The Efficiency of Indigofera Leaves Meal Hydrolysate Utilization on Growth Performance of Leptobarbus hoevenii

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Research Article

The Efficiency of Indigofera Leaves Meal Hydrolysate Utilization on Growth Performance of *Leptobarbus hoevenii*

Rakhmawati Rakhmawati[1*], Anjar Sofiana[2], Nur Indariyanti[3], Agung Kurniawan[3]

[1] Departement of Aquaculture, Politeknik Negeri Lampung, Indonesia
[2] Departement of Poultry Production Technology, Politeknik Negeri Lampung, Indonesia
[3] Departement of Fish Hatchery Technology, Politeknik Negeri Lampung, Indonesia

** Corresponding author: rakhmawati@polinela.ac.id*

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ABSTRACT

This study was conducted to evaluate the efficacy of indigofera leaves meal hydrolysate (ILMH) on growth *Leptobarbus hoevenii* growth. This study employed five dietary treatments with varying levels of ILMH. Cellulase enzymes were used to hydrolyze Indigofera leaf meal, which was then combined with other feed ingredients. Three replicates of feed without ILMH, 10% ILMH, 20% ILMH, 30% ILMH, and 35% ILMH were included in the treatment-examination feed. Average initial fish weight was 1.27±0.01 g, and initial length was 4.28±0.07 cm. Weight observation was also conducted every two weeks. After six weeks of rearing, survival, final biomass, specific growth rate, and feed efficiency were observed. At the beginning and the end of maintenance, temperature, pH, and dissolved oxygen measurements were taken to determine the water's quality. Results that that the utilization of ILMH in *L. hoevenii* feed could substitute the use of soybean meal for 10-35% of the feed. Among all treatments, 10% ILMH-containing feed resulted in the highest growth and feed efficiency. This treatment had a 100% survival rate, a 1.78±0.05% specific growth rate, and a 53.28 ± 1.59% feed efficiency. According to the findings of this study, the utilization of 10% hydrolysate of indigofera leaf meal in feed was effective in enhancing *L. hoevenii's* growth performance.

Keywords: Efficiency, indigofera leaves, *Leptobarbus hoevenii*, hydrolysate, growth.

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi efektifitas pemanfaatan hidrolisat tepung daun indigofera terhadap ikan jelawat (*Leptobarbus hoevenii*). Penelitian ini menggunakan lima jenis pakan uji dengan kandungan hidrolisat tepung daun indigofera (HTDI) yang berbeda. Tepung daun indigofera dihidrolisis menggunakan enzim selulase dan dicampurkan dengan bahan pakan lainnya. Pakan uji perlakuan meliputi pakan tanpa HTDI, pakan mengandung 10% HDTI, pakan mengandung 20% HDTI, pakan mengandung 30% HDTI dan pakan mengandung 35% HDTI dengan tiga kali ulangan. Berat rata-rata ikan awal 1,27±0,01 g dan panjang rata-rata ikan awal 4,28±0,07 cm. Pengamatan bobot ikan setiap dua minggu sekalian dilakukan. Setelah 6 minggu pemeliharaan, dilakukan pengamatan kelangsungan hidup, biomassa akhir, laju pertumbuhan spesifik, dan efisiensi pakan. Pengamatan kualitas air selama pemeliharaan dilakukan pada awal dan akhir perlakuan meliputi suhu, pH, dan oksigen terlarut. Hasil peneltian menunjukkan bahwa pemanfaatan HDTI dalam pakan *L. hoevenii* dapat mensubstitusi penggunaan tepung bungkil kedelai dengan kisaran 10-35% pakan. Pakan mengandung 10% HDTI menghasilkan pertumbuhan dan efisiensi pakan paling tinggi dibandingkan semua perlakuan. Kelangsungan hidup perlakuan ini mencapai 100%, laju pertumbuhan harian 1,78±0,05% dan efisiensi pakan 53,28±1,59%. Kesimpulan penelitian ini adalah pemanfaatan hidrolisat tepung daun indigofera pada pakan sebanyak 10% efektif meningkatkan kinerja pertumbuhan pada *L. hoevenii*.

Kata kunci: Efektifitas, daun indigofera, *Leptobarbus hoevenii*, hidrolisat, pertumbuhan

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1. Introduction

The issue of fish feed in Indonesia is presently dominated by import dependence on feed raw materials, particularly fish meal and soybean meal. Protein content is a significant factor in the growth and selling price of fish feed (Subandiyono & Hastuti*.,* 2020); consequently, the use of these two categories of raw materials is vital. Feed accounts for 60-80% of the total cost of production in fish or shrimp aquaculture. Along with the depreciation of the rupiah in recent years and the increase in the price of soybean meal, production costs have risen. In addition, it is difficult to increase the price of fish because the COVID 19 pandemic that has been ongoing since the beginning of 2020 continues to have a negative impact on the purchasing power of the general population. Thus, the profits of farmers are getting smaller (Sari et al., 2020).

