

GROWTH AND HISTOLOGY OF CATFISH LIVER (*Clarias* sp) WITH THE ADMINISTRATION OF KATUK LEAVES SIMPLICIA (*Sauropus androgynus*)**PERTUMBUHAN DAN HISTOLOGI HATI IKAN LELE (*Clarias* sp) DENGAN PEMBERIAN SIMPLISIA DAUN KATUK (*Sauropus androgynus*)****Awaludin*, Siti Jumariah, Rukisah**

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ABSTRACT

Catfish (*Clarias* sp) production has not been stable from year to year. Increasing catfish production has been achieved in various ways, including adding ingredients rich in natural nutrients, such as katuk leaves. Katuk leaves (*Sauropus androgynus*) are herbal plants that contain high fatty acids and can increase the metabolism and growth of catfish (*Clarias* sp). This study aims to determine the effect of adding katuk leaf powder (*S. androgynus*) as an alternative ingredient to commercial fish feed mixtures on catfish growth (*Clarias* sp). This study was conducted for 30 days using a completely randomized design (CRD) with three treatments and three replications: P1 (control), P2 (commercial feed and katuk leaves powder at 100 grams/kg), and P3 (commercial feed and katuk leaves powder at 150 grams/kg). Based on the results of the study, the provision of katuk leaves had a significant effect on length and weight growth, SGR (Specific Growth Rate), and FCR (Feed Conversion Ratio) in catfish. The Number of hepatocytes observed histologically showed that the highest number was in treatment P3 (225,636), followed by P2 (224,560), and the lowest in P1 (218,756). The best feed to increase growth is P3. Based on the research results on commercial feed mixed with katuk leaves powder (*S. androgynus*) containing fatty acids, it can increase the growth of catfish (*Clarias* sp) observed from the growth and morphology of liver cells.

Keywords: katuk leaves, histology, catfish, growth.

INTRODUCTION

Catfish aquaculture production in 2019 was 1,088,945.20 tons; in 2020, it decreased by 993,768.29 tons. However, catfish fishery production in Indonesia increased again in 2021, to 1,041,574.48 tons (KKP, 2021). The increase in catfish production continues to be developed in various ways. One of the determinants of the success of fish farming is the provision of feed. Feed is a vital factor in the success of a fishery business. However, there is also a weakness: the inefficiency of feeding, which can increase the cost of commercial feed production. Commercial feed production is often faced with various problems related to

the availability of difficult-to-obtain materials and their high prices (Yashni *et al.*, 2020).

Freshwater fish farming business actors can spend commercial feed costs reaching 75% of the total cultivation costs. So it is necessary to have alternative materials at affordable prices, with good availability and high nutritional content. Using natural ingredients is an option; one is katuk leaves (*Sauropus androgynus*), which are abundantly available and rich in nutrients. Natural ingredients such as katuk leaves can reduce production costs and increase fish growth. Katuk leaves (*S. androgynus*) can also increase the growth of fish and shrimp (Julianti, 2020) and tiger

shrimp (Awaludin, 2020). Katuk leaves powder contains 12% water, 8.91% ash, 26.32% fat, 23.13% protein, 29.64% carbohydrates, 372.42 mg/100g carotene, and 447.96 cal energy (Sadi, 1983). Awaludin *et al* (2020) reported the results of the proximate test of katuk protein extract (4.8-6.4), crude fat (1.0), crude fibre (1.5), ash content (1.7), and extract content without nitrogen (BETN) (26.56). This shows that katuk leaves (*S. androgynus*) contain high fatty acids. A lack of fatty acids can cause disorders in fish health, including reduced fecundity and the ability to form embryos, larval death and abnormal growth, incorrect pigmentation, impaired vision, inability to eat at low light intensity, abnormal behaviour, and decreased membrane function at low temperatures (Tocher, 2003). Fatty acids that enter the body can provide energy for metabolism, allowing most of the protein from the feed to be utilized to support fish growth (Mukti *et al.*, 2014). Based on the description above, it is necessary to conduct research to enrich the feed by adding katuk leaf powder to the growth of catfish (*Clarias* sp.).

