Postprint: Optimization of Solid-State Fermentation Conditions for Banana Stem-Leaf Powder and Its Nutrient Utilization Efficiency in Geese

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Date: 2017-10-23T00:00:00+00:00

Abstract

This study aimed to investigate the process parameters of solid-state fermentation technology for improving the nutrient composition of banana stem and leaf powder and the nutrient utilization efficiency by geese. Aspergillus oryzae and Candida utilis were selected as fermentation strains to systematically investigate the effects of five factors (single-strain fermentation, ammonium sulfate supplementation level, inoculation ratio of Aspergillus oryzae to Candida utilis, and mixed culture inoculum amount) on the protein content of fermented banana stem and leaf powder. Orthogonal design was employed to screen for the optimal combination of fermentation temperature, substrate moisture content, and fermentation time. The results showed that both Aspergillus oryzae and Candida utilis single-strain fermentation could significantly or extremely significantly increase the crude protein content of fermented banana stem and leaf powder ($P<0.05$ or $P<0.01$). Ammonium sulfate addition significantly or extremely significantly increased the true protein content of fermented banana stem and leaf powder ($P<0.05$ or $P<0.01$), with the 2% ammonium sulfate addition group achieving the highest true protein content. The net protein gain in banana stem and leaf powder fermented with an Aspergillus oryzae to Candida utilis inoculation ratio of 2:1 was significantly or extremely significantly higher than that of treatments with ratios of 1:1, 1:3, and 3:2 ($P<0.05$ or $P<0.01$). Orthogonal test results revealed that the optimal conditions were: 4% inoculum amount, inoculation ratio of 2:1 (Aspergillus oryzae to Candida utilis culture), 2% ammonium sulfate addition, substrate moisture content of 50%, fermentation at 30 °C for 4 days. Following fermentation with this process, the crude protein content of banana stem and leaf powder increased by 33.82%. Amino acid analysis results demonstrated that, except for lysine and arginine, the contents of the remaining 15 amino acids were increased to varying degrees. Metabolism trial results with Ma Gang geese showed that the crude protein utilization efficiency of fermented

banana stem and leaf powder increased by 52.66%, which was extremely significantly higher than that before fermentation (P<0.01). Additionally, both metabolizable energy and energy utilization efficiency were slightly improved. These results indicate that the nutritional value of banana stem and leaf powder was not only improved through this fermentation process, but also promoted nutrient digestion and absorption in geese.

Full Text

Study on Optimization of Solid-State Fermentation Conditions for Banana Stem and Leaf Powder and Its Nutrient Utilization by Geese

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Abstract

This study was conducted to investigate the process parameters for improving the nutrient composition of banana stem and leaf powder through solid-state fermentation technology and to evaluate its nutrient utilization by geese. *Aspergillus oryzae* and *Candida utilis* were selected as fermentation strains to systematically explore the effects of five factors (single-strain fermentation, ammonium sulfate addition, inoculation ratio of *A. oryzae* to *C. utilis*, and mixed inoculum amount) on the protein content of fermented banana stem and leaf powder. An orthogonal design was employed to identify the optimal combination of fermentation temperature, substrate moisture content, and fermentation time. The results demonstrated that both single-strain fermentations with *A. oryzae* or *C. utilis* significantly or extremely significantly increased the crude protein content of banana stem and leaf powder (P<0.05 or P<0.01). Ammonium sulfate addition significantly or extremely significantly enhanced the true protein content (P<0.05 or P<0.01), with the 2% addition group achieving the highest true protein content. The treatment with an *A. oryzae* to *C. utilis* inoculation ratio of 2:1 produced a significantly or extremely significantly higher net protein increase compared to ratios of 1:1, 1:3, and 3:2 (P<0.05 or P<0.01). Orthogonal test results revealed that the optimal conditions were: 4% inoculum (mixed strain ratio of 2:1), 2% ammonium sulfate addition, 50% substrate moisture, fermentation at 30 °C for 4 days. Under these conditions, the crude protein content of banana stem and leaf powder increased by 33.82%. Amino acid analysis showed that 15 of the 17 amino acids increased to varying degrees, except for lysine and arginine. Metabolic trials with Magang geese indicated that the crude protein utilization rate of fermented banana stem and leaf powder increased by 52.66% compared to the unfermented material (P<0.01), while metabolizable energy and energy utilization also improved slightly. These findings

demonstrate that this fermentation process not only improves the nutritional value of banana stem and leaf powder but also enhances nutrient digestion and absorption in geese.

