B-5295
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Dietary Seaweed Supplementation on Growth, Feed Utilization, Digestibility Co-efficient, Digestive Enzyme Activity and Challenge Study against *Aeromonas hydrophila* of Nile Tilapia *Oreochromis niloticus*

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10.18805/IJAR.B-5295

ABSTRACT

Background: The present study aimed to determine the growth, digestibility co-efficient, digestive enzyme activity and disease resistance of juvenile GIF tilapia fed with *Ulva* seaweed meal supplemented diet.

Methods: The study was undertaken with different inclusion levels of *Ulva* seaweed meal (USM) such as raw (UR) and fermented (UF) *Ulva* meal (UR-5, UR-10, UR-15, UF-5, UF-10 and UF-15 % of diet) and control feed (C) for a period of 60 days.

Result: GIFT fed with fermented *Ulva* meal at a 10% inclusion level in their diet exhibited the highest growth performance (41.43 g) followed by UF5 (41.07±0.43 g), UR5 (39.65±0.36 g), control (39.09±0.20 g), UF15 (38.67±0.26 g), UR10 (38.47±1.47 g) and UR15 (35.77±0.35 g) diet. The highest apparent digestibility coefficient (ADC) with crude protein (85.14±0.91 %) was observed in fishes fed with fermented *Ulva* meal at 10 % inclusion rate than that of other treatments group for dry matter, ether extract and gross energy. The digestive enzymatic activities, amylase (0.029±0.00 units/mg protein/min), protease (0.147±0.0 units/mgprotein/min) and Lipase (0.273±0.05 units/mg protein/min), showed higher level in fishes fed with diet containing 10% fermented *Ulva* meal compared to other treatment groups including control. Moreover, at the end of the trial, increased survival rate (86.66%) was observed with fishes fed with *Ulva* based diets challenged against *Aeromonas hydrophila*. The histopathological study showed a lower level of infection in fishes fed with 10% fermented *Ulva* meal supplementation. The present study concluded that the requirement of *Ulva* meal in fish diet shows the following trend 10% of *Ulva* Fermented (UF) and UF-5, *Ulva* Raw (UR) 5%, control (C), UF-15, UR-10 and UR-15 diet that can improve the health status of the GIF tilapia.

Key words: *Aeromonas hydrophila,* Digestibility coefficient, Digestive enzyme activity, Growth performances, *Ulva*.

INTRODUCTION

The role of nutrition, feed and feed management is significant in advancement of sustainable aquaculture. Feed constitutes predominant and recurring expenditure in aquaculture, it is essential that artificial feed should be scientifically formulated, processed and delivered to fish farming system. Marine macroalgae, referred to as seaweed, offer an alternative option to plant meal and provide unique and valuable dietary component in formulated fish diets. Global cultivation of algae, dominated by marine macroalgae known as seaweeds, grew by half a million tonnes in 2020, up by 1.4% from 34.6 million tonnes in 2019 (FAO, 2022). The classification "macroalgae" encompasses three distinct taxonomic groups: *Rhodophyta* (red), *Chlorophyta* (green) and *Phaeophyta* (brown). The nutritional composition of macroalgae can differ significantly among species, genera, divisions, seasons and locations. Seaweeds have the capacity to act as an additional source of food in the nutrition of fish. Nakagawa *et al*. (1984) conducted one of the earliest documented research studies on the incorporation of macroalgae in manufactured diets for finfish followed by numerous studies have been undertaken on various fish species, investigating a range of seaweed species. These studies have demonstrated that there is considerable potential for the incorporation of seaweed in fish diets, particularly when protein level in the seaweed is sufficiently high and it possesses a balanced amino acid profile comparable to that of the main plant protein ingredients (Angell *et al*., 2016).The present investigation is to evaluate the efficacy of incorporating seaweed into the diet on the growth performance, digestibility, digestive enzymes activity and disease resistance against *Aeromonas hydrophila* during intensive farming of GIF tilapia.

