Jurnal Akuakultur Indonesia 25 (1), 138–150 (2026)

Original article DOI: 10.19027/jai.25.1.138-150

Utilization of mung bean sprout waste *Vigna radiata* hydrolyzed cellulase enzyme in feed on the digestibility of Nile tilapia *Oreochromis* sp.

Pemanfaatan limbah kecambah kacang hijau *Vigna radiata* dihidrolisis enzim selulase pada pakan terhadap kecernaan ikan nila *Oreochromis* sp.

Grenda Audia Wuryas Pradita Negara, Mia Setiawati*, Julie Ekasari, Dedi Jusadi

Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia
*Corresponding author: miasetia@apps.ipb.ac.id

(Received March 22, 2024; Revised February 6, 2025; Accepted August 10, 2025)

ABSTRACT

This study evaluated the utilization of mung bean sprout waste flour hydrolyzed by cellulase enzyme (LT_e) as the feed ingredient of red Nile tilapia weighing 10.00 ± 0.01 g/seed and 7.00 ± 0.15 in length. This study used two stages, each consisting of four treatments and four replicates. The first step was performed by evaluating LT_e flour added with cellulase enzyme of 0 g/kg (control), 0.4 g/kg, 0.8 g/kg, and 1.2 g/kg. The second step was the digestibility test of LT_e and growth performance on red Nile tilapia seeds. The results showed that the addition of cellulase enzyme at a 1.2 g/kg was significantly able to reduce the crude fiber of LT_e with 78.19% %, hemicellulose at 19.22%, neutral detergent fiber at 41.69%, acid detergent fiber at 61.85%, lignin 64.06%, cellulose 62.47% besides having the best value of ingredient, protein, and energy digestibility. The test results on the growth performance of red Nile tilapia seeds fed with LT_e feed with a dose of 1.2 g/kg cellulase enzyme have the highest value significantly different from the control feed based on the value of daily growth rate (SGR), ratio efficiency protein (REP), protein retention (PR), and improvement of feed conversion ratio (RKP).

Keywords: cellulase enzyme, digestibility, growth performance, mung bean sprout waste, tilapia

ABSTRAK

Penelitian ini mengevaluasi pemanfaatan tepung limbah kecambah kacang hijau yang dihidrolisis enzim selulase (LT_e) sebagai bahan baku pakan pada benih ikan nila merah dengan bobot 10,00 ± 0,01 g/ekor dan panjang 7,00 ± 0,15 cm. Penelitian ini menggunakan dua tahap dan masing-masing tahap terdiri dari empat perlakuan dan empat ulangan. Tahap pertama dilakukan evaluasi tepung LT_e sebesar 0 g/kg (kontrol), 0,4 g/kg, 0,8 g/kg, dan 1,2 g/kg. Tahap kedua dilakukan uji kecernaan bahan LT_e dan kinerja pertumbuhan benih ikan nila merah. Hasil penelitian menunjukkan bahwa penambahan enzim selulase pada dosis 1,2 g/kg signifikan mampu menurunkan serat kasar LT_e sebesar 78,19 %, hemiselulosa 19,22%, *neutral detergent fiber* 41,69%, *acid detergent fiber* 61,85%, lignin 64,06%, selulosa 62,47% dan memberikan nilai tertinggi terhadap kecernaan bahan, kecernaan protein, dan kecernaan energi. Hasil uji terhadap kinerja pertumbuhan benih ikan nila merah yang diberi pakan LT_e dengan enzim selulase dosis 1,2 g/kg memiliki nilai tertinggi berbeda nyata terhadap pakan kontrol berdasarkan nilai laju pertumbuhan harian (SGR), retensi protein (PR), rasio efisiensi protein (REP) dan perbaikan nilai rasio konversi pakan (RKP).

Kata kunci: enzim selulase, ikan nila merah, kecernaan, kinerja pertumbuhan, limbah kecambah kacang hijau

INTRODUCTION

Global tilapia production in 2020 reached approximately 4.5 million tons, with the majority of production coming from China, Taiwan, Indonesia, and Egypt. This makes tilapia one of the most widely cultivated freshwater fish species (FAO, 2022). However, the development of tilapia aquaculture in Indonesia remains constrained by the high cost of feed, which is one of the key determinants of aquaculture success. Indonesia's feed production nearly reached 1.7 million tons in 2020 (Suprayudi *et al.,* 2023). The high price of fish feed in Indonesia is mainly due to the fact that about 85% of its ingredients are imported (Suprayudi *et al.,* 2023). In 2020, soybean meal imports reached 2.67 million tons (BPS, 2019). Therefore, alternative feed ingredients that are locally available, non-competitive with human consumption, economically viable, and consistently accessible are needed as protein sources.

One potential local raw material is mung bean (*Vigna radiata*) sprout waste, a by-product of mung bean sprout processing, which can be utilized as an alternative feed ingredient. This approach can be realized by optimizing the availability of local feed resources in Indonesia, an agrarian country rich in natural resources that can support the supply of feed materials such as mung bean-based food products. Mung bean (MB) grows easily in most regions of Indonesia due to its adaptability to the country's climate and soil conditions (Trustinah *et al.,* 2014). According to the Ministry of Agriculture (2021), the total cultivated area of mung bean was 193,221 ha, with a harvested area of 184,020 ha. In 2020, mung bean production reached 222,108 tons, yielding approximately 1,776,864 tons of sprouts and generating around 710,745 tons of sprout waste. The production of mung bean sprouts produces roughly 40% waste, a ratio estimated from mung bean production data (BPS, 2015) and sprout processing reports (Rahayu *et al.,* 2010).

The potential for sprout waste generation corresponds with the geographic distribution of sprout production across Java, Sumatra, Bali, Sulawesi, and West Nusa Tenggara (Kementan, 2013). In addition to sprouts the largest processed form of mung bean other mung bean–based food and beverage processing industries, which account for around 30% of total production, also generate waste. Thus, mung bean sprout waste can be sourced from sprout-processing industries, sprout cultivators, and agro-industrial sectors. According to Hernowo *et al.* (2020), mung bean sprout waste contains 30.21% protein, 2.29% fat, 29.03% carbohydrates, 5.63% ash, 11.38% moisture, and 21.46% crude fiber, indicating its potential as an alternative raw material for fish feed formulation.

The use of mung bean sprout waste meal without raw material modification to replace soybean meal in fish feed has been investigated by several researchers, including for Nile tilapia (*Oreochromis niloticus*) and giant gourami (*Osphronemus gouramy*) (Hernowo *et al.,* 2020; Pinandoyo *et al.,* 2021). The inclusion of sprout waste meal with high crude fiber content has been reported to affect fish growth. However, previous studies have not provided sufficient data or information regarding the optimized dosage of enzymatically treated mung bean sprout waste meal, indicating the need for further research. The issue of high crude fiber content can be addressed by processing raw materials using cellulase enzymes (Jefry *et al.,* 2021).

