

## Utilization of jack bean flour *Canavalia ensiformis* as feed ingredient for tilapia *Oreochromis* sp.

### Pemanfaatan tepung kacang koro pedang *Canavalia ensiformis* sebagai bahan pakan ikan nila *Oreochromis* sp.

Benediktus Anugerah Kalyanaputra Pamungkas<sup>1</sup>, Ichsan Achmad Fauzi<sup>1\*</sup>, Dedi Jusadi<sup>1</sup>,  
Maria Mojena Gonzales-Plasus<sup>2</sup>

<sup>1</sup>Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia

<sup>2</sup>Faculty, College of Fisheries and Natural Sciences, Western Philippines University, Puerto Princesa Campus, Rafols rd, Brgy. Sta Monica, Puerto Princesa City, Palawan, 5300, Philippines

\*Corresponding author: [ichsanfauzi@apps.ipb.ac.id](mailto:ichsanfauzi@apps.ipb.ac.id)

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#### ABSTRACT

The local plant jack bean has a high nutrient content, but this plant is still underutilized. The high protein and carbohydrate content in this plant can be potentially utilized as raw material for fish feed. Thus, this study aims to evaluate the utilization of unpeeled jack bean flour as a feed ingredient on the growth of tilapia (*Oreochromis* sp.). The study consisted of four treatments with three replications using a completely randomized design, namely TK0 (amount of jack bean flour 0%), TK5 (amount of jack bean flour 5%), TK15 (amount of jack bean flour 15%), and TK25 (amount of jack bean flour 25%) added to the feed formulation. Tilapia of 15 fish were kept in an aquarium measuring 50×50×40 cm<sup>3</sup> for 60 days and fed three times a day until apparent satiation. The results showed that treatments with inclusions of jack bean flour in feed had a significantly lower growth performance, feed intake, and intestine morphometrics compared to control treatment ( $P<0.05$ ). There is no significant difference between TK5 and control on feed conversion ratio, protein retention, and survival rate. However, higher inclusion of jack bean flour (TK15 & TK25) demonstrates significantly lower value of those parameters compared to control treatment.

Keywords: feed formulation, growth, jack bean, Nile tilapia

#### ABSTRAK

Tanaman lokal kacang koro pedang memiliki kandungan nutrisi yang tinggi, namun tanaman ini masih kurang dimanfaatkan. Kandungan protein dan karbohidrat yang tinggi pada tanaman ini dapat berpotensi menjadi bahan baku pakan ikan. Penelitian ini bertujuan mengevaluasi pemanfaatan tepung kacang koro pedang dengan kulit ari sebagai bahan baku pakan terhadap pertumbuhan ikan nila (*Oreochromis* sp.). Penelitian terdiri dari empat perlakuan dengan tiga kali ulangan menggunakan rancangan acak lengkap yaitu TK0 (jumlah tepung kacang koro pedang 0%), TK5 (jumlah tepung kacang koro pedang 5%), TK15 (jumlah tepung kacang koro pedang 15%), dan TK25 (jumlah tepung kacang koro pedang 25%) yang ditambahkan pada formulasi pakan. Pemeliharaan ikan nila sebanyak 15 ekor dilakukan di akuarium berukuran 50×50×40 cm<sup>3</sup> selama 60 hari dan diberi pakan tiga kali sehari secara *at satiation*. Hasil penelitian menunjukkan bahwa perlakuan dengan inklusi kacang koro pedang menghasilkan kinerja pertumbuhan, konsumsi pakan, dan nilai morfometris dari usus yang lebih rendah dibandingkan dengan perlakuan ( $P<0,05$ ). Sementara itu, tidak ada perbedaan yang nyata antara perlakuan TK5 dan kontrol pada parameter konversi pakan, retensi protein, dan kelangsungan hidup, walaupun perlakuan dengan inklusi tinggi kacang koro pedang (TK15 & TK25) menunjukkan nilai yang secara signifikan lebih rendah dibandingkan dengan perlakuan kontrol.

Kata kunci: formulasi pakan, ikan nila, kacang koro pedang, pertumbuhan

## INTRODUCTION

Tilapia (*Oreochromis* sp.) is a leading aquaculture commodity, with a total production that increasing by 9.86%, from 1,125,149 tonnes in 2018 to 1,642,544 tonnes in 2022 (DJPB, 2022). The increasing trend of total tilapia production should be supported by the availability of production inputs in tilapias aquaculture activity. One production input that considered to be detrimental in tilapia farming is feed (Nurhalisa *et al.*, 2022), which is known to account for 50-60% of total production costs (Suprayudi *et al.*, 2016). Feed has an important role in fish growth, so the feed provided must be of good quality (Manurung & Mose, 2018). Good quality feed contains complete nutrients according to the needs of fish, is easily digestible by fish, and does not contain substances that are harmful to fish (Yunaidi *et al.*, 2019).

