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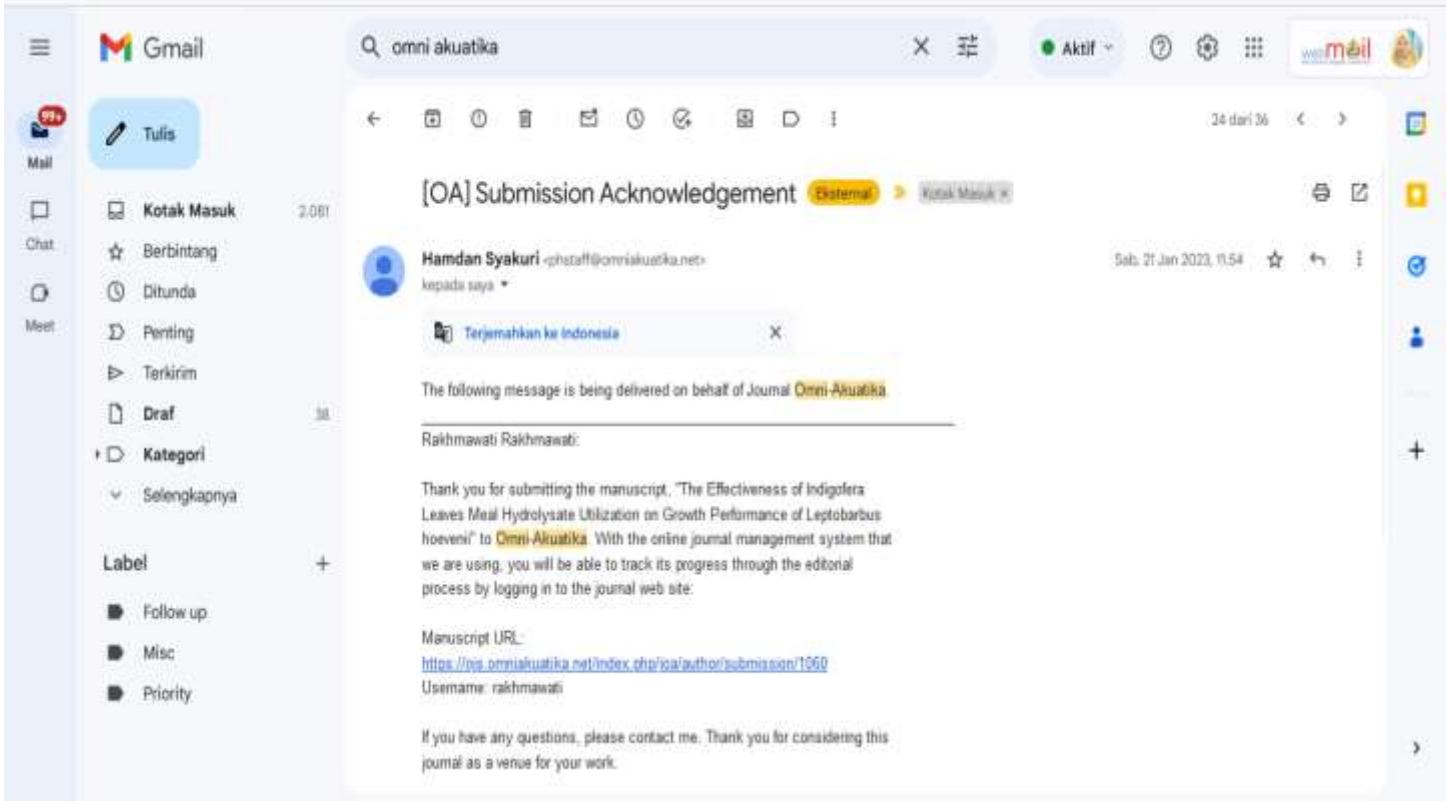

