Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 213 – 234 (2025)
www.ejabf.journals.ekb.eg

Utilization of Pretreated Jack Bean *Canavalia ensiformis* as Raw Material for Tilapia *Oreochromis* sp. Feed

Azizi Putri Nurlita Hidayat, Mia Setiawati, Julie Ekasari, Talita Shofa Adestia, Ichsan Achmad Fauzi*
Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University

***Corresponding Author:** ichsanfauzi@apps.ipb.ac.id

ARTICLE INFO
Article History:
Received: April 10, 2025
Accepted: June 25, 2025
Online: Sep. 20, 2025

Keywords:
Antinutrients,
Digestibility,
Growth,
Jack bean,
Pretreatment,
Tilapia

ABSTRACT
Tilapia (*Oreochromis* sp.) production continues to increase, requiring adequate nutritional support through feed. Jack bean (*Canavalia ensiformis*) is a promising legume with high nutrient content; however, its utilization is limited due to antinutritional factors such as phytic acid, trypsin inhibitors, and tannins. Therefore, this study aimed to evaluate the effects of jack bean meal pretreatment on tilapia performance. Peeled jack beans were pretreated by soaking (6h; 1:10 w/v ratio) and autoclaving (30min at 121°C). Proximate analysis showed that both soaking and autoclaving increased protein and fat content, while all antinutritional factors decreased after pretreatment. A total of 15 tilapia fingerlings (8.19 ± 0.01 g) were stocked in each of 20 aquaria (50 × 50 × 40 cm³) for 60 days, with diets assigned in triplicate. Five isonitrogenous diets (28% crude protein) were formulated using processed and unprocessed jack bean seed at inclusion levels of 0, 5, and 15%. Chromium oxide (Cr_2O_3) was added as an indicator for digestibility tests. Feces and blood samples were collected, and fish were analyzed for growth performance, digestive enzyme activity, and intestinal histology. The highest values of total feed digestibility (51.71%), final biomass (571 g), specific growth rate (2.56%), feed conversion ratio (1.69), plasma protein (4.51 g dL^{-1}), and protein retention (32.27%) were observed with 5% inclusion of processed jack bean. These findings indicate that processed jack bean meal can be effectively incorporated into tilapia feed at a 5% inclusion level.

INTRODUCTION

Tilapia (*Oreochromis* sp.) production increased from 2019–2023 by 1.20% **(DJPB, 2023)**. These data are in line with total fish consumption in Indonesia in 2024, which reached 59kg per capita per year **(KKP, 2024)**, with tilapia consumption per capita per week reaching 617g in Bogor City **(BPS, 2024)**. Based on these data, the aquaculture sector, as one of the high-protein food sources, is recognized as fulfilling food needs. Therefore, food demand can be met through intensive aquaculture production. Intensive cultivation refers to high-density methods used to increase production **(Oke & Goose, 2019)**.

The development of tilapia aquaculture must be supported by adequate nutrition through sustainable feed, with 85–98% of fish production costs absorbed by feed expenses **(Suprayudi *et al.*, 2023)**. According to **Sorensen *et al.* (2009)**, fish feed production is dominated by plant-based protein sources, mainly soybean meal. Fish feed companies in Indonesia still rely heavily on imported soybean meal, with imports reaching 5.33 million tons **(BPS, 2023)**. Consequently, local raw materials such as pollard and bran are increasingly incorporated into fish feed **(Farahiyah *et al.*, 2016)**.

Jack bean (*Canavalia ensiformis*) is a type of legume cultivated in Indonesia as a side crop or hedge crop **(Darini *et al.*, 2021)**. Jack bean production can reach 3–5 tons ha^{-1}, with advantages such as high productivity, a short cultivation period, and resilience to environmental changes **(Darini & Kusdiarti, 2017)**. According to **Hudiyanti *et al.* (2015)**, jack bean has potential as a feed ingredient, containing 34.6% protein, 2.4% fat, 1.2% crude fiber, and 2.8% ash. However, the use of plant-based raw materials, particularly jack bean, is constrained by antinutrient content. Jack bean contains phytic acid (325.47mg 100 g^{-1}) and tannins (0.35g 100 g^{-1}) **(Arise *et al.*, 2022)**. These components can inhibit enzymatic nutrient absorption **(Nolan *et al.*, 1987)**. Additionally, jack bean contains trypsin inhibitor activity of 1682.7 U g^{-1} **(Fagbenro *et al.*, 2008)**. Thus, it reduces protein digestibility in fish **(Liener, 1976)**.

To address this issue, methods of inactivating antinutrients in plant-based raw materials must be developed to maximize their utilization **(Martinez-Palacios *et al.*, 1998)**. Pretreatment methods should follow modern technologies; for instance, **Akande and Fabiyi (2010)** highlighted that autoclaving, a pressure-cooking process, as effective. This method can eliminate thermolabile antinutrients in jack bean within a short time **(Udedibie & Nwaiwu, 1988)**. Similarly, **Doss *et al.* (2011)** reported reduced trypsin inhibitor activity after autoclaving. Moreover, **Ramadan (2012)** noted that phytic acid levels can be reduced by soaking, with additional suppression achieved through autoclaving.

Research on jack bean utilization is most common in catfish (*Clarias gariepinus*) feed. For instance, raw jack bean can be included at 5% **(Solomon *et al.*, 2017)**. With pretreatment such as boiling for 30 minutes, inclusion levels of 12% are possible **(Fagbenro *et al.*, 2008)**, while fermentation allows up to 10.9% incorporation **(Michael *et al.*, 2023)**. However, studies indicate that information on the optimum inclusion level of jack bean meal in tilapia (*Oreochromis* sp.) feed remains limited. Therefore, it is necessary to assess pretreatment methods that reduce antinutrient levels, enabling jack bean to be a viable local feed ingredient. This study thus aimed to evaluate the effects of pretreatment (soaking and autoclaving) on jack bean in tilapia feed formulations and their impact on growth performance.

MATERIALS AND METHODS

Research stages

This research consisted of two stages:

1. Improving the nutrient quality of jack bean through pretreatment.
2. Evaluating feed digestibility and growth performance of tilapia fed diets containing unprocessed and processed jack bean.

Stage 1: Improving the nutrient quality of jack bean through pretreatment

Soaking and autoclaving of jack bean

Jack beans were collected from Bogor, West Java, peeled, and subjected to pretreatment. Soaking was conducted for 6h in water at a 1:10 (w/v) ratio **(Sheahan, 2012)**, followed by autoclaving at 121°C for 30min **(Arise *et al.*, 2022)** and oven-drying at 60°C for 12h.

Proximate analysis of jack bean

The pretreated jack beans were ground into flour and analyzed for proximate composition (moisture, ash, crude fiber, protein, and fat). Moisture content was determined by oven drying at 110°C, ash by furnace at 600°C, crude fiber by sequential acid–alkali digestion with heating, protein by the Kjeldahl method, and fat by Soxhlet extraction **(AOAC, 1997)**.