The feed manufacturing process still relies on the use of soybean meal and pollard as one of the sources of raw materials. However, the soybean meal used is relatively expensive and still imported, so alternative sources of local raw materials are needed (Centyana *et al.*, 2014). Alternative feed ingredients must fulfill several criteria, including continuous and abundant availability, appropriate nutrient composition to meet fish requirements, and ease to procurement (Centyana *et al.*, 2014; Minggawati *et al.*, 2019). An alternative local raw material source that can be used is jack bean (*Canavalia ensiformis*), which is known to have nutrient content in the form of protein and carbohydrates, similar to soybean meal.

Jack bean belongs to the genus *Canavalia* which consists of 48 species of legumes that are classified as underutilized (Okomoda *et al.*, 2016). Jack bean is one of the local plants that can be cultivated in tropical areas such as Sumatra, Lampung, West Nusa Tenggara, Banten, West Java, Central Java, East Java, and Yogyakarta with a productivity level of 12-24 tons per year (Priharyanto *et al.*, 2022; Pertiwi *et al.*, 2018). Odedeji *et al.* (2020) reported that the nutrient content of jack bean flour is 32.02% protein content, 5.01% crude fiber, 46.42% carbohydrate, 4.43% ash content, and 3.80% fat content. Jack bean can be utilized in two forms, namely peeled and unpeeled. The use of unpeeled jack bean can be an option because it produces more flour, costs less to process, and the flouring process is faster when compared to the use of peeled jack, which

requires more processing time and produces waste in the form of husk (Santosa & Yuliati, 2017).

Several studies related to the utilization of jack bean beans in fish feed have been conducted. Based on Hidayat (2023), it was found that the addition of unpeeled jack bean flour with a ratio of 75% commercial feed and 25% unpeeled jack bean flour processed by soaking for 24 hours, resulted in protein digestibility of 76.37%, fat 38.13% and carbohydrates 47.67% which was not significantly different from commercial feed in tilapia. Another study showed that the utilization of raw *C. ensiformis* as much as 5% in catfish feed to substituting soybean meal did not affect fish growth (Solomon *et al.*, 2017). However, it has been reported that the presence of antinutrients in sword beans poses an obstacle to their utilization (Ojo *et al.*, 2018), that can have a negative impact (Arise *et al.*, 2022). Furthermore, research was found that by utilizing the processed jack bean in the form of roasting to substitute soybean meal by 5% (Tiamiyu *et al.*, 2016) and 25% which showed the most optimal growth in catfish (Michael *et al.*, 2023). Based on this description, it is necessary to conduct research to evaluate inclusion levels of soaked and unpeeled jack bean flour as a raw material for tilapia feed.

## MATERIALS AND METHODS

### Experimental design

This research was conducted using an experimental method using a complete randomized design (CRD) with four feed treatments. Each treatment was applied three replications. The research data obtained were processed using Microsoft Excel 2019 software. The treatments involved the use of unpeeled jack bean flour as a substitute for soybean meal and pollard in the feed composition at inclusion levels of 0%, 5%, 15%, and 25%.

### Experimental diets

The experimental diet was formulated from various raw materials at the Fish Nutrition Feed Laboratory, Faculty of Fisheries and Marine Science. Unpeeled Jack bean as a raw material was soaked for 24 hours and drained with water as a process to reduce water-soluble antinutrients. After that, unpeeled jack bean was ground into puree and proximate test was conducted. The proximate test results of jack bean flour are presented in Table 1. The treatments in this study consisted of adding unpeeled jack bean flour

at levels of 0%, 5%, 15%, and 25% to the feed formulation as a substitute for soybean meal and pollard.

Feed preparation was carried out by mixing all raw materials that had been weighed according to the formulation (Table 2). The mixed ingredients were then pelleted using a pellet machine to produce feed pellets with a diameter of 1 mm. The pellets were subsequently dried in an oven at

60°C for 2 hours. After cooling, the experimental feed was placed in plastic container and stored at room temperature to maintain its quality. The feed was then used for the feeding trial.

#### Fish maintenance

The experimental fish used in this study were red tilapia (*Oreochromis* sp.) from farmers in Ciampea, Bogor Regency, West Java. Prior to the

Table 1. Proximate analysis results of jack bean flour (*Canavalia ensiformis*).

Composition	Proximate analysis results (% dry matter)					
	Protein	Lipid	Ash	Crude fiber	NFE <sup>1</sup>	GE <sup>2</sup> (Kcal/kg)
Unpeeled Jack bean	28.49	1.94	4.08	6.62	56.87	4380.70
Peeled Jack bean	36.56	2.46	3.84	0.41	54.49	4528.96

Note: <sup>1</sup>Wet nitrogen-free extract (NFE) = 100 (protein + lipid + ash + crude fiber), <sup>2</sup>Gross energy (GE) composition of dry feed was calculated based on protein = 5.64 kcal/g protein; lipid = 9.44 kcal/g lipid; and carbohydrates or NFE = 4.11 kcal/g carbohydrate (Watanabe, 1998).