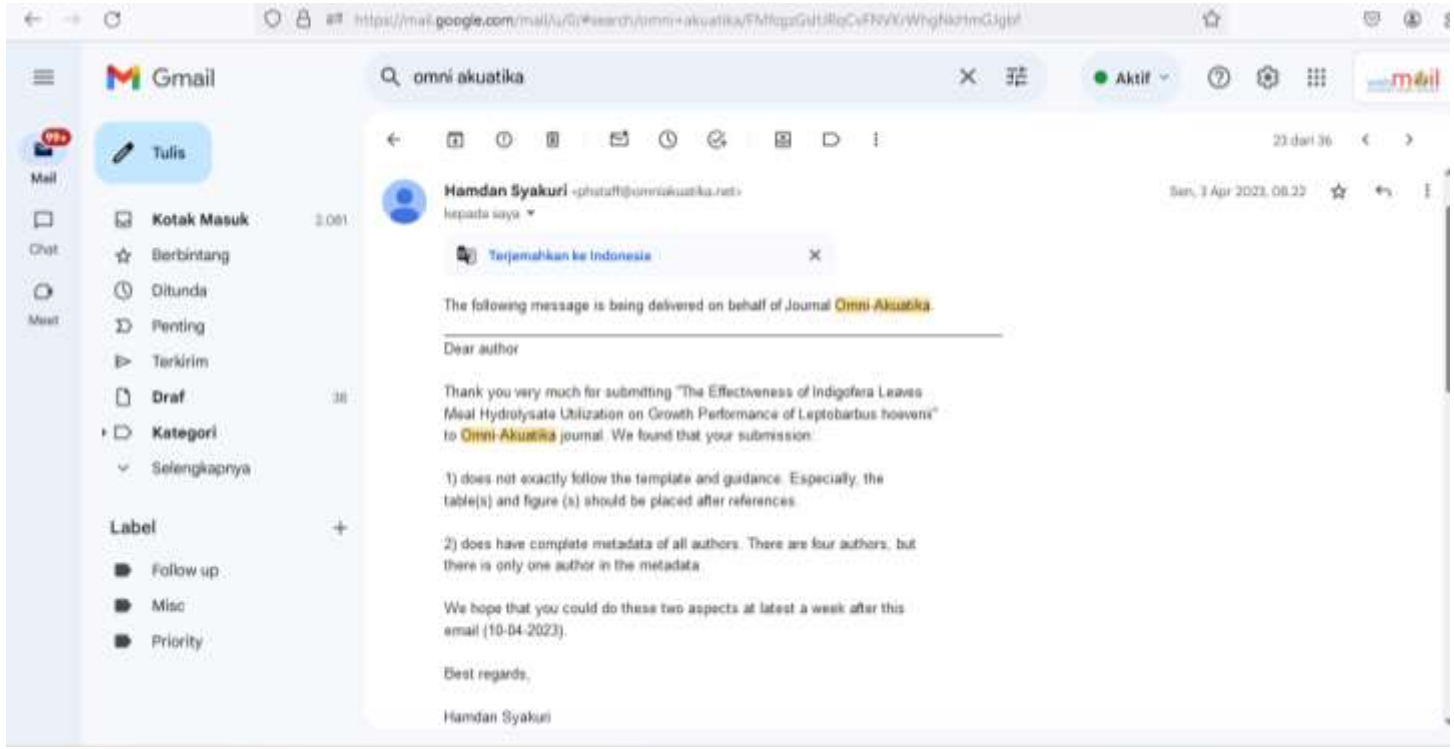
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We hope that you could do these two aspects at latest a week after this email (10-04-2023).
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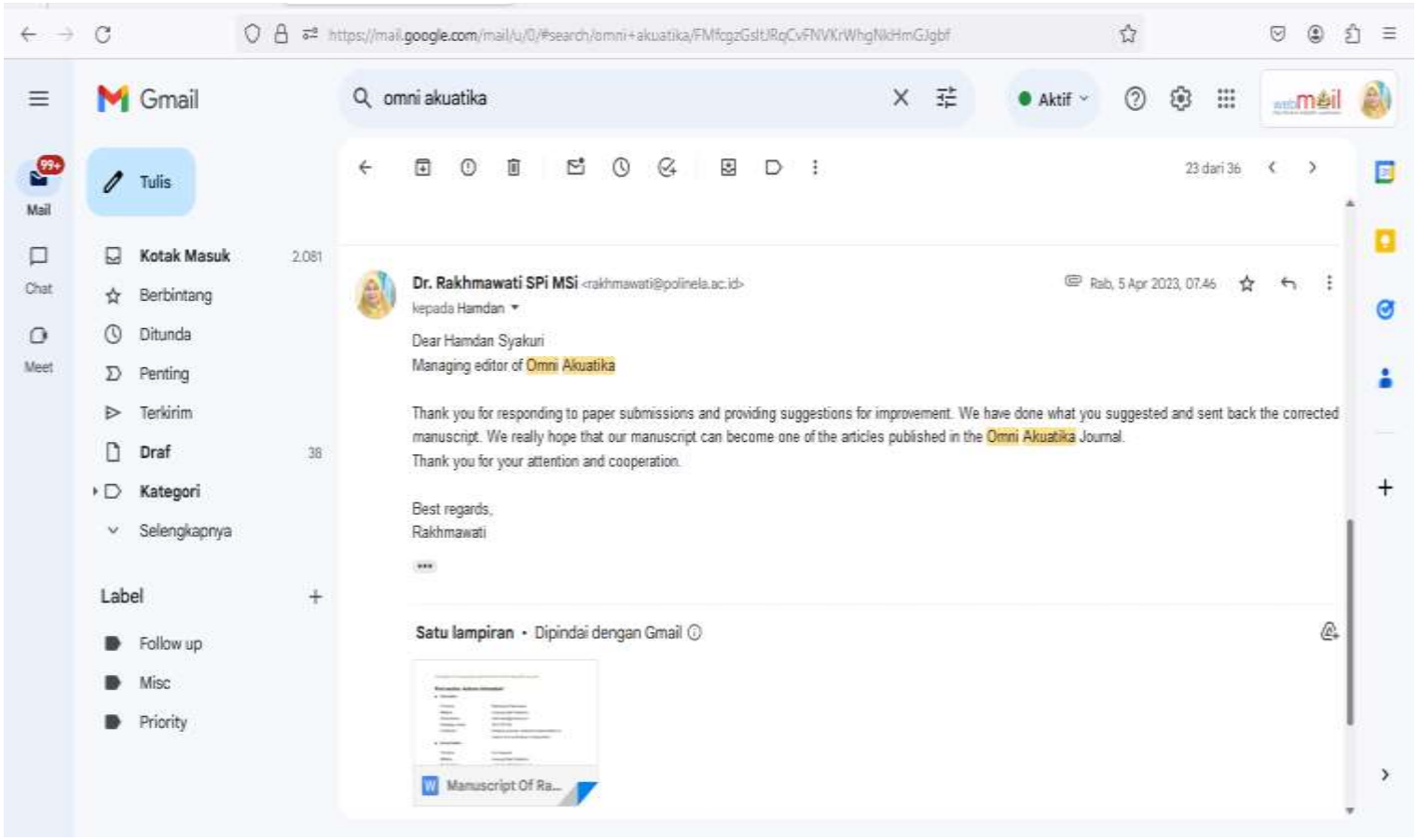
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kepada Hamdan
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Dear Hamdan Syakuri
Managing editor of Omni Akuatika
Thank you for responding to paper submissions and providing suggestions for improvement. We have done what you suggested and sent back the corrected manuscript. We really hope that our manuscript can become one of the articles published in the Omni Akuatika Journal.
Thank you for your attention and cooperation.
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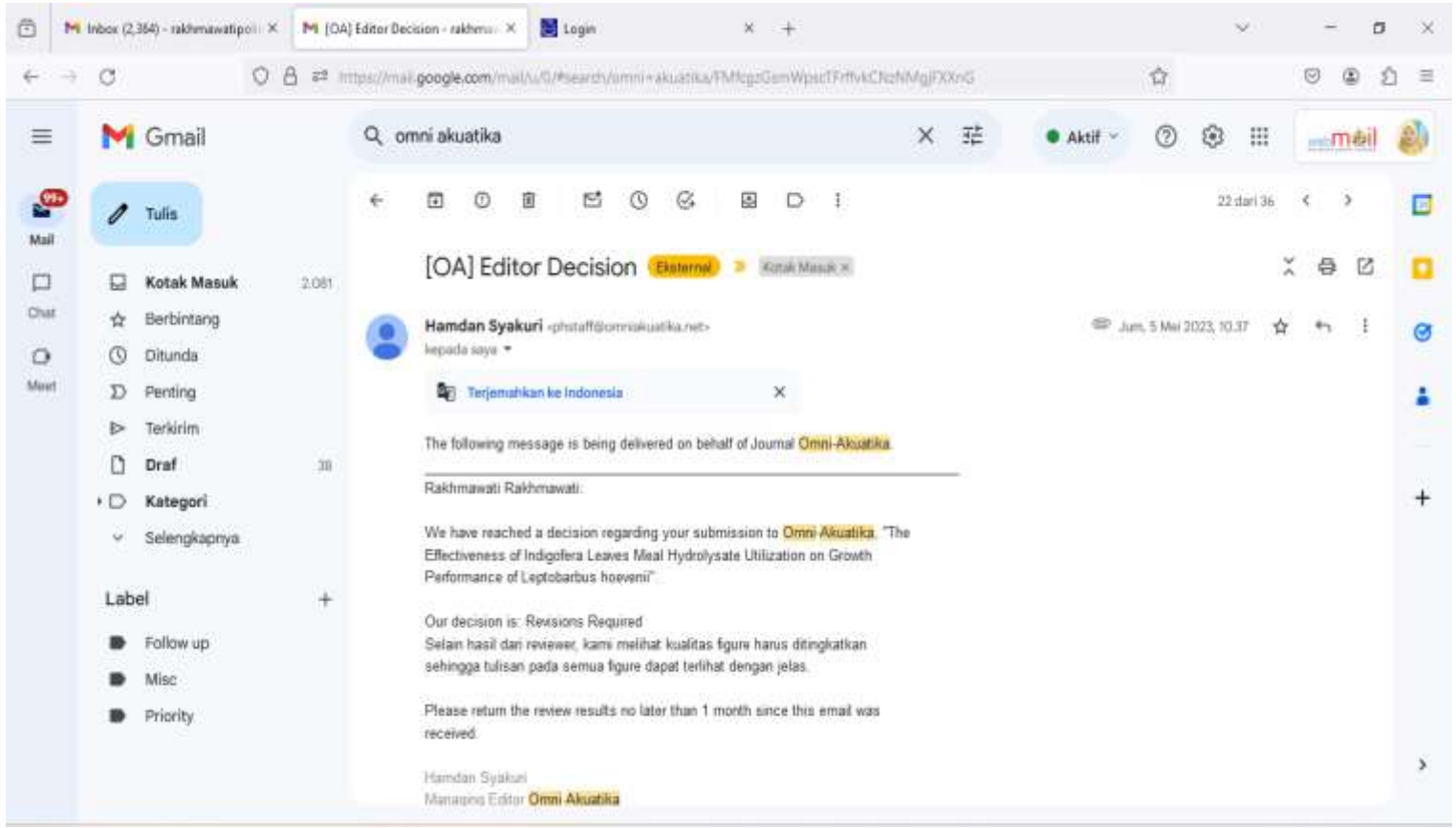
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We have reached a decision regarding your submission to Omni Akuatika, "The Effectiveness of Indigofera Leaves Meal Hydrolysate Utilization on Growth Performance of Leptobarbus hoevenii".
Our decision is: Revisions Required
Selain hasil dari reviewer, kami melihat kualitas figure harus ditingkatkan sehingga tulisan pada semua figure dapat terlihat dengan jelas.
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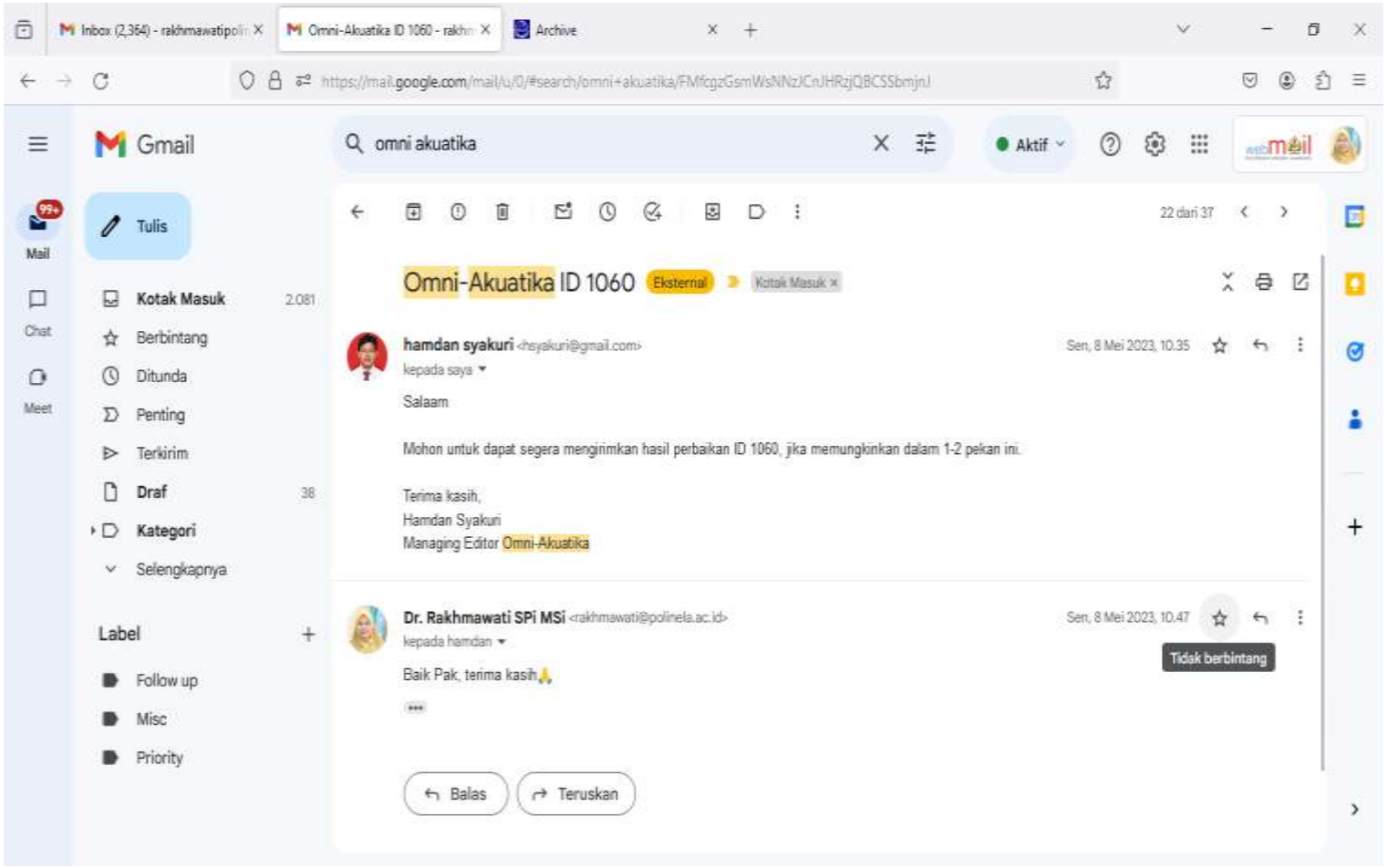
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The Effectiveness of Indigofera Leaves Meal Hydrolysate Utilization on Growth Performance of *Leptobarbus hoevenii*

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Abstract

This study was conducted to evaluate the effectiveness of indigofera leaves meal hydrolysate (ILMH) on the growth performance of *Leptobarbus hoevenii*. This study used five dietary treatments with different levels of ILMH. Indigofera leaves meal was hydrolyzed using cellulase enzymes and mixed with other feed ingredients. The treatment examination feed included feed without ILMH, feed containing 10%, 20%, 30% and 35% ILMH with three replications. Average of initial weight 1.27±0.01 gr and initial length 4.28±0.07 cm. Length and weight observations were also conducted every two weeks. After six weeks of rearing, observations of survival, final biomass, specific growth rate, feed efficiency and feed conversion ratio were conducted. Observations of water quality during maintenance were carried out at the beginning and end of treatment including temperature, pH, and dissolved oxygen. The results showed that the utilization of ILMH in *L. hoevenii* feed could substitute the use of soybean meal in the range of 10-35% of the feed. Feed containing 10% ILMH resulted in the highest growth and feed efficiency compared to all treatments. This study concluded that the utilization of hydrolysate of indigofera leaves meal in feed was effective in increasing growth performance in *L. hoevenii.*

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Keywords: Effectiveness, indigofera leaves, *Leptobarbus hoevenii*, hydrolysate, growth

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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 awal 1.27±0.01

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gr dan panjang rata-rata awal 4.28±0.07 cm. Pengamatan panjang dan bobot setiap dua minggu sekalian dilakukan. Setelah 6 minggu pemeliharaan, dilakukan pengamatan kelangsungan hidup, biomassa akhir, laju pertumbuhan spesifik, efisiensi pakan dan feed convertion ratio. Pengamatan kualitas air selama pemeliharaan dilakukan pada awal dan akhir perlakuan meliputi suhu, pH, dan oksigen terlarut. Hasil peneltiian menunjukkan bahwa pemanfaatan HDTI dalam pakan ikan jelawat 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. Kesimpulan penelitian ini adalah pemanfaatan hidrolisat tepung daun indigofera pada pakan efektif meningkatkan kinerja pertumbuhan pada ikan jelawat.

