Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(3): 395 – 410 (2021)
www.ejabf.journals.ekb.eg

Effects of partial substitution of fish meal with different levels of marine macroalgae species on growth indices and RNA/DNA ratio of hybrid red tilapia

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ARTICLE INFO

Article History:
Received: March 29, 2021
Accepted: April 22, 2021
Online: June 3, 2021

Keywords:
Red tilapia,
Ulva lactuca,
Laurencia obtuse,
Cystoseira myrica,
growth performance,
RNA/DNA ratio.

ABSTRACT

A 90-days experiment was conducted to determine the impact of partial replacement of dietary fish meal (FM) with different concentrations of ultra-fine powder of *Ulva lactuca, Laurencia obtusa and Cystoseira myrica* on growth performance, feed utilization and RNA/DNA ratio of the hybrid red tilapia "Florida strain". A total number of 600 fish of *Oreochromis* sp., with an average weight of 5.67 ± 0.89 g and an average length of 6.4 ± 0.41 cm, were randomly divided into 30 aquaria (20 fish/ aquarium in triplicates). Ten experimental diets were formulated as control (C) that contains FM as the major source of protein and other dietsin which the FM was partially substituted (on the protein basis). Substitution was achieved with 5% *Ulva lactuca* (U5), 10 % *U. lactuca* (U10), 15% *U. lactuca (*U15), 5% *Laurencia obtusa* (L5), 10% *L. obtusa* (L10), 15% *L. obtusa* (L15), 5% *Cystoseira myrica* (C5), 10% *C. myrica* (C10) and 15% *C. myrica* (C15). Results of survival rate (SR) in all treatments were not significantly different but the control treatment showed the best SR%, while the lowest SR% was shown in C15 treatment. In case of *Ulva lactuca*, all growth parameters were at its highest values in U5 and decreased with the increase of inclusion levels. The growth values ,when FM was partially replaced with *C. myrica*, were at their maximum at C15%. In *Laurencia* groups, the growth improved gradually with the increase in concentration and reached the highest level in *Laurencia* 15%. Moreover, the percentage of RNA/DNA increased significantly in *Laurencia* treatment compared with other treatments. In general, the results revealed that marine macroalgae could be considered as an acceptable alternative which can replace dietary FM protein in diets of the hybrid red tilapia. In addition, results showed that, the most effective group to be used is *Laurencia obtusa* which induced better growth performance with replacement levels up to 15% compared to the control diet.

INTRODUCTION

The continuous increase in populations all over the world have led to a continual demand for novel resources to cover the growing needs for food **(Paiva *et al.*, 2018)**. Recently, fish and other products of fisheries are the most consumed protein sources being a precious source of the cheapest animal protein and other different major and micronutrients to all socioeconomic classes **(Mahmoud *et al.*, 2019)**. In addition, it is rich in minerals, vitamins and essential fatty acids, especially long-chained polyunsaturated fatty acids, which are not easily replaced by other food stuffs **(Béné *et al*., 2015; Chan *et al*., 2019)**. Because of the limitation of fish supply, aquaculture is one of the best product to fill the gap between fish stock and demand **(Calheiros *et al.*, 2019)**. Of all the known farmed species, the tilapia is known for its easy adaptation to different environments, high growth rates and acceptance of different types of commercial feeds. These aspects made the tilapia the most productively cultured fish species that compres a radical source of protein for human consumption **(Silva *et al*., 2015)**. Studies also mentioned that, feeding on the euryhaline tilapia increased due to their tasteful flesh and slight fishy taste compared to the freshwater tilapia. The Mozambique tilapia (*Oreochromis mossambicus)* and its hybrids are of the major salt tolerance species in aquaculture. Moreover, the Florida strain hybrid red tilapia *(Oreochromis mossambicus x Oreochromis niloticus*), as one of the hybrids of *O. mossambicus*, is suitable for breeding in saltwater, a phenomenon determined by increased growth rates and feed conversion ratios **(Sallam *et al*., 2017)**. Beside the mentioned advantages of saltwater tilapia, the lack of freshwater in the world and the competition between aquaculture and agriculture and other urban activities, it would be of great interest to culture tilapia in brackish or saline water to ensure a high-quality low-priced animal protein in the future **(Abdelrahman *et al.*, 2020).** In addition to the freshwater shortage, the raised prices of fish feed and feed ingredients is a further problem that affects aquaculture. Fish feeds represent the main operating costs, approximately 60%, for most commercial fish farms, with protein source being the most high-priced dietary constitute **(Silva *et al.*, 2015).**

