



Review

Improving the Nutritional Value of Plant Protein Sources as Poultry Feed through Solid-State Fermentation with a Special Focus on Peanut Meal—Advances and Perspectives

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Abstract: The poultry industry has been and is still suffering considerable challenges because of the increasing price of soybean meal. Therefore, it is imperative to find alternative, high-quality plant protein sources. Peanut meal (PNM), a by-product of peanut oil extraction, is abundant in crude protein (40.1–50.9%), making it a potential plant protein source. However, nutritional and non-nutritional limitations are detrimental to its application in poultry diets, such as an imbalance in amino acid composition, phytate and the risk of aflatoxins pollution. As a processing technique, solid-state fermentation has been used to reduce phytate and improve the nutrient availability of plant protein sources in the feed industry. It is a promising approach to improving the application of PNM in poultry diets. There are several advantages to the solid-state fermentation of PNM, such as low-cost equipment, high productivity, the stability of the product and the minimization of energy consumption. Currently, there is still a lack of synthesized information on the application of solid-state fermented PNM in poultry. This review summarized the limiting factors for PNM application in poultry feed and the improvement of solid-state fermentation on the nutritional value of plant protein sources so as to evaluate the feasibility of improving the nutritional value of PNM as poultry feed through solid-state fermentation. We hope to shed some light on the selection of protein resources in future research.

Keywords: peanut meal; solid-state fermentation; amino acid; anti-nutritional factor; plant protein source; poultry



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1. Introduction

As one of the world's five major oil seeds, peanuts are widely cultivated around the world. China is the largest peanut producing country with the production of 18.3 million tons in 2022, followed by India, Nigeria and the United States with 6.7, 4.5 and 2.5 million tons, respectively [1]. However, there are great differences in the utilization of peanuts in different regions due to the differences in dietary habits. In the United States, nearly 60% of peanuts are used for direct consumption, and in Europe, the proportion is more than 90%. In the case of China, the proportion of peanuts used for food is less than 40%, and more than 50% (9 million tons) are used for the extraction of peanut oil because the Chinese prefer to stir-fry their food with it [2]. The peanut oil is produced mainly by

high temperature pressing and solvent extraction, which will produce 50–65% of residue (peanut meal, PNM), which appears as massive, powdery or flake-like (Figure 1) [3]. Thus, 4.5–5.9 million tons of PNM per year will be obtained in China, and it will bring huge economic benefits if this by-product is fully utilized. The nutritional value of PNM has been well clarified, with 40.1–50.9% crude protein, 0.7–6.0% fat and 5.8–12.6% fiber, as well as a rich array of vitamins, minerals and antioxidant components. Furthermore, it also contains some active components, such as resveratrol and peanut lectin [4,5]. However, there are many limitations when PNM is used as a poultry feed ingredient, including the imbalanced amino acid profiles, anti-nutritional factors (the phytate content is about 1.5%), and vulnerability to contamination by mycotoxins and pathogenic bacteria [6–8]. These factors seriously limit the use of PNM as a high-quality protein raw material in poultry feed.

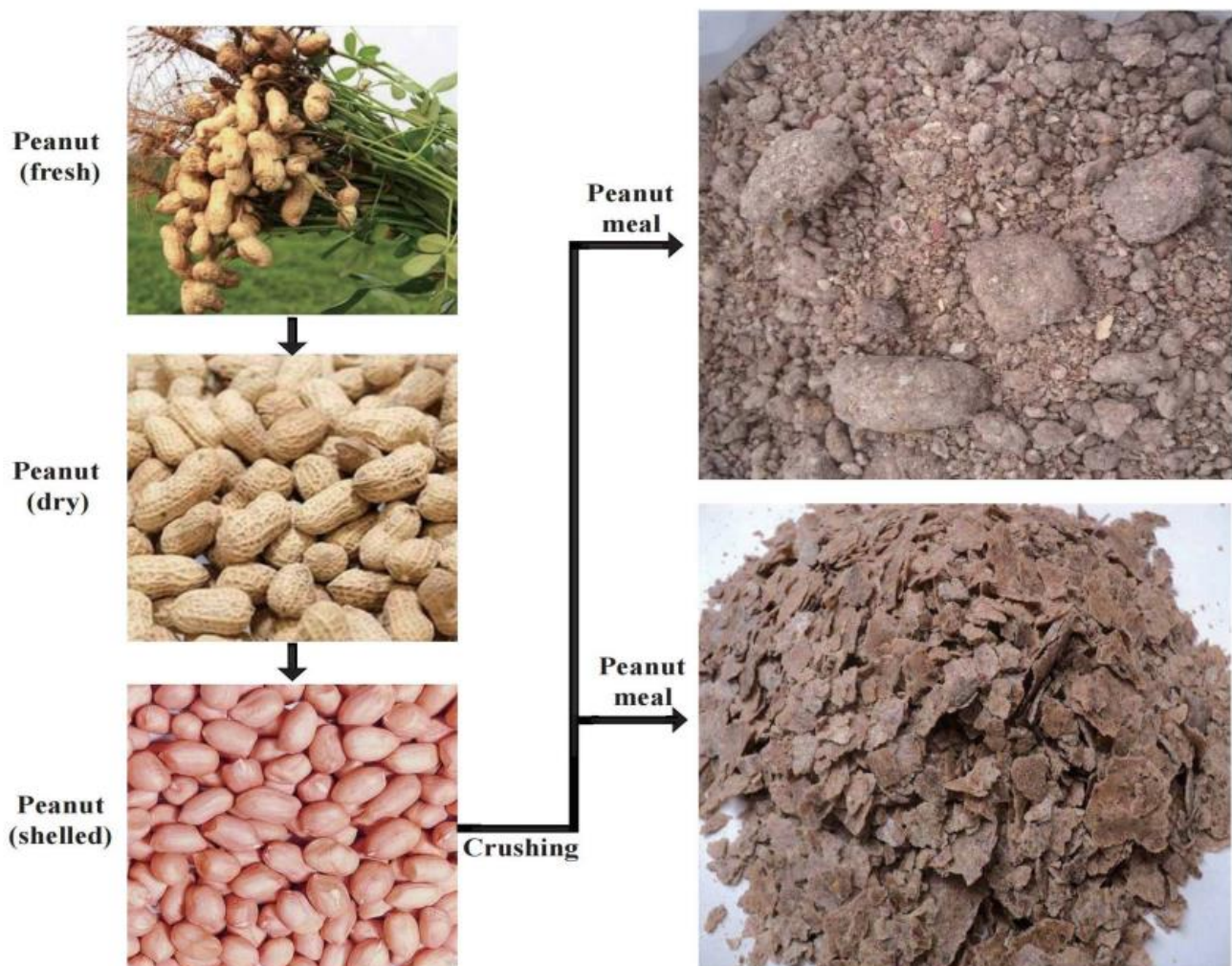


Figure 1. The production process and two main morphological forms of PNM.

