM and FSBM, but FSBM had greater AME and AMEn values than SBM ($p > 0.05$).

Table 4. Metabolizable and nitrogen-corrected metabolizable energy of SBM and FSBM (dry matter basis, MJ/kg).

Items	SBM	FSBM	SEM	*p*-Value
AME	10.29	10.62	0.17	0.12
AMEn	9.09	9.23	0.13	0.29
AME/AMEn	0.88	0.87	-	-

3.4. Ileal Digestibility of Amino Acids in SBM and FSBM

In experiment 2, the NFD was formulated to measure ileal endogenous amino acid losses. Table 5 shows the results of the endogenous amino acid losses of the broilers fed the NFD. The ileal digestibility values of AA in SBM and FSBM are shown in Table 6. For essential AA, the AID and SID of isoleucine, leucine, phenylalanine, and valine were greater in FSBM than in SBM ($p < 0.05$). There was a tendency toward increased AID and SID values of methionine ($p = 0.09$) and arginine ($p = 0.07$). However, no significant differences in the AID and SID values of lysine, threonine, and histidine were observed. For non-essential AA, the AID and SID values of cysteine, tyrosine, and aspartic acid were higher in FSBM than in SBM ($p < 0.05$), but for the remaining non-essential AA, no significant differences in the AID and SID values were observed between SBM and FSBM.

Table 5. Concentration of the ileal endogenous amino acid losses of the broilers fed a nitrogen-free diet.

Amino Acid	Ileal Endogenous Amino Acid Concentration (mg/kg, Dry Matter Intake)
Essential amino acids	
Arg	153.80
His	80.64
Lys	157.07
Met	65.45
Thr	315.31
Ile	156.47
Leu	248.29
Phe	144.80
Val	235.77
Non-essential amino acids	
Cys	80.85
Gly	219.51
Ala	177.46
Tyr	154.69
Asp	401.29
Ser	304.23
Pro	253.66
Glu	459.58

Table 6. Apparent ileal digestibility and standardized ileal digestibility values of amino acids in SBM and FSBM (dry matter basis, %).

Items	AID, %				SID, %			
	SBM	FSBM	SEM	*p*-Value	SBM	FSBM	SEM	*p*-Value
Essential amino acids								
Arg	85.44	87.55	1.02	0.07	86.41	88.52	1.02	0.07
His	82.58	83.59	1.10	0.38	84.70	85.01	0.92	0.75
Lys	84.13	85.12	0.98	0.35	85.96	86.31	0.92	0.71
Met	85.92	87.94	1.08	0.09	88.13	90.15	1.12	0.09
Thr	75.23	77.17	1.38	0.19	78.27	80.75	1.39	0.11
Ile	80.78	85.17	1.18	0.04	83.10	86.74	1.04	<0.01
Leu	81.31	84.71	1.12	<0.01	83.51	86.15	0.96	0.02
Phe	82.93	87.31	0.94	<0.01	84.19	88.58	0.94	<0.01
Val	79.29	83.58	1.20	<0.01	82.40	85.90	1.01	<0.01
Non-essential amino acids								
Cys	67.84	72.15	1.85	0.04	71.27	75.58	1.89	0.04
Gly	75.16	77.52	1.31	0.10	77.46	79.82	1.31	0.13
Ala	81.06	82.55	1.08	0.20	83.55	84.35	0.91	0.41
Tyr	80.72	83.64	1.22	0.03	83.61	85.73	0.99	0.06
Asp	76.09	79.67	1.36	0.03	77.65	81.23	1.36	0.02
Ser	78.51	77.52	1.30	0.47	81.11	80.13	1.30	0.47
Pro	78.09	80.44	1.14	0.13	80.36	82.70	1.41	0.13
Glu	81.84	80.58	1.31	0.36	82.33	81.66	1.26	0.61