Antinutrient analysis

- **Phytic acid**

  Phytic acid levels were analyzed using the method of **Davies and Reid (1979)** with modifications from **Perdani and Utama (2020)**. Briefly, 0.5g of sample was extracted with 20mL of 0.5 M HNO_3, shaken in a water bath for 4h, and diluted 10–20×. One mL of filtrate was mixed with 0.4mL distilled water and 1mL $FeCl_3$ in a screw-cap tube, then heated in boiling water for 20min. After cooling, 5mL n-amyl alcohol and 0.1mL ammonium thiocyanate were added, vortexed, and allowed to stand for 15min. Absorbance was measured at 465nm, using n-amyl alcohol as blank and Na-ferrate (MW: 660.04 g mol^{-1}) as standard.

- **Trypsin inhibitor**

  Trypsin inhibitor activity was determined spectrophotometrically following prior research **(Nwosu *et al.*, 2011)**. Ten g of sample was dispersed in 50mL of 0.5 M NaCl, stirred for 30min at 25°C, centrifuged, and filtered. The extract was tested using benzoyl-DL-arginine-p-nitroanilide (BAPA) as substrate. Two mL of extract was mixed with 10mL of BAPA solution, followed by the addition of 2mL standard trypsin. A blank contained only substrate. Absorbance was read at 430nm. One unit of inhibitory activity was defined as a 0.01 decrease in absorbance.

- **Tannins**

  Tannins were determined using the Folin–Denis spectrophotometric method **(Ezegbe, 2012)**. One g of sample was extracted in 10mL of water, stirred, and left for 30min with intermittent shaking before centrifugation. Then, 2.5mL of extract or standard tannic acid solution was placed in 50mL flasks, mixed with 1mL Folin–Denis reagent and 2.5mL saturated Na_2CO_3, diluted to 50mL, and incubated for 90min. Absorbance was measured at 250nm against a reagent blank.

Stage 2: Feed digestibility and growth performance of tilapia

Experimental diet preparation

Experimental feeds were formulated by incorporating unprocessed and pretreated jack bean at inclusion levels of 5 and 15%. Two sets of diets were prepared: (1) diets for digestibility trials containing chromium(III) oxide (Cr_2O_3) as an inert marker (Table 1), and (2) diets for growth performance evaluation.

Table 1. Digestibility test feed formulation containing jack bean at 28% protein content

Composition Raw Material (%)	Treatments[1]				
	0	**nPr5**	**nPr15**	**Pr5**	**Pr15**
Fish meal	5.00	5.00	5.00	5.00	5.00
Meat bone meal	7.00	7.00	7.00	7.00	7.00
Poultry by-product meal	8.00	8.00	8.00	8.00	8.00
Wheat flour	6.00	6.00	6.00	6.00	6.00
Cassava flour	9.00	9.00	9.00	9.00	9.00
Soybean meal	22.00	18.32	16.08	20.00	15.61
Pollard	18.60	17.28	5.45	15.60	6.14
Wheat bran	20.00	20.00	24.07	20.00	23.85
Unpretreated jack bean	-	5.00	15.00	-	-
Pretreated jack bean	-	-	-	5.00	15.00
Fish oil	1.00	1.00	1.00	1.00	1.00
Crude palm oil	1.20	1.20	1.20	1.20	1.20
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Vitamin mix	0.20	0.20	0.20	0.20	0.20
Mineral mix	0.20	0.20	0.20	0.20	0.20
Polymethylolcarbamide	0.30	0.30	0.30	0.30	0.30
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

[1]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%).

The second set of feed preparations was used for the growth performance test, with the feed formulation presented in Table (2).

Table 2. Test feed formulation containing jack bean at 28% protein content

Composition Raw Material (%)	**Treatments[1]**				
	0	**nPr5**	**nPr15**	**Pr5**	**Pr15**
Fish meal	5.00	5.00	5.00	5.00	5.00
Meat bone meal	7.00	7.00	7.00	7.00	7.00
Poultry by-product meal	8.00	8.00	8.00	8.00	8.00
Wheat flour	6.00	6.00	6.00	6.00	6.00
Cassava flour	9.00	9.00	9.00	9.00	9.00
Soybean meal	22.00	18.06	15.72	20.00	15.25
Pollard	19.10	18.04	6.73	16.10	7.41
Wheat bran	20.00	20.00	23.65	20.00	23.44
Unpretreated jack bean	-	5.00	15.00	-	-
Pretreated jack bean	-	-	-	5.00	15.00
Fish oil	1.00	1.00	1.00	1.00	1.00
Crude palm oil	1.20	1.20	1.20	1.20	1.20
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Vitamin mix	0.20	0.20	0.20	0.20	0.20
Mineral mix	0.20	0.20	0.20	0.20	0.20
Polymethylolcarbamide	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100

[1]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%).

Proximate analysis of the test feeds was conducted to confirm that the formulations met the nutritional requirements of tilapia. The results are presented in Table (3).

Table 3. Nutrient composition of the formulated feed containing unpretreated and pretreated jack bean

Proximate Feed	Treatments[1]				
	0	**nPr5**	**nPr15**	**Pr5**	**Pr15**
Dry matter (%)	90.00	90.75	90.75	89.75	90.50
Crude protein (%)	27.99	27.97	27.99	27.98	27.99
Crude lipid (%)	6.24	6.23	6.25	6.24	6.25
Ash (%)	11.00	10.25	10.00	11.00	10.75
Crude fiber (%)	8.24	6.88	5.86	8.32	8.49
NFE[2] (%)	36.53	39.42	40.65	36.21	37.02
Carbohydrate[3] (%)	44.77	46.30	46.51	44.53	45.51
Gross energy[4] (kcal kg^{-1})	3990	4050	4062	3979	4021
C/P[5] (kcal g^{-1})	14.25	14.48	14.51	14.22	14.37
Total	100	100	100	100	100

[1]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%)
[2]Nitrogen Free Extract (NFE) = 100 - (water + protein + lipid + ash + fiber)
[3]Carbohydrate = 100 - (water + protein + lipid + ash)
[4]Gross energy (GE) in feed was calculated based on protein: 5.64 kcal g $protein^{-1}$, lipid: 9.44 kcal g lipid $^{-1}$, and carbohydrate: 4.11 kcal g $carbohydrate^{-1}$ **(Watanabe, 1998)**
[5]Calorie/Protein (C/P) = GE/protein

Experimental fish

Tilapia (*Oreochromis* sp.) with an initial weight of 8.19 ± 0.01 g were obtained from farmers in Bogor, West Java. Fish were acclimatized for one week before the feeding trials. Fifteen fingerlings were stocked into each of 20 aquaria (50 × 50 × 40 cm^3), each equipped with a thermostat (28 °C) and a single aeration point, and maintained for 60 days. Fish were fed three times daily at 08:00, 12:00, and 18:00 using the ad libitum method. Water quality was maintained within the optimum range for tilapia growth and survival by performing 60% water exchange every three days.

Fecal collection

Feed digestibility testing was conducted by collecting feces 15 days after the start of the feeding trial, 1– 2h post-feeding. Feces were collected using a siphon hose, filtered, transferred to small containers, and stored at –25°C until sufficient material was available for analysis.

Digestive enzyme activity

Digestive enzyme activity was analyzed by sampling 1cm of the anterior intestine, corresponding to ¾ of the total intestinal length after the stomach, as this section is the main site of chemical and enzymatic digestion.

