fermentation

MDPI

Article

Enhancing the Nutritional Quality of Defatted Cottonseed Meal by Solid-State Fermentation with Probiotic Microbes

Jicong Lin [1,2,3], Jingxian Zhang [1,2], Gen Zou [4], Xiaoling Zhang [1,2], Haihong Shang [5], Boyang Ji [6], Yueyu Bai [5], Lingbo Qu [2,5,*] and Yongjun Wei [1,2,3,*]

1 School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China
2 Laboratory of Synthetic Biology, Zhengzhou Research Base, National Key Laboratory of Cotton Bio-Breeding and Integrated Utilization, Zhengzhou University, Zhengzhou 450001, China
3 Center for Lipid Biosynthetic Engineering, Muyuan Laboratory, 110 Shangding Road, Zhengzhou 450016, China
4 Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Southern Key Laboratory of Edible Fungus Resource Utilization, Ministry of Agriculture, National Engineering Research Center of Edible Fungi, Shanghai 201403, China
5 Zhengzhou Research Base, National Key Laboratory of Cotton Bio-Breeding and Integrated Utilization, School of Agricultural Sciences, Zhengzhou University, Zhengzhou 450001, China
6 BioInnovation Institute, Ole Maaløes Vej 3, DK2200 Copenhagen N, Denmark
* Correspondence: qlb@zzu.edu.cn (L.Q.); yongjunwei@zzu.edu.cn (Y.W.)

Abstract: Defatted cottonseed meal (DCSM), a byproduct of the cotton industry, is highly regarded for its high protein content, making it a source of nutrients in animal feed. Traditional physical and chemical treatments of DCSM can lead to a reduction in nutrient content and the presence of residual organic solvents. Probiotic fermentation of DCSM offers several advantages, including degradation of anti-nutritional factors, an increase in nutrient content, and production of beneficial metabolites. This study employed probiotic fermentation of DCSM using a probiotic microbe collection composed of *Saccharomyces cerevisiae*, *Enterococcus faecium*, and *Lactiplantibacillus plantarum*. This fermentation process significantly enhanced the nutritional quality of DCSM. Specifically, the contents of crude protein, free amino acid, total phosphorus, and moisture increased by 1.14-fold, 1.14-fold, 1.24-fold, and 3-fold, respectively. In the meanwhile, there was a substantial reduction in the content of dry matter, crude ash, and crude fat, with decreases of 27.83%, 25.74%, and 88.23%, respectively. Probiotic fermentation of DCSM resulted in an overall enhancement of the palatability of DCSM. This study provides valuable insights into the potential of mixed probiotic fermentation as a promising approach for improving the nutritional quality of DCSM.

Keywords: Defatted cottonseed meal; Probiotics; Anaerobic fermentation; Amino acids; Nutritional quality

Citation: Lin, J.; Zhang, J.; Zou, G.; Zhang, X.; Shang, H.; Ji, B.; Bai, Y.; Qu, L.; Wei, Y. Enhancing the Nutritional Quality of Defatted Cottonseed Meal by Solid-State Fermentation with Probiotic Microbes. *Fermentation* **2024**, *10*, 429. https://doi.org/10.3390/fermentation10080429

Academic Editor: Katarzyna Pobiega

Received: 8 July 2024
Revised: 9 August 2024
Accepted: 14 August 2024
Published: 17 August 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Cotton, a globally cultivated crop, yields approximately 44 million tons of cottonseed annually. After the extraction of edible oils and the removal of hulls and lint, an estimated 15 million tons of defatted cottonseed meal (DCSM) are generated [1]. DCSM contains amino acids, fiber, and other chemicals [2]. With the increasing demand for sustainable animal feed ingredients, DCSM is highly favored for its wide availability and renewable characteristics. DCSM is rich in a variety of essential and non-essential amino acids. DCSM contains some minerals, such as calcium, phosphorus, and magnesium, which contribute to the skeletal growth and metabolic processes of animals [3].

Due to the increasing demand for animal protein from a growing population and the limited availability of commonly used feedstuffs like soybean meal (SBM), there is great interest in exploring alternative proteins to support the poultry industry [4]. Compared to

SBM and other feed ingredients, DCSM is cost-effective, making it an attractive alternative for animal feed [5]. The presence of anti-nutritional factors has restricted the application of DCSM. These anti-nutritional factors include phytic acid and free gossypol (FG). These factors may inhibit the absorption of nutrients and affecting feed digestibility, which potentially leads to a decrease in growth performance. Additionally, some anti-nutritional factors may interact with other components in the feed, impacting the health and production performance of animals [6].

Probiotics refer to a category of beneficial active microorganisms, typically bacteria or yeast, that can inhabit the host's intestinal tract after ingestion and elicit positive physiological effects [7]. Probiotics are often added to food or feed to promote balance of the gut microbiota, enhance immune system function, improve the absorption of nutrients, and enhance digestive health. Probiotics are widely employed in the production of functional dairy products, which offer significant health benefits, such as enhanced intestinal health and modulation of the immune system, primarily through the action of lactic acid bacteria [8]. The fermentation process not only preserves and enhances the viability of these microorganisms, but also ensures the retention of their probiotic properties. Additionally, probiotic fermentation has been extensively used to increase the value of agricultural byproducts, such as DCSM [9], brewers' spent grains (BSG) [10], and yellow wine lees [11].

Pretreatment of biomass followed by fermentation has proven to be an effective method for producing high-value compounds, including organic acids, amino acids, and biofuels. Moreover, the probiotic fermentation of herbal medicines has been proven to increase bioactive natural product contents and pharmaceutical activities [12–15]. Probiotics possess the capacity to degrade anti-nutritional components, augment nutrient content, and generate advantageous metabolites. *Saccharomyces cerevisiae* exhibits multiple beneficial characteristics, including production of extracellular polysaccharides to promote intestinal health, generation of beneficial metabolites to maintain microbial balance, inhibition of harmful bacterial growth, exertion of antioxidative properties for scavenging free radicals, and enhancement of immune function through immune system modulation. These features make *S. cerevisiae* an excellent probiotic that contributes to the overall health of the host. Furthermore, *S. cerevisiae* exhibits relatively strong tolerance to certain stresses and adverse environmental conditions. This capability enables it to maintain excellent fermentation performance under different conditions [16].

S. cerevisiae has been widely used in feed fermentation due to its ability to produce enzymes that degrade fiber and phytic acid, thereby increasing the digestibility of feed [17]. Moreover, engineered *S. cerevisiae* has been widely used in the production of foods and drugs [18–22]. *Enterococcus faecium*, a lactic acid bacterium, is known for its ability to improve the gut microbiota, enhance the immune response, and increase the digestibility of nutrients in feed. It is an important feed additive in livestock production, showing promising prospects for animal growth. For instance, *E. faecium* SF68 can be used as a probiotic supplement in veterinary medicine [23]. Feeding weaned piglets with fermented SBM using *L. plantarum*, *Bacillus subtilis*, and *S. cerevisiae* improved their growth, immune function, and intestinal health [24]. Utilizing *S. cerevisiae* and lactic acid bacteria like *Enterococcus faecium* effectively enhances feed digestibility and nutrient absorption, leading to improvements in animal growth performance and immune function. Moreover, incorporating these microorganisms into animal husbandry practices enhances feed efficiency and promotes animal health. This indicates that fermented feed represents a highly effective strategy for meeting the nutritional needs of animals and boosting production efficiency.

