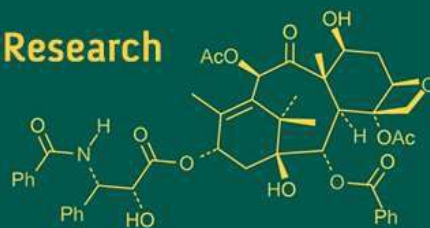


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Effects of dietary fish meal replacement with *Ulva* sp. on growth performance and feed utilization efficiency in Nile tilapia (*Oreochromis niloticus*) Fry

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Abstract

The present study evaluated the effects of dietary fish meal replacement with *Ulva* spp. on growth performance and feed utilization efficiency of Nile tilapia (*Oreochromis niloticus*) fry. A 60-day feeding trial was conducted using four isonitrogenous diets (40% crude protein) containing graded levels of *Ulva* spp. at 0% (control), 5%, 10%, and 15%. Growth performance indices, including final body weight, weight gain (%), specific growth rate (SGR), survival rate, and feed utilization parameters such as feed conversion ratio (FCR) and protein efficiency ratio (PER), were assessed. Results revealed that dietary inclusion of 5% *Ulva* spp. significantly enhanced final weight, weight gain, SGR, and PER, while significantly reducing FCR compared to the control diet ($P < 0.05$). However, higher inclusion levels (10-15%) did not produce additional benefits and resulted in marginal reductions in growth performance. The findings indicate that partial replacement of fish meal with 5% *Ulva* spp. optimizes growth performance and feed utilization in Nile tilapia fry, highlighting its potential as a sustainable ingredient in aquafeed formulations.

Keywords: Nile tilapia, *Ulva* spp., Growth performance, Sustainable aquafeed

Introduction

Aquaculture represents the fastest-expanding sector of global food production and plays an increasingly vital role in meeting the rising demand for animal protein and ensuring food security. Among farmed freshwater species, Nile tilapia (*Oreochromis niloticus*) is of considerable economic importance owing to its rapid growth rate, tolerance to a wide range of environmental conditions, omnivorous feeding behavior, and strong consumer acceptance. The species has been extensively introduced worldwide for aquaculture purposes and is cultivated across diverse freshwater systems, including rivers, lakes, irrigation channels, and earthen ponds. In 2020, global aquaculture production reached a record high, reflecting the growing demand for affordable and nutritionally valuable protein sources (Anonymous, 2021) [3]. Aquaculture has overtaken capture fisheries first time ever in 2020 (Griffin *et al.* 2019) [11]. As aquaculture production continues to expand, the requirement for nutritionally balanced and economically viable feeds intensifies, with feed costs accounting for approximately 30-70% of total production expenses, depending on the cultured species (Rumsey, 1993) [21].

Fish meal (FM) has long been regarded as the benchmark protein ingredient in aquafeeds due to its superior digestibility, balanced amino acid composition, and high palatability. However, increasing constraints related to limited availability, price volatility, and environmental concerns associated with overexploitation of wild fish stocks have raised questions regarding the long-term sustainability of FM use. Consequently, there is growing interest in identifying alternative, eco-friendly protein sources capable of partially or fully substituting FM without adversely affecting growth performance or fish health (Mahmoud *et al.*, 2020) [16].

Macroalgae, commonly referred to as seaweeds, are broadly classified into green (Chlorophyta), red (Rhodophyta), and brown (Ochrophyta) groups, encompassing nearly 10,000 species worldwide, predominantly distributed in marine ecosystems (Kidgell *et al.*, 2019; Sullivan *et al.*, 2010) [15, 23]. Seaweeds are increasingly recognized for their nutritional richness, providing valuable proteins, essential minerals, vitamins, and diverse bioactive

compounds, thereby offering significant potential for both nutritional and functional applications in aquafeed formulations (Azenha, 2019) ^[6].

Within green macroalgae, *Ulva* spp. have attracted considerable attention due to their relatively high protein and mineral content and their suitability as partial replacements for fish meal in aquaculture diets (Jamal *et al.*, 2017) ^[14]. The principal cell wall polysaccharide, ulvan, accounts for approximately 9-36% of the dry biomass and exhibits a wide range of biological activities, including antioxidant, immunomodulatory, anti-inflammatory, anticoagulant, and antiviral effects (Hamed *et al.*, 2015; Pereira, 2018; Usov, 2011) ^[13, 18, 24]. Given its rapid growth rate, widespread distribution along the Gujarat coastline, and year-round availability, *Ulva* spp. were selected in the present study to assess their potential as sustainable feed ingredients for aquaculture.