It is necessary for the aquaculture`s future to find more sustainable and available substitutes to expensive protein sources comprised in aquafeeds which may lower the reliance on fish oil and fish meal **(Sharawy *et al.*, 2016).** Of the most used alternatives, macroalgae and plant-based sources were used extensively to substitute part of the fish meal in fish feed. Macroalgae are rich in variety of nutrients like proteins of high quality, lipids, polysaccharides, dietary fibers, bioactive peptides, essential fatty acids from the omega-3 family, vitamins and phytochemicals such as polyphenols that might have defensive effects against diabetes, digestive disorders, cancers, allergy and oxidative stress (**Lordan *et al*., 2011; Paiva *et al*., 2018)**. The supplementation of fish diets with small amounts of macroalgae meal was found to have advantageous effects on the immune responses, disease resistance, feed utilization and growth performance without causing reversed actions. Several authors have previously shown that numerous species of macro- and microalgae have been included in the formulation of fish feeds to evaluate their nutritional effect, and many were found effective.

To assess the nutritional value of the feed supplemented with macroalgae, different growth parameters for fish in addition to feed utilization are measured **(Rooker *et al*., 1997**; **Ashour *et al.*, 2020)**. One specific measure that has been proved of high accuracy is that of the RNA/DNA ratio. It reflects the metabolic rate, the nutritional state, and the growth of a particular organism because the DNA content is quite constant in the individual and the RNA content is related to the protein synthesis rate **(Tanaka *et al*., 2007)**. Furthermore, this ratio is more sensitive to any change or defect in the physiological state of the fish.

Consequently, the current study was presented to investigate the effect of substitution of fish meal with ultra-fine powder of the three seaweeds *Ulva lactuca, Laurencia obtusa* and *Cystoseira myrica* on the growth performance, feed utilization and RNA/DNA ratio of the Florida strain Red Ttilapia (*Oreochromis* sp.).

MATERIALS AND METHODS

The current study was conducted in the National Institute of Oceanography and Fisheries (NIOF), Suez and Aqaba Gulfs branch to study the effect of using seaweeds as a partial substitute to fish meal on growth of the hybrid red tilapia “Florida strain” {female *Oreochromis mossambicus* (Peters, 1852) × male *Oreochromis niloticus* (Linnaeus, 1758)}.

Seaweeds Preparation:

Three species of marine macroalgae {*Ulva lactuca* Linnaeus 1753 as Chlorophyceae, *Laurencia obtusa* (Hudson) J.V. Lamouroux 1813 as Rhodophycea and *Cystoseira myrica* (Gmelin) C. Agardh 1820 as Phaeophycaea} were collected by snorkeling. Samples were rinsed thoroughly with clean sea water to remove necrotic parts, herbivores and epiphytes, then washed with clean tap water several times. The last wash was with distilled water and then left to dry in the open air till complete dryness. Afterwards, dried samples were grounded into fine powders for nutritional composition and further use.

The Experimental Fish:

The Florida red tilapia fingerlings (5.67±0.89g with 6.4±0.41cm length) were obtained from the General Authority for Fish Resources Development (GAFRD) hatchery at km 21, Alexandria, Egypt. Fish were acclimated to lab conditions for two weeks in a 1.0 m^3 fiber glass tank filled with filterd seawater. At the beginning of the acclimation period, salinity was 35.0±1.0 ppt, a natural photoperiod was obtained, and temperature was (28.0 ± 2.4°C).

