

Effect of Use Fresh Macro Algae (Seaweed) *Ulva fasciata* and *Enteromorpha flaxusa* With or Without Artificial Feed on Growth Performance and Feed Utilization of Rabbitfish (*Siganus rivulatus*) fry

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Abstract

This study a low-cost aquaculture diet with fresh seaweed was tested with the herbivorous rabbitfish (*Siganus rivulatus*) fry. Two fresh seaweed was genera, *Ulva* and *Enteromorpha* (belong to family Ulvaceae) were used to replace the artificial feed by 0, 50 and 100 percent regardless the protein percentage. Initial average weight of fry was 0.18 g. This trial consisted of sixth treatments, the first treatment (T1) fish fed on artificial feed only, the (T2) fish fed of half feeding rate on artificial feed and other fresh *Ulva*, the (T3) fish fed of half feeding rate on artificial feed and other fresh *Enteromorpha*, the (T4) fish fed on fresh *Ulva* only, the (T5) fish fed on fresh *Enteromorpha* only and the (T6) fish fed of half feeding rate on fresh *Ulva* and other fresh *Enteromorpha*. Feeding rat was 7% of biomass and this trial continued for 70 days. There were significant differences between the treatments in all the growth performance parameters. The T3 was the highest in final weight (W_2), total weight gain (TG), average daily gain (ADG), relative growth rate (RGR) and specific growth rate (SGR), followed by both the T2 and T1. And the best feed conversion ratio (FCR) was achieved with T3, T1 and T2 followed by T6 and T5 but the T4 had the worst FCR.

Keywords: Rabbitfish; Macro algae; Feeding rate; Growth performance; Feed utilization

Introduction

Marbled spinefoot rabbitfish *Siganus rivulatus* is a potential for warm water marine aquaculture diversification [1,2]. Rabbitfishes belong to the genus *Siganus* of the family siganidae [3]. Siganids are herbivorous marine and brackish water fishes that are found throughout the indo west pacific [4], and the more common species are the objects of traditional subsistence and commercial fisheries throughout this region. There has been interest in the culture of these fishes in ponds or cages in several areas [5].

Being herbivorous, the *siganus* species need a big quantity of algae feed to assure their biological activities. The stomach of these fishes is an acid medium able to digest marine plants before entering in the digestive tract for complete digestion and thus excreting feces. In addition to algae feed, they can feed accidentally on some non-digestible substances such as mollusk shells and other invertebrates attached to algae [6,7]. Egypt production of rabbitfish was about 1363 ton in 2014, Mediterranean Sea took part in 822 ton production, Red Sea (466 ton) and lakes (75 ton) according to GAFRD [8].

Marine macro algae, or seaweed, are plantlike organisms that generally live attached to rock or other hard substrata in coastal areas. They belong to three different groups, empirically distinguished since the mid-nineteenth century on the basis of thallus color: - red algae (phylum Rhodophyta), brown algae (phylum Heterokontophyta (also known as the Ochrophyta), class Phaeophyceae), and green algae (phylum Chlorophyta, classes Bryopsidophyceae, Chlorophyceae, Dasycladophyceae, Prasinophyceae, and Ulvophyceae). There are about 8,000 species of macro algae (seaweeds) along the world's coast live and they may extend as deep as 270 m [9]. A total of 25 species of green sea weeds, 90 species of brown and 350 species of red seaweeds are found in the world sea area that are commercially important because of their protein, amino acids and mineral contents [10]. The protein content of seaweed differs according to species and seasonal period. Generally, the protein fraction of brown seaweeds is low (3% to 5% of the dry weight)

compared with that of the green or red seaweed (10% to 47% of the dry weight). The content of crude protein, crud lipid, ash and fiber in green meals from 7% to 29%, 0.5% to 4%, 13% to 36% and from 3% to 6% respectively [11,12].

Seaweeds are rarely promoted for the nutritional value of their proteins [13]. Considering the importance of seaweeds, it can be said that, sea weeds can play a vital role in various aspects compared to other aquatic resources. Much attention should be given on seaweed to compensate the food problem to some extent and fulfill the deficiency of nutrition for erecting the economy of several countries [14].

All green seaweed belong to the classes Ulvophyceae, Bryopsidophyceae, and Dasycladophyceae, which include approximately 1,500 species currently referred to eight orders [15]. The genus *Ulva* (Phylum: Chlorophyta, Class: Ulvophyceae, Order: Ulvales, Family: Ulvaceae) was first identified by Linnaeus in 1753 [16]. Since then many taxonomists and phycologists have been involved in the identification of *Ulva* species which are notoriously difficult to classify due to the morphological plasticity expressed by many members as well as the few reliable characters available for differentiating taxa [17].