The sustainable exploitation of *Ulva* as food and feed can contribute to the demand for renewable and novel nutritious food sources, especially with vegetarian and vegan protein, emphasized by the UNSDGs (Assembly U. G. 2015). *Ulva lactuca*, commonly known as sea lettuce, is a green macroalga enriched with bioactive constituents such as polysaccharides, phenolic compounds, and essential amino acids, which contribute to its antioxidant, antimicrobial, and anti-inflammatory properties (El-Baky *et al.*, 2008; Alghazeer *et al.*, 2013; Putra *et al.*, 2024) ^[1, 2, 19]. These characteristics position *U. lactuca* as a promising functional additive in aquafeeds. Recent investigations have reported that dietary incorporation of *U. lactuca* enhances growth performance, feed utilization efficiency, and antioxidant enzyme activity in fish species (Calheiros *et al.*, 2019; Dernekbaşı, 2017) ^[7, 8]. For instance, Dernekbaşı (2017) demonstrated improved growth, digestive enzyme activity, and antioxidant responses in Nile tilapia fed *U. lactuca*-supplemented diets during both fasting and normal feeding periods. Similarly, Calheiros *et al.* (2019) ^[7] observed positive effects of graded *U. lactuca* inclusion levels on growth and antioxidant capacity in tilapia.

Dietary inclusion of *Ulva* spp. in aquafeeds has been increasingly investigated as a sustainable alternative to traditional protein sources, offering bioactive compounds such as sulfated polysaccharides, phenolics, vitamins, and minerals that can positively influence growth performance, antioxidant capacity, and immune function in fish (Valente *et al.*, 2019; Shang *et al.*, 2019; Qi *et al.*, 2012) ^[25, 22, 20]. Moderate dietary inclusion of *Ulva lactuca* (approximately 5-10%) has been shown to enhance antioxidant capacity and favorably modulate hematological responses in tilapia without adversely affecting growth or body composition. Similar benefits have been reported in other finfish species, where seaweed supplementation improves innate immune responses and antioxidant enzyme activities, although growth responses may vary depending on species and environmental conditions. The beneficial effects are largely attributed to bioactive compounds that stimulate glutathione-dependent and related antioxidant pathways. However, excessive inclusion of macroalgae may impair growth performance due to increased dietary fiber content and reduced nutrient digestibility. Despite evidence from juvenile and grow-out stages, information on the effects of

Ulva supplementation during the fry stage remains limited. Therefore, further targeted research is required to establish optimal inclusion levels and to clarify the mechanisms through which *Ulva* influences growth, feed utilization, antioxidant defense, and immune resilience during early developmental stages. The present study was therefore designed to evaluate the effects of replacing fishmeal with graded levels of *Ulva* spp. on growth performance, feed utilization efficiency, and physiological responses in Nile tilapia (*Oreochromis niloticus*) fry. The outcomes are expected to generate evidence for development of nutritionally balanced, cost-effective, and environmentally sustainable aquafeeds.

Materials and Methods

Experimental Fish Husbandry, Rearing Conditions, Diets, and Water Quality Management

Healthy Nile tilapia (*Oreochromis niloticus*) fry were procured from a commercial hatchery and transported to the Inland Fisheries Research Station, Junagadh Agricultural University (JAU), Junagadh, in oxygen-filled polyethylene bags to minimize handling and transport stress. Upon arrival, the fry were acclimatized for a period of seven days in circular fiberglass-reinforced plastic (FRP) tanks under continuous aeration. During the acclimation phase, fish were fed a standard control diet twice daily until they adapted to laboratory conditions.

Following acclimation, a total of 240 fry of uniform size were randomly assigned to four dietary treatments, each conducted in triplicate with 20 fry per replicate. The experimental feeding regimes were formulated as follows:

- **T₀ (Control):** fish meal-based diet (100% fish meal)
- **T₁:** 5% replacement of fish meal with *Ulva*
- **T₂:** 10% replacement of fish meal with *Ulva*
- **T₃:** 15% replacement of fish meal with *Ulva*

Fish were stocked at a density not exceeding 1 g L⁻¹, consistent with standard laboratory rearing practices. Feeding was carried out twice daily at 10:00 and 16:00 h to apparent satiation throughout the 60-day experimental period. All tanks were covered to prevent escape of fish.

