

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 pencernaan ikan nila *Oreochromis sp.*

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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 $10,00 \pm 0,01$ g/ekor dan panjang $7,00 \pm 0,15$ 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 pencernaan 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 pencernaan bahan, pencernaan protein, dan pencernaan 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, pencernaan, 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) *Trichoderma 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 (Cr₂O₃) as an inert marker for digestibility determination. The reference diet consisted of 98.80% commercial feed, 0.6% PMC, and 0.6% Cr₂O₃, 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 (Cr₂O₃) 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 Cr₂O₃ 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 (NO₂⁻) 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: ¹Essential amino acids (EAA); ²Non-essential amino acids (NEAA); ³Total amino acids (TAA); ⁴Nitrogen-free extract (NFE); ⁵Feed 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):

FI (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
LT ^{e1}	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: ¹LT^e = mung bean sprout waste meal hydrolyzed with different cellulase enzyme doses (0, 0.4, 0.8, and 1.2 g/kg); ²Nitrogen-free extract on a dry matter basis (NFE) = 100–(protein + lipid + ash + crude fiber); ³Gross 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); ⁴C/P = calories-to-protein ratio.

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

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

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):

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

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):

$$\text{FCR} = F1 - (W_t + W_0)$$

Note:

FCR = Feed conversion ratio
 $F1$ = 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):

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

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):

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

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):

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

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 $74.29 \pm 0.91\%$, $83.78 \pm 0.13\%$, and $80.20 \pm 0.17\%$, respectively. In contrast, the lowest digestibility values were observed in the LTe 0 g/kg treatment, which recorded $64.72 \pm 0.69\%$ for dry matter digestibility, $78.48 \pm 0.13\%$ for protein digestibility, and $73.53 \pm 0.14\%$ 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: ¹Nitrogen-free extract (NFE); ²Mean \pm standard deviation ($n = 4$); ³Different 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: ¹Neutral detergent fiber (NDF); ²Acid detergent fiber (ADF); ³Feed Science and Technology Laboratory, Faculty of Animal Science, IPB University; ⁴Mean \pm standard deviation ($n = 4$); ⁵Different 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 LTe (%)	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: ¹LTe = mung bean sprout waste hydrolyzed with different cellulase enzyme doses of 0, 0.4, 0.8, and 1.2 g/kg; ²Mean ± standard deviation (n = 4); ³Different 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 ^c
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 ^c
Hepatosomatic performance					
HSI	3.05 ± 0.04 ^c	2.68 ± 0.07 ^d	2.49 ± 0.05 ^c	2.35 ± 0.07 ^b	2.07 ± 0.05 ^a

Notes: ¹Biomass at the beginning (B₀), biomass at the end (B_t), initial individual weight (W₀), final individual weight (W_t), 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). ²Values are presented as mean ± standard deviation (n = 4). ³Different 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 $74.29 \pm 0.91\%$. 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 $83.78 \pm 0.13\%$, while the lowest value ($78.48 \pm 0.13\%$) 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 ($80.20 \pm 0.17\%$). 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 $80.20 \pm 0.17\%$, 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.

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