Experimental Diets:

Experimental diets were formulated to contain almost 31.89±0.43% crude protein (CP) and 7.95±0.32% crude lipid, meeting the growth requirements for the hybrid red tilapia (Table 1). Ten test diets were formulated as control macroalgae-free diet (C) and varying levels of macroalgae meals in partial replacement of fish meal: *Ulva lactuca* 05% (U5), *U. lactuca* 10% (U10), *U. lactuca* 15% (U15), *Cystoseira myrica* 05% (C5), *C. myrica* 10% (C10), *C. myrica* 15% (C15), *Laurencia obtusa* 05% (L5), *L. obtusa* 10% (L10) and *L. obtusa* 15% (L15).

Table 1: Ingredients (g) of different experimental diets.

	Experimental Diets									
Ingredients (g)	**C**	**U5**	**U10**	**U15**	**C5**	**C10**	**C15**	**L5**	**L10**	**L15**
Fish Meal 65% CP	80	76	72	68	76	72	68	76	72	68
Soy Bean 47% CP	560	560	560	560	560	560	560	560	560	560
Ulva lactuca	-	12.49	24.99	37.48	-	-	-	-	-	-
Cystoseira myrica	-	-	-	-	21.81	43.62	65.44	-	-	-
Laurencia obtusa	-	-	-	-	-	-	-	17.65	35.30	52.95
Rice Bran	95	95	95	95	95	95	95	95	95	95
Wheat Bran	100	100	100	100	100	100	100	100	100	100
American Gluten 60%CP	60	60	60	60	60	60	60	60	60	60
Corn	80	80	80	80	80	80	80	80	80	80
Salt + Calcium carbonate	20	20	20	20	20	20	20	20	20	20
Premix	5	5	5	5	5	5	5	5	5	5

Experimental Design:

After acclimation, fish were divided randomly in ten triplicates of 20 fish per replicate in 80L glass aquaria with certain dimensions (30 cm x 40 cm x 80 cm) filled with approximately 70 L of 35.18±1.11 ppt saline water and provided with continuous aeration. Approximately, one third of the water was daily replaced by freshly stored water. Feces and excess feed were removed daily before feeding. Throughout the experiment, fish were fed twice per day at 9.00 am and 2.00 pm. At the beginning of the 12 experimental weeks, fish were fed 7% of its body weight during the first 4 weeks, then with 5% during the next 4 weeks and finally 3% till the end of the experiment.

Growth Performance:

Fish were weighed individually at the beginning of the experiment and biweekly during the 12- weeks experimental period. Growth and feed utilization were measured in terms of the final body weight (g) of the fish, body weight gain (g), relative growth rate (%), specific growth rate (g/day), survival rate (%), feed conversion ratio and protein efficiency ratio according to the following equations:

Body weight gain (BWG) g/fish = BW_F (g) –BW_I (g)
Relative growth rate (RGR) = {BW_F (g) –BW_I (g)}/ BW_I (g)* 100
Specific growth rate (SGR) %/day = (ln BW_F – ln BW_I) / T * 100
Feed conversion ratio (FCR)= Total feed intake (g) /body weight gain (g)
Protein efficiency ratio (PER)=BWG (g) /Protein intake (g)
Survival rate (SR) %= N_F/ N_I *100

RNA/DNA Ratio:

Quantitative determination of nucleic acids in tissue was performed by pentose analysis following the method of **Schneider (1976)**. It was calculated as follows:

DNA content of the nucleic acid extract is presented by the following equation:

µg DNA m/L = OD at 600 nm/0.019

The RNA content of the nucleic acid extract is presented by the following equation:

µg RNA m/L= [(OD at 600 nm+0.008) – (µg DNA m/L × 0.013)] /0.116

Statistical analysis:

All the results were represented as mean values ± SD "standard deviation" of means and subjected to two-way analysis of variance (ANOVA) to test the effects of the experimental diets using the statistical package IBM SPSS statistics v25.0. Duncan's multiple range test at a level of significance of P≤ 0.05 was used to compare differences among treatments.

