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

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Abstract: Peanut meal (PNM) is a byproduct of the peanut oil extraction process, but its application is seriously limited by the presence of anti-nutritional factors, imbalance in amino acid profiles, and susceptibility to mycotoxin contamination. This study was conducted to investigate the effects of solid-state fermentation on the nutritional quality of PNM, as well as the effects of PNM and fermented peanut meal (FPNM) on the ileal digestibility of amino acids and apparent metabolizable energy (AME) of broiler chickens. The results indicated that the fermentation improved the quality of PNM by increasing the crude protein, TCA-soluble protein, and L-lactic acid concentration ($p < 0.05$), and decreasing the crude fiber, phytic acid, and aflatoxin B_1 concentration ($p < 0.05$). Solid-state fermentation also increased the free amino acids level and improved the balance of hydrolyzed amino acids of PNM. A nitrogen-free diet was used to determine the loss of endogenous amino acid in birds, and the PNM or FPNM as the only protein source to formulate semi-purified diets. The result showed that feeding on FPNM resulted in higher apparent ileal digestibility (AID) and standardized ileal digestibility (SID) values of the essential amino acids of methionine, lysine, leucine, and phenylalanine ($p < 0.05$). Moreover, the AID and SID values of the non-essential amino acids of FPNM were both higher than those of PNM, except for proline ($p < 0.05$). The AME was determined by the classic substitution method, and the results showed that fermentation had no effect on the AME value ($p > 0.05$). In conclusion, solid-state fermentation improved the nutritional value of PNM, and FPNM was a potential ingredient as an alternative protein source for broilers.

Keywords: fermented peanut meal; nutritive value; ileal digestibility of amino acids; metabolizable energy; protein source; broilers

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1. Introduction

Corn and soybean meal are the traditional component of poultry diets used to meet energy and protein requirements; however, the price continues to rise due to an imbalance between demand and production. Nutritional expenditure determines 50–70% of the cost of poultry production; an increase in the cost of feedstuff would lead to an increase in production costs, driving more public focus on cost-effective protein feedstuff [1]. Studies have shown that many locally available ingredients can be used as soybean meal substitutes to meet the nutritional requirements of poultry, such as sunflower meal, rapeseed meal and cottonseed meal, which are commonly used [2–4]. Peanut meal (PNM) is a byproduct of

peanut oil production by high temperature pressing, which is also a promising plant protein. It has a powdery or blocky morphology, with a strong peanut flavor that is comprised of 40.1–50.9% protein, 0.7–6.0% fat, 5.8–12.6% crude fiber, and also rich in vitamins, minerals, and antioxidant components (polyphenols, flavone). The nitrogen-corrected metabolic energy of broiler chickens is 9.6–12.6 MJ/kg [5]. Although PNM can be fed to poultry directly, the imbalance in amino acid profiles limits its use in poultry feed, such as lower levels of lysine, threonine, and methionine and an excessively high arginine/lysine ratio (3.49), which is much higher than the National Research Council Nutrient Requirements of Poultry recommended ratio (1.10–1.18) [6]. Studies have shown that excessive arginine may have negative effects on animal health and productivity [7]. Additionally, phytic acid is the main anti-nutritional factor with a content of 1.50–2.00% in PNM, and it not only binds to metal ions, but connects to amino acid residues, which affects the absorption of nutrients by the organism [8]. *Aspergillus flavus* and *Aspergillus parasiticus* infections cause aflatoxin contamination in peanuts, which also limits the application of PNM in feed [9].

Solid-state fermentation is the growth of microorganisms on solid materials under controlled conditions, which can also change the chemical or physical-chemical properties of the substrate [10]. Recent studies have shown that solid-state fermentation of plant protein raw materials can improve nutritional properties. For instance, the contents of harmful compounds such as glucosinolates, non-starch polysaccharides, and phytates in rapeseed meal can be reduced by fermentation, and the indispensable nutrients are not lost in the process [11,12]. Jazi et al. [13] also revealed crude fiber and free cotton phenol in cottonseed meal could be degraded by fermentation. Shi et al. [14] reported that solid-state fermentation of corn-soybean meal mixed diets reduced the concentration of anti-nutritional factors β-soybean globulin and phytic acid while increasing the content of soluble protein. Li et al. [15] reported the total amino acids content of fermented soybean meal increased by 2.56%, crude fiber and trypsin inhibitor decreased by 7.56% and 67.80%, respectively, and the standardized ileal digestibility (SID) of amino acids of broiler chickens increased significantly. Regarding PNM, previous studies found that solid-state fermentation improved the nutrient composition, and also degraded aflatoxin B_1 (AFB_1) [16,17]. Specifically, we hope to tackle the problem of imbalance in amino acid and anti-nutritional factors in PNM via solid-state fermentation, which requires the fermentation bacteria to have the capacity to hydrolyze proteins and depolymerize phytates. In our laboratory's previous work, *Bacillus velezensis* LB-Y-1 and *Pediococcus acidilactici* LC-9-1 were screened out, and they have potential in this regard [18].

