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Efficacy of dietary engkabang oil (*Shorea macrophylla*) on growth performance of fingerling malayan mahseer (*Tor tambroides*)

Aidil Ikhwan Redhwan[1], *Sim* Chun Hock[2], *Asmad* Kari[1], *Siew* Ing Nguang[1], *Hou* Chew Ha[1], and *Connie* Fay Komilus[1,*]

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia
[2]Masheer Hatchery, The Sims Aquaculture Sdn Bhd, 326 Jalan Semaba, 93250 Kuching, Sarawak, Malaysia

Abstract. The use of *Shorea macrophylla* oil (SMO) as a substitute for fish oil in enhancing the growth performance of Malayan Mahseer (*Tor tambroides*) has received limited attention. This study aimed to evaluate the proximate composition fatty acid profiles of SMO and assess its impact as an alternative lipid source for replacing fish oil in the diet of *Tor tambroides*. A 2-month feeding trial was conducted using 75 fingerlings, averaging 17.9±0.1 g in weight, distributed randomly across five triplicated feed treatments. These dietary treatments consisted of 0% SMO (control), 1.25% SMO (T-1.25smo), 2.5% SMO (T-2.5smo), 3.75% SMO (T-3.75smo), and 5% SMO (T-5smo). The fatty acid composition of *Shorea macrophylla* oil and the proximate composition of the treatment diets were analyzed. Growth indices (BWG, FI, FCR, and SR) were recorded at 10-day intervals. The results indicate that the incorporation of 2.5% SMO led to an 80.89% increase in BWG with the lowest FCR (2.61) and feed intake. This suggests that including 2.5% *Shorea macrophylla* oil in the diet may positively impact the growth performance of *Tor tambroides* fingerlings.

1 Introduction

The cultivation of aquatic organisms for human consumption, aquaculture, has emerged as a progressively vital industry, significantly contributing to addressing global food and protein demands. With the rising global population, the demand for protein food has also increased considerably, and aquaculture is forecasted to experience substantial growth in the coming years, primarily driven by the growing demand for aquatic foods among consumers [1]. The popularity of aquaculture, particularly for its provision of protein-rich fish, is on the rise within the fresh food production industry. This trend is driven by the growing consumer awareness of the health advantages of consuming fish and other seafood. Due to this, the aquaculture industry faces forthcoming challenges of finding suitable fish feeds for cultured fish from larvae to spawner stages. The search for alternative feed

* Corresponding author: conniekomilus@unisza.edu.my

ingredients has been the subject of extensive recent research to identify new, locally available ingredients that can serve as essential replacements for traditional feed ingredients. This quest has led to the discovery of several promising alternatives, examples of which are the black soldier fly, crab shell waste, and rubber seed [2,3,4,5]. It's 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.

Malayan Mahseer, also known as "Ikan Empurau" locally, is a highly valued freshwater fish species belonging to the Cyprinidae family in Malaysia. The economic significance of this species cannot be overemphasized, as it commands a high market value in the country. According to [6], the price of Ikan Empurau can reach up to RM800, depending on the grade. Generally, wild Empurau tends to command a higher market price than semi-wild stock[7,8]. Ikan Empurau has the potential to become a premium commercial food product and also has value in the ornamental fish industry. The continuous high demand for this species has resulted in overfishing, leading to a decrease in the wild fish population. Captive breeding is therefore identified as the feasible strategy to address the decreasing population of Ikan Empurau to meet the escalating demand for food fish by Empurau consumers. Captive breeding of Ikan Empurau poses a gigantic challenge to many nutritionists and aquaculturists to develop an understanding of the optimal dietary requirements for commercial propagation of this wild fish in captivity. Similar diets to its natural environment need to be investigated to sustain the flesh quality of Ikan Empurau, which is well known as a premium fish in Borneo, especially Sarawak.

Shorea macrophylla, also known as “buah engkabang” or “buah meranti,” is a fruit that is primarily distributed on Borneo Island [9]. This fruit is highly valued due to its lipid-rich content, predominantly composed of triglycerides with consistent efficacy. The main fatty acids found in this fruit are saturated (18:0, 18:1n-9) and monounsaturated (16:0), according to [10]. In Sarawak, the fruit is frequently available in wet markets and utilized as cocoa butter or a cooking oil. In addition, the lipid found in this fruit is also known for its excellent oxidative stability, rendering it an appropriate component for averting the oxidation of crucial fatty acids within animal tissues. It should be incorporated into animal feed [11]. To promote optimal tissue development in Malaysian mahseers, their diet should contain a substantial quantity of saturated (SFA) and monounsaturated (MUFA) fatty acids. At the same time, the percentage of n-3 PUFAs should be kept to a minimum [12]. Therefore, the lipid-rich content of *Shorea macrophylla* can potentially serve as a beneficial addition to the diet of Ikan Empurau.

