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Research

Replacement of Fish Oil with Engkabang Butter Oil (*Shorea macrophylla*): Effects on Growth Performance of Javan Mahseer (*Tor tambra*)

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ABSTRACT

The effectiveness of *Shorea macrophylla* butter oil (SMBO) as a replacement for fish oil on the growth performance of Javan Mahseer (*Tor tambra*) is still understudied. The purpose of this research was to examine the proximate composition, fatty acid profile, and efficacy of SMBO as an alternative lipid source to replace fish oil on the growth performance of *Tor tambra* (Javan mahseer). A 1-month feeding trial was done using 150 tails of Javan Mahseer fingerlings with an average weight of 3.06±0.17 g, using 5 feed treatments randomly assigned in triplicates. Dietary treatments included (0%) fish oil based as control (C) and SMBO inclusion of 1.25% (T1), 2.5% (T2), 3.75% (T3) and 5% (T4) respectively. The fatty acid composition of *Shorea macrophylla* oil and the proximate composition of treatment diets were determined while growth indices (BWG, FCR & FI) were measured every 10 days. Water quality measurements like pH, dissolved oxygen, and temperature were taken every week. Data were analyzed using One-Way ANOVA. Results showed that BWG was higher at 45.51% after day-10 in C (5% fish oil & 0% *Shorea macrophylla* butter oil) while FCR were C (0.86), T1 (1.17), T2 (1.14), T3 (1.18), and T4 (1.09). It can be concluded that 5% *Shorea macrophylla* oil in the diet may improve the growth performance of *Tor tambra* fingerlings.

Key words: Engkabang, fatty acid, growth, lipid, mahseer, *Tor tambra*

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INTRODUCTION

Aquaculture is one of the industries that encourages the consumption of aquatic foods and is a significant contributor to the world's food and protein sources. This industry is forecasted to grow even more in the coming years as a direct result of increased consumer demand brought on by a rising global population (Edwards *et al*., 2019). The future of the aquaculture industry, on the other hand, will be dependent not only on the population of the world but also on changing phenomena like COVID-19 that impact aquaculture. COVID-19 has impacted the lives and living conditions of over six million people across the globe and has resulted in huge societal and economic ramifications (WHO, 2020, Wu *et al*., 2020). The status of the aquaculture industry in Malaysia was also impacted because of the movement control order, as higher levels of live fish stocks as a result of unsold production led to increased expenses involving fish nutrition and fish mortality for farmers (Azra *et al*., 2021; FAO 2021). Due to that, extensive research on fish nutrition needs more attention to help farmers understand the importance of good aquaculture practices.

Tor tambra, also known as the Javan Mahseer species of Tor is often found in Peninsular Malaysia (Walton *et al*., 2017). Unfortunately, this species is listed as an endangered species in the IUCN Red List of Threatened SpeciesTM (Version 2020-3), which resulted from a declining population of this species in their natural habitat due to human activity, including existing construction of dams in rivers, deforestation, overfishing, pollution, and agricultural development (Pinder *et al*., 2019; Jaafar *et al*., 2021). To meet the ever-increasing demand for *T. tambra* (Javan mahseer), production and conservation efforts have also been ramped up to guarantee that this population can be maintained for the foreseeable future (Muchlisin *et al*., 2016; Pinder *et al*., 2019). By supplying feed supplies that can meet the optimal nutritional requirements of this species, it is possible to produce this species with a quality that is comparable to those that dwell in their nature.

Tor tambra and the *Tor tambroides* consume a wide variety of foods. Mollusks, freshwater crustaceans, and plants are among the scavenged food items that they consume (Kamarudin *et al*., 2018). *Tor tambroides* are fish that can ingest wild fruits that grow along the riverbanks of Sungai Kapit (Redhwan *et al*., 2022a) According to natives who live close to Kapit River in Sarawak, *T. tambroides* feed not only on *S. macrophylla*, which is also known as “buah engkabang” by villagers of Sarawak, but this fish also appears to consume other fruits such as ensurai (*Dipterocarpus oblongifolius*), kelampok or jambu air (*Syzygium aqueum*), buah ara (*Ficus* sp.), and even oil palm fruit (*Elaeis guineensis*) (Redhwan *et al*., 2022a).