Ulva is a good source of protein, pigments, minerals and vitamins, and is especially rich in vitamin C [18,19] and, in recent years, *Ulva* species have become important macro algae, which have been investigated as a dietary ingredient for a wide range of fish species.

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Enteromorpha (Phylum: Chlorophyta, Class: Ulvophyceae, Order: Ulvales, Family: Ulvaceae) have also been used as a source of bioactive compounds similar to those which cause an inhibitory effect against the bacterium *Xanthomonas oryzae*, which causes leaf blight disease in paddy crops [20]. People in the Philippines and Japan also use *Enteromorpha* spp. as food [21,22].

The study aimed to evaluate the effect of use fresh macro algae including feeding rate with artificial feed on growth performance, feed efficiency and feed cost of rabbitfish fry. Whereas the most of the cultured marine fish are carnivorous species, and seldom are herbivorous or omnivorous species, and the higher prices and uncertainty of availability of fishmeal (FM) is limiting the development of mariculture industry, especially the culture of carnivorous fish. So FAO (food and agriculture organization) quite canonizes the culture of herbivorous or omnivorous species [23]. From this point, the present study presented some information for the development of low-cost diets with fresh macro algae as dietary ingredient for the culture of marine fish such as herbivorous rabbitfish fry.

Materials and Methods

The present study was conducted using the research facilities of Shakshouk Fish Research Station, Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt. Rabbitfish (*Siganus rivulatus*) fry were obtained from (Mediterranean Sea) National Institute of Oceanography and Fisheries (NIOF), Alexandria Governorate, Egypt, initial average weight for this fry was 0.18 ± 0.012 g (SE standard error) and initial average length was 2.76 ± 0.057 cm.

Fish acclimatization and diet preparation

Fry were acclimatized to be adapted to water salinity of Lake Qaroun 33 part per thousand (ppt) for one week. An artificial diet was formulated by hand, the diet formulated to be almost containing 36% crude protein (Table 1) and two genres of macro algae belong to family Ulvaceae, the first genus *Ulva* (*Ulva fasciata*) was collected from Mediterranean sea, Alexandria Governorate, Egypt and the second genus *Enteromorpha* (*Enteromorpha flaxusa*) was collected from Qaroun lake, Fayoum Governorate, Egypt. After operation collection,

Ingredients	(g/100 g)
Fish meal (72% CP)	22
Extruded full fat Soybean meal (37% CP)	43
Wheat bran fine	28
Fish oil	4
Super yeast	1
Starch	1.7
Vit. & Min. & premix	0.3
Total	100
Chemical analysis % on dry matter basis	
Moisture (M)	6.94
Dry matter (DM)	93.06
Crude protein (CP)	36.44
Ether extract (EE)	13.78
Crude fiber (CF)	3.10
Nitrogen free extract (NFE)	39.02
Ash	7.66
Gross energy (GE, Kcal/g)*	5.09
Chemical analysis was determined according to AOAC [26] and NFE was calculated by difference.	
*Calculated according to NRC [27].	

Table 1: Ingredients and a proximate chemical analysis of the experimental diet.

Items	<i>Ulva</i> (<i>Ulva fasciata</i>)	<i>Enteromorpha</i> (<i>Enteromorpha flaxusa</i>)
Moisture (M)	76.10	78.60
Dry matter (DM)	23.90	21.40
Crude protein (CP)	27	25.03
Ether extract (EE)	0.57	1.74
Crude fiber (CF)	9.81	4.61
Nitrogen free extract (NFE)	42.56	38.43
Ash	20.06	30.19
Gross energy (GE, Kcal/g)*	3.73	3.00
Chemical analysis was determined according to AOAC [26] and NFE was calculated by difference		
*Calculated according to NRC [27].		

Table 2: A proximate chemical analysis % on dry matter basis of the fresh macro algae (seaweed).

the seaweed were kept in colder and used freshly in fish feeding (Table 2). Feed was offered by hand.

The trial began at 25/7/2015 and ended 3/10/2015, (70 days). An average initial weight (W_1) of fry was 0.18 ± 0.012 g and initial average length (L_1) was 2.76 ± 0.05 cm.

Trial design and distribution of fish in ponds

The indoor ponds laboratory were made of concrete, this trial consists of twelve concrete ponds. The dimensions of each pond were 1 m length \times 1 m width \times 1 m height and the water volume of each pond was 0.95 m^3 .

