



The combination of tofu dregs and *Rhodopseudomonas palustris* on *Oreochromis niloticus* growth performances

Aufal A. Robbani, Onie Ariyanti, Dimas Calvin, Mujiono, Ganjar A. Sutarjo, Hany Handajani, Soni Andriawan

Department of Aquaculture, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Malang, East Java, Indonesia. Corresponding author: S. Andriawan, soniandriawan@umm.ac.id

Abstract. Tofu dregs are a solution to the high feed prices of soybean meal. However, as it is, it cannot be compared with soybean meal, which has a great protein concentration. Single-cell protein bacteria, *Rhodopseudomonas palustris*, could be added to overcome this problem. These bacteria work to improve protein levels in feed through fermentation. This study aimed to evaluate the effect of *R. palustris* supplemented in handmade feed for promoting *Oreochromis niloticus* growth and nutrient absorption. This research was carried out from May to August 2021 at the University of Muhammadiyah Malang Fisheries Laboratory of Indonesia. The research used a Completely Randomised Design (CRD) with five treatments and three replications. The treatments included: T0⁺ - commercial feeds (HIPROVITE 781-1, containing 33% protein); T0 - handmade feed (without tofu pulp); T1 - handmade feed + 5% fermented tofu dregs; T2 - handmade feed + 10% fermented tofu dregs; T3 - handmade feed + 15% fermented tofu dregs. Absolute weight (Wm), absolute length (Lm), daily growth (SGR), total feed consumption (TFC), survival rate (SR), feed conversion ratio (FCR), protein retention (PR), the protein efficiency ratio (PER), fat retention (FR), and energy retention (ER) were assessed to identify the effect of the treatments for 30 days. Adding fermented tofu dregs to the handmade feed formulation increased growth and nutrient retention compared to the control groups. The measurement results revealed that all handmade feeds did not differ from the commercial feed ($p > 0.05$) for Wm, Lm, SGR, FCR, PR, PER, FR, and ER. In contrast, the handmade feed supplemented with fermented tofu dregs showed better data than the control group (T0).

Key Words: fermentation, handmade feed, single-cell bacteria, tilapia feed.

Introduction. Tilapia (*Oreochromis niloticus*) is from Africa (Prabu et al 2019; Ndobe et al 2021). However, these days, tilapia has become one of the potential commodities in Indonesia. Indonesia's Directorate-General for Aquaculture's 2020-2024 Strategic Plan calls for increased production of *O. niloticus* as a priority aquaculture product, making it one of the country's most widely farmed freshwater fish (Ndobe et al 2021). Increasing *O. niloticus* production leads to more costs for commercial feed. Moreover, it is a challenge every fish farmer must deal with because the price of fish feed is rising while the selling price of produced fish remains stagnant (Armen et al 2019). Feed plays a vital role in fish farming activities. 60 to 70% of production costs come from feed (Yulianingrum et al 2017). Unfortunately, homemade meals cannot yet replace commercial feeds. Approximately 70% of the raw materials needed to produce feed depend on imports, including fish meal, soy flour, and pollard hulls (Yakin et al 2019; Handajani et al 2021). Therefore, alternative, cheaper, easily accessible meals can reduce production costs (Vita 2017). Tofu dregs (TD) could be an alternative feed ingredient as a source of vegetable protein (Yakin et al 2019). The tofu industry in Indonesia has a productivity of 2.56 million tons per year, which results in 25 to 35% solid waste (Mathius & Sinurat 2001; Sadzali 2010). TD have been widely used as processed food and animal feed (Hikmah et al 2019). However, its utilization is not optimal due to its high water and fibre content. TD contain 23.5% protein and 22.65% crude fiber (Anggraeni & Rahmiati 2016). Dietary fiber (DF) has been viewed as an anti-

nutritional attribute because of its adverse effects on the uptake of nutrients of several fishes (Jha et al 2010; Zhong et al 2020; Staessen et al 2021).

