

# Quality of fermented duckweed *Lemna* spp. and the effect in the growth response of striped fish *Pangasianodon hypophthalmus*

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**Abstract.** Duckweed (*Lemna* spp.) is an aquatic plant that has a high nutritional content, so it can be utilized as an alternative source of feed ingredients. This study aims to determine how the fermentation process can improve the physico-chemical quality of duckweed and analyze the dosage of fermented duckweed meal (FDM) that can support the growth response of striped catfish (*Pangasianodon hypophthalmus*). The research method using a completely randomized design. This study consisted of 4 treatments and 4 replicates with D0 (control), D10 (addition of 10% FDM), D20 (addition of 20% FDM), and D30 (addition of 30% FDM). This study was conducted for 40 days with several observation parameters, nutritional quality improvement, changes of physico-chemical parameters, specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), and water quality. Data were analyzed using ANOVA test. Significant differences were conducted at  $p < 0.05$ . Post hoc comparison using Duncan's test was utilized to determine the differences between the treatments. The fermentation process improved the physico-chemical quality of FDM by reducing the particle size, increasing the nutritional quality of FDM without changing the functional group composition of duckweed meal. Treatment D20 showed the best results where SGR is 3.19% body weight per day, FCR is 1.18, and SR is 97.5% respectively.

**Key Words:** feed efficiency, nutritional value, seed performance.

**Introduction.** Striped catfish (*Pangasianodon hypophthalmus*) is one of the leading commodities in Indonesia's freshwater aquaculture sector, even based on the Ministry of Marine Affairs and Fisheries of Indonesia data in 2022 (Ministry of Marine Affairs and Fisheries of Indonesia 2022), it is known that striped catfish cultivation covers 2% of total aquaculture production with total striped catfish cultivation production reaching 332,022.98 tons in 2021. Striped catfish has several advantages including that it is easy to cultivate, resistant to disease, has fast growth, and high economic value (Khan et al 2017; Mat Nawang et al 2019). These advantages have led to high production of striped catfish in Indonesia, which has even become one of the countries with the largest striped catfish production in Asia (Tien Thong et al 2017). However, the high market demand for striped catfish needs to be balanced with sufficient production. Increased production through accelerated growth response needs to meet the increasing demand for fish.

Fish growth response is obtained from the utilization of energy and essential amino acids obtained from fish feed (Konnert et al 2022). Increasing the growth response of striped catfish retrieve with various methods including tank coloration (Mat Nawang et al 2019; Ferosekhan et al 2020), feed nutritional value (Vu & Huynh 2020; Haque et al 2021; Khan et al 2018; Le Xuan et al 2022) and genetics (Vu et al 2019; Phuc et al 2017). In addition to the various efforts that have been made, increasing the growth response of striped catfish can also be achieved by utilizing natural ingredients which then undergo a material modification process to obtain feed ingredients that have good

physical and chemical quality. One of the natural ingredients that can be utilized as fish feed is duckweed (*Lemna* spp.).

Duckweed is an aquatic plant that can be found throughout the world and consists of only 5 genus (*Spirodela*, *Landoltia*, *Lemna*, *Wolffia* and *Wolffiella*) and 37 species (Cao et al 2020). *Lemna* spp. is one of the duckweeds that is easily available and has a high nutritional content which includes 28-40% protein, 5% crude fiber, high mineral content (Flores-Miranda et al 2015; Pinandoyo et al 2019; Herawati et al 2020). The use of *Lemna* spp. as fish feed has several obstacles, namely the high fiber content and the presence of antinutrients such as phytic acid and tannin that can inhibit fish growth (Flores-Miranda et al 2015) and the possibility of heavy metals, pesticides, phenols, and pathogens absorbed from polluted waters (van der Spiegel et al 2013). Several studies that have been conducted on the utilization of fermented *Lemna* spp. as fish feed have proven to be able to improve the growth performance of rohu fish, *Labeo rohita* (Bairagi et al 2002), climbing perch, *Anabas testudineus* (Akbar et al 2016), Nile tilapia, *Oreochromis niloticus* (Herawati et al 2015), rainbow trout, *Oncorhynchus mykiss* (Fiordelmondo et al 2022) and even shrimp, Pacific white shrimp, *Litopenaeus vannamei* (Flores-Miranda et al 2015). However, until now there has been no research on the utilization of fermented *Lemna* spp. as striped catfish feed.

