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Influence of The Inclusion of Soaked and Peeled Jack Bean Meals in The Broiler Ration on Internal Vital Organs, Digestive Tract Organs, and Intestinal Villi Morphology

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ABSTRACT. *Jack bean (Canavalia ensiformis L.) is a potential alternative protein source to replace soybean meal in local feed formulations. While jack beans are high in protein, their high levels of anti-nutritional factors limit their use. Identifying effective processing techniques to improve the quality of jack beans is therefore essential. This study examines the effects of incorporating soaked and peeled jack bean meal into broiler diets on internal organs, digestive organs, and intestinal villi morphology. The jack beans were processed by soaking for 3 hours, peeling, oven drying, and grinding into meal form. A completely randomized design was used, with three levels of processed jack bean inclusion (0% JBM0, 5% JBM5, and 10% JBM10), each replicated six times. Data were analyzed using analysis of variance (ANOVA), followed by a post hoc Tukey test for significant findings. The results indicated that soaking for 3 hours and peeling effectively lowered the hydrogen cyanide (HCN) content from 207.61 ppm to 51.21 ppm. The inclusion of processed jack bean meal had no significant impact (P>0.05) on internal organs, digestive tract organs, or intestinal villi morphology. These findings suggest that processed jackbean meal can replace soybean meal in poultry feed at levels up to 10% without adverse effects on internal organs.*

Keywords: Intestinal villi, jack bean, morphology, organs.

INTRODUCTION

Feed costs are the main component in livestock maintenance, contributing around 70% of the total maintenance costs (Donohue M and Cunningham, 2009). Soybean meal is the primary protein source for broiler chicken feed in Indonesia, but its supply is constrained due to reliance on imports. As a result, there is a need to explore alternative protein sources that are more accessible, sustainable, and cost-effective. One promising local option is the jack bean (Canavalia ensiformis L.), which is rich in protein and energy (Mutia et al., 2024). Jack beans are cultivated in several regions of Indonesia and exhibit strong potential, yielding at least 7 tons per hectare with a growing period of 4-6 months (Soedarjo, 2021). Their nutritional profile includes crude protein levels between 22.8-35.3%, crude fiber from 4.7-11.4%, crude fat from 1.6-12.1%, ash from 2.3-5.8%, and total starch content ranging from 24.7-36.9%. The energy content varies from 3500 to 4500 kcal/kg, and jack beans also provide vitamins B1 and B2. Additionally, they contain amino acids similar to those found in soybean meal (Sridhar and Seena, 2006).

The use of jack beans as feed for broiler chickens is still faced with anti-nutritional problems, particularly the high content of cyanide acid (HCN), which can harm poultry if consumed in excessive amounts. Several physical processing methods can be used to significantly reduce the concentration of

cyanide acid, including soaking, boiling, and peeling the skin (Alifianty et al., 2023). Therefore, this research aims to investigate the effects of the inclusion of soaked and peeled jack bean meals in the broiler ration on internal vital organs, digestive tract organs, and intestinal villi morphology.

MATERIALS AND METHODS

Jack beans processing

In this study, jack beans underwent a processing technique that included soaking for 3 hours, followed by peeling, oven drying, and grinding into meal form. The quality of the processed jack beans was assessed based on several parameters, such as hydrogen cyanide (HCN) levels, chemical composition, and physical characteristics. HCN content was measured using the Volhard argentometric method. Nutrient composition, including dry matter, ash, crude protein, crude fat, and crude fiber, was analyzed through proximate analysis (AOAC, 2005). The physical properties were evaluated by measuring bulk density and tapped density, following the method outlined by Ridla et al. (2023).