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How to cite this article: Priyatharshni, A., Antony, C., Ahilan, B., Uma, A., Chidambaram, P., Ruby, P. and Prabu, E. (2024). Effect of Dietary Seaweed Supplementation on Growth, Feed Utilization, Digestibility Co-efficient, Digestive Enzyme Activity and Challenge Study against *Aeromonas hydrophila* of Nile Tilapia *Oreochromis niloticus*. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5295.

Submitted: 26-12-2023 **Accepted:** 12-02-2024 **Online:** 28-02-2024

MATERIALS AND METHODS

Experimental diets

The fermentation of seaweed meal was carried out in accordance with methodology delineated by Siddik *et al.* (2018). Following the completion of the fermentation process, seaweed meal underwent drying process in hot air oven at a temperature of 40°C for 48 hours and subsequently served as the test ingredient. The proximate composition of raw and fermented seaweeds is presented in Table 1.

Feed formulation

Six isonitrogenous (320 g/kg) and isocaloric (4395 Kcal/kg) experimental diets were prepared utilizing *Ulva* seaweeds and a control diet without seaweed. The proximate composition of ingredients and experimental diet are detailed in Table 2.

Experimental fish and feeding trial

420 Nos of GIF tilapia (*Oreochromis niloticus*) used in the experiment. The fishes procured from the Centre for Sustainable Aquaculture (CeSA), TNJFU, Barur, Tamil Nadu. The experimental design consisted of six sets of treatments and one control set, each with three replicates. The 21 FRP tanks with a water volume of 350 liters (length: 94 cm, height: 61 cm and breadth: 70 cm) were used for stocking fishes. Prior to the commencement of the experiment, all experimental tanks underwent thorough cleaning and disinfection. The tanks were filled with water upto 75% of their total volume and provided with suitable aeration facilitated by an oil-free air compressor. The fish seeds were acclimatized in tanks made of fiberglass reinforced plastic (FRP). Prior to the experiment, each fish was graded based on its weight. GIF tilapia juveniles, weighing 2.51 g, were stocked in each experimental tank at a density of 20 individuals/m^2. The fish were provided with food equal to 5% of their total body mass on a daily basis, which was divided into three equal portions and given thrice a day. Uneaten feed and fecal matter were removed daily and water exchange of 10% was done to maintain optimum water quality. Sampling of fish bio-growth was conducted fortnightly throughout the feeding trial, daily monitoring of water quality parameters was performed and average values were recorded according to standard procedure (APHA, 2005) and the recorded values are presented in Table 3.

Fish sampling

The sampling occurred at 15-day intervals. The specific bio-growth indices used are listed below:

Bio-growth parameters

Mean weight gain (g) = Mean final weight - Mean initial weight

Average daily growth (g/day) =

$$\frac{\text{Mean final weight - Mean initial weight}}{\text{Days of culture}}$$

Specific growth rate (%/day) =

$$\frac{\ln\text{ (Final weight) - }\ln\text{ (Initial weight)}}{\text{Number of days}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Initial total number stocked}}{\text{Final numbers obtained}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Total feed fed (g)}}{\text{Total fish weight gained (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Total wet weight gain}}{\text{Dry weight of protein fed}}$$

Digestibility studies

Faecal collection

The GIFT juveniles stocked in FRP tanks were initially fed at 5% of body weight and feeding ration was adjusted to avoid the accumulation of uneaten feed. Feeding and the faeces collection were done twice a day. The tanks were siphoned to remove faeces. The collected faeces were filtered and washed quickly with distilled water, dried and stored in freezer until analysis as per Cruz-Suarez *et al.* (2009).