S. macrophylla also known as “buah engkabang”, and “buah meranti” is distributed on Borneo Island. This fruit has a lipid-rich content, which is primarily made up of consistently efficacious triglycerides, with 18:0,18:1n-9 and 16:0 as main fatty acids (Rakman *et al*., 2021). The fruit is often sold at wet markets in Sarawak eaten as cocoa butter and used as cooking oil. As this lipid has good oxidative stability (Abdul Rahman & Basri 2013), it is suitable to be used in feed to avoid oxidation of beneficial fatty acids in tissues. According to Ramezani-Fard *et al*. (2012), the diet of the Malaysian mahseer has to have a minimal percentage of n-3 PUFAs as well as a massive amount of saturated (SFA) and monounsaturated (MUFA) fatty acids to achieve optimal development in its tissues.

Extensive research has been conducted to identify new local ingredients that can be utilized as essential replacement ingredients in feed nutrition. Black soldier flies and waste from crab shells are two examples of alternative feed ingredients that are gaining popularity as substitutes for traditional ingredients (Zomorni *et al*., 2022; Mohamad-Zuki *et al*., 2022a; Mohamad-Zuki *et al*., 2022b) It is crucial to ensure that nutrition research for fish species meets their nutrient requirements without causing harm to the ecosystem, which can be achieved by incorporating new and sustainable ingredients.

While there has been a study conducted to investigate the effects of *S. macrophylla* on *T. tambroides*, no research has yet examined the dietary impact of *S. macrophylla* on *T. tambra*. As *T. tambra* is not a well-known species as compared to *T. tambroides*, Its specific nutritional needs have not received much attention from nutritionists and researchers.

MATERIALS AND METHODS

Sample analysis

Engkabang butter oil purchased from an Iban’s community at Kanowit, Sarawak was analyzed for its fatty acid profile AOAC 20th Edition, 996.06 / GC-FID. Proximate analysis was conducted at the Nutrition Laboratory, Faculty of Bioresources and Food Industry. AOAC (2016) method was used to determine the crude protein (CP), crude fiber (CF), lipid, ash, and moisture content in both formulated diets with varying concentrations of *Shorea macrophylla* oil and experimental diets.

Fish condition

In total, 150 Javan Mahseer fingerlings were purchased from a local supplier at Paka, Terengganu. Fish were transferred and acclimatized for two weeks in the round tank (1 tonne) with continuous aeration. Fish were fed with a commercial diet containing 40% crude protein before being randomly assigned to 15 rectangular-shaped glass aquariums (61.5 cm × 32 cm × 33.5 cm) with a stocking density of 10 fish per aquarium. For the feeding trial, fish were carefully selected in triplicates for each treatment, with an average weight of 3.06 g ± 0.16. A feeding trial was conducted for 30 days and fish were fed at 5% of body weight twice daily at 7-8 am and 5-6 pm with the respective diets. During the experimental period, fishes were fed with different treatments of diet that included 0% SMBO (C), 1.25% SMBO (T1), 2.50% SMBO (T2), 3.75% SMBO (T3), and 5% SMBO (T4). Fish were also subjected to a photoperiod regime with 12 h of light and 12 h of dark to mimic their natural environment and regulate their circadian rhythm (Abduh *et al*., 2021). Water quality parameters were recorded using a YSI multimeter every 10 days. Water temperatures were monitored daily ranging from 25 °C to 26 °C. Dissolved Oxygen and pH were maintained between 8.03 to 8.81 mg/L^{-1} and 6.98 to 7.36, respectively. All water parameters measured for *T. tambra* were within the optimal range of water quality, as reported in previous studies (Abduh *et al*., 2021; Redhwan *et al*., 2022a). This indicates that the fish were raised in an environment that was well-suited for their growth and development and that the conditions were carefully monitored to ensure their health and well-being. Accumulated faeces in tanks were siphoned every 30 minutes after feeding and 30% of the water was changed every week.

Performance analysis

The following productive performance parameters were evaluated: Body Weight Gain (BWG); Feed Conversion Ratio (FCR); and Feed Intake (FI); according to Redhwan *et al*. (2022a).

$$Body\ Weight\ Gain\ (BWG) = W2 - W1$$ – Equation 1

$$Feed\ Intake = \frac{Feed\ Intake\ per\ replicate\ per\ week}{No.fish\ consumed\ feed\ daily\ during\ the\ week\ period}$$ – Equation 2

$$Feed\ conversion\ ratio = \frac{Feed\ Intake\ (g)}{Fish\ Weight\ gain\ (g)}$$ – Equation 3

Where W2 = Live Body Weight at the end of the same period and W1 = Live Body Weight at the onset of the same period.