RESULTS

Nutritional compoaition of seaweeds:

Nutritional composition of the selected seaweeds are presented in Table (2).

Table 2: Biochemical analyses (% DM) of three selected seaweed.

Nutritional composition	Seaweed species		
	Ulva lactuca	***Laurencia obtusa***	***Cystoseira myrica***
Total Protein	29.12 ± 0.05	12.27 ± 0.04	13.93 ± 0.09
Total Lipids	9.28±0.03	8.49 ± 0.01	8.68 ± 0.04
Total Carbohydrates	25.17± 0.17	46.23 ± 0.01	45.32± 0.16
Fibers	1.69± 0.01	2.41 ± 0.03	2.50± 0.01
Ash	34.75± 0.08	30.61 ± 0.02	29.59± 0.02

Growth Performance:

The averages initial body weight and initial body length of the fingerlings at the beginning of the experiment were 5.67±0.89g and 6.4±0.41cm, respectively. There was no significant differences between the groups for initial body weights and lengths which indicates that the groups were homogenous.

Findings of the current experiment revealed that final body weight (BW_F), body weight gain (BWG), survival rate (SR), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and relative growth rate (RGR) of the hybrid red tilapia were significantly influenced ($P \leq 0.05$) by different seaweeds` substitution levels. In case of *Ulva lactuca* as presented in Table (3), the highest significant values were observed in U5 followed by the control group, while the lowest values were in groups U15 and U10, respectively with no significant difference.

Table 3: Growth performance parameters of hybrid red tilapia (*Oreochromis sp.*) fed at different dietary levels of *Ulva lactuca*.

Growth parameters	C	U5	U10	U15
IBW (g)	5.80 ± 0.87^{a}	5.76 ± 0.93^{a}	5.82 ± 0.99^{a}	5.96 ± 0.87^{a}
FBW (g)	61.50 ± 5.31^{b}	65.23 ± 3.93^{a}	49.35 ± 3.89^{c}	47.84 ± 7.62^{c}
BWG (g)	55.70 ± 4.51^{b}	59.47 ± 3.16^{a}	43.53 ± 2.93^{c}	41.87 ± 6.76^{c}
DWG (g)	0.62 ± 0.05^{b}	0.66 ± 0.035^{a}	0.48 ± 0.03^{c}	0.46 ± 0.075^{c}
SR%	95.00 ± 5.00^{a}	88.33 ± 2.88^{a}	90.00 ± 5.00^{a}	91.67 ± 5.78^{a}
FCR	1.69 ± 0.03^{c}	1.66 ± 0.04^{d}	1.79 ± 0.05^{b}	1.82 ± 0.02^{a}
SGR	2.63 ± 0.09^{b}	2.71 ± 0.13^{a}	2.39 ± 0.10^{c}	2.31 ± 0.04^{d}
PER	1.34 ± 0.03^{b}	1.41 ± 0.04^{a}	1.14 ± 0.02^{c}	1.09 ± 0.11^{d}
RGR%	10.79 ± 0.99^{b}	11.70 ± 1.56^{a}	8.45 ± 0.90^{c}	7.79 ± 0.30^{d}

- Different letters following the means in each row are significantly different ($P \leq 0.05$).
- Initial body weight (IBW), final body weight (FBW), body weight gain (BWG), daily weight gain (DW survival rate (SR%), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency rat (PER) and relative growth rate (RGR%)

Inclusion of 5, 10 and 15% *U. lactuca* in U5, U10 and U15 resulted in a SR% of 88.33%, 90.00% and 91.67%, respectively, compared with the control group (C) with the highest survival percentage of (95.00%). The FCR was affected by the supplementation of fish diet with *Ulva* meals. The highest value of FCR was found in group U15 (1.82±0.02), whereas the smallest value was at U5 (1.66±0.04). This indicates that the highest utilization of feed was observed in U5 group. When *Cystoseira myrica* was used, growth parameters were significantly different ($p \leq 0.05$) between the control group (C) fed the commercial diet and other dietary groups as shown in Table (4).