The utilization of duckweed (*Lemna* spp.) as a feed ingredient for striped catfish certainly has enormous potential given its high nutritional content and abundance in nature. The fermentation process needs to be carried out to ensure that the crude fiber content and antinutrients contained in duckweed are reduced, to maximize the potential contained in the plant. The purpose of this study is to determine how the fermentation process can improve the physical-chemical quality of the material and to analyze the dosage of fermented duckweed meal (FDM) that can support the growth response of striped catfish.

## **Material and Method**

**Experimental diet.** Duckweed (*Lemna* spp.) was fermented using commercial probiotics containing *Saccharomyces cerevisiae*, *Lactobacillus* spp., and *Bacillus* spp. at a dose of 3 mL kg<sup>-1</sup>, which had previously been activated using 3% molasses. The fermentation process was carried out for 9 days at room temperature, dried and floured. Scanning electron microscopy (SEM) tests and Fourier transform infrared (FTIR) tests were conducted to determine the improvement of the physical-chemical quality of FDM through changes in particle size and functional groups. The square methods were used to count the formulation of fish feed with 30% protein content. The experimental diets were supplied to fish three times a day. Four experimental feeds were prepared with 0, 10, 20, 30% addition of fermented duckweed meal (FDM) on the commercial feed.

### **Physico-chemical quality tests of fermented duckweed meal**

**Scanning electron microscopy (SEM) test.** The test was started by preparing duckweed meal samples consisting of 2 samples, fermented and non-fermented, then the samples were placed in the specimen holder using a carbon double tip so that all electrons could be channeled entirely to the sample. SEM tests were carried out using a Hitachi TM3000 tabletop microscope. Furthermore, the sample chamber was vacuumed to ensure that there was no air in the SEM column. The microscope was operated with a work distance (WD) as high as 10 mm because it is expected that this setting can optimize X-Ray detection and enumeration.

**Fourier transform infrared (FTIR) test.** Samples of fermented and non-fermented duckweed meal were prepared and then 10 mg of sample was taken and blended with 100 mg of potassium bromide (KBr) and then molded into a disk shape. Furthermore, the samples were observed in the wavelength range of 4000-450 cm<sup>-1</sup> using FTLA 2000 spectrophotometer.

**Feeding trial.** The study was conducted at the Aquaculture Laboratory, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia, from February to August 2022. Striped catfish fry (*Pangasianodon hypophthalmus*) with a weight of 1 gram and a length of 5 cm approximately were reared in plastic tanks with a capacity of 32 liters of water with a density of 20 fish per tank (SNI 2009) and aerated continuously. The test feed was the addition of fermented duckweed meal to commercial feed with feed test treatments (D0, D10, D20 and D30). Feeding of striped catfish was done 3 times a day at 08.00, 12.00 and 16.00 with a feeding dose of 5% of the test fish biomass. Inedible feed and fish feces were cleaned every 2 days and water was changed by 50% (SNI 2000). Observation of water quality in the form of temperature, pH, dissolved oxygen (DO) and ammonia was conducted every 10 days.

The biological feeding test was conducted for 40 days with four treatments namely D0 (0% FDM), D10 (10% FDM), D20 (20% FDM) and D30 (30% FDM) with 4 replicates of each treatment. During the feeding trial, the water temperature ranged from 25 to 33°C, dissolved oxygen 5.5 to 7.0 mg L<sup>-1</sup>, pH 6.6 to 7.9 and ammonia 0.001 to 0.009 mg L<sup>-1</sup>. Water quality parameters during the biological test process were in the optimal range that could support the growth rate of striped catfish (Table 1).

Table 1  
Water quality parameters ranges measured during the experimental period

Treatment	Parameters			
	Temperature (°C)	DO (mg/L)	pH	Ammonia (mg/L)
D0	25 - 33	5.6 - 6.9	6.7 - 7.9	0.001 - 0.006
D10	26 - 33	5.5 - 7.0	6.8 - 7.8	0.002 - 0.009
D20	26 - 32	5.5 - 6.9	6.7 - 7.6	0.003 - 0.009
D30	27 - 33	5.5 - 7.0	6.6 - 7.9	0.003 - 0.005
Water quality standards	27 - 30	>5 Mg/L	6.5 - 8.5	< 0.02 Mg/L

Note: dissolved oxygen (DO); fermented duckweed meal (FDM); D0 (control), D10 (addition of 10% FDM), D20 (addition of 20% FDM), and D30 (addition of 30% FDM). Water quality standards according to SNI (2000).