- **Amylase activity** was measured using 1% starch solution as substrate in 20mM sodium phosphate buffer (pH 6.9) containing 6mM NaCl **(Worthington, 1993)**.
- **Lipase activity** was assayed using olive oil emulsion as substrate with Tris-HCl buffer following the study of **Borlongan (1990)**.

- **Trypsin activity** was determined using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate according to **Erlanger *et al.* (1961)**.

Histology and morphometry of intestinal villi

At the end of the 60-day trial, tilapia were dissected and samples of the anterior intestine were collected, as this section directly interacts with feed and best reflects the effects of unprocessed and processed jack bean. Tissue segments (1cm) were fixed in buffered neutral formalin for 24h, dehydrated in graded ethanol, cleared with xylene, embedded in paraffin, sectioned, and stained with hematoxylin and eosin **(Kiernan, 2015)**.

Sections were observed under a light microscope, and villus morphometry (height and width) was measured using ImageJ software. For each quadrant of view, ~5 villi in optimal condition were selected and measured. The mean of these measurements was used to calculate the intestinal absorption area (IAA) according to **Mohammady *et al.* (2021)**:

$$IAA\ (\mu m^2) = HV \times WV$$

Where:

IAA : Intestinal absorption area
HV : Height of the villi (µm)
WV : Width of the villi (µm)

Feed digestibility determinations

Total feed digestibility was calculated using the formula of **NRC (2011)** as follows:

$$\text{Total digestibility (\%)} = 1 - (C\ fd/C\ fc) \times 100$$

Where:

C fd : %Cr_2O_3 in feed
C fc : %Cr_2O_3 in feces

Nutrient digestibility in this study includes protein, fat carbohydrate, and energy. Nutrient digestibility was calculated using **NRC (2011)** as follows:

$$\text{Nutrient digestibility (\%)} = 1 - ((C\ fd/C\ fc) \times (N\ fc/N\ fd)) \times 100$$

Explanation:

C fd : %Cr_2O_3 in feed
C fc : %Cr_2O_3 in feces
N fc : %Nutrient in feces
N fd : %Nutrient in feed

Plasma protein assay

Plasma protein analysis was performed at the end of rearing by taking 0.5mL of blood from three test fish in each treatment using 1mL syringe that had been rinsed with anticoagulant (Na-Citrate 3.8%). Fish blood was taken from the vein located at the base of the tail. Blood was taken slowly and collected in an eppendorf that had been rinsed with anticoagulant and then stored in a cool box. After that, the blood was centrifuged at 2500rpm for 15 minutes to separate the serum and blood plasm. Plasma protein measurement was performed by spectrophotometric method with a wavelenght of 545nm based on Labtest total protein kit.

Growth performance

The amount of feed intake (FI) in this study was determined based on the total feed given during the rearing period divided by the total fish.

Specific growth rate (SGR) is defined as the percentage increase in fish weight per day and can be calculated based on the formula of **Huissman (1987)**:

$$SGR\ (\%) = ((\ln W_t - \ln W_0)/\Delta t) \times 100$$

Explanation:

W_t : Average weight of individual fish at the end of rearing (g)

W_0 : Avergae weight of individual fish at the beginning of rearing (g)

Δt : Length of rearing time (days)

The calculation of feed conversion ratio (FCR) is defined as the amount of feed needed to produce 1kg of meat in fish. The calculation refers to **Goddard (1996)**:

$$FCR = FI/((B_t - B_0) + B_d)$$

Explanation:

FI : Feed intake (g)

B_t : Fish biomass at the end of rearing (g)

B_0 : Fish biomass at the beginning of rearing (g)

B_d : Biomass of that died during rearing (g)

Protein retention (PR) and lipid retention (LR) were determined through proximate analysis of whole-body protein and lipid content at the beginning and end of the rearing trial. The amount of protein consumed was calculated based on total feed intake. Protein retention was then calculated following the formula of **Takeuchi (1988):**

$$PR\ (\%) = ((P_t - P_0)/P_c) \times 100$$

Explanation:

P_t : Fish body protein at the end of rearing (g)

P_0 : Fish body protein at the beginning of rearing (g)

P_c : The amount of protein consumed by fish (g)

$$LR\ (\%) = ((L_t - L_0)/L_c) \times 100$$

Explanation:

L_t : Fish body lipid at the end of rearing (g)

L_0 : Fish body lipid at the beginning of rearing (g)

L_c : The amount of lipid consumed by fish (g)

Calculation of survival rate (SR) was done with the following formula **(Effendi 2004)**:

$$SR\ (\%) = Nt/N_0 \times 100$$

Explanation:

Nt : Number of fish at the end of rearing

N_0 : Number of fish at the beginning of rearing

Data analysis

The data obtained were tabulated and analyzed according to the research parameters using Microsoft Excel 2019. Data were analyzed by analysis of variance (One-Way ANOVA to determine significant differences between treatments and Two-Way ANOVA to determine the effect of treatments and their interactions) at a 95% confindence interval. If there are significantly different values in the ANOVA test, analysis was followed by Duncan's multiple range test (DMRT).

RESULTS

Stages 1: Improving the nutrient quality of jack bean through pretreatment process

Proximate analysis and antinutrient content of jack bean

Proximate analysis and antinutrient content of jack bean are presented in Table (4). Based on these results, it is clear that the preatreatment process of soaking for 6 hours and followed by autoclaving for 30 minutes improved nutrient content, but there was a decrease in antinutrients.

Table 4. Effect of soaking and autoclaving on nutrient and antinutrient factors on jackbean

Proximate[1] (%)	Treatments[3]	
	Non Pretreatment	**Pretreatment**
Crude protein	36.56	37.65
Crude lipid	2.46	2.90
Ash	3.84	2.71
Crude fiber	0.44	1.31
Nitrogen free extract[2]	56.71	55.43
Antinutrient content		
Tannin (%)	3.80	2.17
Phytic acid (g kg^{-1})	5.85	5.47
Trypsin inhibitor (mg g^{-1})	0.65	0.37

[1]Results are presented in dy matter condition, moisture content of non pretreatment (2.25%) and pretreatment (22.07%)

[2]Nitrogen Free Extract (NFE) = 100 - (water + protein + lipid + ash + fiber)

[3]Non pretreatment (peeled jack bean) and pretreatment (peeled jack bean, soaked in water for 6 hours,a nd autoclaved for 30 minutes at 121°C).

Stages 2: Feed digestibility test and growth performance of tilapia by feeding with unpretreated and pretreated jack bean

Digestive enzyme activity

The activity of digestive enzymes in tilapia fed diets containing jack bean is presented in Table (6). Analysis of variance showed that the nPr15 treatment resulted in the highest amylase activity, which was significantly different from the other treatments ($P< 0.05$). The highest lipase activity was observed in the Pr5 treatment, while the highest trypsin activity was recorded in the control group, though it was not significantly different from the Pr5 treatment. Two-way ANOVA indicated that both the type of raw material and the inclusion level had significant effects on enzyme activity; however, no interaction between these two factors was observed.