4. Discussion

SBM is by far the main plant protein source applied in livestock production in China. In a typical corn-SBM broilers diet, SBM protein comprises about 70% of the dietary CP [2]. The presence of several ANFs in SBM (for instance, soybean antigenic protein, trypsin inhibitors, and soy oligosaccharides), however, can reduce the growth performance of broiler chickens if not decomposed or deactivated [4,5]. Fortunately, previous studies have demonstrated that microbial fermentation is an effective and cheap method for reducing the levels of ANFs and improving the nutritional characteristics of SBM [5,6,11]. A wide variety of microorganisms, predominantly *L. plantarum*, *B. subtilis*, *Aspergillus oryzae*, and *S. cerevisiae*, have been used for the solid-state fermentation of SBM [5,29]. In previous results of our work, the fermentation process by a combination of bacterial and fungal species effectively increased the CP concentration from 46.64% to 53.74% [30]. Meanwhile, the crude fiber content significantly decreased from 6.22% to 5.75%. Similarly, Yang et al. [17] reported that the CP in FSBM increased significantly from 48.12% to 53.87% after two-stage fermentation with a mixture of *L. casei*, *B. subtilis*, and yeast. Fermentation with *B. amyloliquefaciens* isolated from grass carp was found to increase the CP content by 8.27% [9]. The increase in CP is mainly associated with the decrease in sucrose and oligosaccharide levels in SBM during fermentation. Microbial decomposition and synthesis by *Bacillus* spp. hardly improve the total nitrogen content, but they can break down cell wall polymers, polysaccharides, and oligosaccharides and utilize these as energy sources for metabolism and proliferation [31]. Notably, the content of TCA-SP, which consists of small peptides with 2–20 amino acid residues, was approximately 10 times higher in FSBM than in SBM. An increase in TCA-SP levels is mainly due to the degradation of macromolecules to form small molecular peptides. A previous study elucidated that dipeptides and tripeptides can be directly absorbed by animal intestinal epithelial cells, and amino acids in the form of small peptides are transported faster than their constituent amino acids in free form [32]. In our previous study, FSBM produced by single *B. amyloliquefaciens* exhibited higher in vitro digestible energy values compared with unfermented SBM [33], which further indicates that SBM can be easily digested by digestive enzymes from microorganisms.

Our results of this work demonstrate that 90.53% of glycinin and 84.19% of β-conglycinin were decomposed after two-stage fermentation [30]. Chen et al. [11] demonstrated that *B. velezensis* and *L. plantarum* hydrolyzed 78.60% of glycinin and 72.89% of β-conglycinin, respectively, by 24 h of aerobic fermentation and 72 h of anaerobic fermentation. By contrast, the inoculation of SBM with a mixture of *B. subtilis*, *L. plantarum*, *L. acidophilus*, and *A. oryzae* only eliminated 59.60% of glycinin and 58.43% of β-conglycinin [5]. These varying results might be attributable to the different types of microorganisms applied for fermentation and the various techniques used to process FSBM [30]. In our recent proteomics study, we found that *B. amyloliquefaciens* secretes many kinds of extracellular hydrolytic enzymes during the fermentation of SBM, including not only aminopeptidases but also cellulose-degrading enzymes, hemicellulose-degrading enzymes, lignocellulolytic enzymes, superoxide dismutases, catalases, and phytase, which break down cell wall polymers and facilitate the decomposition of the antigenic protein (unpublished data). In addition, in the current study, it was observed by SEM that the surface structure of FSBM showed a rough, irregular, and porous structure compared with SBM after fermentation. This revealed that the lignocellulose component on the surface of SBM may be disintegrated, and such changes in the surface structure in SBM may be driven by extracellular hydrolytic enzymes secreted during solid-state fermentation.

Amino acid levels and metabolizable energy are two important parameters to be considered when precisely formulating broiler chicken diets, especially with protein sources such as SBM [34]. In the current experiment, the determined AME and AMEn values were 10.29 and 9.09 MJ/kg (DM basis) for SBM, respectively, which is in agreement with the previously published literature. The AME value of SBM for broilers reported by the Feed Composition and Nutritive Values (2020) in China is 10.58 MJ/kg. Ravindran et al. [2] determined the AME values of SBM from Brazil, Argentina, the United States, and India, which ranged from 6.55 to 10.63 MJ/kg for broilers (as-received basis), and SBM from the United States had the highest average AME values. The AME and AMEn values were 10.62 and 9.23 MJ/kg for FSBM (DM basis) in our study, respectively. We observed no significant differences in AME and AMEn values between SBM and FSBM. The substitution method was carried out to measure the AME of test feedstuffs. When 300 g/kg SBM or FSBM was incorporated into a corn-SBM basal diet, dramatically higher dietary CP levels were observed in the FSBM diet compared to the SBM diet. Nieot et al. [35] demonstrated that CP levels of diets of up to 23% can be well digested and absorbed without any negative effects on the metabolizable energy; however, higher CP levels lead to the excessive excretion of nitrogen, with significant energy consumption. Clearly, energy excretion is greater for higher-CP diets than for the common CP level of 20–23%, which resulted in a lower energy availability and, consequently, lower AME values. Therefore, this partly explained the lack of a difference in the AME and AMEn values between FSBM and SBM in our work. Moreover, the content of oligosaccharide has an effect on AME and AMEn determination. Perryman et al. [36] suggested that SBM with low raffinose and stachyose concentrations had greater AME values compared with conventional SBM because this small molecule cannot be hydrolyzed and utilized by broilers due to a lack of endogenous α-1,6 galactosidase.