The high crude fiber concentration (>5%) will affect the digestibility of feed (Handajani et al 2021). According to Van Doan et al (2019), fish can eat up to 23% of crude fiber before their growth slows down. Therefore, a different process is needed to reduce the fiber content in TD. Fermentation has been shown to improve crude protein content and reduce crude fiber content, anti-nutritional factors (ANFs), and hazardous materials in feed ingredients (Nkhata et al 2018; Dawood & Koshio 2020). Fermentation is the application of microbial metabolism to convert raw materials into higher-value products, such as organic acids, single-cell proteins, biopolymers, and antibiotics (Anasih et al 2021). It had been proved by Granito et al (2002) that those processes could improve *Phaseolus vulgaris* quality by reducing its crude fiber, including insoluble and soluble fiber (25 and 95%, respectively). On the other hand, TD cannot be compared with soybean meal in terms of protein content. Soybeans are the most significant crop, with 35 to 40% protein (Gui et al 2008). As a result, TD need to be improved to increase its protein level. Regarding this, *Rhodopseudomonas palustris* might potentially increase TD protein content. According to Wu et al (2019), many high-value biochemical compounds are found in *R. palustris*, including single-cell proteins, carotenoids (Car), and bacteriochlorophyll. Moreover, they are a photosynthetic bacteria (PB) representative strain studied as a probiotic in aquaculture to increase fish growth, water quality, and health (Zhang et al 2019). According to Alloul et al (2021), adding *R. palustris* (5 and 11%) improved the shrimp feed protein content by 5 to 10% through batch fermentation. Furthermore, they could reduce the feed conversion ratio and stimulate the better growth of *Litopenaeus vannamei* than commercial feed after 30 days of cultivation. This study aims to evaluate fermented TD's effect using single-cell protein to promote *O. niloticus* growth and feed intake.

Material and Method

Fish and tofu dregs preparation. This research was carried out for three months, starting from June to August 2021, at the Fisheries Laboratory of the University of Muhammadiyah Malang, Indonesia. The experiment lasted 30 days. The test fish, *O. niloticus*, had 5 cm and were provided by the fisheries laboratory. They were acclimated for seven days. During the acclimatization process, *O. niloticus* was fed with the commercial feed (HIPROVITE 781-1, containing 33% protein) with 3% of total biomass. TD samples were obtained from a tofu factory in Karangploso, Malang Regency, Malang. TD were washed and pressed to reduce the water content before being steamed for 30 to 60 min, then cooled. Steaming aimed to sterilize the TD.

The present study used 15 aquariums (60×30×30 cm²) to run the experiment. The aquarium was filled with water; 80% of the total volume was provided with aerators. 15 *O. niloticus* were added to each aquarium treatment. Fish were fed two times daily at 08.00 and 17.00 with handmade feed.

Experimental design. The research used a completely randomized design (CRD) with five treatments and three replications. The treatments were represented by the addition of TD enriched with *R. palustris* combined in handmade feed. The details of the treatments are as follows: T0⁺ - commercial feed (HIPROVITE 781-1, containing 33% protein); T0⁻ - handmade feed without fermented TD; T1 - handmade feed plus 5% fermented TD; T2 - handmade feed plus 10% fermented TD; T3 - handmade feed plus 15% fermented TD.

Bacteria starter preparation and tofu dregs fermentation. The bacteria were obtained from an aquaculture company in Pasuruan, East Java, Indonesia. The bacteria starter was made by mixing banana peel, coconut water, molasses, aquades, yeast, and *R. palustris*. After stirring, the mixture was placed into a tightly closed container and fermented for seven days. Afterwards, the bacterial starter solution was tested at the protein level employing Lowry methods provided by Biotechnology Laboratory, University

of Muhammadiyah Malang, Indonesia. The protein analysis followed Waterborg (2009) using the Biuret reaction and the Folin reagent. Following the next step, the bacteria starter was added to tofu dregs, mixed, and then put in a plastic bag for seven days. Before and after fermentation, a proximate test was employed to see if there was an increase in TD protein content. The fermented TD were mashed and formulated in tilapia feed (Ramadhan 2019).