Scientists have expended much effort to make improvements and increase utilization of PNM, such as by supplementing essential amino acids (usually lysine and methionine), enzymolysis and fermentation [9–11]. Solid-state fermentation is a biological treatment technology that has a long application history in the food and feed industries. The process involves microorganisms growing on solid materials under controlled conditions; in the absence of free water, the water required for microbial growth and metabolism is in an absorbed state within the solid matrix [12]. Many studies have described how solid-state fermentation can improve the nutritional value of plant protein sources and

show good prospects for reducing anti-nutritional factor levels and promoting nutrient utilization [13–15]. It is also a promising approach to improving the nutrient availability of PNM by solid-state fermentation, which significantly improves the crude protein content, acid-soluble oligopeptide content and *in vitro* digestibility [11]. However, there are few comments about the potential of fermented PNM as a feed ingredient to formulate poultry diets. This article briefly describes the current application and limitations of PNM as an ingredient in poultry feed and introduces the nutritional value enhancement of other plant protein sources through solid-state fermentation. The purpose is to analyze the feasibility of solid-state fermentation in improving the nutritional value of PNM as poultry feed.

2. The Application of PNM as Poultry Feed

Early attempts at using PNM as a source of protein for poultry indicated that its nutritional value was inferior to that of soybean meal. Coupled with its limited yield at that time, a general addition to the diets of broilers in the United States was 3–4% [6]. As it may be gathered from Table 1, the effects of PNM on the production performance of poultry vary in different reports. As early as 1946, Heuser et al. [16] reported that the use of PNM and soybean meal in the diets of broilers at a ratio of 1:1 would not affect their performance. The recent study by Saleh et al. [17] also found that the growth performance and protein digestibility of broilers were not affected when 5% or 10% PNM was added to corn-soybean meal-based diets. However, a previous study found that when PNM replaced 50% of soybean meal, the growth performance of broilers was reduced [18]. El Boushy and Raterink reported that when the proportion of PNM in the diet increased from 5% to 15%, the growth performance and feed conversion of broilers decreased even if the lysine and methionine in the diet were sufficient [19]. Costa et al. [20] also observed that, under the premise of meeting the minimum amino acid requirements of broilers, increasing the addition ratio of PNM (0%, 10%, 20% and 32%) showed a decreasing trend in growth performance.

In terms of application to laying hens, Pesti et al. [21] found that PNM addition at 21.4–35.5% would reduce the egg weight of 22–28-week laying hens compared to corn-soybean meal-based diets. Research on egg ducks obtained similar results: replacing soybean meal with PNM at a ratio of 100% had a negative effect on feed intake, feed conversion and egg weight [22]. These conflicting results may be due to differences in the production process, chemical composition, levels of PNM addition and anti-nutritional factor of PNM. What is certain is that different sources of PNM directly affect the application effect in broilers. Studies have shown that when feeding broilers with high-oleic acid PNM, their growth performance and breast meat yield are lower than on corn-soybean meal-based diets, but the content of unsaturated fatty acids in breast muscle is significantly increased [23,24]. A clear understanding of the limiting factors for the use of PNM in poultry is therefore essential.

Table 1. List of the effects of PNM on the production performance of poultry.

Years	Species	Control Diet	PNM Proportion	Production Performance	Other Effects	Reference	
1946	Broilers	Comb White Leghorn broilers	Corn-soybean	50% substitute soybean meal	No differences	—	Heuser et al. [16]
1959	Broilers	Vantress × White Plymouth broilers	Corn-soybean	50% substitute soybean meal	Decreased BW	—	Douglas and Harms [18]
1989	Broilers	Hybro broilers	Corn-soybean	10% of diet	Decreased BW	—	EL Boushy and Raterink [19]
1989	Broilers	Hybro broilers	Corn-soybean	15% of diet	Decreased BW	—	EL Boushy and Raterink [19]
2001	Broilers	Ross 208 broilers	Corn-soybean	10% of diet	No differences	—	Costa et al. [20]
2001	Broilers	Ross 208 broilers	Corn-soybean	20% of diet	No differences	—	Costa et al. [20]
2001	Broilers	Ross 208 broilers	Corn-soybean	32% of diet	Decreased BW; increased F:G ratio	—	Costa et al. [20]
2009	Broilers	Vencob broilers	Corn-soybean	25% substitute soybean meal	Increased BW	—	Ghadge et al. [25]
2009	Broilers	Vencob broilers	Corn-soybean	50% substitute soybean meal	Increased BW; decreased F:G ratio	—	Ghadge et al. [25]
2009	Broilers	Vencob broilers	Corn-soybean	75% substitute soybean meal	Increased BW; decreased F:G ratio	—	Ghadge et al. [25]
2009	Broilers	Vencob broilers	Corn-soybean	100% substitute soybean meal	Increased BW; decreased F:G ratio	—	Ghadge et al. [25]
2016	Broilers	Lohman broilers	Corn-soybean	50% substitute soybean meal	No differences	—	Ata [26]
2016	Broilers	Lohman broilers	Corn-soybean	100% substitute soybean meal	Increased BW	—	Ata [26]
2020	Broilers	Ross 708 broilers	Corn-soybean	12% of diet	Decreased BW, carcass and breast meat yields	Increased PUFA in breast meat	Toomer et al. [23,24]
2022	Broilers	Cobb 500 broilers	Corn-soybean	10% of diet	No differences	Decreased serum TC, TG and LDL-cholesterol	Saleh et al. [17]
2003	Hens	Hyline W-36 White Leghorn hens	Corn-soybean	3.8 g/hen per d	Decreased egg weight (first 6 weeks)	—	Pesti et al. [21]
2013	Hens	Rugao laying hens	Corn-soybean	5.3% substitute soybean meal	No differences	Decreased egg yolk cholesterol content	Lu et al. [27]
2013	Hens	Rugao laying hens	Corn-soybean	10.6% substitute soybean meal	No differences	Decreased egg yolk cholesterol content	Lu et al. [27]
2013	Hens	Rugao laying hens	Corn-soybean	15.9% substitute soybean meal	No differences	—	Lu et al. [27]
2022	Ducks	Longyan laying ducks	Corn-soybean	25% substitute soybean meal	No differences	Decreased serum GSH	Xia et al. [22]
2022	Ducks	Longyan laying ducks	Corn-soybean	50% substitute soybean meal	Decreased feed intake	Decreased serum GSH	Xia et al. [22]
2022	Ducks	Longyan laying ducks	Corn-soybean	75% substitute soybean meal	Decreased feed intake	Decreased serum GSH	Xia et al. [22]
2022	Ducks	Longyan laying ducks	Corn-soybean	100% substitute soybean meal	Decreased FI and egg weight; increased F:G ratio	Decreased serum GSH; increased serum MDA	Xia et al. [22]

