Macroalga *Ulva* sp. and malunggay *Moringa oleifera* as dietary additives in the growth, survival, and feed efficiency of Mozambique tilapia (*Oreochromis mossambicus*) juvenile in pond culture

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Abstract. The macroalga *Ulva* sp. serves as an alternative nutrient source for aquafeeds and has become a focus of research globally due to its significant economic value. Similarly, *Moringa oleifera* leaves are widely utilized as an alternative food source for human nutrition and animal feeds across various countries. In this study, we investigated the effect of *Ulva* sp. (US) and *M. oleifera* (MO) by working synergically in a commercial diet for the growth, survival, and feed efficiency of juvenile Mozambique tilapia (*Oreochromis mossambicus* (Peters, 1852)). Twelve (12) experimental diets were prepared: Diet 1 (as control diet); Diet 2 (0% US; 10% MO); Diet 3 (10% US; 0% MO); Diet 4 (10% US; 10% MO); Diet 5 (20% US; 10% MO); Diet 6 (30% US; 10% MO); Diet 7 (10% US; 20% MO); Diet 8 (20% US; 20% MO); Diet 9 (30% US; 20% MO); Diet 10 (10% US; 30% MO); Diet 11 (20% US; 30% MO); Diet 12 (30% US; 30% MO). The culture was done for a 90-day culture period in the fishpond. The results indicated that diets with varying inclusion levels of SU and MO in commercial feed diet significantly influenced ($p < 0.05$) the specific growth rate (SGR) of juvenile *O. mossambicus*. Diets 4, 7, and 9 showed significant differences ($p < 0.05$) compared to the control diet. Weight gain was notably higher ($p < 0.05$) in Diets 4 and 2. Regarding survival, Diets 9, 11, and 12 exhibited significantly higher rates ($p < 0.05$) than the control group. Meanwhile, no significant differences ($p > 0.05$) were observed among the experimental diets in terms of feed intake (FI). However, the feed conversion ratio (FCR) for Diet 3 was significantly different ($p < 0.05$) from the other experimental diets. Hence, this study indicated that the synergic application of different inclusion levels of US and MO in a commercial feed diet enhanced the growth, survival, and feed utilization of juvenile *O. mossambicus.*

Keywords: alternative protein sources, aquafeed formulation, functional feed additives, nutrient digestibility, sustainable aquaculture.

Introduction. Aquaculture has undergone remarkable expansion over the past decades, becoming one of the fastest-growing food production sectors globally. According to the Food and Agriculture Organization (FAO), global fisheries and aquaculture production reached a record 223.2 million metric tons in 2022, with aquaculture alone contributing 130.9 million metric tons, surpassing capture fisheries for the first time in history and highlighting its increasing role in global food security (FAO 2024). This growth reflects a continuous upward trend in aquatic food production driven by rising global demand, population growth, and the shift toward sustainable protein sources. Among the widely cultured species, Mozambique tilapia (*Oreochromis mossambicus* (Peters, 1852)) plays an important role due to its adaptability, fast growth, and economic value. Often referred to as the "aquatic chicken" because of its ease of culture and high productivity, this species is widely farmed for commercial purposes, supplying importers, retail markets, and restaurants (Lertwanakarn et al 2023). Tilapia ranks as the third most widely cultured fish species globally, with major production coming from countries such as China, Indonesia, Egypt, Brazil, and Thailand (Prabu et al 2019). Originally native to Africa and the Middle East, tilapia species have been successfully introduced to many parts of the world, including the Philippines, due to their tolerance to a wide range of environmental conditions (El-Sayed & Fitzsimmons 2023).

In the Philippines, Nile tilapia (*Oreochromis niloticus*) production is primarily derived from freshwater ponds, cages, brackishwater ponds, and fish pens. Freshwater pond culture remains the dominant production system, with production increasing significantly from approximately 14,000 metric tons in 1985 to about 270,000 metric tons in 2023 (FAO 2024). Despite this growth, the industry continues to face challenges, particularly in feed costs, disease management, and sustainability issues associated with intensive aquaculture practices. The reliance on commercial feeds, which account for more than 50% of production costs, and the widespread use of antibiotics raise concerns regarding economic sustainability, environmental impacts, and the development of antimicrobial resistance in aquaculture systems (Cabello et al 2013; Okocha et al 2018; Milijasevic et al 2024).