As far as we know, few investigations have been performed on the nutritional value evaluation of FPNM in broiler chickens. In order to better understand the potential application of FPNM in broiler diets, it is necessary to comprehensively evaluate the nutritional value of it. Therefore, the purpose of this study is to analyze the changes of nutritional composition of PNM after solid-state fermentation. Additionally, we hypothesize that fermentation can improve the SID of amino acid and apparent metabolizable energy (AME) of PNM.

2. Materials and Methods

2.1. Ethics Statement

All experimental procedures were approved 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 (AEC-CAAS-20191106, Beijing, China).

2.2. Preparation of FPNM

The commercial PNM was purchased from Xunda Grain and Oil Co. LTD (Puyang, Henan, China). FPNM was prepared by two-stage solid-state fermentation of PNM using strains of *Bacillus velezensis* LB-Y-1 (3.0×10^8 CFU/mL) and *Pediococcus acidilactici* LC-9-1 (1.0×10^8 CFU/mL). The two strains were obtained by specific screening and preserved in

a China General Microbiological Culture Collection Center, numbered as CGMCC 2.1344 and CGMCC 2.1345, respectively. The fermentation was performed as described by Wang et al. [19] with minor modification as follows: (1) The pretreatment of raw material: the PNM was crushed and passed through a 0.88 mm sieve, and was sterilized at 121 °C for 15 min, and cooled down to room temperature (26 °C); (2) First-stage: the fermentation was performed by inoculating 6.0×10^9 CFU/kg PNM of the LB-Y-1 inoculum, and adding distilled water to make the moisture content reach 37.0%. Transferred the fully mixed PNM to the fermentation box (40 × 20 × 10 cm), with the depth of material of 5 cm and a sterile membrane was added to the fermentation box. The PNM were fermented for 54 h at 38 °C in quasi-aerobic conditions, remixed every 4 h; (3) Second-stage: the fermentation was performed by inoculating 2.0×10^9 CFU/kg PNM of the LC-9-1 inoculum, and adding distilled water to make the moisture content reach 40.0%. Mixed PNM were transferred into a polyethylene bag with a one-way valve (to permit carbon dioxide release during fermentation), and sealed immediately, fermented for 18 h at 37 °C in quasi-anaerobic conditions; (4) The fermented samples were dried at 48 °C after fermentation till the moisture content was 10%, and then the dried samples were ground to pass through a 0.88 mm sieve and stored at room temperature (26 °C).

2.3. Sample Preparation and Chemical Analysis

The analysis of routine nutrients followed the guidelines of the Association of Official Analytical Chemists (AOAC). Samples were dried at 105 °C for 5 h in a drying oven to determine the content of dry matter (DM) (method 934.01, AOAC 2006). The total nitrogen content was determined with a combustion analyzer (Dumatherm, Gerhardt, Germany). Crude protein (CP) was calculated as N × 6.25. The ash content was determined by placing samples in a temperature-controlled furnace preheated to 600 °C for 2 h (method 942.05, AOAC 2006). The content of crude fat (EE, method 920.39, AOAC 2006, without HCL hydrolysis), and crude fiber (CF, method 978.10, AOAC 2006) was also analyzed [20]. Gross energy (GE) was determined using a bomb calorimeter (Parr 6300 Calorimeter; Parr Instrument Company, Moline, IL, USA). The level of TiO_2 was determined following the report of Tigemeyer et al. [21]. The Trichloroacetic-acid-soluble protein (TCA-SP) was determined as described by Sriket et al. [22] and the Agricultural Industry Standard of the People's Republic of China (NY/T 3801-2020). Briefly, 5 g samples were placed into a 250 mL conical bottle, and added 100 mL trichloroacetic acid solution (15%, w/v) to dissolve and mixed evenly. The solution was filtered with medium speed qualitative filter paper, and the filtrate was transferred to a centrifuge tube after a 5 min standing. Then, the samples were centrifuged at 4000× g for 20 min at 4 °C, and, finally, the CP concentration was determined according to the method of AOAC. The TCA-SP concentration was calculated as the ratio of CP. The concentration of L-lactic acid was determined as that described by Tejero-Sariñena et al. [23]. The amino acids concentration was determined using an amino acid analyzer (Hitachi L-8800, Tokyo, Japan) after hydrolyzing the samples with 6 M HCl (containing phenol) for 24 h at 110 °C in glass tubes sealed under vacuum, and the cysteine and methionine were analyzed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 h at 0 °C and neutralization with hydrobromic acid prior to hydrolysis according to the method, as described by AOAC (method 994.12, AOAC 1990) [24]. The phytic acid and AFB_1 content of PNM and FPNM samples were measured using a commercial kit (K-PHYT, Megazyme, Wicklow, Ireland) and (Longkefangzhou Bio-Engineering Technology Company, Beijing, China), respectively. Three independent samples were measured in triplicate.

2.4. Scanning Electron Microscopy of PNM and FPNM

The microstructure of PNM and FPNM was analyzed using a field-emission scanning electron microscope (ZEISS Merlin, Oberkochen, Germany), and the samples were gilded in argon by ion sputter coater before observation (Technex, Tokyo, Japan).