Handmade feed preparation. The treatment feed was produced based on the proximate analysis of the ingredients (Table 1). The nutrition Laboratory at the University of Muhammadiyah Malang, Indonesia, provided the proximate analysis.

Table 1
Proximate analysis of feed ingredients

Feed ingredients	Ash (%)	CP (%)	F (%)	CF (%)	NE (%)	Energy (kcal g ⁻¹)
Fish flour	6.73	52.57	7.67	6.34	21.01	3102.4
Soybean meal	2.82	35.1	8.1	5.21	34.61	3082.87
Cornstarch	2.76	10.54	5.39	3.63	67.66	3237.02
Rice bran	17.4	5.75	3.68	32.64	31.86	1706.48

Note: CP - crude protein; F - crude fat; CF - crude fiber; NE - nitrogen-free extract.

Handmade feed was made by combining the ingredients of the feed formulation with fermented tofu dregs according to the treatment (Table 2). The meal mixed with the other ingredients was sun-dried for three days.

Table 2
Handmade feed formulation and proximate analysis

Feed ingredients	Treatment			
	T0-	T1	T2	T3
Fish flour	40	38	36	34
Soybean Meal	25	23.75	22.6	21.25
Cornstarch	24	22.8	21.6	20.4
Fine bran	5	4.75	4.5	4.25
Fish oil	2	1.9	1.8	1.7
Mineral Mix	1	0.95	0.9	0.85
Multivitamins	2	1.9	1.8	1.7
CMC	1	0.95	0.9	0.85
Fermented tofu dregs	0	5	10	15
Total	100	100	100	100
The calculation results				
Ash (%)	11.97	11.77	11.53	11.19
CP (%)	25.83	27.63	32.66	32.85
F (%)	5.86	6.55	6.92	6.87
CF (%)	8.45	8.29	7.76	7.47
NE (%)	44.35	42.43	37.98	38.5
Energy (kcal g ⁻¹)	2.798	2813	2.828	2.853

Note: CMC - carboxy methyl cellulose; CP - crude protein; F - crude fat; CF - crude fiber; NE - nitrogen-free extract.

Observed parameters. Parameters in the form of absolute weight, absolute length, specific growth rate (SGR), survival rate (SR), total feed consumption (TFC), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), fat retention (FR), energy retention (ER), and water quality, including temperature, pH, dissolved oxygen (DO), and ammonia (NH₃).

The growth performances were determined after 30 days of cultivation using the applicable formula from other studies (Dewi & Tahapari 2018; Nazlia 2019; Restianti et al

2016; Sonavel et al 2020; Sukmaningrum et al 2014). Meanwhile, the water quality parameters were measured every three days using various tools, such as a thermometer (temperature), pH pen (pH), and a kit for ammonia level determination.

$$W_m = W_t - W_0 \text{ (Sonavel et al 2020)}$$

$$L_m = L_t - L_0 \text{ (Sonavel et al 2020)}$$

$$TFC = F_1 - F_2 \text{ (Sonavel et al 2020)}$$

$$SR = \frac{S_t}{S_0} \times 100 \text{ (Dewi \& Tahapari 2018; Nazlia 2019)}$$

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100 \text{ (Restianti et al 2016 Nazlia 2019)}$$

$$FCR = \frac{\sum F}{(W_t - W_0)} \text{ (Dewi \& Tahapari 2018; Nazlia 2019)}$$

$$PER = \frac{W_t - W_0}{P_i} \times 100 \text{ (Restianti et al 2016)}$$

$$PR = \frac{P_t - P_0}{P_t} \times 100 \text{ (Dewi \& Tahapari 2018; Nazlia 2019)}$$

$$FR = \frac{F_t - F_0}{F_i} \times 100 \text{ (Dewi \& Tahapari 2018)}$$

$$ER = \frac{(W_t \times E_t) - (W_0 \times E_0)}{FE} \times 100 \text{ (Sukmaningrum et al 2014)}$$

