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Research Paper

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EVALUATION OF SACHA INCHI MEAL (*Plukenetia volubilis*) AS DEFATTED SOYBEAN MEAL REPLACEMENT ON DIGESTIBILITY AND GROWTH PERFORMANCE OF CATFISH

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ARTICLE HIGLIGHTS

- The increasing scale of catfish aquaculture is expected to raise the overall demand for fish feed.
- Catfish feed remains expensive due to the dominance of imported protein ingredients.
- Sacha inchi meal offers a promising plant-based protein alternative for aquaculture diets.
- Replacing 50% of soybean meal with sacha inchi meal improves digestibility, fish growth, and feed efficiency.

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ABSTRACT

Catfish (*Clarias gariepinus*) production has increased, necessitating adequate nutritional support through feed. However, the expensive price of feed, primarily due to reliance on imported ingredients such as soybean meal (SBM), remains a major challenge. Sacha inchi meal (SIM), derived as a by-product from the oil extraction of *Plukenetia volubilis*, is rich in protein and essential amino acids, making it a promising local alternative. This study aimed to determine the feasibility of SIM as a substitute for defatted soybean meal (SBM) in catfish feed. Fifteen juvenile catfish (11.01 ± 0.01 g) were reared in 15 aquariums measuring 90 cm × 45 cm × 45 cm for 60 days, with three replicates for each of five isonitrogenous (30% crude protein) feed formulations containing 0%, 25%, 50%, 75%, and 100% replacement levels of SBM with SIM. Chromium III oxide (Cr_2O_3) was added as an indicator for digestibility analysis. Feces and blood were collected, and fish samples were analyzed to assess growth performance, digestive enzyme activity, and histological characteristics of the intestine and liver. The 50% replacement SBM by SIM demonstrated the highest total digestibility, digestive enzyme activity, and plasma total protein levels, followed by high protein retention and a protein efficiency ratio. Enhanced protein utilization in the 50% replacement SBM by SIM resulted in the highest average final weight, specific growth rate, and optimal feed conversion ratio.

Keywords: catfish, feed, growth performance, sacha inchi meal

INTRODUCTION

Catfish (*Clarias gariepinus*) is the second leading commodity in freshwater aquaculture, with Indonesia's production in 2020 reaching 993 thousand tonnes and experiencing an increase of 14.37% in 2023, reaching 1.14 million tonnes (Ministry of Marine Affairs and Fisheries of Indonesia 2024). The development of aquaculture businesses has resulted in increased demand for feed, raw materials, and feed prices. Catfish is an omnivorous species that tends toward carnivory, thus relying on artificial feed as a growth stimulant. Almost 65 – 75% of a fish's dry body mass consists of protein, therefore, fish require protein for growth, which can only be supplied through feed (Hardy & Kaushik 2022). This dependency carries a high risk for business sustainability because approximately 85 – 98% of fish production costs are absorbed by feed costs (Suprayudi *et al.* 2023). The expensive price of feed is due to the fact that its raw materials are still largely obtained through imports, such as fish meal, shrimp meal, soybean meal, and fish oil (Hildayanti *et al.* 2016).

Soybean meal is a protein-rich plant product that is highly digestible (73.3% dry matter, 96.5% protein) (Elesho *et al.* 2021) and the raw material is the lowest cost per unit weight of protein compared to animal protein. Based on data from BPS (2023), soybean meal was Indonesia's fourth largest imported commodity in 2023, reaching 5.33 million tonnes. This increase in import volume was followed by an increase in soybean meal price from IDR 5,501 in 2019 to IDR 8,900 in 2023, or an increase of 61.8% (Indexmundi 2024) influenced by the exchange rate of the dollar, which resulted in higher feed prices. To reduce import dependence, exploring local plant-based alternatives like the *Plukenetia volubilis* is essential.

Plukenetia volubilis (Sacha inchi, SIc), the Euphorbiaceous family is a climbing plant that can be harvested six months after planting and subsequently harvested every month with a long-term period of up to 15 – 20 years (Yusra *et al.* 2022). In the experimental garden of the Southeast Asian Regional Center for Tropical Biology (SEAMEO BIOTROP) Bogor, the average production of this plant per tree is 535 g/month in open areas, 295 g/month in intercropping areas, and 100 g/month in agroforestry areas (Supriyanto *et al.* 2022). The seed yield in the first year is 0.7 – 1.0 tonnes per hectare, the second-year yield 2 – 3 tonnes per hectare, and the third-year yield stabilizes at 3 – 4 tonnes per hectare (Van *et al.* 2023). Currently, SIc oil marketed for medicinal uses in skincare and as a nutritional supplement for human health because it contains 42.67% omega-3 (alpha-linolenic acid), 39.18% omega-6 (linoleic acid), and 9.82% omega-9 (oleic acid) (Supriyanto *et al.* 2022). The processing of this oil produces, by-products or residue in the form of meal (constituting 70% – 80% of the seed volume) (Singto *et al.* 2021). Sacha inchi meal (SIM) contains high protein (53% – 56.61%) and therefore potential as a substitute for soybean meal in fish feed (Goyal *et al.* 2022).