According to Irawati *et al.* (2017) and Jha and Mishra (2021), crude fiber is composed of neutral detergent fiber (NDF), lignin, cellulose, and acid detergent fiber (ADF) fractions. Cost-effective and readily available biological treatments to reduce crude fiber fractions have been explored, such as: (1) *Tricoderma reesei* (Ranjan *et al.,* 2018), (2) *Rhizopus oryzae* (Ranjan *et al.,* 2019), and (3) *Aspergillus niger*, which can decrease crude fiber, phytic acid, and trypsin inhibitor levels in feed ingredients, thereby improving nutrient absorption in fish (Jannathulla *et al.,* 2018). The application of cellulase enzyme at a concentration of 1.2 g/kg with different raw materials has been shown to influence the crude fiber fraction and enzymatic efficiency, depending on the substrate used (Jefry *et al.,* 2021). Therefore, the objective of this study was to evaluate the digestibility efficiency of mung bean sprout waste meal hydrolyzed with various cellulase enzyme doses in red tilapia (*Oreochromis* sp.) feed.

MATERIALS AND METHOD

Preparation of mung bean sprout waste (tauge) raw material

Mung bean sprout waste was collected from Kampung Babakan Kemasan, Sukaraja District, Bogor, West Java. The types of sprout waste used included sprout skins, whole sprouts, sprout heads, and sprout stems and tails. The sprout skin refers to the seed coat of mung beans that detaches during the germination process. Whole sprouts discarded due to not meeting food quality standards consisted of sprout heads, stems, tails, and plumules (embryonic leaves). Pre-treatment of the mung bean sprout waste meal involved several steps: washing, oven-drying to prevent spoilage during hydrolysis, grinding into fine powder, sieving, and conducting a proximate analysis of the raw material.

The chemical parameters analyzed included vitamins E and C, determined using high-performance liquid chromatography with a photodiode array detector (HPLC-PDA) for both mung bean sprout waste and soybean meal. The fiber fraction composition of the sprout waste comprising neutral detergent fiber (NDF), hemicellulose, cellulose, lignin, and acid detergent fiber (ADF) was analyzed using the *in sacco* method. Amino acid profiles of soybean meal and mung bean sprout waste were determined by high-performance liquid chromatography (HPLC). Table 1 presents the comparison of proximate composition, vitamin E, vitamin C, and amino acid contents between soybean meal and mung bean sprout waste.

Experimental design

The experimental design used in this study was a completely randomized design (CRD). The research was conducted in two stages. The first stage aimed to improve the nutritional quality of mung bean sprout waste through a pre-treatment process using different cellulase enzyme dosages (0 g/kg, 0.4 g/kg, 0.8 g/kg, and 1.2 g/kg), consisting of four dietary treatments and four replicates. The second stage involved evaluating the digestibility of the hydrolyzed mung bean sprout waste from Stage 1 using four dietary treatments and four replicates, as well as conducting a growth performance trial in Nile tilapia with five dietary treatments and four replicates.

In Stage 1, the pre-treatment of mung bean sprout waste meal was carried out through washing, drying, grinding, and sieving of the raw material. The cellulase enzyme was weighed according to the designated treatment dosage. Subsequently, the enzyme (at different doses) was dissolved in 300 mL of water for each treatment and mixed thoroughly with the mung bean sprout waste. The hydrolyzed mung bean sprout waste was then placed in plastic containers, tightly sealed to prevent contamination, and incubated at room temperature for 24 hours. After incubation, the hydrolyzed material was oven-dried at 40°C for 1.5 hours.

The second phase of the study involved the preparation of experimental diets for digestibility analysis. The test diet was formulated using 68.80% commercial feed, 30% cellulase-hydrolyzed mung bean sprout waste meal as the test ingredient, 0.6% polymethylolcarbamide (PMC) as a binder, and 0.6% chromium oxide (Cr_2O_3) as an inert marker for digestibility determination. The reference diet consisted of 98.80% commercial feed, 0.6% PMC, and 0.6% Cr_2O_3, with the addition of water during preparation. The diets were pelleted using a pellet machine equipped with a 1.0 mm die, then oven-dried at 40°C for 4 hours. After drying, the pellets were cooled at ambient temperature and categorized according to the cellulase enzyme dosage (four treatments with four replicates each: 0 g/kg, 0.4 g/kg, 0.8 g/kg, and 1.2 g/kg). The composition and proximate analysis of the experimental diets with varying cellulase doses (0 g/kg, 0.4 g/kg, 0.8 g/kg, and 1.2 g/kg) are presented in Table 2.

Proximate nutrient analysis of the diets included measurements of crude protein, lipid, ash, moisture, crude fiber, and nitrogen-free extract (NFE), following the AOAC (2012) procedures. Collected fecal samples were oven-dried and analyzed for protein content using the Kjeldahl method (Watanabe, 1998). Chromium oxide (Cr_2O_3) content in the diets was determined spectrophotometrically according to McGinnis and Kasting (1964). The cellulase enzyme used in this study was a 70% purity product from Focus Herb LLC, Shanghai, China, with an enzyme activity of ≥20,000 U/g and batch number FH20220316. The Cr_2O_3 used was chromium (III) oxide anhydrous, Technipur™ (catalog number 102483, Merck KGaA, Germany). The PMC used was a commercial product under the trademark SUNNY (China), catalog number 98.1.109888.8.

Maintenance and fecal collection of nile tilapia

Nile tilapia used for the digestibility test had an average body weight of 10.00 ± 0.01 g. The fish were reared in aquaria (90×40×45 cm³, water height 30 cm, volume 108 L) at a stocking density of 15 fish per aquarium for 60 days. Feed was provided three times daily at 07:00, 12:00, and 17:00 using a satiation feeding method. Digestibility measurements were conducted using the fecal collection method. Collected feces were stored in a freezer at −20 °C until further analysis.

Throughout the study, the total water volume in each aquarium was maintained uniformly, and continuous aeration was provided. Water quality parameters during the rearing period were as follows: temperature 28–31°C, pH 6.7–7.5, dissolved oxygen (DO) 4.9–5.4 mg/L, total ammonia nitrogen (TAN) <0.1 mg/L, and nitrite (NO_2^-) 0.04–0.10 mg/L.

Table 1. Proximate composition, vitamin E, vitamin C, and amino acid profiles of soybean meal and mung bean sprout waste meal.