This trial consists of six treatments, the first treatment, fish fed on artificial feed only. The second treatment, fish were fed of half feeding rate on artificial feed and other fresh *Ulva fasciata*, the third treatment, fish were fed of half feeding rate on artificial feed and other fresh *Enteromorpha flaxusa*, the fourth treatment, fish fed of feeding rate on fresh *Ulva fasciata* only, the fifth treatment, fish fed on fresh *Enteromorpha flaxusa* only and sixth treatment fish fed of half feeding rate on fresh *Ulva fasciata* and other fresh *Enteromorpha flaxusa*. It did not take into consideration the percentage of protein diet, but was taking the variety feed. Feeding rate was 7% of fish body weight, 40 fish were stocked in each pond ($n = 80$ fry for each treatment) and the feeding was twice daily. A random sample was taken every three week for change of feed quantity without any mortality for fish.

The system of running water in experimental units

The system contained on water pump, sand filter unit and two large tanks (10000 liter/tank) used to storage the water at a point between the water source (Lake Qaroun water) and experimental units. The water pump was raising the water from water source to the sand filter unit then to the large tanks and hence to experimental units.

The system of aeration in experimental units.

The system contained on Blower connected to a network of plastic pipes this pipes transport the air to each experimental unit, the air was controlled by tap of each pond or tank and the air diffusers was used to distribute of air in all experimental unit trends.

Water quality

Some water quality parameters were measured of each treatment, temperature, pH and salinity were measured daily at 1 pm by centigrade thermometer, Orion digital pH meter model 201 and Refractometer (VITAL Sine SR-6, China) respectively. Dissolved oxygen (DO) was

Items	Treatments					
	T1	T2	T3	T4	T5	T6
	Artificial Feed Only	Artificial feed with <i>Ulva</i>	Artificial feed with <i>Enteromorpha</i>	<i>Ulva</i> Only	<i>Enteromorpha</i> Only	<i>Ulva</i> with <i>Enteromorpha</i>
Temperature (°C)	28.502 ± 0.159	28.369 ± 0.174	28.466 ± 0.162	28.488 ± 0.163	28.487 ± 0.164	28.341 ± 0.179
pH	8.418 ± 0.079	8.447 ± 0.0746	8.436 ± 0.0782	8.478 ± 0.0497	8.455 ± 0.068	8.523 ± 0.039
Salinity (‰)	32.772 ± 0.401	32.710 ± 0.421	32.622 ± 0.443	32.791 ± 0.685	32.523 ± 0.396	32.635 ± 0.415
DO (mg/L)	7.300 ± 0.129	7.425 ± 0.165	7.100 ± 0.208	6.700 ± 0.147	7.400 ± 0.168	8.000 ± 0.258
Nitrite (mg/L)	0.078 ± 0.001	0.107 ± 0.003	0.081 ± 0.002	0.082 ± 0.002	0.074 ± 0.002	0.066 ± 0.001
Nitrate (mg/L)	0.210 ± 0.002	0.143 ± 0.002	0.116 ± 0.007	0.135 ± 0.006	0.154 ± 0.004	0.136 ± 0.005
Total ammonia (mg/L)	0.418 ± 0.008	0.537 ± 0.007	0.362 ± 0.004	0.382 ± 0.002	0.741 ± 0.008	0.467 ± 0.004

Table 3: Mean (± SE) of water quality parameters.

Items	Treatments						SED*
	T1	T2	T3	T4	T5	T6	
	Artificial Feed Only	Artificial feed with <i>Ulva</i>	Artificial feed with <i>Enteromorpha</i>	<i>Ulva</i> Only	<i>Enteromorpha</i> Only	<i>Ulva</i> with <i>Enteromorpha</i>	
Initial weight (w_1), (g)	0.18	0.18	0.18	0.18	0.18	0.18	-
Final length (L_2), (cm)	6.20 ^a	6.26 ^a	6.40 ^a	3.83 ^c	3.93 ^c	4.21 ^b	0.090
Final weight (W_2), (g)	2.49 ^b	2.95 ^b	3.41 ^a	0.73 ^d	0.67 ^d	0.92 ^c	0.070
Total weight gain (TG, g)	2.31 ^b	2.41 ^b	3.23 ^a	0.55 ^d	0.49 ^d	0.73 ^c	0.070
Average daily gain (ADG, g/day)	0.0330 ^b	0.0340 ^b	0.0460 ^a	0.0075 ^c	0.0070 ^c	0.0100 ^c	0.001
Relative growth rate (RGR), (%)	1283.30 ^b	1338.80 ^b	1794.40 ^a	315.55 ^{cd}	272.2 ^d	408.33 ^c	41.950
Specific growth rate (SGR/day, %)	3.75 ^b	3.80 ^b	4.20 ^a	1.99 ^d	1.87 ^d	2.31 ^c	0.118
Survival rate (SR, %)	75 ^a	65 ^{ab}	55 ^b	25 ^c	28.75 ^c	26.20 ^c	5.460

(a, b, c and d) Average in the same row having different superscripts significantly different at ($P \leq 0.05$).