Sacha inchi meal (SIM) has been studied as a raw material and protein source in fish feed. In rainbow trout, it showed the high digestibility, including a 98.0% protein digestibility coefficient, 4,150 cal/kg dry matter digestible energy, and a 73.5% dry matter digestibility coefficient (Chura *et al.* 2018). The use of naturally dried sacha inchi meal did not succeed in enhancing the growth performance of Nile tilapia (*Oreochromis niloticus*), which is influenced by the negative effect of anti-nutritional substances (Muichanta *et al.* 2020). SIM can be included at more than 10% in the diet of whiteleg shrimp (*Litopenaeus vannamei*) (Renteria *et al.* 2022). Recent studies show that extruded SIM can be replaced soybean meal in red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) and common carp (*Cyprinus carpio*) (Khieokhajonkhet *et al.* 2021; Singto *et al.* 2021). The use of SIM in catfish has never been conducted, therefore it is necessary to test of SIM in relation to catfish growth (*Clarias gariepinus*).

MATERIALS AND METHODS

Sacha Inchi Meal Preparation

Sacha inchi meal (SIM) was obtained from Cianjur, Bogor, Central Java Province, then processed using extrusion with 12% moisture at temperature of 80 ± 5 °C (the temperature during extrusion not exceeding 100 °C) and dried at 70 °C by oven for 2 hours, then ground and stored until used (Khieokhajonkhet *et al.* 2022). SIM was subsequently analyzed for proximate composition, and analysis of anti-nutritional content was conducted before and after the extrusion process (Table 1).

Table 1 Nutrient and anti-nutritional composition of SIM

Composition	SIM before extrusion	SIM after extrusion	SBM[1]
Nutrient composition			
Moisture content	8.71	11.52	11.40
Protein (%)	56.49	54.68	45.00
Ash content (%)	5.6	4.64	8.00
Fat (%)	6.86	6.23	0.60
Crude fiber (%)	5.5	5.25	9.40
Anti-nutritional substances			
Phytic acid (mg/g)	7.28	2.30	1.27
Tannins (mg/g)	5.50	1.50	0.005
Saponins (mg/g)	3.05	1.13	2.43
Trypsin inhibitor (mg/g)	0.10	0.05	1.8

Note: SBM[1] = Soybean Meal (Simawan *et al.* 2023).

Table 2 Formulation of Catfish Feed with Sacha Inchi Meal (SIM) Substitution

Items (%)	Treatmentsa				
	SIM0	SIM25	SIM50	SIM75	SIM100
Fish Meal	3.70	3.70	3.70	3.70	3.70
Meat Bone Meal	8.00	8.00	8.00	8.00	8.00
Poultry By-product Meal	9.80	9.40	9.00	8.60	8.20
Soybean Meal	27.60	20.70	13.80	6.90	0.00
Sacha Inchi Meal	0.00	5.50	11.00	16.50	22.00
Wheat Flour	17.75	18.20	18.65	19.10	19.55
Pollard	18.75	20.25	21.75	23.25	24.75
Gaplek	5.00	5.00	5.00	5.00	5.00
Fish Oil	2.40	2.40	2.40	2.40	2.40
Palm Oil	2.15	2.00	1.85	1.70	1.55
Vitamin Premix	1.80	1.80	1.80	1.80	1.80
Mineral Premix	1.75	1.75	1.75	1.75	1.75
Polymethylolcarbamide	0.30	0.30	0.30	0.30	0.30
Monocalcium Phosphate	1.00	1.00	1.00	1.00	1.00
Total (%)	100.00	100.00	100.00	100.00	100.00
Nutrient, energy and C/P ratio of feed[b]					
Moisture (%)	10.15	9.85	10.08	10.05	9.05
Protein (%)	30.23	30.31	30.21	30.29	30.37
Lipid (%)	6.19	6.32	6.06	6.20	6.09
Crude Fiber (%)	2.60	2.76	2.84	3.36	3.59
BETN (%)	41.56	41.58	41.97	41.61	42.70
Ash (%)	8.84	8.87	8.29	8.01	7.79
GE[2] (kcal GE/kg)	4,019.03	4,031.64	4,026.95	4,027.12	4,055.81
C/P Ratio (kcal/kg)	13.29	13.30	13.33	13.29	13.36

Notes: [a] SIM0 = 0% Sacha Inchi Meal substituting Soybean Meal/12% protein SBM; SIM25 = 25% Sacha Inchi Meal substituting Soybean Meal/3% protein SIM; SIM50 = 50% Sacha Inchi Meal substituting Soybean Meal/6% protein SIM; SIM75 = 75% Sacha Inchi Meal substituting Soybean Meal/9% protein SIM; SIM100 = 100% Sacha Inchi Meal substituting Soybean Meal/12% protein SIM.

[b] BETN = Free extract without nitrogen; GE = Gross Energy; 1 g protein = 5.6 kcal; 1 g fat = 9.4 kcal;. 1 g carbohydrate = 4.1 kcal (Watanabe 1988).

Experimental Diets and Design

The treatments in this study consisted of the inclusion levels of Sacha Inchi Meal (SIM) at 0%, 25%, 50%, 75%, and 100% replacing soybean meal (SBM) protein in the feed formulation. The basal diet (SIM0) was developed using SBM as the protein source (276 g/kg). Feed production was carried out twice: for digestibility testing by adding cromium III oxide (Cr_2O_3) to the diet as an indicator, and for growth performance testing by mixing all ingredients weighed according to the formulation (Table 2) and adding 300 mL/kg of water, stirred until homogeneous. The mixture was then extruded using an extruder with a 2 mm feed diameter and dried in an oven at 50 °C for 4 hours.