Parameters	Results (%)	
	Soybean meal	Soybean meal and mung bean sprout waste meal
Proximate analysis		
Protein (%)	42.83	31.57
Fat (%)	1.33	4.54
Ash content (%)	12.00	11.43
Crude fiber (%)	5.59	22.05
Nitrogen-free extract (%)	38.25	30.41
Phytochemical analysis		
Vitamin C (mg/kg)	0.00**	180.41**
Vitamin E (mg/kg)	0.83*	200.60**
Essential amino acids		
Phenylalanine (%)	1.98	1.76
Isoleucine (%)	2.18	2.18
Leucine (%)	3.76	2.21
Valine (%)	2.13	1.85
Threonine (%)	2.14	1.77
Lysine (%)	3.23	2.13
Histidine (%)	1.05	0.50
Arginine (%)	2.83	3.05
Methionine (%)	0.20	0.36
Non-essential amino acids		
Serine (%)	2.73	1.91
Glutamic acid (%)	5.21	3.27
Aspartic acid (%)	4.74	3.20
Alanine (%)	2.04	2.00
Glycine (%)	1.73	1.78
Tyrosine (%)	1.06	1.03
Proline (%)	2.30	2.62
∑ EAA[1]	19.80	15.81
∑ NEAA[2]	19.80	15.81
∑ TAA[3]	39.60	31.57
EAA/NEAA Ratio (%)	100.00	100.00
EAA/TAA Ratio (%)	50.00	50.00

Note: [1]Essential amino acids (EAA); [2]Non-essential amino acids (NEAA); [3]Total amino acids (TAA); [4]Nitrogen-free extract (NFE); [5]Feed Science and Technology Laboratory, Faculty of Animal Science, IPB University; *PT. Saraswanti Indo Genetech Laboratory, Yasmin, Bogor, Indonesia; **Food Science Laboratory, Gadjah Mada University, Yogyakarta.

Tested parameters

The digestibility test of mung bean sprout waste meal hydrolyzed with different cellulase enzyme doses in Nile tilapia was conducted once sufficient fecal samples were collected for analysis. Digestibility parameters of the hydrolyzed mung bean sprout waste meal (on a dry matter basis), including protein digestibility, dry matter digestibility, and energy digestibility, were calculated using the following equation (Takeuchi, 1988):

$$\text{Dry matter digestibility} = \frac{\text{ADT} - (0.7\text{AD})}{0.3}$$

Note:
ADT = Total digestibility percentage of the test diet
AD = Total digestibility percentage of the reference diet

Growth performance parameters included specific growth rate, feed intake, survival rate, protein efficiency ratio, average daily growth, feed conversion ratio, protein retention, lipid retention, and liver performance measured by the hepatosomatic index. The specific growth rate (SGR) was calculated as the percentage increase in body weight of fish, measured by weighing samples from each treatment using a digital balance (Plasus *et al.,* 2019):

$$\text{SGR (\%/day)} = \left[\sqrt[t]{\frac{W_2}{W_1}} - 1\right] \times 100$$

Note:
SGR = Specific growth rate
W_1 = Initial body weight
W_2 = Final body weight
t = Rearing period

Feed intake (FI) was calculated as the total amount of feed consumed by Nile tilapia during the rearing period (Wardani *et al.,* 2021):

F1 (g) = Total feed consumed by tilapia during rearing

Note:
FI = Feed intake

The survival rate (SR) was calculated using the formula (Mello *et al.,* 2023):

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

Note:
SR = Survival rate
N_t = Final number of fish
N_0 = Initial number of fish

Table 2. Composition and proximate analysis of digestibility test diets containing mung bean sprout waste meal hydrolyzed with different cellulase enzyme doses (0 g/kg, 0.4 g/kg, 0.8 g/kg, and 1.2 g/kg).

Feed composition (%)	Dietary treatments of cellulase-hydrolyzed mung bean sprout waste meal (%)				
	Reference diet	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Commercial feed	98.8	68.80	68.80	68.80	68.80
LTe[1]	0	30.00	30.00	30.00	30.00
Polymethylolcarbamide	0.60	0.60	0.60	0.60	0.60
Chromium oxide	0.60	0.60	0.60	0.60	0.60
Total	100	100	100	100	100
Proximate composition of the diet on a dry matter basis (%)					
Protein (%)	31.92	31.89	31.08	31.26	31.11
Nitrogen-free extract[2] (%)	41.28	39.66	41.38	42.67	43.71
Fat (%)	7.44	7.90	7.64	7.21	6.99
Crude fiber (%)	5.36	8.41	7.05	6.09	5.18
Ash content (%)	13.99	12.13	12.84	12.76	13.01
Gross Energy (kcal/kg)[3]	4.199,56	4.174,96	4.174,77	4.198,63	4.210,80
C/P (kcal/kg)[4]	13.15	13.09	13.43	13.42	13.53

Note: [1]LTe = mung bean sprout waste meal hydrolyzed with different cellulase enzyme doses (0, 0.4, 0.8, and 1.2 g/kg); [2]Nitrogen-free extract on a dry matter basis (NFE) = 100−(protein + lipid + ash + crude fiber); [3]Gross energy (GE) of dry feed was calculated based on the energy conversion factors: protein = 5.64 kcal/g, lipid = 9.44 kcal/g, and carbohydrate/NFE = 4.11 kcal/g (Watanabe, 1998); [4]C/P = calories-to-protein ratio.

The protein efficiency ratio (PER) was determined as follows (Zhang *et al.,* 2023):

$$PER = \frac{B_t - B_0}{P_i} \times 100$$

Note:
PER = Protein efficiency ratio
B_t = Final fish biomass
B_0 = Initial fish biomass
P_i = Protein content of the feed

The average daily growth (ADG) was calculated according to Kemal *et al.* (2023):

$$ADG\ (g/day) = \frac{W_2 - W_1}{t}$$

Note:
ADG = Average daily growth
W_1 = Average initial individual weight
W_2 = Average final individual weight
t = Rearing duration

The feed conversion ratio (FCR) was defined as the amount of feed required to produce 1 kg of fish biomass (Cai *et al.,* 2022):

$$FCR = F1 - (W_t + W_0)$$

Note:
FCR = Feed conversion ratio
FI = Feed intake
W_t = Final fish biomass
W_0 = Initial fish biomass

Protein retention (PR) was calculated based on the difference between the final and initial body protein content relative to the total dietary protein consumed (Ramena *et al.,* 2020):

$$PR\ (\%) = \frac{P_t - P_0}{P_p} \times 100$$

Note:
PR = Protein retention
P_t = Total body protein at the end of rearing
P_0 = Total body protein at the start of rearing
P_p = Total protein consumed from feed

Lipid retention (LR) was determined as the difference between the final and initial body lipid content relative to the total dietary lipid consumed (Wardani *et al.,* 2021):

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

Note:
LR = Lipid retention
F = Final body lipid content
I = Initial body lipid content
P = Total lipid consumed from feed

The hepatosomatic index (HSI) was used as an indicator of liver condition and calculated as follows (Chen *et al.,* 2016):

$$HSI\ (\%) = \frac{\text{hepatic organ weight (g)}}{\text{final body weight (g)}}$$

Note:
HIS = Hepatosomatic index

Sample collection for the growth study

Body weight measurements of red Nile tilapia were conducted at the beginning and end of the experiment. The measured weight represented the biomass, which was then averaged for each individual fish. A total of 15 fish were weighed to determine the initial biomass weight. Prior to weighing, the fish were fasted for 24 hours. On day 0, five fish per aquarium were sampled for initial whole-body proximate composition analysis.