Statistical analysis

The software package SPSS Windows 27 was used for statistical analysis. All experiments were in triplicates. Data was analyzed using one-way analysis of variance (ANOVA) and the mean value was compared by Tukey's multiple comparison tests. P values less than 0.05 were considered statistically significant. Results were reported as mean values ± standard deviation (S.D) (Kamarudin *et al*., 2018).

RESULTS AND DISCUSSION

Fatty acid profile of SMBO

Table 1 illustrates the fatty acid profile of *Shorea macrophylla* obtained from Kanowit, Sarawak. The result shows that saturated acid dominates total fatty acid at more than 65% while unsaturated fatty acid was nearly 35%. Saturated acids consist of stearic acid, oleic acid, and palmitic acid at approximately 45%, 33%, and 16% respectively.

Table 1. Fatty acid profile *Shorea macrophylla* butter oil (SMBO)

Fatty Acid of SMBO	Total Fatty Acid
Lauric acid	0.0088
Myristic acid	0.0545
Pentadecanoic acid	0.0192
Palmitic acid	16.8075
Palmitoleic acid	0.0518
Heptadecanoic acid	0.01541
Stearic acid	45.8739
Oleic acid	33.5900
Linoleic acid (Cis)	0.7297
α-Linolenic acid	0.1297
Arachidic acid	1.8836
Behenic acid	0.1253
Lignoceric acid	0.3378
Nervonic acid	0.2340
Saturated Fatty Acid	65.2647
Unsaturated Fatty Acid	34.7352

#Total Fatty Acid for SMBO = 99.99%

Composition of feeds

It is essential to ensure that feed samples are analyzed properly to avoid any contamination or alteration of their nutrient content. Once the sample is obtained, it should be properly labeled and sealed to prevent any moisture or foreign matter from affecting the results. Table 2 shows the ingredients of the formulated feeds including the proximate composition of each diet.

Crude protein in all diets was between 27.76 to 30.95% while lipid content was between 8.52 to 10.78% as shown in Table 2. Crude fibers were between 2.97 to 3.42%, ash (12.15 to 19.24%) and moisture (7.52 to 11.19%) respectively.

Table 2. Feed and proximate composition (% as a fed basis) of experimental treatment varying level of SMBO

Ingredient	Dietary *Shorea macrophylla* oil (SMBO)				
	(C) 0.00	(T1) 1.25	(T2) 2.50	(T3) 3.75	(T4) 5.00
Fishmeal[1]	30.00	30.00	30.00	30.00	30.00
Krill Meal	20.00	20.00	20.00	20.00	20.00
Wheat Meal	14.58	14.58	14.58	14.58	14.58
Sago Starch	10.42	10.42	10.42	10.42	10.42
Fish Oil (FO)	5.00	3.75	2.50	1.25	0.00
SMBO	0.00	1.25	2.50	3.75	5.00
Vitamin Premix[2]	5.00	5.00	5.00	5.00	5.00
Mineral Premix[3]	5.00	5.00	5.00	5.00	5.00
Vitamin C	5.00	5.00	5.00	5.00	5.00
Cellulose	5.00	5.00	5.00	5.00	5.00
Proximate Composition					
Crude Protein	29.47±0.28^{ab}	30.12±0.15^{b}	30.95±0.93^{b}	27.76±0.99^{a}	28.24±0.49^{a}
Crude Lipid	8.52±0.42^{a}	10.38±0.83^{ab}	10.78±0.50^{b}	8.66±0.08^{a}	10.22±0.29^{ab}
Crude Fiber	2.97±0.07^{a}	3.18±0.14^{a}	3.40±0.23^{a}	3.40±0.21^{a}	3.42±0.22^{a}
Ash	19.24±011^{a}	14.75±0.10^{a}	12.15±6.58^{a}	14.37±0.72^{a}	15.78±0.18^{a}
Moisture	7.52±0.18^{a}	10.09±0.19^{d}	7.99±0.01^{b}	11.19±0.21^{e}	9.60±0.14^{c}