Alternative raw materials with comparable protein content provide a solution to the dependence on soybean meal flour. *Indigofera zollingeriana* leaves are presently a viable alternative raw material option. These leaves contain 28-30% protein, which is comparable to the protein content of soybeans and is simple to locate or cultivate (Putri et al., 2019). According to Abdullah & Suharlina (2010), the protein of the leaves is higher than that of the stems. Since 2019, the development of this protein source has been encouraged in the province of Lampung, especially in the Pringsewu and Tulang Bawang areas (Lampost, 2019). Thus, indigofera leaves are readily available, inexpensive, and rich in protein. This can be optimized for fish feed use.

The utilization of indigofera leaves in livestock feed (Palupi et al., 2014) as well as in fish feed has been documented. Catfish, carp, and tilapia have utilized fish feed in the past. Nonetheless, the proportion remains low (Tampubolon, 2017; Putri et al., 2019; Mukti et al., 2019; Jefry, 2020). The issue with fish when using plant-based protein sources, especially forage, is that it contains high crude fiber (Tarigan et al., 2018). This issue must be resolved by the employinh technologies that reduce the amount of crude fiber. The hydrolysis of cellulose enzymes in cultivated fish will be one of the methods utilized in this research. This method is considered the most effective for degrading crude fiber into sugar (Setyoko & Utami, 2016). According to Rakhmawati et al. (2022), indigofera leaves meal hydrolyzed with cellulase enzymes contained less crude fiber. It is anticipated that the application of this technology to the use of Indigofera zollingeriana leaves in fish feed will replace or substitute the use of soybean meal, which is more expensive and dependent on imports.

Leptobarbus hoevenii is a species of fish native in Sumatra, and in the province of Lampung, it is one of the most superior products being developed. This fish is still uncommon on the market, but it is frequently served at family gatherings and traditional events, making it a highly prospective product with a high economic value. This fish is an omnivore, allowing it to utilize more plant-based raw materials. There has never been an investigation into the use of indigofera leaves meal in this fish. To increase aquaculture productivity and feed efficiency, it is necessary to conduct additional research on the use of hydrolyzed Indigofera leaves meal with cellulose enzymes in *Leptobarbus hoevenii*. The objective of this study was to assess the effect of utilizing Indigofera leaves meal hydrolysate in feed composition as a source of plant protein on *L. hoevenii* productivity.

2. Material and methods

2.1. Experimental diets

Table 1 presents the ingredients and composition of the experimental diets. This study utilized five kinds of test diets containing Indigofera leaves meal at varying concentrations. The experimental feed contained indigofera leaves meal hydrolysate at a dose of 0 (control, without indigofera leaves meal); 10%; 20%; 30%, and 35%. Using a commercial cellulase enzyme (Viscozyme Cassava CL), the indigofera leaves meal were hydrolyzed. The indigofera leaves mixture is treated with 10 g/kg of enzymes and 30 % water, then incubated for seven days at room temperature (Rakhmawati et al., 2022).

Following the weighing and uniform mixing of all basic materials, oil and water are added. The feed was molded with a 1 mm diameter, dried in a tumble dryer, and stored until use in plastic containers.

2.2. Experimental fish and rearing activity

Three hundred juveniles *L. hoevenii* (4.28±0.07 g) were obtained from the Center for Freshwater Aquaculture in Jambi Province, Indonesia. Prior to the study, *L. hoevenii* was reared for one week and fed commercial feed (30% protein) to acclimate the fish to the conditions of the experiment. The test fish were raised for a period of six weeks. At the beginning of the study, individual fish were weighed and distributed randomly into 15 aquariums (60 x 80 x 90 cm) at a density of 20 fish per aquarium.

Table 1. Dietary composition Ingredients.

Ingredients	Indigofera leaves meal hydrolysate (ILMH) utilization (%)				
	0%	10%	20%	30%	35%
Fish Meal	18	18	18	18	18
Soybean Meal	35	25	15	5	0
ILMH[1]	0	10	20	30	35
Corn Meal	35	35	35	35	35
Fish Oil	2.5	2.5	2.5	2.5	2.5
Corn Oil	2.5	2.5	2.5	2.5	2.5
CMC[2]	2	2	2	2	2
Vitamin and mineral	5	5	5	5	5

[1] Indigofera leaves meal hydrolisate
[2] Carboxymethyl cellulose

Feeding was performed twice daily (9:00 and 17:00) with a 5% feeding rate. There was continuous aeration and 25% water change every 24 hours. At 16.00 every day, fish feces were siphoned from the aquarium. All experimental media included temperature, dissolved oxygen, total ammonia nitrogen, and pH as water quality parameters. These parameters measured based on the APHA method, 1995. The instruments used were a thermometer, DO meter, spectrophotometer and pH meter, respectively. Temperature and dissolved oxygen were recorded every day, while pH and TAN were measured at beginning and the end of rearing. During the maintenance, the temperature was between 27.6 and 29.5ºC, the pH was between 7.63 and 8.1, and the dissolved oxygen was between 6.5 and 7.7 mg/L.