Keywords: banana stem and leaf powder; solid-state fermentation; protein; nutrient utilization rate; Magang geese; *Aspergillus oryzae*; *Candida utilis*

Introduction

China faces a shortage of feed resources, and developing unconventional feed resources to replace traditional feed ingredients has become a major industry focus. According to statistics, China' s annual banana production exceeds 1$×10^{7}$ tons, generating massive quantities of banana stems and leaves as byproducts. These residues are rich in soluble sugars, minerals, B vitamins, and carotenoids, giving them considerable feeding value and potential as a feed resource. However, current research on feed processing technology for banana stems and leaves is limited, 配套 equipment is lacking, and their low protein content prevents full utilization. If these materials could be effectively incorporated into animal feed, they could reduce feed costs and alleviate resource scarcity. Numerous studies have confirmed that solid-state fermentation (SSF) technology offers advantages including low energy consumption, simple equipment requirements, and zero wastewater discharge. Successful applications have converted waste materials such as corn straw, apple pomace, and soybean residue into microbial protein resources. This study selected *Aspergillus oryzae* and *Candida utilis* as fermentation strains to explore optimal conditions for solid-state fermentation of banana stem and leaf powder, and compared nutrient utilization before and after fermentation through metabolic trials with Magang geese to provide data references for microbial processing of banana stems and leaves.

Materials and Methods

1.1 Test Strains *Aspergillus oryzae* GIM 3.545 and *Candida utilis* were purchased from the Guangdong Microbial Culture Collection Center.

1.2 Test Materials Banana stems and leaves (leaf blades and a short section of pseudostem from the base of the petiole) were collected from a banana orchard in Changban Village, Tianhe District, Guangzhou. The materials were sun-dried, crushed, and passed through a 40-mesh sieve. Ammonium sulfate (analytical grade), potato dextrose agar (PDA), malt extract powder, and yeast extract glucose agar were purchased from Guangzhou Weining Biological Co., Ltd. The required amount of culture medium powder was dissolved in distilled water and sterilized in an autoclave at 121 °C for 20 minutes before use.

1.3 Experimental Procedures 1.3.1 Preparation of *Aspergillus oryzae* Spore Suspension

A. oryzae was cultured on potato dextrose agar for 7 days. Sterile water was used to wash the spores into a conical flask, which was then filtered through four layers of gauze. The suspension was shaken at 28 °C and 200 rpm for 4 hours to disperse the spores. Plate colony counting was used to ensure the spore concentration reached $ 1×10^{8}$ CFU/mL.

1.3.2 Preparation of *Candida utilis* Culture

Malt extract powder was used to prepare liquid culture medium for *C. utilis*, which was incubated at 28 °C and 200 rpm for 24 hours. Plate colony counting confirmed the culture concentration reached $ 1×10^{8}$ CFU/mL.

1.3.3 Single-Strain Fermentation

Fifteen grams of banana stem and leaf powder were placed in 480 mL glass jars, sealed with sealing film, and sterilized at 121 °C for 20 minutes. Prepared inoculum was added at 3% (v/w), substrate moisture was adjusted to 50%, and fermentation proceeded at natural pH in a 28 °C incubator for 60 hours. Jars were shaken every 12 hours to ensure normal strain growth, with six replicates per strain. Fermentation products were dried at 65 °C and analyzed for crude protein content.

1.3.4 Dual-Strain Fermentation

1.3.4.1 Determination of Ammonium Sulfate Addition

Fifteen grams of banana stem and leaf powder were mixed with different amounts of ammonium sulfate (0, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%, w/w) in fermentation jars, sealed, and sterilized at 121 °C for 20 minutes. A mixed inoculum of *A. oryzae* to *C. utilis* at a 1:1 ratio was added at 3% inoculum level. Substrate moisture was adjusted to 50% and fermentation proceeded at 28 °C for 60 hours with shaking every 12 hours. Six replicates were prepared for each ammonium sulfate level. Products were dried at 65 °C and analyzed for true protein content.