Apparent digestibility coefficient (ADC)

The apparent digestibility coefficient (ADC) of the experimental diets was analysed using chromic oxide (Cr_2O_3) as an inert marker at 1 g/kg of the diet. The concentrations of the nutrients considered and the concentration of chromium in the experimental diets and in GIF tilapia faeces were estimated respectively to determine the ADC.

$$\text{ADC dry matter (\%)} = 100 * 1- \frac{\text{\% Faecal nutrient}}{\text{\% Dietery nutrient}}$$

$$\text{ADC nutrient (\%)} = 1- \frac{\text{\% Dietery } Cr_2O_3}{\text{\% Faecal } Cr_2O_3} \times \frac{\text{\% Faecal nutrient}}{\text{\% Dietery nutrient}}$$

Digestive enzyme activity analysis

Intestines from sampled experimental GIFT were removed aseptically added with 0.25 M sucrose solution and homogenised using tissue homogenizer and centrifuged at 8000 rpm for 10 min. The supernatant was separated and stored at -20°C until further analysis. The reducing sugars produced due to gluco amylase and alpha amylase on carbohydrates were estimated using Dinitro salicylic acid (DNS) method (Rick and Stegbauer, 1974). Protease activity was determined following the method of Moore and Stein (1948), using bovine serum albumin as the substrate. The specific activity of protease was expressed as ìg of leucine liberated/mg tissue protein/h at 37°C. The lipase activity was assayed following the method of Cherry and Crandell (1932).

Challenge study against *Aeromonas hydrophila*

At the end of the 90 days experimental trials, fishes from all treatment tanks were challenged with *Aeromonas hydrophila*. The bacteria culture was obtained from State Referral Laboratory for Aquatic Animal Health, Tamil Nadu

Dr. J. Jayalalithaa Fisheries University. The isolate was grown in tryptic soy broth (TSB Hi Media, India) for 24 h (30-31°C) centrifuged at 10,000 rpm for 10 min followed by pellet resuspension in phosphate buffered saline (PBS, pH 7.2). The suspension in sterile PBS was injected intramuscularly (60 µl) in healthy tilapia obtained from all the treatments, delivering 1.92*10^7CFU/fish. The infected moribund fish with typical haemorrhagic wounds at the site of injection were sacrificed for the histopathological study after 10 dpi. Internal organs were dissected, rinsed in normal saline and fixed in 10% formalin buffer for 24 hrs. The fixed tissues were washed in a series of ethyl alcohol of varying concentration (70%, 80%, 90% and 100% respectively) and embedded in paraffin wax. The tissues were sectioned at 5 mm, later stained with hematoxylin-eosin (HandE) (Alyahya *et al*., 2018). The histopathological analysis was performed in the Department of Pathology, Madras Veterinary College, TANUVAS, Chennai.

Statistical analysis

All the data were presented as the mean values ± standard deviation (SD) of three replicates. One-way ANOVA, followed by Tukey's test for multiple comparisons at the significance level of 0.05 was used to compare the differences between the dietary groups. The data were statistically analyzed by SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Influence of supplementation of *Ulva* meal on growth performances of GIF tilapia

The calculated growth parameters for experimental diets are given in Table 4. The present investigation demonstrated that the highest growth performances were observed in GIF tilapia when they were fed with supplementation of fermented *Ulva* meal at 10% in terms of weight gain (41.43±0.38 g) followed by UF5 (41.07±0.43 g), UR5 (39.65±0.36 g), control (39.09±0.20 g), UF15

Table 1: Proximate composition of *Ulva* sp seaweeds.

	Ulva sp.					
	Crude protein (%)	CHO (%)	Crude lipid (%)	Crude fiber (%)	Ash (%)	Moisture (%)
Raw seaweed	11.6	49.26	0.19	11.85	11.2	15.9
Fermented seaweed	15.7	53.35	1.94	2.56	10.05	16.4

Table 2: Formulation and proximate composition of experimental diets with varying inclusion levels of *Ulva* meal (g/kg of diet).