The stocking density of catfish was 15 fish per aquarium. The stocking density of test animals was 125 individuals per cubic meter of rearing water. A total of 15 aquariums measuring 90 cm × 45 cm × 45 cm (120 L of water) were used and equipped with aeration systems and heaters (set on 30 °C) which operated for 24 hours a day. Initial (11 g) and final body weights of the fish were recorded during the rearing period. The fish were anesthetized using "Arowana stabilizer" at a concentration of 1 mL/L by immersion, to prevent stress during weighing. The test feed was administered three times daily at 07:00 AM, 01:00 PM, and 07:00 PM until satiation.

Water quality management was conducted by changing 30% of the water every 3 days for the first 15 days, 50% every 3 days for the following 15 days, and 70% every 3 days until the end of the rearing period. Measurements of temperature and pH were carried out twice daily, DO every 7 days. Total Ammonia Nitrogren (TAN) measurement was taken at the beginning, middle, and end of the rearing period. The monitored water quality results during the catfish rearing period included temperature 28.9 – 30.6 °C, pH 6.52 – 7.45, DO 3.3 – 5.5 mg/L, and TAN 0.21 – 0.69 mg/L.

Digestibility analysis was carried out using the feces collection method (Watanabe 1998). Fish were randomly assigned to 15 containers, each with a volume of 15 L and a stocking density of 4 individuals per container (weight range 11 – 15 g/fish). All rearing containers were equipped with aeration and securely covered to avoid fish jumping. Feces collection began on the 4th day after the fish were given the test feed containing Cr_2O_3 (Watanabe 1988). Feces collection was conducted 60 minutes after feeding. Feces were collected from the bottom of the container by opening the water tap. Prior to feces collection, at approximately 20 – 30 minutes after feeding, the tap was opened to remove leftover feed if present, this was to prevent feed residue from contaminating the feces. The collected feces were placed into sample bottles and then stored in a freezer to avoid spoilage until enough feces were available for analysis. The collected fish feces were then oven-dried at 110 °C for 4 to 6 hours. Cr_2O_3 analysis on the dried feces was carried out using the oxidation method. Then, absorbance values for digestibility were read using an ELISA Reader at a wavelength of 350 nm (Takeuchi 1988).

Sample Collection and Analysis

Digestive Enzyme Activity

The intestine of the fish was collected along its entire length, from the anterior to the posterior section (Gadri *et al.* 2014). The measurement of digestive enzyme activity from the fish's digestive tract was carried out by homogenizing the fish intestine with distilled water at ten times the weight of the sample to produce the crude extract. The crude enzyme extract was preserved at (-20 °C) until it was used for enzyme activity analysis. Amylase activity was measured using a 1% starch solution as the substrate in 20 mM sodium phosphate buffer (pH 6.9) containing 6.0 mM NaCl, following the methodology of Worthington (1993).

One enzyme activity unit was characterized as the amount of amylase that catalyzes the hydrolysis of 1 µg of maltose each minute. Trypsin activity was tested using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as the substrate, as described by the method of Erlanger *et al.* (1961). Olive oil emulsion served as the substrate and Tris-HCl as the buffering agent for determining lipase activity, following the method of Borlongan (1990). One unit of lipase activity (U) was quantified as the volume of 0.01 N NaOH necessary to counteract the fatty acids liberated during 6 hours of substrate incubation, and after correction with the appropriate blank.

Digestibility

Apparent Digestibility Coefficient (ADC) of total dry matter and protein in fish were calculated using the following formula (Watanabe 1988):

$$\text{ADC of dry matter (\%)} = 1 - \frac{\%Cr203 \text{ of diet}}{\%Cr203 \text{ of feces}} \text{ X } 100$$

$$\text{ADC of protein (\%)} = 1 - \left(\frac{\%Cr203 \text{ of diet}}{\%Cr203 \text{ of feces}} \text{ X } \frac{\% \text{ crude protein of feces}}{\% \text{ crude protein of diet}}\right) \text{X } 100$$

The Biological Value (BV) was calculated using formula following Mitchell (1923):

$$BV = \frac{\text{Protein retention}}{\text{ADC of protein}}$$

Histology

At the end of the experimental period, one representative fish was randomly sampled from each aquarium in every treatment for histological analysis, after survival rate data had been recorded. Following anesthetization, the liver and anterior part of the intestine were extracted from one representative fish from each treatment, then the samples were placed in neutral buffered formalin (NBF) for histological slide preparation (n = 15). The samples were then set in paraffin, trimmed to approximately 5 µm thickness, and stained using hematoxylin and eosin dye. Next, the specimens were dried and observed under a microscope (Slaoui & Fiette 2011). Histological slides were observed using an Olympus microscope, and intestinal diameter, villi height, and villi width were measured using ImageJ software.

Hepatosomatic Index (HIS)

The Hepatosomatic Index was calculated using the following formula (Garling & Wilson 1977):

$$HSI (\%) = \frac{\text{Liver Weight (g)}}{\text{Whole body weight (g)}} \text{ X } 100$$

Blood Biochemistry Analysis

A total of 0.5 mL of blood was collected from three fish in each treatment group with a 1 mL syringe flushed with anticoagulant (3.8% Sodium citrate). Blood collection was performed from the vein located at the base of the tail. Blood was drawn slowly, then transferred into an Eppendorf tube that had been rinsed with anticoagulant and stored in a cooling box.