Table 2. Composition and proximate analysis of experimental diets supplemented with unpeeled jack bean *Canavalia ensiformis* (flour) at different inclusion levels (% dry weight).

Composition (%)	Dietary inclusion levels of <i>Canavalia ensiformis</i> flour			
	0%	5%	15%	25%
Fish Meal	5.00	5.00	5.00	5.00
Poultry Meat Meal	6.00	6.00	6.00	6.00
Meat Bone Meal	5.00	5.00	5.00	5.00
Soybean Meal	26.00	24.00	20.00	16.00
Cassava Flour	5.00	5.00	5.00	5.00
Wheat Pollard	25.00	22.00	16.00	10.00
Wheat Flour	23.00	23.00	23.00	23.00
Jack bean flour with skin	0.00	5.00	15.00	25.00
Fish Oil	0.50	0.50	0.50	0.50
Crude Palm Oil	2.20	2.20	2.20	2.20
Vitamin Mix	0.15	0.15	0.15	0.15
Mineral Mix	0.15	0.15	0.15	0.15
Polymethyl carbamide	1.00	1.00	1.00	1.00
Lysine	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50
<b>Proximate nutrient composition</b>				
Water (%)	11.44	12.35	11.00	10.75
Protein (%)	28.80	27.80	27.07	26.98
Fat (%)	6.69	6.48	6.23	5.99
Ash (%)	7.50	6.73	9.50	6.47
Crude fiber (%)	3.41	4.26	2.66	4.49
NFE (%) <sup>1</sup>	42.15	42.38	43.54	45.32
GE (kcal/kg) <sup>2</sup>	4128.80	4096.71	4013.85	4134.49
C/P (kcal/g) <sup>3</sup>	143.35	147.36	148.30	153.26
<b>Total %</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

Note: <sup>1</sup>Wet nitrogen-free extract (NFE) = 100 (protein + lipid + ash + crude fiber), <sup>2</sup>Gross energy (GE) composition of dry feed was calculated based on protein = 5.64 kcal/g protein; lipid = 9.44 kcal/g lipid; and carbohydrates or NFE = 4.11 kcal/g carbohydrate (Watanabe, 1998), <sup>3</sup>C/P = calories/protein.

experiment, the fish were acclimatized for five days in an aquarium measuring 1.00×0.50×0.50 m<sup>3</sup> with a water level of 0.40 m (0.2 m<sup>3</sup> water volume). The fish were then transferred into experimental aquarium measuring 50×50×40 cm<sup>3</sup> with a water level of 30 cm (75 L). Red tilapia used for the growth performance test had a body length of 4–5 cm and an initial weight of 1.92 ± 0.01 g. A total of 15 fish were stocked in each aquarium. The rearing water was stored in a reservoir for 24 hours prior to use to allow sedimentation. After stocking, 0.75 g of Elbayou (Ueno Food Techno Industry) was added to each aquarium at the beginning of the rearing period as a preventive measure against bacterial diseases.

Each aquarium was equipped with aeration and a thermostat set at 30°C to maintain water quality. Water exchange was carried out every three days during the first month and every two days thereafter. Fish were fed to apparent satiation three times daily at 08:00, 12:00, and 16:00 throughout the rearing period. Biomass measurements were conducted every 30 days using a census method. Water quality parameters, including temperature and pH, were measured twice daily to ensure suitable rearing conditions, while dissolved oxygen (DO), nitrite, and total ammonia nitrogen (TAN) were measured every two weeks, as presented in Table 3.

### Chemical analysis

Chemical analyses were conducted on the experimental diets and fish samples collected at the beginning and end of the rearing period. The analyses were performed according to the methods of AOAC (2012), including determination of ash, moisture, crude fat, crude protein, crude fiber, and nitrogen-free extract (NFE) contents. Ash content was determined by incinerating samples in a furnace at 600°C. Moisture content was analyzed by drying samples in an oven at 105–110°C. Wet fat content was determined using the Folch

method, while dry fat content was analyzed using the Soxhlet method.

Protein content was determined using the Kjeldahl method, which consists of three stages: digestion, distillation, and titration. Crude fiber analysis was performed through acid and alkaline digestion followed by furnace heating. Nitrogen-free extract (NFE) content was calculated by subtracting the total percentage of other nutrient components from 100%.

### Experimental parameters

The experimental parameters used in this study were final biomass (Bt), final individual weight (Wt), feed intake (FI), feed conversion ratio (FCR), survival rate (SR), average daily gain (ADG), specific growth rate (SGR), fat retention (FR), protein retention (PR), and intestinal morphometry.

### Feed intake

Feed intake is obtained based on the total amount of feed used during the rearing process from the beginning of stocking to harvest.