Ingredients	Control	UR5	UR10	UR 15	UF 5	UF10	UF15
Fish meal	18	18	18	18	18	18	18
Soy bean meal	22	22	22	22	22	22	22
Corn gluten meal	9	9	9	9	9	9	9
Seaweed meal	-	5	10	15	5	10	15
Wheat flour	18	18	18	18	13	8	3
Corn flour	20	14	9	4	19	19	19
Cassava meal	8	8	8	8	8	8	8
Soy lecithin	1	1	1	1	1	1	1
Fish oil	1	1	1	1	1	1	1
Dicalcium phosphate	1	1	1	1	1	1	1
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	1	1	1	1	1	1	1
Chromium oxide	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100
Proximate composition (%)							
Fibre	9.24	9.60	10.01	9.98	8.63	9.02	9.67
Moisture	2.35	2.00	3.54	3.66	0.17	0.61	2.21
Protein	32.96	32.69	32.90	32.33	32.6	32.61	32.12
Ash	8.54	8.65	9.79	9.97	8.5	8.78	9.38
Fat	1.12	1.19	1.39	1.05	0.77	0.74	0.82
CHO	45.79	45.87	42.37	43.01	49.33	98.8785	45.8

UR- *Ulva* raw, UF- *Ulva* fermented.

(38.67±0.26 g), UR10 (38.47±1.47 g) and UR15 (35.77±0.35 g) diet. GIF tilapia fed with various inclusion levels of *Ulva* supplemented diets were given in Fig 1.

GIF tilapia fed with UF10 diet showed lowest FCR (1.34±0.00), highest SGR (0.99±0.00) and PER (2.37±0.07) followed by UF5 diet, UR5 diet, control diet, UF15 diet, UR10 diet and UR15 diet. Similarly, Nile tilapia fed with *Ulva spp. (Ulva rigida* and *Ulva lactuca*) at 10% inclusion showed improved growth performance (Valente *et al*., 2016). African catfish (*Clarias gariepinus)* fed with *Ulva lactuca* at 10% inclusion showed improved growth performances (Abdel-Warith *et al*., 2015). Nile tilapia fed with *Ulva* meal at 5 to 10% inclusion showed improved growth performances (Guroy *et al*., 2007). Nile tilapia (*Oreochromis niloticus*) juveniles fed with *Ulva* spp. 10% inclusion showed improved growth performance (Silva *et al*., 2015). Gilthead seabream (*S. aurata*) fed with *Ulva* meal at 5% inclusion showed improved growth performances and survival rate (Wassef *et al*., 2005). Gilthead seabream fed with *Ulva* meal at 8% inclusion showed better protein utilization (Kissil and Lupatsch, 1992). European seabass (*Dicentrarchus labrax*) fed with *Ulva lactuca* at 5% inclusion showed improved growth performances, feed utilization, nutrient retention and survival rate (Wassef *et al*., 2013). Nile tilapia, scientifically known as *Oreochromis niloticus*, when provided with *Ulva rigida* at a rate of 5% incorporation, exhibited enhanced growth performance as reported by Guroy (2007). Similarly, Nile tilapia, (*Oreochromis niloticus*), showed improved growth performance when fed with *Ulva lactuca* at 5% inclusion, as documented by Khalafalla and El-Hais (2015). Moreover, Nile tilapia (*Oreochromis niloticus*) demonstrated improved growth performance, feed efficiency, nutrient utilization and body composition when fed with *Ulva* meal at 5% inclusion, as indicated by Ergun *et al.* (2009). Furthermore, Rainbow trout (*Oncorhynchus mykiss*) experienced growth enhancement when fed with *Ulva intestinalis* at 1.5% sulfated polysaccharide inclusion, according to Safavi *et al.* (2019). Dusky kob (*Argyrosomus japonicus*) also displayed improved growth performance without adverse effects when fed with *Ulva* meal at 50 g/kg inclusion, as observed by Madibana *et al.* (2017). In addition, the growth performance of *Solea senegalensis* was found to be improved when fed with *Ulva ohnoi* at 5% inclusion, as reported by Vizcaino *et al.* (2018). Furthermore, juvenile grey mullet (*Mugil cephalus*) exhibited improved growth performance when fed with an extract of *Ulva rigida* at 10 mg kg^{-1} inclusion, as reported by Akbary and Aminikhoei (2018). The presence of sulfated polysaccharides, high protein, mineral contents and a favorable essential amino acid profile in *Ulva* sp stimulates the growth of beneficial intestinal bacteria and promotes antioxidant defense system, as indicated by Wassef *et al.* (2005). Similarly, this study also proved that GIF tilapia fed with fermented *Ulva* meal reflected positive growth performance as per the result given in Table 4.