METHODOLOGY

Materials

The tools used in this study include a water bath, blender, surgical instruments, sieve, plastic jar, Soxhlet, siphon hose, aerator, seser, digital scale, analytical scale, DO meter, pH meter, ruler, microscope, oven, and microtome. The materials used in this study include 5-6 cm catfish seeds, HI-PRO-VITE 781-1 commercial feed, katuk leaves powder, fresh water, progol, paraffin, alcohol, xylol, hematoxylin, and eosin.

Methods

The research design consisted of three treatments and three replications: P1 (without the addition of katuk leaves powder), P2 (commercial feed and katuk leaves powder at 100 grams/kg), and P3

(commercial feed and katuk leaves powder at 150 grams/kg). The commercial feed used is the HI-PRO-VITE 781-1 brand, with a Protein content of 31-33%, Fat of 4-6%, Fibre of 3-5%, water of 9-10%, and a size of 2.0 - 2.3 mm. This feed is made by the mouth opening so that fish can easily digest it. Commercial feed is combined with katuk leaf powder. Feed modification is carried out by weighing the prologue at up to 5 g/kg of feed, dissolving it in 10 ml of water, stirring, adding katuk leaves to the simplicial and commercial feed according to the treatment dose, stirring again until homogeneous, and then drying. The study was conducted at the Mini Hatchery Laboratory, Faculty of Fisheries and Marine Sciences, Borneo Tarakan University. The fish used were catfish measuring 5-6 cm and weighing an average of 1.38 grams. The Number of fish used was 90, and the stocking density in each container was 10 fish/container. Before the fish were spread into the maintenance container, they were acclimatized. Feeding 5% of fish biomass/day with a frequency of 2 times a day, namely in the morning and evening (Erlania, 2010).

Parameter Measurement

The parameters measured include fat content in katuk leaves, measurement of absolute length growth of catfish, the absolute Weight of catfish, Feed Conversion Ratio, Specific Growth Rate, Survival Rate, water quality measurements carried out every day, Hepatosomatic Index, and histology of catfish liver is carried out at the end of the study.

1. Fat Content Testing (Soxhlet Method)

The sample weighed 5 g, then 10 g of Na₂SO₄ was added, and the mixture was stirred until smooth. Please put it in the oven at 105°C for 2 hours, stirring several times. Cool for 1 hour in a desiccator, then place the sample in tissue and place the tissue into the extractor. Prepare the Soxhlet. The bottom flask is filled with 200 mL of diethyl

ether. Place in a water bath for 8-16 hours at 50-70°C. The contents of the bottom flask are transferred to the flash extractor, to the flash extractor is evaporated with a rotary evaporator. The remaining solution is transferred to a beaker of known Weight, then dried and weighed again.

Calculation:

$$\text{Fat (\%)} = (W - W_1) / S \times 100$$

Note:

W = Weight of empty fat flask
 W₁ = Weight of sample (grams)
 S = Weight of fat flask with fat (grams)

2. Absolute Length Growth of Catfish was stated (Herlina, 2016);

$$L_m = L_t - L_o$$

Note:

L_m = Absolute length growth (cm)
 L_t = Average length at the end of the study (cm)
 L_o = Average length at the beginning of the study (cm)

3. Absolute Weight Growth of Catfish (Samad et al., 2014);

$$W = W_t - W_o$$

Note:

W = Absolute weight growth (grams)
 W_t = Final fish weight (grams)
 W_o = Initial fish weight (grams)

4. Feed Conversion Ratio (FCR) (Lozano et al., 2017);

$$\text{FCR} = (\text{Amount of feed used (kg)}) / (\text{Increase in fish weight during cultivation (kg)})$$

5. Specific Growth Rate (Lozano et al., 2017);

Note:

SGR = Specific growth rate (%)
 W_t = Average Weight of worms at the end of the study (g)
 W_o = Average Weight of worms at the beginning of the study (g)
 t = Time of study (days)

6. Survival Rate (Samad et al., 2014)

$$\text{SR (\%)} = N_t / N_o \times 100$$

Note:

SR = Survival (%)
 N_t = Number of fish alive at the end of the experiment (tail)
 N_o = Number of fish alive at the beginning of the experiment (tail)

7. Hepatosomatic index (HSI) (Lozano et al., 2017);

$$\text{HSI (\%)} = 100 \times (\text{liver weight}) / (\text{body weight})$$

8. Histology

The catfish liver was observed histologically (staining) using hematoxylin and eosin (HE) with a thickness of 5 µm at a magnification of 100 µm × 40X at the end of maintenance (Ridwan, 2021). Calculate the Number of hepatocyte cells in the catfish liver using the ImageJ application.