Kata kunci: Efektifitas, daun indigofera, jelawat, hidrolisat, pertumbuhan.

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1. Introduction

The problem of fish feed in Indonesia is currently dominated by import dependence on feed raw materials, especially fish meal and soybean meal. The use of these two types of raw materials is very necessary because the protein contained in them is an important factor in the growth and selling price of fish feed (Rakhmawati et al*.,* 2022). Feed requires 60-80% of the total production cost in fish or shrimp farming. Along with the weakening of the rupiah in recent years, coupled with the higher price of soybean meal, production costs have increased. While the population is increasing, the selling price cannot be increased in line with production costs due to the COVID19 pandemic that has occurred since early 2020 (Sari et al., 2020).

The problem of dependence on imports of these two types of raw materials can be found in alternatives with comparable protein content. One alternative that currently can be chosen is *Indigofera zollingeriana* leaves. These leaves contain 28-30% protein which is close to the protein content of soybeans and is easy to find or cultivate (Putri et al., 2019), Abdullah & Suharlina (2010) stated that the protein in the leaves is higher than in the stems. The growth of this protein source has been encouraged in the province of Lampung, especially in the Pringsewu and Tulang Bawang areas since 2019 (Lampost, 2019). Therefore, this leaf can be optimized for use in the manufacture of fish feed because it is easy to obtain, relatively inexpensive and contains high enough protein.

The use of indigofera leaves have been widely used in livestock feed (Palupi et al., 2014); fish feed has also been conducted such as in catfish, carp, and tilapia, but there is still a small percentage of ingredients in feed that can be used (Tampobolun, 2014; Putri *et al*., 2019; Mukti *et al.,* 2019; Jefry, 2020). The problem with fish when using plant-based protein sources, especially forage, is that it contains high crude fiber (Tarigan et al., 2014). This obstacle must be solved by the application of certain technologies that can reduce the level of crude fiber. One of the methods that will be applied in this research is the hydrolysis of cellulose enzymes in fish to be cultured. This method is considered the most effective for degrading crude fiber into sugar (Setyoko & Utami, 2016). Rakhmawati et al. (2022) stated that indigofera leaves meal hydrolyzed with cellulase enzymes showed a decrease in crude fiber. With the application of this technology to *Indigofera zollingeriana* leaves meal, it is hoped that it can replace or substitute the use of soybean meal which is much higher in price and depends on imports.

Leptobarbus hoevenii is a typical fish in Sumatra and in Lampung Province, it is one of the superior products to be developed. This fish is still rarely found in the market, but it is very popular to be served at family events or traditional events so it is very prospective because it has high economic value. This fish is an omnivore so it can utilize more vegetable raw materials. Research on the use of indigofera leaves meal in this fish has never been done. Therefore, to increase aquaculture productivity and improve feed efficiency, it is necessary to study further the use of hydrolyzed indigofera leaves meal with cellulose enzymes in

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Leptobarbus hoevenii. The purpose of this study was to evaluate the effectiveness of utilization of indigofera leaves meal hydrolysate in feed composition as a source of plant protein on the productivity of *L. hoevenii* culture.

2. Material and methods (Arial 10, bold)

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Experimental diets

The ingredients and composition of the experimental diets are presented in Table 1. This study used five types of examination diets containing indigofera leaves meal at different doses. The experimental feed contained indigofera leaves meal hydrolysate at a dose of 0 (control, without indigofera leaves meal); 10%; 20%; 30%, and 35%. The prepared indigofera leaf meal was hydrolyzed with a commercial cellulase enzyme as much as 10g kg^{-1}. Indigofera leaves meal, added cellulase enzyme and 30% water, then incubated for 7 days at room temperature (Rakhmawati et al., 2022).

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All raw materials are weighed and mixed evenly, followed by the addition of oil and water. The feed was molded with a diameter of 1 mm, dried in a tumble dryer, and stored in plastic containers until use.

Experimental animals and rearing activity.

Three hundred *L. hoevenii* juveniles (4.28±0.07 gr) were obtained from the Center for Freshwater Aquaculture, Jambi Province, Indonesia. Prior to the study, *L. hoevenii* was reared for 1 week and given commercial feed (30% protein) for acclimatization to the research conditions. The test fish were reared for 6 weeks. At the beginning of the study, individual fish were weighed and randomly distributed into 15 aquariums (60 x 80 x 90 cm) with a density of 20 fish/aquarium. Feeding with a feeding rate of 5% twice a day (9:00 and 17:00). Continuous aeration was provided with 25% water change every 24 hours. Fish feces are siphoned from the aquarium at 16.00 every day. Water quality parameters in all experimental media include temperature, dissolved oxygen, total ammonia nitrogen, and pH. The temperature during maintenance was 27.6 – 29.5^{o}C, pH 7.63 – 8.1, and dissolved oxygen 6.5 – 7.7 mg/L.

Growth Evaluation of *Leptobarbus hoevenii*

Observations of individual length and weight growth were conducted every two weeks. After six weeks of rearing, observations of survival rate, final biomass, total feed consumption, specific growth rate, feed efficiency, and feed conversion ratio were conducted.

Statistical analysis

All data presented in the form of figures are the mean ± standard deviation of three replications. Data were analyzed by one-way ANOVA and Tukey's test using the software IBM SPSS Statistics 22. The difference was considered to be significant at $P < 0.05$. In the figure on the results, the difference in letters in the upper right position indicates a significant difference.

3. Results and Discussion (Arial 10, bold)

3.1 Results

Survival rate and feed consumption

Figure 1 shows that the survival rate of *L. hoevenii* that was maintained for six weeks was categorized as good, there was no death with a survival rate of 100%. Likewise, feed consumption during maintenance is almost the same in number, so it does not show a significant difference.

Growth of the average length and weight of juvenile *L. hoevenii*

The growth of the average length of *L. hoevenii* reared for six weeks showed that the use of ILMH resulted in higher length gain at a dose of 10 – 30% of ILMH compared to the control. Meanwhile, the average length gain at the 35% ILMH dose was the same as the control. In contrast to the average weight gain of *L. hoevenii*, the average weight gain indicates that the utilization of ILMH resulted in a higher average weight gain at all utilization doses than the control as shown in Figure 2.

Increase in Biomass and Feed Efficiency

During maintenance, the weight gained of *L. hoevenii* fed with ILHM at a dose of 10% resulted in the best weight gain and feed efficiency among all treatments. While the utilization of ILMH of 20, 30 and 35% showed better growth than the control, they followed higher growth of *L. hoevenii* respectively. Although the weight gain was higher than the control, the higher the utilization of ILMH, the lower the growth (Figure 3).

Spesific Growth Rate dan Feed Convertion Ratio

35.91 ± 1.73^{b}

The specific growth rate of *L.hoevenii* reared for six weeks showed that the utilization of ILMH with a dose of 10% resulted in the highest SGR, followed by treatment with doses of 20%, 30% and 35%. Meanwhile, the feed conversion ratio parameter shows the highest value in the control treatment. Furthermore, the FCR value between treatments with the utilization of ILMH, the lowest was 10%, 20%, 30% respectively, followed by 35% (Figure 4).

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3.2. Discussion

The results showed that the use of ILMH in feed affected the growth of *Leptobarbus hoevenii.* Utilization of ILMH on *L. hoevenii* feed, both 10, 20, 30%, and 35% showed better growth than feed without the use of ILMH. This is in line with other studies on carp that the use of hydrolyzed indigofera leaves meal with cellulase enzymes in feed, increased growth up to a dose of 40% (Jefry et al., 2021). In the same fish, Shulikin et al., (2021) rep 2.79 ± 0.10^{c} ostitution of 20% soybean meal and 20% fish meal with indigofera leaves meal showed the best growth. Mukti et al. (2019) stated that the utilization of indigofera leaves meal resulted in the best growth in the treatment of 20% of the ingredients in the composition of catfish feed. Tilapia showed the best growth in the treatment using indigofera leaves meal as much as 30% (Putri et al., 2019). Likewise, for Sumatran ornamental fish, the addition of 20% indigofera leaves meal provides the best color quality (Pratama et al., 2019).

In this study, indigofera leaves meal was hydrolyzed with the enzyme cellulose (a protein containing living cells that acts as a catalyst in biochemical reactions). This enzyme can hydrolyze crude fiber more effectively than other methods (Setyoko & Utami, 2016). The result of this hydrolysis process is a simpler sugar in the form of cellobiose and glucose (Terri, 1997; Horn et al., 2012). The results showed that the crude fiber content of the feed containing ILMH was up to 35% lower than the control. The use of ILMH in feed reduced crude fiber to 46.2% and increased the carbohydrate content (Table 1) which is used as an energy source that is easily utilized by *L. hoevenii*. The decrease in crude fiber was ~~also obtained by~~ 43.3% which caused total digestibility, protein and fat in gourami fry fed a diet containing indigofera meal which was hydrolyzed by cellulase enzymes (Jefry et al., 2021).

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The results also showed that growth increased as the percentage of ILMH increased to 35%, although the protein content of the feed decreased. The higher growth rate of the fish contained more non-protein energy, namely the C:P ratio of 16.46 – 18.73 kcal/g, while the C:P ratio of the control was 15.73 kcal/g. A balanced ratio of protein and non-protein energy in feed can increase the efficiency of protein metabolism as energy, and increase its use for fish growth (Sankian et al., 2017; Li et al., 2013; Kim *et a*l., 2017). According to Jauralde et al. (2021) that feed with the optimum ratio of protein to energy describes the balance point between the amount of energy required for maintenance and growth. If an appropriate C:P ratio has been achieved, it is possible to reduce the protein level in the feed without reducing fish growth and improving to the growth response (Carneiro *et al*., 2020 & Aboseif et al., 2022).