After a 60-day rearing period, all fish at the end of the experiment were weighed. Subsequently, the hepatosomatic index (HSI) was assessed using five fish per aquarium, where the body and liver weights were recorded. Two fish per aquarium were used for final whole-body proximate composition analysis to calculate protein and lipid retention. The whole-body proximate composition analysis followed AOAC (2012) procedures, and protein and lipid retention values were calculated based on the equations of Takeuchi (1988).

Data analysis

The experimental data were processed and analyzed using Microsoft Excel 2016 and SPSS version 16.0. Normality and homogeneity tests were performed prior to conducting the analysis of variance. A one-way analysis of variance (ANOVA) at a 95% confidence level was used to determine significant differences among treatments. When significant differences were detected, Duncan's multiple range test was applied to identify differences between treatments.

RESULTS AND DISCUSSION

Results

Based on the proximate analysis results (dry matter basis) of mung bean sprout waste hydrolyzed with different doses of cellulase enzyme for 24 hours, as presented in Table 3, the highest reduction in crude fiber was observed in the 1.2 g/kg treatment. The value of nitrogen-free extract (NFE) increased with higher cellulase enzyme doses, while ash, protein, and lipid contents of the cellulase-hydrolyzed mung bean sprout waste flour (LTe) showed no significant differences among treatments. In contrast, crude fiber exhibited a significantly decreasing trend (Table 3). The fiber fraction results of mung bean sprout waste hydrolyzed with different doses of cellulase enzyme are presented in Table 4. The highest reductions in crude fiber, NDF, ADF, hemicellulose, lignin, and cellulose were observed in the treatment with 1.2 g/kg cellulase. In the 1.2 g/kg hydrolysis treatment (LTe 1.2 g/kg), crude fiber decreased by 78.19%, hemicellulose by 19.22%, neutral detergent fiber by 41.69%, acid detergent fiber by 61.85%, lignin by 64.06%, and cellulose by 62.47% compared with the control (P<0.05). The values of NDF, ADF, hemicellulose, lignin, and cellulose declined progressively with increasing cellulase dosage (P<0.05).

The digestibility values of mung bean sprout waste hydrolyzed with different cellulase doses (LTe) are presented in Table 5. The results indicate that all enzymatic treatments differed significantly from the control. The LTe 1.2 g/kg treatment produced the highest digestibility of dry matter, protein, and energy, with values of 74.29 ± 0.91%, 83.78 ± 0.13%, and 80.20 ± 0.17%, respectively. In contrast, the lowest digestibility values were observed in the LTe 0 g/kg treatment, which recorded 64.72 ± 0.69% for dry matter digestibility, 78.48 ± 0.13% for protein digestibility, and 73.53 ± 0.14% for energy digestibility. These findings demonstrate that increasing the cellulase dose used to hydrolyze mung bean sprout waste flour enhances protein, dry matter, and energy digestibility (P<0.05).

The growth performance of Nile tilapia fed mung bean sprout waste hydrolyzed with 1.2 g/kg cellulase enzyme is presented in Table 6. Final biomass (Bt), final individual weight (Wt), daily growth rate (LPH), feed intake (JKP), protein efficiency ratio (REP), protein retention (RP),

Table 3. Proximate composition (dry matter basis) of mung bean sprout waste hydrolyzed with different doses of cellulase enzyme for 24 hours.

Proximate analysis	Mung bean sprout waste hydrolyzed with different cellulase enzyme doses (LTe treatments)			
	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Protein (%)	31.57 ± 0.32[a]	31.58 ± 0.20[a]	31.58 ± 0.29[a]	31.60 ± 0.18[a]
Fat (%)	4.54 ± 0.14[a]	4.57 ± 0.06[a]	4.56 ± 0.32[a]	4.59 ± 0.15[a]
Ash content (%)	11.43 ± 0.54[a]	11.56 ± 0.08[a]	11.07 ± 0.54[a]	11.31 ± 0.6[a]
Crude fiber (%)	22.05 ± 0.46[d]	11.04 ± 0.45[c]	7.96 ± 0.29[b]	4.92 ± 0.3[a]
NFE[1] (%)	30.41 ± 0.21[a]	41.25 ± 0.44[b]	44.82 ± 0.94[c]	47.58 ± 0.39[d]

Notes: [1]Nitrogen-free extract (NFE); [2]Mean ± standard deviation (n = 4); [3]Different lowercase superscript letters within the same row indicate significant differences among treatments (P<0.05).

Table 4. Crude fiber fraction profile of mung bean sprout waste hydrolyzed with different cellulase enzyme doses.

Crude fiber fraction profile[3]	Mung bean sprout waste hydrolyzed with different cellulase enzyme doses (LTe treatments)			
	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
NDF[1] (%)	23.05 ± 0.14[d]	19.28 ± 0.07[c]	17.08 ± 0.29[b]	13.44 ± 0.18[a]
ADF[2] (%)	12.19 ± 0.04[d]	9.37 ± 0.06[c]	7.38 ± 0.12[b]	4.65 ± 0.04[a]
Hemicellulose (%)	10.86 ± 0.13[c]	9.91 ± 0.19[c]	9.70 ± 0.07[b]	8.79 ± 0.18[a]
Lignin (%)	2.81 ± 0.03[d]	2.50 ± 0.04[c]	1.71 ± 0.04[b]	1.01 ± 0.03[a]
Cellulose (%)	8.58 ± 0.08[d]	6.24 ± 0.05[c]	4.31 ± 0.05[b]	3.22 ± 0.05[a]

Notes: [1]Neutral detergent fiber (NDF); [2]Acid detergent fiber (ADF); [3]Feed Science and Technology Laboratory, Faculty of Animal Science, IPB University; [4]Mean ± standard deviation (n = 4); [5]Different lowercase superscript letters within the same row indicate statistically significant differences among treatments (P<0.05).

feed conversion ratio (RKP), and average daily growth (ADG) were all higher in fish receiving the 1.2 g/kg hydrolyzed waste, accompanied by an improvement in RKP values. The incorporation of mung bean sprout waste hydrolyzed with 1.2 g/kg cellulase enzyme resulted in significantly improved growth performance compared with the control treatment (P<0.05).

Discussion

High-quality feed production requires raw materials with good nutritional value, in addition to cost and continuity of supply (Gamboa-Delgado & Marquez-Reyes, 2018; Albrektsen *et al.,* 2022). Protein is utilized by fish for maintenance, growth, and reproduction, and therefore must be supplied continuously (Li & Wu, 2020). Soybean meal is one of the main plant-based protein sources due to its high protein content, balanced amino acid profile, high protein digestibility, and excellent palatability (Tan *et al.,* 2017; Reynaud *et al.,* 2021). The use of alternative feed ingredients requires a good understanding of amino acid requirements and their availability in the

Table 5. Digestibility of mung bean sprout waste flour hydrolyzed with different cellulase enzyme doses.