2.5. Experiment 1: Determination of Ileal Digestibility of Amino Acids

2.5.1. The Birds Management and Sample Collection

The experiment was done at the Nankou Experimental Base of the Chinese Academy of Agricultural Sciences. A total of 120 newly hatched Arbor Acres (AA) male broilers (46.3 ± 0.6 g/bird) were purchased from a local commercial hatchery (Beijing Dafa Chia Tai Co. LTD, Beijing, China). During 1–21 d of age, all the birds were fed a corn-soybean meal diet, which were formulated with reference to the nutrient requirements recommended by the feeding standards of China (NY/T 2004) for broilers (1994). At 22 d of age, the birds were weighed after 6 h of fasting, and the birds of similar weight were selected and randomly divided into 3 groups of 6 replicate cages, with 6 chickens per cage (108 in total). The nitrogen-free diet was used to determine the loss of endogenous ileal amino acids in birds [25]. The semi-purified diets were formulated with PNM and FPNM based on the nitrogen-free diet, respectively; the PNM and FPNM was used as the only protein source to adjust the CP level. The diets were mixed with 0.4% (*w*/*w*) titanium dioxide (TiO_2) as an indicator. Table 1 showed the ingredient composition and nutrient concentration of the reference and experimental diets. According to the management guidelines of commercial AA broiler, all birds were allowed ad libitum purified water via nipple drinkers and feed in pellet form. During the first week, the room temperature was maintained at 33 °C and was gradually decreased, reaching 24 °C at 16 days of age. The lighting program was a period of 16 h of light and 8 h of darkness [26]. On 26 d of age, all birds were euthanized using the intravenous pentobarbital injection method and immediately dissected, and then ileal digesta was collected in a plastic culture dish. The samples from birds within a replicate cage were pooled together and then immediately stored at −20 °C [27]. The ileal digesta was freeze-dried at −50 °C, fully ground, and passed through a 0.5 mm screen and stored in airtight containers at −4 °C for amino acids analysis.

Table 1. Ingredient composition and nutrient concentration of the experimental diets used in the determination of the standardized ilea amino acid digestibility (dry matter basis) [(1)].

Component	NFD	Experiment Diet	
		PNM	FPNM
Ingredient composition, %			
Peanut meal	0	42.45	0
Fermented peanut meal	0	0	39.77
Corn starch	72.37	36.84	39.49
Sucrose	15.43	13.00	13.00
Crystallitic cellulose	5.00	0	0
Soybean oil	2.50	3.50	3.50
Limestone	1.27	1.21	1.21
$CaHPO_4$	2.13	1.71	1.73
NaCl	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10
TiO_2	0.40	0.40	0.40
Mineral premix [(2)]	0.50	0.50	0.50
Total	100.00	100.00	100.00
Nutrient concentrations [(3)]			
Metabolic energy (MJ/kg)	12.96	12.84	12.89
Crude protein	0.20	19.98	20.03
Calcium	0.90	0.90	0.90
Available P	0.35	0.35	0.35

[(1)] Abbreviations: NFD, nitrogen-free diet; PNM, peanut meal; FPNM, fermented peanut meal. [(2)] The premix provided the following per kilogram diet: vitamin A 10,000 IU, vitamin D_3 2000 IU, vitamin E 10 IU, vitamin K_3 2.5 mg, vitamin B_1 1 mg, vitamin B_2 6 mg, vitamin B_3 10 mg, vitamin B_5 40 mg, vitamin B_6 3 mg, vitamin B_{11} 0.3 mg, vitamin B_{12} 0.01 mg, biotin 0.12 mg, Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 60 mg, Zn (as zinc sulfate) 40 mg, Se (as sodium selenite) 0.15 mg, I (as potassium iodide) 0.35 mg. [(3)] Crude protein is the analyzed value (n = 3), and others are the calculated values.

2.5.2. Calculations

The apparent ileal digestibility (AID) values of PNM and FPNM were calculated as followed:

$$\text{AID (\%)} = 1 - (\text{TiO}_2 \text{ in diet}/\text{TiO}_2 \text{ in ileal digesta}) \times (\text{AA in ileal digesta}/\text{AA in diet}) \times 100\% \quad (1)$$

The endogenous ileal amino acids loss (IAA) values of PNM and FPNM were calculated as follows:

$$\text{IAA (mg/kg)} = \text{AA in ileal digesta} \times (\text{TiO}_2 \text{ in diet}/\text{TiO}_2 \text{ in ileal digesta}) \quad (2)$$

IAA is calculated based on the composition content in the nitrogen-free diet.