To date, a study has been conducted to investigate the effects of Engkabang fruits on Ikan Empurau [13]. Still, no research has been done to compare the effectiveness of replacing fish oil with *S. macrophylla* oil for the fingerling stage of Ikan Empurau. Hence, this research aimed to investigate how Shorea macrophylla oil could substitute fish oil and its impact on the growth performance of Ikan Empurau fingerling.

2 Materials and method

2.1 Ethical approval

Before this research, an approval referenced as UAPREC/O/3/374-3(35) was obtained from UniSZA Plant and Animal Research Ethics Committee (UAPREC). This approval was obtained to ensure that the study was conducted ethically and in compliance with the established guidelines and regulations regarding research involving plants and animals.

2.2 Sample analysis

The fatty acid profile of Engkabang butter oil, which was bought from an Iban community from Kanowit, Sarawak, was analyzed using the AOAC 20th Edition, 996.06 / GC-FID method. All diets were formulated to have equal lipid levels, containing varying concentrations of *Shorea macrophylla* oil, and the experimental diets were assessed for their levels of crude protein (CP), crude fiber (CF), lipids, ash, and moisture content according to the [14] method. Five diets were prepared according to Table 1 below.

Table 1. Experimental design.

Treatment	**(% of inclusion *Shorea macrophylla* oil)**
Control (C)	Inclusion with 0% *Shorea macrophylla* oil
T-1.25smo	Inclusion with 1.25% *Shorea macrophylla* oil
T-2.5smo	Inclusion with 2.5% *Shorea macrophylla* oil
T-3.75smo	Inclusion with 3.75% *Shorea macrophylla* oil
T-5smo	Inclusion with 5% *Shorea macrophylla* oil

2.3 Feed formulation

Shorea macrophylla oil from an Iban community from Kanowit, Sarawak, was transported back to the Animal Feed Laboratory, Universiti Sultan Zainal Abidin, which was then processed into five experimental diets. Figure 1 shows *Shorea macrophylla* oil in butter form and using traditional packaging inside bamboo that has been obtained before being processed into oil by Laboratory oven at 45°C. Additionally, FM and other feed ingredient was purchased from a seller in Mersing, Johor, and Rawang, Selangor, and was stored in UniSZA's animal feed storage facility before use. Five different types of feed were prepared according to the information presented in the feed composition in Table 3. Faculty of Bioresources and Food Industry's Nutrition Laboratory conducted a proximate analysis, which involved using [14] methods to determine the moisture content, ash, lipid, crude fiber (CF), and crude protein (CP) levels of both experimental and formulated diets that had varying concentrations of *Shorea macrophylla* oil.

Fig. 1. *Shorea macrophylla* traditionally packaged in bamboo

Table 2. Formatting sections, subsections, and subsubsections.

Ingredient ($g\ kg^{-1}$)	Dietary ***Shorea macrophylla*** oil (SMO)				
	0.00	**1.25**	**2.50**	**3.75**	**5.00**
Fishmeal[1]	300	300	300	300	300
Acetes Meal	200	200	200	200	200
Wheat Flour	140.58	140.58	140.58	140.58	140.58
Sagu Flour	100.42	100.42	100.42	100.42	100.42
Fish Oil (FO)	50	37.5	25	12.5	0
SMO	0	37.5	25	37.5	50
Vitamin Premix[2]	50	50	50	50	50
Mineral Premix[3]	50	50	50	50	50
Vitamin C	50	50	50	50	50
Cellulose	50	50	50	50	50
Proximate Composition					
Crude Protein	290.47±0.28^{ab}	300.12±0.15^{b}	300.95±0.93^{b}	270.76±0.99^{a}	280.24±0.49^{a}
Lipid	80.52±0.42^{a}	100.38±0.83^{ab}	100.78±0.50^{b}	80.66±0.08^{a}	100.22±0.29^{ab}
Crude Fiber	20.97±0.07^{a}	30.18±0.14^{a}	30.40±0.23^{a}	30.4±0.21^{a}	30.42±0.22^{a}
Ash	190.24±011^{a}	140.75±0.10^{a}	120.15±6.58^{a}	140.37±0.72^{a}	150.78±0.18^{a}
Moisture	70.52±0.18^{a}	100.09±0.19^{d}	70.99±0.01^{b}	110.19±0.21^{e}	90.60±0.14^{c}