1.3.4.2 Screening of Strain Ratio

Fifteen grams of banana stem and leaf powder were supplemented with ammonium sulfate based on the previous experiment results, sealed, and sterilized at 121 °C for 20 minutes. Mixed inoculum containing different ratios of *A. oryzae* to *C. utilis* (1:1, 1:3, 3:2, 2:3, and 2:1) was added at 3% inoculum level. Fermentation proceeded at 28 °C and 50% moisture for 60 hours with shaking every 12 hours. Six replicates were prepared for each ratio, and products were dried at 65 °C for true protein analysis.

1.3.4.3 Selection of Mixed Inoculum Amount

Fifteen grams of banana stem and leaf powder were supplemented with ammonium sulfate and sterilized as described above. Different amounts of mixed inoculum (2%, 3%, 4%, 5%, and 6%, v/w) were added based on the optimal strain ratio determined previously. Fermentation conditions were 28 °C, 50%

moisture, and 60 hours with shaking every 12 hours. Six replicates were prepared per treatment, and products were dried at 65 °C for true protein determination.

1.3.5 Orthogonal Experimental Design

Based on the determined ammonium sulfate addition, strain ratio, and mixed inoculum amount, fermentation parameters were optimized using a 3-factor, 4-level orthogonal analysis with fermentation temperature, fermentation time, and substrate moisture as factors. The orthogonal test L16(4^3) factor levels are shown in .

1.4 Measurement of Nutrient Utilization by Magang Geese Thirty-two healthy adult male Magang geese at 120 days of age (body weight 4.00$±$0.50 kg) were randomly divided into 4 groups (basal diet group, fermented banana stem and leaf powder group, unfermented banana stem and leaf powder group, and fasting group), with 8 replicates per group and 1 goose per replicate. The basal diet was a commercial complete pellet feed for geese purchased from Guangzhou Huisheng Grain and Oil Store, composed of corn, soybean meal, wheat middlings, wheat flour, wheat, limestone, dicalcium phosphate, vitamins, minerals, methionine, and soybean oil. Nutrient levels of the basal diet are shown in . Banana stem and leaf powder accounted for 15% of the test diet. Metabolizable energy and nutrient utilization were measured using the forced-feeding excretion method with 60 g feed per goose.

1.5 Analytical Methods 1.5.1 Measurement Methods

Crude protein and true protein contents were determined by the Kjeldahl method (GB/T 6432–1994). Crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed using the Van Soest detergent fiber analysis method. Gross energy was measured with an automatic calorimeter. Amino acid content was determined using an amino acid analyzer. Calcium content was measured by EDTA complexometric titration (GB/T 6436–2002). Phosphorus content was determined by ammonium molybdate spectrophotometry (GB/T 6437–2002). Crude fat content was measured by Soxhlet extraction (GB/T 6433–1994). Crude ash content was determined by the incineration and weighing method (GB/T 6438–1992).

1.5.2 Calculation of Metabolic Energy and Nutrient Utilization

Apparent metabolizable energy (AME) of diet (MJ/kg) = [Gross energy intake (MJ) - Gross energy in excreta (MJ)] / Feed intake (kg)

True metabolizable energy (TME) of diet (MJ/kg) = [Gross energy intake (MJ) - Gross energy in excreta (MJ) + Endogenous energy in excreta (MJ)] / Feed intake (kg)

Apparent metabolizable energy of banana stem and leaf powder (MJ/kg) = (AME of test diet - AME of basal diet × 0.85) / 0.15

True metabolizable energy of banana stem and leaf powder (MJ/kg) = (TME of test diet - TME of basal diet × 0.85) / 0.15

True nutrient utilization of basal diet (%) = 100 × [Total nutrient intake (g) - Fecal nutrient excretion (g) + Endogenous nutrient excretion (g)] / Total nutrient intake (g)

True nutrient utilization of banana stem and leaf powder (%) = 100 - 100 × [Total nutrient excretion (g) - Basal diet nutrient excretion (g) - Endogenous nutrient excretion (g)] / Banana stem and leaf powder nutrient intake (g)

1.6 Statistical Analysis Data were analyzed using SPSS 19.0 software for one-way ANOVA, followed by Duncan' s multiple comparison test. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effect of Single-Strain Fermentation on Crude Protein Content Single-strain fermentation with either *Candida utilis* or *Aspergillus oryzae* significantly increased the crude protein content of banana stem and leaf powder. As shown in , the crude protein content after *C. utilis* fermentation was significantly higher than the blank group ($P<0.05$), while *A. oryzae* fermentation resulted in a highly significant increase ($P<0.01$), indicating both strains are suitable for fermentation.