Routine tank management involved daily manual cleaning, including siphoning of uneaten feed and fecal matter. Following cleaning, an equivalent volume of fresh, aerated water was added to maintain constant water levels. Ambient temperature and photoperiod were maintained under natural conditions without artificial manipulation.

Water quality parameters were monitored regularly throughout the experimental period. Water temperature and dissolved oxygen were recorded daily using a portable multi-parameter meter, while pH was measured with a calibrated digital pH meter. Total ammonia concentration was assessed weekly using standard colorimetric methods. All measurements were taken at consistent times to minimize variability. Any deviations from optimal conditions were promptly corrected through partial water exchange and aeration adjustments. Throughout the study, water quality remained within suitable ranges for tilapia fry: temperature 27-29 °C, dissolved oxygen 5.5-6.5 mg L⁻¹, pH 7.5-8.0, and ammonia levels below 0.25 mg L⁻¹, ensuring a stable and low-stress rearing environment.

Table 1: Composition of experimental diet prepared using macroalgae (*Ulva* sp.)

	Diet prepared with 40% protein				
	T0 (control)	T1 (5% <i>Ulva</i>)	T2 (10% <i>Ulva</i>)	T3 (15% <i>Ulva</i>)	
Fish meal	74	73	71	70	
<i>Ulva</i>	0	4	8	12	
Tapioca Powder	10	9	8	7	
Wheat Flour	10	8	7	5	
Plant oil	2	2	2	2	
Fish oil	2	2	2	2	
Vitamin and Mineral	2	2	2	2	
Total	100	100	100	100	

Growth Performance and Feed Utilization

To monitor growth and feed efficiency, fish from each tank were sampled at 15-day intervals throughout the 60-day feeding trial. Prior to weighing, the fry were starved overnight to ensure accurate measurements, and their body weight was recorded using a precision electronic balance. At the end of the experiment, all fry were carefully weighed to determine final growth parameters.

1. Weight gain (WG; g) = final weight (Wf; g) - initial weight (Wi; g);
2. Weight gain % (WG%) = 100 [WG (g)]/Wi (g);
3. Specific growth rate (SGR; %/day) = 100 × (Ln Wf - Ln Wi)/90;
4. Feed conversion ratio (FCR) = Dry feed intake (g)/Net wet weight gain (g);
5. The survival rate of fish (%) = 100 × (final fish count/initial fish count).

Water Quality Estimation

Water quality parameters were monitored throughout the 60-day experimental period to ensure optimal rearing conditions for Nile tilapia (*Oreochromis niloticus*) fry. Water temperature, dissolved oxygen (DO), and pH were measured daily using calibrated digital instruments, while total ammonia nitrogen (TAN), nitrite, and alkalinity were analyzed at weekly intervals following standard analytical procedures. All chemical parameters were estimated according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2017) [4]. Measurements were taken prior to feeding to minimize diurnal variation, and uniform aeration and periodic water exchange were maintained across all treatments. The monitored water quality parameters remained within the acceptable ranges recommended for tilapia culture.

Statistical Analysis

All data, including growth performance, feed utilization, antioxidant enzyme activities, and hematological parameters, were analyzed using one-way analysis of variance (ANOVA) in SPSS software (version 26, IBM, Richmond, VA, USA). When significant differences were detected ($p < 0.05$), Tukey's HSD test was applied as a post-hoc comparison to identify differences between treatment means. All results are presented as mean ± standard error (SE).

Results

The present study investigated the effects of graded dietary inclusion levels of *Ulva* meal on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) fry over a 60-day feeding trial. The absence of significant differences in initial body weight among treatments

confirms homogeneity at stocking and validates that subsequent variations in growth and nutritional indices were attributable to dietary interventions.