The standardized ileal digestibility (SID) values of PNM and FPNM were calculated as follows:

$$\text{SID (\%)} = \text{AID} + (\text{IAA}/\text{AA in diet}) \quad (3)$$

2.6. Experiment 2: Determination of Apparent Metabolizable Energy

2.6.1. The Birds Management and Sample Collection

The AME and apparent retention of gross energy (ARGE) were measured used the classical substitution method [28]. The experiment was also conducted at the Nankou Experimental Base of the Chinese Academy of Agricultural Sciences. A total of 80 newly hatched AA male broilers (45.7 ± 0.5 g/bird) were purchased from a local commercial hatchery. The management procedures and feeding for birds between 1–21 d of age was the same as in Experiment 1. On 22 d of age, the birds were weighed after 6 h of fasting, and birds with similar weight were selected and randomly divided into 3 groups of 6 replicate cages, with 4 chickens per cage (72 in total), and fed 3 experimental diets, which consisted of a corn-soybean meal diet, and the nutrients were formulated to meet the feeding standard of China (NY/T 2004) for broilers (Table 2). The other two diets included test ingredients, PNM or FPNM proportionally replaced 30% of the energy-yielding components of the basal diet. TiO_2 was added to all diets as an indigestible marker. All birds were allowed ad libitum feed and water during the experiment. The dietary adaption period was four days. During the last four days (26 to 30 d), feed intake of each group was monitored, and the excreta samples were collected (spilled feed and feathers were removed) quantitatively daily and pooled within a cage. The collected excreta samples were dried in a forced air drying oven at 65 °C for 72 h, fully ground, and passed through a 0.5 mm screen and stored in airtight containers at −4 °C for AME and ARGE measurement.

Table 2. Ingredient composition and nutrient concentration of the basal diet used in the determination of the apparent metabolizable energy (dry matter basis).

Component	Basel Diet	PNM Diet	FPNM Diet
Ingredient composition, %			
Corn	56.28	38.62	38.62
Soybean meal, (CP 43%)	30.38	20.85	20.85
PNM	0	30	0
FPNM	0	0	30
Corn gluten meal, (CP 60%)	3.97	2.72	2.72
Soybean oil	4.98	3.42	3.42
$CaHPO_4$	1.73	1.73	1.73
L-Lysine	0.06	0.06	0.06
DL-Methionine	0.12	0.12	0.12
Limestone	1.21	1.21	1.21
NaCl	0.27	0.27	0.27
Choline chloride, (50%)	0.10	0.10	0.10
TiO_2	0.40	0.40	0.40
Mineral premix [(1)]	0.50	0.50	0.50
Total	100.00	100.00	100.00

Table 2. *Cont.*

Component	Basel Diet	PNM Diet	FPNM Diet
Nutrient concentration [2]			
Metabolic energy, MJ/kg	12.96	12.06	12.06
Crude protein, %	20.08	27.85	28.80
Calcium, %	0.90	0.94	0.94
Available P, %	0.35	0.42	0.42
Lysine, %	1.00	1.21	1.28
Methionine + Cysteine, %	0.76	0.83	0.85

[1] The premix provided the following per kilogram diet: 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_5 6 mg, vitamin B_6 6 mg, vitamin B_{11} 0.3 mg, vitamin B_{12} 0.025 mg, nicotinic acid 50 mg, folic acid 1.25 mg, D-pantothenic acid 12 mg, biotin 0.12 mg, Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 100 mg, Zn (as zinc sulfate) 78 mg, Se (as sodium selenite) 0.15 mg, I (as potassium iodide) 0.35 mg. [2] Crude protein, lysine, methionine, and cysteine are the analyzed value (n = 3), and others are the calculated values.

2.6.2. Calculations

The apparent metabolizable energy (AME) values of PNM and FPNM were calculated as follows:

$$\text{IF} = \text{TiO}_2 \text{ in diet}/\text{TiO}_2 \text{ in excreta} \quad (4)$$

$$\text{AME}_{\text{diet}} \text{ (MJ/kg)} = \text{GE in diet} - (\text{GE in excreta} \times \text{IF}) \quad (5)$$

$$\text{AME}_{\text{PNM or FPNM}} \text{ (MJ/kg)} = ((\text{AME of experiment diet} - (\text{AME of control diet} \times 0.70))/0.30 \quad (6)$$

where IF is the indigestibility factor, and GE is the gross energy.

The apparent retention of gross energy (ARGE) values of PNM and FPNM were calculated as follows:

$$\text{ARGE}_{\text{PNM or FPNM}} \text{ (\%)} = \text{AME of the ingredient}/\text{GE of the ingredient} \times 100\% \quad (7)$$

2.7. Statistical Analysis

All experimental data were tested for normality by using the Shapiro-Wilk test of normality and Levene's test for homogeneity of variances. All datasets were distributed normally. Afterward, the data were analyzed by a one-factor ANOVA procedure of the SPSS19.0 software package for Windows (SPSS Inc., Chicago, IL, USA), and the indexes were expressed as means with standard error of mean (SEM), where $p < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Effects of Solid-State Fermentation on the Chemical Composition and Surface Microstructure of PNM and FPNM

The chemical compositions of PNM and FPNM are shown in Table 3. Fermentation of PNM significantly increased the content of CP, TCA-SP and L-lactic acid by 6.70%, 536.09%, and 439.39%, respectively ($p < 0.05$), and decreased the content of CF, phytic acid, and AFB_1 by 14.91%, 66.29%, and 43.68%, respectively ($p < 0.05$). The physical microstructure of PNM and FPNM were shown in Figure 1. The particles of PNM showed a relatively smooth surface. After fermentation, the large particles were broken down into smaller ones, which tightly pack together in irregular shapes. The surface of FPNM particles exhibits more micropores and cracks compared with that of PNM.