In this study, the feed consumption in each treatment was not significantly different (Figure 1), ~~but~~ the growth of *L. hoevenii* containing ILMH was up to 35%. The non-protein energy content contained in the feed, especially carbohydrates, was higher than the others (Table 1). Thus, in this treatment, fish were better able to utilize non-protein energy for their energy needs so that protein could be stored effectively for growth and achieved higher levels of feed efficiency (Figure 3) and lower FCR (Figure 4). This is in line with the opinion of Mohseni et al. (2013) that protein and energy balance can increase growth, feed efficiency, and use of protein.

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4. Conclusions

Utilization of hydrolyzate of indigofera leaves meal are effective to increase growth performance in juvenile *Leptobarbus hoevenii*. Utilization of ILMH on *L. hoevenii* up to 35% in feed resulted in better growth. The best growth and feed efficiency is the utilization of 10% ILMH on *L. Hoevenii.*

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Acknowledgements

This study was funded by Lampung State Polytechnic research grant. We thank our students of Aquaculture Study Program from Lampung State Polytechnic: Agus, Suhendi, Habib, Dimas and Tomy for their participation and assistance during the laboratory experiment.

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Table 1. Composition Ingredient in the Diets

Ingredients	Indigofera leaves meal hydrolyzate (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

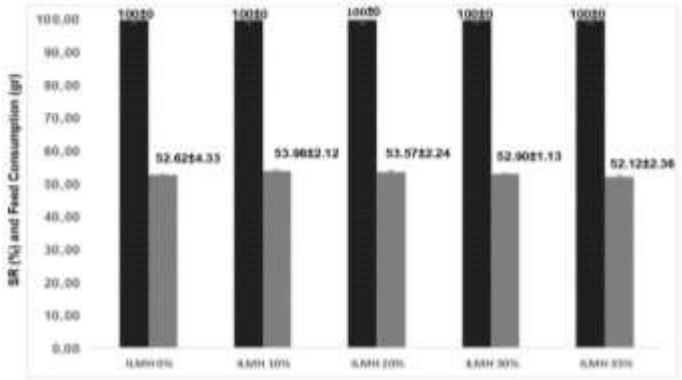
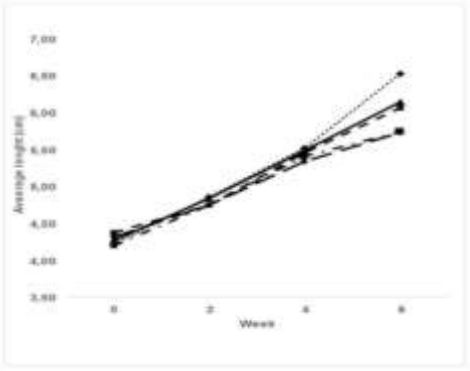


Figure 1. Survival Rate (SR) and Feed Consumption (FC) of *L. hoevenii* reared for 6 weeks with the utilization of indigofera leaves meal hydrolyzate (ILMH). ( ) denote SR and ( ) FC.



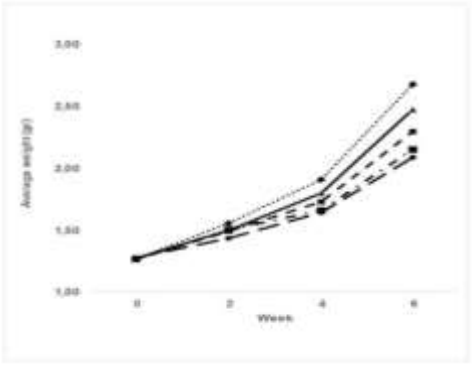


Figure 2. Growth of average weight and length of *L. hoevenii* reared for 6 weeks with the utilization of indigofera leaves meal hydrolyzate (ILMH) treatments. ( ) denote ILMH 0%, ( ) ILMH 10%, ( ) ILMH 20%, ( ) ILMH 30%, ( ) ILMH 35%.

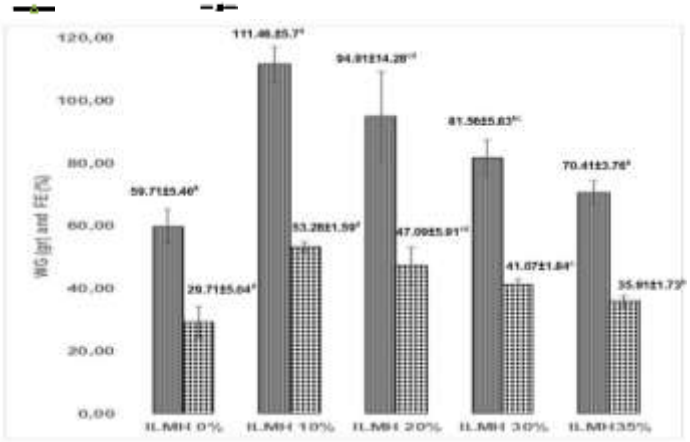


Figure 3. Weight Gain of Biomass (WG) and Feed Efficiency (FE) of *L. hoevenii* reared for 6 weeks with the utilization of indigofera leaves meal hydrolyzate (ILMH). (■) denote WG and ( ) FE.

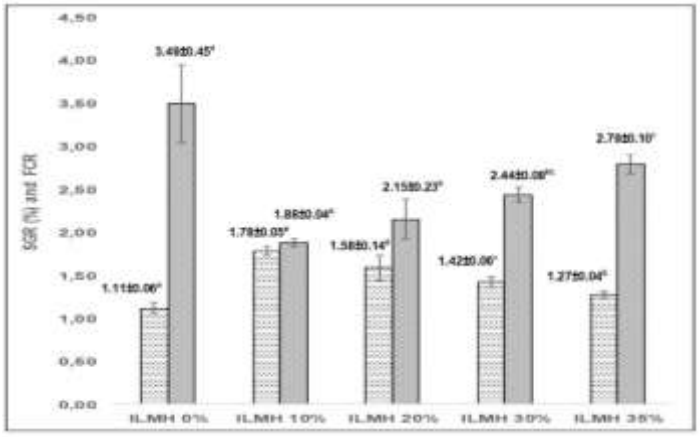


Figure 4. Specific growth rate (SGR) and feed conversion ratio (FCR) of *L. hoevenii* reared for 6 weeks using indigofera leaves meal hydrolyzate (ILMH). ( ) denote SGR and ( ) FCR.

Perbaikan dari Reviewer 2

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

Abstract

This study was conducted to evaluate the effectiveness of indigofera leaves meal hydrolysate (ILMH) on the growth performance of *Leptobarbus hoevenii*. This study used five dietary treatments with different levels of ILMH. Indigofera leaves meal was hydrolyzed using cellulase enzymes and mixed with other feed ingredients. The treatment examination feed included feed without ILMH, feed containing 10%, 20%, 30% and 35% ILMH with three replications. Average of initial weight 1.27±0.01 gr and initial length 4.28±0.07 cm. Length and weight observations were also conducted every two weeks. After six weeks of rearing, observations of survival, final biomass, specific growth rate, feed efficiency and feed conversion ratio were conducted. Observations of water quality during maintenance were carried out at the beginning and end of treatment including temperature, pH, and dissolved oxygen. The results showed that the utilization of ILMH in *L. hoevenii* feed could substitute the use of soybean meal in the range of 10-35% of the feed. Feed containing 10% ILMH resulted in the highest growth and feed efficiency compared to all treatments. This study concluded that the utilization of hydrolysate of indigofera leaves meal in feed was effective in increasing growth performance in *L. hoevenii.*

Keywords: Effectiveness, 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 awal 1.27±0.01 gr dan panjang rata-rata awal 4.28±0.07 cm. Pengamatan panjang dan bobot setiap dua minggu sekalian dilakukan. Setelah 6 minggu pemeliharaan, dilakukan pengamatan kelangsungan hidup, biomassa akhir, laju pertumbuhan spesifik, efisiensi pakan dan feed convertion ratio. Pengamatan kualitas air selama pemeliharaan dilakukan pada awal dan akhir perlakuan meliputi suhu, pH, dan oksigen terlarut. Hasil peneltiian menunjukkan bahwa pemanfaatan HDTI dalam pakan ikan jelawat 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. Kesimpulan penelitian ini adalah pemanfaatan hidrolisat tepung daun indigofera pada pakan efektif meningkatkan kinerja pertumbuhan pada ikan jelawat.

Kata kunci: Efektifitas, daun indigofera, jelawat, hidrolisat, pertumbuhan.

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5. Introduction

The problem of fish feed in Indonesia is currently dominated by import dependence on feed raw materials, especially fish meal and soybean meal. The use of these two types of raw materials is very necessary because the protein contained in them is an important factor in the growth and selling price of fish feed (Rakhmawati et al*.,* 2022). Feed requires 60-80% of the total production cost in fish or shrimp farming. Along with the weakening of the rupiah in recent years, coupled with the higher price of soybean meal, production costs have increased. While the population is increasing, the selling price cannot be increased in line with production costs due to the COVID19 pandemic that has occurred since early 2020 (Sari et al., 2020).

The problem of dependence on imports of these two types of raw materials can be found in alternatives with comparable protein content. One alternative that currently can be chosen is *Indigofera zollingeriana* leaves. These leaves contain 28-30% protein which is close to the protein content of soybeans and is easy to find or cultivate (Putri et al., 2019), Abdullah & Suharlina (2010) stated that the protein in the leaves is higher than in the stems. The growth of this protein source has been encouraged in the province of Lampung, especially in the Pringsewu and Tulang Bawang areas since 2019 (Lampost, 2019). Therefore, this leaf can be optimized for use in the manufacture of fish feed because it is easy to obtain, relatively inexpensive and contains high enough protein.

The use of indigofera leaves have been widely used in livestock feed (Palupi et al., 2014); fish feed has also been conducted such as in catfish, carp, and tilapia, but there is still a small percentage of ingredients in feed that can be used (Tampobolun, 2014; Putri *et al*., 2019; Mukti *et al.,* 2019; Jefry, 2020). The problem with fish when using plant-based protein sources, especially forage, is that it contains high crude fiber (Tarigan et al., 2014). This obstacle must be solved by the application of certain technologies that can reduce the level of crude fiber. One of the methods that will be applied in this research is the hydrolysis of cellulose enzymes in fish to be cultured. This method is considered the most effective for degrading crude fiber into sugar (Setyoko & Utami, 2016). Rakhmawati et al. (2022) stated that indigofera leaves meal hydrolyzed with cellulase enzymes showed a decrease in crude fiber. With the application of this technology to *Indigofera zollingeriana* leaves meal, it is hoped that it can replace or substitute the use of soybean meal which is much higher in price and depends on imports.