*, SED is the standard error of difference ($\sqrt{2 \text{ mean square of error } / \text{ replicates}}$).

Table 4: Effect of use fresh macro algae with or without artificial feed on growth performance of rabbitfish (*Siganus rivulatus*) fry.

measured every week by oxygen meter (Cole Parmer model 5946). Nitrite, nitrate, total ammonia were measured every two week by the chemical methods according to [24,25].

Measurements of growth performance and some of the internal organs

Total weight gain (TG), average daily gain (ADG), Relative growth rate (RGR), specific growth rate (SGR) and survival rate (SR).

These parameters were calculated according the following equations:

$$TG, g = \text{Final Weight } (W_2) - \text{Initial Weight } (W_1)$$

$$ADG, g / \text{day} = \text{Average Weight Gain, } g / \text{Experimental Period, day}$$

$$RGR, \% = [(W_2 - W_1) / W_1] \times 100$$

$$SGR, \% / \text{day} = [(\ln W_2 - \ln W_1) / t] \times 100$$

whereas ln: is the natural log, and t: is the time in days,

$$SR\% = (\text{Number of fish at end} / \text{Number of fish at start}) \times 100$$

Measurements of feed utilization efficiency

Feed intake g/fish (FI), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), protein productive value (PPV), energy efficiency ratio (EER) and energy productive value (EPV).

These parameters were calculated according the following equations:

FI g/fish feed intake during the trial period/ the final number of fish for this trial

$$FCR = \text{Feed Intake, } g / \text{Weight Gain, } g$$

$$FCE\% = (\text{Weight Gain, } g / \text{Feed Intake, } g) \times 100$$

$$PER = \text{Weight Gain, } g / \text{Protein Intake, } g$$

$$PPV\% = (\text{Retained Protein, } g / \text{Protein Intake, } g) \times 100$$

$$EER = \text{Weight Gain, } g / \text{Energy Intake, } Kcal$$

$$EPV\% = (\text{Retained Energy, } Kcal / \text{Energy Intake, } Kcal) \times 100$$

Chemical analysis of feeds and whole body fish

The conversional chemical analysis of diet and whole body fish samples were carried out as described by AOAC [26] and Gross energy (GE) was estimated for formulated diets the factors 5.64 Kcal/g, 9.44 Kcal/g and 4.11 Kcal/g for CP, EE and carbohydrates respectively were used [27], for fish 5.5 Kcal/g and 9.5 Kcal/g for protein and fat respectively [28].

Statistical analysis

The analysis of variance and LSD of Duncan Waller were used to compare treatment means. Data were analysed using stratigraphic package software [29] SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Level of significant was 0.05.

Results and Discussion

Water quality

Some water quality parameters recorded in this trial were shown in Table 3. The averages of water temperature, water pH, water salinity, dissolved oxygen (DO), and nitrite, nitrate and total ammonia values in

all treatments were within the acceptable limits for rabbitfish (*Siganus rivulatus*) fry as reported by [2,30-37].

Growth performance

As shown in Table 4, there were significant differences between the treatments in all the growth performance parameters. The differences between the T1 (fry fed at artificial feed only), the T2 (fry fed at artificial feed with fresh *Ulva*) and the T3 (fry fed at artificial feed with fresh *Enteromorpha*) in final length (L_2) were not significant followed by the T6 (fry fed at fresh *Ulva* and fresh *Enteromorpha*). While the lowest L_2 were obtained by T4 (fry fed at fresh *Ulva* only) and T5 (fry fed at fresh *Enteromorpha* only). The T3 was the highest in W_2 , TG, ADG, RGR and SGR. Followed by both the T2 and T1.

The T6 was the lower in these parameters than both the T2 and T1. But both the T4 and T5 were the lowest in all treatments in these parameters. The highest Survival rate (SR) was achieved with the T1 followed by the T2 and the T3. While insignificant differences between T4, T5 and T6.