The blood was then centrifuged at 2,500 rpm for 15 minutes to isolate the serum and plasma. Plasma protein measurement was conducted using

a spectrophotometric method at a wavelength of 595 nm, based on the measurement method by Bradford (1976), and the results were compared with BSA (Bovine Serum Albumin) standards. From the mathematical equation of the protein standard curve, the concentration of soluble protein contained in the fish plasma solution will be obtained.

The blood was transferred into a microtube and then centrifuged for 15 minutes at 3,000 rpm. The blood plasma samples were then analyzed to determine the levels was triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Triglyceride were determined using the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method. HDL and LDL analysis was conducted using the cholesterol oxidase-p-aminophenazone (CHOD-PAP) method.

Growth Performance

The survival rate (SR) was calculated using formula following Zonneveld *et al.* (1991):

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

where:

Nt = Final number of fish
No = Initial number of fish

The Protein Efficiency Ratio (PER) was calculated using the following formula (Hepher (1998):

$$PER = \frac{Wt - Wo}{Pi}$$

where:

Wt = Total fish biomass at the end of the study (g)
Wo = Total fish biomass at the beginning of the study (g)
Pi = Protein content of the feed given (%) × total feed consumption (g)

The Protein retention (PR) was calculated using a formula following Watanabe (1988):

$$PR\ (\%) = \frac{Fp - Ip}{Pp} \times 100$$

where:

Fp = Final fish protein content (g)
Ip = Initial fish protein content (g)
Pp = Fish protein intake (g)

The Lipid Retention (LR) was calculated using the following formula (Watanabe 1988):

$$LR\ (\%) = \frac{Fi\text{-}Il}{Pl} \times 100$$

where:

Fl = Final fish lipid content (g)
Il = Initial fish lipid content (g)
Pl = Fish lipid intake (g)

The Specific Growth Rate (SGR) was calculated using a formula following Zonneveld *et al.* (1991):

$$SGR\ (\%) = \frac{In\ Wt - In\ Wo}{t} \times 100$$

where:

t = 60 days
Wt = Final body weight
Wo = Initial body weight

The Feed Conversion Ratio (FCR) was calculated using the following formula (Goddard 1996):

$$FCR\ (\%) = \frac{Fl}{(Wt + Wd) - Wo}$$

where:

Wt = Total fish biomass at the end of the rearing period (g)
Wd = Biomass of dead fish (g)
Wo = Total fish biomass at the beginning of the rearing period (g)
FI = Fish feed intake (g)

Data Analysis

The data were tabulated using Microsoft Excel 365. The collected data were analyzed using one-way analysis of variance to determine variations among treatments. Significant differences among treatments were determined using Duncan's multiple range test, performed with IBM SPSS Statistics 26. Data were presented as mean ± SD, with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Feed Digestibility

The inclusion of SIM at levels of 25% – 75% increased total digestibility compared to the control group (SIM0) (Table 3). Digestibility values show the amount of nutrient composition absorbed and utilized by fish for metabolic processes and growth (NRC 2011).

Total and protein digestibility remained high in fish fed diets containing sacha inchi meal (SIM) as a replacement for up to 50% of soybean meal (SBM) protein. The higher digestibility may be attributed to the greater digestibility of SIM compared with SBM. The reported total digestibility of SIM (73.5%) is slightly higher than that of SBM (73.3%) (Chura *et al.* 2018; Elesho *et al.* 2021). Protein digestibility ranged from 76.97 to 80.07%, which is still within the normal range of reported for catfish (> 60%) (NRC 2011). The high protein digestibility may also reflect the increasing proportion of SIM in the diets, as the reported protein digestibility of SIM (94.2% – 98.0%) exceeds that of SBM (92.2%) (Chura *et al.* 2018; Elesho *et al.* 2021; Simawan *et al.* 2023). These results are consistent with those reported by Khieokhajonkhet *et al.* (2021) who observed comparable protein digestibility in Nile tilapia fed diets in which SIM replaced SBM protein. Feed ingredient composition is a major determinant of nutrient digestibility, and ingredients with higher digestibility generally improve the overall digestibility of the diet (Kaushik & Schrama 2022).

Digestive Enzyme Activity

Digestive enzyme activities differed significantly with the substitution of soybean meal by SIM ($P < 0.05$) (Table 4). The high digestibility value in catfish treated with SIM50 was also supported by higher digestive enzyme activities, such as amylase, lipase, and trypsin, compared to the SIM0 treatment.

Amylase plays an important role in the digestive process by hydrolyzing complex carbohydrates into simpler sugars that can be readily absorbed. Amylase can assist the protein digestion process by increasing carbohydrate digestion efficiency, allowing carbohydrates to serve as an energy source needed for the entire digestive process. These data are supported by the findings of Pradhan *et al.* (2020), which stated that there is a direct correlation between protease and amylase enzyme activities, as well as with protein, fat, and carbohydrate digestibility in fish. This illustrates that digestive enzymes not only enhance nutrient digestibility but also interact synergistically with nutrients to improve their effectiveness. Similar results were also acquired when SBM was substituted up to 100% by cashew nut meal in tilapia (Pradhan *et al.* 2020) and sesame seed meal in common carp (Hekmatpour *et al.* 2023), which showed significant increases in digestive enzyme activity. The inclusion of SIM enhances digestive enzyme activity and nutrient digestibility, although this benefit is offset when SIM exceeds the threshold, particularly when SBM replacement exceeds 50% in catfish diets.