9. Water Quality

This study measured water quality parameters from the beginning to the end of maintenance, including temperature, pH value, and dissolved oxygen (DO). Water quality measurements, such as pH and temperature, were conducted daily, while dissolved oxygen (DO) was measured once a week. To reduce organic matter from leftover feed and fish waste, siphoning was performed at the bottom of the container every 3 days, depending on water conditions; water changes were also performed once a week, replacing 30% of the water. (Herdelah, 2019).

Data Analysis

The data, such as Histology, were analyzed descriptively, while absolute growth, absolute Weight, FCR, SGR, SR, and HSI were analyzed statistically using analysis of variance (ANOVA) with a confidence level of 95% in SPSS 26, followed by the Duncan test.

RESULTS AND DISCUSSION

The feed mixed with katuk flour was then tested for fat. The results of the fat content test for commercial feed mixed with katuk leaves powder are shown in Table 1.

Table 1. The fat content of the feed is mixed with katuk leaves powder.

Parameter	Treatment	Treatment	Treatment	SNI 01-4087-2006
Parameter	P1	P2	P3	SNI 01-4087-2006
Fat Content %	9,2%	11,8%	12,4%	Min 5

Description: P1: control, P2: Feed added with 100 g katuk leaves powder, P3: Feed added with 150 g katuk leaves powder.

The results of measuring the fat content in commercial feed mixed with katuk leaves powder obtained the highest fat content in treatment P3 (12.4%) and the lowest in treatment P1 (9.2%). The higher the addition of katuk leaves mixed with artificial feed, the higher the increase in fat content. Good feed for catfish (*Clarias* sp) has a fat content of 4% to 16% (Grasela *et al.*, 2022). This shows that the fatty acid content in the mixed feed of katuk leaves simplicia can meet the fatty acid needs of fish. Fat also serves as an additional energy source for fish survival, as a vitamin solvent, and as a regulator of fish

buoyancy in water. Fat is a very effective energy source and the first energy reserve for fish. Fat is usually used when fish lack food. The study's results showed that providing commercial feed with a mixture of katuk leaves powder affected the growth of length and Weight of tilapia, SGR values, and FCR values. The following are measurement data for absolute length growth, absolute weight growth, FCR, SGR, SR, hepatosomatic index, and histology. The research results on the growth of catfish fed katuk flour for 30 days are shown in Table 2.

Table 2. Results of catfish growth

Treatment	Length (cm)	Growth Weight (gr)	Growth Specific rate (%)	growth Feed conversion rasio
P1	3,70 ± 0,25b	5,12 ± 0,29b	0,17± 0,01b	0,41 ± 0,06b
P2	4,80 ± 0,48a	6,46 ± 0,44a	0,22 ± 0,01a	0,37 ± 0,02ab
P3	5,17 ± 0,44a	7,49 ± 0,87a	0,25 ± 0,02a	0,32 ± 0,03a

Note: P1: Treatment 1 (control), P2: Treatment 2 (commercial feed and katuk leaves powder 100 grams/kg), P3: Treatment 3 (commercial feed and katuk leaves powder 150 grams/kg).

The ANOVA analysis showed that the differences were significant. The length growth of P1 (control) was significantly different from P2 (100 grams of katuk) and P3 (150 grams of katuk), with P3 showing the greatest increase compared to other treatments. Based on Table 2, shows that during the 30-day maintenance period, the highest results were in treatment P3 ($5.17 \text{ cm} \pm 0.44$), followed by treatment P2 ($4.80 \text{ cm} \pm 0.48$), and the lowest treatment was P1 ($3.70 \text{ cm} \pm 0.25$). Treatment P1 (Control) has the lowest value among the other treatments. The average length growth of catfish in treatments P2 and P3 increased; this is thought to be due to the addition of katuk leaves simplicia containing optimal fatty acids in P3, and the feed given has been utilized by the test fish well, so that the absolute length growth of the fish is higher than in other treatments. Fat is a source of energy fish need and plays a vital role in storing feed (Pranata *et al.*, 2023). Although fat cannot dissolve in water, it can dissolve in organic solvents and is an important energy source for fish growth.