From these results, it can be observed that, the total replacement of artificial feed with fresh macro algae had negative effect on growth performance of rabbitfish fry, however, use of the macro algae as half of the feeding rate with artificial feed had positive effect on growth performance of rabbitfish fry and reduce of the feed cost. The positive effects of macro algae on growth performance may be due to the algae are a strongly appreciated source of protein, essential amino acids [38] and vitamins [39].

As well as the positive effect of the used additive algae decrease the cholesterol and fat level and improved lipid metabolism in fish too [40]. And, Seaweeds cannot be considered as a main source of energy but they have nutritional value regarding vitamin, protein and mineral contents [41]. According to Chapman and Chapman [42], 100 g seaweed provides more than the daily requirement of Vitamin A, B1 and B12 and two thirds of Vitamin C. Also seaweeds are natural sources of hydrosoluble and liposoluble vitamins, such as thiamine and riboflavin, *b*-carotene and tocopherols, as well as of long-chain polyunsaturated essential fatty acids from the omega-3 (ω -3) (family such as eicosapentaenoic acid [43]. Moreover, Some dietary macroalge meals are improved the growth, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality of various fish species [44-46]. So gut weed can be used as a direct feed or as ingredient in diets for herbivorous fish according to Teimouri et al. [47].

Over and above, rabbitfish are herbivorous fish and mainly graze on seaweeds. However, they can also feed on formulated feed or trash fishes when seaweeds are absent [48].

Due to seasonal availability of seaweeds used for feed, it is necessary to develop a formulated feed in order to promote the development of an intensive aquaculture industry for rabbitfish. Studies of stomach contents and food preference revealed that rabbitfish feed on fresh green or red algae or benthic marine plants. Studies revealed that among the many different algal species, *Gracilaria* is the preferred species of food [49].

As well as most herbivorous fishes prefer fleshy seaweeds over calcareous coralline and encrusting seaweeds [50].

In view of the economic side, the fishmeal and fish oil remain the main protein and lipid sources of feed for marine fish, and the development and application of formulated feed are restricted by the high cost of these ingredients. Therefore, efforts should focus on

producing commercial feed for rabbitfish at low cost and high efficiency [51]. Such as the use of seaweeds in the development of low-cost, highly nutritive diets for animal nutrition, especially animal nutrition since sea vegetables are able to accelerate the growth of oysters, tilapia, salmon, trout, etc., all of great commercial interest [52,53]. As well as marine macro algae could be a potential low-cost source of protein for fish [54]. Moreover, the economic comparison of feed cost indicated that increasing level of fresh and dried gut weed in alternative feeding treatments, commercial feed used for fish growth was reduced leading to significantly reduction of feed cost. Compare to commercial feed, treatments [55].

Likewise, Xu et al. [51] reported that, the *Gracilaria lemaneiformis* GL is the preferred food for *S. canaliculatus*, it is logical to utilize Dried *Gracilaria lemaneiformis* DGL as a dietary ingredient for developing low-cost feeds for *S. canaliculatus*. Utilization of DGL can perhaps partially replace protein from fish meal in feeds and reduce fishmeal inclusion, and secondly, the high level of carbohydrates in GL can reduce the amount of supplemental starch in formulated feeds and minimize competition with human food sources. Furthermore, application of GL in feed can promote the coordinated development of aquaculture industries for GL and rabbitfish and simultaneously increase incomes of fisherman. Previous studies [56] investigating inclusion of other plant ingredients in the diets of *S. canaliculatus* showed different results, not influencing growth.