Sustainable aquaculture emphasizes the use of eco-friendly, cost-effective, and socially acceptable production systems. One promising strategy is the incorporation of natural, plant- and algae-based feed additives to enhance fish growth, improve feed efficiency, and reduce dependence on synthetic inputs. In this context, the use of locally available resources such as *Moringa oleifera* (malunggay) and *Ulva* sp. (sea lettuce) has gained increasing attention. *M. oleifera* is widely recognized as a "miracle tree" due to its high nutritional value and diverse bioactive compounds. Its leaves are rich in proteins, vitamins, minerals, and natural antioxidants such as flavonoids, phenolics, and carotenoids, which contribute to improved growth performance, immune response, and overall health in aquatic organisms (Siddhuraju & Becker 2003; Fahey 2005; Anwar et al 2007). In addition, its antimicrobial properties make it a potential natural alternative to antibiotics in aquaculture systems (Bondad-Reantaso et al 2023).

Ulva sp., commonly known as sea lettuce, is an abundant green macroalga found in coastal environments worldwide. Although often associated with green-tide events due to eutrophication, *Ulva* sp. is increasingly recognized for its nutritional and functional properties, as it contains essential nutrients, bioactive compounds, and polysaccharides that can enhance feed utilization, growth performance, and immune response in aquatic organisms. Recent studies have highlighted its potential as alternative ingredient in aquafeeds, partially replacing conventional protein sources such as soybean meal (Pereira et al 2012; Wan et al 2019; Ang et al 2021). Given the growing need for sustainable and cost-efficient aquaculture practices, the integration of natural feed additives such as *Ulva* sp. and *M. oleifera* presents a promising approach. However, limited studies have evaluated their combined effects on tilapia, particularly under pond culture conditions. Therefore, this study aims to determine the effects of dietary inclusion of *Ulva* sp. and *M. oleifera* on the growth performance, survival rate, and feed efficiency of juvenile *O. mossambicus* cultured in ponds.

Material and Method

Study site. This study was conducted at Fish Pond Demo Farm of the Mindanao State University Tawi-Tawi College of Technology and Oceanography located at Pasiagan, Bongao, Tawi-Tawi (Figure 1) with a duration of three (3) months from April 17, 2024 to July 17, 2024.

Extraction of Moringa and preparation of Ulva. Fresh *M. oleifera* leaves were collected from mature trees within the vicinity of Mindanao State University–Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Bongao, Tawi-Tawi, Philippines, while *Ulva* sp. samples were harvested from the coastal waters of Bongao, Tawi-Tawi, Philippines. The collected materials were thoroughly washed and oven-dried at a controlled temperature until constant weight was achieved. The dried samples were then pulverized into fine powder using a laboratory blender. For extraction, 20 g of each powdered sample was mixed with 600 mL of hot water (80-90°C) and maintained in a water bath for 2 hours. The mixture was subsequently filtered using Whatman No. 2 filter paper to obtain the filtrate. The extracted solution was transferred into clean flasks and stored under refrigeration until further use. Different concentrations of the extracts were prepared by diluting the stock solution with distilled water. A fixed volume of 10 mL per treatment was prepared at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, and 6000 ppm for experimental application.

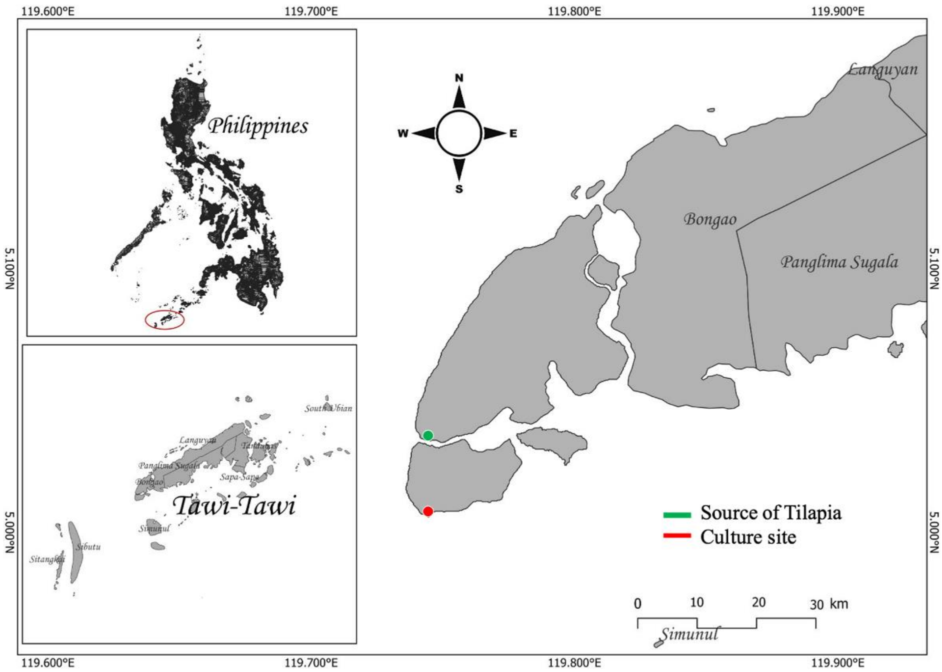

Figure 1. Map showing the location of study site in Tawi-Tawi, Philippines.