Compared to fermentation using a single strain, utilizing a mixture of strains in feed fermentation has various advantages [25]. Fermentation with multiple microbes promotes the degradation of complex organic compounds in DCSM, thereby enhancing fermentation efficiency. Different strains may possess distinct enzyme systems, collectively participating in substrate degradation and generating a more diverse range of beneficial metabolites, thus expanding the applicable scope of fermentation reactions. Additionally, fermentation with multiple microbes helps maintain microbial balance, reduce the growth

of harmful microorganisms, and improve the purity and quality of fermentation products. Through the rational selection and combination of strains, targeted improvements in specific components of DCSM can be achieved, meeting diverse production requirements and enhancing the biological utilization of nutrients. Fermentation of DCSM with multiple microbes exhibits significant advantages in improving fermentation efficiency, optimizing product quality, and broadening the adaptability range of substrate degradation [26]. Therefore, the utilization of mixed strain fermentation has the potential to enhance the nutrient content of DCSM.

In this study, a probiotic microbe collection comprising *S. cerevisiae*, *E. faecium*, and *L. plantarum* was used to ferment DCSM. The impact of mixed probiotic fermentation on the nutritional quality of DCSM was evaluated (Figure 1).

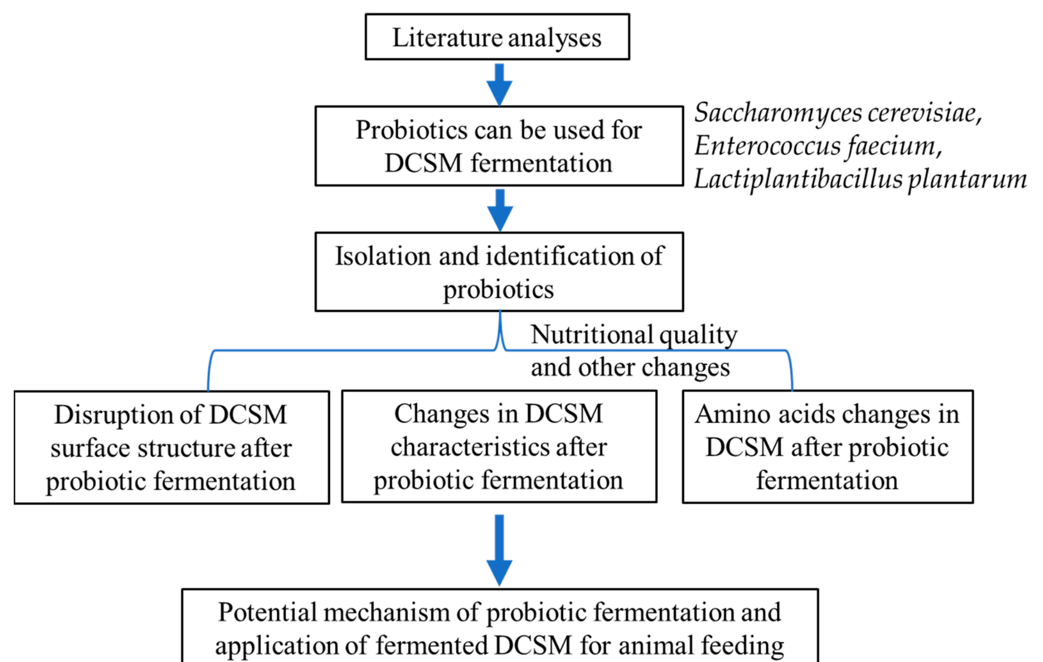

Figure 1. The scheme of this study.

2. Materials and Methods

2.1. Materials and Reagents

The DCSM used in this study was purchased from Western research institute, CAAS. The lysis buffer used for microbial lysis was purchased from Takara Biomedical Technology Co. Ltd. (Beijing, China). The 2 × Magic Green Taq Super Mix was purchased from Tolo Biotech Co. Ltd. (Chu Zhou, China).

2.2. Isolation and Identification of Strains

All the strains used in this study were isolated from the fecal microbiota of healthy cows in Weihui City, Henan Province. The fecal samples were carefully transferred into sterile centrifuge tubes and moved to the laboratory. The samples were stored at −4 °C before use. *S. cerevisiae* was isolated using YPD medium at 30 °C and named *S. cerevisiae* LBC-2. The yeast were then lysed at 80 °C for 15 min using lysis buffer for microorganisms to direct PCR, in order to release genomic DNA for ITS amplification. The ITS amplification program consisted of 95 °C for 3 min, followed by 30 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 42 s, and finally 72 °C for 5 min.

E. faecium and *L. plantarum* were isolated using MRS medium at 37 °C, and they were named *E. faecium* JZ-1 and *L. plantarum* DC-1. The 16S rRNA amplification process (the amplification primers are 27 F and 1492 R) consisted of 95 °C for 3 min, 27 cycles of 95 °C

for 10 s, 55 °C for 10 s, and 72 °C for 90 s, and then 72 °C for 5 min. The PCR fragments were purified and sequenced at Beijing Tsingke Biotech Co., Ltd., (Beijing, China). By conducting BLAST analysis, the species of these two strains were determined. The phylogenetic tree was constructed using MEGA11 software [27].

The obtained ITS and 16S rRNA gene sequences were deposited in the Genbank database with the accession numbers OR910533, OR910572, and OR910603.

2.3. Strains and DCSM Solid-State Fermentation

The *S. cerevisiae* LBC-2 culture was inoculated in YPD liquid medium and incubated at 30 °C for 48 hours at 200 rpm. The *E. faecium* JZ-1 and *L. plantarum* DC-1 cultures were inoculated in MRS liquid medium and cultured at 37 °C for 24 hours. The concentrations of the microbial suspensions were adjusted to 10^9 CFU/mL. The DCSM was then inoculated with 10^9 CFU/mL of the *S. cerevisiae*, *E. faecium*, and *L. plantarum* cultures (v:m = 1:0.5) to create the fermentation experimental group (FCP). The inoculated DCSM was put into fermentation bags with air holes and fermented at 28 °C for 5 days. The unfermented DCSM was served as the control (CT) group, and triplicates were performed for each group.

2.4. Chemical Composition Analysis of DCSM

All samples were pretreated using the acid hydrolysis method specified in China GB/T 18246–2019. Specifically, the samples were pretreated by drying and grinding, mixing with 6 M HCl, and hydrolyzing at 110 °C for 24 h. After hydrolysis, the mixture was rotary-evaporated and re-solubilized in sodium citrate buffer. Samples were taken before and after fermentation, and the moisture content, dry matter content, crude protein content, crude fat content, crude ash content, calcium content, total phosphorus content, and amino acid composition were determined. The content of crude protein was determined using the Kjeldahl method [28]. The content of dry matter and crude fat was determined following the methods described by Sinkovič et al. The sample is firstly digested with concentrated sulfuric acid to convert organic nitrogen into ammonium sulfate. Next, ammonia is released through alkalization and distillation, and absorbed into a boric acid solution. Finally, the absorbed ammonia is titrated with a standard acid solution to calculate the total nitrogen content of the sample. The crude protein content is then determined by multiplying the total nitrogen content by a conversion factor of 6.25 [29]. The content of crude ash was determined using the methods described by Lee et al. [30]. The sample is placed in a porcelain crucible of known weight, then the crucible is placed in a high-temperature furnace and burned at 600 °C for 4 h. After cooling, the ash weight of the sample is obtained by subtracting the weight of the crucible from the total weight. The amino acid composition was determined using an automated amino acid analyzer. Calcium in the treated filtrate was titrated using a standard solution of ethylenediaminetetraacetic acid (EDTA), and the calcium content of the sample was calculated based on the volume of EDTA consumed.