Where: W_m - absolute weight gain (g); L_m - absolute length (cm); SGR - specific growth rate (g); SR - survival rate (%); TFC - total feed consumption (g); FCR - feed conversion ratio; PER - protein efficiency ratio (%); PR - protein retention (%); FR - fat retention (%); ER - energy retention (%); W_t - final average weight (g); W_0 - initial average weight (g); L_t - final average length (cm); L_0 - initial average length (cm); t - time (day); N_0 - initial number of fish; N_t - final number of fish; F - the number of feed; F_1 - uneaten feed; F_2 - eaten feed; P_i - amount of feed consumed x % feed protein; P_t - final fish protein; P_0 - initial fish protein; F_t - final fish fat; F_0 - initial fish fat; F_i - amount of feed consumed x % of feed fat; E_t - final body energy; E_0 - initial body energy; FE - feed energy.

Statistical analysis. All the data were analyzed using the analysis of variance (ANOVA) function provided by the SPSS software. Furthermore, the data were subjected to Duncan's Multiple Range Test to determine whether there were differences across treatments.

Results and Discussion

The protein content of the bacteria starter. Based on the Lowry analysis, the protein concentration of *R. palustris* culture was 57.87%. Starter bacteria color became red after seven days of fermentation (Figure 1) and smelled like alcohol.



Figure 1. The bacteria starter after fermentation.

Proximate analysis of tofu dregs. The proximate results of TD before and after fermentation using *R. palustris* are presented in Table 3. Some data showed a decrease after fermentation, such as ash content, crude fat, crude fiber, nitrogen-free extract, and energy, while the protein value increased three times, from 5.27% to 15.43%. Moreover, there was a considerable decline in crude fiber content from 21.8% to 14.64%, but this content could still affect *O. niloticus* growth.

Table 3

Tofu dregs before and after fermentation

Feed ingredients	Ash (%)	CP (%)	F (%)	CF (g)	NE (%)	Energy (kcal g ⁻¹)
Tofu dregs	2.94	5.27	13.71	21.8	53.76	2.734
Fermented tofu dregs	2.66	15.43	7.57	14.64	39.07	2.665

Note: CP - crude protein; F - crude fat; CF - crude fiber; NE - nitrogen-free extract.

Tilapia growth performances. Table 4 shows the growth performances and feed consumption of handmade feed compared to the commercial feed. The data showed a significant difference for five measurements. Overall, the commercial feed and T3 shared the best parameters, while T0⁻ is not recommended. In the case of absolute weight, the addition of 15% fermented TD (T3) and commercial feed shared the highest level, accounting for 4.3±0.37 g and 4.05±0.35 g, respectively, compared to the control group (T0⁻).

Table 4

The growth rate, survival rate, and feed utilization of *Oreochromis niloticus* after 30 days of feeding trial

Treatment	Absolute weight (g)	Absolute length(cm)	SR (%)	TFC (g)	FCR
T0+	4.05±0.35 ^b	2.11±0.35 ^{ab}	66.67±13.33 ^a	67.33±1.52 ^a	1.12±0.1 ^{ab}
T0-	3.22±0.41 ^a	1.87±0.41 ^a	66.67±6.67 ^a	62.67±8.02 ^a	1.30±0.23 ^b
T1	3.84±0.26 ^{ab}	1.94±0.26 ^{ab}	64.44±10.18 ^a	64.67±3.05 ^a	1.12±0.21 ^{ab}
T2	4.02±0.31 ^{ab}	2.16±0.31 ^{ab}	78.00±15.40 ^a	68.00±2.00 ^a	1.13±0.06 ^{ab}
T3	4.30±0.37 ^b	2.45±0.37 ^b	66.67±17.64 ^a	68±5.29 ^a	1.06±0.14 ^a

Note: SR - survival rate; TFC - total feed consumption; FCR - feed conversion ratio; different superscripts show significant differences (p<0.05).