In the light of the results were shown in Table 4 it can be observed that, the (T3) fish fed at artificial feed with fresh *Enteromorpha* was better in growth performance than the (T2) fish fed at artificial feed with fresh *Ulva*, this may be due to the fresh *Ulva* contained the higher percentage of fiber than fresh *Enteromorpha* as shown in Table 2 this was supported by Leary and Lovell [57] high levels of fiber in the diets of many finfish species, have been shown to reduce growth. For tilapia, Anderson et al. [58] concluded that dietary fiber levels above 5% reduce food utilization and digestibility and protein utilization were reduced with excess fiber levels in the diets. As well as Ortiz et al. [18] reported that *U. lactuca* contains about 60% fiber this might reduce its value in aqua feeds. Early studies of stomach contents and food preference revealed that among the many different algal species and vascular plants eaten, the presence of *Enteromorpha* was high and was the preferred species. This preference for *Enteromorpha* by siganids is not directly related to the calorific value of the algae but is related to the texture of its thallic which is crispy and thin [59,60]. Moreover, the results confirmed that, the replacement at 50% of artificial diet with fresh macro algal (*Enteromorpha* or *Ulva*) from rabbitfish fry feed led to an increase in the growth performance parameters compared with 100% artificial diet. But the total replacement of artificial feed with fresh macro algal (*Enteromorpha* or *Ulva*) led to a decrease in the growth performance parameters, because protein percentage of the artificial feed was high level (35% CP) in addition to artificial feed contained on fish meal (FM) which is high protein content and balanced essential amino acids EAA profile. Fish meal FM is also an excellent source of essential fatty acids (EFA), digestible energy, minerals and vitamins and it is well known as being highly palatable and digestible to fish [56,61]. The T6 (fish fed at *Ulva* with *Enteromorpha*) was the better in the growth performance and survival rate than T5 or T4, this may be due to both the *Ulva* with *Enteromorpha* has more a balanced amino acid composition than *Ulva* alone or *Enteromorpha* alone, in addition to the varying and mixture between *Ulva* and *Enteromorpha* resulted in highly palatable and digestible to fish.

In general, these results are in agreement with Costa et al. [62]

Items	Treatments						SED*
	T1	T2	T3	T4	T5	T6	
	Artificial Feed Only	Artificial feed with <i>Ulva</i>	Artificial feed with <i>Enteromorpha</i>	<i>Ulva</i> Only	<i>Enteromorpha</i> Only	<i>Ulva</i> with <i>Enteromorpha</i>	
Feed intake (FI), g/ fish	3.54 ^f	4.05 ^e	4.70 ^c	6.1 ^a	4.34 ^d	5.04 ^b	0.083
Feed conversion ratio (FCR)	1.53 ^c	1.68 ^c	1.45 ^c	11.22 ^a	8.85 ^b	7.74 ^b	0.752
Feed conversion efficiency (FCE, %)	65.25 ^b	59.50 ^c	68.72 ^a	9.01 ^e	11.29 ^e	14.55 ^d	1.260
Protein utilization							
Protein efficiency ratio (PER)	1.79 ^c	1.87 ^b	2.23 ^a	0.33 ^f	0.46 ^e	0.56 ^d	0.0116
Protein productive value (PPV, %)	86.32 ^c	97.29 ^b	107.56 ^a	20.46 ^f	25.32 ^e	32.34 ^d	0.0141
Energy utilization							
Energy efficiency ratio (EER, g/Kcal)	0.128 ^b	0.134 ^b	0.170 ^a	0.24 ^d	0.38 ^{cd}	0.43 ^c	0.0059
Energy productive value (EPV, %)	77.44 ^b	75.32 ^c	94.27 ^a	12.86 ^f	18.94 ^e	22.30 ^d	0.0141

(a, b, c, d, e and f) Average in the same row having different superscripts significantly different at (P ≤ 0.05).
 *, SED is the standard error of difference ($\sqrt{2 \text{ mean square of error } / \text{ replicates}}$).

Table 5: Effect of use fresh macro algae with or without artificial feed on feed utilization efficiency of rabbitfish (*Siganus rivulatus*) fry.

reported that fresh and dried gut weed can be used as a feed to substitute commercial feed for herbivorous fish such as spotted scat, (*Scatophagus argus*), red tilapia (*Oreochromis* sp.) and giant gourami (*Osphronemus goramy*) juveniles.

In the same trend, Siddik et al. [55] found that, the lowest final body weight and SGR were observed in treatments feeding fresh and dried gut weed as single feeds.

Moreover, herbivorous fish like tilapia generally accept plant originated ingredients better than animal originated ingredients in their diet. For example, Swain and Padhi [63] reported that tilapia grew better fed diet replacing 75% fish meal with okra meal (a by-product of soybean meal) than fed diet containing 100% fish meal. Moreover, the rabbitfish were fed a control diet with addition of a known weight of fresh *Enteromorpha* placed in plastic baskets at the bottom of the rearing tanks were the higher in final body weight and SGR than fish fed at the control diet or the other treatments [56].

The results were in partial disagree with El-Tawil [64] who reported that, specific growth rate of red tilapia (*Oreochromis* sp) improved significantly (P<0.05) with increasing *Ulva* level in the diet up to 15%. Increasing *Ulva* level beyond 15% had no significant effects on growth. Elmorshedy [65] showed that final body weight, weight gain and specific growth rate of gray mullet *Liza ramada* 0.094 g initial body weight were increased significantly with increasing seaweeds level (*Ulva* sp.) up to 28% in the fish diet. And Xu et al. [51] recommend a level of less than 33% DGL in the diet for *S. canaliculatus* and the optimum level require to be investigated in future studies.