Parameters regarding specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were calculated by following the formulas (Effendie 1979):

$$SGR (\% \text{ day}^{-1}) = (\ln W_t - \ln W_0) \times 100 / t;$$

$$FCR = \text{feed intake} / (W_t + D) - W_0 ;$$

$$SR (\%) = (\text{final fish count} / \text{initial fish count}) \times 100$$

Where t is the total of testing days, lnW<sub>0</sub> is the natural logarithm of the initial weight of experimental fish, lnW<sub>t</sub> is the natural logarithm of the final weight of experimental fish, and D is the weight (g) of fish that died during the feeding trial.

**Intestine histological analysis.** After the 40th day, 10 fish from each replicate were taken randomly according to the treatment and then dissected using a dissecting kit. Next, intestinal samples were taken at the anterior and posterior parts along 2 cm, then the samples were fixed using Bouin's fluid and soaked for 24 hours. After that, the object glass was placed on a hotplate to be heated for 15 minutes until all the water evaporated, then the sample was stained with hematoxylin and eosin for three minutes and rinsed using distilled water. The preparations were then placed on a Zeiss Primo Star Microscope for further observation by measuring the length and width of the intestinal villi at 40x magnification.

**Statistical analysis.** One-way variance (ANOVA) test was conducted to determine the level of difference in SGR, FCR and SR. If there is a significant difference from the ANOVA test results, it will be continued with Duncan's multiple range test with p<0.05 to determine the value between differences.

## Results

### **Physico-chemical quality improvement of duckweed meal**

**Duckweed particle structure.** Improvement of physical quality of FDM particles are the result of the fermentation process. The structure and shape of duckweed meal particles can be determined after SEM testing (Figure 1).

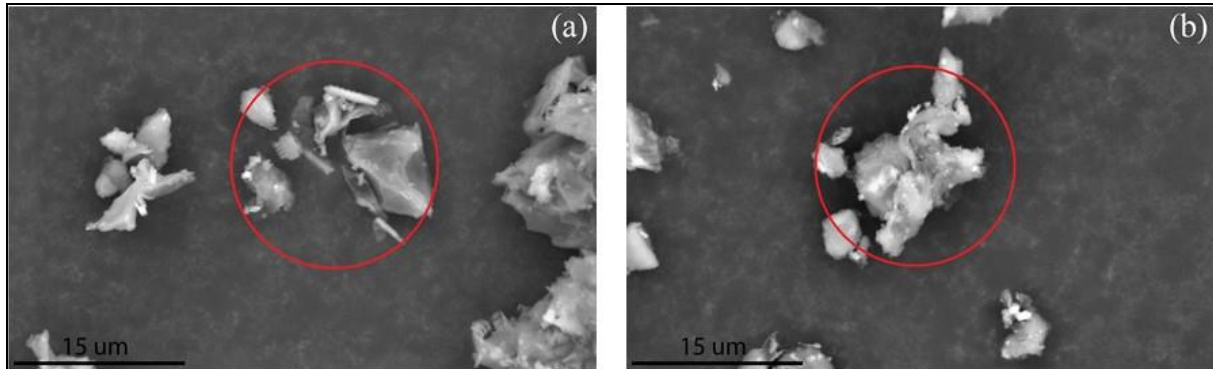


Figure 1. Morphology of duckweed meal: (a) fermented duckweed meal; (b) non-fermented duckweed meal (control).

Fermented duckweed meal (Figure 1a) showed dispersed, smaller, and broken particles. Meanwhile, non-fermented duckweed meal (Figure 1b) showed particles that were still fused, bound, and clumped together.