### Feed conversion ratio

Feed conversion ratio (FCR) is defined as the amount of feed required to produce 1 kg of fish biomass. Feed conversion was calculated by the following formula (Goddard, 1996):

$$FCR = \frac{F}{Wt - W0 + Wd}$$

Note:

- FCR = Feed conversion ratio
- Wt = Total fish biomass at the end of rearing (g)
- W0 = Total fish biomass at the beginning of rearing (g)
- Wd = Total weight of dead fish during rearing (g)
- F = Total of feed consumed (g)

Table 3. Water quality measurement results during rearing.

Parameters	Unit	Dietary inclusion level of <i>Canavalia ensiformis</i> flour				Reference
		TK0	TK5	TK15	TK25	
Temperature	°C	28.3-30.9	28.8-30.6	28.5-30.8	29.8-30.8	28-31 <sup>1</sup>
pH		6.85-7.95	6.58-7.98	6.76-8.26	7.70-7.98	6.5-8 <sup>2</sup>
DO	mg/L	3.3-4.4	3.0-4.2	3.2-4.4	3.6-4.6	≥3 <sup>2</sup>
TAN	mg/L	0.00-0.85	0.03-0.92	0.00-0.60	0.00-0.79	0.25-0.94 <sup>1</sup>
Nitrite	mg/L	0.27-0.81	0.18-0.69	0.01-0.63	0.02-0.67	<1 <sup>3</sup>

Note: <sup>1</sup>(Yusuf *et al.*, 2016); <sup>2</sup>(SNI, 2009); <sup>3</sup>(Deswati *et al.*, 2022).

### Survival rate

Survival rate was calculated using the following formula (Effendie, 2002):

$$SR (\%) = \frac{N_t}{N_0} \times 100$$

Note:

- SR = Survival rate (%)  
 Nt = Number of test fish at end of rearing  
 N0 = Number of test fish at the beginning of rearing

### Average daily gain

Average daily gain define as the weight gain of fish per day. Average daily gain was calculated using the formula used by Witoko *et al.* (2018):

$$ADG = \frac{\text{final weight} - \text{initial weight}}{\text{days}}$$

Note:

- ADG = Average daily gain  
 Wt = Final weight (g)  
 W0 = Initial weight (g)  
 T = Observation period (days)

### Specific growth rate

Specific growth rate is the average amount of fish weight obtained based on the weighing of samples from each treatment carried out. Specific growth rate was calculated using the formula (Huisman, 1987):

$$SGR = \left( \frac{\ln W_t - \ln W_0}{t} \right) \times 100$$

Note:

- SGR = Specific growth rate  
 Wt = Final weight (g)  
 W0 = Initial weight (g)  
 T = Observation period (days)

### Fat retention

Fat retention (FR) is the difference between body fat levels at the end and beginning of rearing compared to the amount of feed fat consumed by fish. Fat retention (FR) was calculated based on the formula (Dewi & Tahapari, 2017):

$$LR (\%) = \frac{F - I}{P} \times 100$$

Note:

- FR = Fat retention (%)  
 F = Total fish fat at the end of rearing (g)

I = Total fish fat at the beginning of rearing (g)

P = Total fat consumed by fish (g)

### Protein retention

Protein retention (RP) is the difference between body protein levels at the end and the beginning of maintenance compared to the amount of feed protein consumed by fish. Protein retention (RP) was calculated based on the formula (Dewi & Tahapari, 2017):

$$PR (\%) = \frac{F - I}{P} \times 100$$

Note:

- PR = Protein retention (%)  
 F = Total fish protein at the end of rearing (g)  
 I = Total fish protein at the beginning of rearing (g)  
 P = Total of protein consumed by fish (g)

### Intestinal morphometry

Histological procedures were performed according to Kiernan (2015). Midgut tissues fixed in neutral buffered formalin for 24 hours were dehydrated through a graded alcohol series and subsequently cleared with xylene. Samples were embedded in paraffin, then tissue samples were sectioned and stained with hematoxylin and eosin. The tissue sections were observed using a light microscope equipped with a camera. ImageJ application was used to measure the height and width of each villus. Before starting the measurement, the scale bar was set at 100  $\mu\text{m}$  and 'straight line selection' was selected from the toolbar. One representative villus with the best morphology was selected from each replicate for measurement. The dimensions of the selected villi were then averaged to obtain the final value. The absorptive surface area was calculated using the following formula (Mohammady *et al.*, 2021):

$$\text{Absorption area } \mu\text{m}^2 = \text{height of villi} \times \text{width of villi}$$

### Data calculation and analysis

The research data were processed and analyzed using Microsoft Excel 2019 and SPSS version 26.0. Prior to analysis, the data were tested for normality and homogeneity of variance. Analysis of variance (ANOVA) was performed at a 95% confidence level to determine the effect of treatments on the measured parameters. When

significant differences were detected, Duncan's multiple range test was subsequently conducted to identify differences among treatments.