2.5. Analysis of Changes in Fermented DCSM Surface Features

After applying gold deposition treatment to CT and FCP samples, a Zeiss Sigma 300 field-emission scanning electron microscope (SEM) and Oxford Energy Spectroscopy were used to observe their surface features.

2.6. Statistical Analysis

The experiments were performed in triplicate, and GraphPad Prism 8.0 was used to analyze the data. The results are expressed as mean ± standard deviation (S.D.). Student's t test was used to compare the two groups, and a value of * $p < 0.05$ was considered significant.

3. Results

3.1. Isolation of Microbial Strains Used for DCSM Fermentation

The ITS sequences and 16S rRNA sequences of the isolated microorganisms were aligned with selected known gene sequences in the Genbank database. Following BLAST analysis, LBC-2 showed the highest homology with *S. cerevisiae*, JZ-1 exhibited the highest homology with *E. faecium*, and DC-1 showed the highest homology with *L. plantarum*. The homology of all strains reached 100%, with an E-value of 0, indicating statistical significance. In the phylogenetic tree, strains from different genera clustered together, with LBC-2 being closest to *S. cerevisiae*, JZ-1 closest to *E. faecium*, and DC-1 closest to *L. plantarum*. Based on sequence homology comparison and phylogenetic analysis, these three strains were identified as *S. cerevisiae*, *E. faecium*, and *L. plantarum*, respectively (Figure 2).

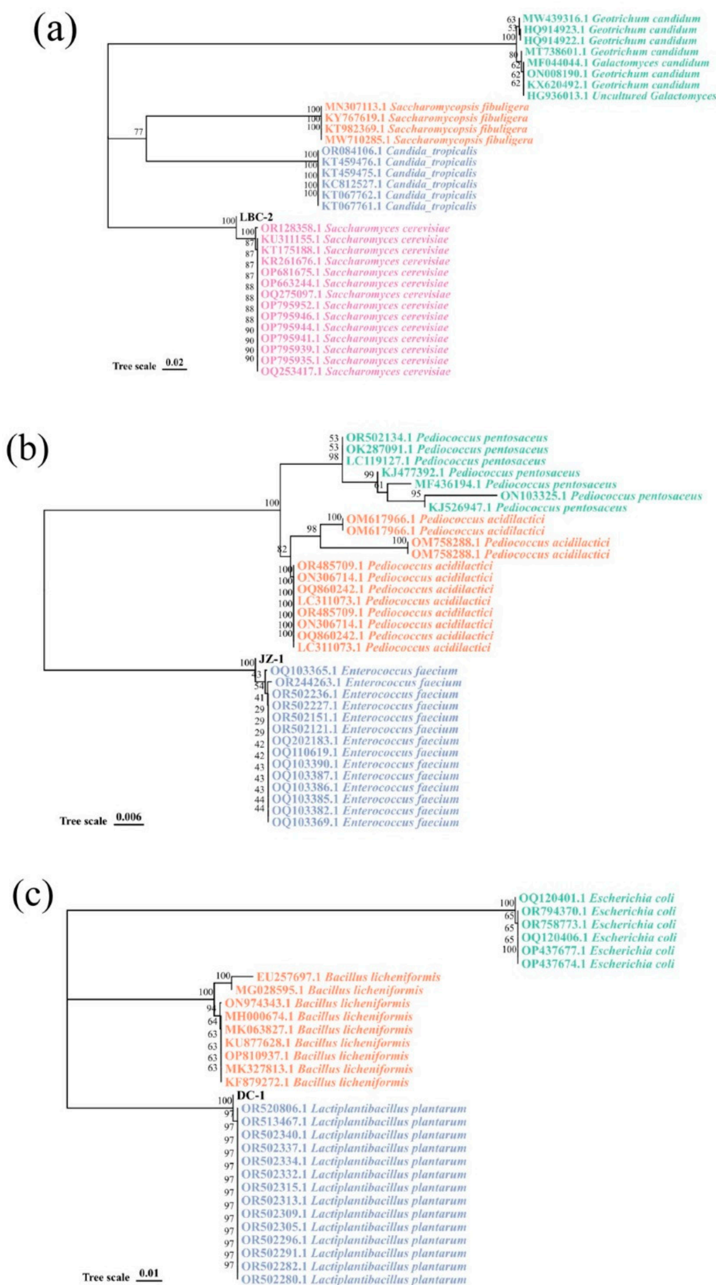

Figure 2. Phylogenetic trees of the isolated microbial strains. (**a**) *SS. cerevisiae* LBC-2; (**b**) *E. faecium* JZ-1; (**c**) *L. plantarum* DC-1.

3.2. Changes in DCSM Surface Features

The SEM images of the DCSM before and after fermentation were different (Figure 3). At 1000× magnification, the unfermented group consisted of laminated, bar-shaped structures that had smooth surfaces and relatively neat edges. There was a clear separation between nearby structures (Figure 3a). In contrast, at 1000× magnification, the surface and edges of the structures in the fermented group looked irregular. The probiotics might break down certain components of the DCSM during the fermentation process, especially the soluble fibers, leading to changes in the microstructure of the fiber surface. Some bumps with different shapes were distributed between and on the fibers, which might be due to fermentation by the probiotic microbe collection (Figure 3b). At 5000× magnification, the unfermented DCSM displayed a uniform and flat microstructure with clear contours and relatively regular morphology (Figure 3c). The surface of DCSM fermented with probiotics had depressed and swollen areas, and the fermentation process may have led to the structural changes, suggesting that probiotic fermentation might degrade the biomass in DCSM and disrupt the fundamental structure of DCSM (Figure 3d). The main differences between unfermented and fermented DCSM were surface texture and overall structural integrity. Unfermented DCSM maintained a neater and stronger fiber structure, whereas fermented DCSM exhibited a surface that had become irregular and had reduced connectivity between fibers. This difference further suggests that the DCSM structure had been disrupted.

