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Research Article
Modify the Chemical Composition of Jack Bean to be Used as Alternative Feedstuff in Poultry Diets

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Abstract

Background and Objective: Jack bean (*Canavalia ensiformis* L.) has the potential to be developed as a raw material in place of soybean meal for the manufacturing of poultry feed, but the presence of anti-nutritional compounds constitutes an obstacle to the utilization of jack bean as a raw material for poultry feed. This study analyzed the effects of different inoculum doses of *Rhizopus oligosporus* (*R. oligosporus*) and the fermentation time with the aim of increasing and decreasing the nutritional and anti-nutritional contents of jack bean, respectively. **Materials and Methods:** The main materials used in this study were jack beans and *R. oligosporus*. The experiment was conducted using a completely randomized design with a nested pattern (3×3) in triplicate. The treatment variables consisted of the *R. oligosporus* inoculum doses (0.1, 0.2 and 0.3%) and fermentation times (24, 48 and 72 h). The data were analyzed by analysis of variance and Duncan's multiple-range differential test was used to determine the differences in the effects among the treatments. **Results:** This research showed that increase in the inoculum doses and fermentation times significantly increased the nutrient content, particularly the protein content and significantly decreased the anti-nutritional content (hydrocyanic acid, phytic acid and tannin) in jack beans. **Conclusion:** A fermentation process using a 0.2% (b/b) dose of *R. oligosporus* inoculum with a 72 h fermentation time yielded the best effects. This optimized process increased the nutritional content by more than 50%, decreased the anti-nutritional content by more than 50% and reduced the content of hydrocyanic acid and tannin by more than 75%.

Key words: Anti-nutrients, fermentation, jack bean, *Rhizopus oligosporus*, poultry feed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jack bean (*Canavalia ensiformis* L.) is a type of legume with the potential to be developed as an alternative to soybean meal as a raw material in poultry feed production because it has good nutritive quality for feed formulation and utilization[1]. Several studies found that the nutrient content of mung beans consists of 10-11% water, 23-30% protein, 2-3% fat, 45-53% carbohydrate, 5-10% crude fiber and 3-4% ash[2]. In addition, jack bean has adaptive properties that allow its growth in various soil and environmental conditions and the jack bean kernel can grow in marginal soils and dry to semi-arid areas that are unsuitable for other pea plants.

The presence of anti-nutritional compounds in the form of cyanide/hydrocyanic acid (HCN), phytate and tannin constitutes an obstacle to the utilization of jack bean as a raw material for the production of poultry feed. A high hydrocyanic acid content, when administered directly to livestock without treatment, might lead to stunted growth, diarrhea and abnormalities in the poultry leg joint[3]. Hydrocyanic acid can interfere with the availability of iodine, which is needed for formation of the thyroid hormone. Decreased iodine levels can be obtained through the detoxification of HCN and this process produces goitrogenic thiocyanate, thereby disrupting protein metabolism and resulting in impaired tissue formation and stunted growth[4].

Tannin is an anti-nutrient because it can bind to proteins to form insoluble complex compounds. This effect can reduce protein digestion and the binding of tannin to an enzyme produced by the digestive system will decreased enzyme activity[5]. Tannin is also known to bind organic iron and B vitamins and this binding reduces the amounts of iron and vitamin B that can be absorbed by the body from feed ingredients[6].

Phytic acid is an antioxidant and anti-nutritional compound similar to tannin. Phytic acid can bind to proteins as well as minerals to form bonds that cause a decrease in the solubility of these compounds. This effect leads to decrease in the bioavailability (absorption) of minerals and proteins in the body, which would decrease the nutritional quality of feed ingredients. Phytic acid is relatively resistant to heating; thus, the heat treatment of feed ingredients is ineffective when used to reduce the phytic acid levels. Afify *et al.*[7] reported that immersion, fermentation and germination are effective means for reducing the levels of phenol and phytic acid compounds in seeds or pods.

It is important to eliminate or reduce the anti-nutritional compounds contained within pea legumes in order to improve their nutrient quality and increase their efficiency for the generation of poultry feed. Thus, several processing techniques are required to ensure optimal utilization[8]. One of the processing techniques used for improving the nutritional value of jack bean is fermentation. Fermentation techniques can be applied to nut and bean feed ingredients to increase their nutrient value, decrease their levels of anti-nutrient substances and exert good effects on livestock growth. The fermentation using single starter culture mold can be used to improve the nutritional quality of jack bean[9].