Leptobarbus hoevenii is a typical fish in Sumatra and in Lampung Province, it is one of the superior products to be developed. This fish is still rarely found in the market, but it is very popular to be served at family events or traditional events so it is very prospective because it has high economic value. This fish is an omnivore so it can utilize more vegetable raw materials. Research on the use of indigofera leaves meal in this fish has never been done. Therefore, to increase aquaculture productivity and improve feed efficiency, it is necessary to study further the use of hydrolyzed indigofera leaves meal with cellulose enzymes in

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Commented [L34]: Indigofera plant?

Leptobarbus hoevenii. The purpose of this study was to evaluate the effectiveness of utilization of indigofera leaves meal hydrolysate in feed composition as a source of plant protein on the productivity of *L. hoevenii* culture.

6. Material and methods (Arial 10, bold)

Experimental diets

The ingredients and composition of the experimental diets are presented in Table 1. This study used five types of examination diets containing indigofera leaves meal at different doses. The experimental feed contained indigofera leaves meal hydrolysate at a dose of 0 (control, without indigofera leaves meal); 10%; 20%; 30%, and 35%. The prepared indigofera leaf meal was hydrolyzed with a commercial cellulase enzyme as much as 10g kg^{-1}. Indigofera leaves meal, added cellulase enzyme and 30% water, then incubated for 7 days at room temperature (Rakhmawati et al., 2022).

All raw materials are weighed and mixed evenly, followed by the addition of oil and water. The feed was molded with a diameter of 1 mm, dried in a tumble dryer, and stored in plastic containers until use.

Experimental animals and rearing activity.

Three hundred *L. hoevenii* juveniles (4.28±0.07 gr) were obtained from the Center for Freshwater Aquaculture, Jambi Province, Indonesia. Prior to the study, *L. hoevenii* was reared for 1 week and given commercial feed (30% protein) for acclimatization to the research conditions. The test fish were reared for 6 weeks. At the beginning of the study, individual fish were weighed and randomly distributed into 15 aquariums (60 x 80 x 90 cm) with a density of 20 fish/aquarium. Feeding with a feeding rate of 5% twice a day (9:00 and 17:00). Continuous aeration was provided with 25% water change every 24 hours. Fish feces are siphoned from the aquarium at 16.00 every day. Water quality parameters in all experimental media include temperature, dissolved oxygen, total ammonia nitrogen, and pH. The temperature during maintenance was 27.6 – 29.5^{o}C, pH 7.63 – 8.1, and dissolved oxygen 6.5 – 7.7 mg/L.

Growth Evaluation of *Leptobarbus hoevenii*

Observations of individual length and weight growth were conducted every two weeks. After six weeks of rearing, observations of survival rate, final biomass, total feed consumption, specific growth rate, feed efficiency, and feed conversion ratio were conducted.

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Why did you measure Protein Efficiency?

Statistical analysis

All data presented in the form of figures are the mean ± standard deviation of three replications. Data were analyzed by one-way ANOVA and Tukey's test using the software IBM SPSS Statistics 22. The difference was considered to be significant at P < 0.05. In the figure on the results, the difference in letters in the upper right position indicates a significant difference.

7. Results and Discussion (Arial 10, bold)

3.1 Results

Survival rate and feed consumption

Figure 1 shows that the survival rate of *L. hoevenii* that was maintained for six weeks was categorized as good, there was no death with a survival rate of 100%. Likewise, feed consumption during maintenance is almost the same in number, so it does not show a significant difference.

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Growth of the average length and weight of juvenile *L. hoevenii*

The growth of the average length of *L. hoevenii* reared for six weeks showed that the use of ILMH resulted in higher length gain at a dose of 10 – 30% of ILMH compared to the control. Meanwhile, the average length gain at the 35% ILMH dose was the same as the control. In contrast to the average weight gain of *L. hoevenii*, the average weight gain indicates that the utilization of ILMH resulted in a higher average weight gain at all utilization doses than the control as shown in Figure 2.

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Increase in Biomass and Feed Efficiency

During maintenance, the weight gained of *L. hoevenii* fed with ILHM at a dose of 10% resulted in the best weight gain and feed efficiency among all treatments. While the utilization of ILMH of 20, 30 and 35% showed better growth than the control, they followed higher growth of *L. hoevenii* respectively. Although the weight gain was higher than the control, the higher the utilization of ILMH, the lower the growth (Figure 3).

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Spesific Growth Rate dan Feed Convertion Ratio

35.91±1.73[b]

The specific growth rate of *L.hoevenii* reared for six weeks showed that the utilization of ILMH with a dose of 10% resulted in the highest SGR, followed by treatment with doses of 20%, 30% and 35%. Meanwhile, the feed conversion ratio parameter shows the highest value in the control treatment. Furthermore, the FCR value between treatments with the utilization of ILMH, the lowest was 10%, 20%, 30% respectively, followed by 35% (Figure 4).

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3.2. Discussion

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The results showed that the use of ILMH in feed affected the growth of *Leptobarbus hoevenii.* Utilization of ILMH on *L. hoevenii* feed, both 10, 20, 30%, and 35% showed better growth than feed without the use of ILMH. This is in line with other studies on carp that the use of hydrolyzed indigofera leaves meal with cellulase enzymes in feed increased growth up to a dose of 40% (Jefry et al., 2021). In the same fish, Shulikin et al., (2021) rep $^{2.79\pm0.10^{c}}$ ostitution of 20% soybean meal and 20% fish meal with indigofera leaves meal showed the best growth. Mukti et al. (2019) stated that the utilization of indigofera leaves meal resulted in the best growth in the treatment of 20% of the ingredients in the composition of catfish feed. Tilapia showed the best growth in the treatment using indigofera leaves meal as much as 30% (Putri et al., 2019). Likewise, for Sumatran ornamental fish, the addition of 20% indigofera leaves meal provides the best color quality (Pratama et al., 2019).

In this study, indigofera leaves meal was hydrolyzed with the enzyme cellulose (a protein containing living cells that acts as a catalyst in biochemical reactions). This enzyme can hydrolyze crude fiber more effectively than other methods (Setyoko & Utami, 2016). The result of this hydrolysis process is a simpler sugar in the form of cellobiose and glucose (Terri, 1997; Horn et al., 2012). The results showed that the crude fiber content of the feed containing ILMH was up to 35% lower than the control. The use of ILMH in feed reduced crude fiber to 46.2% and increased the carbohydrate content (Table 1) which is used as an energy source that is easily utilized by *L. hoevenii*. The decrease in crude fiber was also obtained by 43.3% which caused total digestibility, protein and fat in gourami fry fed a diet containing indigofera meal which was hydrolyzed by cellulase enzymes (Jefry et al., 2021).

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The results also showed that growth increased as the percentage of ILMH increased to 35%, although the protein content of the feed decreased. The higher growth rate of the fish contained more non-protein energy, namely the C:P ratio of 16.46 – 18.73 kcal/g, while the C:P ratio of the control was 15.73 kcal/g. A balanced ratio of protein and non-protein energy in feed can increase the efficiency of protein metabolism as energy, and increase its use for fish growth (Sankian et al., 2017; Li et al., 2013; Kim *et a*l., 2017). According to Jauralde et al. (2021) that feed with the optimum ratio of protein to energy describes the

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balance point between the amount of energy required for maintenance and growth. If an appropriate C:P ratio has been achieved, it is possible to reduce the protein level in the feed without reducing fish growth and improving to the growth response (Carneiro *et al*., 2020 & Aboseif et al., 2022).

In this study, the feed consumption in each treatment was not significantly different (Figure 1), but the growth of *L. hoevenii* containing ILMH was up to 35%. The non-protein energy content contained in the feed, especially carbohydrates, was higher than the others (Table 1). Thus, in this treatment, fish were better able to utilize non-protein energy for their energy needs so that protein could be stored effectively for growth and achieved higher levels of feed efficiency (Figure 3) and lower FCR (Figure 4). This is in line with the opinion of Mohseni et al. (2013) that protein and energy balance can increase growth, feed efficiency, and use of protein.

8. Conclusions

Utilization of hydrolyzate of indigofera leaves meal are effective to increase growth performance in juvenile *Leptobarbus hoevenii*. Utilization of ILMH on *L. hoevenii* 10% up to 35% in feed are better growth than 0% (without ILMH). The best growth and feed efficiency is the utilization of 10% ILMH on *L. hoevenii.*

Acknowledgements

This study was funded by Lampung State Polytechnic research grant. We thank our students of Aquaculture Study Program from Lampung State Polytechnic: Agus, Suhendi, Habib, Dimas and Tomy for their participation and assistance during the laboratory experiment.

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Table 1. Composition Ingredient in the Diets

Ingredients	Indigofera leaves meal hydrolyzate (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

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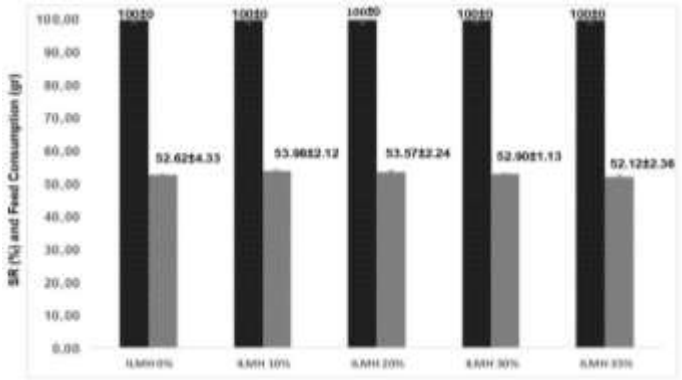

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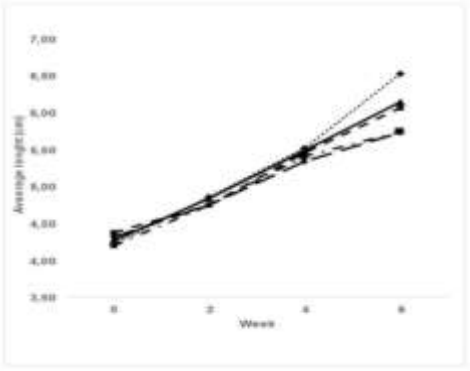