Dietary inclusion of *Ulva* at 5% (T₁) resulted in a statistically significant enhancement ($p < 0.05$) of final body weight, weight gain percentage, and specific growth rate compared to the control and higher inclusion levels. This improvement indicates that low-level *Ulva* supplementation positively modulates growth performance in tilapia fry. The observed growth stimulation at 5% inclusion may be attributed to the presence of biologically active compounds in *Ulva*, including sulfated polysaccharides, essential minerals, vitamins, and antioxidant molecules, which are known to enhance metabolic efficiency and physiological performance in fish.

The intermediate response observed in fish fed the 10% *Ulva* diet (T₂), which was statistically comparable to both T₁ and the control, suggests a dose-dependent response with diminishing returns beyond the optimal inclusion level. In contrast, the 15% *Ulva* diet (T₃) did not improve growth performance and yielded values comparable to the control, indicating that excessive inclusion may adversely affect nutrient availability. This reduction in efficacy at higher inclusion levels is likely associated with increased dietary fiber and complex non-starch polysaccharides, which can impair nutrient digestibility and limit energy utilization in juvenile fish.

Feed conversion ratio was significantly reduced in fish fed the 5% *Ulva* diet, reflecting improved feed efficiency. Enhanced feed conversion may be indicative of improved digestive capacity, possibly mediated through stimulation of digestive enzyme activity or favorable modulation of intestinal microflora. Macroalgal polysaccharides have been reported to act as prebiotic substrates, promoting gut health and improving nutrient assimilation, particularly in early life stages.

Protein efficiency ratio followed a similar trend, with significantly higher values observed in the 5% *Ulva* group, suggesting improved dietary protein utilization and nitrogen retention. The lack of improvement in PER at higher inclusion levels may be related to the presence of antinutritional components or reduced protein digestibility due to excessive structural carbohydrates, which can interfere with amino acid absorption.

Collectively, the results indicate that *Ulva* meal functions more effectively as a functional feed ingredient rather than as a major nutrient replacement when included at low dietary levels. Moderate inclusion enhances growth performance and feed utilization, whereas excessive incorporation negates these benefits due to reduced digestibility and metabolic efficiency.

Overall, the growth performance and feed utilization data indicate that 5% *Ulva* supplementation (T₁) was optimal for promoting growth in *O. niloticus* fry, whereas higher levels (15%) did not confer additional benefits. Detailed mean $\pm$ SE values and statistical differences are summarized in Table 2 & Fig. 1 and 2.

Water Quality Parameters

Throughout the 60-day experimental period, key water quality parameters were regularly monitored and maintained within acceptable ranges for Nile tilapia (*Oreochromis niloticus*) fry culture. Water temperature, dissolved oxygen, pH, total ammonia nitrogen, nitrite, and alkalinity did not show significant variation ($p > 0.05$) among the dietary

treatments, indicating that the inclusion of *Ulva* meal at different levels had no adverse effect on the rearing environment. Dissolved oxygen levels remained consistently above the critical threshold required for optimal tilapia growth, while pH values were maintained within the slightly alkaline range suitable for freshwater aquaculture. Nitrogenous waste metabolites were maintained at sub-lethal concentrations throughout the experimental period, reflecting effective husbandry practices and adequate water exchange. These stable water quality conditions ensured that the observed differences in growth performance and feed utilization were primarily attributable to dietary treatments rather than environmental variability.

Table 2: Growth performance of *O. niloticus* fry fed diets supplemented with different levels of *Ulva*. Values are mean $\pm$ SE (n = 3). Different superscript letters within a row indicate significant differences ($p < 0.05$).

Parameter	T0 (Control)	T1 (5% <i>Ulva</i>)	T2 (10% <i>Ulva</i>)	T3 (15% <i>Ulva</i>)
Initial Weight (g)	1.232 $\pm$ 0.045 ^a	1.226 $\pm$ 0.049 ^a	1.229 $\pm$ 0.049 ^a	1.218 $\pm$ 0.046 ^a
Final Weight (g)	4.685 $\pm$ 0.187 ^a	5.948 $\pm$ 0.238 ^b	5.421 $\pm$ 0.216 ^{ab}	4.496 $\pm$ 0.180 ^a
Weight Gain (%)	380.1 $\pm$ 15.2 ^a	478.6 $\pm$ 19.1 ^b	425.2 $\pm$ 17.0 ^{ab}	369.8 $\pm$ 14.8 ^a
SGR (%/day)	2.23 $\pm$ 0.089 ^a	2.63 $\pm$ 0.105 ^b	2.47 $\pm$ 0.099 ^{ab}	2.18 $\pm$ 0.087 ^a
FCR	1.94 $\pm$ 0.077 ^b	1.44 $\pm$ 0.058 ^a	1.79 $\pm$ 0.072 ^b	1.93 $\pm$ 0.077 ^b
PER	1.292 $\pm$ 0.052 ^a	1.742 $\pm$ 0.070 ^b	1.386 $\pm$ 0.055 ^a	1.284 $\pm$ 0.051 ^a