[1]Fishmeal contains approximately 71.5% crude protein, 10% fat, 7% moisture, and 3.7% salt.
[2]Vitamin Premix: Retinol,0.005 ; Cholecalciferol 0.001 ; Tocophenol 0.130 ; Menadione 0.001 ; Thiamine 0.001 ; Riboflavin 0.0016 ; Pyridoxine 0.01 ; Cyanocobalamin, 0.05 ; Niacin 0.02 ; $C_9H_{17}NO_5$, 0.0056 ; $C_{19}H_{19}N_7O_6$ 0.0008 ; Biotin 0.05 ; Anticake 0.002.
[3]Mineral Premix: Cu, 0.75 ; Fe,1.25 ; Mn,25 ; Zn,1.25 ; Co,0.05 ; I,0.1.75 ; Se,0.03 ; and Flow aids,1.0.
Values represent the average ± standard deviation of two measurements. Significant differences among the treatment samples labeled as a, b, and c in the same row ($p < 0.05$).

2.4 Fish condition

75 fingerling of Malayan Mahseer (*Tor tambroides*) with an average weight of 17.9±0.1g were obtained from a fish farm at Serian, Sarawak, and transported alive to Sim Aquaculture Hatchery located in Kuching, Malaysia. The fish were initially nourished with the 'Tropicarp' commercial diet, which consists of 40% crude protein. Subsequently, the fish were randomly distributed into fifteen rectangular glass aquariums (measuring 615mm x 320mm x 335mm) in triplicate, each accommodating five fish. The feeding trial extended over a 60-day period, where the fish received two daily feedings equivalent to 5% of their body weight. Simultaneously, continuous monitoring of water quality was conducted. The tanks adhered to a 12-hour light and 12-hour dark cycle as part of their photoperiod schedule. [15] to imitate conditions in the wild. Diets used during the experimental period include control (C), 1.25% (T-1.25smo), 2.5% (T-2.5smo), 3.75% (T-3.75smo), and 5.00% (T-5.00smo).

In monitoring water quality, commercial strip water quality multimeters “TOGAWA” were used to record water quality parameters every ten days. Water temperature was monitored daily and maintained at 25°C to 26°C while dissolved oxygen and pH levels were maintained between 8.00 mg/L-1 and 6.5, respectively, as recommended for *Tor* spp. in previous studies [16]. This ensures that fish are raised in an optimal environment for their growth and development and that their health and well-being are carefully monitored.

Accumulated feces were siphoned every 30 minutes after feeding and replaced with clean water. They were also done weekly to maintain the cleanliness of aquariums. This was done to minimize the harmful substances in the water that could adversely affect the fish's growth and health.

2.5 Growth Performance Analysis

Fish sampling on body weight and number of fish was done every ten days. Growth performance parameters like Body Weight Gain (BWG), Feed Conversion Ratio (F CR), Feed Intake (FI), and Survival Rate (SR) were taken as basic analyses for growth performance. [17,18]:

Body Weight Gain (BWG)

$$\text{Body Weight Gain (BWG)} = [(FW-IW)/IW]\times 100 \quad (1)$$

Where :
FW = Final Weight
IW = Initial Weight

Feed Intake (FI)

$$\text{Feed Intake (FI)} = FC/FD \quad (2)$$

Where :
FC= Feed consumed per replicate per week
FD= No.fish consumed feed daily during the week

Feed Conversion Ratio (FCR)

$$\text{Feed Conversion Ratio (FCR)} = TFC/TWPP \quad (3)$$

Where :
TFC= Total feed consumed
TWPP= Total weight of product produced

Survival Rate (SR)

$$\text{Survival Rate (SR)} = [(FTF \times 100) / ITF] \quad (4)$$

Where :
FSF= Final total of fish
ISF= Initial total of fish

2.5 Statistical analysis

The data was analyzed through a one-way variance analysis (ANOVA), and the mean values were compared using Tukey's multiple comparison tests conducted in Minitab 21 version 21.3.[13]. P-values below 0.05 were deemed to have statistical significance.