Absolute weight was almost the same in all treatments ($p>0.05$), except $T0^-$ and $T3$, which had a big gap (1.87 ± 0.41 g versus 2.45 ± 0.37 g, $p<0.05$) in absolute length. For SR and TFC, the data presented insignificant differences. As a result, the feed treatment did not affect SR and TFC during the 30-day maintenance. Finally, the FCR values were significantly different among treatments. $T3$ and $T0^-$ presented a noticeable difference ($p<0.05$), but they were not different from other treatments in the case of FCR.

The SGR in different treatments showed significant differences ($p<0.05$). Data analysis revealed that $T3$ was the best treatment to increase the SGR value of *O. niloticus*, while the lowest level belonged to $T0^+$ containing commercial feed. In the first week, $T0^+$, $T0^-$, and $T3$ had the best SGR values, reaching 3.06, 3.14, and 3.08 g, respectively. In contrast, the lowest SGR was obtained in $T1$ and $T2$, with 1.62 and 1.77 g, respectively. Furthermore, the SGR values were evenly distributed among treatments in the 2nd week. There were only two treatments, $T2$ and $T3$, which showed differences ($p<0.05$) in the SGR value compared to the control group ($T0^-$), but the values were not different from those of $T0^+$ and $T1$, with 2.52 and 2.55 g, respectively. Following the next week, all SGR data leveled off. Statistically, that means the treatment did not impact *O. niloticus* growth. Finally, handmade feed completed with fermented TD stimulated the SGR level of *O. niloticus* better than the control group (commercial feed), which only increased *O. niloticus* SGR by 1.34 g (Figure 2). Regarding our discoveries, the present study suggests that handmade feed supplemented with fermented TD (5 to 15%) could compete with commercial feed. Therefore, it might be applied in aquaculture production and replace the industrial proteic meal for some species.

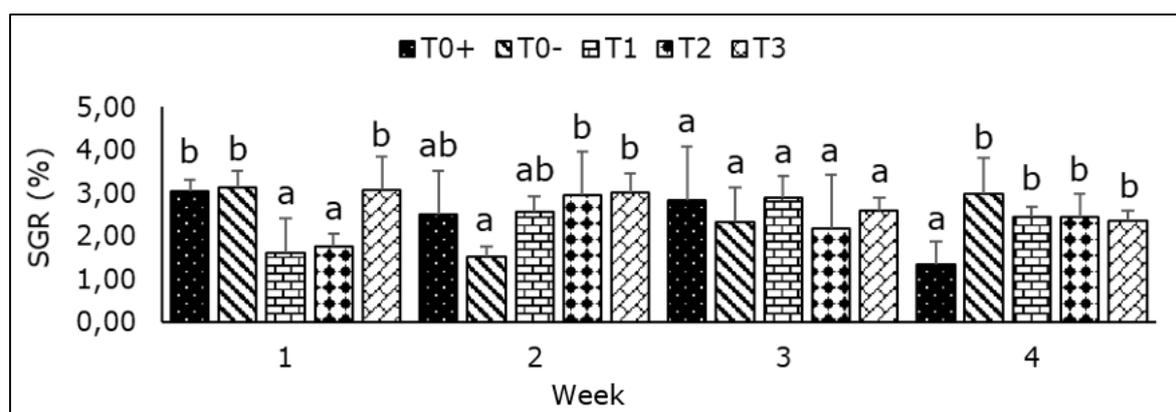


Figure 2. The weekly growth rate of tilapia for 30 days; different letters above columns show significant differences ($p<0.05$).