Abbreviations: BW, body weight; F:G ratio, feed intake: weight gain; FI, feed intake; PUFA, polyunsaturated fatty acids; TC, total cholesterol; TG, triglycerides; GSH, glutathione; MDA, malonaldehyde. “—”: no relevant content.

3. The Limiting Factors of PNM as Poultry Feed

The factors limiting PNM as a poultry feed ingredient can be summarized as nutritional and non-nutritional issues.

3.1. Imbalance of Amino Acid Composition

The imbalance in amino acid composition is one of the nutritional limitations. Table 2 shows the contents of amino acids in PNM and soybean meal reported in various literature. Obviously, although there are some slight differences in the content of amino acids in PNM from different resources, their trends are similar. Compared with amino acids in soybean meal, arginine, glutamine and glycine in PNM are relatively high, while essential amino acids such as methionine, lysine, threonine, isoleucine and valine are relatively low. In addition, the ratio of arginine to lysine in PNM is 3.27–3.55, far exceeding the recommended range of 1.10–1.18 proposed by the National Research Council Nutrient Requirements of Poultry [28]. Studies have shown that excessive arginine can inhibit the absorption of lysine, leading to a further deficiency of lysine in the body [29]. Driggers and Tarver [9] found that by adding lysine to broiler diets, PNM could replace 50% of the soybean meal. Shortly afterwards, researchers demonstrated that lysine was the first limiting amino acid in the corn-PNM-based diet, followed by methionine and threonine [18,30]. Robbins [31] further discovered that the content of cystine was low in PNM and established a model of cystine deficiency in broilers by supplementing other amino acids in a corn starch-PNM-based diet. In addition, due to the need for heat pressing before solvent extraction of peanut oil, it is possible that overprocessing may reduce the protein quality of PNM. Zhang et al. [32] reported that the amino acid digestibility decreased with the prolongation of the heat pressing time, especially the digestibility of methionine, which decreased from 87% to 57%. As described above, the focus must be placed on the balance of amino acid composition, especially the concentration and proportion of lysine, methionine, threonine and cystine when formulating poultry diets with PNM.

Table 2. The contents of amino acids in PNM and soybean meal reported in various literature.

Items	PNM		Soybean Meal	
	Zhang's Report [32] (USA)	Chinese Feed Database [33] (China)	Cowieson's Report [34] (New Zealand)	Chinese Feed Database [33] (China)
CP, %	47.8	47.8	48.2	47.9
Essential amino acids				
Methionine	0.47	0.41	0.66	0.68
Lysine	1.66	1.40	2.98	2.99
Threonine	1.31	1.11	2.02	1.85
Arginine	5.90	4.88	3.68	3.43
Isoleucine	1.60	1.25	2.11	2.10
Leucine	3.10	2.50	3.52	3.57
Valine	1.91	1.36	2.32	2.26
Histidine	1.10	0.88	1.22	1.22
Phenylalanine	2.35	1.92	2.38	2.33
Non-essential amino acid				
Glycine	2.88	—	2.17	—
Serine	2.56	—	2.72	—
Proline	2.12	—	2.37	—
Alanine	1.91	—	2.11	—
Asparagine	6.36	—	5.91	—
Glutamine	10.15	—	8.44	—
Cysteine	0.69	0.40	0.65	0.73
Tyrosine	1.42	1.39	1.47	1.57

“—”: not determined.

3.2. Phytate

Myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate (IP6), also known as phytate, has an asymmetrical six-carbon ring structure (Figure 2A) [35]. As the major anti-nutritional factor in PNM (its content is about 1.5%), phytate strongly binds to protein molecules by phosphate groups, significantly reducing the digestibility and utilization of protein [36]. In addition, phytate also binds to the basic amino acid residues of enzymes, which in turn inhibits the activities of trypsin, amylase and pepsin, ultimately affecting the digestion and absorption of nutrients [37]. Cowieson et al. [38] believed that phytate increased the excretion of endogenous nitrogen in animals, resulting in nitrogen loss in the body and reducing the utilization of protein by animals. Moreover, phytate can firmly adhere to zinc, copper, calcium, magnesium, iron and other positively charged metal ions in the gastrointestinal tract of animals to form insoluble phytate complexes, resulting in reduced bioavailability of trace mineral elements [39]. Microbial phytase, which belongs to the hydrolase family, can be produced by fungi or bacteria. The degradation of phytate is carried out by catalyzing the hydrolysis of phytate salt into low-grade myo-inositol phosphate and inorganic phosphorus (Figure 2B) [40,41]. Almost no endogenous phytase exists in the digestive tract of poultry, so it is difficult for the body to decompose phytate. The benefits of supplemental phytase in poultry diets are clearly established, including improved nutrient utilization, growth performance, bone mineralization and so on [42–44]. Regarding the application effect of phytase in the PNM diet, Driver et al. [45] proved that adding phytase to a corn-PNM diet increased the nitrogen-corrected apparent metabolizable energy of broilers from 3209 kcal/kg to 3559 kcal/kg.