Preparation of diets. The extracted sap of *M. oleifera* leaves was mixed with the commercial feeds (grower) using a mixer to thoroughly incorporate it, and some amount of distilled water was added. The amount of water added was estimated to ensure that the incorporated *M. oleifera* sap and commercial feeds would easily form dough. The pulverized *Ulva* sp. was mixed into the commercial feeds (grower) by putting it in the mixer, and then adding some water so that it could easily form a dough. The prepared dough has been pelleted using a manual pelletizer. The pelleted feeds were put in a stainless tray with foil, and dried using an oven at 60°C overnight or until ≤ 10% moisture content was attained. Dried feeds were placed in a labeled plastic bag and stored in a refrigerator. Feeds have been weighed in accordance with 10% of the respective average body weight of the organisms.

Preparation of experimental set-up, source of organisms, and experimental design. The experimental set-up consisted of fish cages installed at the water intake area of a pond to ensure continuous water flow throughout the culture period. A total of twelve (12) cages were constructed using bamboo frames and netting materials. The cages were arranged systematically with a spacing of approximately 1.0-1.5 meters between units to promote proper water circulation and ease of management (Figure 2). A total of 400 individuals were collected from the wild in Kalang, near Sanga-Sanga Barangay Hall, using scoop nets and hand nets, and a total of 360 individuals were used as the experimental organisms (*O. mossambicus*), with a weight range from 3.28 to 4.06 g and a total length range from 4.7 to 7.2 cm. After collection, the fish were placed in large containers and transported to the Multi-Species Hatchery of the College of Fisheries for a one-week acclimatization period to reduce stress and ensure uniformity prior to stocking. After conditioning, the fish were transported to the culture site at the Fish Pond Demo Farm in Pasiagan, Bongao, Tawi-Tawi. During transport, the fish were placed in oxygenated plastic bags inside Styrofoam boxes with ice placed at the corners to minimize stress and maintain water temperature. The experiment followed a Completely Randomized Design (CRD) consisting of twelve (12) dietary treatments with varying inclusion levels of *Ulva* sp. and *M. oleifera*, as presented in Table 1. Each treatment was randomly assigned to cages to avoid bias, with thirty (30) fish stocked per treatment, where each cage served as one experimental unit. The experimental layout was designed to maintain uniform environmental conditions across all treatments, ensuring adequate separation between cages to prevent interaction effects. This arrangement facilitated accurate monitoring of fish growth performance, survival rate, and feed utilization throughout the study period.

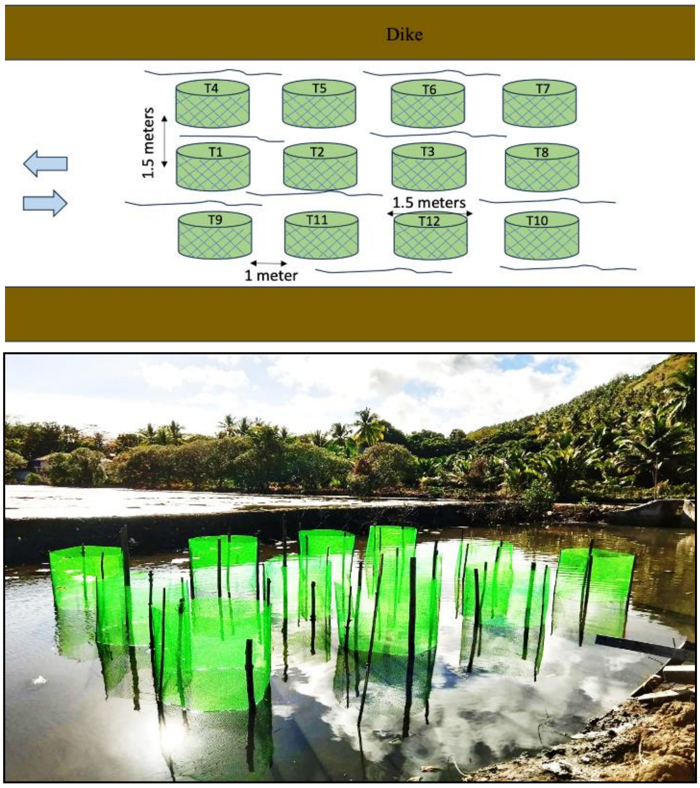

Figure 2. Experimental layout of the study set-up.