## RESULTS AND DISCUSSION

### Result

#### Growth parameters

The effects of incorporating unpeeled jack bean flour into the diets on the growth performance of tilapia over a 60-day rearing period are presented in Table 4. Analysis of variance (ANOVA) revealed significant differences ( $P < 0.05$ ) in several growth parameters among treatments. The inclusion of unpeeled jack bean flour in the feed formulation as a substitute for soybean meal and pollard resulted in decreased final biomass (Bt), average daily gain (ADG), specific growth rate (SGR), survival rate (SR), protein retention (PR), and fat retention (FR) as the inclusion level increased. In contrast, the feed conversion ratio (FCR) increased with higher levels of unpeeled jack bean flour inclusion.

#### Intestinal morphometry

The histological features of tilapia intestine after feeding with different amounts of unpeeled jack bean flour are presented in Figure 1. The results of the calculation of intestinal morphometry of tilapia fish fed with feed containing unpeeled jack bean flour (TK) at different doses are presented in

Table 5. The results of villi width measurements showed that the control treatment (without TK) showed significant different results ( $P < 0.05$ ) when compared to other treatments that used the addition of unpeeled jack bean flour in feed.

### Discussion

Based on the results of proximate analysis (% dry weight), unpeeled jack bean flour (TK) in this study contained 28.49% protein, 1.94% fat, 4.08% ash, 6.62% crude fiber, and 56.87% NFE (Table 1). The protein content of unpeeled jack bean flour was lower than that of huskless jack bean flour, but Aurelia *et al.* (2019) reported that 1.5 kg of jack bean will produce 200 grams of husk, so when its utilization without skin increases, it will produce a large amount of unused jack bean husk. Aurelia *et al.* (2019) reported that jack bean husk contains 4.76% ash, and the crude fiber content of jack bean flour increases when the husk is included. However, research by Hidayat (2023) proved that the digestibility of unpeeled jack bean can be utilized as one of the raw materials for feed constituents in tilapia.

Final biomass (Bt) and final individual weight (Wt) generally decreased as the amount of unpeeled jack bean flour increased in feed. The results for the final biomass (Bt) parameter showed a significant difference between the control treatment (without unpeeled jack bean flour) and the TK25 treatment. The control

Table 4. The results of the growth performance of tilapia, *Oreochromis* sp. fed with diets containing unpeeled jack bean flour (TK) at different inclusion level.

Experimental parameters <sup>1</sup>	Dietary inclusion of <i>Canavalia ensiformis</i> flour			
	TK0	TK5	TK15	TK25
B <sub>0</sub> (g)	28.72 ± 0.10	28.67 ± 0.15	28.82 ± 0.13	28.88 ± 0.03
B <sub>t</sub> (g)	148.59 ± 3.10 <sup>a</sup>	79.60 ± 8.16 <sup>b</sup>	13.65 ± 2.11 <sup>c</sup>	12.17 ± 0.40 <sup>c</sup>
W <sub>0</sub> (g)	1.91 ± 0.01	1.91 ± 0.01	1.92 ± 0.01	1.93 ± 0.01
W <sub>t</sub> (g)	10.64 ± 0.54 <sup>a</sup>	6.46 ± 0.36 <sup>b</sup>	2.57 ± 0.43 <sup>c</sup>	2.50 ± 0.48 <sup>c</sup>
ADG (g/day)	0.15 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>c</sup>	0.01 ± 0.01 <sup>c</sup>
SGR (%/day)	2.86 ± 0.08 <sup>a</sup>	2.03 ± 0.08 <sup>b</sup>	0.47 ± 0.26 <sup>c</sup>	0.41 ± 0.33 <sup>c</sup>
SR (%)	93.33 ± 6.67 <sup>a</sup>	88.89 ± 7.70 <sup>a</sup>	35.55 ± 3.85 <sup>b</sup>	33.33 ± 6.67 <sup>b</sup>
FI (g)	215.52 ± 6.48 <sup>a</sup>	120.08 ± 6.36 <sup>b</sup>	33.41 ± 1.26 <sup>c</sup>	32.91 ± 1.86 <sup>c</sup>
FCR	1.74 ± 0.09 <sup>a</sup>	1.77 ± 0.01 <sup>a</sup>	3.51 ± 0.86 <sup>b</sup>	3.90 ± 0.18 <sup>b</sup>
PR (%)	32.00 ± 2.44 <sup>a</sup>	25.90 ± 1.58 <sup>a</sup>	15.53 ± 4.40 <sup>b</sup>	13.61 ± 4.93 <sup>b</sup>
FR (%)	72.75 ± 5.85 <sup>a</sup>	44.23 ± 2.44 <sup>b</sup>	36.34 ± 6.19 <sup>b</sup>	33.82 ± 6.20 <sup>b</sup>