Discussion

Growth Performance and Feed Utilization

The present study demonstrated that dietary inclusion of *Ulva* significantly influenced the growth performance of *Oreochromis niloticus* fry. Among the tested inclusion levels, 5% *Ulva* (T₁) consistently produced the highest final weight, weight gain, and specific growth rate (SGR), indicating that this is the optimal level for growth enhancement. Fish fed 10% *Ulva* (T₂) showed moderate growth improvement, whereas 15% *Ulva* (T₃) did not provide additional benefits compared to the control. These findings suggest a clear dose-dependent response, with moderate supplementation being most effective while higher levels may adversely affect growth.

A probable reason for the enhanced growth at 5% inclusion is that *Ulva* is a rich source of minerals, vitamins, and protein, which are critical for the growth and metabolic processes in fish (Jamal *et al.*, 2017) [14]. Fish in T₁ exhibited a significantly higher weight gain (%) of 477.95 $\pm$ 6.56%, compared to 380.30 $\pm$ 1.79% in the control, highlighting the positive effect of moderate *Ulva* supplementation. Conversely, T₃, which contained 15% *Ulva*, recorded the lowest weight gain (370.47 $\pm$ 1.50%), likely due to reduced palatability, lower feed intake, or the presence of non-digestible polysaccharides at higher inclusion levels.

The weight gain trend also varied over the experimental period. Although growth did not show a consistent difference among treatments initially, significant variation was observed at the end of the 60-day trial. This indicates that the effect of *Ulva* supplementation may be time-dependent, with benefits becoming evident only after prolonged feeding. Such a delayed effect is likely related to the gradual adaptation of the digestive system and gut microbiota to dietary seaweed components, improving

nutrient absorption over time.

Feed utilization parameters further corroborate these findings. Fish in T₁ displayed the lowest feed conversion ratio (FCR) and the highest protein efficiency ratio (PER), indicating superior feed efficiency. Moderate *Ulva* supplementation likely optimized the protein-to-energy ratio, improved nutrient digestibility, and enhanced metabolic utilization of feed. In contrast, higher inclusion (T₃) did not improve FCR or PER, supporting the hypothesis that excessive levels of seaweed may introduce indigestible components or anti-nutritional factors, limiting growth.

The results of this study are consistent with previous research. Ergun *et al.* (2009) [10] reported that Nile tilapia fed 5% *Ulva* meal exhibited improved growth compared to non-supplemented diets. Similarly, Natify *et al.* (2015) [17] observed that moderate inclusion of *Ulva* as a feed additive enhanced weight gain in tilapia, while higher inclusion levels were less effective. These findings indicate that the positive effects of *Ulva* supplementation are maximized at moderate inclusion levels and are likely species-specific, as nutrient digestibility can vary depending on fish physiology and diet composition.

The enhanced growth observed at moderate *Ulva* inclusion may also be attributed to bioactive compounds present in the algae, including polysaccharides, polyunsaturated fatty acids, and antioxidants, which can stimulate digestive enzyme activity, improve gut health, and modulate nutrient absorption. Furthermore, the improved palatability of moderate inclusion diets may encourage higher feed intake, thereby contributing to increased weight gain and growth rates. Conversely, at higher inclusion levels, reduced feed intake due to taste, texture, or the presence of anti-nutritional components may explain the diminished growth observed in T₃.