Table 3 Total digestibility and protein digestibility of catfish fed diets

Digestibility (%)	Treatment (Protein substitution level of SBM with SIM)[a]				
	SIM0	SIM25	SIM50	SIM75	SIM100
Total	57.28 ± 0.71[b]	58.50 ± 0.14[a]	59.23 ± 0.59[a]	58.37 ± 0.64[a]	56.66 ± 0.63[b]
Protein	77.82 ± 0.37[bc]	78.86 ± 0.63[ab]	80.07 ± 1.39[a]	79.34 ± 1.29[ab]	76.97 ± 0.75[c]

Note: a Different letter in the same row show significantly different treatment effects ($P < 0.05$).

Table 4 Digestive enzyme activity in catfish fed diets containing different levels of SIM during a 60-day rearing period

Activity (IU/mL)	Treatment (Protein substitution level of SBM with SIM)[a]				
	SIM0	SIM25	SIM50	SIM75	SIM100
Amylase	6.75 ± 0.35[c]	7.79 ± 0.09[b]	8.70 ± 0.09[a]	8.02 ± 0.13[b]	6.90 ± 0.14[c]
Lipase	0.358 ± 0.005[d]	0.408 ± 0.005[c]	0.511 ± 0.009[a]	0.425 ± 0.005[b]	0.368 ± 0.008[d]
Trypsin	0.444 ± 0.015[c]	0.496 ± 0.009[b]	0.623 ± 0.011[a]	0.512 ± 0.004[b]	0.458 ± 0.014[c]

Note: a Different letter in the same row show significantly different treatment effects ($P < 0.05$).

Table 5 Blood biochemical profile of catfish after feeding diets with different SIM inclusion levels for 60 days of rearing

Parameter	Treatment (Protein substitution level of SBM with SIM)[a]				
	SIM0	SIM25	SIM50	SIM75	SIM100
Protein (g/dL)	3.51 ± 0.10[b]	3.64 ± 0.14[b]	3.71 ± 0.22[a]	3.47 ± 0.05[bc]	3.23 ± 0.13[c]
Triglyceride (mg/dL)	294.33 ± 17.14[c]	274.92 ± 8.79[bc]	267.73 ± 8.45[b]	255.45 ± 8.07[b]	203.78 ± 12.13[a]
High density lipoprotein (mg/dL)	42.33 ± 1.53[c]	47.00 ± 2.65[b]	54.33 ± 2.08[a]	48.00 ± 2.65b	48.67 ± 3.06[b]
Low density lipoprotein (mg/dL)	46.67 ± 1.53[b]	40.33 ± 3.06[a]	39.67 ± 3.51[a]	38.67 ± 1.53[a]	41.33 ± 3.06[a]

Note: a Different letter in the same row show significantly different treatment effects ($P < 0.05$).

Blood Biochemistry

The results of the blood profile analysis in catfish, including plasma protein, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), showed that the use of SIM to replace SBM affected the blood biochemical levels of catfish ($P < 0.05$) (Table 5).

Substitution up to SIM50 level was able to increase plasma protein, which then decreased again with higher inclusion levels. This decrease is suspected to be related to the lower digestibility value of the feed. A similar finding was reported by Pradhan *et al.* (2020), in which higher protein digestibility positively correlated with increased plasma protein in Nile tilapia fed cashew nut meal as a replacement for SBM.

LDL is a lipoprotein that transports cholesterol to tissues that require it, while HDL is a lipoprotein that carries cholesterol from tissues to the liver (NRC 2011). Increasing the proportion of SIM in the feed significantly reduced triglyceride (TG) levels in catfish. A decrease in blood TG levels may lead to a decrease in LDL levels (Rohmah *et al.* 2023). SIM is high in omega-3 fatty acids and additional biologically active compounds, which are suspected to contribute to the reduced TG and LDL levels observed in this study. A similar effect has also been reported in Nile tilapia fed SIM, where liver function improved with reductions in cholesterol and TG levels (Khieokhajonkhet *et al.* 2021; Khieokhajonkhet *et al.* 2022). LDL levels are related with the ability of omega-3 fatty acids to inhibit the production of apo-B. When the production of apo-B is inhibited, the conversion process of very low-density lipoprotein into LDL is also inhibited, resulting in a decrease in LDL levels (Scorletti & Byrne 2013). TG production is reduced through the mechanism by which omega-3 inhibits TG synthesis by blocking the enzymes diacylglycerol transferase and phosphatidic acid phosphohydrolase (Rohmah *et al.* 2023). Low HDL content may be caused by high levels of LDL and TG in the blood, which increases the TG content in HDL. The rise in TG levels will enhance the catabolism of HDL, thus increasing cholesterol levels in the blood.

Histological Assessment

The histological picture of the catfish intestine with different SIM substitution levels is shown in Figure 1. Replacing SBM with more than 50% SIM caused a linear decrease in villi height and width, leading to a reduction in the absorptive surface area (Table 6). The high activity of digestive enzymes correlated with the development of villi and the midgut area (Fig. 1; Table 6).