**Functional groups of duckweed meal.** The functional groups of a material can be known by conducting an FTIR test. The graph of FTIR test results (Figure 2) shows the peak vibrations of  $2920.33\text{ cm}^{-1}$  in non-fermented samples and  $2921.83\text{ cm}^{-1}$  explains the presence of C-H  $\text{sp}^3$  stretching (1), O-H stretching (2) is found at the peak vibrations of  $3271\text{--}3272\text{ cm}^{-1}$  in both samples, N-H bending (3) is seen at the peak vibrations of  $1634.21\text{ cm}^{-1}$  and  $1546.08\text{ cm}^{-1}$  in non-fermented samples and  $1596.42\text{ cm}^{-1}$  in fermented samples, O-H bending (4) is seen at  $1400\text{--}1413\text{ cm}^{-1}$  in both samples, C-N bending (5) can be seen in the vibration peak of  $1319.51\text{ cm}^{-1}$  in non-fermentation and  $1317.74\text{ cm}^{-1}$  in fermented samples, vibration peaks at wave numbers  $1018.15\text{ cm}^{-1}$  and  $1023.21\text{ cm}^{-1}$  show the presence of C-O stretching (6) and vibration peaks at  $599\text{ cm}^{-1}$  in both samples indicate the presence of C-H bending (7).

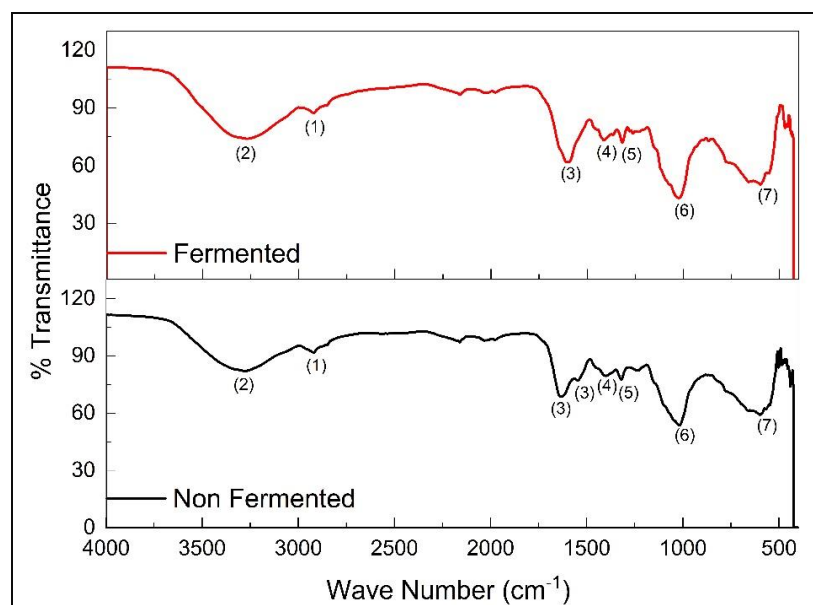


Figure 2. FTIR results of duckweed meal.

**Nutritional quality improvement of duckweed meal.** The fermentation process carried out on duckweed is an effort to improve the nutritional quality and reduce the antinutrient content (Figure 3).

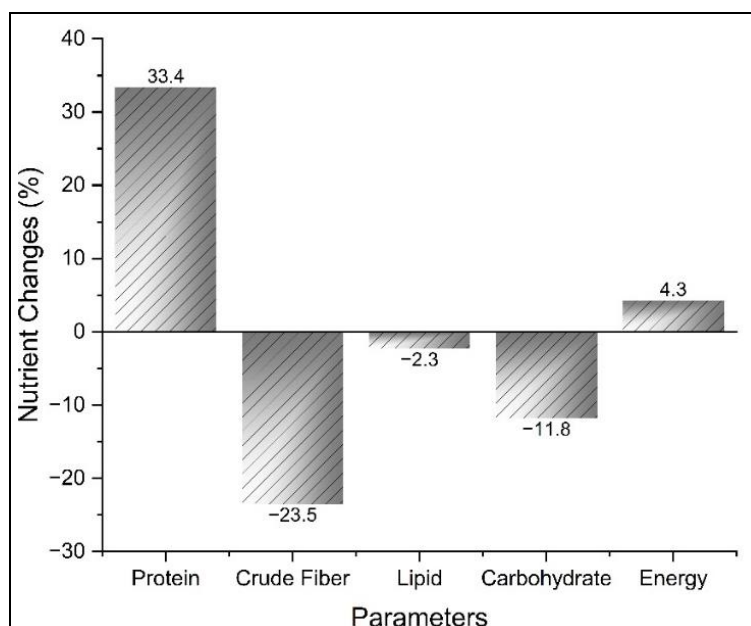


Figure 3. Nutritional improvement after fermentation process.

After the fermentation process, the protein and energy content increase to 33.4% and 4.3%, crude fiber, lipid and carbohydrate decrease 23.5%, 2.3%, and 11.8% respectively.