Protein, fat, energy retention, and protein efficiency ratio. Figure 3 presents the PER, PR, FR, and ER levels of *O. niloticus* after the feeding treatment for 30 days. There are significant differences among treatments ($p<0.05$). In general, it could be said that $T3$ is the best treatment, reaching the highest level of the four measurements. Meanwhile, $T0^-$ was last in terms of value levels. PER, PR, and FR had similar values statistically. $T3$ had the optimum dosage of TD to stimulate *O. niloticus* PER, PR, and FR, accounting for 3.22%, 10.47%, and 6.31%, respectively. On the opposite side, $T0^-$ had the smallest percentage for these values, of 2.70%, 8.18%, and 4.95%, respectively. $T0^+$, $T1$, and $T2$ revealed no differences. The ER analysis showed that $T0^+$ and $T3$ shared the highest ER percentage at the end of the period, of 82.82% and 84.79%, respectively. $T1$ and $T2$ had 77% and 77.24% ER levels, respectively. Our study recommends the handmade feed supplemented with fermented TD for promoting *O. niloticus* nutrient retention at the same levels as commercial feed.

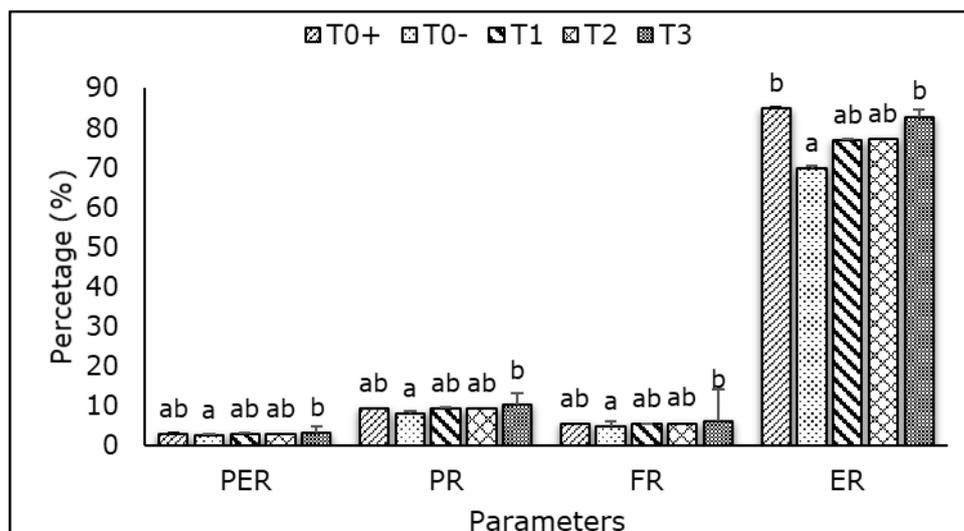


Figure 3. The protein, fat, energy retention, and protein efficiency ratio of *O. niloticus* after feeding trial for 30 days.

Water quality indicators. The water quality values, including temperature, pH, DO, and ammonia during the research can be seen in Table 5. Overall, the water quality during the study was suitable for supporting *O. niloticus* growth performance in all treatments.

Table 5
Water quality during the study

Parameter	T0+	T0-	T1	T2	T3	Indonesian standard 6141:2009
Temperature (°C)	25-29	26-28	26-29	26-29	25-29	25-30
pH	7.5-8	7.5-8	7.5-8.1	7.5-8.1	7.5-8.1	6.5-8.5
DO (mg L ⁻¹)	4.8-8	4.9-8	4.6-6.5	5-7	4.5-6.9	>5
Ammonia (mL L ⁻¹)	0.25-1	0.25-1	0.25-0.5	0.25-0.5	0.25-1	<1

Note: T0+ - commercial feed (control); T0- - 100% formulated feed; T1 - feed formula 95% + fermented tofu dregs 5%; T2 - feed formula 90% + fermented tofu dregs 10%; T3 - feed formula 85% + fermented tofu dregs 15%.

