The impact of enzyme addition on the metabolizable energy and protein digestibility of peeled Jack bean meal (*Canavalia ensiformis* L.)

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Abstract. This research sought to explore how different processing methods, including soaking and peeling the beans, and the introduction of protease enzyme and non-starch polysaccharides (NSP) enzymes, impact the nutrient content, metabolizable energy, and protein digestibility of processed Jack bean meal. The study utilized a completely randomized design, with four treatments and four replications. A total of 36 animals were involved, with 32 used for measuring metabolizable energy and protein digestibility, and 4 broiler chickens for endogenous measurement. Treatments included processed Jack bean meal alone, with protease enzyme, with NSP enzymes, and with both enzymes combined. Results showed a significant increase ($P<0.05$) in metabolizable energy and protein digestibility with enzyme supplementation. This improvement was attributed to the protease enzyme breaking down proteins into smaller peptides and NSP enzymes facilitating the breakdown of non-starch polysaccharides, enhancing digestion and absorption. In conclusion, supplementing peeled Jack bean meal with protease and NSP enzymes enhances its metabolizable energy and protein digestibility, suggesting its potential as an alternative protein source for broiler chickens.

1 Introduction

Broiler chickens play a crucial role in meeting Indonesia's animal protein needs due to their affordability and rapid growth, typically ready for meat production at 5-6 weeks of age. High-quality feed significantly enhances their performance, supporting health, growth, and energy supply [1].

However, the soaring cost of soybean meal and increasing imports burden farmers, with feed expenses accounting for about 70% of total maintenance costs. This raises concerns about meeting the rising demand for chicken meat and threatens farmer livelihoods. Thus, there's a pressing need for locally available, sustainable, and cost-effective feed alternatives, such as Jack beans [2].

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Jack beans contain high protein have the potential as a protein source in poultry feed rich also in phenolic compounds and flavonoids, and are easier to cultivate, making them a promising option for feed substitution [3]. Although they contain anti-nutritional substances like cyanide acid (HCN), pre-treatment methods can mitigate these risks [2, 3]. Additionally, the supplementation of exogenous enzymes like protease enzymes and NSP enzymes can enhance amino acid digestibility and overall productivity [4].

Integrating alternative feed ingredients like Jack beans with appropriate enzymes offers a practical solution to mitigate the challenges posed by expensive soybean meal, ensuring sustainable poultry farming and continued provision of protein-rich chicken meat in Indonesia. Rigorous testing through chemical and biological analyses is essential to validate the quality and effectiveness of Jack bean-based feed formulations.

2 Materials and methods

2.1 Samples preparation and examination

The Jack beans used for this study were processed, to reduce the antinutrient content, by soaking them for three hours, followed by peeling and drying them in an oven at a temperature of 65 °C for 48 hours. Subsequently, the dried beans were finely ground to form a mash of about 850 microns. The processed Jack bean samples underwent further processing for laboratory analysis.

The nutrient composition of the Jack bean meal, encompassing parameters such as moisture content, crude ash, crude protein, ether extract, and crude fiber, was evaluated following the AOAC [5] methodology. Additionally, the energy content was determined using a bomb calorimeter. Furthermore, the content of Cyanide acid in the Jack bean meal was measured by the Volhard argentometric method.

2.2 Metabolizable energy measurement

The assessment of metabolizable energy involved 36 broiler chickens, aged 35 days, which were adapted to the treatment feed for 4 days. Before treatment, all the chickens fasted for 24 hours. After this fasting period, 4 chickens, used for collecting endogenous samples, underwent an additional 24-hour fasting period but were allowed to drink water. The remaining 32 chickens were divided into 4 treatments, with 4 replications each containing 2 chickens. Feed was administered via force-feeding, allowing for an examination of 50 grams.

All chickens were placed in metabolic cages. Excreta was collected every 6 hours. H_2SO_4 (1N) was sprayed on the excreta to bind nitrogen. Samples were frozen for approximately 24 hours, thawed for 2 hours, and then dried at 105ºC for 24 hours before analysis. The calculations for metabolizable energy followed the method detailed by Sibbald and Wolynetz [6], using the formula provided by Ridla et al [7] to determine the values of apparent metabolizable energy (AME), apparent metabolizable energy corrected for nitrogen (AMEn), true metabolizable energy (TME), and true metabolizable energy corrected for nitrogen (TMEn).

2.3 Protein digestibility measurement

Protein digestibility (PD) refers to the portion of feed protein that is effectively digested and absorbed by an animal, which is crucial for assessing the nutritional value of feedstuffs, especially those rich in protein. PD can be calculated using the formula:

PD (%) = [(CP Consumption – Excreta protein) / (CP Consumption)] x 100%

where:

• CP Consumption: The amount of crude protein (CP) consumed by the animal, calculated by multiplying feed consumption (in kg) by the percentage of CP in the feed.

• Excreta protein: The quantity of protein excreted in the animal's feces, determined by subtracting endogenous excreta protein (protein secreted by the animal's digestive system) from the total excreta protein.

2.4 Experiment design and statistical analysis

A completely randomized design with four treatments and four replications was used. The treatments included processed Jack bean meal as a control (P0), P0 with the addition of protease enzyme (P1), P0 with the addition of NSP enzymes (P2), and P0 with both protease and NSP enzymes (P3). The enzymes and their doses per kilogram of feed used in this experiment were proteases at 150,000 units and NSP enzymes consisting of xylanase at 7,500 units, ß-glucanase at 125 units, ß-mannanase at 125 units, cellulase at 50 units, pectinase at 125 units, protease at 2,500 units, and amylase at 750 units.