[1]Fishmeal with a crude protein, fat, moisture, and salt at 71.5%,10%,7%, and 3.7% respectively.[2]Vitamin Premix (g kg −1premix): vitamin A,0.05; vitamin D3 0.01; vitamin E 1.30; vitamin K3 0.01; vitamin B1 0.01; vitamin B2 0.016; vitamin B6 0.1; vitamin B12, 0.5; Niacin 0.2; $C_9H_{17}NO_5$, 0.056; $C_{19}H_{19}N_7O_6$ 0.008; biotin 0.5; Anticake 0.02.[3]Mineral Premix (g kg −1 premix): Copper,7.5; Iron,125; Manganese,25; Zinc,125; Cobalt,0.5; Iodine,0.175; Selenium,0.3; and Anticake,10. Values are means ± SD of duplicate measurements. Treatment samples followed by a, b, and c in row were significantly different (p<0.05)

Growth performance

Body Weight Gain (BWG)

BWG of Javan Mahseer (*T. tambra*) is shown in Figure 1. There were significant differences (p<0.05) of BWG among all treatments. All trends of each treatment gradually increased from day 10 until day 30. The highest BWG during day 10 was C (100% of FO & 0% of SMBO) at 45.51%. Other treatments like T1, T2, T3 and T4 recorded BWG of 11.98%, 9.84%, 21.99% and 41.66%, respectively. Body weight gain in T1 (1.25% SMBO & 3.75% FO), T2 (2.5% SMBO & 2.5% FO), and T3 (3.75% SMBO & 1.25% FO) from day 10 to day 30 were lower than C (0% SMBO & 5% FO) and T4 (5% SMBO & 0% FO). However, T4 showed the highest BWG after day 30 with 5% SMBO (*S. macrophylla*) than C (5% of Fish oil). It shows that *S. macrophylla* butter oil has a positive effect on the growth performance of *T. tambra* fingerlings. This is because *S. macrophylla* butter oil is rich in fatty acids with 18 carbons, which are essential for freshwater fish species, while fish oil is a rich source of n-3 fatty acids (Han *et al*., 2018; Rakman *et al*., 2021). *T. tambra* as a freshwater species requires more C18 PUFA but not C20 and C22 HUFAs and this species can naturally produce LC-PUFA from C18 PUFA which is available in vegetable oils (Kamarudin *et al*., 2012; Bami *et al*., 2017). Therefore, these results support Jaya-Ram *et al*. (2018) that freshwater fish have a higher content of n-6 fatty acids while marine fish have higher levels of n-3 fatty acids. According to Craig *et al*. (2017), omnivorous fish species such as *T. tambra* have a lower intake of n-3 fatty acids because they consume fewer fish sources that are rich in n-3 fatty acids and feed more on plant materials. Ng and Addin (2011) are also in agreement that *Tor spp.* required low n-3 fatty acids and higher n-6 fatty acids with ratios of 0.3 and 0.6.

Feed Intake (FI)

Feed intake plays an important role in achieving good growth performance in fish. In Figure 2, results showed that there were significant differences (p<0.05) in FI among all treatments. On day 10, T1, T2, T3, and T4 (1.25% of SMBO, 2.5% of SMBO, 3.75% of SMBO & 5% of SMBO) showed higher feed intake compared to control (C). Feed intake seems to increase on day 20 as well as day 30 for all treatments. However, C with 5% of fish oil showed a lower feed intake from day 10 until day 30 compared to other treatments but it gave good growth as well as T4 with 5% of SMBO. This is because fish oil has high n-3 fatty acids that are essential for the growth and development of fish while n-6 fatty acids are important for fish to activate the immune system in fish but with different roles (Terpstra, 2015). According to observation from a recent study, fish in Treatment 4 (T4) are more interested in feeds with 5% SMBO than fish in Control (C) with 5% FO. This is probably due to the aromatic smell of fruit that attracts fish to consume these feeds and commonly exists in their natural habitat (Kamarudin *et al*., 2018). It is also observed that SMBO is preferred as a fish feed ingredient to *T. tambra* because of its high n-6 that can improve animal and human health. In addition, *T. tambra* requires more n-6 fatty acids than n-3 fatty acids because these species are more likely to consume plant-based foods that have little or no n-3 fatty acids. *T. tambra* has a similar ability to *T. tambroides* to synthesize n-3 fatty acids like EPA and DHA from the n-6 fatty acids available in vegetable oil like SMBO (Redhwan & Komilus, 2021). Janaranjani *et al.* (2018) reported that the mRNA method demonstrated *T. tambra* ability to synthesize n-6 is due to Δ5 Fatty Acyl Desaturase (FADS) enzymes and fatty acyl elongase (Elovl) enzyme that could convert n-6 to PUFA such as arachidonic acid (ARA) and eicosapentaenoic acid (EFA). The conversion rate from this enzyme shows that 20% of n-6 fatty acids were converted to n-3 fatty acids, thus automatizing the conversion of SMBO to ARA and EFA.