Table 1

Ratio of the feed inclusions per formulation of 1000 grams

Treatments	*Ulva (g)*	*Moringa (mL)*	*Grower commercial feeds (g)*
Diet 1	0	0	1000
Diet 2	0	10	990
Diet 3	10	0	990
Diet 4	10	10	980
Diet 5	20	10	970
Diet 6	30	10	960
Diet 7	10	20	970
Diet 8	20	20	960
Diet 9	30	20	950
Diet 10	10	30	960
Diet 11	20	30	950
Diet 12	30	30	940

***Stocking of experimental organisms**.* The initial length and weight of the experimental fish were measured prior to stocking. Individual fish length was measured in a transparent plastic container placed over a ruler for standardization. For weight measurement, a plastic container filled with water was placed on an analytical balance and tared to zero. Fish were then gently placed into the container, and their initial weights were recorded. After measurement, the fish were carefully released into the experimental cages. Feeding was initiated 24 hours after stocking to allow acclimatization. Initial water quality parameters were also recorded prior to the start of the feeding trial.

***Feeding and maintenance**.* Juvenile *O. mossambicus* were fed at a rate of 10% of their average body weight (ABW) using the broadcast feeding method, wherein the feed was evenly scattered across the water surface to ensure uniform feed distribution and equal access to feed among the fish. Feeding was carried out twice daily, at 7:00 AM and 5:00 PM. Routine maintenance of the culture system was conducted throughout the experimental period. This included cleaning of cages and removal of waste materials during each sampling period to maintain water quality, reduce organic accumulation, and minimize turbidity within the culture system.

***Sampling**.* Sampling was conducted at 15-day intervals throughout the study period. Fish were carefully collected from each cage for biometric measurements. Body weight was determined using an analytical balance, following the same procedure as the initial measurement (container with water tared to zero before weighing). All measurements were consistently recorded throughout the experimental period to monitor growth performance and survival.

Survival rate. The survival rate (%) was calculated every 15 days following the equation below (Robles et al 2023):

$$\text{Survival Rate} = \frac{\text{Final number of individuals in every sampling}}{\text{Initial number of individuals}} \text{x} 100$$

Specific growth rate. The specific growth rate (SGR, % day^{-1}) was defined below (Hairol et al 2022):

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\ln \text{Wf} - \ln \text{Wi}}{\text{DOC}} \text{X } 100$$

where: Wi = initial weight (g);
Wf = final weight (g);
DOC = days of culture.

Feed utilization. Feed conversion ratio (FCR) was determined using the formula:

$$FCR = \frac{FI}{WG}$$

where: FCR = feed conversion ratio;
FI = feed intake (g);
WG = weight gain (g).

Feed intake (FI, g) = feed given – uneaten feed

Weight gain (WG, g) = final weight – initial weight

***Data analysis**.* All collected data were analyzed using one-way analysis of variance (ANOVA) with SPSS version 25. When significant differences were detected among treatments, Duncan's Multiple Range Test (DMRT) was applied as a post hoc test to compare means. Growth performance and survival data were also organized and initially processed using Microsoft Excel prior to statistical analysis.

Results

Growth and survival. The specific growth rate (SGR) in the diet of juvenile *O. mossambicus* cultivated in a fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera* is shown in Figure 3. The SGRs of Diet 1 to Diet 12 were 0.014±0.001 day^{-1}, 0.016±0.001 day^{-1}, 0.014±0.001 day^{-1}, 0.018±0.001 day^{-1}, 0.015±0.001 day^{-1}, 0.013±0.001 day^{-1}, 0.017±0.001 day^{-1}, 0.016±0.001 day^{-1}, 0.018±0.001 day^{-1}, 0.015±0.001 day^{-1}, 0.016±0.001 day^{-1}, 0.015±0.001 day^{-1} respectively after 90 days of culture period. Analysis revealed that Diet 4, Diet 7, and Diet 9 were significantly different ($p < 0.05$) than the control diet.