The technique used for the fermentation of jack bean peas in this study was conducted using *Rhizopus oligosporus*. This mold was selected because the microbe is easily bred, grows rapidly on solid substrate media and is relatively inexpensive. The *R. oligosporus* strains do not produce mycotoxins (rhizonins A and B), which can cause mycotoxicosis[10]. Kovac and Raspor[11] reported that tempeh fermentation using *R. oligosporus* decreases the phytic acid levels in soybeans and the same effect has also been observed in the cyanide ion, which results in a decrease or loss of HCN[12].

This study aimed to analyze the effect of the *R. oligosporus* inoculum dose and the fermentation time on the increase in the nutrient content and the decrease in the anti-nutrient content of jack beans, which will be used as a quail feed ingredient.

MATERIALS AND METHODS

The materials used were jack beans as the fermented substrate, *R. oligosporus* and rice as a source of energy for the mold during the manufacturing of the inoculum. The other materials were distilled water, alcohol, sodium chloride and water.

The experiments started with the production of agar medium: 100 g of blended potatoes and 500 mL of distilled water were heated in a beaker glass to yield a potato extract and the extract was filtered into an Erlenmeyer flask with a gauze-coated funnel. The resulting potato extract was combined with 10 g of sugar and 30 g of agar bar and the mixture was heated until these materials were dissolved, sterilized with an autoclave at a pressure of 1 atm and a temperature of 121°C for 15 min, cooled in a 3.0 mL reaction tube, sealed with cotton and placed in a slanted position.

Ready-to-use agar media were used for mold growth. Pure *R. oligosporus* culture was streaked with a needle against the slanted aseptic agar medium in the reaction tube and incubated for 3 days at room temperature. The *R. oligosporus* inoculum used for the jack bean fermentation, was then prepared.

The jack beans were first milled and then added to 1% rice flour and water (to a concentration as high as 60% (v/w)) and the mixture was achieved, sterilized and then drained until the temperature reached 30°C. The substrate to be fermented was inoculated with the prepared *R. oligosporus* inoculum at doses of 0.1, 0.2 and 0.3% (b/b). Each sample mass of 100 g was placed in a plastic bag (with a thickness of 2 cm) that was perforated to create an aerobic atmosphere. These plastic bags were incubated in a fermentor chamber at 30°C for 24, 48 or 72 h. Each treatment was repeated three times. To maintain a constant humidity throughout the fermentation process, the bottom of the fermentor rack was installed with a plastic tray filled with water. Each treatment was continued for the indicated incubation time and sterilized for 15 min and the sample was then dried.

The nutrient content of the fermented jack bean products was determined through an assessment of crude protein, crude fiber and crude fat using proximate analysis[13] and an analysis of the anti-nutrient content using the following method.

Hydrocyanic acid (HCN) analysis: The HCN analysis was performed according to Sudarmadji *et al.*[14]. One hundred milliliters of distilled water were added to a 20 g pulverized bean sample in an Erlenmeyer flask and the mixture was incubated for 2 h. Then, 100 mL of distilled water was added and the mixture was distilled with steam. The distillates were channeled into an Erlenmeyer flask filled with 20 mL of 2.5% NaOH. The distillation process was stopped when the distillation volume in the Erlenmeyer flask reached 150 mL. Five milliliters of 5% KI and 8 mL of NH_4OH were added to the distillation mixture and the resulting mixture was titrated with 0.02 N $AgNO_3$ solution until a cloudy mixture was obtained. The hydrocyanic acid content was then calculated using the following Eq.:

$$HCN = \frac{mL\ AgNO_3 \times 0.54}{\text{Material mass}} \times 1000 \frac{mg}{k}$$

Phytic acid analysis: The content of phytic acid was analyzed according to Narsih *et al.*[15], with some modifications to the preparation of the fermented jack bean flour samples and the creation of the phytic acid standard curve. One gram of jack bean flour was suspended in 50 mL of 0.5 M HNO_3 and the resulting suspension was stirred using a magnetic stirrer or shaker for 2 h at room temperature. After the suspension was filtered, 0.5 mL of the resulting filtrate, 0.9 mL of 0.5 M HNO_3 and 1 mL of $FeCl_3$ were added to a test tube. The reaction tube was sealed and immersed in boiling water for 20 min. Once the tube cooled down, 5 mL of amyl alcohol and 1 mL of ammonium thiocyanate solution were added and the tube was then centrifuged at a rate of 3000 rpm for 5 min. Fifteen minutes after the addition of ammonium thiocyanate, the absorbance of the amyl alcohol solution was measured using a Rayleigh UV-Vis spectrophotometer at a wavelength of 465 nm and an amyl alcohol blank. The results were compared with a 0.04 mM Na-phytic acid standard curve.