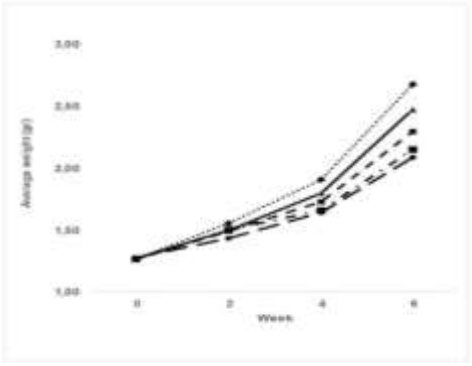

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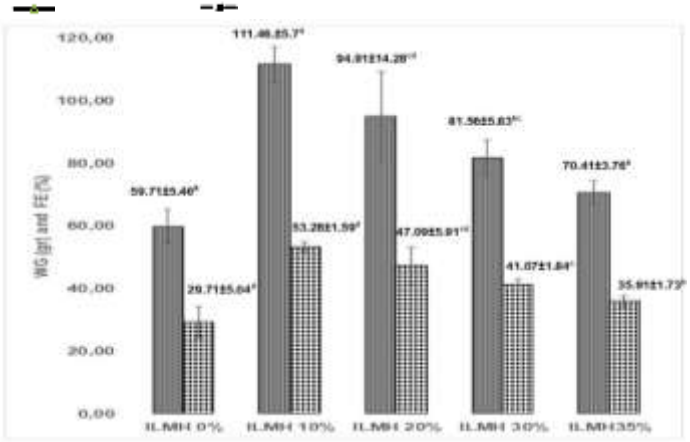

Figure 3. Weight Gain of Biomass (WG) and Feed Efficiency (FE) of *L. hoevenii* reared for 6 weeks with the utilization of indigofera leaves meal hydrolyzate (ILMH). (■) denote WG and ( ) FE.

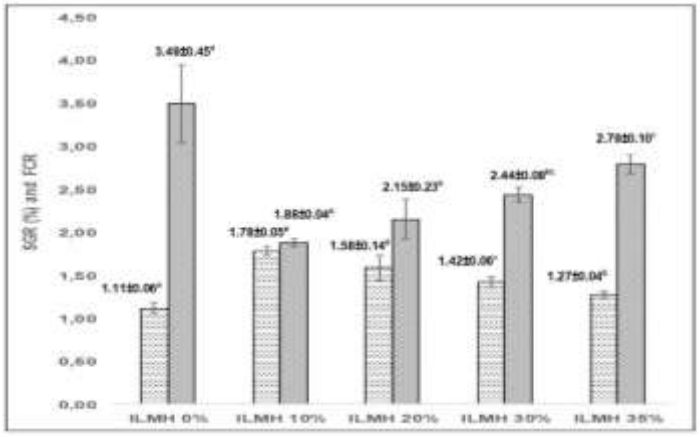

Figure 4. Specific growth rate (SGR) and feed conversion ratio (FCR) of *L. hoevenii* reared for 6 weeks using indigofera leaves meal hydrolyzate (ILMH). ( ) denote SGR and ( ) FCR.

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Manuskrip yang telah direvisi

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

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

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.

9. 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 (Tampobolun, 2014; 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., 2014). 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.

10. Material and methods

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.

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. Feeding 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 29.5^{o}C, the pH was between 7.63 and 8.1, and the dissolved oxygen was between 6.5 and 7.7 mg/L.

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) = [(ln Final mean body weight − ln 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).

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.

11. Results and Discussions

3.1 Results

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%.

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.

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).

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).

3.2. Discussions

35.91 ± 1.73^{b}

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 me 2.79 ± 0.10^{c} (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).

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). This hydrolysis process results in the formation of cellobiose and glucose, two simpler sugars (Terri, 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, compated 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.

12. 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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Table dan Figures

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

Figure 1.

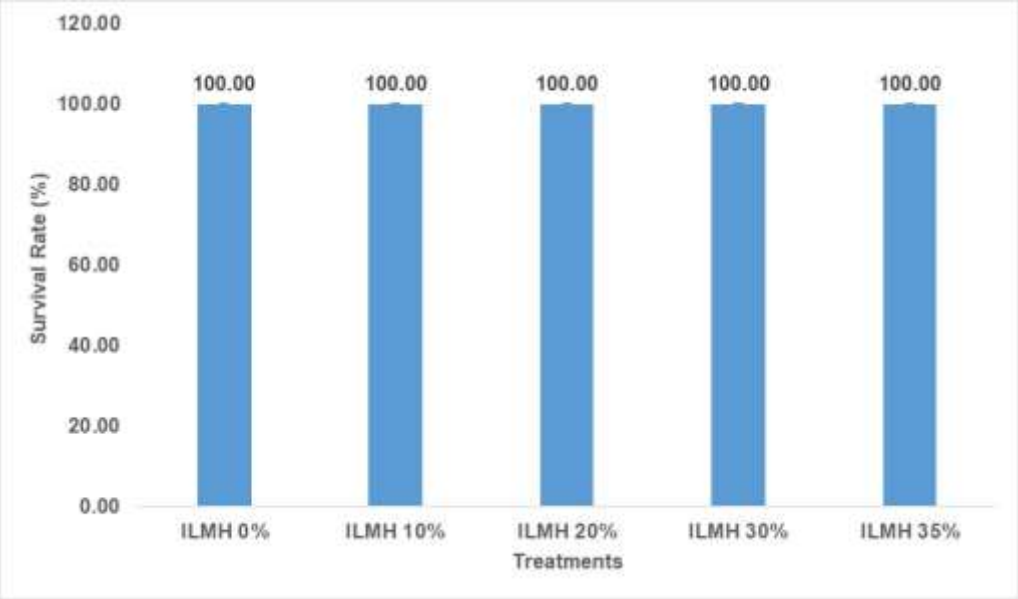


Figure 1. Survival Rate (SR) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote SR.

Figure 2.

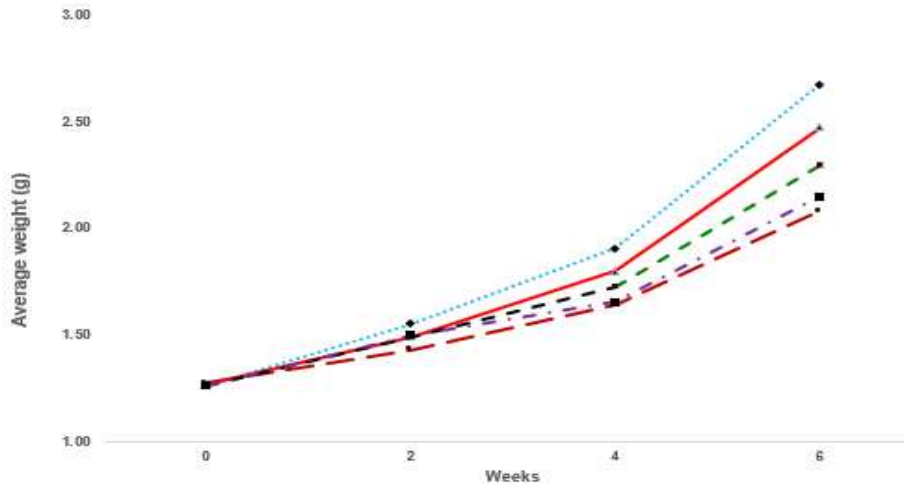


Figure 2. Average weight growth (g) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) treatments for 6 weeks. ( ) denote ILMH 0%, ( ) ILMH 10%, ( ) ILMH 20%, ( ) ILMH 30% and ( ) ILMH 35%.

Figure 3.

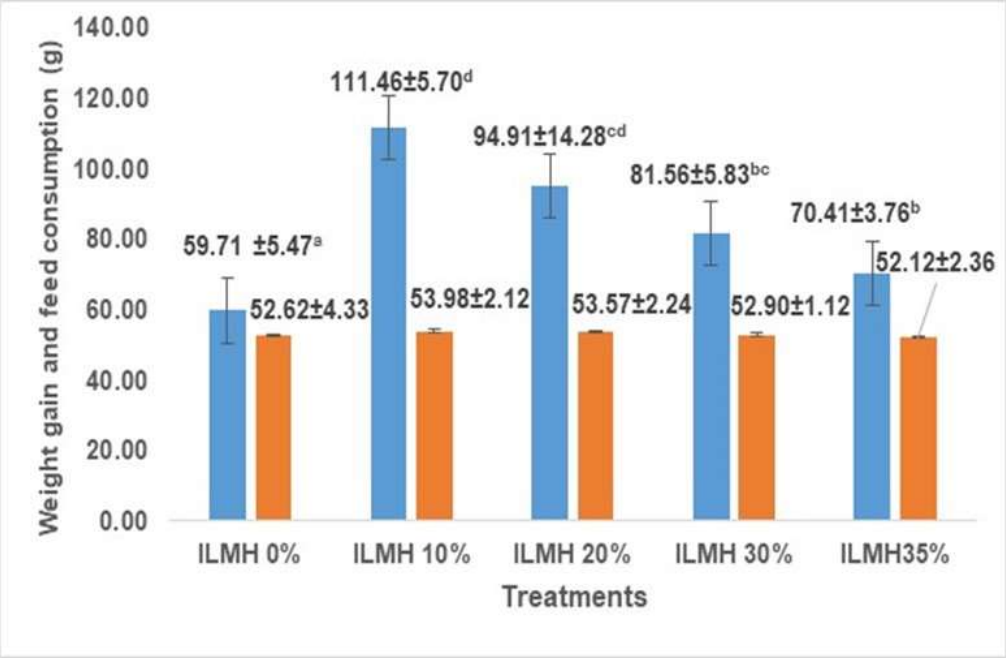


Figure 3. Weight gain of biomass (WG) and feed consumption (FC) of *L. hoevenii* reared with indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote WG and (■) FC.

Figure 4.

