

Cellulase hydrolyzed *Indigofera zolingeriana* leaf utilization as a feed ingredient for gourami fingerling

Pemanfaatan daun *Indigofera zolingeriana* yang dihidrolisis enzim selulase sebagai bahan baku pada pakan benih ikan gurami

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ABSTRACT

This study was aimed to evaluate the utilization of hydrolyzed *Indigofera zolingeriana* by cellulase enzyme as the feed ingredient of gourami fish. This study used a completely randomized design which contained three steps, whereas each step contained four treatments and four replications. The first step performed by evaluating the *Indigofera* leaf meal (ILM) 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 ILM on gourami seeds. The third step was feed evaluation added with ILM as much as 0% (control), 15%, 30%, and 45% against the growth performance on gourami seeds. The gourami seeds used in the second and third steps with a weight of 13.65 ± 0.39 g/seed and 5.95 ± 0.15 g/seed, respectively. The addition of 0.8 g/kg and 1.2 g/kg cellulase enzyme could significantly decrease the crude fiber of ILM with 43.33%, besides having the best value of total, ingredient, protein, lipid, and energy digestibility. The growth performance of gourami seeds given 15% ILM added feed had the best value and insignificantly different from the control feed without ILM addition based on the specific growth rate (SGR), protein retention (PR), and feed efficiency (FE).

Keywords: Cellulase, feed, hydrolyze, *Indigofera zolingeriana*, *Osphronemus gouramy*.

ABSTRAK

Penelitian ini dilakukan untuk mengevaluasi pemanfaatan daun *Indigofera zolingeriana* yang dihidrolisis enzim selulase sebagai bahan baku pada pakan benih ikan gurami. Penelitian ini menggunakan rancangan acak lengkap yang terdiri atas tiga tahap dan masing-masing tahap terdiri dari empat perlakuan dan empat ulangan. Pada tahap pertama dilakukan evaluasi tepung daun *Indigofera* (TDI) yang ditambahkan enzim selulase sebesar 0 g/kg (kontrol), 0.4 g/kg, 0.8 g/kg, dan 1.2 g/kg. Pada tahap kedua dilakukan uji pencernaan bahan TDI pada benih ikan gurami. Pada tahap ketiga dilakukan evaluasi pakan yang ditambahkan TDI sebesar 0% (kontrol), 15%, 30% dan 45% terhadap kinerja pertumbuhan pada benih ikan gurami. Benih ikan gurami yang digunakan pada penelitian tahap kedua dengan bobot 13.65 ± 0.39 g/ekor dan 5.95 ± 0.15 g/ekor. Penambahan enzim selulase pada dosis 0.8 g/kg dan 1.2 g/kg secara signifikan mampu menurunkan serat kasar TDI sebesar 43.33 % dan memberikan nilai terbaik terhadap nilai pencernaan total, pencernaan bahan, pencernaan protein, pencernaan lemak dan pencernaan energi. Kinerja pertumbuhan benih ikan gurami yang diberikan pakan yang ditambahkan TDI sebesar 15% memiliki nilai terbaik dan tidak berbeda nyata terhadap pakan kontrol tanpa TDI dari aspek laju pertumbuhan spesifik (LPS), retensi protein (RP) dan efisiensi pakan (EP).

Kata kunci: Hidrolisis, *Indigofera zolingeriana*, *Osphronemus gouramy*, pakan, selulase.

INTRODUCTION

Feed is one of the important aspects on intensive fish culture. The most important component in fish feed is protein content. Common plant protein source utilized in feed is soybean meal and fish meal. According to BPS (2019), Indonesian soybean meal import inclined from 1.7 million tons in 2010 to 2.6 million tons in 2019. Fish meal play a huge role in fish diet industry because it holds such a large amount of nutrition (Rahman *et al.*, 2016). In contrast, the cost of using fish meal as the main ingredient of fish diet is relatively high. Therefore, alternative ingredient is required with cheaper price and higher quality. One of the ingredients utilized as plant protein source is *Indigofera* leaf. *Indigofera* plant is a leguminose plant that mostly found in Indonesia and grows fastly and adaptively against low soil fertility (Abdullah, 2014; Fugarasti *et al.*, 2020). According to Herdiawan and Krisnan (2014), *Indigofera* plant spreads mostly in Java, Sumatera, Sumba, and Flores Island with 52 ton/ha/year production capacity.