Note: <sup>1</sup>Initial biomass (B<sub>0</sub>), final biomass (B<sub>t</sub>), individual initial weight (W<sub>0</sub>), individual final weight (W<sub>t</sub>), survival rate (SR), feed intake (FI), feed conversion ratio (FCR), average daily gain (ADG), specific growth rate (SGR), protein retention (PR), and fat retention (FR). <sup>2</sup>Different letters in the same row indicate significant differences ( $P < 0.05$ ). Values shown are the mean and standard deviation.

treatment produced a final biomass of  $148.59 \pm 3.10$  g, whereas the TK25 treatment resulted in a biomass of  $11.89 \pm 2.26$  g. Similar trends were observed for average daily gain (ADG) and specific growth rate (SGR), both of which decreased as the inclusion level of unpeeled jack bean flour in the diet increased.

These results indicate that increasing the inclusion level of unpeeled jack bean flour significantly reduced the growth performance of tilapia. Better growth performance was observed in the TK5 treatment compared to the TK15

and TK25 treatments. Similarly, Olunkule *et al.* (2015) reported that substituting soybean meal with jack bean flour resulted in the highest weight gain (3.62 g) in catfish fed the control diet (0% jack bean seed meal), while weight gain decreased with increasing inclusion levels of jack bean seed meal, reaching 2.50 g in TD1 (5% jack bean seed meal) and the lowest value of 1.49 g in TD4 (20% jack bean seed meal). The same results were revealed in the study of Solomon *et al.* (2017) that the substitution of soybean meal with raw *C. ensiformis* flour. The highest weight

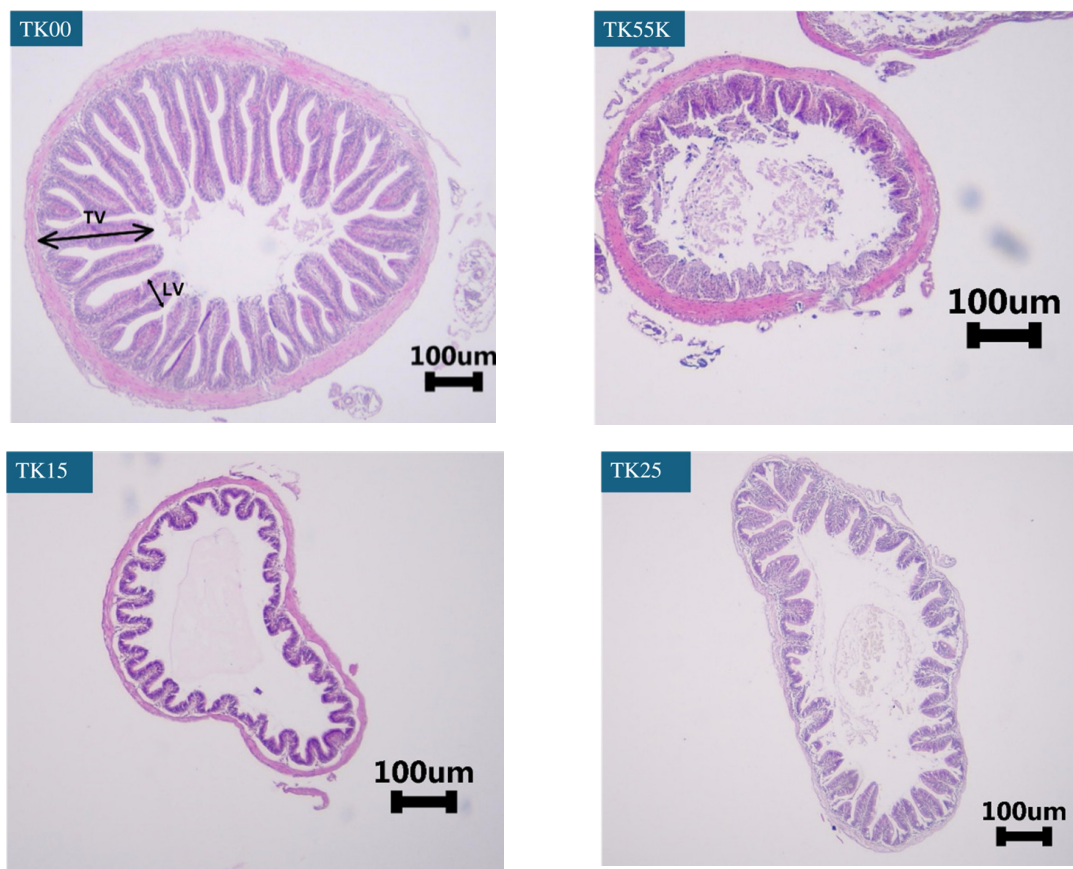


Figure 1. Intestinal morphology of tilapia *Oreochromis* sp. fed diets containing jack bean with skin (TK) at different inclusion level (100× magnification). (TK0) jack bean inclusion 0% treatment in feed, (TK5) jack bean inclusion 5% treatment in feed, (TK15) jack bean inclusion 15% treatment in feed, (TK25) jack bean inclusion 25% treatment in feed, VH=Villi height, VW=Villi width.

