Kansas Soybean Commission Final Report

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I. Objectives

The goal of this proposed research is to change the narrative regarding soybean inclusion in pet foods by enhancing the nutritional value and resolving flatulence issues associated with oligosaccharides, eliminating trypsin inhibitors, while increasing bioactive molecules such as isoflavones, and saponins through fermentation technology. The specific objectives are:

1. To convert oligosaccharides and modify proteins in soybean meal through fermentation to improve its nutritional values for dog food.
2. To study the effect of fermentation on trypsin removal and soybean nutritional values.
3. To study the potential of fermented soy meal as a major ingredient for dog food application.

II. Materials and Methods

Raw Materials

Soybean meal was purchased from MKC (3384 Excel Rd, Manhattan, KS 66502). The soybean meal contains 42.68% protein, 40% carbohydrates, 6% fiber, 2.63% oil, and 7.65% ash. *A. oryzae* pellets (lyophilized, American Type Culture Collection ATCC 42149) was used for soybean meal fermentation. *A. oryzae* pellets was stored at 4 ^{o}C before cultivation.

Preparation of fungal spores and seeds culture

Figure 1 shows the preparation of fungal spores and seeds culture. Approximate 0.5 to 1.0 mL of sterile distilled water is added to an ampoule with *A. oryzae* pellets and stirred to form a suspension. The suspension is transferred to a test tube with ~5 mL sterile distilled water and kept at room temperature (25 ^{o}C) for overnight and mixed well before inoculation. A few drops are removed from test tube and added to liquid medium (made from ATCC medium 200 YM Yeast Mold Agar) for inoculation. The inoculation time is about 2 to 4 days depending on the sign of viability with spore suspension of around 107 spores/mL. Spore suspension is stored at 4°C until used.

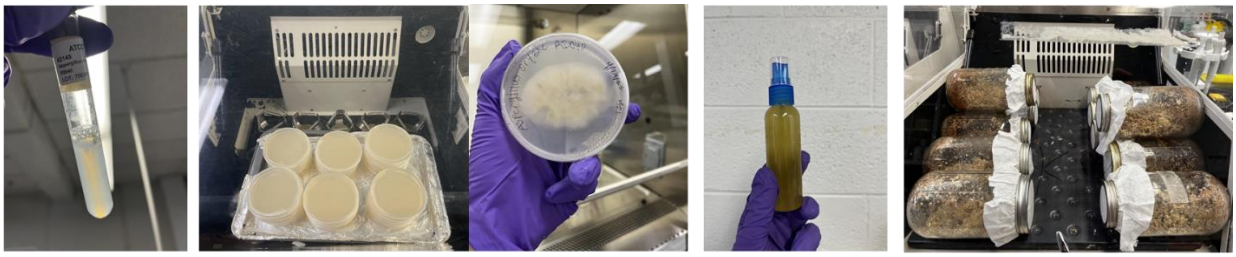

Figure 1. Preparation of fungal spores and seeds culture.

Fermentation

Solid-state fermentation was carried out in large humidity chamber with temperature and humidity ratio control. Autoclave was used to sterilize soybean meal. Before autoclaving, moisture content of soybean meal was adjusted to 50% moisture content (wb) with distilled water using mixer, and autoclaved at 121°C for 2 hours. Then soybean meal is inoculated at ratio of spore seeds culture and soybean meal from 4% to 8% and fermented at 37 °C for 72 hours. **Figure 2** shows the instruments used for mixing, sterilization, and fermentation. At different time intervals, fermented products are harvested, and fermented products were lyophilized and milled with an Udy cyclone mill equipped with a 1.0-mm screen for chemical analyses. **Figure 3** shows the soybean meal during fermentation and after fermentation.

Figure 2. Mixer, autoclave and fermentation chamber used for soybean meal solid-state fermentation.

Figure 3. Soybean meal during fermentation and after fermentation.