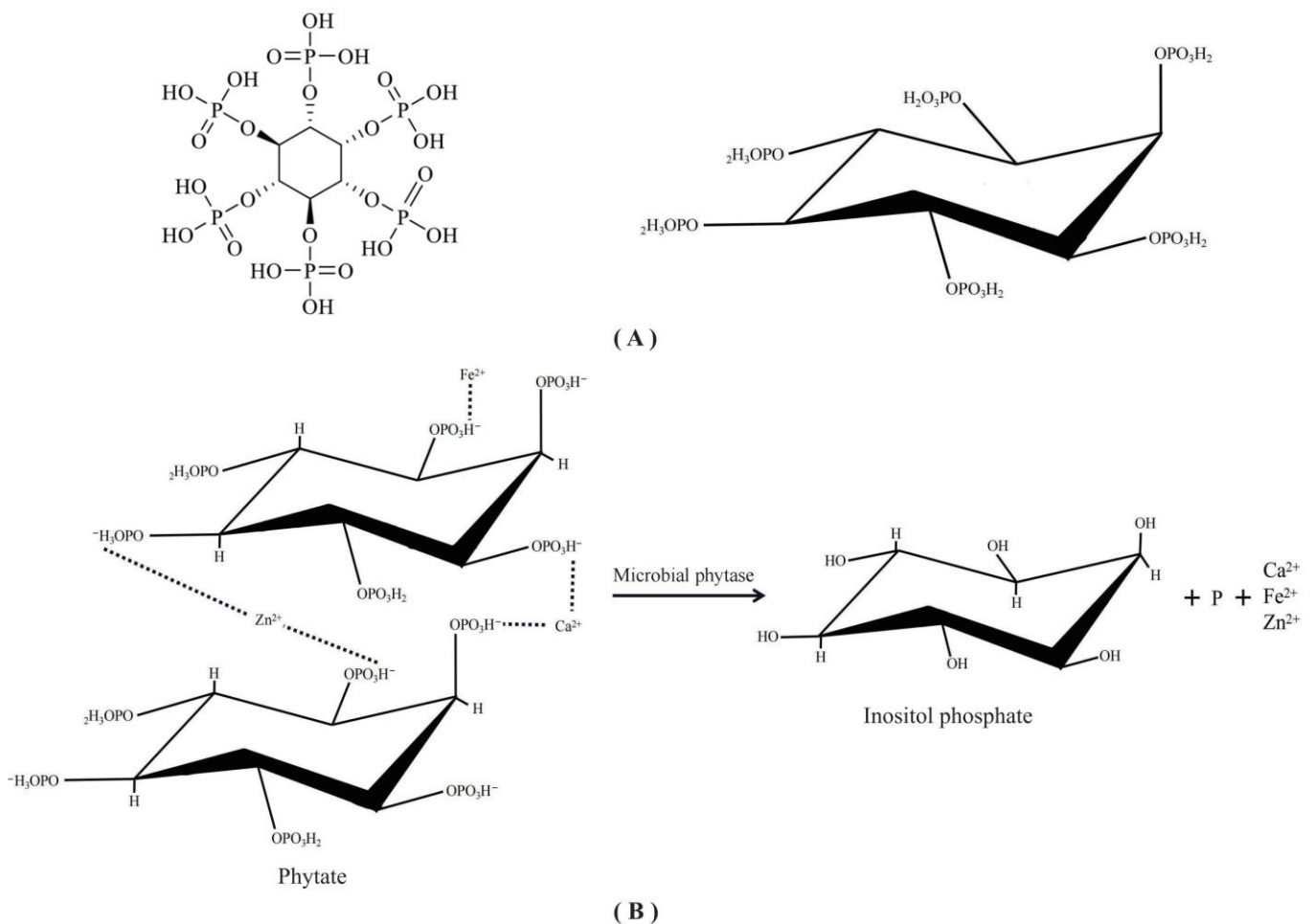


Figure 2. The mechanism of phytase to hydrolyze phytate. (A) Phytic acid; (B) The mechanism of microbial phytase action.

3.3. Risk of Aflatoxin Pollution

Aflatoxin is a toxic metabolite produced by a number of fungi, including *Aspergillus parasiticus* and *Aspergillus flavus*. The earliest report on aflatoxins in PNM dates back to 1960, when an outbreak of “X” disease in turkeys occurred in the UK, resulting in the death of approximately 100,000 birds due to the ingestion of PNM contaminated with aflatoxins [46]. Since then, aflatoxins contamination in PNM has gradually become an increasing concern around the global feed industry. Currently, twenty kinds of aflatoxins have been isolated, such as B₁, B₂, M₁, M₂, G₁ and G₂, with similar structures, of which aflatoxin B₁ (AFB₁) is the most common and carcinogenic one. Kana et al. [47] investigated the contamination of aflatoxins in PNM in Cameroon; the results revealed that the positive rate was 100% and the total aflatoxin (B₁ + B₂ + G₁ + G₂) concentrations ranged from 39 to 950 µg/kg. Chen et al. [48] collected 322 samples of peanut flour from Taiwan, and the detection results showed that the positive rates for AFB₁, AFB₂ and AFG₂ were 100%, 89.6% and 8.3%, respectively. The average concentrations of AFB₁, AFB₂ and AFG₂ were 7.02 µg/kg, 1.54 µg/kg and 0.21 µg/kg, respectively, and AFG₁ was not detected. It can be seen that PNM was widely contaminated with aflatoxins, especially AFB₁. In fact, the accumulation of aflatoxins in the PNM is not accidental, as most peanuts have already been infected with *Aspergillus parasiticus* or *Aspergillus flavus* during the growth process. Moreover, PNM is an excellent culture medium for fungi, and the release of aflatoxins increases with the proliferation of fungi [49]. During oil extraction, small portions of aflatoxins in peanuts are transferred to the oil phase, and most of them remain in the PNM [50]. When poultry are fed diets containing AFB₁, it will be absorbed by the small intestine and bind to plasma albumin. However, it is not AFB₁ itself that is toxic to the host, but its enzymatic transformation products, including AFB₁-8,9-exo-epoxide, AFM₁, AFQ₁ and AFP₁ [51]. Studies have shown that AFB₁-8,9-exo-epoxide is critically important in the acute and chronic toxicity of AFB₁, which can interfere with protein synthesis and lead to cell death, oxidative damage, decreased productivity and even death [52,53]. Furthermore, due to the lipophilicity of AFB₁, it can be absorbed by the intestine and distributed to the liver, muscle, kidney, and fat tissues, posing further threats to human food safety [54]. Previous studies revealed that, when the concentration of AFB₁ in the broiler diet exceeded 1800 µg/kg, 3.84 µg/kg AFB₁ was detected in the liver in the second week [55]. Therefore, when PNM is added to the poultry diet, the mycotoxin content must be monitored as a key variable, and consideration should be given to using mycotoxin adsorbents or binders to control toxin levels [22]. The European Commission has established the most stringent regulations for mycotoxins in feed, with the maximum allowable total AFs (AFB₁ + AFB₂ + AFG₁ + AFG₂) set at 10 µg/kg and 5.0 µg/kg of AFB₁ [56]. In China, based on the Chinese Feed Hygiene Standard (2017 version), the maximum allowable limit of PNM as a feed ingredient is 50 µg/kg, and the maximum allowable limit in broiler feed is 10 µg/kg [57]. Reassuringly, the risk of aflatoxin contamination can be effectively controlled by planting resistant peanut varieties combined with necessary crop management [49]. Through a series of policy formulation and implementation efforts, the contamination of peanuts with AFs has improved [58].