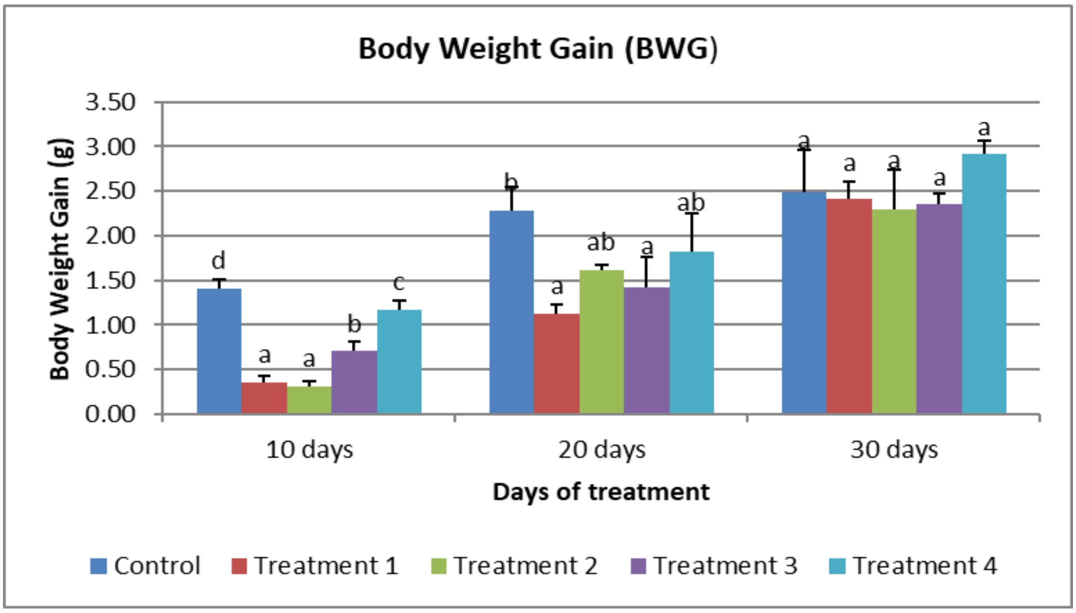

Fig. 1. The Body Weight Gain (BWG) of *T. tambra* fingerling throughout 30 days with different treatments.