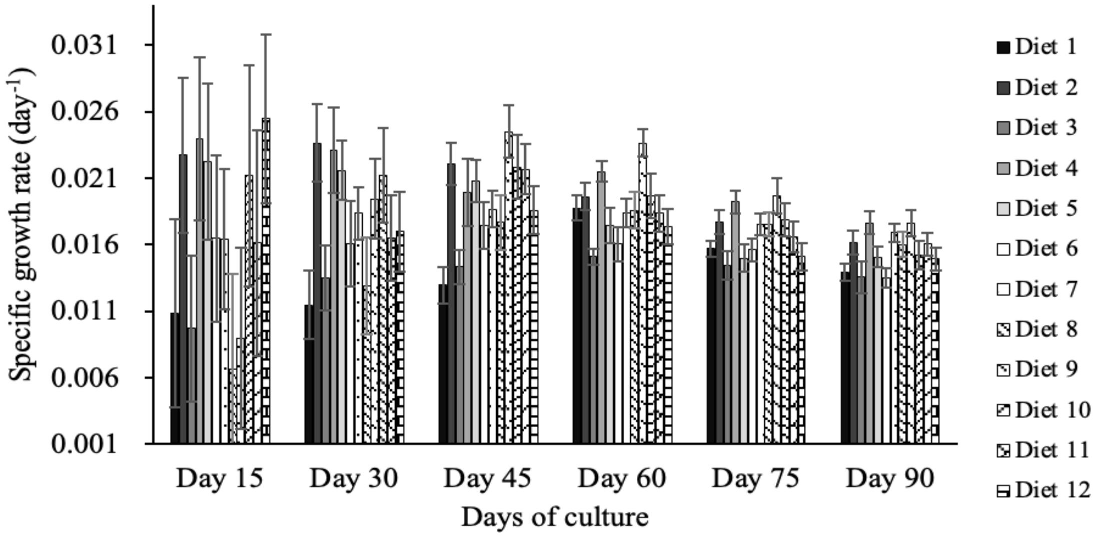

Figure 3. Specific growth rate (SGR, % day^{-1}) of juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera*. Error bars are in standard error mean (SEM).

The weight gain of juvenile tilapia *O. mossambicus* in Diet 4 (13.990±1.402 g) and Diet 2 (13.163±1.152 g) significantly increased ($p < 0.05$) compared to the control diet (10.54±0.817 g) at the end of the culture period (Figure 4).

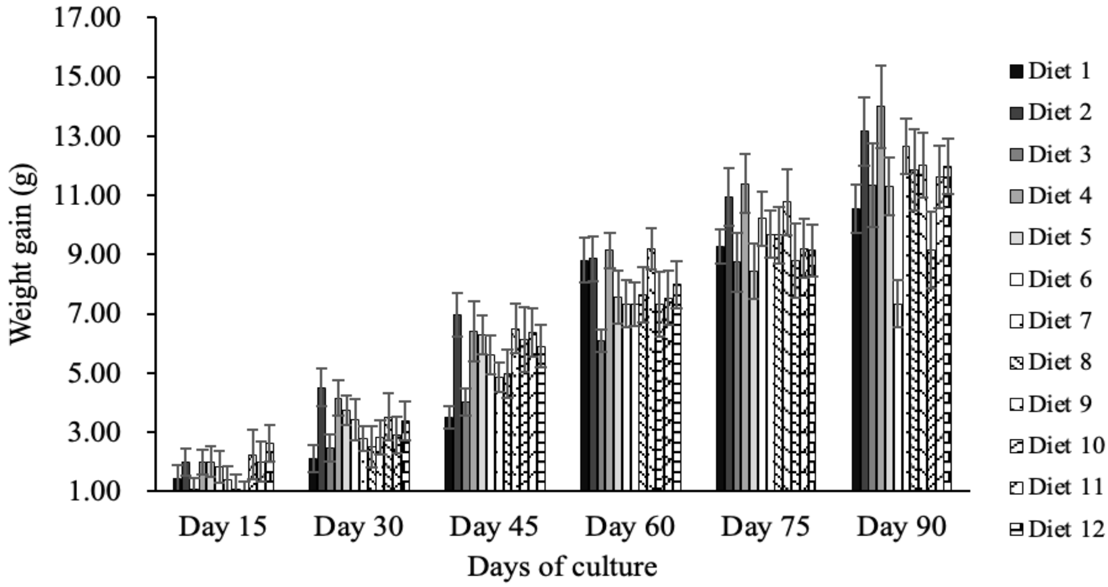

Figure 4. Weight gain (g) of juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera.* Error bars are in standard error mean (SEM).