Parameters	Mung bean sprout waste hydrolyzed with different cellulase enzyme doses (LTe treatments)			
	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Dry matter digestibility of LT_e (%)	64.72 ± 0.69[a]	67.49 ± 0.64[b]	70.56 ± 0.74[c]	74.29 ± 0.91[d]
Protein digestibility (%)	78.48 ± 0.13[a]	80.12 ± 0.09[b]	81.99 ± 0.10[c]	83.78 ± 0.13[d]
Energy digestibility (%)	73.53 ± 0.14[a]	75.27 ± 0.14[b]	77.88 ± 0.05[c]	80.20 ± 0.17[d]

Notes: [1] LTe = mung bean sprout waste hydrolyzed with different cellulase enzyme doses of 0, 0.4, 0.8, and 1.2 g/kg; [2]Mean ± standard deviation (n = 4); [3]Different lowercase superscript letters within the same row indicate significantly different treatment effects (P<0.05).

Table 6. Growth performance of Nile tilapia fed mung bean sprout waste meal hydrolyzed with different cellulase enzyme doses.

Parameters[1]	Mung bean sprout waste hydrolyzed with different cellulase enzyme doses (LTe treatments)				
	Reference diet	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Growth					
B_0 (g)	150.17 ± 0.08[a]	150.18 ± 0.06[a]	150.18 ± 0.03[a]	150.21 ± 0.05[a]	150.21 ± 0.03[a]
B_t (g)	703.39 ± 4.65[b]	671.29 ± 9.85[a]	712.93 ± 13.55[b]	745.79 ± 9.38[c]	758.61 ± 7.50[c]
W_0 (g)	10.00 ± 0.01[a]	10.00 ± 0.01[a]	10.00 ± 0.02[a]	10.00 ± 0.02[a]	10.00 ± 0.01[a]
W_t (g)	46.89 ± 0.31[b]	44.75 ± 0.66[a]	47.53 ± 0.90[b]	49.72 ± 0.63[c]	50.57 ± 0.50[c]
ADG (g/day)	0.61 ± 0.01[b]	0.58 ± 0.01[a]	0.63 ± 0.02[b]	0.66 ± 0.01[c]	0.68 ± 0.01[c]
SR (%)	100.00 ± 0.01[a]	100.00±0.01[a]	100.00 ± 0.01[a]	100.00 ± 0.01[a]	100.00 ± 0.01[a]
FI (g)	715.15 ± 11.26[b]	696.65 ± 7.21[a]	745.38 ± 13.36[c]	754.86 ± 7.60[cd]	765.75 ± 4.84[d]
SGR (%)	2.61 ± 0.01[b]	2.53 ± 0.02[a]	2.63 ± 0.03[b]	2.71 ± 0.02[c]	2.74 ± 0.02[c]
FCR	1.29 ± 0.03[ab]	1.34 ± 0.04[c]	1.32 ± 0.03[bc]	1.27 ± 0.02[a]	1.26 ± 0.01[a]
PER	2.42 ± 0.05[b]	2.35 ± 0.07[a]	2.43 ± 0.06[b]	2.52 ± 0.03[c]	2.55 ± 0.02[c]
PR (%)	39.62 ± 0.75[a]	42.36 ± 0.82[b]	46.03 ± 0.77[c]	48.51 ± 0.58[d]	51.81 ± 0.45[e]
LR (%)	31.44 ± 0.52[b]	28.82 ± 0.79[a]	33.15 ± 0.75[c]	39.43 ± 0.44[d]	46.12 ± 0.34[e]
Hepatosomatic performance					
HSI	3.05 ± 0.04[e]	2.68 ± 0.07[d]	2.49 ± 0.05[c]	2.35 ± 0.07[b]	2.07 ± 0.05[a]

Notes: [1]Biomass at the beginning (B0), biomass at the end (Bt), initial individual weight (W0), final individual weight (Wt), average daily growth (ADG), feed intake (FI), protein efficiency ratio (PER), survival rate (SR), specific growth rate (SGR), protein retention (PR), lipid retention (LR), hepatosomatic index (HSI), and feed conversion ratio (FCR). [2]Values are presented as mean ± standard deviation (n = 4). [3]Different lowercase superscript letters within the same row indicate significant differences among treatments (P<0.05).

ingredient (Masagounder *et al.,* 2016; Glencross *et al.,* 2020).

The quality of a feed ingredient can be evaluated based on its protein quality and amino acid composition. A deficiency in any limiting amino acid can disrupt protein synthesis and consequently inhibit growth. Protein quality can be assessed by comparing the essential amino acid composition of the ingredient with the amino acid requirements of the target species. In this study, soybean meal and mung bean sprout waste flour were used as the reference ingredients. Mung bean sprout waste contains limiting amino acids such as lysine, threonine, and methionine (Yi-Shen *et al.,* 2018). Its amino acids include essential amino acids (phenylalanine 1.76%, isoleucine 2.18%, leucine 2.21%, valine 1.85%, threonine 1.77%, lysine 2.13%, histidine 0.50%, arginine 3.15%, and methionine 0.36%) and non-essential amino acids (serine 1.91%, glutamic acid 3.27%, aspartic acid 3.20%, alanine 2.00%, glycine 1.78%, tyrosine 1.03%, and proline 2.62%).

Meanwhile, the amino acids of soybean meal are composed of essential amino acids (phenylalanine 1.98%, isoleucine 2.18%, leucine 3.76%, valine 2.13%, threonine 2.14%, lysine 3.23%, histidine 1.05%, arginine 2.83%, and methionine 0.20%) and non-essential amino acids (serine 2.73%, glutamic acid 5.21%, aspartic acid 4.74%, alanine 2.04%, glycine 1.73%, tyrosine 1.06%, and proline 2.30%). Essential amino acids in mung bean sprout waste such as arginine, isoleucine, and methionine are higher than in soybean meal, while non-essential amino acids such as proline and glycine are also higher. Mung bean sprout waste flour additionally contains vitamin E (200.60 mg/kg) and vitamin C (180.41 mg/kg). Thus, mung bean sprout waste flour may serve as a potential plant-based protein alternative to soybean meal.

The proximate composition of mung bean sprout waste flour meets the general nutritional requirements for Nile tilapia feed ingredients. Although the material originates from mung bean sprouts, which are rich in protein, it also contains high crude fiber (Pinandoyo *et al.,* 2021). The proximate composition of unhydrolyzed mung bean sprout waste includes 31.57% protein, 4.54% lipid, 11.43% ash, 22.05% crude fiber, and 30.41% nitrogen-free extract (NFE). In the first stage of this study, enzymatic hydrolysis using cellulase was applied to reduce the crude fiber content before its use as a feed ingredient. The high crude fiber, composed largely of lignin and cellulose, is difficult for fish to digest; therefore, hydrolysis using 1.2 g/kg cellulase, which showed the greatest fiber reduction, was applied. After hydrolysis with 1.2 g/kg cellulase, the mung bean sprout waste flour contained 31.60% protein, 4.59% lipid, 11.31% ash, 4.92% crude fiber, and 47.58% NFE. The crude fiber decreased from 22.05% to 4.92%.