Table 3: Optimum water quality parameters observed during the experimental trial.

Parameters	Range
Temperature	27.5-29.0°C
Dissolved Oxygen	5-6 ppm
pH	8.2-8.4
Ammonia	0.01-0.02 ppm
Nitrite	0.03-0.04 ppm
Nitrate	10-12 ppm
Hardness	324-355 ppm
Alkalinity	167-172 ppm

Influence of *Ulva* meal on apparent digestibility coefficient (ADC) of GIF tilapia

The digestibility coefficient of GIF tilapia fed *Ulva* supplemented diets are given in Table 5. The present investigation demonstrated a significant increase in the Apparent Digestibility Coefficient (ADC) in fish that were fed *Ulva* meal diets. The GIF tilapia exhibited a higher level of increased ADC (85.14±0.91%) when fed with fermented *Ulva* meal at a 10% inclusion level, followed by UF5, UR5, control group, UF15, UR10 and UR15 diet.

Likewise, there were noticeable alterations in the morphology of the digestive system in Nile tilapia (*Oreochromis niloticus*) juveniles when *Ulva* was included in their diet (Silva *et al*., 2015). The inclusion of *Ulva fasciata* extract at 100 mg/kg in the diet of Nile tilapia (*Oreochromis niloticus*) resulted in an enhancement of villus length and goblet cell number (Abo Raya *et al*., 2021). Furthermore, *Solea senegalensis* that were fed with *Ulva ohnoi* at 5%

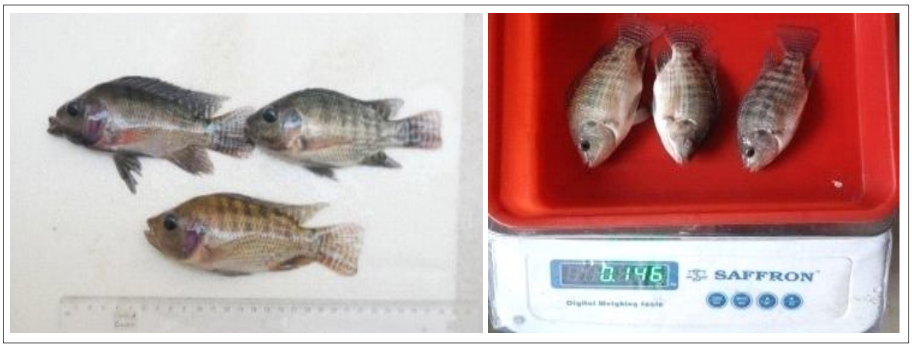

Fig 1: GIF tilapia fed with various inclusion levels of *Ulva* supplemented diets.

inclusion level exhibited an absence of damage in the intestinal mucosa and a significant increase in mucosal absorptive surface (Vizcaino *et al*., 2018). It should be noted that the height and width of intestinal villi and the number of goblet cells directly impact the digestion and nutrient absorption in fish (Elsabagh *et al*., 2018). The presence of antioxidant properties can prevent the oxidation of biological molecules, particularly lipids and proteins, consequently improving the processes of digestion and absorption (Mohan *et al*., 2019 and Ruby *et al*., 2022). Similarly, the results presented in Table 5 of this study also confirm that GIF tilapia fed with fermented *Ulva* meal exhibited positive responses in apparent digestibility coefficient.

Influence of *Ulva* meal on digestive enzyme activity of GIF tilapia

The digestive enzyme activity of GIF tilapia fed *Ulva* supplemented diets are given in Table 6. The present investigation observed higher activities of digestive enzymes, namely Amylase activity (0.029±0.00), Protease activity (0.147±0.00) and Lipase activity (0.273±0.05), in the 10% fermented *Ulva* meal supplemented diet compared to UF5 diet, UR5 diet, control diet, UF15 diet, UR10 diet and UR15 diet.

