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Optimization of processing conditions for solid-state fermented soybean meal and its effects on growth performance and nutrient digestibility of weanling pigs

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ABSTRACT

This study was conducted to investigate the appropriate processing conditions for solid-state fermented soybean meal (FSBM) using a mixed starter culture of *Streptococcus thermophilus*, *Bacillus subtilis* MA139, and *Saccharomyces cerevisae* (Exp. 1), and determine the effect of FSBM (produced with appropriate conditions) as a partial replacement for soybean meal (SBM) on growth performance and nutrient digestibility of weanling pigs (Exp. 2). In Exp. 1, SBM was fermented using different processing conditions, including initial moisture content, incubation temperature and duration, sugar addition, protease supplementation, and neutral protease to acid protease ratio. After fermentation, pH value and contents of crude protein, lactic acid, glycinin, and β-conglycinin in FSBM were determined. Results showed that the appropriate incubation temperature for FSBM production was 40 °C. Greater initial moisture content (60%) and protease supplementation (0.3%) improved nutritional value. The suitable neutral protease to acid protease ratio was 3:1. Five days of incubation was sufficient for production of good quality FSBM. Addition of brown sugar did not affect the glycinin, and β-conglycinin contents in FSBM. In Exp. 2, a total of 72 crossbred piglets (Duroc × Land race × Large White) of an average weight of 8.9 kg were randomly allotted to 2 treatments with 6 replicate pens and 6 piglets per pen. Piglets were fed either a control diet with 24% SBM or a test diet with 6% FSBM added at the expense of SBM. Results from Exp. 2 showed that replacing SBM with 6% FSBM improved average daily gain and average daily feed intake ($P < 0.05$), while feed conversion ratio was unaltered. The nutrient digestibility and plasma urea *N* concentration in piglets on the FSBM diet were not different from those fed the SBM diet. In conclusion, achieving a suitable incubation temperature, greater initial moisture content, and supplementing with protease are essential to obtain good quality FSBM. Feeding a diet containing 6% FSBM can result in greater growth performance in weanling pigs, implying that this new strategy has the potential to be used to produce solid-state FSBM.

1. Introduction

Soybean meal (SBM) is the most commonly used plant derived protein source in the poultry and swine industries (Cromwell, 2012). Its high Lys content, excellent nutrient availability, and favorable palatability make it a good source of protein for use in animal diets. However, a variety of anti-nutritional factors, such as antigenic protein, trypsin inhibitors, oligosaccharides, and phytic acid, interfere with digestion, absorption, and utilization of nutrients. Hypersensitivity to antigenic proteins in SBM, such as glycinin and β-conglycinin, are considered as main reasons for abnormal

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morphology of the small intestine and diarrhea in nursery pigs (Huang et al., 2010; Sun et al., 2008).

Previous research has indicated that fermentation improved the nutritional quality of SBM. Fermentation of SBM can remove trypsin inhibitors, oligosaccharides, and phytic acid, and supply partially digested soybean proteins and live microorganisms, as well as enhance the apparent total tract digestibility (Egounlety and Aworh, 2003; Feng et al., 2007; Hong et al., 2004; Refstie et al., 2005). Immunoglobulin *E* immunoreactivity of SBM also could be reduced by fermentation (Song et al., 2008). Consequently, fermented SBM (FSBM) can be used to replace other high quality protein sources without adversely affecting the performance of nursery pigs (Kim et al., 2010; Zhang et al., 2013).

During our previous studies, multi-layer polythene bags equipped with a gas-pressure opening valve (Patent No. 200610002389.9; State Intellectual Property Office of the PR China, Beijing, China) were used to produce fermented compound pig feed (Hu et al., 2008), fermented wheat bran and SBM mixture (Ying et al., 2009), and fermented rapeseed meal (Chiang et al., 2010). The valve is designed to discharge internal air when the air pressure is increased by carbon dioxide metabolized by the aerobic bacteria, but the valve does not allow external air into the bag. With this technology, it is possible to co-incubate aerobic bacteria with anaerobic bacteria in the production of FSBM. Therefore, a combined starter culture of *Streptococcus thermophilus*, *Bacillus subtilis* MA139, and *Saccharomyces cerevisiae* was used to produce FSBM. *Saccharomyces cerevisiae* is used to consume the oxygen inside the fermentation bag to create an anaerobic condition for *Streptococcus thermophilus* and *Bacillus subtilis* MA139. *Bacillus subtilis* MA139 was successfully isolated in our previous study (Guo et al., 2006) and is capable of secreting several active components such as β-mannanase and β-glucanase (Qiao et al., 2009, 2010). *Bacillus subtilis* MA139 is also able to synthesize anti-microbial substances and prevent the growth of enterobacteriaceae (Ying et al., 2009). *Streptococcus thermophilus*, which is thermoduric homofermentative lactic acid bacteria, was generally used in the production of yogurt. Furthermore, the difficulty of heat release caused by low thermal conductivities of substrate was not a concern with this technology.