On the other hand, carnivorous fish, such as African catfish, would tend to prefer diets with animal ingredients rather than plant feedstuff, and such a conclusion is in accordance with our results showing a decrease in feed intake by fish at the levels 20% and 30% of *U. lactuca* in their diets was increased [66].

The highest survival rate was achieved by the T1 (fish fed at the artificial diet only) and decreased with T2 and T3 while the lowest survival rate was achieved by the T4, T5 and T6. Hence, it can be said that the total replacement or partial of artificial diet with macro algae negatively affected on survival rate of rabbitfish fry this may be due to the important role of fish meal. Whereas, fishmeal has a balanced amino acid composition [56].

But Siddik et al. [55] found that, the equal survival (P>0.05) of

tilapia juvenile in all dietary treatments was in agreement with the study of Rahman and Meyer [67] who observed similar survival of fish fed diet with seaweed and without seaweed.

Feed utilization efficiency

As shown in Table 5 there were significant differences between the treatments in all the feed utilization parameters the highest feed intake (FI, g/fish) was achieved by T4 followed by T6, T3, T5 and T2 respectively. While the lowest FI achieved by T1. The best feed conversion ratio (FCR) was achieved with T1, T2 and T3 followed by T6 and T5 but the T4 had the worst FCR. The highest protein efficiency ratio (EPR) and protein productive value (PPV) were achieved with the T3 Followed by T2, T1, T6, T5 and T4 respectively. In the same trend was energy efficiency ratio (EER), whereas the highest EER was obtained by T3 followed by T2, T1, T6, T5 and T4 respectively. The highest energy productive value (EPV) was obtained by T3 followed by T1, T2, T6, T5 and T4 respectively.

From these results, it can be observed that, the T3 (fish fed at the artificial diet with fresh *Enteromorpha*) was the highest in FCE, PER, PPV, EER and EPV. The best FCR was achieved by T3, T1 and T2 whereas the statistical analysis disappeared any significant difference between these the treatments. This confirmed that, the total replacement of artificial diet by fresh macro algae negatively affected on the feed utilization of rabbitfish fry while the replacement of artificial diet at 50% of feeding rate by fresh macro algae did not only negatively effect on the feed utilization of rabbitfish fry but also the replacement of artificial diet at 50% of feeding rate by fresh macro algae excelled in the feed utilization parameters compared with the use of the artificial diet only in particular the replacement of artificial diet at 50% of feeding rate by *Enteromorpha*.

This may be returned to macro algae improved rabbitfish fry palatable and digestible at the artificial feed as mentioned by Yone et al. [68] who interpreted the effect on growth as due to an acceleration of nutrient absorption of dietary algae. These results are in agreement with Yousif et al. [56] who found that, the fish was fed a control diet with addition of a known weight of fresh *Enteromorpha* placed in plastic baskets at the bottom of the rearing tanks was the best in FCR, PER and PPV than the other treatments, also they added that, Protein productive value (PPV) affected significantly (P<0.05) by different levels of *Ulva* sp. It increased significantly with increasing *Ulva* level in the diet Siddik et al. [55] who found that, tilapia fed alternative1 day

Items	Start	Treatments						SED*
		T1 Artificial Feed Only	T2 Artificial feed with <i>Ulva</i>	T3 Artificial feed with <i>Enteromorpha</i>	T4 <i>Ulva</i> Only	T5 <i>Enteromorpha</i> Only	T6 <i>Ulva</i> with <i>Enteromorpha</i>	
Moisture (M, %)	80.70	70 ^c	73.83 ^{abc}	72.71 ^{bc}	80.43 ^a	79.71 ^{ab}	80.20 ^{ab}	2.88
Dry matter (DM, %)	19.30	30 ^a	26.17 ^{abc}	27.29 ^{ab}	19.57 ^c	20.29 ^{bc}	19.80 ^c	2.88
Crude protein (CP, %)	50.17	48.35 ^b	51.55 ^{ab}	48.07 ^b	58.63 ^a	54.54 ^{ab}	56.03 ^{ab}	3.48
Ether extract (EE, %)	9.75	33.84 ^a	27.50 ^b	29.48 ^{ab}	17.29 ^c	17.63 ^c	18.44 ^c	2.27
Ash, %	34.57	13.35 ^b	15.26 ^b	18.04 ^{ab}	20.36 ^{ab}	18.44 ^{ab}	27.35 ^a	4.08
Gross energy (GE, Kcal/g)	3.68	5.87 ^a	5.45 ^{ab}	5.44 ^{ab}	4.86 ^c	4.67 ^c	4.84 ^c	0.32

(a, b and c) Average in the same row having different superscripts significantly different at (P ≤ 0.05).
*, SED is the standard error of difference ($\sqrt{2 \text{ mean square of error } / \text{ replicates}}$).