Table 5. Digestive enzyme activity in tilapia by feeding with unpretreated and pretreated jack bean

Digestive Enzyme ($U\ mL^{-1}$)	Treatments[1]					Two-Way ANOVA[2]		
	0	nPr5	nPr15	Pr5	Pr15	RM	D	RM*D
Amylase	5.87±0.23[c]	7.61±0.80[b]	9.29±0.33[a]	5.28±0.05[c]	7.14±0.08[b]	*	*	-
Lipase	0.44±0.00[b]	0.37±0.01[d]	0.33±0.00[e]	0.47±0.02[a]	0.42±0.01[c]	*	*	-
Trypsin	0.04±0.00[a]	0.03±0.00[c]	0.02±0.00[d]	0.04±0.00[a]	0.04±0.00[b]	*	*	-

Notes: Different letters in the same row indicate significantly different treatment effects (P<0.05)
[1]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%)
[2]RM (Raw material), D (Dosage), RM*D (Interaction of raw material and dosage)

Histology and morphometry of tilapia intestinal villi

The histological structure of the tilapia intestine in response to diets containing non-pretreated (nPr) and pretreated (Pr) jack bean at inclusion levels of 5 and 15% is shown in Fig. (1). The figure provides a comparison of intestinal microstructures across treatments. In the control group (0%), no damage or detachment of the villous epithelium was observed, and both villus morphology and muscle fiber structure appeared normal. In contrast, fish fed diets containing 5 and 15% non-pretreated jack bean exhibited reduced numbers of goblet cells on the mucosal surface, along with loosely arranged connective tissue in the submucosa. Meanwhile, fish fed 5 and 15% pretreated jack bean displayed improved villus morphology and muscle fiber structure, resembling the control group. In these treatments, numerous goblet cells were observed, and the muscularis mucosa was thickened compared to the other groups.

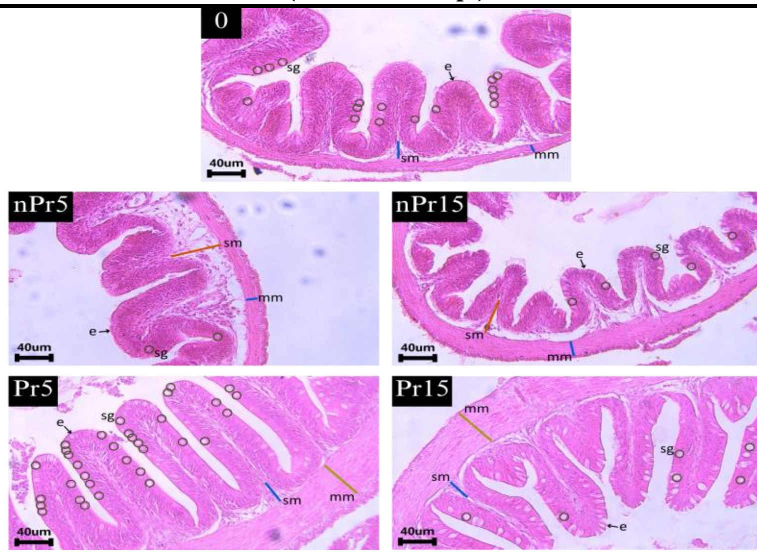

Fig. 1. Intestinal histology of tilapia after 60 days of feeding containing jack bean. Magnification (4x10). 0 (control), nPr5 (addition of 5% non pretreated jack bean), nPr15 (addition of 15% non pretreated jack bean), Pr5 (addition of 5% pretreated jack bean), Pr15 (addition of 15% pretreated jack bean). sg (goblet cells), e (epithelium), sm (submucosa), mm (muscularis mucosa)

The height and width of intestinal villi, as well as the calculated absorption area of tilapia fed diets containing unpretreated and pretreated jack bean, are presented in Table (6). Analysis of variance showed that the highest villus height, villus width, and intestinal absorption area were observed in the treatment with 5% pretreated jack bean, which was significantly different from the other treatments (*P*< 0.05). Increasing the inclusion level or using unpretreated jack bean resulted in reduced morphometric values. Two-way ANOVA further indicated that both the type of raw material and the inclusion level significantly affected intestinal morphometry; however, no interaction between these two factors was observed.

Table 6. Intestinal morphometry of tilapia after 60 days of feeding containing unpretreated and pretreated jack bean

Parameter[1]	**Treatments**[2]					**Two-Way ANOVA**[3]		
	0	**nPr5**	**nPr15**	**Pr5**	**Pr15**	**RM**	**D**	**RM*D**
VH (µm)	168±2.70^{b}	145±2.69^{bc}	94.8±1.83^{c}	277±3.95^{a}	194±3.85^{b}	*	*	-
VW (µm)	82.3±1.37^{ab}	59.4±1.36^{c}	56.3±0.95^{c}	87±1.79^{a}	73.8±1.35^{b}	*	*	-
IAA (µm^2)	1410±41.41^{b}	878±31.22^{bc}	531±13.25^{c}	2454±63.86^{a}	1427±36.41^{b}	*	*	-

Notes: Different letters in the same row indicate significantly different treatment effects (P<0.05)
[1]VH (Villi height), VW (Villi width), IAA (Intestinal absorption area)
[2]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%)
[3]RM (Raw material), D (Dosage), RM*D (Interaction of raw material and dosage).

Digestibility of test feed

Feed digestibility of tilapia during the 15-day trial is presented in Table (7). The highest total, protein, lipid, and energy digestibility values were observed in the Pr5 treatment, which were significantly different from the other treatments ($P < 0.05$) and showed a decreasing trend at higher inclusion levels. In contrast, carbohydrate digestibility increased compared to the control. Two-way ANOVA indicated that both the type of raw material and the inclusion level had significant effects on digestibility, with a significant interaction between these factors for all parameters except carbohydrate digestibility.

Table 7. Digestibility of test feed containing unpretreated and pretreated jack bean in tilapia

Parameter[1]	Treatments[2]					Two-Way ANOVA[3]		
	0	nPr5	nPr15	Pr5	Pr15	RM	D	RM*D
TD (%)	47.00±0.97[b]	40.63±0.47[c]	38.83±2.02[c]	51.71±0.22[a]	45.42±1.82[b]	*	*	*
PD (%)	75.93±1.00[a]	59.16±0.31[c]	53.86±1.96[d]	77.87±0.83[a]	68.73±1.70[b]	*	*	*
LD (%)	55.53±0.91[b]	45.51±0.70[d]	40.59±0.59[e]	64.66±1.13[a]	52.01±1.67[c]	*	*	*
CD (%)	36.21±1.44[c]	41.28±0.76[b]	44.12±1.78[a]	39.16±0.97[b]	40.11±1.55[b]	*	*	-
ED (%)	54.65±0.92[b]	48.15±0.58[c]	44.20±3.16[d]	65.33±0.24[a]	53.34±1.39[b]	*	*	*

Notes: Different letters in the same row indicate significantly different treatment effects (P<0.05)
[1]TD (Total digestibility), PD (Protein digestibility), LD (Lipid digestibility), CD (Carbohydrate digestibility), ED (Energy digestibility)
[2]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%)
[3]RM (Raw material), D (Dosage), RM*D (Interaction of raw material and dosage).