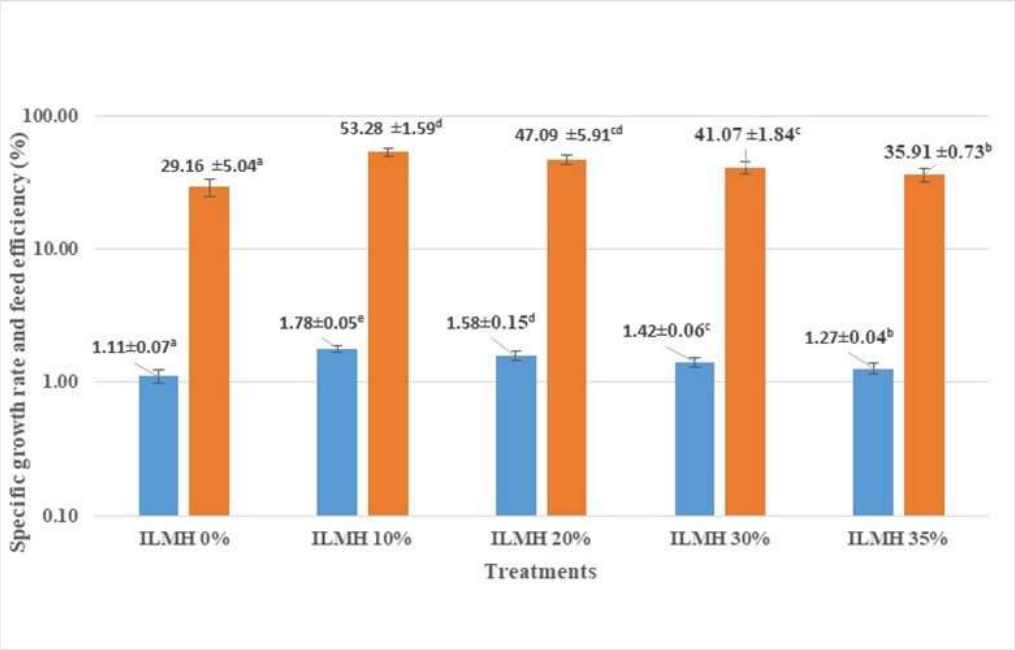
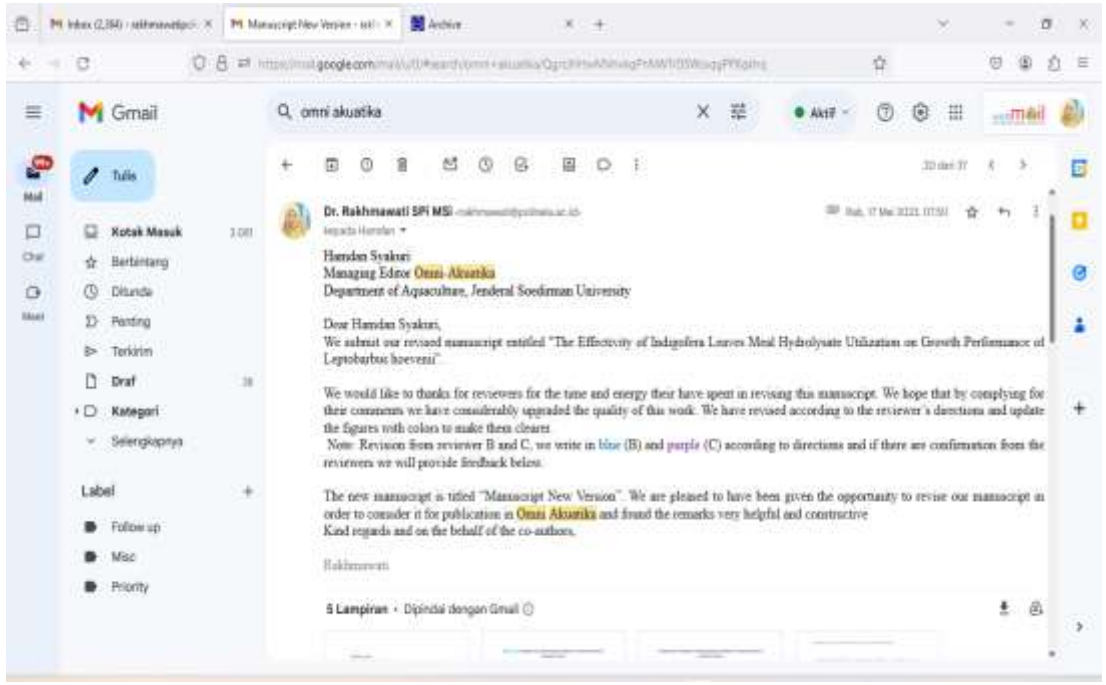


Figure 4. Specific growth rate (SGR) and feed efficiency (FE) of *L. hoevenii* reared using indigofera leaves meal hydrolysate (ILMH) for 6 weeks. (■) denote SGR and (■) FE.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Dear Hamdan Syakuri,
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We would like to thanks for reviewers for the time and energy their have spent in revising this manuscript. We hope that by complying for their comments we have considerably upgraded the quality of this work. We have revised according to the reviewer's directions and update the figures with colors to make them clearer.
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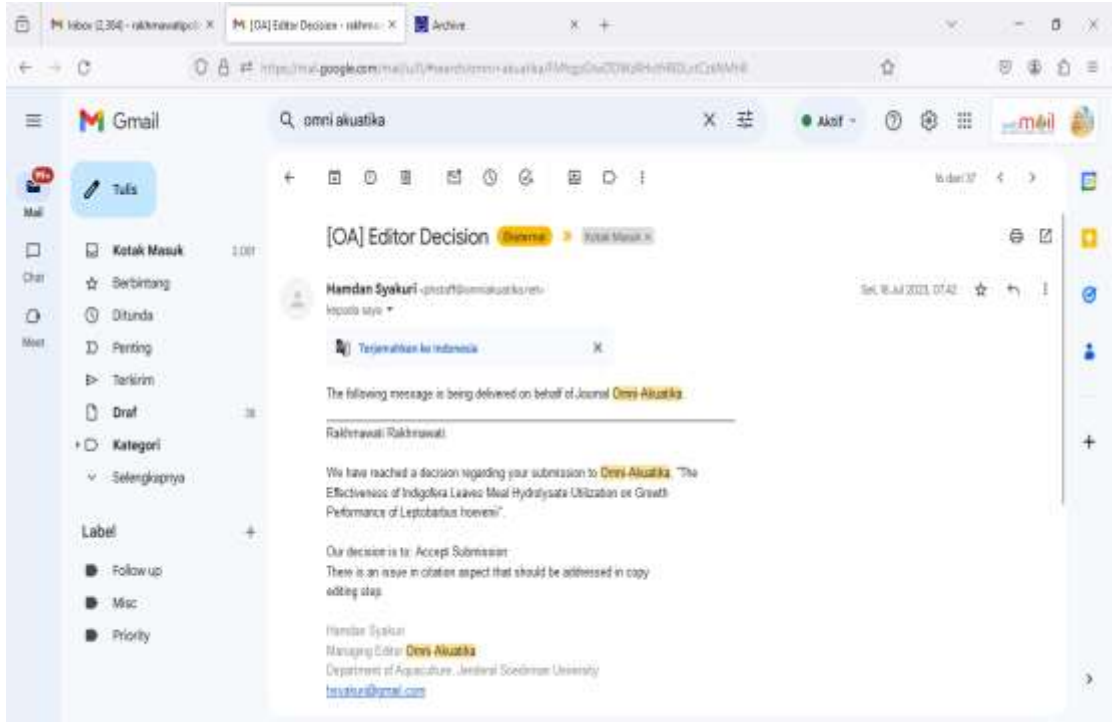
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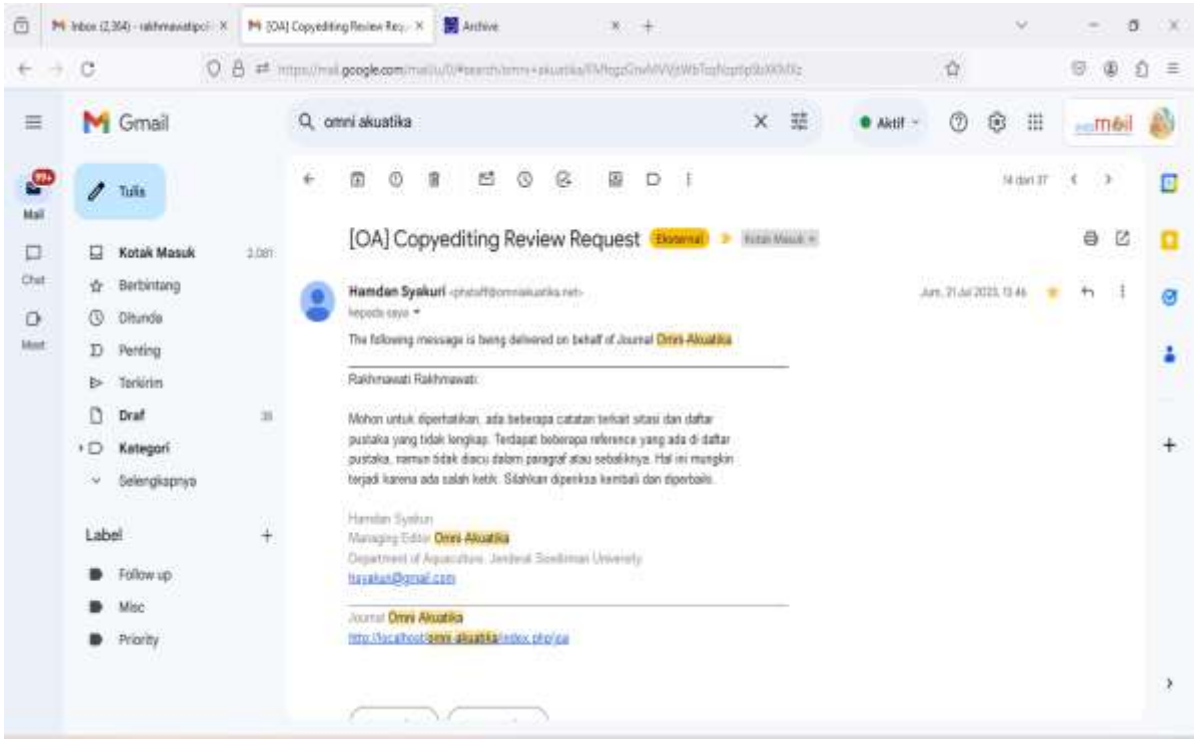
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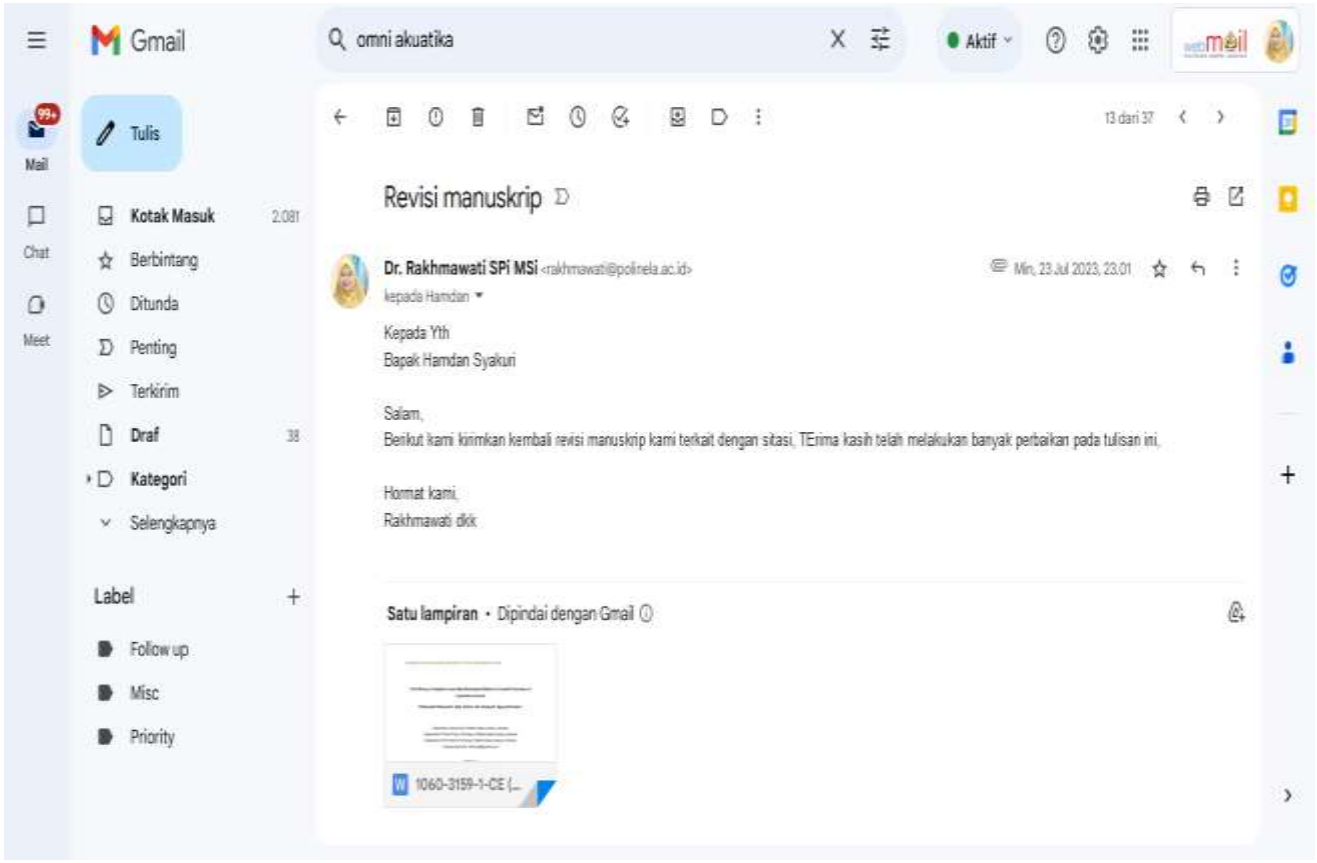
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Dr. Rakhmawati SPi MSi <rakhmawati@polinela.ac.id>
kepada Hamdan
Min, 23 Jul 2023, 23.01
Kepada Yth
Bapak Hamdan Syakuri
Salam,
Berikut kami kirimkan kembali revisi manuskrip kami terkait dengan sitasi, TErima kasih telah melakukan banyak perbaikan pada tulisan ini.
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Rakhmawati dkk
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#1060 Summary