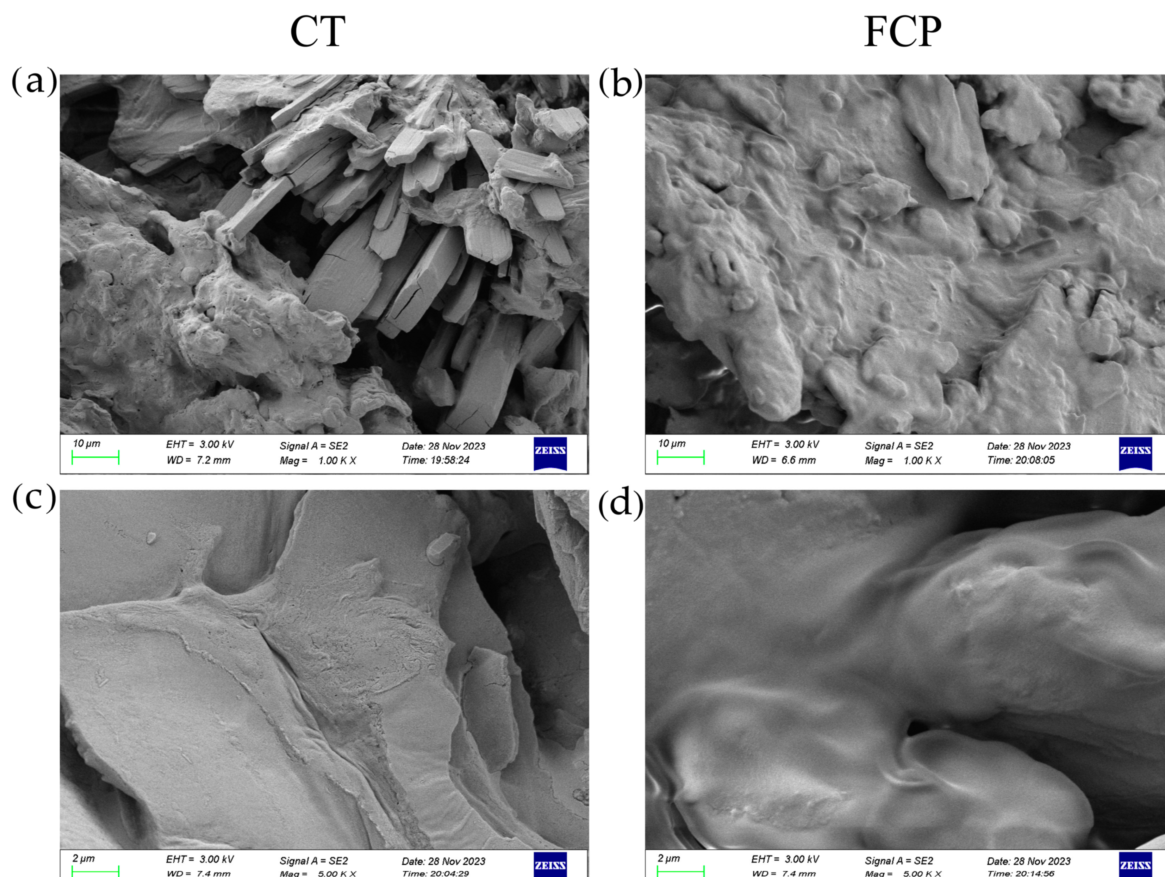

Figure 3. SEM images of the DCSMs. (**a**,**c**) represent the control groups at magnifications of 1000× and 5000×, respectively; (**b**,**d**) represent the fermented groups at magnifications of 1000× and 5000×, respectively.

3.3. Changes in DCSM Characteristics

Compared to the control group, the contents of moisture (339.7 g/kg), crude protein (361.7 g/kg), total phosphorus (9.1 g/kg), and amino acids (310.7 g/kg) of the FCP group increased by 3-fold, 1.14-fold, 1.24-fold, and 1.14-fold, respectively, after 5 days of fermentation with *S. cerevisiae*, *E. faecium*, and *L. plantarum* (Figure 4a,c,g,h). The contents

of dry matter (660.3 g/kg), crude fat (10.7 g/kg), and crude ash (50 g/kg) were reduced by 27.83%, 88.23%, and 25.74% (Figure 4b,d,e), respectively. Meanwhile, there was no significant change in calcium content (Figure 4f).

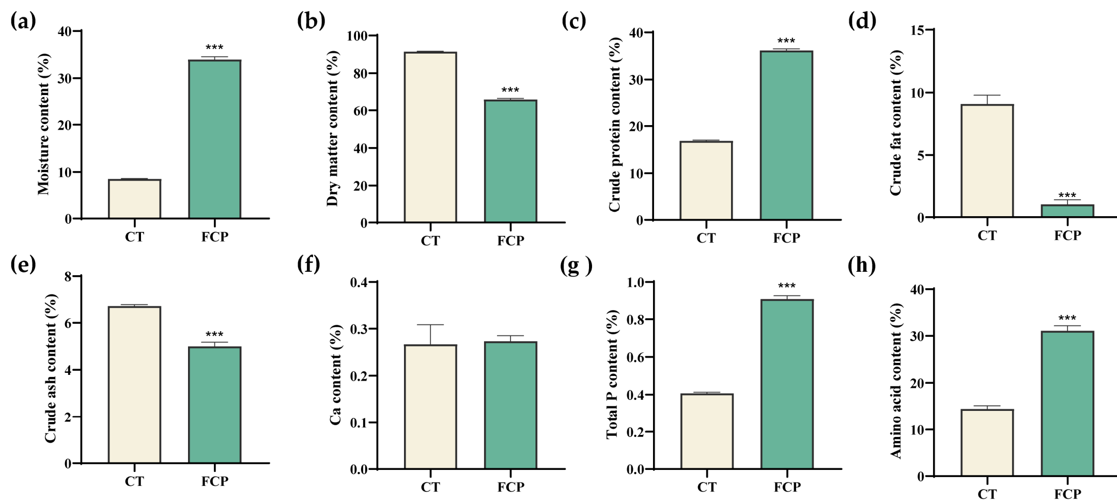

Figure 4. Changes in moisture (**a**), dry matter (**b**), crude protein (**c**), crude fat (**d**), crude ash (**e**), calcium (**f**), total phosphorus (**g**), and amino acid (**h**) content in the control and FCP groups before and after fermentation (*** $p < 0.001$).

3.4. Changes in Amino Acids in FCP

A significant increase in the levels of 17 amino acids after 5 days of fermentation with *S. cerevisiae*, *E. faecium*, and *L. plantarum* was observed compared to the control (CT) group. The total amino acid content increased 1.14-fold, with essential amino acids and non-essential amino acids increasing 1.2-fold and 1.24-fold, respectively. Glutamic acid (6.88%), arginine (3.6%), and aspartic acid (3.22%) were the major amino acids both before and after fermentation (Figure 5b). Especially, the methionine content in the fermented product significantly increased by approximately 1.83-fold compared to the CT group, representing the highest multiplicative change (Figure 5a). Additionally, the levels of phenylalanine (Figure 5a) and tyrosine (Figure 5b) were both increased by 1.34-fold, and this fold increase was the next highest compared to CT.

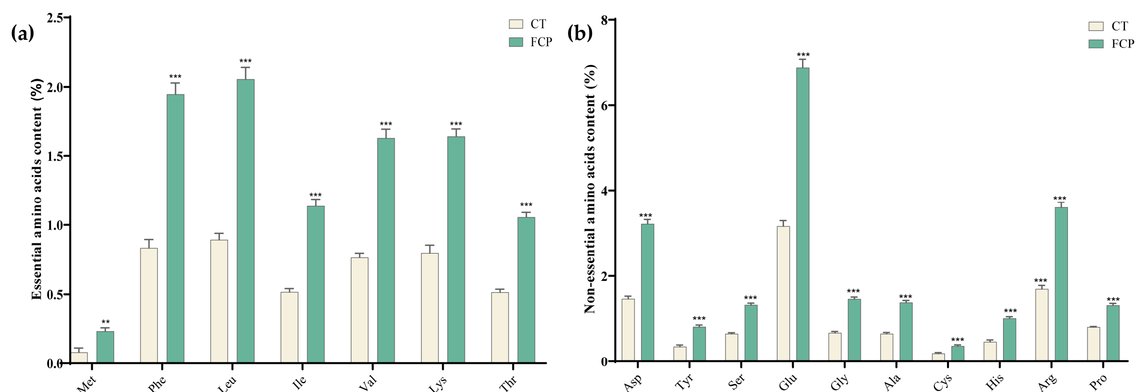

Figure 5. Changes in amino acids in FCP. (**a**) Essential amino acid changes; (**b**) Non-essential amino acid changes (** $p < 0.01$, *** $p < 0.001$).