Table 3. Effects of solid-state fermentation on the chemical composition of PNM and FPNM (dry matter basis, $n = 3$) [1].

Parameter	PNM	FPNM	SEM	*p*-Value	Changes, %
DM, %	88.65	88.73	0.103	0.723	NS
CP, %	46.85 b	49.99 a	0.476	<0.001	6.70
EE, %	1.13	1.11	0.014	0.479	NS
CF, %	6.44 a	5.48 b	0.148	<0.001	−14.91
Ash, %	5.60	5.65	0.018	0.108	NS
TCA-SP, %	3.02 b	19.21 a	2.441	<0.001	536.09
L-lactic acid, %	0.66 b	3.56 a	0.438	<0.001	439.39
Phytic acid, %	1.75 a	0.59 b	0.175	<0.001	−66.29
AFB_1, μg/kg	38.37 a	21.61 b	2.570	<0.001	−43.68

[1] Abbreviations: PNM, peanut meal; FPNM, fermented peanut meal; DM, dry matter; CP, crude protein; EE, crude fat; CF, crude fiber; TCA-SP, Trichloroacetic-acid-soluble protein. Determined in triplicate. "NS" stands for no significant difference. Different letters refer to the significant differences ($p < 0.05$).

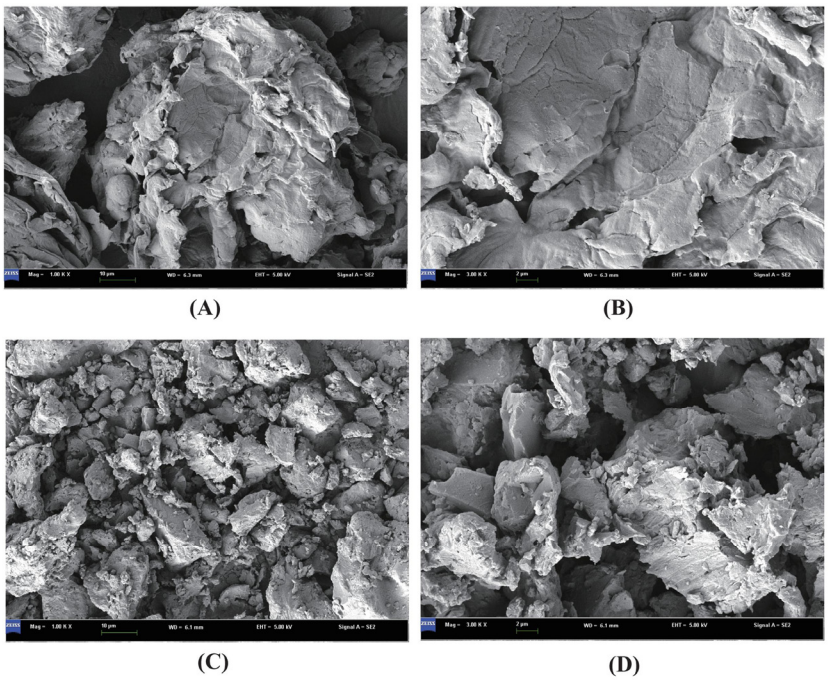

Figure 1. Effects of solid-state fermentation on the surface microstructure of PNM and FPNM. (**A**,**B**), scanning electron microscopy images of PNM (1000× & 3000×); (**C**,**D**), scanning electron microscopy images of FPNM (1000× & 3000×).

3.2. Effects of Solid-State Fermentation on the Amino Acids of PNM and FPNM

The amino acid composition of PNM and FPNM is shown in Table 4. The fermentation of PNM increased the free amino acids level of essential amino acids, except arginine ($p < 0.05$), and also increased all free amino acids' level of non-essential amino acids ($p < 0.05$). The hydrolyzed amino acids of essential amino acids including lysine, leucine, valine, and phenylalanine were increased after fermentation, while the arginine level was decreased ($p < 0.05$). Fermentation decreased the ratio of arginine to lysine from 3.01 to 1.60. The glycine, proline, and alanine level of hydrolyzed amino acids of non-essential amino acids was increased after fermentation, while the serine level was decreased ($p < 0.05$).

Table 4. Effects of solid-state fermentation on the amino acids of PNM and FPNM (dry matter basis, $n = 3$) [1].