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Authors	Rakhmawati Rakhmawati, Nur Indariyanti, Anjar Sofiana, Agung Kurniawan
Title	The Efficiency of Indigofera Leaves Meal Hydrolysate Utilization on Growth Performance of Leptobarbus hoeveni
Original file	1060-2998-1-SM.DOCX 2023-01-21
Supp. files	None
Submitter	Rakhmawati Rakhmawati
Date submitted	January 21, 2023 - 11:53 AM
Section	Research Articles
Editor	Hamdan Syakuri
Abstract Views	178

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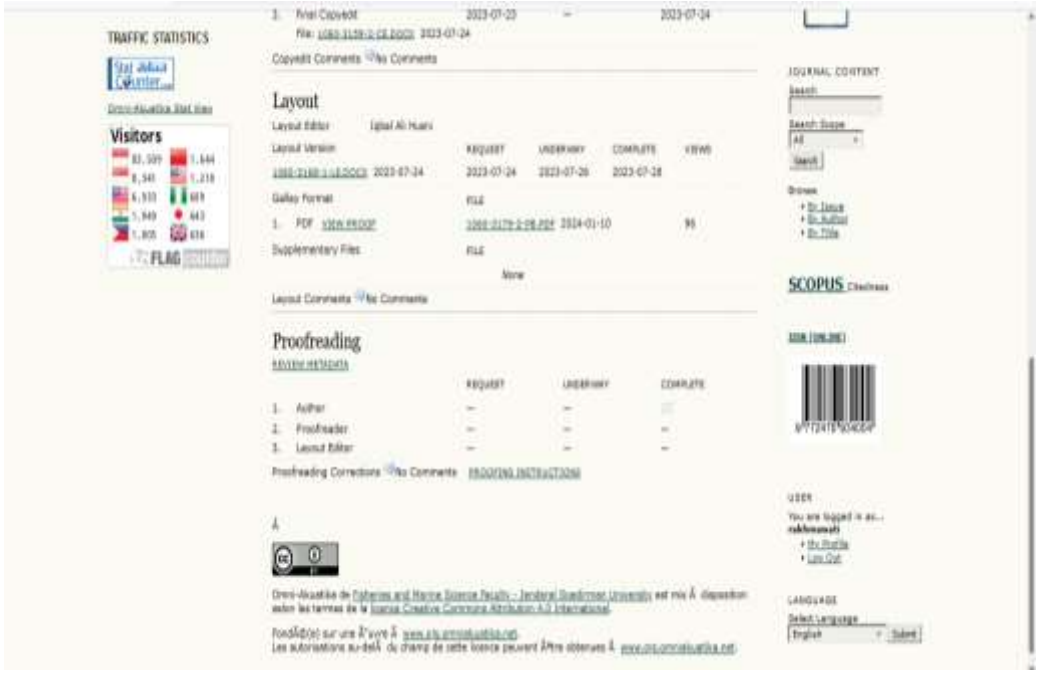

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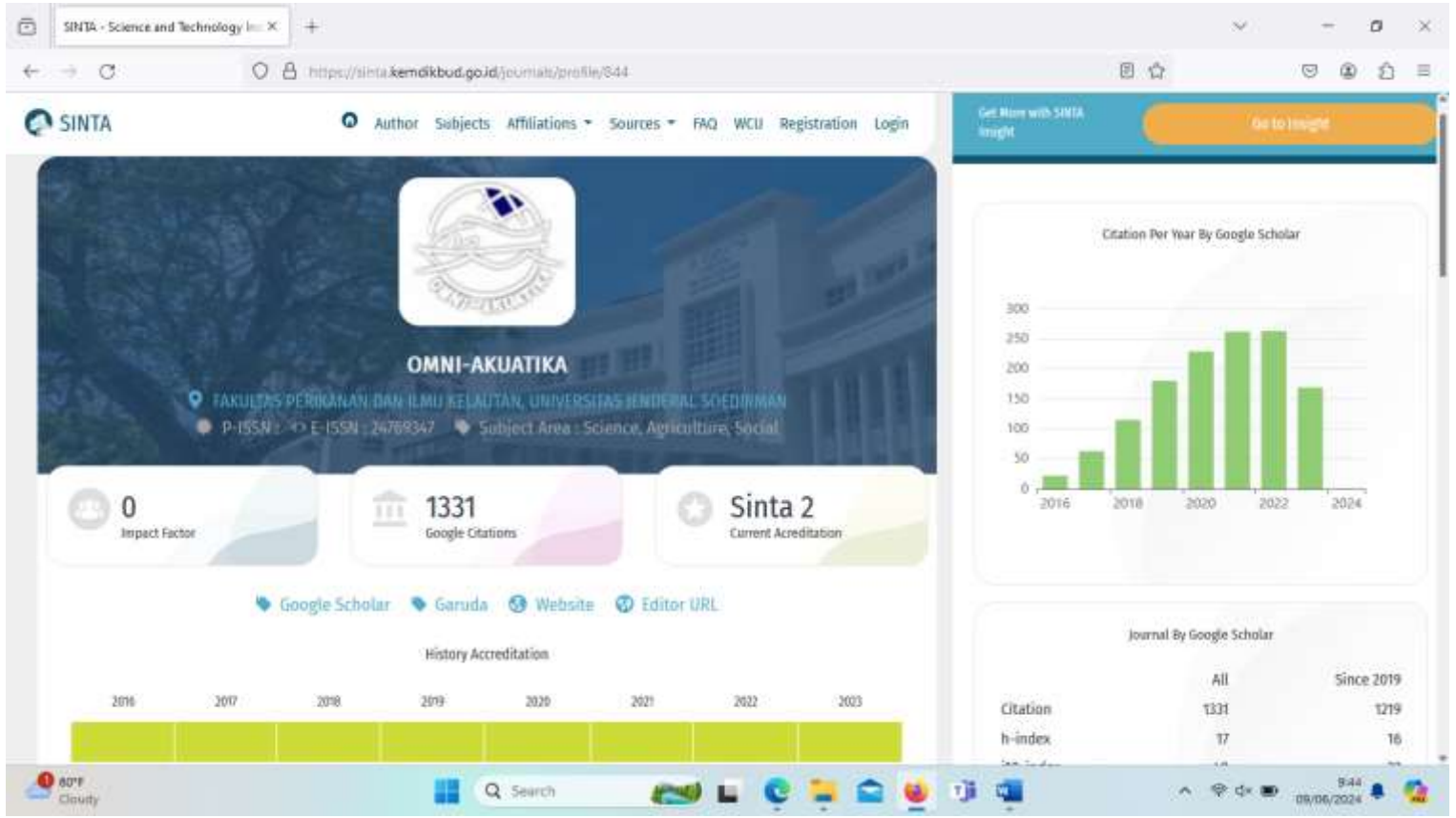
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