To the best of our knowledge, the present study on broilers is the first to report AID and SID for FSBM produced by a mixture of *B. amyloliquefaciens*, *L. acidophilus*, and *S. cerevisiae*. In the current experiment, an NFD method was used to correct the endogenous losses of AA, and the results of the endogenous losses of AA were consistent with the results of Wu et al. [37] and Golian et al. [38] but were lower than the results of Barua et al. [39]. The NFD has been generally formulated with corn starch, dextrose, or sucrose (approximately 85% of NFD) as major energy resources. The corn starch is a polysaccharide consisting of a large amount of glucose interconnected by an α-1,4 and α-1,6 linkage; they would be hydrolyzed into small molecules by several enzymes before absorption [40]. However, the sucrouse can be rapidly digested and absorped with sucrase assimilation in the brush border of the small intestine. Hence, the lower concentrations of corn starch (84.2% vs. 68.1%) and the

higher concentrations of sucrose (19.98% vs. 0%) in the NFD in the present study compared to those of the report by Barua et al. [39] may account for the lower endogenous losses of AA. The lowest AID value of an essential AA in SBM is that of threonine, and the highest is that of methionine (75.23% vs. 85.92%, respectively). In general, the AID values of SBM measured for broilers in our work are within the range in the published literature [2,36,41]. Coca-Sinova et al. [41] investigated the AID values of six batches of SBM imported from Brazil and Argentina and reported AID values from 66.40% to 76.00% for threonine and from 81.90% to 88.80% for methionine. In the present study, the AID and SID values of all the amino acids in the broilers fed an FSBM diet were higher than those in the broilers fed an SBM diet, except for glutamic acid and serine, which indicates that fermentation improves the digestibility of AA. The present findings generally agree with those of Zhang et al. [42], who documented that the AID and SID values of most essential AA, such as lysine, leucine, isoleucine, and histidine, were elevated for weaning piglets fed an FSBM diet compared with piglets fed an SBM diet. Hossain et al. [43] showed that the AID and SID values of all AA in pigs fed an FSBM with 70% solubility potassium hydroxide were greater than those in pigs fed an SBM diet. However, according to Cervantes-Pahm and Stein [23], the AID and SID values of AA in FSBM were not different from those in SBM, except for lysine. The authors further explained that the concentrations of antigenic protein and oligosaccharides in FSBM were not different from the concentrations in SBM, which might explain the lack of a difference in the digestibility of AA between FSBM and SBM. Purified glycinin and β-conglycinin could damage the intestinal mucosa, increase the intestinal permeability, and decrease the nitrogen digestibility in animals [8]. Hence, the reduction in the concentrations of antigenic proteins and trypsin inhibitors in FSBM was expected to contribute to the increased digestibility of AA in FSBM when fed to broiler chickens. Increased TCA-SP concentrations may be another reason accounting for the improved digestibility of AA in FSBM. The absorption and transportation of small peptides are faster than those of free AA [32]. Furthermore, the microorganism species and strains used for fermentation affect the hydrolysis degree of ANFs and the nutritional values of SBM. Kim et al. [44] reported that the AID and SID values of AA in FSBM produced by *B. subtilis* pp6 were higher than those in FSBM fermented by *A. oryzae* GB-107. Therefore, it is necessary to establish an AID and SID database of FSBM produced by different microorganisms and various production processes and to further provide basic data for the application of FSBM in livestock production.

5. Conclusions

In conclusion, fermentation changed the chemical composition of SBM. It resulted in improved crude protein and acid-soluble protein contents, an increase in most amino acid concentrations, and a significant decrease in the glycinin and β-conglycinin levels in SBM. Our results also show that fermentation improved the AID and SID values of some amino acids in SBM but did not affect the energy value.

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