Intestinal villi play a role in the process of nutrient absorption and the transport of absorbed nutrients (Iheanacho *et al.* 2019; Dawood *et al.* 2020). The SIM50 treatment showed villi development (taller, wider, and more complex villus structures) and a better midgut area, supporting optimal nutrient digestion and absorption through increased intestinal surface area (Munglue *et al.* 2019; Jiao *et al.* 2023). This process occurs due to increased proliferation and differentiation of intestinal epithelial cells, which is associated with energy supply from the feed, thus supporting the epithelial function in digestion (Hekmatpour *et al.* 2023). Although the overall intestinal histological architecture appeared similar among treatments, morphometric measurements revealed clear differences between SIM50 and lower SIM inclusion levels ($P < 0.05$; Table 6). Specifically, villi height at SIM50 reached the highest value among treatments, indicating an expanded absorptive surface, while villi width was also significantly increased, contributing to a larger villi area. Conversely, intestinal villi with SIM inclusion levels >50% underwent a reduction

in villus size, thereby reducing enzyme activity, nutrient absorption surface area, and nutrient digestibility in fish.

Physically, the liver condition in fish fed with the SIM0 diet revealed hepatocyte structures that appeared normal, with lobules of hepatocytes arranged orderly and spherical nuclei positioned at the center of the cytoplasm (Fig. 2). Slight enlargement of cytoplasmic vacuoles was noted, possibly attributed to the elevated proportion of plant-derived ingredients in the feed composition (Fig. 2).

No significant changes were observed in hepatocyte morphology in the SIM25 and SIM50 groups compared to the control group. However, higher SIM replacement levels (up to 220 g/kg) increased cytoplasmic vacuolization (SIM75). Additionally, catfish fed SIM100 showed hepatocyte swelling and displacement of the hepatocyte nucleus toward the periphery (edge) (Fig. 2). Histological observation of catfish liver in this study confirmed that liver alterations depended on the SIM dosage, where increasing the proportion of SIM in the fish feed increased fat accumulation and caused hepatocyte hypertrophy (increase of liver cells) with slight degeneration. This indicates the occurrence of fatty liver degeneration in liver cells, the displacement of the cell nucleus to the cell periphery due to excessive fat accumulation in the liver cells, resulting in enlargement of the cytoplasm (jiao *et al.* 2024). Nevertheless, alterations are deemed pathological only when substantial hepatic necrosis is present (Saraiva *et al.* 2015).

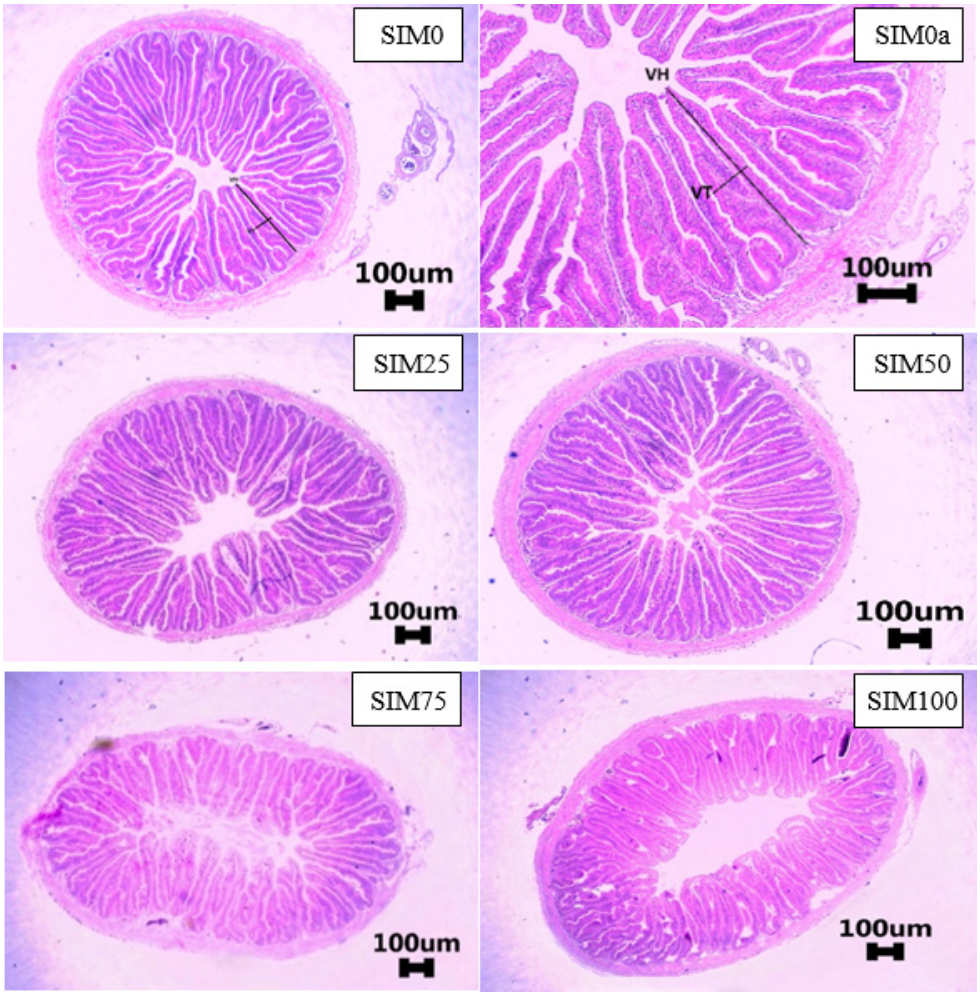


Figure 1 Intestinal histology of catfish

Notes: Images were taken at 40× magnification. Intestinal morphometric parameters are shown in panel SIM0: villi height (VH) and villi thickness (VT) (magnification 100×).