Table 5. Intestinal morphometry of tilapia *Oreochromis* sp. after feeding with different doses of jack bean flour with skin (TK) for 60 days.

Experimental parameters <sup>1</sup>	Dietary inclusion level of <i>Canavalia ensiformis</i> flour			
	TK0	TK5	TK15	TK25
VH (µm)	$368.14 \pm 7.23^a$	$87.36 \pm 9.34^b$	$64.28 \pm 2.71^c$	$56.25 \pm 3.18^c$
VW (µm)	$83.61 \pm 7.53^a$	$42.85 \pm 2.12^b$	$41.55 \pm 1.05^b$	$38.44 \pm 1.84^b$
IAA (µm <sup>2</sup> )	$30757 \pm 2464^a$	$3731 \pm 232^b$	$2672 \pm 180^b$	$2158 \pm 28^b$

Note: <sup>1</sup>Villi height (VH), villi width (VW), and intestinal absorption area (IAA), <sup>2</sup>Different letters in the same row indicate significant differences ( $P < 0.05$ ). Values shown are the mean and standard deviation.

gain was observed in catfish fed the control diet (0% *Canavalia ensiformis*), reaching 3.81 g, and decreased with increasing inclusion levels of *C. ensiformis* flour, with weight gains of 3.78 g at 10% inclusion and the lowest value of 2.69 g at 40% inclusion.

The SR value in the control treatment (TK0) was not significantly different ( $P < 0.05$ ) with TK5, but significantly different in the treatments of TK15 and TK25. The treatment given affects the survival of tilapia. The inclusion of unpeeled jack bean flour in the diet at 5% did not significantly affect the growth performance or survival rate of tilapia. However, inclusion levels of 15% and 25% negatively affected both growth and survival rate. These findings are consistent with the study by Tiamiyu *et al.* (2016) which reported that the survival rate of *Clarias gariepinus* fed diets containing different inclusion levels of jack bean flour decreased as the inclusion level increased, from 64.4% in the control treatment to 20% at the 20% inclusion level.

The feed intake (FI) values showed a decreasing trend with increasing inclusion levels of unpeeled jack bean flour, with the TK5 treatment producing the best result. However, the TK5 treatment was found to be significantly different ( $P < 0.05$ ) from the control treatment. This is related to feed palatability. Feed palatability is affected by feed aroma, and an unappealing aroma may lead to reduced feed consumption in fish (Andriani & Rostika, 2021). This is similar to the research of Tiamiyu *et al.* (2016) FI of *Clarias gariepinus* fish fed with feed with different inclusion levels of raw jack bean flour decreases as the inclusion level of jack bean flour increases: control treatment 8.49 g and 6.65 g at the 20% inclusion level.

Similar results were reported by Solomon *et al.* (2017) using the same fish species and treatment, where feed intake (FI) in the control treatment was 6.24 g and decreased to 5.21 g at the 40% inclusion level. The feed conversion ratio (FCR) of tilapia in the control treatment (without unpeeled jack bean flour) was not significantly different from that of the 5% inclusion treatment ( $P > 0.05$ ), but differed significantly from the 15% and 25% inclusion treatments. According to Shofura *et al.* (2017), feed conversion ratio (FCR) is defined as the ratio between the amount of feed consumed and the weight gain produced by the fish. Efficient feed utilization allows nutrients to be effectively converted into fish biomass. Differences in the ability of fish to digest and absorb nutrients may

result in variations in FCR values. A lower FCR indicates better feed quality and greater efficiency of the fish in utilizing feed for growth (Maryam *et al.*, 2019).

This is in accordance with the study of Tiamiyu *et al.* (2016), the feed conversion ratio (FCR) of *Clarias gariepinus* fed diets containing different inclusion levels of raw jack bean flour increased as the inclusion level of jack bean flour increased, from 6.57 in the control treatment to 10.63 at the 20% inclusion level and research by Solomon *et al.* (2017) with the same fish and treatment, FCR on control treatment was 2.06 and 2.76 at 40% inclusion level. Protein retention shows the percentage ratio between the total protein consumed during the rearing period and the protein stored in the fish body (Lubis *et al.*, 2019). Protein in the fish body functions for metabolic processes, repairing damaged cells, and supporting growth (Pitri *et al.*, 2021).