**Growth performance of striped catfish.** Growth is the increase in body weight and length in certain period which is the body's response to the feed given. Striped catfish growth performance variables, including specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) were fit to one-way variance (ANOVA) test (Table 2).

Table 2  
SGR, FCR and SR of striped catfish fingerlings with addition of FDM on fish feed for 40 days

Variables	Diets			
	D0	D10	D20	D30
Final weight (g)	2.75 ± 0.20 <sup>a</sup>	2.63 ± 0.09 <sup>a</sup>	3.24 ± 0.14 <sup>b</sup>	2.59 ± 0.17 <sup>a</sup>
Weight gain (g)	1.82 ± 0.14 <sup>a</sup>	1.63 ± 0.10 <sup>a</sup>	2.34 ± 0.19 <sup>b</sup>	1.72 ± 0.16 <sup>a</sup>
SGR (% day <sup>-1</sup> )	2.72 ± 0.27 <sup>a</sup>	2.43 ± 0.16 <sup>b</sup>	3.19 ± 0.16 <sup>c</sup>	2.72 ± 0.14 <sup>a</sup>
Feed conversion ratio	1.64 ± 0.17 <sup>c</sup>	1.77 ± 0.13 <sup>c</sup>	1.18 ± 0.08 <sup>a</sup>	1.57 ± 0.11 <sup>b</sup>
Survival rate (%)	98.75 ± 0.50 <sup>b</sup>	95 ± 1.15 <sup>b</sup>	97.5 ± 1.00 <sup>b</sup>	88.75 ± 0.50 <sup>a</sup>

Note: Different superscript letters indicate significant differences among treatments (Duncan test;  $p < 0.05$ ).

The growth performance of striped catfish fingerlings was affected by the addition of FDM on fish feed ( $p < 0.05$ ). Through the ANOVA test, the best supplementation of FDM was 20 g kg<sup>-1</sup> for SGR and FCR. Survival rate of striped catfish was similar among the D0, D10 and D20 treatments ranging from 95 to 98.75%, but D30 had the smallest SR of 88.75%.



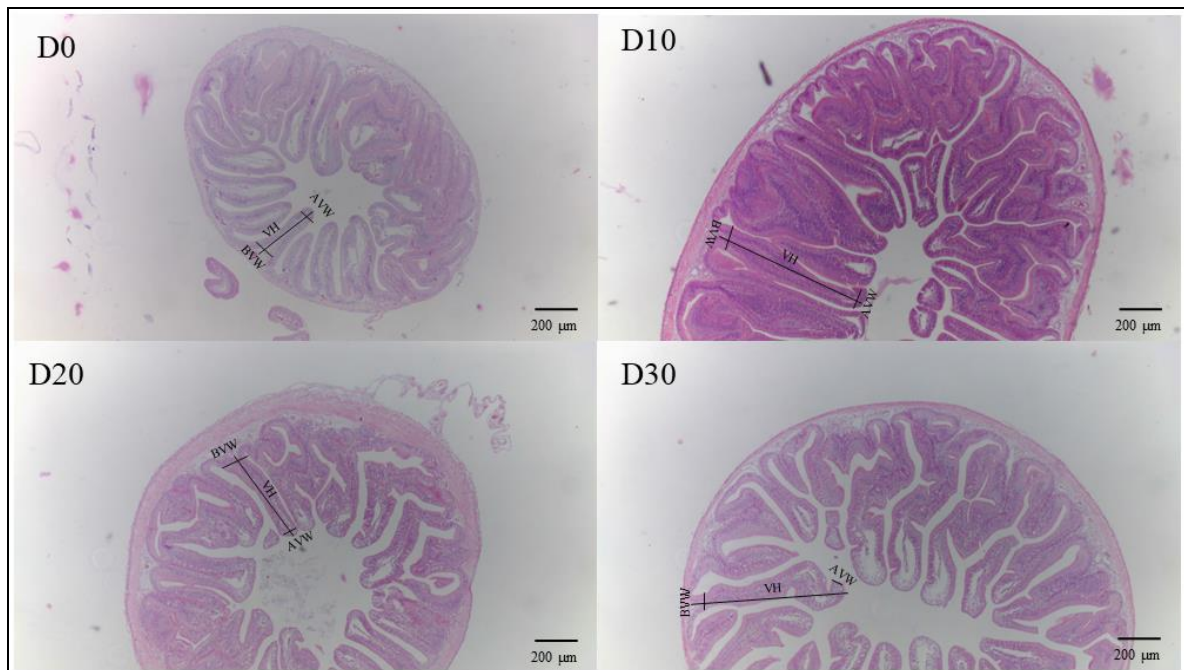


Figure 4. Intestinal villus of striped catfish fed with FDM after 40 days of experimental diets. VH (villi height), AVW (apical villi width), BVW (basal villi width).