2.3. Evaluation of Leptobarbus hoevenii's Growth

Every two weeks, weight of each individual growth was measured. After six weeks of rearing, observations were made of survival rate (SR), weigth gain (WG), feed consumption (FC), specific growth rate (SGR), and feed efficiency (FE). Where calculated as follow SR (%) = (No. of fish survived/ No. of fish released) × 100, WG (g/fish) = Final mean body weight − initial mean body weight (Hassan et al. 2021), FC (g) was calculated on daily basis as the total amount of feed per aquarium divided by the number of fish in the aquarium (Ponzoni et al., 2013), SGR (% body weight/day) = [(In Final mean body weight − Initial mean body weight)/No of days] X 100 (Biswas et al., 2011), FE (%) = [(Weight of fish biomass at the end of rearing – Weight of biomass at the beginning of rearing)/feed consumption during rearing period (g)] x 100 (Watanabe, 1988).

2.4. Statistical analysis

All data displayed as figures are the mean ± standard deviation of three replicates. Using IBM SPSS Statistics 22, one-way ANOVA and Tukey's test were used to analyze the data. At $P < 0.05$, the difference was deemed to be statistically significant. The differences in letters in the upper right corner of the results graph indicates a statistically significant difference.

3. Results

3.1. Survival rate

Figure 1 demonstrates that the survival rate of *L. hoevenii* that was maintained for six weeks was deemed satisfactory, as there was no mortality with a survival rate of 100%.

3.2. Average weight growth of juvenile *L. hoevenii*

As shown in Figure 2, the average weight gain of *L. hoevenii* reared for six weeks indicates that the use of ILMH resulted in a greater average weight gain at all concentrations than the control.

3.3. Increased biomass and feed consumption

Among all treatments, *L. hoevenii* fed 10% gained the most weight during maintenance when compared to other treatments. While the use of ILMH at concentrations of 20, 30 and 35% resulted in greater growth than the control, the growth of *L. hoevenii* was greater. The greater the dose of indigofera leaves utilized, the lower the growth of fish. On the other hand, feed consumption between treatments showed insignificant results (Figure 3).

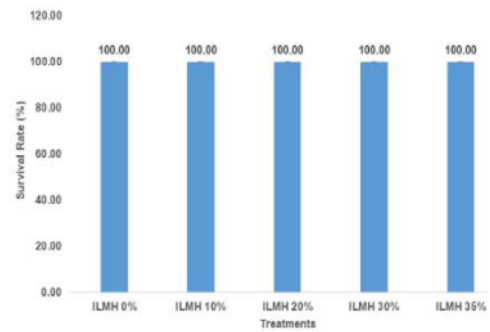

Figure 1. Survival Rate (SR) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote SR.

3.4. Spesific growth rate dan feed efficiency

The specific growth rate of *L.hoevenii* reared for six weeks revealed that 10% ILMH produced the highest SGR, followed by 20%, 30% and 35% dosages. The control treatment has the greatest value for the feed conversion ratio parameter. Furthermore, the feed efficiency value between treatments with 10% ILHM was the highest, followed by treatments with 20%, 30%, and 35% ILHM (Figure 4).

4. Discussions

The results indicated that the use of ILMH in feed affected the growth of *Leptobarbus hoevenii*. Figure 2 demonstrates that the use of ILMH on *L. hoevenii* feed at concentrations of 10, 20, 30%, and 35% resulted in greater growth than feed without ILMH. The use of hydrolyzed indigofera leaves meal with cellulase enzymes in feed increased growth by up to 40%, consistent with previous research (Jefry et al., 2021). In the same fish, Shulikin et al., (2021) reported that the best growth was observed in the same fish when 20% soybean meal and 20% fish meal were replaced with indigofera leaves meal. Mukti et al. (2019) reported that the utilization of indigofera leaves meal as 20% of the ingredients in the composition of catfish feed produced the greatest growth. Tilapia demonstrated the best growth in the treatment using indigofera leaves meal as much as 30% (Putri et al., 2019). Similarly, 20% indigofera leaves meal provides the best color quality for Sumatran ornamental fish (Pratama et al., 2019).