Parameter	Free Amino Acids				Hydrolyzed Amino Acids			
	PNM	FPNM	SEM	*p*-Value	PNM	FPNM	SEM	*p*-Value
Essential amino acids, %								
Methionine	0.01 b	0.22 a	0.049	<0.001	0.50	0.59	0.029	0.120
Lysine	0.02 b	0.59 a	0.126	<0.001	1.70 b	2.05 a	0.080	<0.001
Threonine	0.02 b	0.19 a	0.036	<0.001	1.28	1.33	0.022	0.314
Tryptophan	0 b	0.01 a	0.003	<0.001	0.44	0.48	0.013	0.123
Arginine	0.21 a	0.03 b	0.039	<0.001	5.12 a	3.29 b	0.392	<0.001
Isoleucine	0.01 b	0.39 a	0.086	<0.001	1.64	1.73	0.036	0.272
Leucine	0.01 b	0.94 a	0.209	<0.001	3.02 b	3.34 a	0.089	0.049
Valine	0.01 b	0.87 a	0.191	<0.001	2.00 b	2.30 a	0.077	0.027
Histidine	0.01 b	0.29 a	0.062	<0.001	1.12	1.29	0.049	0.088
Phenylalanine	0.04 b	1.23 a	0.267	<0.001	2.45 b	2.97 a	0.117	0.001
Non-essential amino acids, %								
Glycine	0.01 b	0.54 a	0.118	<0.001	2.70 b	3.02 a	0.081	0.021
Serine	0.03 b	0.08 a	0.012	0.019	2.15 a	1.85 b	0.073	0.008
Proline	0.02 b	0.40 a	0.085	<0.001	2.13 b	2.46 a	0.076	0.001
Alanine	0.03 b	0.95 a	0.206	<0.001	1.91 b	2.63 a	0.162	<0.001
Asparagine	0.05 b	0.67 a	0.139	<0.001	5.43	5.69	0.078	0.093
Glutamine	0.14 b	2.01 a	0.422	<0.001	8.97	9.48	0.237	0.302
Cysteine	0 b	0.15 a	0.034	<0.001	0.55	0.62	0.027	0.171
Tyrosine	0 b	0.45 a	0.099	<0.001	1.48	1.46	0.036	0.845
Arginine/Lysine		—			3.01	1.60	0.308	<0.001

[1] Abbreviations: PNM, peanut meal; FPNM, fermented peanut meal. Different letters refer to the significant differences ($p < 0.05$).

3.3. Ileal Digestibility of Amino Acids of PNM and FPNM

Table 5 shows the results of the ileal endogenous amino acid losses of broilers fed the NFD, and the AID and SID values of amino acids in PNM and FPNM are shown in Table 6. The AID and SID values of essential amino acids (methionine, lysine, leucine and phenylalanine) were higher for FPNM than for PNM ($p < 0.05$). Moreover, the AID and SID values of non-essential amino acids of FPNM were both higher than those of PNM, except for proline ($p < 0.05$).

Table 5. Concentration of the ileal endogenous amino acid losses of the broilers fed a nitrogen-free diet (dry matter basis, $n = 6$) [(1)].

Items	Average, mg/kg	SD, mg/kg
Crude protein	8746.28	545.89
Essential amino acids		
Methionine	125.47	25.08
Lysine	319.26	24.68
Threonine	775.67	33.39
Tryptophan	130.14	32.11
Arginine	353.03	56.65
Isoleucine	341.50	53.18
Leucine	567.42	37.34
Valine	555.74	52.20
Histidine	190.86	38.76
Phenylalanine	409.62	60.18
Non-essential amino acids		
Glycine	544.07	55.87
Serine	670.73	50.89
Proline	613.07	57.51
Alanine	432.20	47.62
Asparagine	873.64	34.65
Glutamine	939.55	45.82
Cysteine	413.91	55.47
Tyrosine	400.73	43.91

[(1)] Abbreviations: SD, standard deviation.

Table 6. Apparent and standardized ileal digestibility values of amino acids in PNM and FPNM (dry matter basis, $n = 6$) [(1)].

Items	AID, %				SID, %			
	PNM	FPNM	SEM	*p*-Value	PNM	FPNM	SEM	*p*-Value
Essential amino acids								
Methionine	85.35	89.95	0.830	0.001	88.12	91.85	0.722	0.003
Lysine	81.87	85.72	0.701	0.001	83.70	87.32	0.672	0.001
Threonine	78.63	80.76	0.678	0.119	84.74	86.31	0.642	0.239
Tryptophan	83.48	86.37	0.749	0.058	86.44	88.91	0.716	0.082
Arginine	90.97	91.40	0.334	0.542	91.68	91.74	0.328	0.917
Isoleucine	84.27	86.73	0.638	0.053	86.38	88.57	0.616	0.071
Leucine	85.80	88.27	0.536	0.012	87.71	89.84	0.519	0.032
Valine	84.44	86.72	0.668	0.087	87.32	89.28	0.644	0.134
Histidine	87.43	89.30	0.463	0.056	89.03	90.65	0.442	0.061
Phenylalanine	88.53	90.96	0.470	0.003	90.14	92.36	0.446	0.005
Non-essential amino acids								
Glycine	67.45	72.40	0.965	0.003	69.51	74.12	0.925	0.005
Serine	81.01	83.88	0.675	0.025	84.25	87.10	0.664	0.023
Proline	84.44	86.43	0.523	0.051	87.36	88.99	0.494	0.100
Alanine	83.51	86.80	0.634	0.003	85.80	88.80	0.601	0.005
Asparagine	83.62	86.44	0.654	0.022	85.27	87.81	0.628	0.035
Glutamine	87.83	89.82	0.411	0.007	88.95	90.83	0.407	0.012
Cysteine	70.71	79.60	1.368	<0.001	79.52	85.77	0.984	<0.001
Tyrosine	88.31	91.29	0.632	0.009	90.77	93.35	0.589	0.019

[(1)] Abbreviations: PNM, peanut meal; FPNM, fermented peanut meal; AID, apparent ileal digestibility; SID, standardized ileal digestibility.