Tannin content: The tannin content was analyzed according to the method described by Nishitani and Osawa[16], with some modifications to the creation of the tannic standard curve and the preparation of jack bean flour sample. The first stage in the analysis of the tannin content was the creation of a tannic acid standard curve. Volumes of 1, 2, 4, 5 and 6 mL of a 25 μg mL^{-1} tannic acid standard solution were separately pipetted into 25 mL volumetric flasks. Three milliliters of iron (III) ammonium disulfate were added to each sample and the resulting solutions were stirred for 20 min. After 3 mL of potassium iron (III) cyanide was added to each solution, the resulting solutions were stirred for 20 min and distilled water was then added to each solution to obtain standard solution concentrations of 1, 2, 4, 5 and 6 μg mL^{-1}. The absorbance of each tannic acid standard solution was then measured with a UV-Vis spectrophotometer at 720 nm to obtain the tannin level standard curve.

Fermented jack bean flour samples were then prepared through the following steps to analyze the tannin content:

- First, the fermented jack bean was milled into fermented jack bean flour with a disc mill pin and 5 g of the resulting sample was dissolved in 100 mL of distilled water until a homogeneous mixture was obtained. In addition, as much as 5 mL of jack bean flour solution was removed and diluted in a 10 mL volumetric flask. To avoid an excessive concentration, a second dilution was prepared by diluting 1 mL of the first diluted solution in a 25 mL volumetric flask
- After the addition of 3 mL of iron (III) ammonium disulfate, the solution was stirred for 20 min
- Three milliliters of a potassium iron (III) cyanide solution was added and the solution was stirred again for 20 min and diluted to a total volume of 25 mL with distilled water. The absorbance of the sample solution was then measured with a UV-Vis spectrophotometer at a wavelength of 720 nm

Observed variables: The observed variables were increased crude protein content, decreased crude fiber content, decreased crude fat content and decreased anti-nutrient (hydrocyanic acid, phytic acid and tannin) content. The changes in the nutrient and anti-nutrient contents were calculated as follows:

$$\text{Change in the increase in nutrient content (\%)} = \frac{[K_2 - K_1]}{K_1} \times 100\%$$

$$\text{Change in the decrease in nutrient content (\%)} = \frac{[K_1 - K_2]}{K_1} \times 100\%$$

Where:
K_1 = Nutrient content prior to fermentation
K_2 = Nutrient content after the fermentation

Experimental design and statistical analysis: The experiment was conducted using a completely randomized design with a nested pattern (3×3) in triplicate. The treatment consisted of two factors: *R. oligosporus* inoculum dose (D_1 = 0.1%, D_2 = 0.2% and D_3 = 0.3%) and fermentation time (T_1 = 24 h, T_2 = 48 h and T_3 = 72 h). Duncan's multiple range test was conducted to assess the difference in effects among the treatments.

RESULTS AND DISCUSSION

The determined nutrient contents of the jack beans used as raw ingredients in this research study were 27.05% crude protein, 42.02% carbohydrate, 5.75% crude fat and 10.07% crude fiber. The fermentation of jack beans by *R. oligosporus* increased the protein content and decreased the crude fat and crude fiber content as well as the amount of anti-nutrient compounds. Omosebi and Otunola[17] reported that fermentation increased the dissolved protein content and yield a better essential amino acid composition. In addition, fermentation also increased the levels of vitamin B and some minerals such as iron, zinc and calcium. The presence of pathogenic microorganisms cannot be detected in fermentation products, making them suitable for consumption or as feed for livestock rations. The experimental results showed an increased nutrient content and a decreased anti-nutrient content in jack beans after fermentation with *R. oligosporus*, as presented in Table 1.