According to our results, the fermentation process and the addition of *R. palustris* improve the nutritional content of TD. The fermentation can reduce crude fiber, ash content, crude fat, NE, and energy. In comparison, the protein content increased from 5.27% to 15.43% after the fermentation process for seven days. According to Mutia et al (2019), the fermented material will be easily digested, and the amino acids and vitamin contents will improve. Fermentation improves proteins and carbohydrates digestibility and provides minerals through phytase synthesis (Samtiya et al 2021). Zhang et al (2013) noted that the protein content of distiller grains enhanced significantly from 11.4 to 33.6% (w/w) after fermentation with *R. palustris*. Moreover, fermentation using *R. palustris* reduces the crude fiber content of TD to 14.64%, which might still inhibit *O. niloticus* growth. According to Ihtifazhuddin et al (2017), fermentation for two to four days can reduce the crude fiber content.

The handmade feed with fermented TD increased tilapia weight and length gain. In the case of the SGR and FCR, the handmade feed supplemented with fermented TD showed a better result than the commercial feed. The fermented TD with *R. palustris* worked well to promote *O. niloticus* growth. As a probiotic, *R. palustris* has been widely used in fish farming to boost fish growth and water quality and promote the health of fish (Wang 2011; Peirong & Wei 2013; Zhang et al 2019). *R. palustris* stimulating the development of *Litopenaeus vannamei* has also been proved by Melgar Valdes et al

(2013). Probiotic bacteria containing *R. palustris* improved pH regulation and decreased nitrate concentration.

(TD) have long been recognized as a suitable pellet substitute because they are nutritionally adequate, particularly in protein content, but also rich in carbohydrates, fat, and fiber (Anshar & Mulyawan 2021). Therefore, their combination in this study have improved protein levels in handmade feed (Table 2) and positively affected *O. niloticus* growth (Table 4). SR could be affected by several factors such as feed management, water quality (Utomo et al 2017), disease, fry quality, and stocking density.

For PR and PER, T3 was the best treatment. It increased the protein retention value above that of the control group (T0). The protein content in the handmade feed is needed for the growth of tilapia. The present study assumed that T3 provided a better content of amino acids, easy to be absorbed. The breakdown of amino acids in the fish body is due to the high-efficiency ratio of the protein (Restianti et al 2016). According to Khalida et al (2017) and Tomaso & Azhari (2019), biologically valuable protein in feed promotes greater protein accumulation in the body. It was proved that the addition of lysine encouraged the feed protein retention of 4.8% by *Colossoma macropomum*. Feeding a diet rich in protein will stimulate the growth of new tissue, increasing the animal's rate of development (Marzuqi et al 2012).

Moreover, Zamani et al (2020) proved that single-cell protein (SCP) promoted PER of *Oncorhynchus mykiss* by substituting 50% fishmeal. Another SCP, *Clostridium autoethanogenum*, also worked well to boost PER considerably compared to the control group replacing 58.2% diet (Chen et al 2020). The PER value is strongly influenced by the levels of protein and other components in food ingredients (Rahmawan & Arini 2014).

The FR value in tilapia increased significantly compared to that of the control group (without fermented TD). Fish FR refers to the ability to store and utilize the fat consumed. The energy provided by feed fat is crucial. Fat also aids the metabolic processes and regulates the fish buoyancy in the water (Arief et al 2014). In this case, T3 was the best treatment, promoting the FR of *O. niloticus*, demonstrating that the assessed diet is of excellent quality regarding fat content (NRC 2011). The increase of FR in each treatment might be caused by the activity of lactic acid bacteria contained in the fermented TD. They could provide an acidic atmosphere to eliminate pathogenic bacteria that cannot tolerate an acidic environment (Turnip et al 2018). Changes in FR values between the control and T3 groups may be due to differences in dietary fatty acid profiles and lipid groups (Morais et al 2004). TD enriched with *R. palustris* may have impacted the digestibility of the diet, as other investigators have documented. For instance, a study conducted by Liu et al (2020) discovered that *R. palustris* increased TOR and NF- κ B signaling pathways, digestion capacity, nonspecific immune function, and antioxidant activity by increasing the expression levels of associated genes.