Table 6: Effect of use fresh macro algae with or without artificial feed on whole body chemical composition and energy content of rabbitfish (*Siganus rivulatus*) fry.

commercial feed and 1 consecutive day fresh or dried gut weed showed similar feed utilization to tilapia feed the commercial feed.

These results clearly indicated that gut weed can be used 1 day after using 1 day commercial feed without affecting feed utilization of tilapia. Also these results are partial in agreement with El-Tawil [64] reported that, supplementation of *Ulva sp.* to the prepared fish diet had a positive effect on FCR except fish fed the diet containing 25% *Ulva* level with the poorest FCR value. As well as, Diler et al. [69] stated that PPV improved significantly with increasing dietary *Ulva* inclusion rate up to 15%. Also, similar results were found by Elmorshedy [65] with gray mullet.

On the other hand, Abdel-Warith et al. [66] reported that, a decrease in feed intake by African catfish at the levels 20% and 30% of *U. lactuca* in their diets was improved FCR and added that, the fish fed the control diet displaying a superior PER, Protein productive values (PPV%) values also showed a decrease when fishmeal was replaced by the *U. lactuca* meal source. Also, Ergün et al. [44] suggested that low-level inclusion of *Ulva* meal can significantly improve growth performance and nutrient utilization of tilapia fed high-lipid diets.

Body chemical composition and energy content

Whole body chemical composition and energy content of rabbitfish fry (*Siganus rivulatus*) at the beginning and the end of the experimental period are shown in Table 6. There were significant differences between the treatments at the end of the experimental period in moisture (M, %), crude protein (CP, %), ether extract (EE, %), ash (%) and gross energy (GE, kcal/g).

The highest moisture (M, %) was achieved by T4 followed by T6, T5, T2 and T3 but the lowest (M) was achieved by T1. the highest value of (CP) was obtained by the T4 and insignificant differences between T6, T5 and T2 while the lowest value of (CP) was obtained by T1 and T3. The highest value of (EE) was achieved by the T1 and T3 followed by T2 while there are not significant differences in EE between the T6, T5 and T4. The highest ash was achieved with the T6 and there are not significant differences in ash between the T5, T4 and T3 while the lowest ash was achieved T1 and T2. The highest value of GE was recorded by T1 followed T2 and T3 while there were insignificant differences in ash between the T4, T6 and T5.

It can be said that, an increase of EE and GE level of whole body rabbitfish fry at the end of the experimental period with the fish fed at the artificial feed or the fish fed at the artificial feed with fresh macro algae, may be due to the artificial feed contained on high level of EE (13.78%) this resulted in increment of lipid level in body fish, this are in agreement with Siddik et al. [55] who found that, the fish which fed at the commercial feed had the highest EE followed by the fish which fed at the fresh and dried gut weed with the commercial feed while the

lowest EE content was noticed with the fish were fed at fresh or dried gut weed alone.

The highest (M, %) was achieved by the fish were fed at fresh macro algae but the lowest (M, %) was achieved by the fish were fed at the artificial feed, this was agree with Siddik et al. [55] found that, The highest moisture content in tilapia carcass was observed in treatments fresh gut weed and dried gut weed while lowest was observed in treatments commercial feed.

While the highest ash was achieved by the fish fed at the diet without artificial feed (fresh macro algae only). This may be due to the fresh macro algae contained on high level of ash content in *Ulva* (20.06%) and *Enteromorpha* (30.19%) this resulted in increment of lipid level in body fish.

Conclusion

The total replacement of artificial feed with fresh macro algae had negative effect on growth performance of rabbitfish fry; however, use of the macro algae as half of the feeding rate with artificial feed had positive effect on growth performance of rabbitfish fry and reduce of the feed cost. Whereas, the T3 was the highest in the final weight (W_2) total weight gain (TG), average daily gain (ADG), relative growth rate (RGR) and specific growth rate (SGR). Followed by both the T2 and T1. Moreover, There are significant differences between the treatments in all the feed utilization parameters. The best feed conversion ratio (FCR) was achieved with T1, T2 and T3 followed by T6 and T5 but the T4 had the worst FCR.

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