The presence of polysaccharides stimulates the secretion of digestive enzymes, leading to enhanced utilization and digestion of nutrients, ultimately resulting in improved health and growth (Mohan *et al*., 2016; Peixoto *et al*., 2016). Sulfated polysaccharides with antioxidant properties have the potential to prevent the oxidation of biological molecules, particularly lipids and proteins, thereby enhancing the processes of digestion and absorption (Mohan *et al*., 2019). Correspondingly, the current research also demonstrated a positive correlation between the consumption of fermented *Ulva* meal by GIF tilapia and the enhancement of digestive enzyme activities, as indicated in Table 6.

Influence of *Ulva* meal on disease resistance against *Aeromonas hydophila* infection in GIF tilapia

In this study, the survivility showed an increasing trend with fishes fed with *Ulva* meal as compared to control after challenged with *Aeromonas hydrophila*. The relative survival percentage of GIF tilapia fed with varying inclusion levels of

Table 4: Bio-growth performances and feed utilization of GIF tilapia juveniles fed varying inclusion levels of *Ulva* supplemented diets.

	Control	UR5	UR10	UR15	UF5	UF10	UF15
IBW (g)	2.46±0.09^{a}	2.47±0.00^{a}	2.55±0.12^{a}	2.50±0.03^{a}	2.42±0.01^{a}	2.49±0.09^{a}	2.38±0.04^{a}
FBW (g)	41.55±0.16^{c}	42.17±0.04^{c}	40.97±1.31^{c}	38.28±0.37^{d}	43.50±0.42^{ab}	43.93±0.44^{a}	41.02±0.24^{c}
WG (g)	39.09±0.20^{c}	39.65±0.36^{bc}	38.47±1.44^{c}	35.77±0.35^{d}	41.07±0.43^{ab}	41.43±0.38^{a}	38.67±0.26^{c}
Survival (%)	98.33±2.89 ^{a}	99.67±0.58 ^{a}	96.67±2.89 ^{a}	95.00±0.00^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	96.67±2.89 ^{a}
Biomas (g)	742.77±3.96^{bc}	797.47±2.08^{ab}	768.47±28.70^{ab}	691.62±18.82^{c}	794.15±27.94^{ab}	814.79±28.07^{a}	772.72±5.21^{ab}
FCR	1.74±0.00^{c}	1.69±0.00^{d}	1.92±0.00^{b}	2.03±0.00^{a}	1.49±0.00^{e}	1.34±0.00^{f}	1.89±0.00^{b}
FER	0.57±0.00^{de}	0.59±0.00^{cd}	0.52±0.00^{bc}	0.549±0.00^{f}	0.67±0.00^{b}	0.74±0.00^{a}	0.53±0.00^{e}
PER	1.89±0.00^{c}	1.83±0.01^{c}	1.62±0.00^{d}	1.59±0.04^{d}	2.17±0.07^{b}	2.37±0.07^{a}	1.65±0.00^{d}
DGR (g/day)	0.65±0.00^{c}	0.66±0.00^{c}	0.64±0.02^{c}	0.59±0.00^{d}	0.68±0.00^{ab}	0.69±0.00^{a}	0.64±0.00^{c}
SGR (%/day)	0.97±0.03^{ab}	0.97±0.00^{ab}	0.93±0.04^{ab}	0.92±0.00^{b}	0.98±0.02^{ab}	0.99±0.00^{a}	0.98±0.01^{ab}

Note: Values were expressed as means±SD of three replicate tank per treatment (n=3) and values with different superscripts indicate significant differences as determined by Tukey's test ($p<0.05$).

Table 5: Apparent digestibility coefficient (ADC) in GIF tilapia juveniles fed varying inclusion levels of *Ulva* supplemented diets.