Table 4: Growth performance parameters of hybrid red tilapia (*Oreochromis sp.*) fed at different dietary levels of *Cystoseira myrica*.

Growth parameters	C	C5	C10	C15
IBW (g)	5.80 ± 0.87^{a}	5.62 ± 0.97^{a}	5.88 ± 1.07^{a}	5.719 ± 1.09^{a}
FBW (g)	61.50 ± 5.31^{a}	57.48 ± 8.86^{ab}	54.90 ± 8.87^{b}	62.40 ± 9.32^{a}
BWG (g)	55.70 ± 4.51^{ab}	51.86 ± 8.03^{bc}	49.01 ± 7.88^{c}	56.68 ± 8.32^{a}
DWG (g)	0.62 ± 0.06^{ab}	0.58 ± 0.09^{bc}	0.54 ± 0.09^{c}	0.63 ± 0.09^{a}
SR%	95.00 ± 5.00^{a}	91.67 ± 10.41^{ab}	88.33 ± 7.64^{ab}	78.33 ± 10.40^{b}
FCR	1.69 ± 0.03^{bc}	1.70 ± 0.04^{b}	1.74 ± 0.03^{a}	1.67 ± 0.03^{c}
SGR	2.63 ± 0.09^{a}	2.58 ± 0.10^{b}	2.49 ± 0.08^{c}	2.66 ± 0.08^{a}
PER	1.34 ± 0.03^{ab}	1.25 ± 0.12^{b}	1.27 ± 0.09^{c}	1.40 ± 0.10^{a}
RGR%	10.79 ± 0.99^{ab}	10.30 ± 1.04^{b}	9.32 ± 0.73^{c}	11.10 ± 0.83^{a}

- Different letters following the means in each row are significantly different ($P \leq 0.05$).
- Initial body weight (IBW), final body weight (FBW), body weight gain (BWG), daily weight gain (DWG), survival rate (SR%), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and relative growth rate (RGR%)

Results of BW_F showed that the highest value was observed in C15 (62.40±9.32 g) and the lowest was observed in C10 (54.90±8.87 g). Concerning BWG and DWG, they were higher in C15 (56.68±8.32 g and 0.63±0.09 g) and lower in C10 (49.01±7.88 g and 0.54±0.088 g), respectively. The survival percentage was also affected but difference showed no significancy. The highest concentration of inclusion with *Cystoseira* resulted in the reduction of survival rates, and the survivability of C15 was 78.33%. Survival rates in C5 and C10 were 91.67% and 88.33%, respectively which were relatively lower compared to that of the control group (C) which recorded the highest survival percentage (95.00%).

In addition, the FCR was affected with supplementation of fish diet with *Cystoseira*. The highest value of FCR was found in group C10 (1.74±0.03) and the smallest value was at C15 (1.67±0.03). SGR was also affected by the inclusion of *Cystoseira* in fish diet. The highest value of SGR was found in C15 (2.66±0.08), whereas the smallest value was found at C10 (2.49±0.08). Concerning the RGR% and PER, results showed that the highest values were in group C15 (11.10±0.83 and 1.40±0.10), respectively, and the smallest values were found in C10 (9.32±0.73 and 1.27±0.09).