Based on the observation results during the 30-day maintenance period, catfish showed different increases in weight growth across treatments. The results of the ANOVA statistical analysis of catfish weight growth were significantly different. The highest biomass growth of catfish weight was shown in the provision of katuk leaves powder treatment P3 ($7.49 \text{ grams} \pm 0.87$), followed by the provision of katuk leaves powder treatment P2 ($6.46 \text{ grams} \pm 0.44$), and then treatment P1 ($5.12 \text{ grams} \pm 0.29$). This shows that the concentration is better than the concentration in the treatment that does not receive katuk leaves powder. The best weight growth of catfish was in the P3 treatment. This is thought to be because the amount of feed given was appropriate and supported by the provision of katuk leaves simplicia, which met the energy needs. The high growth in fish weight across treatments shows that the fish can digest feed well and absorb more nutrients. The increased weight growth of catfish is also

thought to be due to the influence of the fat content in katuk leaves powder with an optimum dose. This is based on Tobuku's (2022) statement that the use of fat in feed must be at the right level or ratio, as excessive or insufficient levels can harm the fish being fed.

Based on observations of the specific growth rate of catfish (*Clarias* sp) during the study period, the results of the ANOVA analysis of variance showed that the growth rate of catfish with the provision of katuk flour in the feed had a significant effect ($P < 0.05$). Treatment P3 had the highest value, namely ($0.25\% \pm 0.02$), then by treatment P2 ($0.22\% \pm 0.01$), and treatment P1 had the lowest growth rate value, namely ($0.17\% \pm 0.01$). The high specific growth rate in treatment P3 is thought to be due to its high fat content (12.4%). The best growth rate data was found in treatment (P3), namely a combination of commercial feed with katuk leaves powder (150 grams). The higher the specific growth rate, the better the fish's growth. The highest percentage of specific growth rate of catfish was achieved in treatment P3 (Feed added with 150 grams of katuk leaves simplicia). This may be because, in treatment P3, the katuk leaf dose has the appropriate nutritional content and is the optimum dose for catfish growth compared to other treatments. The results of the ANOVA statistical analysis of the Feed Conversion Ratio (FCR) in catfish fed katuk flour showed significant differences ($P < 0.05$). The highest FCR was found in P1 ($0.41 \text{ grams} \pm 0.06$), followed by P2 ($0.37 \text{ grams} \pm 0.02$) and P3 ($0.32 \text{ grams} \pm 0.03$). This shows that treatment P3 has the highest feed efficiency, as its FCR is the lowest among treatments P1 and P2.

FCR is a comparison between the amount of feed given and the Weight of the fish produced (Anis and Hariani, 2019); feed in low amounts but can react optimally to fish growth is a sign that the feed is of good quality. If the FCR value is low, it indicates that the fish use feed very efficiently and that the energy obtained from the feed is used for growth. Conversely, if the FCR value is

significant, the effective feed consumed is small. A low FCR value impacts feed conversion, which is very effective for growth. The feed conversion ratio describes the efficiency with which nutrients in fish feed are utilized. The lower the feed conversion ratio value, the higher the efficiency of fish in utilizing feed nutrients. This is suspected based on the difference in fat content between treatments with the addition of katuk simplisia, which indicates efficient feed use. Fat in the feed will be oxidized for energy, thereby fulfilling its role as a protein-sparing agent. This is also suspected because the katuk leaves mixed

with commercial feed are very small, making the feed more easily digestible by the catfish's intestines. In addition to providing energy, quality feed is expected to increase fish digestibility, thereby optimizing fish growth. In the observation results, the increase in Weight from the beginning to the end of maintenance was greater in the treatment with feeding than in the treatment without feeding.

The study's results on the survival rate, hepatosomatic index, and Number of hepatocyte cells of catfish maintained for 30 days with the addition of katuk flour to the feed are shown in Table 3.

Table 3. Performance of catfish liver

Treatment	Survival rate	HSI (%)	Number of hepatocyte cells
P1	100 ± 0 ^a	1,92% ± 0,32 ^a	218.756±4.471 ^a
P2	100 ± 0 ^a	2,03% ± 0,20 ^a	224.560±12.098 ^a
P3	100 ± 0 ^a	2,45% ± 0,39 ^a	225.636±14.679 ^a

Note: P1: Treatment 1 (control), P2: Treatment 2 (commercial feed dan katuk leaves powder 100 grams/kg), P3: Treatment 3 (commercial feed dan katuk leaves powder 150 grams/kg).