Growth performance

The growth performance of tilapia fed diets containing jack bean for 60 days is presented in Table (8). Analysis of variance showed that the Pr5 treatment resulted in the best growth performance, with significantly higher values than the other treatments ($P < 0.05$). This was reflected in the highest final biomass, specific growth rate, and most efficient feed conversion ratio. Protein and lipid retention exhibited also the highest values in the Pr5 group. Survival rate in the 5% pretreated jack bean treatment was 100%, equal to the control group, and higher than the other treatments. Plasma protein concentration was the highest in the Pr5 treatment, followed by the control, and declined with increasing inclusion levels of pretreated jack bean or the addition of non-pretreated jack bean. Two-way ANOVA indicated that both raw material type and inclusion level significantly affected most parameters; however, no interaction was observed for feed conversion ratio, protein retention, and survival rate.

.

Table 8. Growth performance of tilapia with the addition of unpretreated and pretreated jack bean in the diet

Parameter[1]	Treatments[2]					Two-Way ANOVA[3]		
	0	nPr5	nPr15	Pr5	Pr15	RM	D	RM*D
IB (g)	122±0.06	122±0.08	122±0.02	122±0.03	122±0.01			
FB (g)	341±22.93^{b}	106±33.70^{d}	15.79±6.67^{e}	571±13.17^{a}	193±28.77^{c}	*	*	*
IBW (g)	8.19±0.01	8.19±0.01	8.19±0.01	8.19±0.00	8.19±0.01			
FBW (g)	22.77±1.53^{b}	12.73±0.31^{d}	11.88±0.32^{d}	38.12±0.88^{a}	16.28±1.09^{c}	*	*	*
FI (g)	423±27.19^{b}	182±0.98^{d}	27.40±0.82^{e}	758±9.81^{a}	260±5.64^{c}	*	*	*
SGR (%)	1.70±0.11^{b}	0.73±0.04^{d}	0.62±0.04^{d}	2.56±0.04^{a}	1.15±0.11^{c}	*	*	*
FCR	1.94±0.08^{d}	3.57±0.34^{b}	5.18±0.54^{a}	1.69±0.07^{d}	2.73±0.23^{c}	*	*	-
PR (%)	28.77±1.83^{ab}	17.73±3.43^{c}	13.18±3.01^{c}	32.27±0.53^{a}	25.35±4.62^{b}	*	*	-
LR (%)	51.06±0.51^{b}	25.36±4.25^{c}	10.18±3.29^{d}	66.01±1.60^{a}	30.11±2.75^{c}	*	*	*
SR (%)	100±0.00^{a}	55.55±16.78^{b}	8.89±3.85^{c}	100±0.00^{a}	80±17.64^{a}	*	*	-
PP (g dL^{-1})	3.53±0.12^{b}	3.06±0.04^{cd}	2.73±0.08^{d}	4.51±0.28^{a}	3.44±0.43^{bc}	*	*	*

Notes: Different letters in the same row indicate significantly different treatment effects ($P<0.05$)

[1]IB (Initial biomass), FB (Final biomass), IBW (Initial body weight), FBW (Final body weight), FI (Feed intake), SGR (Specific growth rate), FCR (Feed conversion ratio), PR (Protein retention), LR (Lipid retention), SR (Survival rate), PP (Plasma protein)

[2]Treatment 0 (No addition of jack bean), nPr5 (addition unpretreated jack bean 5%), nPr15 (addition unpretreated jack bean 15%), Pr5 (addition pretreated jack bean 5%), Pr15 (addition pretreated jack bean 15%)

[3]RM (Raw material), D (Dosage), RM*D (Interaction of raw material and dosage).

DISCUSSION

Effects of soaking and autoclaving on the antinutritional factors of jack bean

The presence of tannins, phytic acid, and trypsin inhibitors is known to limit the use of legumes in aquaculture feeds **(Meng *et al.*, 2021)**. In this study, pretreatment of jack bean effectively reduced antinutritional compounds. Soaking legumes in water causes tissue swelling and water absorption without cell separation **(Eyaru *et al.*, 2009)**. This process reduces tannin content, as phenolic compounds are water-soluble and leach into the soaking medium **(Bhat *et al.*, 2013)**. The decrease in tannins observed here is consistent with the findings of **Khandelwal *et al.* (2010)**, who reported that soaking Indian pulses (*Cajanus cajan*, *Cicer arietinum*, *Lens esculenta*, *Phaseolus aureus*) for 12h reduced tannin and polyphenol concentrations. Similarly, the reduction in phytic acid content can be attributed to its water solubility, as phytate ions dissolve in water and phytase enzymes become activated during soaking, degrading phytate **(Nakitto *et al.*, 2015)**. Comparable reductions have been reported in soybean, where soaking and autoclaving reduced phytic acid from 0.625 to 0.423%, and in kernels and flours soaked for extended periods, where phytic acid fell below detectable levels **(Helbig *et al.*, 2003; Ramadan, 2012)**.

In addition to soaking, heat treatments also reduce antinutrient content. Tannin levels in jack bean were shown to decrease after roasting for 40min **(Odedeji *et al.*, 2019)**, although reductions were limited under dry heat. This agrees with **Khattab and Arntfield (2009)**, who found that roasting and microwave cooking reduced tannins only modestly in cowpea (*Vigna sinensis*), pea (*Pisum sativum*), and kidney bean (*Phaseolus vulgaris*). For example, tannin content in cowpea decreased by just 12.5% after roasting at 120°C for 30min **(Udensi *et al.*, 2007)**. Similarly, phytic acid decreased by only 35.25% under dry roasting **(Khattab & Arntfield, 2009)**. In contrast, moist heat treatment, such as autoclaving, has been shown to be far more effective **(Carlini & Udedibie, 1997)**, as tannins **(Mubarak, 2005; Rakic *et al.*, 2007)**, phytic acid **(Udensi *et al.*, 2007)**, and trypsin inhibitors **(Ezeagu, 2006; Pedrosa *et al.*, 2021)** which are heat-labile and degrade under high temperature and pressure.

The reduced tannin content in pretreated jack bean in this study suggests safe inclusion in tilapia feed at 5%. This agrees with **Mukhopadhyay and Ray (1999)**, who reported growth depression in tilapia fingerlings when tannin content in copra meal reached 2.4% at inclusion levels of 20–25%. Likewise, the phytic acid levels observed in pretreated jack bean were lower than those in soybean meal (10– 15g kg^{-1}) **(Francis *et al.*, 2001)**. Diets containing phytic acid above 5– 6g kg^{-1} have been shown to reduce growth in the rainbow trout **(Spinelli *et al.*, 1983)**. For trypsin inhibitors, untreated and treated jack bean contained 0.72 and 0.37mg g^{-1}, respectively—lower than the threshold of 0.8mg g^{-1} reported for tilapia **(Wee & Shu, 1989)**. Furthermore, these values are considerably lower than that of the soybean (2– 6mg g^{-1}) **(Synder & Kwon, 1987)**.

Digestive enzyme activity

Enzyme activity reflects the ability of fish to utilize nutrients from plant-based feed ingredients **(Zhao *et al.*, 2016)**. In this study, amylase activity was at its highest in the nPr15 treatment, consistent with findings in broad bean-fed tilapia where increasing carbohydrate levels elevated amylase activity **(Fu *et al.*, 2023)**. Conversely, trypsin activity was reduced in diets containing non-pretreated jack bean due to higher trypsin inhibitor content, which binds to the enzyme and reduces activity through competitive or allosteric inhibition **(Glencross, 2016)**.