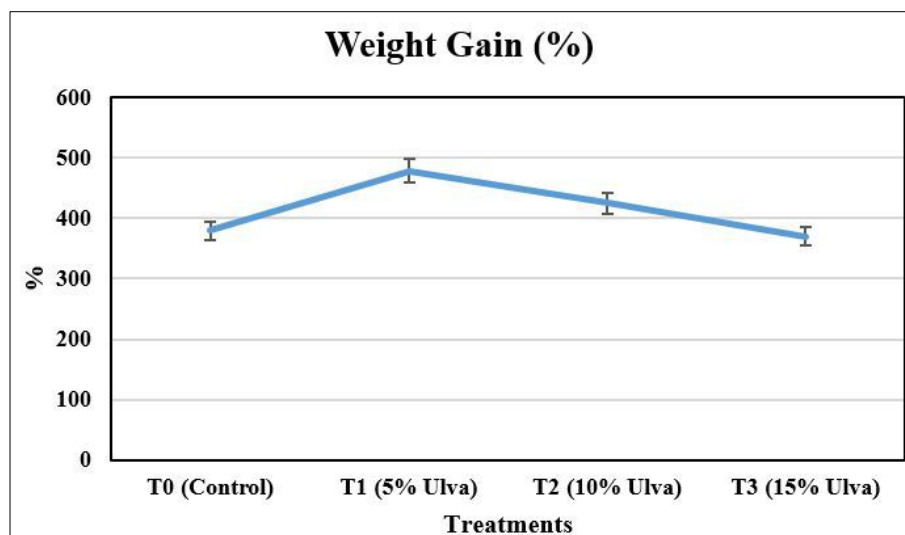


Fig 1: Weight gain (%) *O. niloticus* fry fed diets supplemented with different levels of *Ulva*

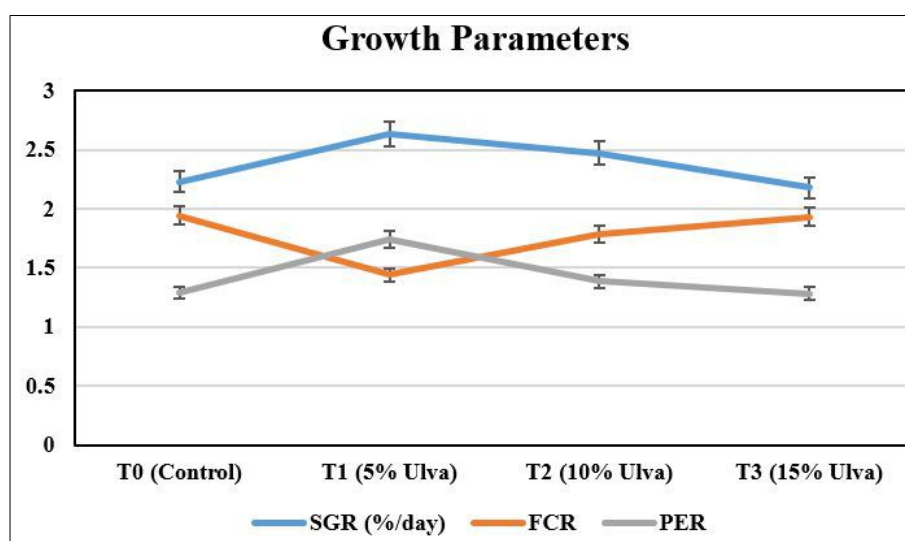


Fig 2: Growth Performance of *O. niloticus* fry fed diets supplemented with different levels of *Ulva*

Conclusion

Overall, these findings demonstrate that dietary inclusion of *Ulva* at 5% replacement of fish meal optimally enhances growth performance, weight gain, SGR, and feed utilization in *O. niloticus* fry. Higher levels (10-15%) do not provide additional benefits and may even reduce growth efficiency. These results provide practical guidance for aquaculture nutritionists seeking to incorporate macroalgae into tilapia diets.

Dietary inclusion of *Ulva* in the diets of *Oreochromis niloticus* fry significantly enhanced growth performance and feed utilization efficiency. Among the tested levels, fish fed a diet containing 5% *Ulva* (T₁) achieved the highest final body weight, weight gain, and specific growth rate, along with improved feed conversion ratio and protein efficiency ratio. In contrast, higher inclusion levels (10-15%) did not result in additional growth benefits, and performance declined slightly at the 15% inclusion level. This reduction may be attributed to decreased feed palatability, reduced feed intake, or the presence of indigestible components at higher *Ulva* concentrations. These findings suggest that moderate dietary inclusion of *Ulva* can effectively promote growth and nutrient utilization in Nile tilapia fry.

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