The changes that occurred in the chemical composition of the simpler ingredients also affected the size of the intestinal villi of the test fish (Figure 4). The size of fish intestinal villi in the treatment with the addition of FDM became larger than the control treatment.

**Discussion.** The structure and shape of duckweed meal particles can be determined after SEM testing. Fermented duckweed meal (Figure 1a) showed dispersed, smaller, and broken particles. Meanwhile, non-fermented duckweed meal (Figure 1b) showed particles that were still fused, bound, and clumped together. The difference in the morphology of the duckweed meal occurs due to the activity of lactic acid bacteria in the fermentation process which causes the breaking of organic chain bonds which will also have an impact on changes in particle structure and morphology (Murtini et al 2016; Lu et al 2005). Changes in the morphological structure of FDM particles have a positive effect on the quality of the meal produced. The smaller the particle size of feed ingredients, the faster the feed will be digested by fish. This is in accordance with the statement of Andriani et al (2019) that a decrease in flour particle size will cause the rate of absorption of nutrients to be faster because the contact surface area that reacts with digestive enzymes becomes larger. The results of this study are in line with research conducted by Andriani et al (2020) and Majzoobi et al (2014) which proved that the fermentation process carried out can reduce particle size. In addition to having a positive impact on the speed of feed digestion, a decrease in particle size will make the process of mixing and combining fermented flour with other feed ingredients easier (Andriani et al 2020; Vukmirović et al 2017).

The functional groups of both fermented and non-fermented duckweed, have no difference based on the FTIR test results. The absence of changes in the composition of functional groups in both samples was due to because that in the processing of fermented duckweed there was no heat treatment that can cause carbon burning and the formation of new compounds. This is in accordance with the statement of Nasution and Rambe (2013) which state that high temperature treatment on a material can result in the loss of absorption of wave numbers and even the formation of new compounds. The results of this study are in line with Andriani et al (2020) in their research on the fermentation process of food waste which states that there are no differences in functional groups after the fermentation process. So, it can be concluded that the fermentation process carried out on the material can improve the physical quality of the

duckweed meal produced without having a negative impact on the composition of the functional groups contained in duckweed meal.

Changes in nutrient content after fermentation can be seen after conducting a proximate test (Figure 3). The 33.4% increase in protein content was due to the presence of single-cell protein sources, including *Bacillus* spp. and *Saccharomyces cerevisiae* from probiotics. *Bacillus* spp. is known to be one of the single-cell protein sources because it has a high protein content, reaching 65% (Inuhan et al 2016; Feliatra et al 2021). In addition, *Saccharomyces cerevisiae* also has a high protein content, reaching 60% (Gervasi et al 2017). Thus, an increase in the number of single-cell protein colonies will affect the increase in crude protein content of FDM. The decrease in crude fiber by 23.5% occurred after the fermentation process, this was due to the activity of cellulolytic and hemicellulolytic enzymes produced by *Lactobacillus* spp. which were able to break down cellulose into lactic acid, energy, and short chain fatty acids (SCFA) (Rahmawati et al 2015; Li et al 2020). Based on the results of this study, it is known that the fermentation process carried out on duckweed can improve the nutritional quality of duckweed meal by increasing protein and reducing crude fiber.

The specific growth rate in treatment D20 with a SGR value of 3.19% body weight day<sup>-1</sup>, was significantly different from treatment D0 at 2.72% body weight day<sup>-1</sup>, D30 at 2.72% body weight day<sup>-1</sup>, and the lowest treatment is D10 at 2.43% body weight day<sup>-1</sup>. Erfanto et al (2013) explained that the fermentation process can convert long chain proteins into short chain peptide bonds so that they will be easily absorbed by fish for growth. The changes that occurred in the chemical composition of the simpler ingredients also affected the size of the intestinal villi of the test fish (Figure 4). The size of fish intestinal villi in the treatment with the addition of FDM became larger than the control treatment. The increased size of the intestinal villi will have an impact on the increased digestion and absorption of nutrients that occur in the fish intestine (Xie et al 2020). The addition of FDM at 20% gives an increase in the size of the largest intestinal villi, this is because the addition of FDM will increase the digestibility of fish to feed to increase the size of intestinal villi. Haetami et al (2022) explained that the increase in the size of intestinal villi is closely related to the ability to digest and absorb nutrients derived from feed.