Histology and morphometry of intestinal villi

The intestine plays a central role in digestion and absorption due to the presence of columnar epithelial cells with microvilli that expand the absorptive surface **(Zhennan *et al.*, 2015)**. Histological analysis is therefore a valuable parameter for evaluating fish metabolism and structural changes in the gut **(Shi *et al.*, 2017)**. In this study, 15% pretreated and all non-pretreated jack bean diets significantly reduced villus height and width ($P< 0.05$), indicating impaired nutrient absorption. Fish fed 5 and 15% pretreated jack bean, however, displayed villus morphology and absorption areas similar to the control, while non-pretreated diets caused severe changes, including submucosal dilation and villus shortening. These findings are consistent with intestinal damage reported in tilapia fed dried faba bean diets **(Chen *et al.*, 2024)**. This, in turn, is an evidence that antinutrients inhibit epithelial cell proliferation **(Miao *et al.*, 2018; daSilva *et al.*, 2022)**. Higher tannin concentrations (1.5–2%) have been shown to cause villus damage in tilapia **(Liu *et al.*, 2025)**.

Feed digestibility

Feed digestibility was highest in the Pr5 treatment (51.71%), but declined at higher inclusion levels or when using non-pretreated jack bean. This trend mirrors findings in tilapia fed chickpea diets, which exhibited lower digestibility than other plant-based feeds due to antinutrients **(Montoya-Mejia *et al.*, 2016)**. Tannin concentrations as low as 0.63% have been shown to reduce nutrient digestibility in tilapia **(Pinto *et al.*, 2000)**, while diets containing faba beans lowered digestibility by 24–36% **(Azaza *et al.*, 2009)**. The reduced digestibility is likely linked to tannins, which form complexes with proteins or bind enzymes, as well as phytic acid, which is indigestible in fish lacking endogenous phytase, and trypsin inhibitors, which have been shown to decrease protein digestibility from 65% to 31% at high inclusion levels **(Lall, 1991; Krogdahl *et al.*, 1994; Mandal & Ghosh, 2010; Omnes *et al.*, 2017)**.

Growth performance and feed utilization

Growth performance was best in the Pr5 treatment, reflecting enhanced palatability and digestibility following antinutrient reduction through soaking and autoclaving **(Okomoda *et al.*, 2016)**. In contrast, non-pretreated jack bean diets impaired growth, similar to observations in tilapia fed coffee grounds containing high tannins **(Moreau *et al.*, 2003)**. Tannins are known to interfere with digestion by binding to enzymes or nutrients **(Francis *et al.*, 2001)**, leading to reduced feed intake, lower protein retention, and poorer feed conversion ratios. Similar reductions in growth and nutrient utilization have been reported in tilapia fed jack bean **(Martinez-Palacios *et al.*, 1988)** due to toxic thermostable compounds such as L-canavanine. Diets containing 0.5–1.5% tannins have also been associated with reduced body protein and lipid content in tilapia and rainbow trout **(Yurkowski *et al.*, 1978; Buyukcapar *et al.*, 2011)**, linked to reduced digestibility and enzyme inhibition. Additionally, reduced plasma protein concentrations observed with increasing jack bean inclusion may reflect impaired nutrient transport due to antinutrient activity **(Liu *et al.*, 2015)**.

CONCLUSION

The results of this study demonstrate that soaking and autoclaving effectively reduce antinutritional factors in jack bean, enabling its safe inclusion in tilapia feed at a 5% level, as evidenced by improved growth performance.

ACKNOWLEDGMENTS

Special thanks go to the research funder, the Indonesian Ministry of Research, Technology and Higher Education through the BIMA 2024 research funding program with contract number 027/E5/PG.02.00.PL/2024 as of June 11, 2024. The authors would also like to thank all colleagues and laboratorty asisstants in the Division of Nutrition and Fish Feed Technology, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

REFERENCES

Akande, K.E. and Fabiyi, E.F. Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding. International Joournal of Poultry Science. 9(10): 996–1001.

[AOAC] Association of Official Analytical Chemist (2012). Official methods of analysis of AOAC Intl. Association of Official Anlytical Chemists, Maryland (US).

Arise, A.K.; Malomo, S.A.; Cynthia, C.I.; Aliyu, N.A. and Arise, R.O. (2022). Influence of processing methods on the antinutrients, morphology and *in-vitro* protein digestibility of jack bean. Food Chemistry Advances. 1(1), 1-7.

Azaza, M.S.; Wassim, K.; Mensi, F.; Abdelmouleh, A.; Brini, B. and Kraiem, M.M. (2009). Evaluation of faba beans (*Vicia faba* L. var. minuta) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus*. Aquaculture. 287(1), 174-179.

Bhat, T.K.; Kannan, A.; Singh, B. and Sharma, O.P. (2013). Value addtion of feed and fodder by allevating the antinutritional effects of tannins. Agricultural Researh. 2(1), 189–206.

Borlongan, I.G. (1990). Studies on the digestive lipases of milkfish (*Chanos chanos*). Aquaculture. 89(3), 312–325.

[BPS] Central Statistics Agency. (2023). Soybean Imports by Main Country of Origin 2022. Central Statistics Agency, Jakarta (ID).

[BPS] Central Statistics Agency. (2024). Average Weekly Per Capita Consumption by Fish Group by District/City. Central Statistics Agency, Jakarta (ID).

Buyukcapar, H.M.; Atalay, A.I. and Kamalak, A. (2011). Growth performance of Nile tilapia (*Oreochromis niloticus*) fed with diets containing different levels of hydrolysable and condensed tannin. J. Agr. Sci. Tech. 13(1), 1045-1051.

Carlini, C.R. and Udedibie, A.B. (1997). Comparative effects of processing methods on hemagglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. J. Agric. Food Chem. 45(1), 4372-4377.

Chen, M.; Li, Q.; Yang, L.; Lin, W.; Qin, Z.; Liang, S.; Lin, L. and Xie, X. (2024). Effects of diet containing germinated faba bean (*Vicia faba* L.) on the intestinal health and gut microbial communities of Nile tilapia (*Oreochromis niloticus*). Aquaculture Reports. 36(1), 1–11.

Darini, M.T.; Susilaningsih, S.E.P. and Sunaryo, Y. (2021). The potential of Jack Bean (*Canavalia ensiformis* L.) developed in suboptimal soil to succeeding food suffiency. International Journal of Current Science Research and Review. 4(7), 740–744.

Darini, M.T. and Kusdiarti, L. (2017). Growth and yield of jack bean (*Canavalia ensiformis* L.) under different rhizobium inoculants and urea doses in sandy soil. Jurnal Agroteknologi. 1(2), 113-122.

daSilva, R.C.; Teixeira, C.S.; Pretto, A.; Costa, T.S.; deSiqueira, J.C.; Pantoja, B.T.S.; Baldisserotto, B. and Lopes, J.M. (2022). Antinutritional effect of lectin from faveira (*Parkia platycephala*) seeds in tambatinga (*Colossoma macropomum X Piaractus brachypomus*). Boletim do Instituto de Pesca. 48(1), 1–10.

Davies, N.T. and Reid, H. (1979). An evaluation of phytate, zinc, copper, iron and availability from soy based textured vegetable protein meat substitutes or meat extruders. British Journal of Nutrition. 41(1), 579–589.