Table 6 Villi length, width, and area of catfish after feeding with diets containing different levels of SIM for 60 days of rearing

Measured Parameter	Treatment (Protein substitution level of SBM with SIM)[a]				
	SIM0	SIM25	SIM50	SIM75	SIM100
Villi length (µm)	708.14 ± 33.47^{bc}	718.78 ± 20.23^{bc}	769.18 ± 17.14^{a}	748.96 ± 11.68^{ab}	690.61 ± 27.19^{c}
Villi width (µm)	118.16 ± 2.39^{c}	125.43 ± 1.75^{b}	131.74 ± 2.73^{a}	126.54 ± 2.08^{b}	117.24 ± 1.88^{c}
Villi area (×10^{3}µm^{2})	83.73 ± 5.59^{d}	90.13 ± 1.29^{c}	101.32 ± 2.34^{a}	94.77 ± 1.83^{b}	81.00 ± 4.42^{d}

Note: a Different superscript letters within the same row show significant differences among treatments ($P < 0.05$).

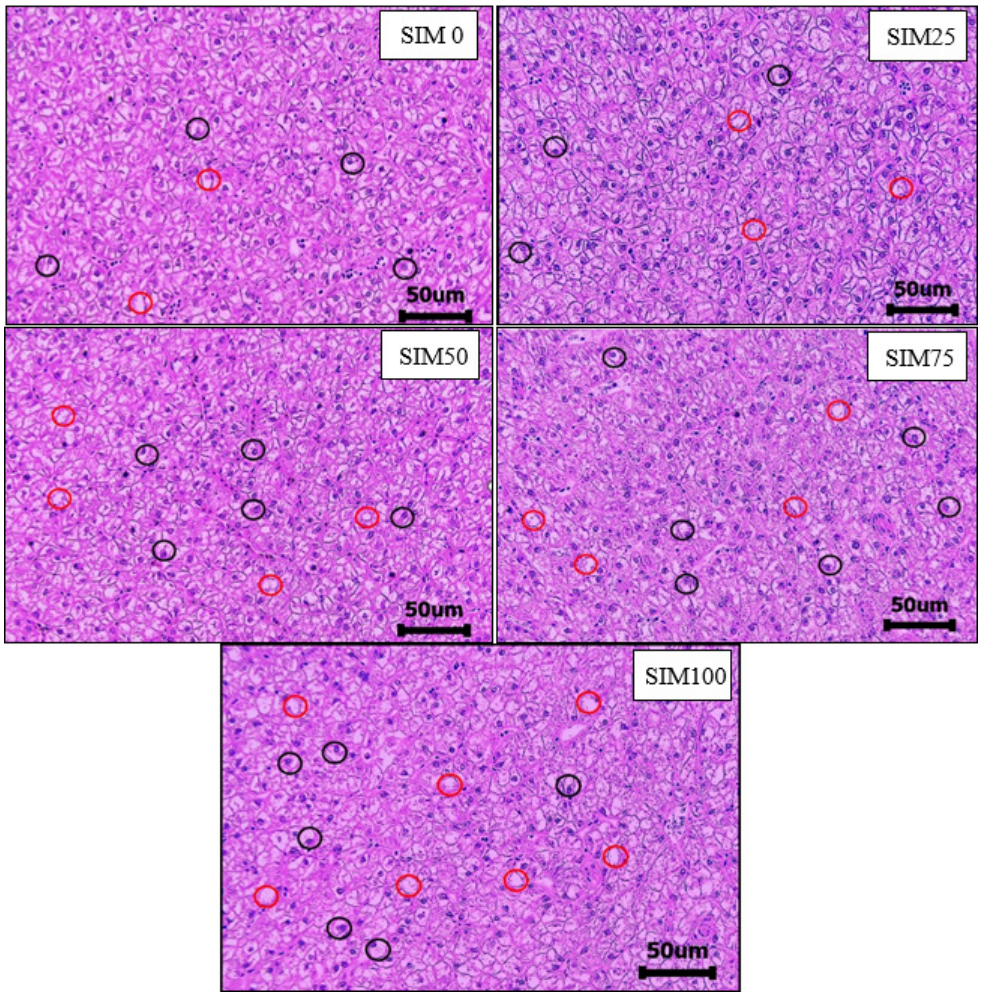


Figure 2 Liver histology of catfish

Notes: Images were taken at 400× magnification. The red circles (**O**) show hepatocytes vacuolation, while the black circles (**O**) show nuclear displacement.

Table 7 Growth performance of catfish after feeding with feed containing different levels of soybean meal substitution with SIM during a 60-day rearing period