Indigofera zollingeriana is a plant containing approximately 29.06% of protein source (Pangentasari, 2018). Kumalasari *et al.* (2017) also stated that *Indigofera zollingeriana* contained 25.17%–26.44%. According to Palupi *et al.* (2014), the amino acid contents in *Indigofera* leaf are histidine 0.67%, threonine 1.14%, arginine 1.67%, tyrosine 1.05%, methionine 0.43%, valine 1.56%, phenylalanine 1.60%, isoleucine 1.35%, leucine 2.26%, and lysine 1.57%. The study also reported that the crude fiber in *Indigofera* leaf ranged from 27.68% or 28.98%. However, it has a limiting factor to be utilized as a protein ingredient source in fish feed due to high crude fiber. Sun *et al.* (2019) also confirmed that crude fiber is hardly digested and absorbed by fish. According to SNI 7473:2009, the tolerable crude fiber content in fish feed is around 5–8%. It inevitably limits the utilization for feed (Caruso, 2015; Daniel, 2018) because it will lead to low digestibility (Pandey, 2013). According to Melati and Sunarno (2016), crude fiber contains ADF (acid detergent fiber), NDF (neutral detergent fiber), cellulose, hemicelluloses, and lignin fraction. One attempt that can be done to reduce the crude fiber content and anti-nutrient compounds is by using hydrolysis enzyme (Olude *et al.*, 2016).

Hydrolysis is commonly known as cellulose breakdown process (Ketut *et al.*, 2017).

Hydrolysis enzyme can eliminate the excess crude fiber content and anti-nutrient compounds (tannins and phytic acid) on bran, wheat, rice plants, and seeds (Dhingra *et al.*, 2012; Raveendran *et al.*, 2018). This particular process is possible to improve the nutritive value of each feed ingredients (Bae *et al.*, 2019). This condition is also expected to have similar effect in *I. zollingeriana* leaf meal. Therefore, it can conceivably maintain the digestibility level (Zuraida *et al.*, 2012; Pamungkas, 2012). One of hydrolysis enzyme types can be found in cellulase enzyme. The commercial product of cellulase enzyme contains 20 000 U/g enzyme activity. According to Nurliana *et al.* (2019), crude fiber content in feed ingredient can be declined using cellulase enzyme by hydrolyzing the cellulose macromolecules, then transformed into easily digested simple molecules.

As the *I. zollingeriana* leaf meal can be used as an alternative feed ingredient after being hydrolyzed with commercial cellulase enzyme product, this feed ingredient should be tested its digestibility and effect on the fish growth. The fish growth test can be performed in herbivore fish such as gourami, *Osphronemus gouramy*. This particular species is one of the most excellent freshwater fish commodities with high economical value and relatively stabilized cost. Based on DJPB (2017), the production of gourami in 2017 reached 149 552.70 tons, increased from 118 776.39 tons in 2014. However, the protein content in gourami feed is still low, specifically the plant protein content, as good protein level for gourami feed is 30% (Mithun *et al.*, 2018). This study was aimed to evaluate the utilization of cellulase hydrolyzed *I. zollingeriana* as plant protein source feed ingredient on gourami fingerling. Thus, further studies are necessary to determine the proper content of plant protein source using *I. zollingeriana* leaf meal in gourami feed.

MATERIALS AND METHODS

Period and location

This study was performed on October 2019 until March 2020 in the Laboratory of Fish Nutrition, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. Feed preparation and production activity were performed in the laboratory of feed processing. Proximate analysis was performed

in the chemical laboratory of fish nutrition. The tested fish was reared in the laboratory of fish nutrition, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor.