Broiler chickens rearing

Processed jack bean meal was incorporated into broiler diets at levels of 0% (JBM0), 5% (JBM5), and 10% (JBM10) for feeding trials. The feed formulations adhered to the standard nutritional requirements for broilers based on BSN (2015), with details outlined in Table 1. Cobb broiler chickens were used as the experimental subjects, with 180 female broilers divided into three treatment groups. Each group had six replications, with ten chickens per replication. Commercial feed was provided during the pre-starter phase. The experiment began on day 8 and continued until day 35, targeting optimal performance. Throughout the trial, chickens were fed a crumble-form diet, with water available ad libitum. Chaff replacement occurred twice a week or as needed based on cage conditions. Weekly measurements of body weight gain and feed intake were taken. After 35 days, assessments were conducted on the chickens, focusing on the performance of internal organs (liver, heart, kidney), digestive organs (pancreas, bile, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and colon), and histological examination of intestinal villi.

Broiler chicken organ measurements

A total of 18 chickens were sampled, with one chicken selected from each replication. The evaluation focused on three categories of organs: vital organs (heart, liver, and kidneys), and digestive tract organs (pancreas, bile, pylorus, gizzard, duodenum, jejunum, ileum, cecum, and colon).

Intestinal villi histology

Histopathological analysis was conducted following the method outlined by Bancroft and Gamble (2008). Intestinal samples were taken to observe villi morphology, with a 3 cm section extracted from the middle of the ileum. The samples were cleaned and preserved in formalin-filled tubes to prevent degradation.

Data analysis

Data were analyzed using analysis of variance (ANOVA), and when significant differences were detected, a post hoc Tukey test was applied. The analysis was performed with IBM SPSS version 25 (IBM Corp, 2017). For the data related to hydrogen cyanide (HCN) content, as well as the physical and chemical properties of processed jack beans and the interpretation of histopathological images, a descriptive analysis was utilized.

RESULT AND DISCUSSION

Data Table 2 presents the results of the study, which investigated the effects of soaking and peeling treatments on hydrogen cyanide

(HCN) content, as well as the physical and chemical properties of processed jack beans. The results demonstrated a decrease in HCN content with both soaking and peeling treatments. The initial HCN content of 207.61 ppm in fresh jack beans decreased to 51.21 ppm with soaking and peeling treatments. This reduction was attributed to HCN deposition in the bean skin and the release of HCN during soaking and peeling. The findings align with Damayanti et al. (2019), highlighting the potential of water soaking in reducing anti-nutritional factors like HCN.

The study also found that processed jack bean meals had higher bulk density and tapped density values compared to their unprocessed counterparts. This indicates that the soaking and peeling treatments affected the density, likely due to changes in nutrient content. Ridla et al. (2023) noted that increases in bulk and tapped densities are associated with variations in crude protein and crude fiber content. The experiment's results indicated a rise in nutritional content attributed to the processed jack bean meal. Processed treatment reduced fiber content from 8.98% to 1.86% and led to increased crude protein levels from 26.35% to 32.37%, likely due to the removal of the peel containing these components.

Vital organs

The internal vital organs examined included the liver, heart, and kidneys, which are particularly sensitive to toxic substances and anti-nutrients in broiler blood. Table 3 indicates that incorporating 0% (JBM), 5% (JBM5), and 10% (JBM10) processed jack bean meal into broiler diets did not have a significant effect ($P > 0.05$) on the percentage weights of the liver, heart, and kidneys.

The function of the liver is to neutralize toxins in the body. In this study, the liver size ranged from 2.31% to 2.53%. According to Putnam (1991), a normal liver size falls within the range of 1.7% to 2.8%. A normal liver demonstrates healthy growth in broiler chickens, ensuring that it does not adversely affect liver performance and that its size remains relatively stable.

The heart functions as a pump for blood circulation in the body, making it highly susceptible to toxins and anti-nutrients. If toxins accumulate in the heart muscle, it can lead to heart enlargement. In this study, the percentage value of heart weight ranged from 0.42% to 0.46%. The research findings indicate that incorporating processed jack bean meal into the ration did not adversely impact the metabolism of the livestock's body. Therefore, the heart performed its function normally, consistent with Akhadiarto (2010), who reported a range of 0.42% to 0.62%.

The kidneys are crucial for filtering plasma from the blood, selectively reabsorbing water and essential elements from the filtrate, and removing excess and waste products. In this study, the average kidney weight percentage ranged from 0.49% to 0.59%. Hermana et al. (2005) reported that the normal range for kidney weight in broiler chickens is approximately 0.43% to 0.84%.