Many physical, chemical and biological methods have been verified to be able to inactivate or detoxify the aflatoxins in contaminated feedstuffs. Among them, the biological method is a very promising measure due to its specificity, irreversibility and efficiency in detoxification [59]. Some specific microorganisms can play a role in the adsorption and degradation of AFB₁. The physical adsorption of AFB₁ by lactic acid bacteria is related to the cell wall, which is a rapid and reversible process, and the adsorption capacity varies with different bacterial species and doses [60]. In addition, AFB₁ can also be degraded by extracellular enzymes secreted by microorganisms, such as *Bacillus* spp. or white rot fungi [61–63]. According to the structure analysis of the degradation products, the epoxide ring, carbonyl and unsaturated carbon-carbon double bonds of AFB₁ are generally attacked, forming water-soluble salts without toxicity [64].

3.4. Other Limiting Factors

PNM can provide good sources of carbon, nitrogen and energy for bacterial growth and reproduction, which makes it prone to contamination by pathogenic bacteria such as *Salmonella* and *Escherichia coli* during storage. Exposure to pathogenic bacteria poses numerous and diverse threats to developing animals [65]. In addition, PNM contains large amounts of highly unsaturated fatty acids, especially linoleic and linolenic acids, which are oxidized to produce free radicals and peroxynitrite. This makes PNM prone to oxidation deterioration, resulting in decreased shelf life, poor palatability and nutritional losses [66]. These are also a limiting factor for the use of PNM as a feed ingredient for poultry.

4. Solid-State Fermented Plant Protein Sources in the Diets of Poultry

In general, the production process of plant protein sources mainly consists of pretreatment, microbe inoculation, fermentation procedure and product collection (Figure 3). It is worth noting that there will be one-step fermentation or multi-step fermentation according to the raw materials and purposes [11,12]. It has been shown that many beneficial compounds have been proven to be produced by fermentation, such as enzymes, organic acids, flavor compounds and bioactive substances. Table 3 summarizes the nutritional changes of common plant protein sources during fermentation and their application effects in poultry.

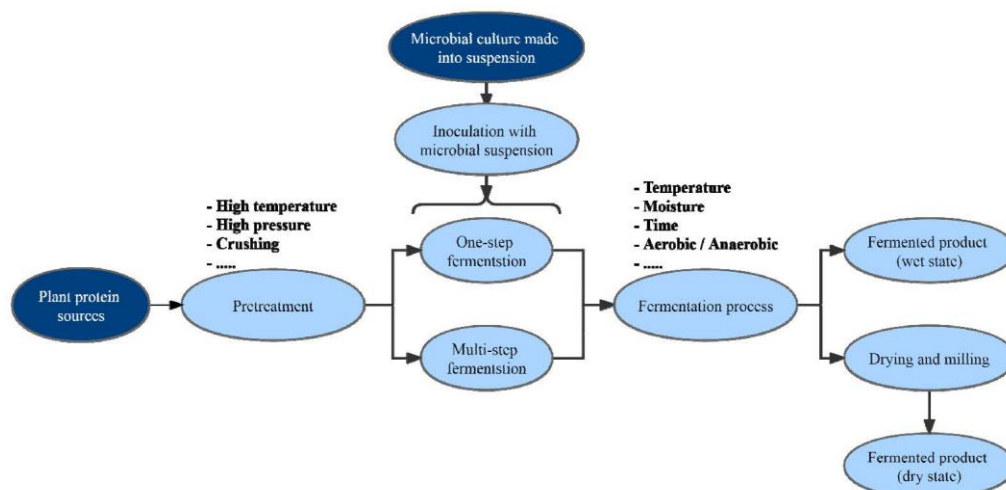


Figure 3. Schematic representation of steps involved in the solid-state fermentation of plant protein sources.

Proteins in animals are digested and decomposed into various kinds of amino acids and peptides, which are then absorbed by animals [67–69]. The modern protein nutrition theory holds that only part of the proteins are absorbed by animals in the form of amino acids, while most of the proteins are absorbed in the form of peptides. The peptide transport system has the characteristics of fast speed, low energy consumption and non-saturation of transport carriers [70]. Therefore, the peptide content is an important index for evaluating the fermentation products of plant protein sources. Trichloroacetic-acid-soluble protein (TCA-SP) is usually used as an indicator for plant protein source fermentation, which consists of peptides with a molecular weight less than 10 kDa, free amino acids and small amounts of non-protein nitrogen compounds [14,71]. Other evaluation indices include the degree of degradation of anti-nutritional factors or the increase in organic acids (generally lactic acid). Next, we will briefly review the related research on the solid-state fermentation of soybean meal, rapeseed meal and cottonseed meal, as they are similar to PNM in terms of nutritional composition, in the hope of providing some basic guidance on the PNM fermentation.