Analysis methods

The nutritional profiles of unfermented and fermented soy meal were analyzed. Moisture content was determined using air force oven at 105 ^{o}C for 4 hrs. Protein content was measured using nitrogen combustion method via a Leco FP-2000 nitrogen determinator (St. Joseph, MI) according to AOAC method 990.03. Nitrogen content was converted to protein using a conversion factor of 6.25. Oil content was determined by using the Soxhlet petroleum ether extraction method according to AOAC method 920.39C for oil and expressed as the weight percentage on a dry basis. Crude fiber was determined according to AOCS approved procedure Ba 6a-05. Total Dietary Fiber was determined by kit (TDF-100A kit; Megazyme International Ireland Limited, Ireland). Ash content was measured using muffle furnace at 575 ± 25 ^{o}C and dried to a constant weight. The amount of trypsin inhibitor was measured according to the AOCS method. Oligosaccharides content was measure by using HPLC. Separation and analysis of the amino acids will be performed on HPLC. Amino acids were determined with a fluorescence detector after pre-column derivatization with o-phthaldialdehyde.

Extrusion to produce dog pellets

Each one of the experimental diets was extruded in a single screw extruder and dried in a forced air drier and coated with topical chicken fat and flavor enhancer as needed to produce consistent nutritional kibbles. After the extrusion, dietary treatments were evaluated for nutritional composition and anti-nutritional factors (e.g. urease inhibitor, trypsin inhibitor, oligosaccharides composition).

Metabolizable energy and Digestible energy of the fermented soybean meal

A total of 12 Beagle dogs were used to evaluate the nutrition values of the fermented sorghum meal with other ingredients. The experimental diets in a 4 x 4 replicated (r = 3) Latin Square designed experiment. Animals were individually housed with constant access to water. Lights will be on a 12 h cycle with lights out from 1900 to 0700 each night. The Food amounts were estimated to maintain body weight throughout the duration of the study using the NRC (2006) equations to estimate the ME of the food and food amounts as $130 \times BW^{0.75}$ for dogs. Dogs were fed twice a day and excess food will be determined 1 hour after each feeding.

Within each of the four experimental periods, dog was allowed to adapt to experimental diets for 9 days followed by 5 days of sample (120h). During the sampling period feces were collected after each meal, and whenever observed during routine animal checks. Feces were scored according to a 5-point scale, then collected into a whirlpak bag and frozen for later analysis. Separate samples of fresh feces will also be collected (approximately 5 grams) and stored (-80 ^{o}C) in microcentrifuge tubes to be banked for later analysis of dog fecal microbiome if results indicate.

At the culmination of the feeding assay fecal samples were weighed, dried, and ground to pass a 1 mm screen. Feces and food samples were analyzed for dry matter, organic matter, crude protein, crude fat, crude fiber, and total dietary fiber according to AOAC international approved analytical methodologies. Food and feces were also be analyzed for gross energy by bomb calorimetry. Additionally, feed and feces will be analyzed for indirect marker (titanium dioxide).

Metabolizable energy (ME) is calculated using the following equation:

$$ME = digestible\ energy - (digestible\ protein \times correction\ factor\ for\ energy\ lost\ in\ urine)$$

$$Crrection\ factor = 1.25\ kcal/g\ in\ dogs$$

Digestible energy (DE) is calculated using the following equation:

$$DE = 1 - \frac{gross\ energy\ of\ feces \times \%\ Ti\ in\ food}{gross\ energy\ of\ food \times Ti\ in\ feces} \times gross\ energy\ of\ food$$

III. Results and Discussion

Protein content increased significantly through fermentation

A. aryzae fermentation can change the physical and nutritional characteristics of soybean meal through by converting the nonstructural polysaccharides, and oligosaccharides into valuable nutrients, especially increasing (microbial) protein content, reducing or eliminating the trypsin inhibitor and other antinutritional factors. **Table 1.** shows that the fermentation significantly increased soybean meal protein content from 42.68% to 56.97% with increase rate of 33.48%. This result indicates that fungal fermentation of soy meal is powerful way to increase soy meal nutrition value and feeding value. In addition, the fermentation also increased the fat content from 2.63% to 2.75% with increase rate of 4.5%, while ash decreased ash from 7.65% to 7.16% with decrease rate of -6.4%.