3.4. Apparent Metabolizable Energy of PNM and FPNM

The AME and ARGE values of PNM and FPNM are shown in Table 7. There was no significant difference in AME and ARGE between PNM and FPNM ($p > 0.05$).

Table 7. Apparent metabolizable energy and apparent retention of gross energy in PNM and FPNM (dry matter basis, $n = 6$) [(1)].

Items	PNM	FPNM	SEM	*p*-Value
AME, MJ/kg	10.68	10.89	0.152	0.508
ARGE, %	57.70	58.51	0.810	0.640

[(1)] Abbreviations: PNM, peanut meal; FPNM, fermented peanut meal; AME, apparent metabolizable energy; ARGE, apparent retention of gross energy.

4. Discussion

China is currently the largest producer, consumer, and exporter of peanuts in the world. According to the report of the Food and Agriculture Organization of the United Nations of 2020, the annual peanut production was about 18 million tons in China, of which more than 50% (9 million tons) was used for crushing, which produced about 4.5–5.9 million tons of PNM [29,30]. In early studies, some negative effects of PNM were found, which limit its application. Costa et al. [31] found that, due to the imbalance of amino acids, broilers fed on corn-peanut meal diets required additional supplementation of lysine, threonine, and methionine to achieve the same growth performance as corn-soybean meal diets. Xia et al. [32] also found that replacing soybean meal with peanut meal in the diet reduced the egg production and antioxidant capacity of egg ducks.

Solid-state fermentation has been studied and applied in the food and feed industry for a long time. Its application can improve the nutritional value of the substrate, as well as produce organic acids, enzymes, and other beneficial metabolites in the body [10,33]. The present study found that fermentation changed the chemical composition of PNM. Yang et al. [16] reported that the CP content increased by 6.96% after fermentation with

Bacillus licheniformis, which is close to 6.70% in this study. However, this was not an actual increase in protein content since no additional nitrogen sources were supplemented during fermentation. Hu et al. [12] considered that the increase in CP content was mainly associated with a decrease in the concentration of non-structural carbohydrates, and the proportion of CP that was compensated for increased due to a decrease in dry matter content. Additionally, except for proteases, strains of *Bacillus* spp. produce fiber-degrading enzymes, such as cellulases, hemicellulases, and β-glucanases [34]. The decrease in CF content may be related to the fiber-degrading enzymes secreted by the bacteria used for fermentation.

TCA-SP is a parameter that indicates the degree of protein decomposition, mainly including small peptides with molecular weight less than 10 kDa; free amino acids; and small amounts of non-protein nitrogen compounds [35]. Yang et al. [16] reported that the content of TCA-SP in fermented PNM was increased by 400.37%, which was consistent with our findings. The positive effect of fermentation on protein of PNM facilitated protein utilization, and amino acids in the form of small molecular peptides were more easily transported by intestinal epithelial cells than their free form. The increase of L-latic acid content can lower the pH value of raw materials and resist the proliferation of mold and harmful microorganisms, which can prolong the storage time of the product and solve the problem that PNM is not easy to store [36]. PNM contains many anti-nutritional factors such as phytic acid, trypsin inhibitor and plant hemagglutinin, among which phytic acid is an important factor for inhibiting the utilization of nutrients in PNM. *Bacillus subtilis*, *Bacillus cereus*, and other *Bacillus* spp. can secrete phytase, which can hydrolyze phytic acid into inositol and phytate, and release nutrients such as amino acids and mineral elements combined with phytic acid in the process of enzymatic hydrolyzation, thus improving the utilization rate of nutrients in raw materials [37,38]. Our study found that the level of phytic acid in PNM was significantly reduced after the solid-state fermentation, which may be related to the production of phytase by microorganism. The study of Castro-Alba et al. [39] showed that the fermentation of pseudocereal flours by *Lactobacillus* degraded phytic acid and thus improved the utilization of mineral elements. Concomitantly, researchers also reported that phytase produced by microorganisms was selective for the degradation of phytic acid, and not all raw materials can achieve the same degradation efficiency, which suggested that we need to use effective microorganisms for fermentation. In this study, the degradation of phytic acid by microorganisms was not only due to the phytase, but also because the L-lactic acid produced by the *Pediococcus acidilactici* reduced the pH value of PNM, and the change in pH value can provide conditions for the activation of microbial phytase and endogenous plant phytase [40]. AFB_1 is the most carcinogenic of all natural toxins, and with a detection rate of nearly 100% in PNM [41]. Through our experiments, we found that fermentation of PNM by *Bacillus velezensis* and *Pediococcus acidilactici* significantly reduced the level of AFB_1 in PNM and improved the quality of PNM. Probiotics generally clear AFB_1 by inhibiting spore formation of *Aspergillus flavus* and *Aspergillus parasiticus*, which produces AFB_1, or by enzymatic cleavage of the lactone ring of AFB_1 [42,43]. For this study, both effects may be present simultaneously. Wang et al. [44] have reported that *Bacillus velezensis* has great potential as a feed additive to remove mycotoxins in animal feed. What's more, the fermentation strain *Pediococcus acidilactici* LC-9-1 used in this experiment has the function of inhibiting the proliferation of some pathogenic microorganisms [18].