3 Results and discussion

3.1 Fatty acid profile

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Table 3. Fatty acid profile *Shorea macrophylla* oil

Fatty Acid	***Shorea macrophylla* (%)**
12:0	0.0088
13:0	0.0000
14:0	0.0545
14:1	0.0000
15:0	0.0192
15:1	0.0000
16:0	16.8075
16:1	0.0518
17:0	0.1541
17:1	0.0000
18:0	45.8739
18:2n6t	0.0000
18:1n9c	33.5900
18:2n6c	0.7297
18:3n3	0.1297
18:3n6	0.000
20:0	1.8836
20:1n9	0.0000
20:2	0.0000
20:3n3	0.0000
20:3n6	0.000
20:4n6	0.0000
20:5n3	0.0000
22:0	0.1253
22:1n9	0.0000
22:6n3	0.0000
24:0	0.3378
24:1	0.2340
SFA	65.2647
USFA	34.7352

3.2 Performance analysis

3.2.1 Body Weight Gain (BWG)

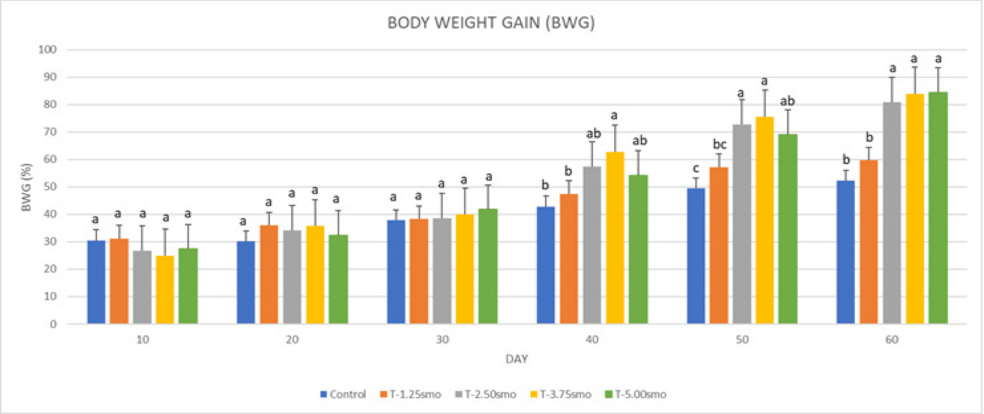

Fig. 2. Body Weight Gain (BWG) of *T. tambroides* throughout 60 days with five treatments.

Figure 2 illustrates the Body Weight Gain (BWG) of *T. tambroides*. All treatments demonstrated incremental growth starting from day 20 to day 30 without any significant differences among treatments. On day 40, the BWG of T-3.75smo, which contains 75% of *S. macrophylla* and 25% fish oil, significantly differed ($p<0.05$) from other treatments at 62.79%. Control, T-1.25smo, T-2.5smo, and T-5smo recorded BWG of 42.77%, 47.45%, 57.39%, and 54.19%, respectively without any significant differences. However, control (0% of *S. macrophylla* and 100% of fish oil) and T-1.25smo (25% *S. macrophylla* and 75% of fish oil) growth performance showed slower growth compared to treatments with higher inclusion of SMO starting from day 50 until day 60. The BWG trend, as demonstrated in this result, indicates that *T. tambroides* fingerlings can digest diets containing 50% to 100% *S. macrophylla* oil, strongly suggesting that SMO may be one of the fish's natural diets in the wild. This species is also known as a river fish that consumes riverine fruits, which are normally rich in lipids and saturated fatty acids, which may explain their favorable response to the oil-based diet [19,6]. The incremental growth performance of *T. tambroides* fingerlings to more than 50% inclusion of SMO can be positively influenced by *S. macrophylla* oil, abundant in 18-carbon fatty acids, vital for freshwater fish species.