Parameter[1]	Treatment (Protein substitution level of SBM with SIM)a				
	SIM0	SIM25	SIM50	SIM75	SIM100
Initial body weight (g)	11.01 ± 0.003	11.01 ± 0.002	11.01 ± 0.002	11.01 ± 0.004	11.01 ± 0.004
Final body weight (g)	98.71 ± 1.03^{cd}	101.14 ± 2.00^{c}	110.68 ± 1.12^{a}	103.82 ± 1.04^{b}	96.74 ± 1.37^{d}
Feed intake (kg)	1.58 ± 0.03^{b}	1.62 ± 0.01^{b}	1.68 ± 0.03^{a}	1.63 ± 0.04^{b}	1.58 ± 0.02^{b}
Survival rate (%)	91.11 ± 3.85	97.78 ± 3.85	91.11 ± 7.70	95.56 ± 3.85	91.11 ± 3.85
PER (%)	2.56 ± 0.06^{c}	2.69 ± 0.06^{b}	2.84 ± 0.02^{a}	2.72 ± 0.04^{b}	2.48 ± 0.06^{c}
FCR	1.27 ± 0.05^{b}	1.23 ± 0.03^{b}	1.16 ± 0.01^{a}	1.22 ± 0.02^{b}	1.33 ± 0.03^{c}
SGR (%/day)	3.65 ± 0.02^{c}	3.70 ± 0.03^{b}	3.85 ± 0.02^{a}	3.74 ± 0.02^{b}	3.62 ± 0.02^{c}
Biological value	53.94 ± 1.73^{c}	57.78 ± 2.03^{b}	62.33 ± 1.59^{a}	59.18 ± 0.58^{b}	53.67 ± 2.76^{c}
Protein retention (%)	41.98 ± 1.34^{c}	45.56 ± 1.39^{b}	49.92 ± 2.10^{a}	46.95 ± 0.88^{b}	41.30 ± 1.76^{c}
Lipid retention (%)	104.94 ± 4.54b	115.17 ± 5.13^{a}	122.93 ± 2.52^{a}	117.79 ± 5.41^{a}	103.65 ± 4.16^{b}
HSI	1.79 ± 0.11	1.83 ± 0.08	1.92 ± 0.17	1.82 ± 0.10	1.88 ± 0.25

Note: [a] PER = protein efficiency ratio; FCR = feed conversion ratio; SGR = specific growth rate; HIS = hepatosomatic index. Data are presented as mean ± standard deviation. Different superscript letters within the same row show significant differences ($P < 0.05$).

[1] SIM0 = 0% SBM protein substituted with SIM; SIM25 = 25% SBM protein substituted with SIM; SIM50 = 50% SBM protein substituted with SIM; SIM75 = 75% SBM protein substituted with SIM; SIM100 = 100% SBM protein substituted with SIM.

Growth Performance

The growth performance of catfish during the 60-day rearing period showed that the replacement of soybean meal with SIM affected final body weight, feed intake (FI), protein efficiency ratio (PER), specific growth rate (SGR), biological value (BV), feed conversion ratio (FCR), protein retention (PR), and lipid retention (LR) ($P < 0.05$) (Table 7). The feeding of SIM increased final weight, JKP, RP, RL, REP, LPS, and BV, and decreased RKP up to the SIM50 treatment. The results of the study showed that the hepatosomatic index (IHS) was not affected by SIM inclusion.

The SGR value increased in line with the decrease in the FCR. The FCR value decreased up to the SIM50 treatment, reaching 1.16, then increased with further inclusion (Table 7). An efficient FCR shows higher retention of protein, lipid, and energy, indicating that the fish more efficiently convert feed nutrients into body weight (Elvy *et al.* 2022). This finding is consistent with the increased protein and lipid retention values observed up to the SIM50 treatment, which then declined with increasing proportions of SIM. The increase in protein retention contributed to the improved growth performance of the fish. In the SIM75 and SIM100 treatments, a decrease in protein retention was observed, which is suspected to result from the presence of excess phytate and tannin, which can interfere with the nutrient absorption process of the feed.

Phytate contains six reactive phosphate groups, which confer strong chelating properties. Through this mechanism, phytate is able to form insoluble complexes with metals, thereby inhibiting the absorption of essential minerals such as Fe^{2+}, Ca^{2+}, Mg^{2+}, and Zn^{2+}, potentially leading to mineral deficiencies (Krogdahl *et al.* 2022).

Deficiencies of these minerals may result in reduced protein retention and decreased plasma protein levels due to impaired metabolic functions and protein synthesis. Tannins present in feed ingredients are also considered anti-nutritional factors that interfere with digestion by forming complexes with proteins and minerals. The interaction between the hydroxyl groups of tannins and the carbonyl groups of proteins results in the formation of tannin–protein complexes that are resistant to digestion, and these interactions may further inhibit the activity of digestive enzymes (Nikmaram *et al.* 2017). Consequently, amino acid availability is reduced, negatively affecting protein retention and plasma protein levels in the body.

Protein retention reflects the percentage ratio between the protein consumed by fish and the amount of protein stored in the body, providing an illustration of the efficiency of nutrient absorption by fish in utilizing the available protein sources (Bai *et al.* 2022). As the proportion of SIM in the feed increases, the levels of antinutritional factors such as tannins and phytates also rise (Table 1).

The biological value of protein shows effectively the protein is used for growth and metabolic processes. The BV value was calculated by measuring the percentage of absorbed nitrogen, considering the nitrogen content in fish feces, showing the amount of nitrogen retained or available for the body (Simon *et al.* 2019). The results of this study showed that BV increased up to the SIM50 treatment and then declined toward SIM100. Fish treated with SIM50 showed the highest final body weight, better blood biochemical parameters, and improved intestinal histological structure compared to those fed SIM0. The extrusion processing of SIM was an essential factor in the effective presence of SIM in this study and recent studies in rainbow trout and tilapia (Chura *et al.* 2018; Khieokhajonkhet *et al.* 2021).

CONCLUSION

Sacha inchi meal is capable of replacement up to 50% of soybean meal in catfish feed formulation without disrupting its growth and health.

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