[DJPB] Directorate General of Aquaculture. (2023). Performance Report 2024. Directorate General of Aquaculture, Jakarta (ID).

Doss, A.; Pugalenthi, M.; Vadivel, V.G.; Subhashini, G. and Anitha Subash, R. (2011). Effects of processing technique on the nutritional composition and antinutrients content on under-utilized food legume *Canavalia ensiformis* L.DC. International Food Research Journal. 18(3), 965–970.

Effendie, M.I. (2002). Fisheries Biology, first ed. Yogyakarta, Indonesia.

Erlanger, B.F.; Kokowsky, N. and Cohen, W. (1961). The preparation and properties of two new chromogenis substrates of trypsin. Archive of Biochemistry and Biophysics. 95(2), 271–278.

Eyaru, R.; Shrestha, A.K. and Arcot, J. (2009). Effect of various processing techniques on digestibility of starch in Red kidnet bean (*Phaseolus vulgaris*) and two varieties of peas (*Pisum sativum*). Food Research International. 42(1), 956–962.

Ezeagu, I.E. (2006). Efficiency of inactivation of trypsin inhibitory activity in some selected tropical plant seeds by autoclaving. Nigerian Society for Experimental Biology. 18(1), 21 – 24.

Ezegbe, C.C. (2012). *Nutritional Quality and Physico-Chemical Properties of the Seed and Oil of Chinese Fan Palm*. Nigeria: Department of Food Science and Technology, Federal University of technology Owerri MSc Thesis.

Fagbenro, O.A.; Adeparusi, E.O. and Jimoh, W.A. (2008). Evaluation and nutrient quality of detoxified jackbean seeds, cooked in distilled water or trona solution, as a subtitute for soybean meal in nile tilapia *Oreochromis niloticus* diets. Journal of Applied Aquaculture. 19(2), 83-100.

Farahiyah, I.J.; Zainal, A.A.R.; Ahmad, A.; Mardhati, M.; Thayalini, K. and Yong, S.T. (2016). Evaluation of local feed ingredients based diets on growth performance of african catfish, Clarias gariepinus. Malaysian Journal of Animal Science. 19(2), 39–46.

Francis, G.; Makkar, H.P.S. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and thei effects in fish. Aquaculture. 1(1), 197–227.

Fu, B.; Peng, K.; Chen, B.; Peng, Z.; Zhao, H.; Wang, G.; Huang, W.; Cao, J.; Lu, M. and Cao, J *et al.* (2023). Effects of faba bean supplementation on growth performance, intestinal digestive enzyme activities, intestinal morphology and intestinal flora of tilapia (*Oreochromis niloticus*). Chinese Journal of Animal Nutrition. 35(1), 494–504.

Glencross, B. (2016). Understanding the nutritional and biological constraints of ingredients to optimize their application in aquaculture feeds. Aquafeed Formulation. 1(1), 39–42.

Goddard, S. (1996). Feed Management in Intensive Aquaculture. New York (US): 194 10hal. Chapman and Hall.

Hudiyanti, D.; Arya, A.P.; Siahaan, P. and Suyati, L. (2015). Chemical composition and phospholipids content of Indonesia Jack Bean (*Canavalia ensiformis* L.). Oriental Journal of Chemistry. 31(4), 2043-2046.

Helbig, E.; Oliveira, A.C.; Queiroz, K.S. and Reis, S.M.P.M. (2003). Effect of soaking prior to cooking on the levels of phytate and tannin of the common bean (*Phaseolus vulgaris*, L.) and the protein value. J. Nutr. Sci. Vitaminol. 49(1), 81-86.

Huissman, E.A. (1987). The Principles of Fish Culture Production. The Netherland (NL): Wageningen Agriculture University.

Jansman, A.J.M. (1993). Tannins in feedstuffs for simple-stomached animals. Nutr. Res. Rev. 6(1), 209-236.

Khandelwal, S.; Udipi, S.A. and Ghugre, P. (2010). Polyphenold and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. Food Research International. 43(1), 526-530.

Khattab, R.Y. and Arntfield, S.D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. LWT Food Science and Technology. 42 (1), 1113-1118.

[KKP] Ministry of Marine Affairs and Fisheries. (2024). Performance Report Yeay 2024, Jakarta (ID).

Krogdahl, A.; Lea, T.B. and Olli, J.J. (1994). Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 107(1), 215-219.

Lall, S.P. (1991). Digestibility, metabolisme and excretion of dietary phosphorus in fish. In: Cowey, C.B.; Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Wastes. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Wastes. University of Gieplh, Guelph, Ontario, Canada. 21-26.

Liener, I.E. (1976). Legume toxins in relation to protein digestibility-a review. Journal of Food and Science. 41(1), 1076–1081.

Liu, X.H.; Ye, C.X.; Zheng, L.M.; Ou, C.C.; Wang, A.L.; Ye, J.D. and Kong, J.H. (2015). Dietary maize starch influences growth performance, apparent digestibility coefficient, and hepatic enzyme activities of carbohydrate metabolism in obscure puffer *Takifugu obscurus* (Abe). Journal of the World Aquaculture Society. 46(1), 102–113

Liu, J.; Zhang, X.; Lu,Q.; Zhang, H.; Lin, L. and Li Q. (2025). Tannic acid reduced the growth performance, antioxidant, and immune function of the Nile tilapia (*Oreochromis niloticus*). Aquaculture. 596(1), 1-8.

Mandal, S. and Ghosh, K. (2010). Inhibitory effect of *Pistia* tannin on digestive enzymes of Indian major carp: an in vitro study. Fish Physiol. Biochem. 36(1), 1171-1180.

Martinez-Palacios, C.A.; Galvan, C.R.; Olvera-Novoa, M.A. and Charves-Martinez, C. (1988). The use of jackbean (*Canavalia ensiformis* Leguminosae) meal as a partial substitute for fish meal in diets for tilapia (*Oreochromis mossambicus* Cichlidae). Aquaculture. 68(1), 165-175.

Meng, Z.; Liu, Q.; Zhang, Y.; Chen, J.; Sun, Z.; Ren, C.; Zhang, Z.; Cheng, X. and Huang, Y. (2021). Nutritive value of faba bean (*Vicia faba* L.) as a feedstuff resource in livestock nutrition: A review. Food Science and Nutrition. 9(9), 5244–5262.

Michael, K.G.; Sogbesan, O.A. and Onyia, L.U. (2023). Growth performance of *Clarias gariepinus* fingerlings fed fermented *Canavalia ensiformis* seed meals. Asian Journal of Biological Sciences. 16(1), 71-88.

Miao, S.; Zhao, C.; Zhu, J.; Hu, J.; Dong, X. and Sun, L. (2018). Dietary soybean meal affects intestinal homoeostasis by altering the microbiota, morphology and inflammatory cytokine gene expression in northern snakehead. *Scientific Reports*. 8(1), 1–10

Montoya-Mejia, M.; Hernandez-Llamas, A.; Garcia-Ulloa, M.; Nolasco-Sori, H.; Gutierrez-Dorado, R. and Rodriguez-Gonzalez, H. (2016). Apparent digestibility coefficient of chickpea, maize, high-quality protein maize, and beans diets in juvenile and adult Nile tilapia (*Oreochromis niloticus*). Revista Brasileira de Zootecnia. 45(8), 427–432.