Figure 5 shows the survival performance of juvenile tilapia *O. mossambicus.* It has been revealed that Diet 9 (92.22±1.111 %), Diet 12 (88.89±1.111 %), and Diet 11 (82.22±1.111 %) were significantly higher than the survival performance of the control diet (75.56±1.111 %) in juvenile tilapia *O. mossambicus* cultivated in a fishpond fed with different inclusion levels of *Ulva* sp. and *Moringa oleifera* after 90 days of culture*.*

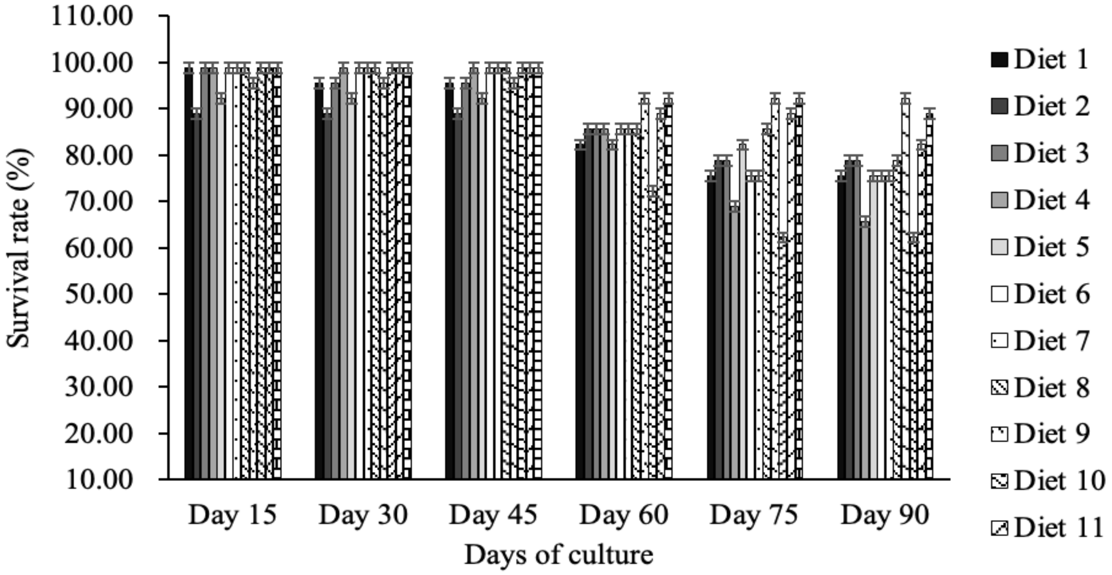

Figure 5. Survival rate (%) of juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera.* Error bars are in standard error mean (SEM).

Feed utilization*.* The feed utilization, particularly the feed intake (FI) of juvenile tilapia *O. mossambicus* cultivated in a fishpond fed with different inclusion levels of *Ulva* sp. and *Moringa oleifera*, is shown in Figure 6. The FI of Diet 1 to Diet 12 were 1.45±0.812 g, 1.69±0.115 g, 1.54±0.141 g, 1.73±0.140 g, 1.49±0.097 g, 1.43±0.090 g, 1.60±0.094 g, 1.51±0.137 g, 1.48±0.110 g, 1.19±0.129 g, 1.49±0.105 g, and 1.59±0.094 g, respectively. Results revealed that no significant difference ($p > 0.05$) in the experimental diets was observed among experimental groups.