The results of the statistical analysis showed that both the inoculum dose and the fermentation time had significant ($p<0.05$) effects on the increase in the nutrient content and the decrease in the anti-nutrient content obtained by jack bean fermentation with *R. oligosporus* (Table 1). To some extent, the crude protein content of the fermented jack bean products increased proportionally in line with the increase in the inoculum dose and the fermentation time. According to Aisjah and Abun[18], the inoculum dose is related to the microbial population: The amount of inoculum is likely to determine the rate of microbial development because the inoculum is responsible for the production of enzymes that break down the available substrate and thus, affect the final product. A higher inoculum dose is associated with the presence of a higher number of microbial populations and an increased number of bioconverted substrate components. The incubation time is related to the time that will be used by the mold to grow and multiply. During this period, the mold continues to use substrate components for its survival.

As shown in this study, the optimal increase in the protein content of jack bean can be obtained by fermentation using a 0.2% dose of *R. oligosporus* inoculum with a fermentation time of 72 h (D_2T_3). These optimal fermentation conditions were identified based on several findings. First, the crude protein content of jack bean with a 0.3% inoculum dose tended to be lower than that obtained with a 0.2% inoculum dose. With a 0.3% *R. oligosporus* inoculum dose, a longer

Table 1: Average increase and decrease in the nutrient and anti-nutrient contents, respectively, obtained by the bioconversion of jack beans using *R. oligosporus*

	Nutrient and anti-nutrient content (%)					
Treatments	Crude protein	Crude fiber	Crude fat	Hydrocyanic acid	Phytic acid	Tannin
D_1T_1	5.48±0.76^a	41.90±0.62^a	7.53±0.26^a	37.45±0.18^a	24.11±1.31^a	71.50±1.37^a
D_1T_2	11.98±0.49^a	44.61±3.29^a	9.10±0.95^a	41.49±1.23^a	31.41±6.00^a	72.07±1.50^a
D_1T_3	18.77±2.29^a	44.62±3.29^a	10.02±1.91^a	47.96±3.45^a	35.01±0.96^a	73.92±0.42^a
D_2T_1	26.24±0.18^a	47.63±1.09^a	10.66±0.43^a	44.92±12.38^a	46.33±0.96^a	74.92±0.65^a
D_2T_2	33.67±0.13^b	49.21±0.12^{ab}	12.40±0.35^{ab}	57.83±6.19^a	51.15±0.96^a	74.78±0.85^a
D_2T_3	42.15±0.31^c	51.63±1.95^b	15.30±3.41^b	76.32±1.87^a	54.92±0.96^a	75.78±3.26^a
D_3T_1	26.24±0.18^a	46.27±2.98^a	10.54±0.35^a	82.75±5.45^a	62.05±4.88^a	76.63±2.74^b
D_3T_2	17.24±2.65^a	48.72±0.44^a	8.86±1.71^a	88.68±2.16^a	74.88±3.14^b	77.91±3.25^b
D_3T_3	7.33±3.09^a	45.47±1.93^a	8.11±0.26^a	98.30±0.02^b	82.80±1.92^c	80.91±4.61^b

D: Inoculum dose, T: Fermentation time, D_1: 0.1%, D_2: 0.2%, D_3: 0.3%, T_1: 24 h, T_2: 48 h and T_3: 72 h

fermentation time tended to be associated with a lower increase in the jack bean crude protein content tends. A reduced increase in the crude protein content of jack bean after fermentation with the higher inoculum dose was obtained because a high inoculum amount causes rapid depletion of food substance energy by the high number of microbes, resulting in a suboptimal fermentation process; excessive microbe numbers also cause sporulation to occur too rapidly, resulting in some energy not being used for cell multiplication during the fermentation process.

The increased jack bean protein content obtained after fermentation was also affected by the proteolytic enzymes produced by *R. oligosporus*. Proteins are broken down by proteolytic enzymes into amino acids, causing an increase in the dissolved N concentration[19]. The presence of proteolytic enzymes from this mold speeds up the hydrolysis of proteins into amino acids, which can improve digestibility[20,21].

For use as a raw ingredient for livestock rations, the jack bean should have a high protein content and low crude fiber and crude fat contents because a feed ingredient with high crude fiber and crude fat contents would affect the digestion and growth of livestock. The jack bean fermentation process using a *R. oligosporus* inoculum dose of 0.2% and a fermentation time for 72 h yielded the highest crude fiber and crude fat content loss rates of 51.63% (from 10.07-4.87%) and 15.30% (from 5.75-4.87%), respectively.