With the replacement of fish meal with *Laurencia obtusa* results of BW_F that are introduced in Table (5) showed that, the highest BW_F was observed in L15 (69.77±11.87 g) and the lowest was in group L5 (43.73±6.52 g). Regarding the BWG and DWG, both were higher in L15 (64.25±11.04 g and 0.71±0.12 g), while the lowest values were observed in C (55.70±4.51 g and 0.42±0.06 g). Inclusion of *Laurencia obtusa* in L5, L10 and L15 resulted in the survival percentages of 95.00%, 96.67% and 96.67%, respectively, compared to the control group (C) with a survival percentage of (93.33%). Additionally, the FCR was affected by the supplementation of fish diet with *Laurencia* meals. The highest value of FCR was found in L5 (1.86±0.04) and the smallest value was at L15 (1.62±0.02). This indicates that the highest utilization of feed was observed in L15 group. The SGR was also affected by inclusion of *Laurencia* in fish diet. The highest value of SGR was found in L15 (2.82±0.05), and the smallest value was found at L5 (1.36±0.03). Results of the RGR% and PER were similar to SGR. The highest values of RGR% and PER were found in group L15 (12.93±0.68 and 1.46±0.08), respectively and the smallest values were found in L5 (7.33±0.58 and 1.00±0.11), respectively.

RNA/DNA Ratio:

Selected samples to determine the RNA/DNA ratios were almost similar in its size to avoid differences that may occur due to the size or age, as most of the experimented fish were of the same batch. The mean values of the RNA/DNA ratios of the different treatments and SGR are introduced in Table (6). The ratios at the groups fed on *Laurencia obtusa* increased obviously compared to other macroalgae species. Analysis of variance (One-Way ANOVA) revealed that groups fed L5, L10 and L15 were highly significant (p≤0.05) compared to the control one. The highest ratio was observed in the L15 group while the lowest in U10 group.

Table 5: Growth performance parameters of hybrid red tilapia (*Oreochromis sp*.) fed at different dietary levels of *Laurencia obtusa*.

	C	L5	L10	L15
IBW (g)	5.80±0.87[a]	5.80±1.05[a]	5.70±1.07[a]	5.52±0.87[a]
FBW (g)	61.50±5.31[b]	43.73±6.52[c]	66.42±8.72[ab]	69.77±11.8[a]
BWG (g)	55.70±4.51[b]	37.93±5.58[c]	60.716±7.68[a]	64.25±11.0[a]
DWG (g)	0.62±0.06[b]	0.42±0.06[c]	0.67±0.09[a]	0.71±0.12[a]
SR%	95.00±5.00[a]	91.67±7.64[a]	90.00±5.00[a]	93.33±7.64[a]
FCR	1.69±0.03[b]	1.86±0.04[a]	1.65±0.03[c]	1.62±0.02[d]
SGR	2.63±0.09[c]	2.25±0.08[d]	2.74±0.08[b]	2.82±0.05[a]
PER	1.34±0.03[c]	1.00±0.11[d]	1.42±0.05[b]	1.46±0.08[a]
RGR%	10.79±0.99[c]	7.33±0.58[d]	11.99±1.03[b]	12.93±0.68[a]

- Different letters following the means in each row are significantly different (P ≤ 0.05).

- Initial body weight (IBW), final body weight (FBW), body weight gain (BWG), daily weight gain (DWG), survival rate (SR%), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and relative growth rate (RGR%)

Table 6: RNA/DNA ratio and specific growth rate (SGR) of hybrid red tilapia (*Oreochromis sp*.) fed on diets with different levels of substitution with macroalgae

Treatment	RNA/DNA Ratio	SGR
C	1.67±0.010 [c]	2.631±0.08[b]
U5	1.76±0.192[c]	2.71±0.13[bc]
U10	1.56±0.109[c]	2.39±0.104[g]
U15	1.64±0.023[c]	2.31d±0.037[h]
C5	1.657±0.029[c]	2.583±0.103[e]
C10	1.687±0.044[c]	2.485±0.076[f]
C15	1.712±0.042[c]	2.660±0.078[cd]
L5	3.34±0.26[ab]	2.249±0.078[i]
L10	2.72±0.10[b]	2.738±0.084[b]

Different letters following the means in each column are significantly different (P ≤ 0.05).