Digestive Tract Organs

Digestion involves both physical and chemical transformations of feed ingredients within the digestive tract. This process is crucial for nutrient absorption, such as protein, which is essential for muscle and meat growth (Sturkie, 2000). In this study, the size of the digestive organs in broiler chickens fed diets containing jack bean meal was similar to the size of the digestive organs in broilers fed the control diet.

The weight percentage of the pancreas does not increase unless the organ is under greater demand for digestive enzyme production. An increase in pancreatic weight reflects an adaptation to meet higher digestive enzyme needs. In this study, pancreatic weight ranged from 0.24% to 0.29%. This falls within the normal range reported by Sturkie (2000), which is 0.25% to 0.40% of body weight. The pancreas's weight suggests that the body's metabolic system and digestive enzyme secretion are functioning normally.

Bile is essential for absorbing fats and removing toxins from metabolic waste. The gallbladder holds bile salts, cholesterol, pigments, fats, organic salts, and lecithin. In this study, the bile weight percentage ranged between 0.05% and 0.07%, closely matching the 0.029% to 0.097% range found by Faria et al. (2019). This stable bile percentage generally suggests that there are no major disruptions in the metabolic system of broiler chickens.

The proventriculus is a gland that produces amylase, lipase, and pepsin enzymes in the stomach (Amrullah, 2004). Ukim et al. (2012) conducted research testing the weight of the proventriculus, obtaining an average percentage result of 0.40% to 0.58%, which is considered normal. In the results obtained in this study, the average percentage of proventriculus weight ranged from 0.58% to 0.66%, categorizing it as normal weight.

The gizzard, also known as the muscle stomach, is situated between the proventriculus and the upper small intestine. This muscular stomach organ functions to grind incoming feed, and larger feed particles can lead to faster contractions, causing the gizzard to enlarge. In this study, the gizzard ranged between 1.67% and 1.73%, still within the normal range for gizzards according to Sturkie (2000), which is 1.6% to 2.3%. A higher percentage of gizzard weight, as indicated by Amrullah (2004), suggests that the gizzard is working harder to break down particle sizes, facilitating their digestion in the small intestine.

The small intestine is divided into three sections: the duodenum, jejunum, and ileum. It is essential for absorbing digestive products and serves as a defense mechanism against microorganisms, toxins, and antigens (Dwijayanti et al., 2021). The duodenum is the primary site for active digestion, where the hydrolysis of crude nutrients such as starch, fat, and protein occurs. Digestion and absorption then continue in the jejunum until only indigestible material remains. The ileum's function is to absorb the remaining nutrients not absorbed by the jejunum, as well as mineral salts and B vitamins (Gao et al., 2008).

In this study, the average weight percentage of the duodenum ranged from 0.61% to 0.64%, the jejunum from 1.40% to 1.44%, and the ileum from 0.96% to 1.04%. The relative lengths of these sections were as follows: duodenum ranged from 1.70% to 1.82%, jejunum from 4.82% to 4.93%, and ileum from 4.24% to 4.30%. These values fall within the normal range for weight percentage according to Has *et al*. (2014), who reported duodenum weight percentages of 0.50% to 0.73%, jejunum percentages of 1.24% to 1.51%, and ileum percentages of 0.96% to 1.32%. The relative lengths also align with the research of Pertiwi *et al*. (2017), who found relative lengths of the duodenum ranging from 1.61% to 2.01%, jejunum from 4.46% to 5.14%, and ileum from 3.68% to 4.60%.

The cecum, located between the small and large intestines, contributes to protein and carbohydrate digestion and assists in water absorption with the help of resident bacteria (Sharifi et al., 2012). According to Citra et al. (2019), the typical cecum percentage ranges from 0.37% to 0.50%, and its length ranges from 1.84% to 2.34%. In this study, no significant differences were found, with cecum percentages ranging from 0.37% to 0.39% and relative lengths between 1.99% and 2.23%.