Experimental design

First step: Indigofera leaf hydrolysis

The first step was a hydrolysis process of *I. zollingeriana* meal using a completely randomized experimental design containing four treatments and four replications. Hydrolysis process was started from preparing the indigofera leaf meal ingredient (ILM) and cellulase enzyme as well as proximate analysis of ILM ingredient before and after hydrolysis. The ingredients were milled, grinded, and mixed with cellulase enzyme based on each treatment as much as 0.4, 0.8, and 1.2 g/kg. After that, the mixture was dissolved in water as many of 30% of the ingredient, then thoroughly mixed. The hydrolysis process was performed in a plastic container at room temperature for 24 hours. Thereafter the hydrolyzed ILM ingredient was dried using an oven at 60°C for 1.5 hours. The indigofera leaf meal hydrolysis test is presented below:

- ILM : *Indigofera* leaf meal without cellulase enzyme
- 0.4 ILM : *Indigofera* leaf meal + 0.4 g/kg cellulase enzyme
- 0.8 ILM : *Indigofera* leaf meal + 0.8 g/kg cellulase enzyme

- 1.2 ILM : *Indigofera* leaf meal + 1.2 g/kg cellulase enzyme

Parameters

Ingredient chemical analysis performed in this study step were NDF (neutral detergent fiber) and ADF (acid detergent fiber) analysis using *In Sacco* method (Van Soest, 1976), as well as hydrolyzed and non-hydrolyzed ILM proximate analysis. The proximate analysis contained crude protein, moisture, crude lipid, crude fiber, and ash. Crude protein analysis used Kjeldahl method, moisture and ash content were performed using Gravimetric method and crude lipid used Soxhlet method (AOAC, 1995).

Second step: hydrolyzed Indigofera ingredient digestibility test

Ingredient digestibility test was performed using a completely randomized design containing four treatments and four replications. Ingredient test used was commercial feed, non-hydrolyzed ILM, hydrolyzed ILM on certain dozes, and 0.3% Cr2O3 as digestibility test indicator. The tested fish was fed using commercial feed with 32% of protein content. The tested fish used with the total average length 7.75 ± 0.18 cm of and weight of 13.65 ± 0.39 g were obtained from gourami fish hatchery in Ciseeng, Bogor. The stocking density used was 12 fish/90/L and fed with satiation method three times a day at satiation. The feed formulation for ingredient digestibility test is presented in Table 1.

Table 1. Feed formulation for ingredient digestibility test on gourami fingerlings

Ingredient	Reference diet (%)	Feed treatment			
		0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Commercial feed	99.1	69.1	69.1	69.1	69.1
ILM		30	30	30	30
Cr ₂ O ₃	0.6	0.6	0.6	0.6	0.6
CMC	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
Proximate analysis (dry base %)					
Protein	31.36	30.12	30.23	30.79	30.51
Lipid	6.73	6.31	6.08	6.20	6.24
Crude fiber	3.93	5.88	5.66	5.53	5.16
Ash	11.73	11.84	11.67	11.6	11.18
NFE	46.25	45.85	46.36	45.87	46.91
GE (Kcal/100 g)	450.50	438.19	439.08	440.74	444.74

Note: ILM = *Indigofera* leaf meal; CMC= Carboximethyl cellulase; NFE = Nitrogen free extract; GE= Gross energy

Parameters

Parameters determined on digestibility test contained of total digestibility using the method of Takeuchi (1988), total digestibility, nutrient digestibility (protein, lipid, and energy), and ingredient digestibility based on Watanabe (1988) method.

Third step: growth performance test of gourami fingerling

The third step was growth performance test on gourami fingerlings containing four treatments and four replications. Treatments from this study was the total ILM hydrolysis added in feed formulation as a replacement of soybean meal, namely 0%, 15%, 30%, and 45%. The test fish used had 5.88 ± 0.15 cm length and 5.95 ± 0.15 g weight. The stocking density used was 15 fish/90 L water and fed three times a day at satiation. Feed production was performed by mixing all measured ingredients based on the formulation (Table 2), then stirred and pelleted using a pelleting machine. Pelleted feed was then

dried in an oven at 60°C for 2 hours and moved into a plastic container. Feed formulation used for growth performance test can be seen in Table 2.

Parameters

Growth performance test parameters determined contained: feed intake (FI), survival rate (SR), specific growth rate (SGR), protein efficiency ratio (PER), protein retention (PR), and enzyme activity (protease, amylase, and lipase).

RESULTS AND DISCUSSION

Results

The first study step presented that *Indigofera* leaf meal (ILM) with different cellulase enzyme dozes for 24 hours was significantly different ($P < 0.05$) against declined crude fiber. The 1.2 g/kg cellulase enzyme doze had the most declined crude fiber, NDF, and hemicellulose on ILM hydrolysis process. The proximate analysis result on ILM ingredient can be seen in Table 3.