Table 3. The nutritional changes of common plant protein sources during fermentation and their application effects in poultry.

Years	Substrates	Microorganisms	Animals Applied	Nutritional Improvement/Beneficial Effects	Reference
1998	Soybean meal	<i>Aspergillus usarii</i>	Broilers	Decreased phytate phosphorus (complete degradation). Fermented soybean meal improved BW, retained phosphorus and femoral phosphorus in broilers	Hirabayashi et al. [13]
2006	Soybean meal	<i>Aspergillus niger</i>	Broilers	Fermented soybean meal improved BW, ileum villi length and width in broilers	Mathivanan et al. [72]
2007	Soybean meal	<i>Aspergillus oryzae</i>	Broilers	Fermented soybean meal improved ADG, FI, serum phosphorus, IgM and IgA; decreased serum urea nitrogen in broilers	Feng et al. [73]
2016	Soybean meal	<i>Bacillus amyloliquefaciens</i>	—	Decreased trypsin inhibitor, raffinose and stachyose; increased antioxidant activity and metal-chelating ability	Chi and Cho [74]
2016	Soybean meal	<i>Bacillus subtilis</i>	—	Decreased trypsin inhibitor and β -conglycinin	Seo and Cho [75]
2020 & 2022	Soybean meal	<i>Bacillus amyloliquefaciens</i> , <i>Lactobacillus acidophilus</i> and <i>Saccharomyces cerevisiae</i>	Broilers	Decreased glycinin and β -conglycinin; increased CP and TCA-SP. Fermented soybean meal improved energy digestibility and SID of amino acids in broilers	Li et al. [14,76]
2023	Soybean meal	<i>Bacillus</i> spp. yeast, <i>Lactobacillus</i> spp. and <i>Clostridium</i> spp.	Laying hens	Increased the CP, amino acids and organic acids; decreased NDF and ADF. Fermented soybean meal improved the laying performance, egg quality, intestinal barrier function and follicle development in hens	Lu et al. [67]
2001	Rapeseed meal	<i>Rhizopus oligosporus</i>	—	Increased nitrogen and protein contents; decreased glucosinolates, thiooxazolidones, phytate and CF	Vig and Walia [77]
2016	Rapeseed meal	<i>Bacillus subtilis</i> , <i>Candida utilis</i> and <i>Enterococcus faecalis</i>	Broilers	Increase CP and small peptides; decreased CF, glucosinolate, isothiocyanate, tannin and phytate. Fermented rapeseed meal improved antioxidant level and intestinal morphology of broilers	Hu et al. [15]
2017	Rapeseed meal	<i>Lactobacillus acidophilus</i> , <i>Bacillus subtilis</i> , and <i>Aspergillus niger</i>	Broilers	Fermented rapeseed meal decreased colonization of <i>Salmonella</i> and <i>Typhimurium</i> ; improved growth performance	Ashayerizadeh et al. [78]
2019	Rapeseed meal	<i>Bacillus licheniformis</i> , Yeast and <i>Lactobacillus</i>	Broilers	Improved the sensory properties, CP, lactic acid and total amino acid; decreased glucosinolate and NDF. Fermented rapeseed meal improved productivity performances of broilers	Wang et al. [69]
2022	Rapeseed meal	<i>Bacillus subtilis</i>	Japanese quail	No significant differences were found	Wengerska et al. [79]
2022	Rapeseed meal	<i>Bacillus subtilis</i> and <i>Aspergillus niger</i>	Laying hens	Increased lactic acid bacteria and CP; decreased pH, dry matter, CF and anti-nutritional factors. Fermented rapeseed meal improved egg production and egg mass in hens	Taheri et al. [80]
2012	Cottonseed meal	<i>Bacillus subtilis</i>	Broilers	Decreased free gossypol. Fermented cottonseed meal improved growth performance and immunity in broilers	Tang et al. [81]
2017	Cottonseed meal	<i>Bacillus subtilis</i> , <i>Aspergillus niger</i> and <i>Aspergillus oryzae</i>	Broilers	Decreased CF and free gossypol; increased CP and lactic acid bacteria. Fermented cottonseed meal improved intestinal development and growth performance; decreased abdominal fat yield in broilers	Jazi et al. [82]
2019	Cottonseed meal	<i>Candida tropicalis</i>	Broilers	Fermented cottonseed meal decreased abdominal fat yield and subcutaneous fat thickness in broilers	Niu et al. [83]

Abbreviations: BW, body weight; F:G ratio, feed intake: weight gain; ADG, average daily gain; FI, feed intake; CP, crude protein; CF, crude fiber; NDF, neutral detergent fiber; ADF, neutral acid detergent fiber; TCA-SP, trichloroacetic-acid-soluble protein; SID, standardized ileal digestibility. “—”: no animal tests were conducted.

4.1. Soybean Meal

Soybean meal is the most widely used feedstuff as a plant protein source. The anti-nutritional factors, such as protease inhibitors, soybean agglutinin, antigen protein, phytate and soybean oligosaccharides in soybeans, are difficult to remove in the process of oil extraction by heating, pressing and leaching, so they are largely left in the soybean meal [84]. However, most of these anti-nutritional factors can be degraded by solid-state fermentation. Hirabayashi et al. [13] found that fermentation of soybean meal with *Aspergillus usarii* can efficiently degrade the phytate and provide more available phosphorus. Trypsin inhibitor is a protein-based anti-nutritional factor in soybean meal that inhibits the activity of trypsin and chymotrypsin [85]. Reports from Chi and Seo [74,75] showed that fermented by *Bacillus* spp. can effectively degrade this component in soybean meal. Furthermore, Wang et al. [86] achieved a 94.2% degradation efficiency of trypsin inhibitor by using *Bacillus subtilis* to conduct two-stage solid-state fermentation of soybean meal. At the same time, the contents of soybean globulin and β -conglycinin, which are antigens that trigger hypersensitivity reactions, were also effectively reduced. Chi et al. [74] also reported that raffinose and stachyose, as indigestible oligosaccharides in soybean meal, were almost completely degraded after fermentation by *Bacillus amyloliquefaciens*. Solid-state fermentation can also optimize the composition of nutrients in soybean meal. Hong et al. [87] showed that after fermentation by *Aspergillus oryzae*, the amount of macromolecular protein content (>20 kDa) in soybean meal decreased from 76.9% to 17.7%. In addition, Li et al. [14] reported that the macromolecular proteins in soybean meal were degraded into small peptides and free amino acids by *Bacillus amyloliquefaciens* fermentation, the total amino acid content increased by 2.56%, and the crude fiber decreased by 7.56%. They also found that the standardized ileal digestibility of amino acids in broilers was significantly increased. In vivo tests showed that dietary fermented soybean meal supplementation had beneficial effects on improving growth performance, promoting intestinal development, reducing the colonization rate of pathogenic bacteria in the intestine, and enhancing the immune responses of poultry; the effects on laying birds included improving laying performance, egg quality and follicle development [67,73,88,89].