In this study, fermentation significantly increased the concentration of free amino acids in PNM, excluding arginine. The increase is based on the activity of peptidases, and is highly dependent on the microorganisms involved in fermentation [45]. However, we also found a decrease in the concentration of serine and arginine as hydrolyzed amino acids, which may be related to the utilization of microorganisms. Similar to our results, in a study on solid-state fermentation of soybean meal, serine and arginine decreased by 22.04% and 21.73%, respectively [15]. Compared to fish meal (0.76) and soybean meal (1.15), the ratio of arginine to lysine in PNM is 3.01, which is seriously imbalanced [46]. Excessive arginine will lead to the antagonism of amino acids in animals and inhibit the absorption of

lysine, and affect the normal growth and development of animals. We found that the lysine content of hydrolyzed amino acids in the FPNM was significantly increased by probiotics fermentation and the ratio of arginine to lysine was reduced to 1.6, making the amino acid composition of FPNM closer to the ideal amino acid composition.

The endogenous amino acid losses in broilers were corrected by the classical NFD method, and the result was similar to that in the report of Barua et al. [47], which proved that the method was reasonable. Our experiment showed that the proportion of main endogenous amino acids such as glutamate, aspartic, threonine, and serine in ileum increased in broilers fed with FPNM [48]. There are limited studies on the effect of FPNM on ileal amino acid digestibility in broiler chickens, but we have found in other studies on plant-based protein feedstuffs that rapeseed meal and soybean meal fermented by probiotics increase the AID and SID of most amino acids in broiler chickens, which is similar to our research in which the AID and SID of partial essential and non-essential amino acid were both increased in broilers fed FPNM [15,27]. Additionally, it was found that the digestibility of dry matter of PNM in vitro was increased by 10.18% after fermentation by *Bacillus licheniformis* [16]. The reason for the increase in amino acid digestibility may be related to the improvement of protein molecules in PNM after fermentation; more peptides and free amino acids were absorbed by the animal intestine, and the peptides also promoted the utilization of amino acids by the animal intestine [49]. Furthermore, the fermentation by probiotics reduced the interference of crude fiber on the absorption of nutrients in PNM; specific manifestations include the decreased viscosity of digesta and better binding between digestive enzymes and substrates. These also promoted the digestibility of amino acids [50]. Additionally, the decrease in the phytic acid level can also promote the digestion of amino acids. It's well known that phytic acid combines with endogenous proteases (such as pepsin and trypsin) to form complexes that reduce amino acids digestibility, especially the AID of cysteine, aspartic acid and glycine, ultimately leading to lower feed conversion rates [51–53]. As the limiting amino acids in livestock diets, the improvement in the digestibility of methionine and lysine can promote amino acid balance and utilization efficiency in diets [54]. Moreover, amino acids balance can accurately predict the nutritional value of feedstuff and reduce the impact of unnecessary nitrogen emissions on environmental pollution, and also provide a new theory and method for the study of low protein diets. In this study, we obtained an AME of 10.68 MJ/kg for PNM, which was close to the value of 10.88 MJ/kg published in the Chinese Feed Composition and Nutritional Value (2019 version) [55]. However, no significant difference was derived between PNM and FPNM for AME and ARGE. Similar to our results, Li et al. [15] did not find a significant difference in AME between soybean meal and fermented soybean meal in broilers. The availability of energy in PNM and FPNM mainly depends on the balance of energy-yielding components in the feed and the factors that inhibit its utilization. Reduction in CF and anti-nutritional factors would favor the utilization of nutrients, and degraded CF was converted into disaccharides and eventually into glucose, which was more readily utilized by the body [56–58]. However, fermentation itself is an energy-consuming process, in which the microorganisms consume carbon sources such as monosaccharides, disaccharides, oligosaccharides, starch, and cellulose from the substrate for proliferation and biotransformation [59]. Thus, the energy that can be utilized by the body may not increase, which we believe has led to the absence of any difference in the AME of PNM and FPNM.

5. Conclusions

Solid-state fermentation significantly improved the quality of PNM by increasing the concentration of CP, TCA-soluble protein, and L-lactic acid, decreasing the concentration of CF, phytic acid, and AFB_1; and fermentation improved the composition of amino acids including free and hydrolyzed amino acids. Our study also demonstrated that fermentation improved the AID and SID of most amino acids in PNM but did not affect the AME or

ARGE. The present study provides basic data and theory for the study of FPNM and provides references for the design of broiler formulations.

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