Table 2. Feed composition used for gourami fish fingerling using *I. zollingeriana* leaf meal hydrolysis.

Ingredient	Ingredients ILM hydrolyzed in feed			
	0%	15%	30%	45%
Fish meal	10.00	10.00	10.00	10.00
Meat bone meal (MBM)	16.00	16.00	16.00	16.00
Soybean meal	31.00	23.00	16.00	8.00
Hydrolyzed ILM	0.00	15.00	30.00	45.00
Pollard	29.00	22.00	15.00	7.00
Tapioca	7.67	7.67	7.67	7.67
Fish oil	1.50	1.50	1.50	1.50
Corn oil	1.50	1.50	1.50	1.50
Vitamin dan mineral mix	3.00	3.00	3.00	3.00
Phytase	0,03	0,03	0,03	0,03
CMC	0,30	0,30	0,30	0,30
Total	100.00	100.00	100.00	100.00
Proximate analysis (%)				
Protein	32.63	32.42	32.56	32,34
Lipid	7.30	7.61	7.94	8.21
Crude fiber	4.24	4.73	5.15	5.64
Ash	11.54	12.03	12.54	13.03
NFE	40.96	39.88	38.52	37.45
GE (kcal/100 g)	422.47	419.78	421.65	415.06
C/P ratio	12.91	12.91	12.79	12.79

Note: ILM= *Indigofera* leaf meal; CMC= Carboximethyl cellulase; NFE= Nitrogen free extract; GE= Gross energy; C/P Ratio= Caloric part protein ratio

The most declined crude fiber value was resulted in the 1.2 g/kg cellulase enzyme doze, followed by The declined neutral detergent fiber and hemicellulose value at 16.49% in the same enzyme doze. Meanwhile, the acid detergent fiber (ADF) value on 0.8 g/kg and 1.2 g/kg doze decreased significantly (P<0.05). The second study step results (Table 4) showed that the total, ingredient, protein, lipid, and energy digestibility on 0.8 g/kg and 1.2 g/kg doze had the best values to incline digestibility in gourami fingerlings.

The production performance of gourami fingerlings on third study step result was significantly different (P<0.05) among treatments (Table 5). The utilization of cellulase enzyme hydrolyzed ILM on feed formulation more than 15% (30% and 45%) had protein efficiency ratio (PER), protein retention (PR), specific growth rate (SGR), and protease enzyme activity value lower than control treatment with 100% plant protein source of soybean meal (P<0.05, Table 5).

Protease enzyme activity on control treatment and 15% doze also showed a higher result than 30% and 45% doze (P<0.05; Table 5). Increased ILM addition in feed (30% and 45%) made the protease enzyme tended to decrease.

Discussion

The most declined crude fiber value was resulted in the 1.2 g/kg cellulase enzyme doze. Reasonably, it was caused by the cellulase enzyme that hydrolyze the complex components, such as cellulose and hemicellulose, into simply digestible glucoses. Cellulase enzyme is composed of endo-β-1.4-gluconase, exo-β-1.4-gluconase, and β-1.4-glucosidase enzyme (Crueger & Crueger, 1984). Endo-1.4-β-D-glucanase enzyme has a function to degrade cellulose polymer randomly on internal bond of α-1.4-glycosides into oligodextrines with various chain lengths, as well as exo-β-1.4-gluconase and β-1.4-glucosidase enzyme that degrade cellulose from the reductor and non-reductor to produce cellobiose or glucose (Co *et al.*, 2021).

Acid detergent fiber (ADF) value on 0.8 g/kg and 1.2 g/kg doze decreased significantly (P<0.05). This condition was caused by the cellulose degradation process (ADF process), so that the higher cellulose was degraded by cellulase enzyme, the ADF value would be more declined (Kumar *et al.*, 2019). The same result was also shown by Melati and Sunarno (2016), as the ADF value on cassava skin added with

Table 3. Proximate analysis of cellulase hydrolyzed *I. zolingeriana*.