The survival rate of catfish (*Clarias* sp.) was determined by direct observation and by calculating the Number of fish at the beginning and end of maintenance for 30 days, yielding 100% in all treatments. The ANOVA analysis showed that adding katuk leaf powder at different doses to fish feed had no significant effect on any treatment. However, the survival data showed that treatment with katuk leaf powder resulted in good survival. Survival is the comparison of the Number of organisms that live at the end of the period and the Number of organisms that live at the beginning of the period (Ramadhan *et al.*, 2021). Increasing fish survival is primarily determined by several factors, namely providing sufficient, high-quality feed and maintaining well-maintained environmental conditions. However, a lack of feed and poor environmental conditions can impact fish health and reduce the survival of the fish

being kept (Akbar, 2012). The high average percentage of catfish survival in each treatment is thought to be influenced by the quality of water maintained through regular water changes; the quantity and quality of feed given have met the nutritional needs of catfish so that the feed given can be utilized optimally by the fish, so that the fish can survive (Indra *et al.*, 2021). Adequate quantities and types of feed can also support fish life and prevent cannibalism (Niode *et al.*, 2016). The ANOVA results showed that the average HSI values for treatments P1, P2, and P3 were not significantly different. HSI is often used as a parameter to assess various metabolic activities of fish (Pyle *et al.*, 2015); the average HSI value of catfish in treatment P1 was (1.92% ± 0.32). The average HSI value tends to increase in treatments P2 and P3, which are (2.03% ± 0.20) % and (2.45% ± 0.39), respectively. Increasing digestion can reduce the portion

of feed that is not digested and is wasted as feces. The health of the fish's liver affects its physiology. Awaludin *et al* (2025) reported that catfish given curcumin were able to reduce SGPT (serum glutamic pyruvic transaminase) and SGOT (serum glutamic oxaloacetic transaminase) levels.

Furthermore, the high availability of simple nutrients in the digestive tract triggers their absorption into the blood and then into cells. The marked increase in absorption in the combination treatment with

mixed microorganisms is evident in the hepatosomatic index. An increase in the hepatosomatic index indicates an increase in the amount of nutrients absorbed, so that the amount of nutrients accumulated in the liver increases. Du *et al.* (2005) stated that an increase in lipids follows an increase in lipids in the hepatosomatic index in juvenile gourami fish. Increasing lipid levels in feed can increase lipid deposits (Martino *et al.*, 2002).