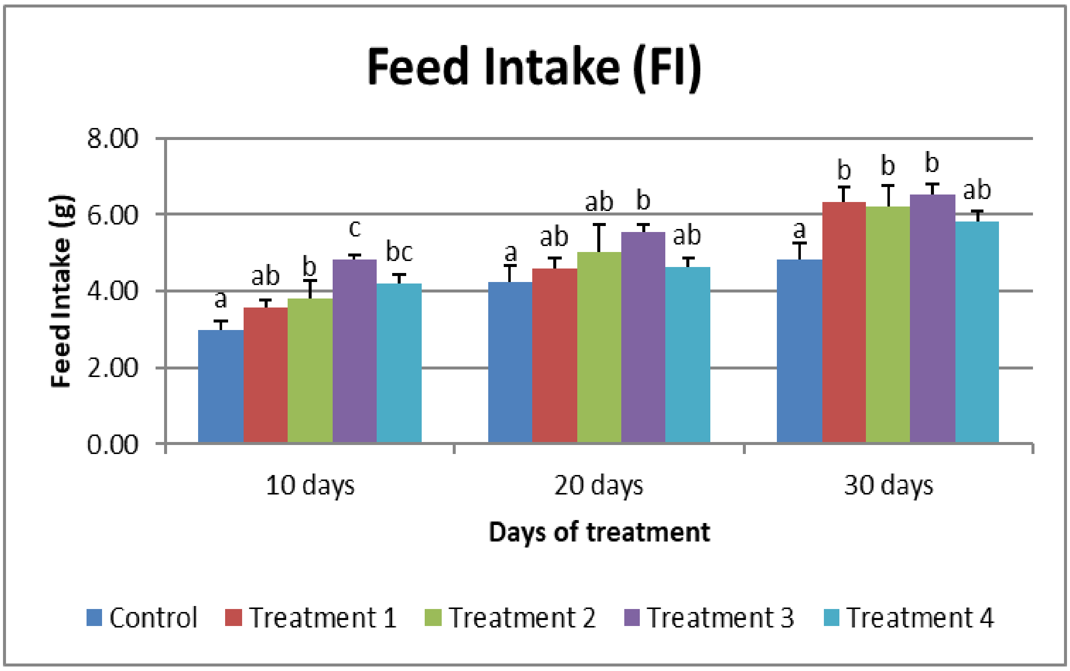

Fig. 2. Feed Intake of *T. tambra* fingerling throughout 30 days with different treatments.

Feed Convention Ratio (FCR)

Based on the results in Figure 3, there were no significant differences among treatments for 30 days. During the feeding trial, Treatment 4 (T4) showed higher BWG (2.49 g) but lower FCR (1.09 g). Lower FCR with higher BWG are important positive indicators in the feed industry. According to Fry *et al.* (2018), feed costs are frequently the largest budget item for fish farms. As a result, trustworthy indications are required to assess the level of performance attained from feed or additives. Therefore, FCR is an indicator that represents the effectiveness of a feed plan.

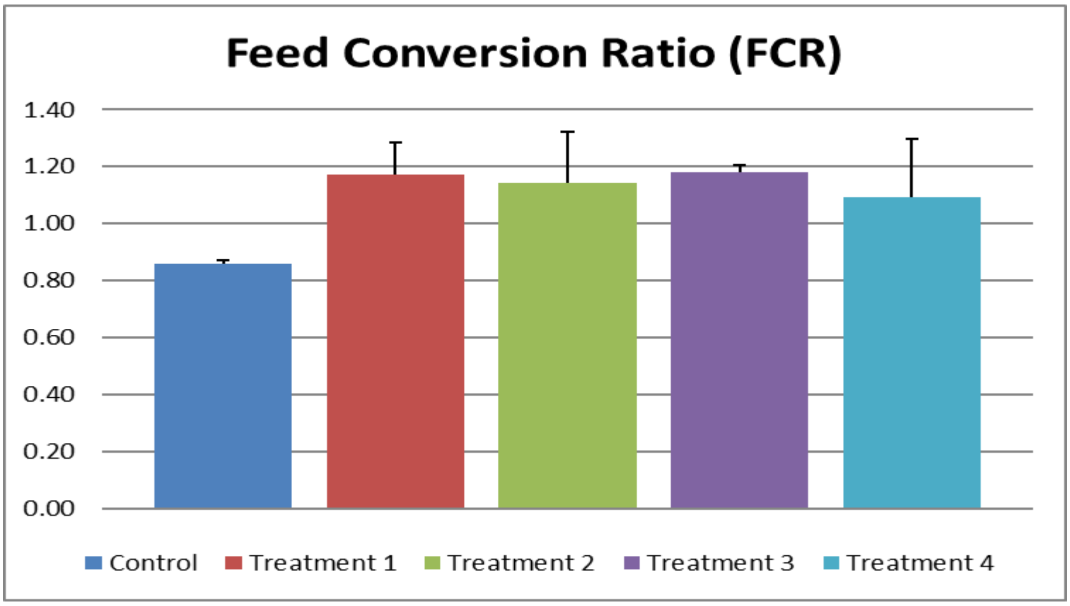

Fig. 3. The Feed Conversion Ratio (FCR) of *T. tambra* fingerlings for 30 days with different treatments.

CONCLUSION

It can be concluded that 5% of *S. macrophylla* butter oil (SMBO) as a lipid source showed positive effects towards *T. tambra* fingerling as an alternative lipid source. However, T4 (5% of *S. macrophylla* butter oil) shows a similar efficiency to Control (5% fish oil) that gives good growth performance for *T. tambra* fingerling. T4 gave the best BWG (2.49 g) and FCR (1.09) meanwhile C gave the best FI (4.81 g). It's advisable to conduct a dietary feeding trial with a longer trial period in the future to gain a deeper understanding of the effects and potential applications of *S. macrophylla* butter oil as a feed ingredient.

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ETHICAL STATEMENT

This study was approved by the ethical committee Ref: UAPREC/O/3/374-3(35) from UniSZA Plant and Animal Research Ethics Committee (UAPREC).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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