Proximate analysis	Cellulase enzyme doze			
	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Protein (%)	30.35 ± 0.38 ^a	30.91 ± 1.97 ^a	31.12 ± 0.19 ^a	30.25 ± 1.60 ^a
Lipid (%)	4.57 ± 0.50 ^a	4.79 ± 0.43 ^a	4.84 ± 0.30 ^a	5.04 ± 0.40 ^a
Ash (%)	9.12 ± 0.15 ^a	9.28 ± 0.26 ^a	9.19 ± 0.08 ^a	9.21 ± 0.35 ^a
Crude fiber (%)	17.03 ± 0.11 ^d	15.30 ± 0.50 ^c	10.90 ± 0.37 ^b	9.65 ± 0.32 ^a
NFE (%)	38.93 ± 0.75 ^b	39.72 ± 1.64 ^b	43.95 ± 0.59 ^a	45.85 ± 2.21 ^a
NDF (%)	36.38 ± 0.10 ^d	33.57 ± 0.14 ^c	31.67 ± 0.14 ^b	30.38 ± 0.34 ^a
ADF (%)	26.19 ± 0.18 ^c	25.47 ± 0.09 ^b	24.93 ± 0.11 ^a	24.88 ± 0.11 ^a
Hemicellulose (%)	10.19 ± 0.25 ^d	8.11 ± 0.14 ^c	6.74 ± 0.12 ^b	5.50 ± 0.32 ^a

Note: NFE= Nitrogen free extract; NDF= Neutral detergent fiber; ADF= Acid detergent fiber

Table 4. Total, ingredient, protein, lipid, and energy digestibility of *Indigofera* leaf meal on gourami fingerling feed

Parameter (%)	Cellulase enzyme treatment doze			
	0 g/kg	0.4 g/kg	0.8 g/kg	1.2 g/kg
Total digestibility	71.64 ± 0.81 ^b	72.15 ± 0.50 ^b	73.50 ± 0.33 ^a	73.82 ± 0.75 ^a
Ingredient digestibility	61.75 ± 2.61 ^b	65.57 ± 1.60 ^b	69.02 ± 1.08 ^a	70.82 ± 2.44 ^a
Protein digestibility	85.58 ± 0.67 ^b	86.04 ± 0.42 ^b	87.24 ± 0.61 ^a	87.26 ± 0.46 ^a
Lipid digestibility	76.25 ± 0.78 ^b	76.37 ± 1.21 ^b	78.31 ± 0.61 ^a	78.87 ± 0.95 ^a
Energy digestibility	78.91 ± 0.50 ^b	78.90 ± 0.52 ^b	79.89 ± 0.27 ^a	80.24 ± 0.53 ^a

Note: Different letters on the same line show a significant difference (P<0.05). Values are presented in average ± standard deviation.

cellulase enzyme could decline until 1.65%. The neutral detergent fiber and hemicellulose value on 1.2 g/kg doze declined significantly until 16.49%. Significantly, declined NDF value was suspected due to the reaction between cellulase enzyme and hemicellulose contained in ILM. According to Jamarun *et al.* (2017), NDF is composed of ADF and hemicellulose which has smaller molecules than cellulose, therefore easily degraded by cellulase enzyme.

The second study step results (Table 4) showed that the total, ingredient, protein, lipid, and energy digestibility on 0.8 g/kg and 1.2 g/kg doze had the best values to incline digestibility in gourami fingerlings. This condition was caused as crude fiber content on feed ingredient declined, therefore inclining the digestibility value of gourami fingerlings. The digestibility of the feed is determined by various factors, e.g. feed chemical composition and feed preparation (Mayulu, 2014). Supporting Mayulu (2014), Suprayudi *et al.* (2011), digestibility value is influenced by some factors as one of which are crude fiber content. The higher crude fiber value, the lower feed digestibility value. Digestibility value has a positive correlation against fish growth. The higher digestibility value of fish, the better feed digested by fish as energy source and growth (Bokau *et al.*, 2018; Hassaan *et al.*, 2019; Azaza *et al.*, 2020). Based on the results (Table 4), the digestibility value on each ILM treatment in a commonly normal fish range is 75–95% for protein digestibility and 70–85% for energy digestibility (NRC, 1993). Vinasyam *et al.* (2016) identified tilapia treated with rELGH performed

a higher protein digestion, and retention in the body. It contributed in a lower ammonia excretion rate that enhanced growth of the fish.