The decrease in the crude fiber and crude fat contents obtained during the fermentation process due to the presence of *Rhizopus* mold can reduce the content of nutrients such as carbohydrates and fats, which are used by molds for growth and breeding. Mold can synthesize proteins by using carbohydrates (glucose and sucrose), inorganic materials and minerals from the substrates as carbon substrates[22,23]. This finding is supported by the opinion of Munguia-Perez *et al.*[24], who stated that the fermentation of soybeans using *R. oligosporus* produces fermented tempeh products with improved nutritional characteristics because this process reduces the energy value associated with a decrease in fat content yields a product with a better texture. The decrease in the crude fiber and crude fat contents obtained in this study indicated that product will be more palatable as poultry feed.

The results showed that the percent decrease in anti-nutrient compounds (hydrocyanic acid, phytic acid and tannins) contained in jack bean after fermentation with *R. oligosporus* was proportional to the increase in the inoculum dose and the fermentation time (Table 1). The highest decrease in the hydrocyanic acid, phytic acid and tannin levels in jack bean was obtained with the 0.3% (3 g kg^{-1} substrate) *R. oligosporus* inoculum dose and a 72 h fermentation time: 98.30% (from 80.8-1.36%) decrease in the hydrocyanic acid level, 82.80% (from 1.59-0.27%) decrease in the phytic acid level and 80.91% (from 2.34-0.45%) decrease in the tannin level. The decrease in the hydrocyanic acid content in fermented jack bean was due to the ability of the mold to utilize N elements from not only proteins but also HCN compounds present in the jack bean during the fermentation process. Therefore, the mold was more likely to degrade the N content of HCN found in jack bean during a fermentation with a higher inoculum dose and a longer fermentation time. *R. oligosporus* as a fermentation agent can degrade anti-nutritional compounds in soybeans and is able to reduce the harmful substances to improve the final product[25].

The decrease in the phytic acid level in fermented jack beans is caused by the production of the phytase enzyme during the fermentation process. The phytase enzyme plays an important role in the dephosphorylation of phytic acid to yield organic inositol and inorganic phosphate, which have a lower chelating capacity and higher solubility and thereby, eliminates the inhibitory effect of phytic acid on mineral absorption[26]. The fermentation process significantly enhances the nutritional value, particularly the protein content and lysine availability and decreases the amount of phytic acid[27]. Egounlety and Aworh[28] reported that a fermentation process using *R. oligosporus* reduced the phytic acid level by 30.7% in soybeans, 32.6% in cowpeas and 29.1% in ground beans and the fermentation process can also decrease the levels of phytic acid and canavanine in jack beans[29].

Similarly, the reduction of the tannin content in fermented jack beans is caused by the use of the mold *R. oligosporus* as the inoculum in the fermentation process because this mold can produce the enzyme tannase, which breaks down tannins. Anwar and Burhanuddin[30] reported that *R. oligosporus* is able to produce tannase with a higher activity than that generated by *Aspergillus niger*. The *R. oligosporus* mold isolated from cocoa shells can reduce the tannin content by 79.28%[31]. Gabriel *et al.*[9] reported that the fermentation of jack beans with *Rhizopus oryzae* can decrease the content of tannins and phytic acid.

CONCLUSION

A fermentation process using the *R. oligosporus* can increase the nutrient content and decrease the anti-nutrient content of jack beans such that these can be used as a raw material for poultry feed. A fermentation process using a 0.2% (b/b) *R. oligosporus* inoculum dose and a 72-h fermentation time yielded the best effects: Increased the nutritional content

by more than 50%, decreased the anti-nutritional content by more than 50% and reduced the hydrocyanic and tannic acid contents by more than 75%.

SIGNIFICANCE STATEMENT

This study discovered that fermentation of jack beans using *R. oligosporus* can increase and decrease their nutritional and anti-nutritional contents, respectively, which can be beneficial for the use of the product as a raw material for the preparation of poultry feed. This study will help researchers to uncover critical areas of the jack bean fermentation process that have previously not been explored. Thus, these results present new insights regarding the appropriate R. *oligosporus* dose and fermentation time needed to increase the nutritional content and decrease the anti-nutritional content of jack beans.

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