T3 elevated the ER compared to other handmade treatments, even though it did not differ from the commercial feed (33% protein). According to Sukmaningrum et al (2014), treated fish are usually more efficient in using energy for daily growth and metabolism. Energy content in T3 was suitable for the needs of *O. niloticus*, so that fish could optimally absorb the feed. The feed composed of ingredients with the right protein-energy balance, with proper feeding, results in good growth and feed conversion (Khalida et al 2017). The present study also assumed that changes in the protein level affect the amount of protein retained, leading to differences in the energy value produced. Haryati & Pranata (2011) stated that energy retention is closely related to the protein content of the feed, because it is the main energy source for the growth of fish.

Finally, water quality parameters, such as temperature, pH, dissolved oxygen, and ammonia, were at good levels for *O. niloticus* growth based on the Indonesian Aquaculture Standard 6139:2009 (Indonesian National Standard 2019). According to Azhari & Tomaso (2018), the optimal temperature range in freshwater fish farming is 25 to 30°C, in line with research results. Moreover, low oxygen levels can cause a decrease in fish appetite, affecting the growth rate and survival of fish (Noviana 2014). The oxygen content of this study was maintained between 4.6 and 8 mg L⁻¹. The pH values ranged from 7.5 to 8.1. These results included optimal conditions, tilapia preferring pH between 6.5 and 9 (Nugroho et al 2014). Acidity (pH) that is not optimal causes stress

for fish, susceptibility to disease, and low productivity and growth. Finally, ammonia levels in the study ranged from 0.25 to 1 mg L⁻¹, which was still optimal because it did not exceed 1 mg L⁻¹. Khalida et al (2017) stated that, in general, the concentration of ammonia in water should not exceed 1 mg L⁻¹.

Conclusions. The study results concluded that enriching tofu waste using single-cell bacteria *R. palustris* positively affected *O. niloticus* growth and nutrient utilization, except SR and FCR, compared to handmade control feed (without fermented TD). Moreover, the handmade feed in this study has the potential to be produced on a large scale, because it could compete with the control feed based on a commercial feed. Further study might be needed to evaluate the optimum dosage for boosting the growth performances of fish.

Acknowledgements. The authors would like to thank the Director-General of Higher Education from the Ministry of Education, Culture, Research, and Technology, who has provided funding for this study through the Student Innovation Program and to the Laboratory of Fisheries, Faculty of Agriculture and Animal Science, the University of Muhammadiyah Malang, which facilitated equipment for this research.

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

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Received: 10 January 2022. Accepted: 19 March 2022. Published online: 22 August 2022.

Authors:

Aufal Ahdi Robbani, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: aufalaar@gmail.com

Onie Ariyanti, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: oniarisyanti3@gmail.com

Dimas Calvin, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: dimascalvin99@gmail.com

Mujiono, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: mujiyono0802@gmail.com

Ganjar Adhywirawan Sutarjo, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jalan Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: ganjar@umm.ac.id

Hany Handajani, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: handajani@umm.ac.id

Soni Andriawan, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Aquaculture, Jl. Raya Tlogomas 246, 65144 Malang, East Java, Indonesia, e-mail: soniandriawan@umm.ac.id

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How to cite this article:

Robbani A. A., Ariyanti O., Calvin D., Mujiono, Sutarjo G. A., Handajani H., Andriawan S., 2022 The combination of tofu dregs and *Rhodopseudomonas palustris* on *Oreochromis niloticus* growth performances. *AAFL Bioflux* 15(4):2113-2124.