	Control	UR5	UR10	UR15	UF5	UF10	UF15
Crude protein (%)	78.35±1.13^{b}	78.15±1.21^{b}	73.40±2.19^{c}	68.51±1.95^{d}	83.62±1.35^{a}	85.14±0.91^{a}	75.91±0.91^{bc}
Dry matter (%)	91.62±0.63^{b}	91.45±0.15^{b}	91.53±0.53^{c}	91.55±0.06^{d}	91.33±0.10^{a}	91.90±0.73^{a}	91.59±0.50^{c}
Ether extract (%)	87.82±0.11^{b}	88.66±0.08^{a}	85.48±0.01^{e}	83.27±0.01^{f}	92.51±0.05^{a}	92.88±0.10^{a}	86.75±0.24^{dc}
Gross energy (Kcal/kg)	91.44±0.11^{c}	91.88±0.10^{b}	87.52±0.20^{e}	85.46±0.24^{f}	95.54±0.12^{a}	96.46±0.10^{a}	89.39±0.08^{d}

Note: Values were expressed as means±SD of three replicate tanks per treatment (n=3).

Table 6: Digestive enzyme activities in GIF tilapia juveniles fed varying inclusion levels of *Ulva* supplemented diets.

	Control	UR5	UR10	UR15	UF5	UF10	UF15
Protease activity	0.146±0.00^{a}	0.144±0.00^{a}	0.143±0.00^{a}	0.140±0.02^{a}	0.150±0.01^{a}	0.147±0.00^{a}	0.144±0.00^{a}
Amylase activity	0.021±0.00^{ab}	0.026±0.00^{ab}	0.017±0.00^{ab}	0.015±0.00^{b}	0.028±0.00^{a}	0.029±0.00^{a}	0.019±0.00^{ab}
Lipase activity	0.267±0.00^{a}	0.273±0.00^{a}	0.263±0.00^{a}	0.257±0.00^{a}	0.272±0.00^{a}	0.273±0.05^{a}	0.265±0.00^{a}

Note: Protease, amylase and lipase values are expressed in unit/mg protein/min. Values were expressed as means±SD of three replicate tanks per treatment (n=3).