T. tambroides have a higher demand for C18 HUFAs but not C20 and C22 HUFAs. Additionally, it can naturally synthesize LC-PUFAs from C18 PUFAs in vegetable oils. Similar observations were reported by [20], who observed that freshwater fish consume elevated levels of Omega 6 fatty acid LC-PUFA, which could be sourced from Arachidonic Acid (ARA), Linoleic Acid, and α-Linolenic Acid that exist in SMO. The small amount of 1.88% of ARA found in SMO could have acted as a precursor for producing LC-PUFA, which this fish requires to grow optimally in captivity. According to [21], The addition of ARA in the range of 0.3% to 0.9% could potentially enhance the growth, immune system, and hematological characteristics of olive flounder, while [22] stated that at least 1% ARA in the maturation diet of *T. trichopterus* broodstock, an asynchronous fish improves its reproductive performance. A study by [23] Employing the mRNA approach revealed that *T. tambra* possesses the capacity to produce n-6 fatty acids as a result of the existence of Δ5 Fatty Acyl Desaturase (FADS) enzymes and fatty acyl elongase (Elovl) enzymes. These enzymes can transform n-6 fatty acids into significant PUFAs, including arachidonic acid (ARA) and eicosapentaenoic acid (EPA). The conversion efficiency of these enzymes is estimated at around 20%, signifying that approximately 20% of n-6 fatty acids can be transformed into n-3 fatty acids. This may suggest that *S. macrophylla,* rich in n-6 fatty acids, can be converted to ARA and EPA by *T. tambroides,* although no analysis was done in this study. This suggests further studies related to in vivo synthesis in fish about SMO.

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The result shows that treatments with high fish oil between 75% and 100% may not favor T. tambroides in indoor culture after more than 30 days. This is perhaps due to the low intake of omnivorous fish species like *T. tambroides* on n-3 fatty acids due to their consumption of fewer fish sources and higher intake of plant materials [24]. Based on [25], the ideal fatty acid ratio for *Tor* spp. is low in omega three fatty acids and high in omega six fatty acids, with a ratio of 0.3 to 0.6. In this study, it is observed that a ratio of 1:1 gives optimal growth performance to *T. tambroides*.

3.2.2 Feed Intake

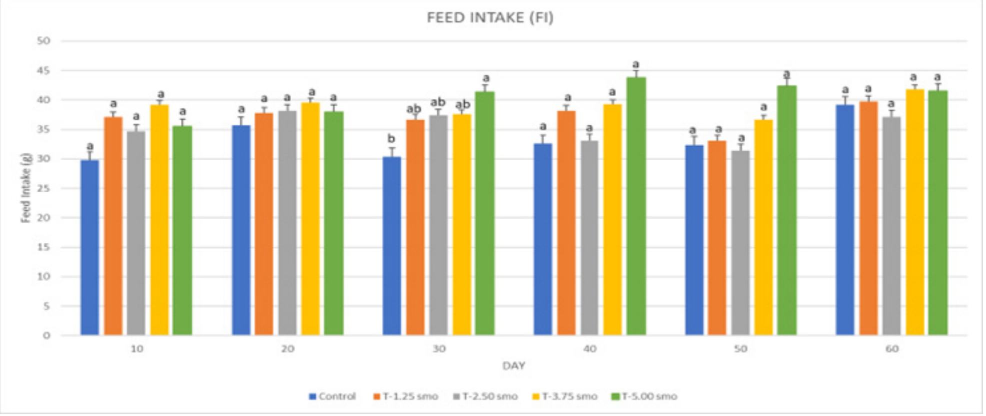

Fig. 3. Feed Intake of *T. tambroides* fingerling for 60 days with different treatments.

The significance of feed intake in optimizing fish growth performance cannot be emphasized enough. The quantity and quality of food consumed by fish directly influence their growth and development. As depicted in Figure 3, the data revealed a notable difference in feed intake (FI) among treatments on day 30 only, with fingerlings displaying a preference for feed containing 100% SMO (more than 40%). In comparison, those fed with 100% FO exhibited lower consumption. This preference may be attributed to a limited in vivo fatty acid metabolism rate, especially in converting C18 PUFA into LC-PUFA. This observation aligns with prior research [26], suggesting that dietary fatty acids play a pivotal role in accumulating body fatty acids. At the same time, environmental temperatures primarily govern the in vivo fatty acid composition during the endogenous biosynthesis of LC-PUFA in Nile tilapia. Consequently, a diet composed of 100% FO may not be an ideal lipid source for T. tambroides fingerlings.

The fruit's aroma could also affect feed intake, which attracts the fish to consume these feeds and commonly exists in their natural habitat [13]. SMO used in diets may produce some volatile aromatic fragrances with a pleasant scent, which attracts feeding. This adheres to [27] findings that essential oil isolated from *Shorea roxburghii* has a pleasant, unique scent similar to fresh flowers.