The colon plays a crucial role in water absorption and maintaining fluid balance, containing fermenting bacteria that help digest crude fiber and break down food ingredients through fermentation. The by-products of this process are utilized to meet nutritional needs, while the remaining softer food residues are expelled (Grist, 2006). The weight and length of the colon are affected by factors such as water intake and age. In this study, the average colon weight percentage ranged from 0.41% to 0.50%,

with a relative length of 0.19% to 0.29%. These values are within the normal range for weight percentages and are somewhat longer compared to the findings of Lestari et al. (2020), who reported an average weight percentage of 0.15% to 0.92% and a length of 0.64% to 0.67%.

Histology of Intestinal Villi

The environment and the composition of the food ingested are key factors influencing the growth and development of the intestinal tract. A well-developed digestive system in broiler chickens is marked by appropriate weight and length increases, as well as optimal growth of intestinal villi, which enhances nutrient absorption (Pertiwi et al., 2017). The development of the digestive tract and intestinal villi is critical for ensuring the nutritional health of broiler chickens.

The intestinal villi found in the intestine have a finger-like shape, serving as absorbers of food essence. The nutritional content of a well-balanced ration can stimulate the growth of villi in the intestine. The effects of providing rations with 0% (JBM0), 5% (JBM5), and 10% (JBM10) on intestinal morphology, including histological features, are presented in Figure 1.

The histological observations of the ileum in broiler chickens, conducted through Hematoxylin and Eosin staining at 4X magnification, revealed no differences. The images depicted mild inflammatory cell infiltration and mild desquamation, indicating that the provided feed ingredients did not induce excessive activity in the small intestine, particularly the ileum, during the absorption of produced food substances.

Table 1. Feed composition and nutrient content of broiler chicken ration

Ingredients (%)	JBM0	JBM5	JBM10
Corn	52.52	50.51	48.70
Processed Jack Bean meal	0.00	5.00	10.00
Soybean meal	28.91	23.91	18.91
Rice bran	7.85	7.85	7.85
Crude Palm Oil	3.10	3.10	3.10
Meat Bone Meal	5.00	7.00	8.81
DL-Methionine	0.35	0.35	0.35
L-Lysin	0.30	0.30	0.30
Calculated nutrient content			
Dry matter, %	88.00	89.06	88.90
Crude protein, %	20.93	22.35	21.49
Crude fiber, %	5.08	3.89	3.82
Crude fat, %	6.52	6.87	6.52
Ash, %	6.02	6.26	6.74
Metabolizable Energy, kcal/kg	3273	3598	3521
Calcium, %	0.80	1.06	1.27
Phosphor available, %	0.40	0.50	0.58
Dig. Lysine, %	1.31	1.56	1.49
Dig. Methionine, %	0.62	0.98	0.67

Noted: JBM0 = Control feed, JBM5 = Feed containing 5% processed jack bean meals, and JBM10 = Feed containing 10% processed jack bean meals.

Intestinal desquamation serves as an indication of the intestine's defensive response to antigen infection, while infiltration (inflammation) arises from disease agents or continuous exposure to foreign agents. Desquamation involves the release of epithelial cells from the tissue surface and serves as a protective function against the tissue's defensive response to irritants, preventing further damage. Inflammation functions to

destroy, dilute, or limit harmful agents, initiating processes aimed at restoring and replacing damaged tissue (Herdiani and Putri, 2018).

Berata et al. (2018) explained that infiltration and desquamation can result from two causes: microorganisms, including viruses, bacteria, fungi, protozoan worms, and other microorganisms; and non-microorganisms, such as chemicals, extreme temperatures, trauma, incision, surgery, and other factors.

Table 2. Hydrogen cyanide (HCN) content, physical and chemical properties of jack beans

Item	Unprocessed	Processed
HCN, ppm	207.61	51.21
Dry matter, %	90.78	90.85
Crude ash, % DM	7.34	7.13
Crude protein, % DM	26.35	32.37
Ether extract, % DM	3.36	2.95
Crude fiber, % DM	8.98	1.86
Non-fiber carbohydrate, % DM	53.97	55.69
Gross energy, kcal kg^{-1}	3658.42	3948.33
Bulk density, g l^{-1}	507.13	528.32
Tapped density, g l^{-1}	740.12	765.24

Note: Processed = Soaked for 3 hours followed by peeling, DM=dry matter.