Based on the results (Table 3), cellulase hydrolysis (LTe) reduced crude fiber by 78.19% at a dose of 1.2 g/kg. This reduction was associated with decreases in the structural fiber components NDF and ADF (Table 4), leading to an increase in NFE from 30.41% to 47.58%, representing more digestible carbohydrates. Protein, lipid, and ash contents did not differ significantly among the hydrolyzed and non-hydrolyzed treatments because cellulase specifically hydrolyzes cellulose, not protein or lipid. Reduced NDF and ADF indicate increased soluble glucose content, reflecting greater cellulose breakdown. Decreases in NDF and ADF suggest effective degradation of cell wall components by extracellular cellulase enzymes. Similar findings were reported by Imran *et al.* (2016) and Jefry *et al.* (2021), who noted that cellulase hydrolysis lowers crude fiber, ADF, NDF, lignin, hemicellulose, and cellulose without altering protein content.

In the second stage, the highest digestibility value of the hydrolyzed ingredient reached 74.29 ± 0.91%. The improved digestibility is attributed to cellulase breaking down the complex fiber in mung bean sprout waste into simpler components more easily digested by tilapia. High digestibility of plant-based ingredients is advantageous because simpler carbohydrates can serve as an energy source. Digestibility values help optimize fish growth by considering nutrient requirements and metabolic excretion. High crude fiber lowers digestibility because fiber is not digestible by fish and accelerates intestinal transit, reducing nutrient absorption such as protein (Wang *et al.,* 2017; Sarkar *et al.,* 2021).

Cellulase is an extracellular enzyme complex consisting of endo-β-1,4-glucanase, exo-β-1,4-glucanase, and β-1,4-glucosidase. It breaks cellulose into cellobiose and finally into glucose. Endoglucanase hydrolyzes internal bonds in cellulose, producing oligosaccharides; exoglucanase converts oligosaccharides and cellulose into cellobiose; and β-glucosidase converts cellobiose into glucose. The activity of cellulase at 1.2 g/kg thus enhances the breakdown of cellulose, improving protein and energy digestibility. Higher digestibility values indicate that nutrients are more easily absorbed (Heinitz *et al.,* 2016; Schrama *et al.,* 2018; Suryaningrum & Samsudin, 2020; Phan *et al.,* 2021; Azaza *et al.,* 2020). Conversely, excess fiber can disrupt metabolism by hindering nutrient absorption, reducing energy utilization, and impairing growth (Adewumi & Ola-Oladimeji, 2016; Rawski *et al.,* 2020; Shao *et al.,* 2021).

Protein digestibility of the hydrolyzed flour reached 83.78 ± 0.13%, while the lowest value (78.48 ± 0.13%) occurred in the unhydrolyzed treatment. Higher protein digestibility reflects increased availability of digestible protein in the feed. Factors that influence protein digestibility include feed intake, water temperature, feed particle size, and protein content (Marzuqi *et al.,* 2020). High crude fiber in the unhydrolyzed treatment increased fecal output, reducing protein digestibility. Lower crude fiber after hydrolysis increases retention time in the digestive tract, improving nutrient absorption (Gilannejad *et al.,* 2019).

Different fish species vary in digestive capacity. Carnivorous fish have stronger proteolytic enzymes, while herbivorous species such as Nile tilapia possess higher capability to digest plant fiber (An & Anh, 2020). Therefore, tilapia can utilize the hydrolyzed ingredient more efficiently. Energy digestibility was also highest in the hydrolyzed treatment (80.20 ± 0.17%). Energy digestibility reflects the proportion of digestible protein, lipid, and carbohydrate. Higher values indicate more energy available for metabolism and activity. Lower digestibility values were associated with high crude fiber, which increases peristalsis and shortens intestinal contact time.

Growth trial results showed that replacing soybean meal with mung bean sprout waste hydrolyzed using 1.2 g/kg cellulase improved several growth parameters including feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR). Improved feed efficiency was evidenced by lower FCR and higher PER. Increased PR indicates more protein retained in the body relative to intake. Higher lipid retention was associated with carbohydrate storage through lipogenesis. Hydrolyzed flour (1.2 g/kg) also increased final weight, specific growth rate, and average daily growth while decreasing the hepatosomatic index (HSI), compared with the unhydrolyzed control. The reduction in HSI suggests lower lipid accumulation in the liver, indicating more efficient carbohydrate utilization. According to Faria *et al.* (2021) and Li *et al.* (2021), lower HSI reflects better conversion of dietary carbohydrates into energy rather than storage as glycogen and lipid in the liver.

CONCLUSION

The utilization of mung bean sprout waste flour hydrolyzed with 1.2 g/kg cellulase improved digestibility and growth performance of red Nile tilapia. The energy digestibility of the hydrolyzed flour at 1.2 g/kg reached 80.20 ± 0.17%, representing the highest value among treatments. The high energy digestibility indicates that the protein, lipid, and carbohydrate fractions of the feed were efficiently digested by the fish. Proximate analysis showed that hydrolysis with 1.2 g/kg cellulase resulted in a low crude fiber content, thereby enhancing digestive efficiency in the fish.

REFERENCES

Adewumi AA, Ola-Oladiimeji FA. 2016. Performance characteristics and feed utilization of African catfish (*Clarias gariepinus*) fed varying inclusion levels of fermented mulberry leaf. Journal of Agriculture and Food Science 4: 87–93.

Albrektsen S, Kortet R, Skov PV, Ytteborg E, Gitlesen S, Kleinegris D, Mydland LT, Hansen JO, Lock EJ, Morkore T, James P. 2022. Future feed resources in sustainable salmonid production: A review. Reviews in Aquaculture 14: 1790–1812.

An BNT, Anh NTN. 2020. Co-culture of Nile tilapia (*Oreochromis niloticus*) and red seaweed (*Gracillaria tenuistipitata*) under different feeding rates: effects on water quality, fish growth and feed efficiency. Journal of Applied Phycology 32: 2031–2040.

[AOAC] Association of Official Analytical Chemists. 2012. Official Methods of Analysis of AOAC International, Volume 1. Maryland, United States: AOAC International.

Azaza MS, Saidi SA, Dhraief MN, El-feki A. 2020. Growth performance, nutrient digestibility, hematological parameters, and hepatic oxidative stress response in juvenile Nile Tilapia, *Oreochromis niloticus*, fed carbohydrates of different complexities. Animals 10: 1913.