Moreau, Y.; Arredondo, J.L.; Gaime, I.P. and Roussos, S. (2003). Dietaru utilisation of protein and energy from fresh and ensiled coffe pulp by the Nile tilapia, *Oreochromis niloticus*. Brazilian Archives of Biology and Technology. 46(2), 223-231.

Mubarak, A.E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chemistry. 89(1), 489-495.

Mukhopadhyay, N. and Ray, A.K. (1999). Utilization of copra meal in the formulation of compound diets for rohu, *Labeo rohita*, fingerlings. J. Appl. Ichthyol. 15(1), 127-131.

Nakitto, A.M.; Muyonga, J.H. and Nakimbugwe, D. (2015). Effects of combinated traditional processing methods on the nutritional quality of beans. Food Science and Nutrition. 3(3), 233–241.

Nolan, K.B.; Duffin, P.A. and McWeeny, D.J. (1987). Effects of phytate one mineral bioavailability in vitro studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} (also Cd^{2+}) solubilities in the presence of phytate. Journal of The Science of Food and Agriculture. 40(1), 79–85.

Nwosu, J.; Ubbaonu, C.; Banigo, E. and Uzomah, A. (2011). The effects of processing on the anti-nutritional properties of Oze Bosqueia angolensis seed. Journal of American Science. 7(1), 1–6.

Odedeji, J.O.; Akande, E.A.; Fapojuwo, O.O. and Olawuyi, O.J. (2019). Influence of roasting on nutritional and antinutritial factors of jackbean, *Canavalia ensiformis* (L) D.C. Flour. Journal of Underutilized Legumes. 1(1), 112-121.

Oke, V. and Goosen, N.J. (2019). The effect of stocking density of profitability of African catfish (*Clarias gariepinus*) culture in extensive pond systems. Aquaculture. 507, 385 – 392.

Okomoda, V.T.; Tiamiyu, L.O. and Uma, S.G. (2016). Effects of hydrothermal processing on nutritional value of *Canavalia ensiformis* and its utilization by

Clarias gariepinus (Burchell, 1822) fingerlings. Aquaculture Reports. 3(1), 214–219.

Omnes, M.H.; Le Goasduff, J.; Le Delliou, H.; Le Bayon, N.; Quazuguel, P. and Robin J.H. (2017). Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). Aquaculture Resports. 6(1), 21-27.

Pedrosa, M.M.; Guillamon, E. and Arribas, C. (2021). Autoclaved and extruded legumes as a sources of bioactive phytochemicals: A review. Foods. 10(2), 379.

Perdani, A.W. and Utama, Z. (2020). Correlation of phytic acid and soluble protein content of local yellow (*Glycine max*) and black (*Glycine soja*) soybean tempeh flour during fermentation. Jurnal Universitas Negeri Yogyakarta. 15(1), 1–11.

Pinto, L.G.Q.; Pezzato, L.E.; Miranda, E.C.; Barros, M.M. and Furuya, W.M. (2000). Acao do tanino na digestibilidade de dietas pela tilapia-do-nilo (*Oreochromis niloticus*). Acta Scientiarum. 22(3), 677-681.

Rakic, S.; Petrovic, S.; Kukic, J.; Jadranin, M; Tesevic, V.; Povrenovic, D. and Marinkovic, S.S. Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorn from Serbia. Food Chemistry. 104(1), 830-834.

Ramadan, E. (2012). Effect of processing and cooking methods on the chemical composition, sugars, and phytic acid of soybeans. Food and Public Health. 2(1), 11–15.

Sorensen, M.; Stjepanovic, N.; Romarheim, O.H.; Krekling, T. and Storebakken, T. (2009). Soybean meal improves the physical quality of extruded fish feed. Animal Feed Science and Technology. 149(1), 149–161.

Sheahan, C. (2012). Plant guide for jack bean (*Canavalia ensiformis*). USDA-Natural Resources Conservation Service. Cape May (NJ): Cape May Plant Materials Center.

Shi, X.; Luo, Z.; Chen, F.; Wei, C.C.; Wu, K.; Zhu, X.M. and Liu, X. (2017). Effect of fish meal replacement by *Chlorella* meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for crucian carp *Carassius auratus*. Aquac. Res. 48(6), 3244-3256.

Solomon, S.G.; Okomoda, V.T. and Oguche, O. (2017). Nutritional value of raw Canavalia ensiformis and its utilization as partial replacement for soybean meal in the diet of Clarias gariepinus (Burchell, 1822) fingerlings. Journal of Food Science and Nutrition. 1(1), 1–7.

Spinelli, J.; Houle, C.R. and Wekell, J.C. (1983). The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture. 30(1), 71–83.

Suprayudi, M.A.; Jusadi, D.; Setiawati, M.; Ekasari, J. and Fauzi, I.A. (2023). Improved protein and feed efficiency of aquatic organisms, first ed. Bogor, Indonesia.

Synder, H.E. and Kwon T.W. (1987). Soybean Utilization. Van Nostrand Reinhold, New York.

Takeuchi, T. (1988). Laboratory Work Chemical Evaluation of Dietary Nutrients. Tokyo (JP): Japan Kanagawa International Fisheries Training Center. 179–233.

Udedibie, A.B.I. and Nwaiwu, J. (1988). The potential of jackbean (*Canavalia ensiformis*) as animal feed. Nigeria Agricultural Journal. 23(1), 130–143.

Udensi, E.A.; Onwuka, G.I. and Okoli, E.G. (2008). Effect of processing on the levels of some antinutritional factors in *Mucuna utilis*. Plant Products Research Journal. 8(1), 1-6.

Watanabe, T. (1988). Fish Nutrition and Marine Culture. Tokyo (JP): Kanagawa Fisheries Training Center, Japan International Cooperation Agency

Wee, K.L. and Shu, S.W. (1989). The nutritive value of boiled full-fat soybean in pelleted feed for Nile tilapia. Aquaculture 81, 303–314.

Worthington, V. (1993). Worthington Enzyme Manual: Enzymes and Related Biochemicals. New Jersey (US): Worthington Biochemical Corporation.

Yurkowski, M.; Baily, J.K.; Evans, R.E.; Tabachek, J.A.L; Ayles, G.B. and Eales, J.G. (1978). Acceptability of rape seed proteins in diets of rainbow trout (*Salmo gairdnerii*). J. Fish. Res. Board Can. 35(1), 951-962.

Zhao, Z.X.; Song, C.Y.; Xie, J.; Ge, X.P.; Liu, B.; Xia, S.L.; Yang, S.; Wang, Q. and Zhu, S.H. (2016). Effects of fish meal replacement by soybean peptide on growth performance, digestive enzyme activities, and immune responses of yellow catfish *Pelteobagrus fulvidraco*. Fisheries Science. 82(1), 665–673.

Zhennan, Z.; Ermeng, Y.; Jun, X.; Guangjun, W.; Deguang, Y.; Zhifei, L.; Wangbao, G.; Haiying, W.; Nan, W. and Yun, X. (2015). Intestinal microflora dynamic change, serum enzyme and growth performance of the grass carp (*Ctenopharyngodon idellus*) at different stages of feeding broad bean (*Vicia faba*). Journal of Agriculture Biotechnology. 23(2), 151–160.