4.2. Rapeseed Meal

The crude protein content of rapeseed meal is 35–40%, and the annual production is more than 10 million tons in China, making it a potential substitute for soybean meal in poultry feed [69,90]. However, the application of rapeseed meal is restricted due to its poor palatability and anti-nutritional factors such as glucosinolates, phytate and tannin, especially the high content of glucosinolates, which can cause thyroid enlargement, growth inhibition and a decrease in production [91]. Early research showed that solid-state fermentation of rapeseed meal with *Rhizopus oligosporus* reduced the content of glucosinolates, phytate and crude fiber by 43.1%, 42.4% and 25.5%, respectively, without loss of essential nutrients [77]. Subsequent reports have shown that fermentation of rapeseed meal with mixed microorganisms (*Bacillus licheniformis*, *Yeast* and *Lactobacillus*) can degrade most rapeseed meal proteins into small peptides with molecular weights less than 9.5 kDa, which significantly improves the sensory properties and bioavailability of rapeseed meal [69]. In the study of the fermentation of rapeseed meal with lactic acid bacteria, an increase in organic acid content was also found [78,90]. Xu et al. and Taheri et al. [80,92] reported that dietary fermented rapeseed meal supplementation had beneficial effects on the growth performance and egg quality of poultry. These changes indicate increased nutrient utilization of the rapeseed meal. There is evidence suggesting that fermented rapeseed meal had a higher apparent metabolizable energy value and standardized ileal digestibility of amino acids compared to unfermented rapeseed meal in broilers [90]. Moreover, Ashayerizadeh et al. [78] found that fermented rapeseed meal could effectively reduce the colonization of *Salmonella typhimurium* in broilers and also showed potential as an anti-stress product.

4.3. Cottonseed Meal

Cottonseed meal is a byproduct of cottonseed oil extraction and is also considered to be a potential source of plant protein. However, its application in poultry diets is greatly limited by the presence of gossypol [93]. Solid-state fermentation is recognized as an effective method for reducing gossypol [81,82]. Additionally, the metabolites such as vitamins and organic acids produced in the fermentation process can improve the nutritional value of cottonseed meal [82]. Previous research has shown that fermented cottonseed meal has positive effects on the antioxidant capacity, intestinal development, growth performance and immune function of poultry [81]. Niu et al. [83] further demonstrated that fermented cottonseed meal can reduce abdominal fat content and subcutaneous fat thickness in broilers by regulating the metabolism of organic acids, fatty acids and amino acids.

5. Improving the Nutritional Value of PNM through Solid-State Fermentation

According to the previous description, we aim to achieve the following objectives by solid-state fermentation of PNM: (1) increase the application ratio of PNM in poultry feed while reducing the supplementation of commercial amino acids; (2) reduce the content of crude fiber and phytate in PNM; (3) reduce the contamination of pathogenic microorganisms in PNM during storage; and (4) obtain metabolites or bioactive substances with beneficial effects on poultry health. According to the nutritional characteristics of PNM and the reports of other plant protein sources, we believe that the expected objectives can be achieved by certain technical means.

5.1. Bio-Transformation and Bio-Conversion of PNM

PNM can provide good nutritional and environmental conditions for the reproduction of bacteria as a solid-state medium. Currently, there are few reports about the solid-state fermentation of PNM, but some research on the bio-modification or bio-conversion of PNM may provide some insights. Table 4 summarizes the related reports. PNM is a rich carbon source and can be used as a substrate to produce high-value products such as rhamnolipids through bio-conversion [94]. Using nitrogen sources from PNM to produce D-lactic acid and succinic acid is considered to be a more economical and efficient method [95,96]. In addition, many reports have shown that the conversion of peanut proteins into bioactive peptides is mainly achieved by hydrolysis or fermentation methods and that the resulting products have excellent antioxidant, immunomodulation, antimicrobial and anti-cancer properties [97,98]. Regarding the molecular weight size of peptides, 1–10 kDa had the highest antioxidant activity [99,100]. Wang et al. [101] fermented PNM with lactic acid bacteria and also found an increase in antioxidant activity, but they suggested that the mechanism might be related to the transformation of rutin in PNM to quercetin. Yang et al. [11] attempted to improve the nutritional and functional properties of PNM by liquid-state fermentation (80% moisture), and the results showed that the content of crude protein, acid-soluble oligopeptides, organic acids and *in vitro* digestibility of PNM were significantly improved after fermentation, and the content of amino acids was balanced by fermentation. In future studies, we hope to achieve the same effect through solid-state fermentation.

Table 4. Bio-transformation and bio-conversion of PNM.