The results showed that the retention value in the control treatment (without unpeeled jack bean flour) was not significantly different ( $P > 0.05$ ) from that in the TK5 treatment, but differed significantly ( $P < 0.05$ ) from those in the TK15 and TK25 treatments. Fat retention describes the ability of fish to store and utilize feed fat (Supati *et al.*, 2021). The results showed the retention value in the control treatment (without TK) showed significantly different results ( $P < 0.05$ ) in all treatments of TK addition. According to Yanto *et al.* (2019), Low feed intake may reduce the amount of carbohydrate-derived energy consumed by the fish. As a result, protein and fat are utilized as alternative energy sources to meet metabolic needs, leading to lower protein retention and fat retention values.

These findings are consistent with the study by Purnamasari *et al.* (2020) which reported that reduced feed intake could result in lower protein and fat retention. In addition, the presence of antinutritional compounds in alternative feed ingredients may inhibit nutrient metabolism, thereby decreasing protein and fat retention values (Kushayadi, 2019; Annisa *et al.*, 2020). Intestinal histology in tilapia showed severe changes such as reduced submucosal width, shrunken and stunted villi. The villi height was significantly shorter as the amount of unpeeled jack bean flour in the feed increased. Longer intestinal villi were observed in the control treatment, followed by the TK5, TK15, and TK25 treatments. These findings are consistent with the study by Hlophé & Moyo,

which reported that *Clarias gariepinus* fed diets containing higher inclusion levels of moringa flour (>50%) exhibited significantly shorter villi.

According to Da Silva *et al.* (2012) longer intestinal villi in fish indicate greater efficiency of nutrient absorption. The size of the villi is also closely related to intestinal morphology, the larger the size of the villi, the higher the absorption area, allowing the intestine to absorb and digest nutrients better (Mohammady *et al.*, 2021). Digestive inefficiency increases due to the presence of antinutrients that interfere with optimal nutrient absorption (Lepcha *et al.*, 2017). This was also reflected in the better growth performance observed in the control and TK5 treatments compared to the other treatments. The results of the present study showed that the inclusion of unpeeled jack bean flour at a level of 5% produced the highest intestinal absorptive surface area, which differed significantly from the other jack bean treatments. A reduction in villus height may decrease the absorptive surface area for nutrient uptake, which could also contribute to poorer liver condition (Da Silva *et al.*, 2012). This phenomenon is known to be related to the relatively high sensitivity of the distal intestine to antinutrient compounds (Wang *et al.*, 2016).

This study showed that increasing the inclusion level of unpeeled jack bean flour in the diet resulted in decreased growth performance of tilapia. The reduction in growth performance is likely associated with the presence of antinutritional compounds in unpeeled jack bean. Pal *et al.* (2017) reported that jack bean husk contains relatively high levels of antinutrients; therefore, peeling is commonly performed to reduce their presence. As mentioned in Utami (2022) and Tiamiyu *et al.* (2016), Jack bean contains antinutrients in the form of canavanine, saponins, crude fiber, phytic acid, tannins, and cyanide (HCN). The presence of antinutrients in legumes limits their use as feed ingredients in aquaculture (Meng *et al.*, 2021). Soaking jack bean seeds can reduce a substantial amount of soluble cyanide through leaching into the soaking water, thereby making the processed material safer for use as a feed ingredient. However, the effectiveness of cyanide reduction depends on the type of soaking solution used and the duration of soaking (Ramli *et al.*, 2021).

According to Adam and Hermanto (2024), soaking jack bean seeds for 24 hours can reduce cyanide content by 33%, decreasing it from 41.07 mg/kg to 27.64 mg/kg. Canavanine has thermostable and toxic properties, the presence

of thermostable antinutritional compounds may lead to reduced growth performance, lethargy, and mortality in fish (Okomoda *et al.*, 2016). The beans also contain saponins that are toxic or inhibit the growth of fish that leading the content of antinutrients in the raw material of jack bean causes most of the performance growth to decrease during treatment. This is in accordance with the study of Lestari *et al.* (2023), where fish fed high doses of mahogany seed extract exhibited reduced growth performance and eventually mortality due to the presence of saponin as an antinutritional compound in mahogany seeds. In addition, fish lack the phytase enzyme, which limits their ability to efficiently utilize feed containing phytate (Kosim *et al.*, 2016).

In addition, tilapia experienced a decrease in survival rate in the TK treatment caused by the content of antinutrients in the form of cyanide acid (HCN). According to Hardy and Kaushik (2021), cyanide will reduce its capacity to bind and distribute oxygen when absorbed into circulation and bind to hemoglobin. The respiration process of fish will be disrupted if these conditions persist to cause fish stress which is then followed by a decrease in appetite to death. Most of the enzyme systems in the fish body will be inactivated due to the cyanide contained in the feed (Kavasoglu & Usyal, 2023). Based on study by Suprayudi *et al.* (2016), tilapia has the ability to adapt to feed containing HCN up to 23 mg/kg.

## CONCLUSION

The results of this study indicate that the inclusion of unpeeled jack bean (*Canavalia ensiformis*) flour in tilapia diets is not recommended due to its adverse effects on intestinal morphometry and subsequent reduction in growth performance.

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