Tabel 3. Broiler chicken vital organs

Organs	JBM0	JBM5	JBM10
	---------- (% Body weight) ----------		
Liver	2.45±0.21	2.53±0.29	2.31±0.09
Heart,	0.42±0.03	0.43±0.04	0.46±0.03
Kidneys	0.52±0.12	0.49±0.07	0.59±0.17

Note: JBM0 = Control feed, JBM5 = Feed containing 5% processed jack bean meals, and JBM10 = Feed containing 10 processed jack bean meals.

Table 4. Broiler chicken digestive tract organs

Organs	**JBM0**	**JBM5**	**JBM10**
	---------- (% Body weight) ----------		
Pancreas	0.29±0.07	0.24±0.07	0.26±0.04
Bile	0.06±0.00	0.07±0.04	0.05±0.03
Proventriculus	0.58±0.10	0.57±0.14	0.52±0.09
Gizzard	1.67±0.14	1.73±0.21	1.68±0.21
Duodenum	0.64±0.11	0.61±0.11	0.61±0.04
Jejunum	1.44±0.22	1.40±0.20	1.40±0.14
Ileum	1.04±0.23	0.96±0.17	1.00±0.19
Cecum	0.39±0.05	0.37±0.07	0.38±0.06
Colon	0.19±0.25	0.20±0.08	0.24±0.04
	---------- (Relative length, cm/100 g BW) ----------		
Duodenum	1.78±0.11	1.82±0.17	1.70±0.22
Jejunum	4.82±0.35	4.93±0.50	4.90±0.58
Ileum	4.30±0.41	4.29±0.35	4.24±0.52
Cecum	1.99±0.35	2.23±0.28	2.15±0.25
Colon	0.50±0.13	0.41±0.17	0.49±0.13

Note: JBM0 = Control feed, JBM5 = Feed containing 5% processed jack bean meals, and JBM10 = Feed containing 10% processed jack bean meals.

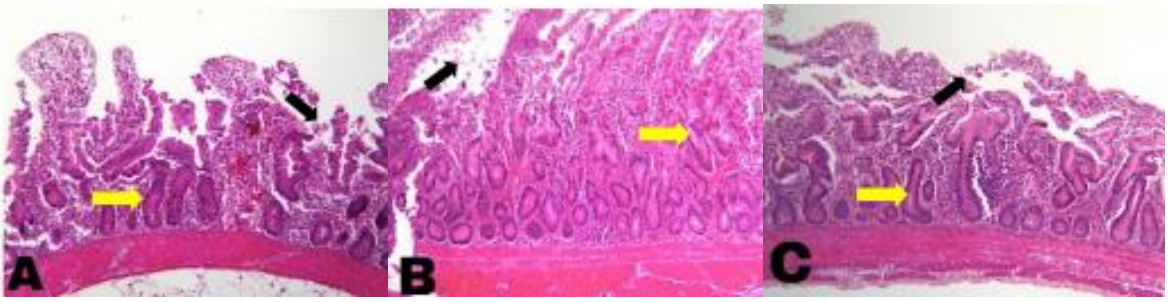

Figure 1. Mild inflammatory cell infiltration, mild desquamation of the ileum, epithelial desquamation (➡), inflammatory cell infiltration (⇨). A=JBM0, B=JBM5, C=JBM10

CONCLUSION

Processed jack beans through soaking for 3 hours followed by peeling effectively reduces HCN content and improves nutrient content. Utilizing processed jack bean meal as a substitute for soybean meal in poultry feed, up to a concentration of 10%, yields benefits without any adverse effects on vital organs, digestive tract organs, or the histology of intestinal villi.

CONFLICT OF INTEREST

There are no conflicts of interest with any financial organizations concerning the materials discussed in this manuscript.

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