The production performance of gourami fingerlings on third study step result was significantly different ($P < 0.05$) among treatments (Table 5). The utilization of cellulase enzyme hydrolyzed ILM on feed formulation more than 15% (30% and 45%) had protein efficiency ratio (PER), protein retention (PR), specific growth rate (SGR), and protease enzyme activity value lower than control treatment with 100% plant protein source of soybean meal ($P < 0.05$, Table 5). Mukti *et al.* (2019) stated that the best growth rate was recorded in 20% of ILM. In contrast, higher content of ILM (30%) tended to reduce the growth rate. Antinutritional compound was suspected to decrease the growth rate (Samtiya *et al.*, 2020). Phytic acid and trypsin inhibitor, common antinutritional compounds, can adversely affect the feed taste, intake, and nutrient absorption, resulting in a diminishing in fish growth (Burr *et al.*, 2012; Haghbayan & Mehrgan, 2015). Higher concentration of tannin and saponin in the higher ILM content presumably diminished the digestibility and nutrient absorption. This condition was also suspected due to ILM amino acid content in the form of arginine had 1.67% lower than soybean meal with 3.48%, therefore suspected to cause amino acid deficiency that impacted the gourami fish fingerling growth. However, the production performance value on 15% ILM feed doze was similar to control feed ($P < 0.05$). This indicates that the utilization of 15% ILM can be utilized well by fish, therefore

Table 5. Production performance of gourami fingerlings on growth test added with ILM on a certain doze in feed.

Parameter	ILM hydrolysis utilization in feed			
	0%	15%	30%	45%
FI (g/fish)	46.68 ± 2.44 ^a	47.49 ± 0.94 ^a	44.44 ± 0.59 ^b	44.44 ± 1.46 ^b
PER	2.03 ± 0.07 ^a	2.03 ± 0.05 ^a	1.63 ± 0.05 ^b	1.41 ± 0.07 ^c
PR (%)	38.16 ± 0.96 ^a	38.04 ± 1.10 ^a	29.50 ± 0.98 ^b	24.50 ± 1.54 ^c
SGR (%)	3.05 ± 0.10 ^a	3.10 ± 0.07 ^a	2.73 ± 0.08 ^b	2.53 ± 0.11 ^c
HSI	1.70 ± 0.11 ^a	1.70 ± 0.01 ^a	1.77 ± 0.08 ^a	1.73 ± 0.09 ^a
FE	66.25 ± 2.16 ^a	65.51 ± 1.60 ^a	53.31 ± 1.49 ^b	45.58 ± 2.24 ^c
SR (%)	100	100	100	100
Protease (unit/mL/min.)	0.0990 ± 0.0078 ^a	0.0917 ± 0.0030 ^a	0.0727 ± 0.0126 ^b	0.0682 ± 0.0070 ^b
Lipase (unit/mL/min.)	0.0105 ± 0.0014 ^a	0.0100 ± 0.0017 ^a	0.0105 ± 0.0014 ^a	0.0100 ± 0.0017 ^a
Amylase (unit/mL/min.)	0.4427 ± 0.0294 ^c	0.4874 ± 0.0238 ^b	0.5233 ± 0.0158 ^a	0.5508 ± 0.0024 ^a

Note: FI= Feed intake; PER= Protein efficiency ratio; PR= Protein retention; SGR= Specific growth rate; HSI= Hepatosomatic index; FE= Feed efficiency; SR= Survival rate

feed nutrient can be preserved efficiently for fish growth performance.

Protease enzyme activity on control treatment and 15% doze also showed a higher result than 30% and 45% doze ($P < 0.05$; Table 5). According to Santos *et al.* (2020), declined protease enzyme activity is caused by some factors, such as protein ingredient source and high value of glucose/monosaccharides in feed. Therefore, the amino acid transport in digestive tract is inhibited due to this condition. Increased ILM addition in feed (30% and 45%) made the protease enzyme tended to decrease. This was suspected because glucose content on 30% and 45% ILM feed doze increased. A similar result was found in Li *et al.* (2020) as 16% glucose diet decreased the protease enzyme activity in *Rhynchocypris lagowskii* Dybowski.

CONCLUSION

The utilization of hydrolyzed *I. zollingeriana* leaf meal by cellulase enzyme with 1.2 g/kg doze as much as 15% in feed composition can produce an optimum gourami fingerling growth performance.

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