3.2.3 Feed Conversion Ratio (FCR)

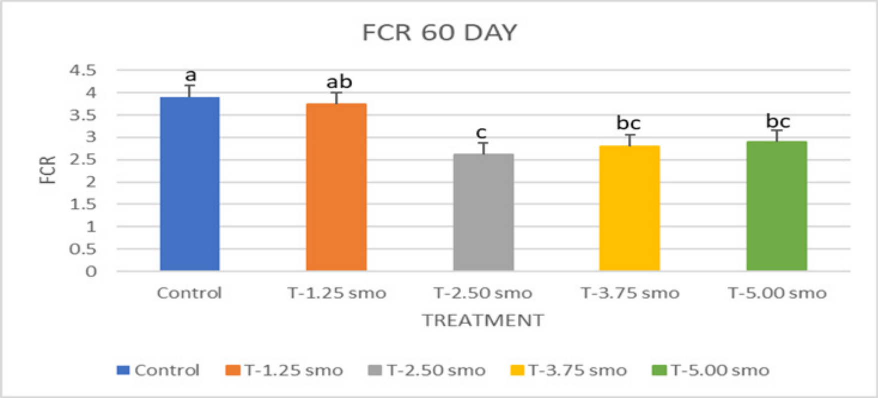

Fig. 4. The Feed Conversion ratio (FCR) of *T. tambroides* fingerling for 60 days with different treatments.

Figure 4 illustrates that the inclusion of 50% inclusion of SMO showed high BWG (80.89%) with the lowest FCR at 2.61. This result conforms to an observation reported by [28] that T. tambroides fed with 50% cod liver oil (CLO) and 50% palm oil gave the best result on feed conversion ratio (FCR). According to [29], Feed expenses often represent the most substantial portion of fish farm budgets. Hence, a reliable metric such as FCR is essential for evaluating the performance achieved with a particular feed or additive.

3.2.3 Survival Rate

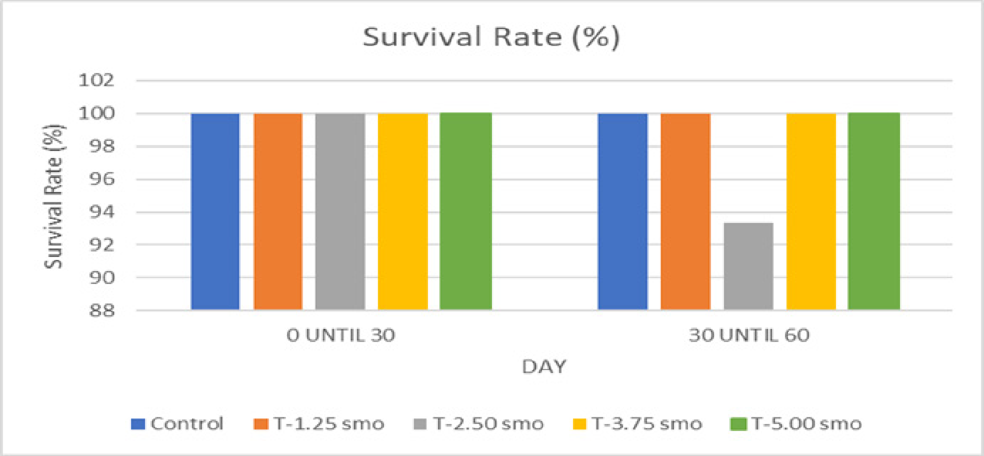

Fig. 5. Survival rate (SR) of *T. tambroides* fingerling for 60 days with five treatments, including *S. macrophylla oil*.

Figure 5 illustrates that there were no notable distinctions observed among the treatments. The average survival rate (SR) data in the concluding days ranged from 93% to 100%. SR is a crucial indicator for evaluating the success of water quality management and feeding protocols. Due to the high sensitivity of this species, particularly those bred in captivity, maintaining optimal water quality and favorable environmental conditions is imperative.

4 Conclusion

It can be inferred that incorporating 2.5-5.0% of S. macrophylla oil as a lipid source has demonstrated beneficial outcomes for T. tambroides fingerlings, presenting it as a viable alternative lipid source. Specifically, a diet containing 2.5% S. macrophylla oil exhibited improved Feed Conversion Ratio (FCR), satisfactory Feed Intake (FI), and notably higher Body Weight Gain (BWG). However, further dietary investigations are warranted, involving longer trial periods exceeding three months, to gain a more comprehensive understanding of the effects and potential applications of S. macrophylla oil as a dietary component for T. tambroides."

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