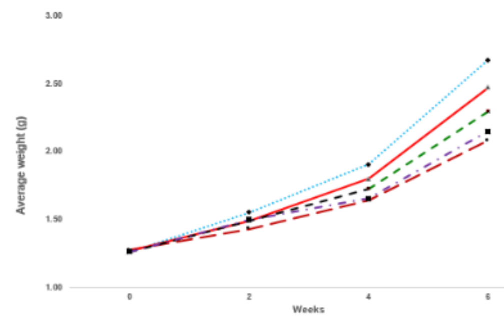

Figure 2. Average weight growth (g) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) treatments for 6 weeks. ( ) denote ILMH 0%, ( ) ILMH 10%, ( ) ILMH 20%, ( ) ILMH 30% and ( ) ILMH 35%.

In this study, the enzyme cellulose was used to hydrolyze indigofera leaves meal. Enzymes are proteins composed of living cells that catalyze biochemical reactions (Saha & Pathak, 2021). This method more effective than other methods at hydrolyzing crude fiber (Setyoko & Utami, 2016).

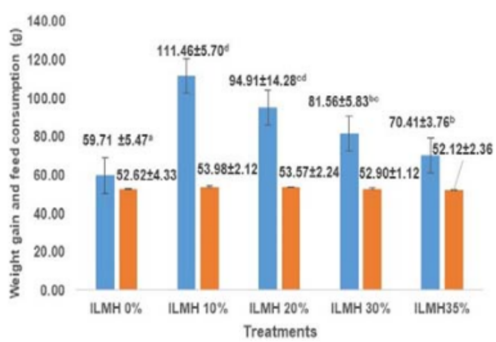

Figure 3. Weight gain of biomass (WG) and feed consumption (FC) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote WG and (■) FC.

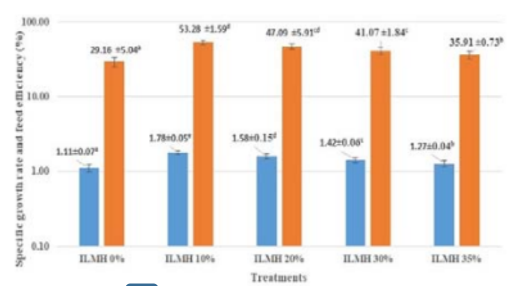

Figure 4. Specific growth rate (SGR) and feed efficiency (FE) of *L. hoevenii* reared using indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote SGR and (■) FE.

This hydrolysis process results in the formation of cellobiose and glucose, two simpler sugars (Teeri 1997; Horn et al., 2012). The results demonstrated that the crude fiber content of the feed containing ILMH was as much as 35% less than the control. The use of ILMH in feed decreased the crude fiber content to 46.2% and increased the carbohydrate content (Rakhmawati et al., 2022). Carbohydrates are a readily utilized energy source by *L. hoevenii*. Jefry et al. (2021) found that a decrease in crude fiber by 43.3% in indigofera leaves meal hydrolyzed with cellulase enzymes increased total digestibility, protein digestibility, and lipid digestibility in gourami larvae fed ILHM-containing feed.

The results also showed that growth increased as the percentage of ILMH increased to 35%, despite the decrease in protein content of the feed. The higher growth rate of the fish contained more non-protein energy, as measured by the C:P ratio of 16.46 – 18.73 kcal/g, compared to the C:P ratio of 15.73 kcal/g for the control. A balanced ratio of protein and non-protein energy in feed can increase the efficiency of protein metabolism as an energy source and its utilization for fish growth (Sankian et al., 2017; Li et al., 2013; Kim *et al*., 2017). The optimal ratio of protein to energy, according to Jauralde et al. (2021), describes the point of equilibrium between the amount of energy required for maintenance and growth. If an appropriate C:P ratio is obtained, it is possible to reduce the protein level in the feed without impairing fish growth and enhancing the growth response (Carneiro *et al*., 2020 & Aboseif et al., 2022).

In this study, feed consumption did not differ significantly between treatments (Figure 1), but *L. hoevenii* containing ILMH grew by up to 35%. The non-protein energy content contained in the feed, notably carbohydrates, was higher than the others. The percentage of ILMH is increasing, while soybean meal is decreasing (Table 1). Thus, in this treatment, fish were better able to utilize non-protein energy for their energy requirements, allowing protein to be effectively stored for growth, resulting in increased feed efficiency (Figure 3) and decreased FCR (Figure 4). According to Mohseni et al. (2013), protein and energy balance can increase growth, feed efficiency, and protein utilization.

5. Conclusions

Hydrolyzed indigofera leaf diet increases the growth performance of juvenile *Leptobarbus hoevenii*. Utilization of ILMH on *L. hoevenii* up to 35% in feed resulted in improved growth. Utilizing 10% ILMH the the diet of *L. Hoevenii* maximizes growth and feed efficiency.

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