2.2.1 Effect of Ammonium Sulfate Addition on True Protein Content As presented in , ammonium sulfate addition significantly or highly significantly increased the true protein content of fermented banana stem and leaf powder compared to the group without ammonium sulfate ($P<0.05$ or $P<0.01$). True protein content initially increased then decreased with higher ammonium sulfate levels, peaking at 2% addition. Therefore, 2% ammonium sulfate was selected as the optimal level.

2.2.2 Effect of Strain Ratio on True Protein Content Different inoculation ratios of the two microorganisms affected both true protein content and net protein increase. According to , when the *A. oryzae* to *C. utilis* ratio was 2:1, both true protein content and net protein increase reached maximum values, establishing this as the optimal strain ratio.

2.2.3 Effect of Mixed Inoculum Amount on True Protein Content As shown in , true protein content and net protein increase gradually rose with increasing inoculum amount, reaching maximum values at 4% inoculum. Thus, 4% was selected as the optimal mixed inoculum level.

2.3 Orthogonal Test Results Experiments were conducted according to the combinations in , with six replicates per group and results expressed as means. The range (R) values for net protein increase in the intuitive analysis indicated that fermentation time had the greatest effect, followed by substrate moisture, with fermentation temperature having the smallest effect (B>C>A). Based on the R values for net protein increase, the optimal combination was A3B4C2: fermentation temperature 30 °C, fermentation time 4 days, and substrate moisture 50%.

Variance analysis of the orthogonal design () showed no significant differences (P>0.05) among fermentation temperature, time, and substrate moisture on net protein increase, though factor B (fermentation time) had a greater influence than factors C (moisture) and A (temperature). Similarly, revealed no significant differences (P>0.05) among these factors on net crude fiber reduction, with factors A (temperature) and C (moisture) showing greater influence than factor B (time).

2.4 Amino Acid Content Analysis Based on the optimized fermentation process (2% ammonium sulfate, 2:1 strain ratio, 4% inoculum, 30 °C, 50% moisture, 4 days), amino acid contents are presented in . Except for lysine, which decreased by 13.56%, all other essential amino acids increased to varying degrees, with methionine, isoleucine, and threonine showing the most prominent increases (40.00%, 32.00%, and 23.21%, respectively). Among non-essential amino acids, cysteine and histidine increased markedly by 100.00% and 78.79%, respectively.

2.5 Nutrient Utilization by Magang Geese Nutrient contents before and after fermentation are shown in . Compared to the unfermented material, fermented banana stem and leaf powder showed 33.82% higher crude protein content and 9.38% lower tannin content.

As shown in , true metabolizable energy increased by 7.64% (P>0.05), while true crude protein utilization increased by 52.66% (P<0.01) after fermentation.

Discussion

3.1 Single-Strain Fermentation Effects Microbial growth and reproduction require nutrients from the substrate. The single-strain fermentation results demonstrated that both *A. oryzae* and *C. utilis* could grow using banana stem and leaf powder as the sole nutrient source. The higher crude protein content achieved with *A. oryzae* may be attributed to its more rapid consumption of soluble sugars and cellulose in the substrate, leading to protein concentration.

3.2 Effect of Ammonium Sulfate Addition Nitrogen is essential for microbial growth as a component of proteins and nucleic acids. In this study, all ammonium sulfate addition levels significantly increased true protein content

compared to the control, confirming that both *A. oryzae* and *C. utilis* can utilize ammonium sulfate as a nitrogen source, consistent with findings by Rosma et al. [4], Liu et al. [5], and Liu et al. [6]. Research indicates that when microorganisms use ammonium sulfate, ammonium ion (NH_4^+) utilization lowers substrate pH [7]. Excessive ammonium sulfate may cause rapid pH decline, inhibiting microbial growth, while high sulfate concentrations also hinder fermentation [8].

3.3 Strain Ratio and Inoculum Amount Effects During fermentation, *A. oryzae* and *C. utilis* exist as an integrated system with both cooperative and competitive relationships. Yeasts primarily utilize sugar sources and produce substantial microbial protein, while molds secrete cellulase to degrade crude fiber [9]. The results showed that at a fixed strain ratio, inoculum amount did not significantly affect true protein increase, consistent with previous research [10], indicating that inoculum amount has relatively minor impact on fermentation. Slight variations in prepared inoculum concentration between batches precluded inter-batch comparisons.