4. Discussion

During probiotic fermentation, *L. plantarum* may secrete ferulic acid esterase and other hydrolases with similar functions to act on the cell wall, cutting off the cross-links between

polysaccharide–polysaccharide and polysaccharide–lignin in the cell wall and destroying the original surface features [31]. Consequently, probiotic fermentation of DCSM offers a possible method for enhancing its nutritional value through the release of bioactive substances and the degradation of anti-nutrient compounds. Thus, irregular changes on the surface of DCSM may be probiotic hydrolysis of polysaccharide components such as cellulose, hemicellulose, and lignin. This enzymatic degradation not only increases the nutrient density of DCSM, but also potentially improves its digestibility in the animal digestive system and enhances the overall feed value of DCSM.

DCSM contains high nutritional substances, but the presence of anti-nutritional factors and crude fiber has a negative impact on animal growth performance and nutrient digestibility [32]. Probiotic solid-state fermentation of DCSM can enhance its nutritional value and improve feed palatability [5]. During solid-state fermentation, microorganisms secrete hydrolytic enzymes such as cellulases, which utilize carbohydrates in DCSM as a nutrient source for their growth and reproduction [33]. The fermentation process significantly reduces the dry matter content of DCSM and increases the moisture content of DCSM (mainly derived from the inoculated medium). Despite the moisture content of FCP tripling, the crude protein content (361.7 g/kg) increased by 1.14-fold. While there may be some protein in the medium, the levels are relatively low, showing the increased protein content directly originating from the growth of microorganisms utilizing the nutrients in the DCSM as a substrate. Decomposition of crude fat into free fatty acids in DCSM further improves digestion and absorption by animals. The increase in free fatty acids provides an additional source of energy, contributing to the maintenance of daily metabolic activities in animals [34].

Reducing crude ash content in feeds can effectively improve the absorption efficiency of nutrients in the digestive system of animals, reduce the intestinal tract burden caused by high crude ash, improve the health of the digestive tract, improve the taste of feeds, and increase the appetite and intake by animals [35,36]. The increase in total phosphorus after fermentation may be due to phosphorus in the MRS medium. The total phosphorus content of the feed promotes bone growth and density, contributing to the overall health and growth of the animal [37]. The characteristics of different probiotics vary, and the conditions and methods of fermenting DCSM are different. We employed anaerobic co-fermentation of yeast and lactic acid bacteria to process DCSM. In this situation, yeast metabolites, such as amino acids, play a promoting role in the growth and accumulation of prebiotic metabolites in lactic acid bacteria. Lactic acid bacteria have the ability to hydrolyze lactose into galactose and glucose, with galactose serving as a novel carbon source for the growth of yeast [38]. The symbiotic interaction between yeast and lactic acid bacteria in the probiotic microbe collection forms a beneficial cycle, resulting in the production of various metabolites and a significant improvement in anaerobic fermentation efficiency [39].

During the fermentation process of DCSM, *S. cerevisiae* may synthesize specific proteins, including extracellular enzymes released into the fermentation medium. This process results in an increased crude protein content in the feed. The amino acid levels in FCP surpassed those in CT, with essential and non-essential amino acids exhibiting 1.2-fold and 1.24-fold increases, respectively. Additionally, *L. plantarum* has the capability to synthesize proteinases that facilitate protein degradation and amino acid generation. This suggests that the presence of probiotics enhances the rate of protein degradation in DCSM, leading to the release of a greater quantity of amino acids [40,41]. The increase in amino acid content in fermented feed brings numerous benefits to animals. Arginine can regulate vascular generation and development in sows, thereby influencing potential placental vascular formation to provide more nutrients and oxygen supply to the fetus. Adding L-arginine to the diet of pregnant sows significantly increases the average weight of piglets and reduces the concentration of triglycerides in the pregnant sow's body [42]. Adding glutamic acid and aspartic acid to the diet can influence the glucose and lipid metabolic pathways in piglets, improve liver lipid metabolism, and provide greater support for piglet growth and development after weaning [43].

The synergistic action of probiotics and hydrolases has a beneficial impact on plant biomass fermentation. DCSM contains pectin and hemicellulose, which are the main components of cellulose found in the cell walls. Pectinolytic enzymes can break down complex pectin molecules into various oligomeric pectins, fructose, and other monosaccharides [44]. Xylanase, through the hydrolysis of 1,4-β-xylose linkages in the xylan backbone, results in the production of xylo-oligosaccharides (XOSs) [45]. The increased production of monosaccharides serves as a carbon source for the growth of probiotics. XOSs act as one kind of prebiotic with various bioactivities, such as anti-inflammatory, antioxidant, and anti-tumor effects [46]. Lignocellulose can be effectively degraded by diverse lignocellulases, including pectinolytic enzymes and xylanases [47]. The combined use of probiotics and lignocellulases would accelerate substrate degradation and release a more abundant pool of nutrients. Furthermore, sulfite pretreatment can alter lignin structure, and the use of lignin blockers enhances enzymatic hydrolysis of lignocellulosic biomass, thereby improving the subsequent fermentation process and increasing the yield of desired product [48]. Therefore, degradation of pretreated biomass will be accelerated and richer nutrients will be released through the combined use of probiotics and lignocellulases. Furthermore, the collaborative action of probiotics and lignocellulases degrades complex substrate structures, facilitating a more comprehensive breakdown and yielding a diverse array of metabolites, thus expanding the diversity of fermentation products.

Incorporating mixed fermented feed with *E. faecium* and *B. subtilis* into the diet of fattening pigs significantly enhances the average daily weight gain of sows and increases levels of flavor amino acids and unsaturated fatty acids in pork. This indicates that supplementing fermented feed can improve pork quality [49]. Feeding sows with probiotic fermented liquid can increase the levels of IgA, IL-10, and interferon-α in the offspring piglets [50]. Different combinations of probiotics may have varying probiotic effects, and future efforts should explore diverse combinations to comprehensively enhance the application potential of probiotic-fermented DCSM.

In the future, the identification and exploration of probiotics within traditional fermented food microbiota, as well as the genetic engineering of yeast or other probiotics [51–55], holds the potential to facilitate the efficient biotransformation of DCSM and other plant biomass [56]. This bio-transformative process would result in the production of nutritional and safe feedstocks for animals. Utilizing fermented DCSM or other fermented plant biomass as feed would contribute to the healthy development of cultivated animals, promoting their overall well-being and growth.