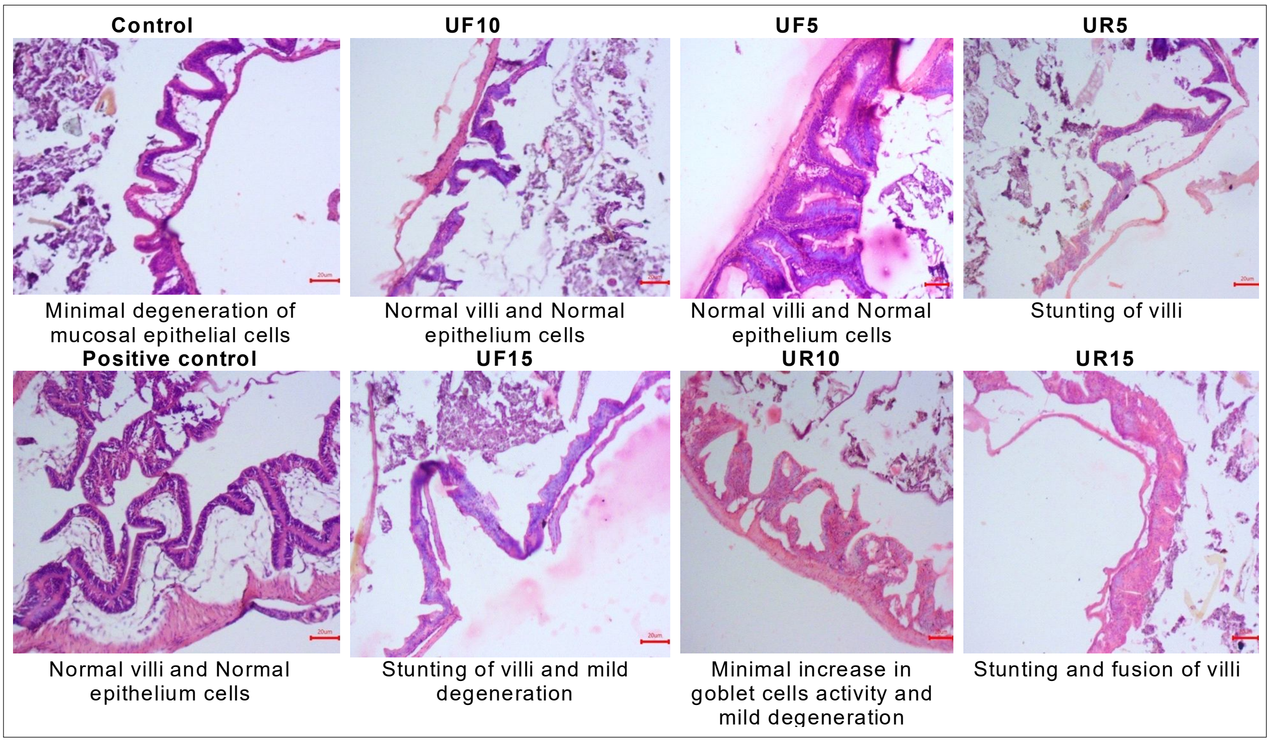

Fig 2: Histopathological changes in GIF tilapia fed varying inclusion levels of *Ulva* meal supplemented diets.

Table 7: Relative survival percentage of GIF tilapia juveniles fed varying inclusion levels of *Ulva* supplemented diets.

Treatment	Relative survival %
Control	46.666
UR5	46.666
UR10	33.333
UR15	33.333
UF5	73.33
UF10	80.00
UF15	40.00

Ulva meal are given in Table 7. The highest relative survival percentage was recorded in UF10 group (80.00%) followed by UF5, control diet, UF15, UR10 and UR15 diet.

Histopathological changes in GIF tilapia fed with varying inclusion levels of *Ulva* meal supplemented diets were given in Fig 2. Results of histopathological study showed that the supplementation of 10% of fermented *Ulva* meal had presence of lower degree levels of infection than other treatments and control diet fed fishes. Similarly, Nile tilapia fed with *Ulva fasciata* extract at 100 mg/kg diet inclusion showed improved histomorphology of intestine with an increase in villi length and villi width and goblet cells numbers (AboRaya *et al*., 2021). Juvenile grey mullet (*Mugil cephalus)* fed with *Ulva rigida* extract inclusion showed decreased mortality against Photobacterium *damselae* (Akbary and Aminikhoei, 2018). Healthy morphometric structure of intestine increases the feed utilization and considered a good indicator of a healthy fish (BananKhojasteh, 2012). Goblet cells produce mucous to protect the mucosal layer from dehydration, injury and pathogenic microorganisms along the course of intestine (Lauriano *et al*., 2016). Similarly this study also proved that GIF tilapia fed with fermented *Ulva* meal reflected health improvement against *A. hydrophila* bacterial infection and increased relative survival percentage as per the result given in Table 7.

CONCLUSION

The utilization of *Ulva* seaweed meal in the diets of juvenile GIF tilapia resulted in improved growth performance and a better feed utilization. The inclusion of seaweed supplementation had a significant impact on digestibility, digestive enzyme activities and an improved immune enhancement against *A. hydrophila* as well as an improved relative survival percentage. This study concluded that inclusion of *Ulva* meal in the diet of GIF tilapia at a UF-10% of the diet, followed by UR5 diet, UR5 diet, control diet, UF15 diet, UR10 diet and UR15 diet improves growth and health status of fish.

ACKNOWLEDGEMENT

The authors sincerely thank Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India for the grants and facilities offered. We also thank the Department of Aquaculture, Dr. M.G.R. Fisheries College and Research Institute, TNJFU for providing the Indoor aquaculture facility to conduct the feeding trial.

Conflict of interest

The authors declare that they have no conflict of interest.

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