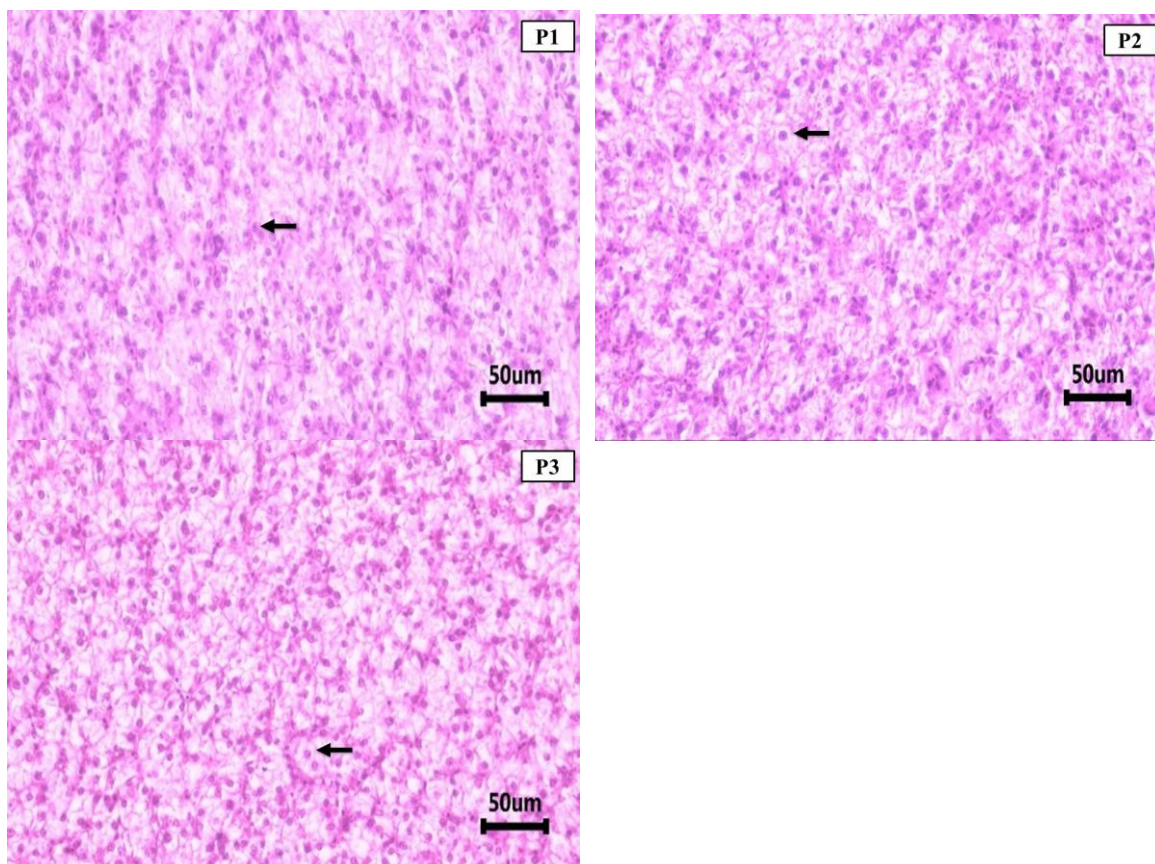


Figure 1. Histological structure of catfish liver (*Clarias* sp) with 40x magnification. Description: P1: Catfish liver (Control), P2: Catfish liver (added with 100 g katuk leaves powder), P3: Catfish liver (added with 150 g katuk leaves powder). Arrow: hepatocyte cells.

The liver is one of the most important vital organs in the body. It is located on the side of the stomach and is the largest organ in the fish's body. The liver functions to neutralize toxins and can secrete chemicals used in the digestion process. The liver also plays an important role in the body's metabolism. The main structure of the liver is composed of liver cells, or hepatocytes (Setyowati *et al.*, 2010). Catfish hepatocyte

cells at the end of the study are shown in Figure 1. The Number of hepatocytes observed in histological sections is shown in Table 3. Based on the results of observations, the histological structure of the catfish liver (*Clarias* sp) microscopically in the treatment that did not give katuk leaves powder and the treatment that was given katuk leaves powder showed an increase in hepatocyte cells. Statistically, the number of

hepatocytes in the fish liver was not significantly different across treatments. The highest number of hepatocytes was found in treatment P3 ($225,636 \pm 14,679$), followed by P2 ($224,560 \pm 12,098$), and the lowest in P1 ($225,636 \pm 14,679$). Hepatocytes play a central role in liver metabolism and also metabolize or detoxify fat. Hepatocytes are very active in synthesizing proteins and lipids for secretion.

Water quality observation data during the 30-day maintenance period under optimal conditions for catfish growth. Water quality parameters measured during the maintenance period were temperature, pH and DO. Temperature and pH were measured daily, while DO was measured every 7 days. Data on the quality of catfish maintenance water are shown in Table 4.

Table 4. Maintenance of water quality.

Parameter	Range	Range Optimum
Temperature (°C)	24,8°C-28,7°C	22-34°C
pH	7-7,4	6,5-8,5
DO (mg/L)	6,6-8,1	>3

Note: *SNI 2014 *Afifi, 2014

The results of temperature parameter measurements during the research period for each treatment did not show significant differences. The temperature range obtained during the study was 24.7°C – 28.7°C, pH 7-7.4, and DO 6.6-8.1 mg/L. This shows that the aquarium's temperature is still optimal.

CONCLUSION

Based on the results of the study, the provision of commercial feed mixed with katuk leaves powder (*S. androgynus*) containing fatty acids can increase the growth of catfish (*Clarias* sp) as observed from the growth and morphology of liver cells, so that katuk leaves can be used as organic raw materials in making feed. The optimal dose of adding katuk extract to feed for optimal growth is 150 g/kg.

Acknowledgements

Thanks to LPPM Universitas Borneo Tarakan for funding the 2023 Lecturer Competency Research (RKD) scheme with Number 128/UN51/KPT/2023.

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