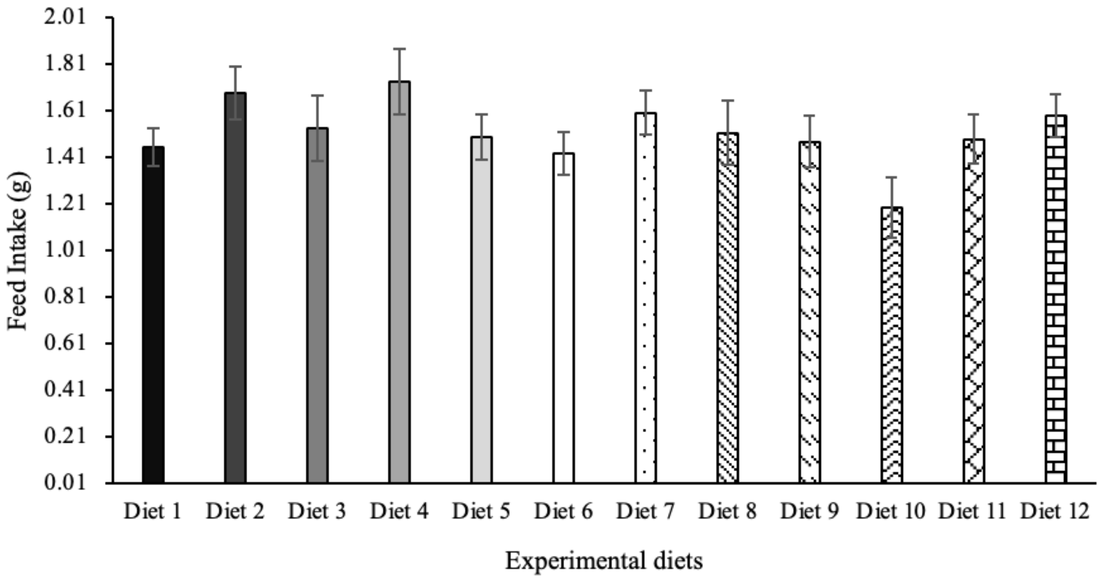

Figure 6. Feed intake (FI) (g) of juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera.* Error bars are in standard error mean (SEM).

In terms of the feed conversion ratio (FCR), Diet 4 was significantly different ($p < 0.05$) among experimental diets for juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *Moringa oleifera* (Figure 7).

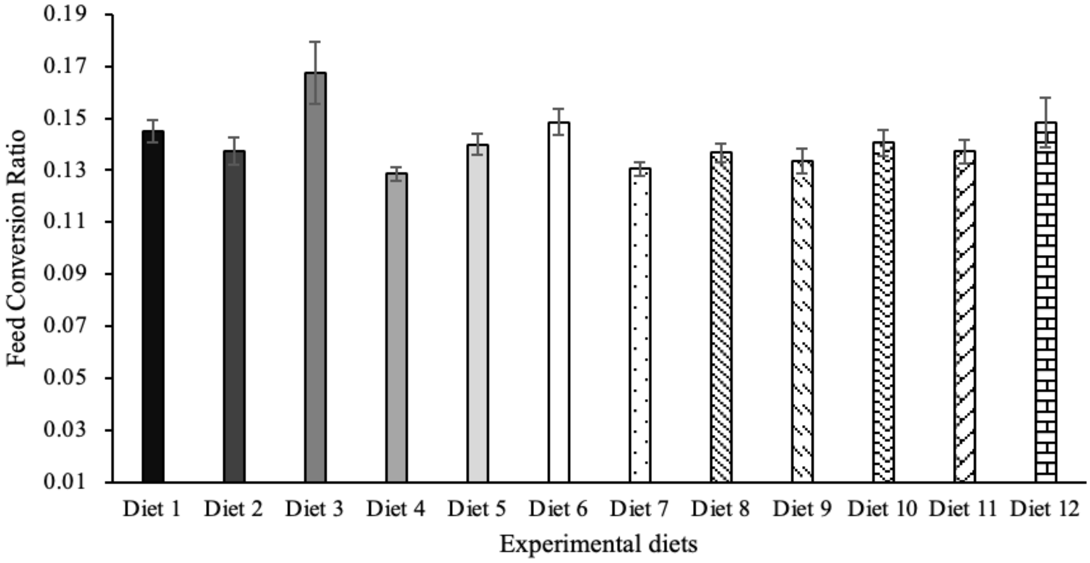

Figure 7. Feed conversion ratio (FCR) of juvenile tilapia *O. mossambicus* cultivated in fishpond fed with different inclusion levels of *Ulva* sp. and *M. oleifera.* Error bars are in standard error mean (SEM).

Discussion. The results showed that the SGR of juvenile *O. mossambicus* cultivated in fishponds was significantly influenced ($p < 0.05$) by different levels of *Ulva* sp*.* and *M. oleifera* added to their diet. The highest growth rate was observed in fish fed with a diet containing 10% *Ulva* sp*.* and 10% *M. oleifera*, which was significantly greater ($p < 0.05$) than that of the control diet after 90 days of culture. Furthermore, diets with 10% *Ulva* sp. combined with 20% *M. oleifera*, as well as 30% *Ulva* sp. with 20% *M. oleifera*, also led to a marked improvement in the growth performance of *O. mossambicus*. The weight gain of *O. mossambicus* improved significantly ($p < 0.05$) when their diet was supplemented with different levels of *Ulva* sp*.* and *M. oleifera*. Notably, fish fed with 10% *Ulva* sp. and 10% *M. oleifera*, as well as those given 10% *Moringa* alone, showed better weight gain compared to those on the control diet. These findings suggest that supplementing commercial tilapia feed with the right combination of *Ulva* sp. and *M. oleifera* can significantly improve growth performance, making it a promising approach for more efficient and sustainable aquaculture. These results align with findings from other studies. For instance, El-Tawil (2010) reported that supplementing the diet of *Oreochromis* sp. with 10% *Ulva* sp. significantly enhanced the SGR, achieving 3.53 % day^{-1}. Similarly, Richter et al (2003) found that the inclusion of 12% and 24% *M. oleifera* leaves resulted in an SGR of 1.0 % day^{-1} and 1.2 % day^{-1} in fish respectively. Compared to these studies, the present research demonstrated a superior growth response, as the combined inclusion of *Ulva* sp. and *M. oleifera* significantly improved the SGR of juvenile *O. mossambicus* beyond the levels previously reported. Furthermore, Mansour et al (2018) reported that dietary inclusion of 5%, 10%, and 15% *M. oleifera* significantly enhanced the weight gain of gilthead seabream (*Sparus aurata*). Similarly, Richter et al (2003) observed that a 24% inclusion of *M. oleifera* leaves led to an increased weight gain of 27.37 g compared to the control. In the present study, juvenile tilapia fed with diets containing 10% *Ulva* sp. combined with 10% *M. oleifera*, as well as those fed with 10% *Moringa* alone, exhibited superior weight gain compared to fish on the control diet.