5. Conclusions

In this study, DCSM underwent synergistic fermentation with *S. cerevisiae*, *E. faecium*, and *L. plantarum*. The fermented DCSM exhibited an increase in crude protein, calcium, total phosphorus, and amino acid levels. The levels of all 17 amino acids exhibited a significant increase, with essential and non-essential amino acids showing 1.2-fold and 1.24-fold increases, respectively. Through optimized solid-state anaerobic fermentation with a probiotic microbe collection, we have elevated the nutritional quality of DCSM, a low-value byproduct of cotton (Figure 6). To further improve our understanding of the potential applications of the fermented DCSM, it is necessary to evaluate the degradation rate of its anti-nutritional factors and explore its antioxidant activity. In addition, the digestibility and absorption rates post-consumption by animals should be investigated (Figure 6). To summarize, this study highlights the significant potential of mixed probiotic fermentation for improving the nutritional quality of DCSM. This study not only contributes to a better understanding of innovative fermentation approaches, but also identifies a valuable use for DCSM or other agricultural byproducts in the animal feed industry. These findings offer valuable insights that could have significant implications for the development of sustainable agricultural practices and related industries.

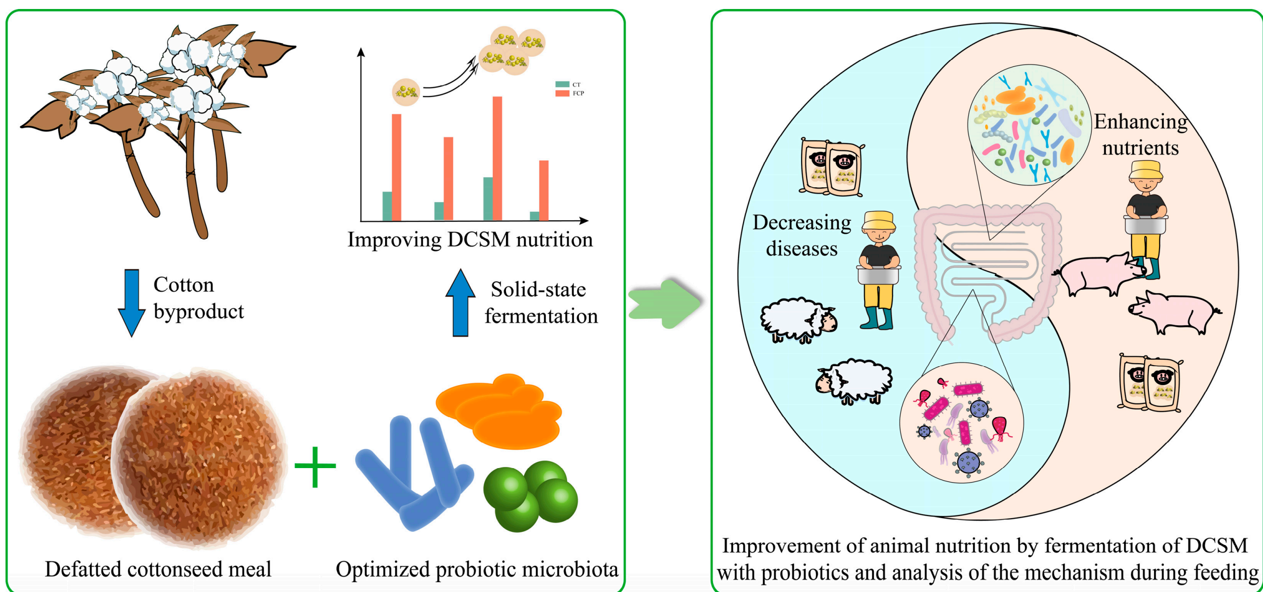

Figure 6. Improved nutritional quality of defatted cottonseed meal fermented by optimized probiotic flora, and analysis of the mechanism.

Author Contributions: Y.W., Y.B., B.J. and L.Q. conceived the study. J.L., J.Z., X.Z., Y.B., G.Z. and Y.W. performed the experiments and drafted the manuscript. H.S. provided the cottonseed meal and discussed the results. Y.W., B.J., Y.B., G.Z. and X.Z. revised and polished the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Zhengzhou Research Base, National Key Laboratory of Cotton Bio-breeding and Integrated Utilization (No. CBIU2023ZZ06, CB2024A30, and CBIU2023ZZ09), Muyuan Laboratory (No. 12106022401), and the National Natural Science Foundation of China (No. 32111530179).

Data Availability Statement: All of the sequence data from this study have been submitted to the Genbank database or presented within the manuscript itself.

Acknowledgments: We thank Henan Muyi Animal Pharmaceutical Co., Ltd. for fermentation help. During the preparation of this work, the authors used ChatGPT to polish the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Prawirodigdo, S.; Gannon, N.J.; Leury, B.J.; Dunshea, F.R. Basal diet and indigestible marker influence apparent digestibilities of nitrogen and amino acids of cottonseed meal and soybean meal in pigs. *Anim. Nutr.* **2019**, *5*, 234–240. [CrossRef] [PubMed]
2. He, Z.; Zhang, H.; Olk, D.C. Chemical Composition of Defatted Cottonseed and Soy Meal Products. *PLoS ONE* **2015**, *10*, e0129933. [CrossRef] [PubMed]
3. Xu, X.A.; Yang, H.M.; Yang, Z.; Wang, Z.Y. Effect of heating time of cottonseed meal on nutrient and mineral element digestibility in chicken (based on cottonseed meal replaced with all soybean meal). *Animals-Basel* **2022**, *12*, 883. [CrossRef] [PubMed]
4. Olukomaiya, O.; Fernando, C.; Mereddy, R.; Li, X.; Sultanbawa, Y. Solid-state fermented plant protein sources in the diets of broiler chickens: A review. *Anim. Nutr.* **2019**, *5*, 319–330. [CrossRef] [PubMed]
5. Yusuf, H.A.; Rehemujiang, H.; Ma, T.; Piao, M.; Huo, R.; Tu, Y. Fermented total mixed ration with cottonseed meal or rapeseed meal improved growth performance and meat quality of Hu lamb compared to total mixed ration with soybean meal. *Fermentation* **2022**, *8*, 576. [CrossRef]
6. Ma, M.; Ren, Y.; Xie, W.; Zhou, D.; Tang, S.; Kuang, M.; Wang, Y.; Du, S.K. Physicochemical and functional properties of protein isolate obtained from cottonseed meal. *Food Chem.* **2018**, *240*, 856–862. [CrossRef]
7. Zendeboodi, F.; Khorshidian, N.; Mortazavian, A.M.; da Cruz, A.G. Probiotic: Conceptualization from a new approach. *Curr. Opin. Food. Sci.* **2020**, *32*, 103–123. [CrossRef]