There is a difference in the best treatment in the addition of FDM, and this can occur due to differences in the composition of commercial feed, probiotic brands and probiotic doses used, allowing for differences in observation results. This is in line with the statement of Yanuar (2017), that the growth rate of fish is influenced by the type and quality of feed given and environmental conditions. Fish growth occurs due to excess energy, or that the amount of digested feed is greater than the fish needs for body maintenance, metabolism, and activity (Volkoff & Rønnestad 2020; Assan et al 2021; Yilmaz et al 2004). Hamre et al (2013) also stated that fish growth can occur if the amount of feed nutrients digested and absorbed by fish is greater than the amount needed for body maintenance, while slow growth occurs if the feed nutrients provided are inadequate.

The lowest feed conversion ratio is D20 at 1.18, followed by D30 at 1.57, then D0 with commercial feed only at 1.64 and the highest value is D10 at 1.77 (Table 1). The smaller the feed conversion ratio is, the better the feed will support fish growth. According to Rivaie et al (2023), feed conversion ratio in experimental fish is 1.5, meaning that the feed conversion value in all treatments can be said to be good, because in general it is still in the range. Lutful Kabir (2009) stated that the value of feed conversion ratio is influenced by feed protein in accordance with the nutritional needs of fish resulting in more efficient feeding. This shows that the provision of fermented duckweed in the feed mixture given can still be utilized for catfish which are classified as omnivorous fish with carnivorous tendencies, but some treatments are not more efficient or almost the same as the treatment with commercial feed. The high feed conversion value is thought to be caused by the remaining feed that is wasted and not utilized by fish (Iskandar et al 2019). A high conversion ratio is an indication that the feed used is not good for aquaculture production, while the smaller the feed conversion ratio, the better the quality of the feed given (Rahman et al 2020).

Feed efficiency depends on species (feeding habits and size or stadia of fish), water quality (oxygen, temperature, pH, and ammonia) and type of feed (quantity and quality) (Pěnka et al 2023). Striped catfish in the fry phase itself is an omnivorous fish that tends to be carnivorous (Wulansari & Ardiansyah 2013). The use of feed in this research containing fermented duckweed, which is a vegetable material with a high crude fiber and carbohydrate content, allows striped catfish fry to be required to adapt to the feed switch. In line with the statement of Rahardja et al (2011) that carnivorous fish generally have a lower ability to utilize feed carbohydrates than omnivores or herbivores, the fermentation process is needed when feeding using duckweed forage.

The survival rate of experimental fish in each treatment with a maintenance period of 40 days shows values ranging from 88.75% to 98.75% (Table 2). Fermentation of feed ingredients has a survival rate value above 80% because the ingredients processed through fermentation have the advantage of being easily digested by fish because the energy used by fish to digest is less, so that energy can be used to increase vitality. Fermented feed ingredients have higher nutritional value and become soft to increase digestibility, allowing fish to absorb more energy. This is in accordance with the statement of Sandra et al (2019) that the survival value will increase in line with the maintenance of fish health which is an effect that arises when the specific growth rate and feed conversion ratio are good. Arief et al (2011) state that the survival rate of fish is influenced by good aquaculture management by paying attention to stocking density, feed quality and water quality and disease. Water quality has a big role in supporting the survival rate of fish. Good feed that has good nutritional value can also keep survival rates high, especially if the feed goes through the fermentation stage so that it is of better quality.

**Conclusions.** The fermentation process of duckweed using commercial probiotics can reduce the particle size of meal production, improve the nutritional quality of duckweed (*Lemna* spp.) meal without changing the functional groups of fermented duckweed meal produced. The addition of 20% FDM (D20) had the best growth rate (3.19% day<sup>-1</sup>), feed conversion ratio (1.18), and survival rate (97.5%). So, it can be concluded that the use of FDM as a fish feed ingredient can support the growth rate of striped catfish (*Pangasianodon hypophthalmus*) and can support aquaculture activities.

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**Conflict of interest.** The authors declare that there is no conflict of interest.

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