This study revealed that dietary combinations of 30% *Ulva* sp. with 20% *M. oleifera*, 20% *Ulva* sp. with 30% *M. oleifera*, and 30% *Ulva* sp. with 30% *M. oleifera* significantly enhanced the survival rate of juvenile tilapia compared to the control group. Various studies have reported that different inclusion levels of *Ulva* sp. and *M. oleifera* significantly enhance the survival performance of fish species such as Nile tilapia

(*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) (Dongmeza et al 2006; Azaza et al 2008; Saleh et al 2014; Gbadamosi & Osungbemiro 2016). Different inclusion levels of *Ulva fasciata* meal, specifically 25% and 50%, did not result in significant differences in the feed intake of red hybrid tilapia (Saleh et al 2014). Additionally, Mansour et al (2018) reported that the inclusion of 5% and 10% *M. oleifera* leaves did not significantly affect the culture performance of seabream (*S. aurata*). Similarly, 10% and 15% *M. oleifera* did not significantly influence the feed intake of juvenile red hybrid tilapia (*Oreochromis* sp.) as observed by Farahiyah & Nor Maisarah (2020). Their findings are consistent with the present study, where varying inclusion levels of *Ulva* sp. and *M. oleifera* also showed no significant differences in feed intake among the experimental diets. This suggests that the inclusion of these plant-based ingredients does not negatively affect the palatability or acceptance of the feed. Maintaining consistent feed intake is crucial, as it ensures that fish receive the required nutrients for optimal growth and health, thereby supporting the potential of *Ulva* sp. and *M. oleifera* as sustainable feed additives in aquaculture. The present study revealed that the FCR of juvenile *O. mossambicus* was significantly lower in experimental diets containing 10% *Ulva* sp. and 10% *M. oleifera* compared to the control experiment. However, in the study by Farahiyah & Nor Maisarah (2020), the inclusion of 10% and 15% *M. oleifera* in the diet of juvenile red hybrid tilapia (*Oreochromis* sp.) significantly increased the FCR compared to the control group. This finding highlights the advantage of combining *Ulva* sp. and *M. oleifera* at appropriate levels, as a lower FCR indicates more efficient feed utilization, meaning less feed is required to produce the same amount of fish biomass. This is crucial for improving cost-effectiveness and sustainability in aquaculture production, making the present study a valuable contribution to the development of efficient alternative feed strategies.

Conclusions. The findings of this study demonstrate that incorporating *Ulva* sp. and *Moringa oleifera* into commercial diets can effectively enhance the performance of juvenile *Oreochromis mossambicus*. The synergistic use of these alternative nutrient sources significantly improved key growth indicators, particularly specific growth rate and weight gain, while also increasing survival rates at higher inclusion levels. Although feed intake remained unaffected, improvements in feed efficiency were evident in selected diet combinations. Overall, the results confirm that the combined inclusion of *Ulva* sp. and *M. oleifera* is a promising, sustainable strategy for improving growth performance, survival, and feed utilization in tilapia culture, with specific diet formulations (notably moderate inclusion levels) showing the most beneficial effects.

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Authors contributions. JHS: Conceptualization, Data Curation, Analysis, Interpretation of the Data, Writing - Original Draft, Review & Editing; ANHK and RKH: Data Curation, Analysis and Interpretation of the Data, Writing - Original Draft; FNTE, MDH, EMT, WMJ, RJFR, and IPM: Writing - Review & Editing. All authors approved the final manuscript.

Conflicts of interest. The authors declare that there is no conflict of interest.

Data availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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