8. Kaur, H.; Kaur, G.; Ali, S.A. Dairy-Based Probiotic-Fermented Functional Foods: An Update on Their Health-Promoting Properties. *Fermentation* **2022**, *8*, 425. [CrossRef]
9. Zhang, Z.; Yang, D.; Liu, L.; Chang, Z.; Peng, N. Effective gossypol removal from cottonseed meal through optimized solid-state fermentation by *Bacillus coagulans*. *Microb. Cell Fact.* **2022**, *21*, 252. [CrossRef]
10. Mitri, S.; Salameh, S.-J.; Khelfa, A.; Leonard, E.; Maroun, R.G.; Louka, N.; Koubaa, M. Valorization of Brewers' Spent Grains: Pretreatments and Fermentation, a Review. *Fermentation* **2022**, *8*, 50. [CrossRef]
11. Zhang, Y.; Wang, C.; Su, W.; Jiang, Z.; He, H.; Gong, T.; Kai, L.; Xu, H.; Wang, Y.; Lu, Z. Co-fermented yellow wine lees by *Bacillus subtilis* and *Enterococcus faecium* regulates growth performance and gut microbiota in finishing pigs. *Front. Microbiol.* **2022**, *13*, 1003498. [CrossRef] [PubMed]
12. Zhang, X.; Miao, Q.; Pan, C.; Yin, J.; Wang, L.; Qu, L.; Yin, Y.; Wei, Y. Research advances in probiotic fermentation of Chinese herbal medicines. *iMeta* **2023**, e93. [CrossRef]
13. Zou, G.; Nielsen, J.B.; Wei, Y.J. Harnessing synthetic biology for mushroom farming. *Trends Biotechnol.* **2023**, *41*, 480–483. [CrossRef]
14. Zhang, X.; Tang, B.; Wen, S.; Wang, Y.; Pan, C.; Qu, L.; Yin, Y.; Wei, Y. Advancements in the Biotransformation and Biosynthesis of the Primary Active Flavonoids Derived from *Epimedium*. *Molecules* **2023**, *28*, 7173. [CrossRef]
15. Huang, S.; Shao, L.; Chen, M.; Wang, L.; Liu, J.; Zhang, W.; Huang, W. Biotransformation differences of ginsenoside compound K mediated by the gut microbiota from diabetic patients and healthy subjects. *Chin. J. Nat. Med.* **2023**, *21*, 723–729. [CrossRef]
16. Brandt, B.A.; García-Aparicio, M.D.P.; Görgens, J.F.; van Zyl, W.H. Rational engineering of *Saccharomyces cerevisiae* towards improved tolerance to multiple inhibitors in lignocellulose fermentations. *Biotechnol. Biofuels* **2021**, *14*, 173. [CrossRef] [PubMed]
17. Schlabitz, C.; Lehn, D.N.; de Souza, C.F.V. A review of *Saccharomyces cerevisiae* and the applications of its byproducts in dairy cattle feed: Trends in the use of residual brewer's yeast. *J. Clean. Prod.* **2022**, *332*, 130059. [CrossRef]
18. Peng, J.J.; Wang, L.; Wang, M.G.; Du, R.; Qin, S.S.; Jin, C.Y.; Wei, Y.J. Yeast synthetic biology for the production of *Lycium barbarum* polysaccharides. *Molecules* **2021**, *26*, 1641. [CrossRef]
19. Wei, Y.J.; Ji, B.Y.; Ledesma-Amaro, R.; Chen, T.; Ji, X.J. Editorial: Engineering yeast to produce plant natural products. *Front. Bioeng. Biotechnol.* **2021**, *9*, 798097. [CrossRef]
20. Lu, C.J.; Du, R.; Fu, H.; Zhang, J.Z.; Zhao, M.; Wei, Y.J.; Lin, W. Heterologous biosynthesis of medicarpin using engineered *Saccharomyces cerevisiae*. *Syn. Syst. Biotechno.* **2023**, *8*, 749–756. [CrossRef]
21. Wei, Y.J.; Ji, B.Y.; Siewers, V.; Xu, D.Y.; Halkier, B.A.; Nielsen, J. Identification of genes involved in shea butter biosynthesis from *Vitellaria paradoxa* fruits through transcriptomics and functional heterologous expression. *Appl. Microbiol. Biot.* **2019**, *103*, 3727–3736. [CrossRef] [PubMed]
22. Wang, M.G.; Wei, Y.J.; Ji, B.Y.; Nielsen, J. Advances in metabolic engineering of *Saccharomyces cerevisiae* for cocoa butter equivalent production. *Front. Bioeng. Biotech.* **2020**, *8*, 594081. [CrossRef] [PubMed]
23. Krawczyk, B.; Wityk, P.; Galecka, M.; Michalik, M. The Many aaces of *Enterococcus spp.*-commensal, probiotic and opportunistic pathogen. *Microorganisms* **2021**, *9*, 1900. [CrossRef] [PubMed]
24. Zhu, J.; Gao, M.; Zhang, R.; Sun, Z.; Wang, C.; Yang, F.; Huang, T.; Qu, S.; Zhao, L.; Li, Y.; et al. Effects of soybean meal fermented by *L. plantarum*, *B. subtilis* and *S. cerevisieae* on growth, immune function and intestinal morphology in weaned piglets. *Microb. Cell Fact.* **2017**, *16*, 191. [CrossRef] [PubMed]
25. Crognale, S.; Russo, C.; Petruccioli, M.; D'Annibale, A. Chitosan Production by Fungi: Current State of Knowledge, Future Opportunities and Constraints. *Fermentation* **2022**, *8*, 76. [CrossRef]
26. Zhang, M.Z.; Pan, L.Q.; Fan, D.P.; He, J.J.; Su, C.; Gao, S.; Zhang, M.Y. Study of fermented feed by mixed strains and their effects on the survival, growth, digestive enzyme activity and intestinal flora of *Penaeus vannamei*. *Aquaculture* **2021**, *530*, 735703. [CrossRef]
27. Xie, J.M.; Chen, Y.R.; Cai, G.J.; Cai, R.L.; Hu, Z.; Wang, H. Tree visualization by one table (tvBOT): A web application for visualizing, modifying and annotating phylogenetic trees. *Nucleic Acids Res.* **2023**, *51*, W587–W592. [CrossRef]
28. Fu, M.; Jahan, M.S.; Tang, K.; Jiang, S.Z.; Guo, J.X.; Luo, S.W.; Luo, W.L.; Li, G.H. Comparative analysis of the medicinal and nutritional components of different varieties of *Pueraria thomsonii* and *Pueraria lobata*. *Front. Plant Sci.* **2023**, *14*, 1115782. [CrossRef]
29. Sinkovic, L.; Dez, M.; Kopinc, R.; Meglic, V. Macro/microelements, nutrients and bioactive components in common and Tartary buckwheat (*Fagopyrum spp.*) grain and stone-milling fractions. *LWT-Food Sci. Technol.* **2022**, *161*, 113422. [CrossRef]
30. Lee, J.; Jo, N.Y.; Shim, S.Y.; Linh, L.T.Y.; Kim, S.R.; Lee, M.G.; Hwang, S.G. Effects of Hanwoo (Korean cattle) manure as organic fertilizer on plant growth, feed quality, and soil bacterial community. *Front. Plant Sci.* **2023**, *14*, 1135947. [CrossRef]
31. Xu, D.M.; Ding, Z.T.; Bai, J.; Ke, W.C.; Zhang, Y.X.; Li, F.H.; Guo, X.S. Evaluation of the effect of feruloyl esterase -producing *Lactobacillus plantarum* and cellulase pretreatments on lignocellulosic degradation and cellulose conversion of co -ensiled corn stalk and potato pulp. *Bioresource. Technol.* **2020**, *310*, 123476. [CrossRef]
32. Yusuf, H.A.; Piao, M.Y.; Ma, T.; Huo, R.Y.; Tu, Y. Effect of lactic acid bacteria and yeast supplementation on anti-nutritional factors and chemical composition of fermented total mixed ration containing cottonseed meal or rapeseed meal. *Anim. Biosci.* **2022**, *35*, 556–566. [CrossRef] [PubMed]
33. Gupta, S.; Lee, J.J.L.; Chen, W.N. Analysis of improved nutritional composition of potential functional food (Okara) after probiotic solid-state fermentation. *J. Agric. Food Chem.* **2018**, *66*, 5373–5381. [CrossRef]
34. Guzmán, M. Fatty acids fuelling astroglia and beyond. *Nat. Metab.* **2023**, *5*, 1253–1254. [CrossRef]