[BPS] Badan Pusat Statistik. 2015. Produksi kacang hijau (ton): 1993-2020. https://www.

bps.go.id/id/statistics-table/2/MjMjMg==/produksi.html. [8 October 2021].

[BPS] Badan Pusat Statistik. 2019. Impor kedelai menurut negara asal utama 2017. https://www.bps.go.id/id/statistics-table/1/MjAxNSMx/impor-kedelai-menurut-negara-asal-utama--2017-2024.html. [8 October 2021].

Cai Y, Huang H, Yao W, Yang H, Xue M, Li X, Leng X. 2022. Effects of fish meal replacement by three protein sources on physical pellet quality and growth performance of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Reports 25: 101210.

Chen Q, Zhao H, Huang Y, Cao J, Wang G, Sun Y, Li Y. 2016. Effects of dietary arginine levels on growth performance, body composition, serum biochemical indices and resistance ability against ammonia-nitrogen stress in juvenile yellow catfish (*Pelteobagrus fulvidraco*). Animal Nutrition 2: 204–210.

Co R, Hug LA. 2021. A need for improved cellulase identification from metagenomic sequence data. Applied and Environmental Microbiology 87: 1–10.

Faria DLJM, Guimarães LN, da Silva VC, de Oliveira Lima EC, de Sabóia-Morais SMT. 2021. Recovery trend to co-exposure of iron oxide nanoparticles (γ-Fe_2O_3) and glyphosate in liver tissue of the fish *Poecilia reticulata*. Chemosphere. 282: 130993.

[FAO] Food and Agricultural Organization. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. https://doi.org/10.4060/cc0461en. [8 November 2023].

Gamboa-Delgado J, Márquez-Reyes JM. 2018. Potential of microbial-derived nutrients for aquaculture development. Reviews in Aquaculture 10: 224–246.

Gilannejad N, Silva T, Martinez-Rodriguez G, Yufera M. 2019. Effect of feeding time and frequency on gut transit and feed digestibility in two fish species with different feeding behaviours, gilthead seabream and Senegalese sole. Aquaculture 513: 734438.

Glencross BD, Baily J, Berntssen MH, Hardy R, MacKenzie S, Tocher DR. 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Reviews in Aquaculture 12: 703–758.

Heinitz MC, Lemme A, Schulz C. 2016. Measurement of digestibility in agastric fish based on stripping method–apparent nutrient, energy and amino acid digestibilities of common feed ingredients for carp diets (*Cyprinus carpio*). Aquaculture Nutrition 22: 1065–1078.

Hermawan D, Suprayudi MA, Jusadi D, Alimuddin, Ekasari J. 2021. Evaluation of corn steep powder as a protein source of Nile tilapia *Oreochromis niloticus* diet. Jurnal Akuakultur Indonesia 20: 115–129.

Hernowo IA, Pinandoyo, Hutabarat J, Herawati VE. 2020. The effect of substitution addition of Indian copperleaf flour and taoge flour combination in artificial feed on feed utilization efficiency and growth of tilapia (*Oreochromis niloticus*). Aquacultura Indonesiana 21: 32–41.

Houfani AA, Anders N, Spiess A, Baldrian P, Benallaoua S. 2020. Insights from enzymatic degradation of cellulose and hemicellulose to fermentable sugars–a review. Biomass and Bioenergy 134: 105481.

Imran M, Anwar Z, Irshad M, Asad MJ, Ashfaq H. 2016. Cellulase production from species fungi and bacteria from agricultural wastes and its utilization in industry: a review. Advances in Enzyme Research 4: 44–45.

Irawati E, Fitri L, Adelina T, Elviriadi. 2017. Fraksi serat kulit ubi kayu (*Manihot utilissima*) yang difermentasi dengan ragi tape (*Saccharomyces cerevisiae*). Jurnal Peternakan 14: 48–53. (In Indonesian).

Jannathulla R, Dayal JS, Ambasankar K, Muralidhar M. 2018. Effect *Aspergillus niger* fermented soybean and sunflower oil cake on growth, carcass composition and haemolymph indices in *Penaeus vannamei* boone, 1931. Aquaculture 486: 1–8.

Jefry J, Setiawati M, Jusadi D, Fauzi IA. 2021. Cellulase hydrolyzed *Indigofera zolingeriana* leaf utilization as a feed ingredient for gourami fingerling. Jurnal Akuakultur Indonesia 20: 139–147.

Jha R, Mishra P. 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. Journal of Animal Science and Biotechnology 12: 1–16.

Kemal R, Fauzi IA, Nuryati S, Wardani WW, Suprayudi MA. 2023. Evaluation of Selenoprotein Supplementation on Digestibility, Growth, and Health Performance of Pacific White Shrimp Litopenaeus vannamei. Aquaculture Nutrition 1: 2008517.

[Kementan] Kementerian Pertanian. 2013. Prospek pengembangan agribisnis kacang hijau. https://repository.pertanian.go.id/server/api/core/bitstreams/ce165ddd-576a-41ad-b53d-6062dea451ee/content. [24 April 2022].

[Kementan] Kementerian Pertanian. 2021. Laporan kinerja direktorat jenderal tanaman pangan tahun 2021. https://ppid.pertanian.go.id/doc/14/LAKIN%20DITJEN%20TP%2020211.pdf. [24 April 2022].

Laca A, Laca A, Díaz M. 2019. Hydrolysis: From cellulose and hemicellulose to simple sugars. In: Basile A, Dalen F. Second and Third Generation of Feedstocks. Rende, Italy: Elsevier. pp. 213–240.

Li P, Wu G. 2020. Composition of amino acids and related nitrogenous nutrients in feedstuffs for animal diets. Amino Acids 52: 523–542.

Li LY, Wang Y, Limbu SM, Li JM, Qiao F, Chen LQ, Zhang ML, Du ZY. 2021. Reduced fatty acid β-oxidation improves glucose catabolism and liver health in Nile tilapia (*Oreochromis niloticus*) juveniles fed a high-starch diet. Aquaculture. 535: 36392.

Marzuqi M, Wayan N, Astuti W, Giri NA, Mahardika K. 2020. Performa pertumbuhan dan nilai kecernaan pakan pada yuwana kerapu hibrid “cantik” (*Epinephelus fuscoguttatus* x *Epinephelus polyphekadion*) dengan pemberian bakteri probiotik dan/atau enzim papain dalam pakan. Media Akuakultur 15: 29–37. (In Indonesian).

Masagounder K, Ramos S, Reimann I, Channarayapatna G. 2016. Optimizing nutritional quality of aquafeeds. Aquafeed Formulation in Academic Press 239–264.

Mcginnis AJ, Kasting R. 1964. Chromic oxide indicator method for measuring food utilization in a plant-feeding insect. Science 144: 1464–1465.