Years	Preparation Method	Strain/Enzyme	Objective	Characteristic	Reference
2016	Fermentation	<i>Bacillus licheniformis</i>	Enhancement of nutritional and antioxidant properties	The nutritional properties and antioxidant capacity of PNM were enhanced	Yang et al. [11]
2011	Fermentation	<i>Bifidobacterium longum</i> <i>Lactobacillus casei</i> <i>Lactobacillus acidophilus</i> <i>Lactobacillus plantarum</i>	Produce fermented peanut flour	Antioxidant activity was increased	Wang et al. [101]
2012	Fermentation	<i>Aspergillus oryzae</i> <i>Aspergillus niger</i>	Produce antioxidant peptides	Peptide fraction of 3–10 kDa showed the highest antioxidant activity	Wei et al. [99]
2013	Fermentation	<i>Bacillus subtilis</i>	Produce antioxidant peptides	High antioxidant peptide activity was obtained	Zhang et al. [97]
2015	Fermentation	<i>Actinobacillus succinogenes</i>	Produce succinic acid	PNM can be used as an efficient and economical source of nitrogen	Shen et al. [96]
2023	Fermentation	<i>Pseudomonas aeruginosa</i>	Produce rhamnolipid	Produced rhamnolipid exhibited good physicochemical and antimicrobial activities	Zhao et al. [94]
2010	Hydrolysis	<i>Sporolactobacillus</i> sp.	Produce D-lactate	High D-lactate production	Wang et al. [95]
2011	Hydrolysis	Crude enzyme obtained from <i>Aspergillus oryzae</i>	Produce antioxidant peptides	Peptide fraction of 1–3 kDa showed the highest antioxidant activity	Su et al. [100]
2013	Hydrolysis	Alcalase from <i>Bacillus licheniformis</i>	Produce bioactive peptides	Bioactive peptides have a potential benefit for blood pressure regulation	White et al. [102]

5.2. Selection of Strains for Solid-State Fermentation of PNM

The screening of fermentation strains must comply with the principle of safety, and the selected strains cannot destroy the inherent balance of the ecological environment or produce toxic and harmful substances. In addition, strains for fermenting PNM must have the potential to decompose proteins and cellulose, degrade phytate and inhibit pathogenic microbial pollution, which requires a specific screening process to select the strains.

Bacteria and fungi are commonly used in the fermentation of plant protein sources. Studies have shown that bacteria usually have genes coding for plant cell wall-degrading enzymes and are more easily capable of utilizing cellulose as a carbon source than fungi, which is beneficial for the decomposition of fiber in PNM. In addition, bacteria are more suitable for industrial applications than fungi due to their advantages, such as fast growth rates, good resistance and ease of operation [103]. *Bacillus* spp. are famous for their production of various metabolic products, such as cellulase, amylase, lipase, vitamins and antimicrobial peptides, and especially for their important role in degrading peanut proteins. After entering the intestine, *Bacillus* spp. will consume a large amount of oxygen to maintain the anaerobic environment of the intestine, thus inhibiting the growth of aerobic pathogenic bacteria and ultimately maintaining the balance of microflora in the animal intestine [11]. Lactic acid bacteria are generally regarded as safe strains (GRAS), so they are often used for health-promoting purposes as probiotics, as well as widely used in the fermentation of plant protein sources [104]. During the fermentation process, organic acids are produced to reduce the pH of the feed and inhibit the growth of pathogenic bacteria [105]. Forestie et al. [106] found that lactic acid bacteria can inhibit the adhesion of pathogenic bacteria such as *Salmonella* and *Escherichia coli* to intestinal cells, modulate the immune response and protect the intestinal barrier.

5.3. Framework for Future Research

For the purpose of improving the nutritional value of PNM by solid-state fermentation, we provide an action framework and strategies (Figure 4). The following steps were designed and completed in sequence: (1) screening and characterization of lactic acid bacteria based on acid production capacity, antibacterial activity against selected pathogens and antioxidant capacity; (2) screening and characterization of *Bacillus* spp. based on the ability to produce multi-enzymes, including protease, cellulase and phytase. It should be noted that the screened strains need to be comprehensively tested to ensure that they do not have adverse biological characteristics, such as harmful biochemical effects, antibiotic resistance and virulence factors; (3) the fermentation process and nutritional value evaluation of PNM in broilers. Specifically, analysis of the changes in the nutritional composition of PNM after solid-state fermentation and determination of the effect of feeding broilers a diet of PNM and FPNM on ileal amino acid digestibility values and apparent metabolizable energy; and (4) the application of FPNM in the diets of broilers, particularly its effect on growth performance and meat quality.

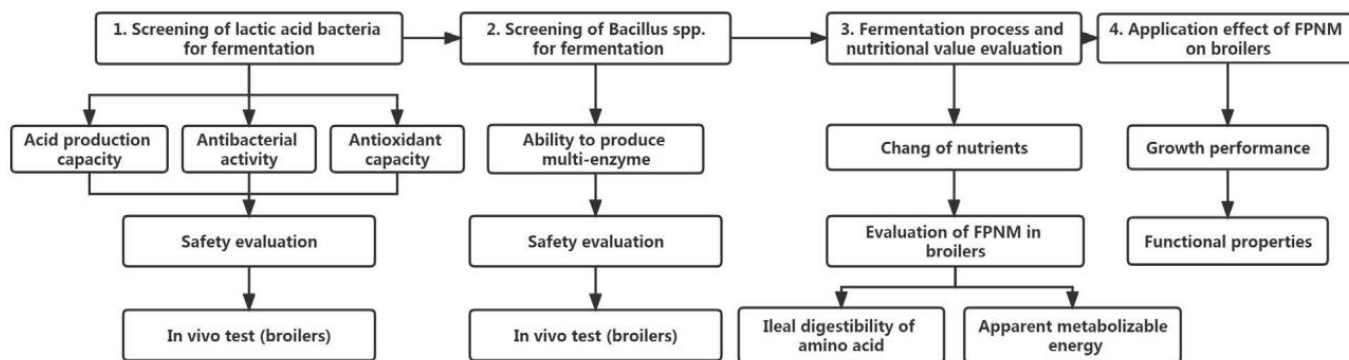


Figure 4. The technical route of the solid-state fermentation process of PNM and its application in broilers.

6. Conclusions and Perspectives

As reported in this review, enhancing the nutritional value of PNM through solid-state fermentation is technically challenging but feasible. It should be noted that further studies should primarily focus on improving amino acid imbalance, reducing phytate content and preventing microbial contamination. Obviously, the quality of the final fermented product is closely related to the fermentation microorganisms used. This suggests that we need to screen microorganisms based on the characteristics of PNM and ensure their biosafety. We expect that after solid-state fermentation, PNM will have a higher nutrient availability in poultry and an enhanced shelf life, while not excluding some enhanced functional characteristics, such as antioxidant capacity.

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