The present study was conducted to investigate the effects of initial moisture content, incubation temperature and duration, sugar addition, protease supplementation, and neutral protease to acid protease ratio on the biochemical characteristics of FSBM, especially on the content of glycinin and β-conglycinin. Then, we determined the effect of solid-state FSBM produced with the appropriate fermentation conditions on growth performance, nutrient digestibility, and plasma urea *N* concentration in weanling pigs.

2. Materials and methods

2.1. Exp. 1

2.1.1. Preparation of starter cultures

Streptococcus thermophiles (CGMCC No. 1.2471) and *Saccharomyces cerevisiae* (CGMCC no. 2.1793; Microbial Institute of the Chinese Academy of Sciences, Beijing, China) and *Bacillus subtilis* MA139 (National Key Laboratory on Animal Nutrition of China, Beijing, China) were obtained accordingly. *Streptococcus thermophilus* was cultured in de Man, Rogosa and Sharp media at 37 °C for 24 h. *Saccharomyces cerevisae* and *Bacillus subtilis* MA139 were grown in yeast peptone dextrose and mixed nutrition broth in a rotary shaker (225 rpm) at 30 °C for 24 h, respectively. After incubation, cells were washed twice with sterile saline solution and inoculated to have a final inoculant of 1×10^7 CFU/mL. The liquid starter culture was prepared by mixing 3 inoculants before fermentation.

2.1.2. Fermentation of soybean meal

Soybean meal was utilized as the substrate for fermentation after being milled through 1.0-mm screen. Neutral protease and acid protease in powder form had an activity of 50,000 IU/g (Finnico; Bosar Biotechnology, Beijing, China). Neutral protease was mixed with acid protease at 1:1 to prepare the protease mixture. Natural brown sugar, which contained 96.2% sucrose, was obtained (Fortune; China Oil & Foodstuffs Corporation, Beijing, China) and used as a sugar additive. Liquid starter culture (10% v/w) was added to SBM, which was fortified with different concentrations of brown sugar (0, 0.5, 1.0, 1.5, 2.0, and 2.5% w/w) and protease mixture (0, 0.15, 0.30, 0.45, and 0.60% w/w). Sterile distilled water was added to achieve different initial moisture content (20, 30, 40, 50, and 60%, w/w). Fermentation of SBM was performed in multi-layer polythene bags (500-g capacity) equipped with a gas pressure opening valve at various temperatures (20, 30, 40, 50, and 60 °C) for 1, 2, 3, 4, 5, 6 and 7 d. All experiments were performed 6 times. After fermentation, samples of FSBM were dried at 60 °C and ground to 0.25 mm particle size prior to analysis.

2.1.3. Initial moisture content, and incubation temperature and duration

To study the effects of initial total moisture content, fermentations were conducted under various initial moisture contents (20, 30, 40, 50, and 60%, w/w), adjusted with distilled water. The other conditions were set at 2.0% sugar, 0% protease supplementation and the fermentation was carried out for 5 d at 40 °C.

The incubation temperatures tested ranged from 20 to 60 °C and were kept constant by a regulated temperature chamber (Shanghai Boxun Industry & Commerce Company, Shanghai, China). The other conditions such as initial moisture content, sugar addition level, and protease supplementation were set at 40, 2.0, and 0%, respectively. The fermentation was run for 5 d.

Different incubation durations (1–7 d) were employed to study their effect on the nutritional quality of FSBM. The initial moisture content, sugar addition level, protease supplementation, and incubation temperature were set at 40%, 2.0%, 0%, and 40 °C, respectively.

2.1.4. Sugar addition and protease supplementation

Substrates were adjusted at different sugar addition levels (0, 0.5, 1.0, 1.5, 2.0, and 2.5% w/w) with brown sugar to investigate the effect of sugar addition on the nutritional