3.4 Orthogonal Test Analysis The results showed maximum net crude fiber reduction at 28 °C. El-Ghonemy et al. [11] reported that the optimal temperature for *A. oryzae* cellulase production is 28 °C, corresponding to our findings. Appropriate moisture content for solid-state fermentation generally ranges from 40% to 70% [12]. Excessive moisture reduces inter-particle spaces and oxygen permeation, while insufficient moisture hinders nutrient dissolution and microbial growth [13]. Bhanja et al. [14] found that *A. oryzae* grew most vigorously at a substrate dry/wet ratio of 1:1 (50% moisture), matching our result of maximum crude fiber reduction at 50% moisture. Protein content increase depends on microbial proliferation using organic and inorganic nitrogen sources. Previous studies indicate suitable SSF temperatures of 25–35 °C [15-16], as microbial nucleic acids and proteins are temperature-sensitive, and temperature affects enzyme systems governing nutrient absorption and accumulation. *C. utilis* grows optimally at 30 °C and 50–125% moisture [17-18]. Our results showed increasing crude fiber reduction during the first 3 days, while net protein increase continued throughout the 4-day period, suggesting *C. utilis* continued proliferating using ammonium sulfate after *A. oryzae* activity stabilized.

3.5 Changes in Amino Acid and Proximate Nutrient Composition *A. oryzae* and *C. utilis* synthesize proteins by converting ammonium sulfate into required amino acids, altering the amino acid profile. Chen [19] demonstrated that *A. oryzae* consumes lysine during growth, while Yu et al. [20] showed that *C. utilis* requires arginine, explaining the reduced lysine and arginine contents after fermentation. Microbial metabolism converted soluble sugars to water and carbon dioxide, decreasing nitrogen-free extract by 8.65%. Although *A. oryzae* cellulase partially degraded cellulose, the consumption of soluble sugars resulted in increased crude fiber, NDF, and ADF contents. Crude protein in-

creased by 4.18 percentage points through microbial synthesis of protein from ammonium sulfate and protein concentration from sugar consumption, consistent with Hong et al. [21]. Despite energy consumption for microbial activities, gross energy increased slightly after fermentation, similar to Yu et al.' s [20] finding that fermented soybean meal had 5.43% higher gross energy, indicating nutrient concentration during fermentation.

3.6 Nutrient Utilization Analysis The crude protein utilization of fermented banana stem and leaf powder was extremely significantly higher than that of the unfermented material. Banana leaf proteins partially bind with tannins that hinder utilization, as dietary tannins form insoluble complexes with intestinal mucosal proteins, causing nitrogen loss [22-23]. *A. oryzae* secretes tannase that degrades tannins [24-26], and our results showed 9.38% lower tannin content after fermentation, contributing to improved protein utilization. Fermentation converted some proteins to microbial protein and degraded others into peptides and amino acids that are readily absorbed. Microbial protein synthesized from ammonium sulfate also became available for digestion, collectively enhancing protein utilization. Although crude fiber content was lower in unfermented powder, utilization was slightly higher after fermentation, indicating that fermentation made fiber more digestible. Niu et al. [27] reported that *A. oryzae* growth damages fiber structure, breaks cellulose chains, and alters crystalline structure, making it more susceptible to microbial degradation in the goose intestine. Improved protein and fiber utilization, combined with slightly higher gross energy, resulted in increased metabolizable energy and energy utilization after fermentation.

Conclusions

Based on the results of this study, three main conclusions can be drawn. First, the optimal fermentation process involves adding 2% ammonium sulfate, inoculating with 4% mixed culture of *A. oryzae* and *C. utilis* at a 2:1 ratio, adjusting moisture to 50%, and fermenting at 30 °C for 4 days. This process slightly increased gross energy while raising protein content by 33.82% and increasing 15 amino acids to varying degrees. Second, Magang geese showed high utilization of energy, neutral detergent fiber, and crude fiber from banana stem and leaf powder, though the raw material' s low protein content resulted in poor protein utilization. Third, fermentation improved metabolizable energy, energy utilization, and crude fiber utilization slightly, while protein utilization increased extremely significantly compared to unfermented material, demonstrating that fermentation enhances the nutritional value and digestibility of banana stem and leaf powder for geese.

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Note: Figure translations are in progress. See original paper for figures.