The observed variables included AME, AMEn, TME, TMEn, and PD. Data analysis involved analysis of variance (ANOVA), with post-hoc testing, specifically the Tukey test, conducted using SPSS 19 software [8] in cases of significant differences.

3 Results and discussion

3.1 Chemical composition

Table 1 presents the nutrient content of both processed and unprocessed Jack beans. Processed Jack beans exhibit higher levels of crude protein and gross energy, while conversely, they show lower levels of crude fiber (1.80%) and HCN (38.34 ppm). The reduction in crude fiber and HCN content could be attributed to the soaking and peeling process, suggesting that the peel of Jack beans contains significant amounts of crude fiber as well as HCN. Similarly, soaking the beans for 3 hours has led to the dissolution of some of the HCN content. As per the European Commission Regulation [9], the maximum allowable levels of hydrocyanic acid are established for various forms of linseed and almonds. For unprocessed whole, ground, milled, cracked, and chopped linseed not sold directly to consumers, the limit is 250 ppm. For linseed sold to consumers, the limit is 150 ppm. For unprocessed whole, ground, milled, cracked, and chopped almonds intended for consumers, the maximum level is 35 ppm. The decrease in crude fiber content had increased the percentage of crude protein content. These findings align with those reported by Alifianti et al. [2], who observed an improvement in the nutrient quality of Jack beans following soaking and peeling treatment.

Table 1. Nutrient content of Jack bean.

Nutrient content	Unprocessed Jack bean	Processed Jack bean
Dry matter, %	92.47	94.89
Crude ash, % DM	7.34	7.13
Crude protein, % DM	25.58	33.99
Ether extract, % DM	3.36	2.95
Crude fiber, % DM	7.21	1.80
Nonfiber carbohydrate, % DM	53.97	54.13
Gross Energy, kcal kg^{-1}	3865.35	4126.56
Cyanide acid, ppm	207.11	38.34

3.2 Metabolizable energy

Based on Table 2, the research results demonstrate a significant effect (P<0.05) on Apparent Metabolizable Energy (AME) and True Metabolizable Energy (TME) values when protease enzyme and NSP enzymes were utilized in peeled Jack bean meal. The addition of these enzymes (protease enzyme, NSP enzymes, and a combination of both) in treatments P1, P2, and P3 respectively, resulted in an increase in the metabolizable energy value of peeled Jack bean meal compared to the control (P0). The increasing values were recorded as follows: for nitrogen-corrected Apparent Metabolizable Energy (AMEn), 651.08 kcal kg^{-1} for P1, 675.53 kcal kg^{-1} for P2, and 824.32 kcal kg^{-1} for P3, respectively.

The average metabolizable energy value across the four treatments of peeled Jack bean meal for broiler chickens was approximately 3704.47 kcal kg^{-1}, notably higher than the value of soybean meal metabolizable energy reported by Xavier Junior et al. [10], which ranged from 2494 to 2677 kcal kg^{-1} for the treatments with and without enzymatic addition.

The improvement in metabolizable energy observed in this research may be attributed to the protease enzyme breaking down proteins into smaller peptides [4] and NSP enzymes facilitating the breakdown of non-starch polysaccharides [11], thereby enhancing the digestion and absorption of energy and protein in the feed by the animals. Additionally, Mahardhika et al. [4] emphasize that enzymes could mitigate the negative effects of HCN present in the feed.

3.3 Protein digestibility

The protein digestibility values of peeled Jack beans are detailed in Table 2. The obtained protein digestibility values in this study increased significantly (P<0.05), ranging from 79.7% to 92.85%. This increase in protein digestibility can be attributed to enzyme supplementation, as indicated by studies such as that by Kemigabo et al. [12], which noted that protein digestibility was more efficient with protease enzymes in high-protein diets. Similarly, NSP enzymes can enhance the nutrient value and digestibility of feed ingredients, aiding better protein digestion compared to feeds without enzyme supplementation [13].

Protein digestibility in poultry is influenced by various factors, including protein intake, environmental temperature, anti-nutritional factors, and the physiological condition of the animals [14]. In poultry, protein digestion primarily occurs in the proventriculus through the action of pepsin and continues in the small intestine, where enzymes secreted by the pancreas play a crucial role [15].

Table 2. Metabolizable energy of Jack Bean.

Parameter	P0	P1	P2	P3	SEM	P-Value
AME, kcal kg^{-1}	$2812,82^{a}$	$3469,02^{b}$	$3493,17^{b}$	$3643,55^{b}$	13.53	P<0.01
AMEn, kcal kg^{-1}	$2806,01^{a}$	$3457,09^{b}$	$3481,54^{b}$	$3630,33^{b}$	12.74	P<0.01
TME, kcal kg^{-1}	$3034,88^{a}$	$3691,07^{b}$	$3715,23^{b}$	$3865,61^{b}$	10.51	P<0.01
TMEn, kcal kg^{-1}	$3028,07^{a}$	$3679,15^{b}$	$3703,60^{b}$	$3852,39^{b}$	10.74	P<0.01
DP, %	79.75^{a}	85.15^{a}	83.36^{a}	92.85^{b}	3.95	P<0.01

4 Conclusion

Soaking and peeling Jack bean meal, along with the inclusion of protease enzymes, NSP enzymes, or a combination of both, can improve metabolizable energy and protein

digestibility in broiler chickens. These methods can also reduce the impact of cyanide acid on feed digestibility. Additionally, processed Jack bean meal serves as a viable alternative to soybeans as a protein source in broiler chicken feed formulations.

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