35. Naseem, S.; Bhat, S.U.; Gani, A.; Bhat, F.A. Perspectives on utilization of macrophytes as feed ingredient for fish in future aquaculture. *Rev. Aquacult.* **2021**, *13*, 282–300. [CrossRef]
36. Prawirodigdo, S.; Gannon, N.J.; Leury, B.J.; Dunshea, F.R. Acid-insoluble ash is a better indigestible marker than chromic oxide to measure apparent total tract digestibility in pigs. *Anim. Nutr.* **2021**, *7*, 64–71. [CrossRef] [PubMed]
37. Rieger, H.; Ratert, C.; Wendt, M.; Schwennen, C.; Kamphues, J. Comparative study on the chemical composition of different bones/parts of bones in growing pigs differently supplied with inorganic phosphorus and phytase. *J. Anim. Physiol. Anim. Nutr.* **2021**, *105*, 106–118. [CrossRef] [PubMed]
38. Sieuwerts, S.; Bron, P.A.; Smid, E.J. Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT-Food Sci. Technol.* **2018**, *90*, 201–206. [CrossRef]
39. Loh, L.X.; Ng, D.H.J.; Toh, M.Z.; Lu, Y.Y.; Liu, S.Q. Targeted and nontargeted metabolomics of amino acids and bioactive metabolites in probiotic-fermented unhopped beers using liquid chromatography high-resolution mass spectrometry. *J. Agric. Food Chem.* **2021**, *69*, 14024–14036. [CrossRef]
40. Li, X.; Tian, J.; Zhang, Q.; Jiang, Y.; Hou, J.; Wu, Z.; Yu, Z. Effects of applying *Lactobacillus plantarum* and Chinese gallnut tannin on the dynamics of protein degradation and proteases activity in alfalfa silage. *Grass Forage Sci.* **2018**, *73*, 648–659. [CrossRef]
41. Zhang, L.H.; Zha, M.M.; Li, S.F.; Zong, W. Changes on some quality characteristics of jujube juice with enzymatic hydrolysis prior to *Lactobacillus plantarum* fermentation. *J. Food Meas. Charact.* **2022**, *16*, 3196–3207. [CrossRef]
42. Fonseca, L.D.; Lanferdini, E.; Moreira, R.H.R.; Chaves, R.F.; Perazolli, P.H.; Paula, Y.H.D.; Renn, L.N.; Garbossa, C.A.P.; Cantarelli, V.D.; De, L.T. Arginine supplementation in the feed of gestating sows. *Livest. Sci.* **2022**, *263*, 104999. [CrossRef]
43. Qi, M.; Wang, J.; Tan, B.E.; Li, J.J.; Liao, S.M.; Liu, Y.H.; Yin, Y.L. Dietary glutamine, glutamate, and aspartate supplementation improves hepatic lipid metabolism in post-weaning piglets. *Anim. Nutr.* **2020**, *6*, 124–129. [CrossRef] [PubMed]
44. Miao, Q.; Zhang, X.; Wang, Y.; Li, X.; Wang, Z.; Tian, L.; Qu, L.; Wei, Y. Characterization of novel pectinolytic enzymes derived from the efficient lignocellulose degradation microbiota. *Biomolecules* **2022**, *12*, 1388. [CrossRef] [PubMed]
45. Zhang, X.; Miao, Q.; Tang, B.; Mijakovic, I.; Ji, X.-J.; Qu, L.; Wei, Y. Discovery of novel alkaline-tolerant xylanases from fecal microbiota of dairy cows. *Biotechnol. Biof. Biop.* **2023**, *16*, 182. [CrossRef] [PubMed]
46. Huang, C.X.; Yu, Y.X.; Li, Z.; Yan, B.W.; Pei, W.H.; Wu, H. The preparation technology and application of xylo-oligosaccharide as prebiotics in different fields: A review. *Front. Nutr.* **2022**, *9*, 996811. [CrossRef] [PubMed]
47. Wang, J.; Liang, J.W.; Li, Y.H.; Tian, L.M.; Wei, Y.J. Characterization of efficient xylanases from industrial-scale pulp and paper wastewater treatment microbiota. *Amb Express* **2021**, *11*, 19. [CrossRef] [PubMed]
48. Huang, C.; Li, R.; Tang, W.; Zheng, Y.; Meng, X. Improve Enzymatic Hydrolysis of Lignocellulosic Biomass by Modifying Lignin Structure via Sulfite Pretreatment and Using Lignin Blockers. *Fermentation* **2022**, *8*, 558. [CrossRef]
49. Liu, S.Q.; Du, M.; Tu, Y.; You, W.J.; Chen, W.T.; Liu, G.L.; Li, J.Y.; Wang, Y.Z.; Lu, Z.Q.; Wang, T.H.; et al. Fermented mixed feed alters growth performance, carcass traits, meat quality and muscle fatty acid and amino acid profiles in finishing pigs. *Anim. Nutr.* **2023**, *12*, 87–95. [CrossRef]
50. Wang, W.L.; Zijlstra, R.T.; Gänzle, M.G. Feeding *Limosilactobacillus fermentum* K9–2 and *Lacticaseibacillus casei* K9–1, or *Limosilactobacillus reuteri* TMW1.656 Reduces Pathogen Load in Weanling Pigs. *Front. Microbiol.* **2020**, *11*, 608293. [CrossRef]
51. Gao, X.; Zheng, T.; Yuan, X.L.; Li, Z.K.; Wei, Y.J. Draft genome sequence of an epiphytic strain, *Bacillus sp*. strain WL1, isolated from the surface of *Nostoc flagelliforme* Colonies in Yinchuan, Ningxia, China. *Microbiol. Resour. Announc.* **2021**, *10*, e0038221. [CrossRef] [PubMed]
52. Zou, G.; Li, B.; Wang, Y.; Yin, X.; Gong, M.; Shang, J.J.; Wei, Y.J.; Li, X.L.; Bao, D.P. Efficient conversion of spent mushroom substrate into a high value-added anticancer drug pentostatin with engineered *Cordyceps militaris*. *Green Chem.* **2021**, *23*, 10030–10038. [CrossRef]
53. Yuan, Y.Z.; Yang, Y.T.; Xiao, L.L.; Qu, L.B.; Zhang, X.L.; Wei, Y.J. Advancing insights into probiotics during vegetable fermentation. *Foods* **2023**, *12*, 3789. [CrossRef] [PubMed]
54. Wei, Y.; Li, X.; Ji, B.; Qu, L. Recent advances on the recovery, modulation and synthetic biology of gut microbiota and hosts. *Sci. Sin. Vitae* **2022**, *52*, 249–265. [CrossRef]
55. Lin, J.; Zou, G.; Liu, H.; Wei, Y. Application of CRISPR/Cas genome editing technology in the synthesis of secondary metabolites of filamentous fungi. *Synth. Biol. J.* **2023**, *4*, 738–755. [CrossRef]
56. Liu, Y.F.; Li, X.; Yang, Y.D.; Liu, Y.; Wang, S.J.; Ji, B.Y.; Wei, Y.J. Exploring gut microbiota in patients with colorectal disease based on 16S rRNA gene amplicon and shallow metagenomic sequencing. *Front. Mol. Biosci.* **2021**, *8*, 703638. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.