Table 1. Effect of fermentation on chemical composition of soybean meal.

	Dry matter %	Crude protein (%, db)	Fat (%, db)	Ash (%, db)
Soy meal	91.06	42.68	2.63	7.65
Fermented soy meal	96.59	56.97	2.75	7.16
Difference %		33.48%	4.5%	-6.4%

Energy content of fermented soybean meal

The energy content (kcal/kg) is an important quality factor of soybean meal, which affects its feeding and nutrition values. In general, the non-dehulled soybean meal has gross energy content of ~4,120 kcal/kg dry matter and fermented soybean meal has gross energy content of ~4500 kcal/kg dry matter [1]. Figure 4 shows fermentation effectively increased energy content from 4,598 to 4,873 kcal/kg dry mater with increase rate of 6%. The energy content of fermented soybean meal also higher that previous reported results.

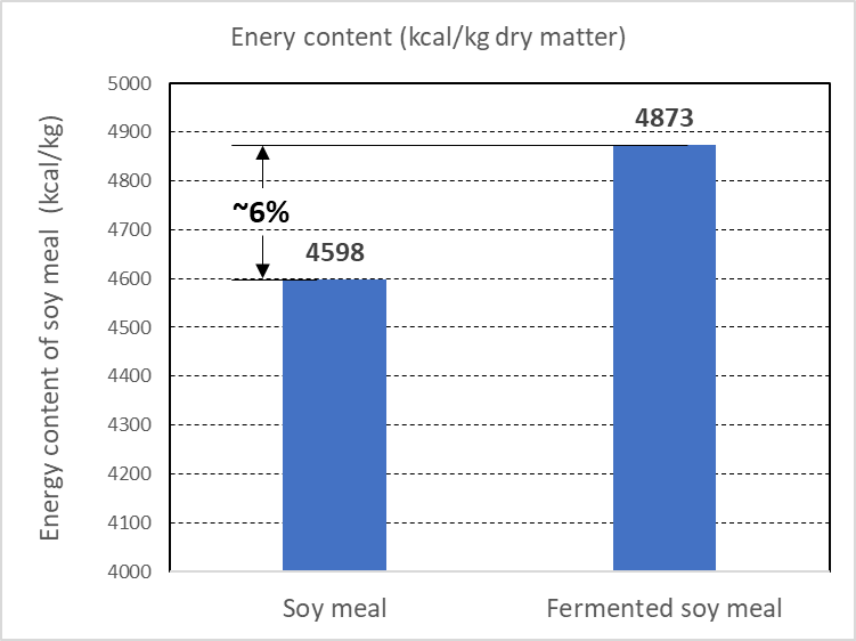

Figure 4. Fungal fermentation increased soybean meal energy content.

Metabolizable energy and Digestible energy

The energy content of SBM has been reported as 3619 kcal/kg digestible energy (DE) and 3294 kcal/kg metabolizable energy (ME) [2]. We assumed that through fermentation and extrusion process, the DE and ME values are expected to increase significantly. However, due to that Prairie AquaTech Technology continuedly delated to provide us SoyOS as we planned, the ME and DE evaluation using dog have not completed yet. We will find other alternatives and complete the ME and DE evaluation in the fall semester.

Reference

[1] UIUC. https://nutrition.ansci.illinois.edu/sites/default/files/SwineFocus004.pdf.

[2] Cemin, H.S., Williams, H.E., Tokach, M.D. et al. Estimate of the energy value of soybean meal relative to corn based on growth performance of nursery pigs. J Animal Sci Biotechnol 11, 70 (2020). https://doi.org/10.1186/s40104-020-00474-x