Mello N, Konig IFM, Ríos-Duran MG, Navarrete-Ramírez P, Martínez-Palacios CA, Murgas LDS, Martínez-Chavez CC. 2023. Feeding frequency has a determinant role in growth performance, skeletal deformities, and body composition in the Mexican pike silverside (*Chirostoma estor*), an agastric short-intestine fish (Teleostei: Atheriniformes). Aquaculture 562: 738766.

Mulyasari, Widarnani, Suprayudi MA, Junior MZ, Sunarno MTD. 2016. Screening of probiotics from the digestive tract of gourami (*Osphronemus goramy*) and their potency to enchance the growth of tilapia (*Oreochromis niloticus*). AACL Bioflux 9: 1121–1132.

Phan LTT, Masagounder K, Mas-Muñoz J, Schrama JW. 2021. Differences in energy utilization efficiency of digested protein, fat and carbohydrates in snakehead (*Channa striata*). Aquaculture 532: 736066.

Pinandoyo, Herawati VE, Hutabarat J, Windarto S. 2021. Application of Indian nettle (*Acalypha indica*) and mung bean sprouts (*Vigna radiata*) as a source of plant protein to improve gourami (*Osphronemus goramy*) production. AACL Bioflux 14: 141–150.

Plasus MMG, Kondo H, Hirono I, Satoh S, Haga Y. 2019. Cysteamine dioxygenase as enzymes for taurine synthesis and negative effect of high dietary cysteamine on growth and body shape of the common carp, *Cyprinus Carpio*. Aquaculture Science 67: 95–108.

Rahayu S, Diapari D, Wandito DS, Ifafah WW. 2010. Survei potensi ketersediaan limbah taoge sebagai pakan ternak alternatif di kodya bogor. Laporan Penelitian. Bogor, Indonesia: Institut Pertanian Bogor. (In Indonesian).

Ramena Y, Rawles SD, Lochmann R, Gaylord TG, McEntire ME, Farmer BD, Baumgartner W, Webster CD, Beck BH, Green BW, Barnett LM. 2020. Growth, nutrient retention, innate immune response, and intestinal morphology of juvenile, soy-naïve hybrid striped bass, *Morone saxatilis* x *M. chrysops* fed commercial-type, soy-based, ideal protein, fish meal replacement diets. Aquaculture 522: 735150.

Ranjan A, Sahu NP, Deo AD, Kumar S. 2018. Comparative growth performance, in vivo digestibility and enzyme activities of *Labeo rohita* fed with DORB based formulated diet and commercial carp feed. Turkish Journal of Fishieries and Aquactic Science 18: 1025–1036.

Ranjan A, Sahu NP, Deo AD, Kumar S. 2019. Solid state fermentation of de-oiled rice bran: Effect on in vitro protein digestibility, fatty acid profile and anti-nutritional factors. Food Research International 119: 1–5.

Rawski M, Mazurkiewicz J, Kierończyk B, Józefiak D. 2020. Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in Siberian sturgeon nutrition: The effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization. Animals 10: 2119.

Reynaud Y, Buffiere C, Cohade B, Vauris M, Liebermann K, Hafnaoui N, Lopez M, Souchon I, Dupont D, Rémond D. 2021. True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAASs) of plant-based protein foods. Food Chemistry 338: 128020.

Roques S, Deborde C, Richard N, Skiba-Cassy S, Moing A, Fauconneau B. 2020. Metabolomics and fish nutrition: a review in the context of sustainable feed development. Reviews in Aquaculture. 12: 261–282.

Sarkar MM, Rohani MF, Hossain MAR, Shahjahan M. 2021. Rvaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. Biology Trace Rlement Research 200: 844–854.

Schrama JW, Haidar MN, Geurden I, Heinsbroek LT, Raushik SJ. 2018. Rnergy efficiency of digestible protein, fat and carbohydrate utilisation for growth in rainbow trout and Nile tilapia. British Journal of Nutrition 119: 782–791.

Shao XY, Wu P, Feng L, Jiang WD, Liu Y, Ruang SY, Tang L, Zhou XQ. 2021. Growth performance, digestive and absorptive capacity of on-growing grass carp (*Ctenopharyngodon idellus*) fed with graded level of dietary fibre from soybean hulls. Aquaculture Nutrition 27: 198–216.

Suprayudi MA, Jusadi D, Setiawati M, Rkasari J, Fauzi IA. 2023. Peningkatan efisiensi protein dan pakan organisme akuatik. Bogor, Indonesia: IPB Press. (In Indonesian).

Suryaningrum LH, Samsudin R. 2020. Nutrient digestibility of green seaweed Ulva meal and the influence on growth performance of Nile tilapia (*Oreochromis niloticus*). Rmirates Journal of Food and Agriculture 32: 488–494.

Tan Q, Song D, Chen X, Xie S, Shu X. 2017. Replacing fish meal with vegetable protein sources in feed for juvenile red swamp crayfish, *Procambarus clarkii*: Rffects of amino acids supplementation on growth and feed utilization. Aquaculture Nutrition 24: 858–864.

Takeuchi T. 1988. Laboratory Work Chemical Rvaluation of Dietary Nutriens In: Fish Nutrition and Mariculture. Tokyo: JICA.

Trustinah. Radjit BS. Prasetiaswati N. Harnowo D. 2014. Adopsi varietas unggul kacang hijau di sentra produksi. IPTRR Tanaman Pangan 9: 24–38. (In Indonesian).

Wang JH, Guo H, Zhang TR, Wang H, Liu BN, Xiao S. 2017. Growth performance and digestion improvement of juvenile sea cucumber *Apostichopus japonicus* fed by solid-state fermentation diet. Aquaculture Nutrition 23: 1312–1318.

Wardani WW, Alimuddin A, Junior MZ, Setiawati M, Nuryati S, Suprayudi MA. 2021. Growth performance, fillet quality and immunity after challenge with *Streptococcus agalactiae* of red tilapia (*Oreochromis* sp.) fed diet containing cysteamine and creatine. Aquaculture Research 52: 4237–4248.

Watanabe T. 1998. Fish Nutrition and Mariculture. Tokyo, Japan: Ranagawa Fisheries Training Center, Japan International Cooperation Agency.

Yi-Shen Z, Shuai S, Fitzgerald R. 2018. Mung bean proteins and peptides: Nutritional, functional, and bioactive properties. Food and Nutrition Research 62: 1290.

Zhang F, Li L, Li P, Meng X, Cui X, Ma Q, Wei Y, Liang M, Xu H. 2023. Fish oil replacement by beef tallow in juvenile turbot diets: Rffects on growth performance, body composition and volatile flavor compounds in the muscle. Aquaculture 564: 739070.

Zhao Y, Zhang TR, Li Q, Feng L, Liu Y, Jiang WD, Wu P, Zhao J, Zhou XQ, Jiang J. 2020. Rffect of dietary L-glutamate levels on growth, digestive and absorptive capability, and intestinal physical barrier function in Jian carp (*Cyprinus carpio* var. Jian). Animal Nutrition 6: 198–209.