DISCUSSION

The principal objectives in formulation of aquafeeds are to: (a) fulfil all the required nutrients for the growth of the species; (b) reduce feed price; and (c) minimize wastage by selecting ingredients that will be readily utilized.

Seaweeds can secure the required nutrients essential in aquafeeds even more than what is attained in fish meal and soy powder. Some bioactive compounds present in seaweeds can significantly improve fish performance **(Kiadaliri *et al.*, 2020).**

Growth performance:

There are few numbers of systematic studies of using algae as an ingredient in fish feed **(Zhu *et al.*, 2015; Sharawy *et al.*, 2020)**. Partial substitution of fish meal by *Ulva sp*. can cause positive impacts on growth, body composition, resistance to diseases and stress and feed utilization (**Silva *et al.*, 2015**; **Shpigel *et al.*, 2017)**. Studies performed on *Ulva rigida* as a feed additive of different fish species, reported alike results, where the best performance was obtained for the 5% level of inclusion and reduced at levels of 10 to 20% **(Güroy *et al.*, 2007**; **Ergün *et al.*, 2009)**. The same ideal rates of 5% inclusion were reported in the red tilapia **(El-Tawil, 2010**; **Saleh, 2014)** and in the Nile tilapia **(Khalafalla *et al.*, 2015**; **Natify *et al.*, 2015)**.

Stadtlander *et al.*, (2013) found that the growthof theNile tilapia as well as feed utilization improved when 15% of FM was substituted with the red algae *Porphyra yezoensis*. However, **Soler-Vila *et al.* (2009)** stated that using *Porphyra dioica (*Rhodophyta) in different inclusion levels as the rainbow trout (*Oncorhynchus mykiss*) feed resulted in a quite minor performance of the fish compared to the fish fed on the control diet. Nevertheless, the used algae can be productively included in its feeds up to 10%, with no negative significant effects on growth performance and weight gain.

Markedly, brown macroalgae (Phaeophyta) are of lower nutritional rate than red (Rhodophyta) and green algae (Clorophyta), because of their poor content of protein and superior content of minerals **(Makkar *et al.*, 2016)**, despite that the brown algae have some bioactive compounds. To illustrate, **Güroy *et al.* (2007)** studied the effect of feeding the juvenile of the Nile tilapia (*Oreochromis niloticus*) with feeds supplemented by *Cystoseira* meal (5%, 10%, or 15%) on growth rate, feed utilization and feed intake. They detected positive effect on growth performance up to 5% level of inclusion but not beyond, but the differences in values were not significant ($P > 0.05$).

Previous studies discovered that the addition of high levels of different seaweeds led to minor growth and nutrient utilization compared to the control group. Those findings are in agreement with the current results, reporting a decrease in all growth and feed utilization parameters in diets containing 10% and 15% of the *U. lactuca* compared with control and 5% diets. **Madibana *et al.* (2017)** suggested that elevated levels of seaweeds in the diet reduced the nutritional quality of the experimented diets. The decline in fish growth and nutrient's utilization at inclusion concentrations of *Ulva* that was higher than 5% might be clarified by the occurrence of anti-nutritional factors in the algae composition including tannins, saponins, and phytic acid that are stated to present in many plant vegetative tissues **(Francis *et al.*, 2001)** and may have an effect on fish growth **(Azaza *et al.*, 2008)**. The occurrence of indigestible fibers can also weaken the ability to digest protein **(Azaza *et al.*, 2008),** which might be responsible to the growth decline recorded during the experiment. **Lech & Reigh (2012)** stated that fish growth might be compromised by the decline in protein content and the raised fiber content. The addition of plant protein in fish diets has been described to decrease feed intake per day **(Davies *et al.*,1997)** this could be another cause for the reduction in growth performance and feed utilization.

The increase in algae concentrations may reduce the homogeneity of the diets which cause leaching of some constituents of the artificial diets into water, and this can explain the negative growth results.

The variation in optimum substitution level of algae meal is probably caused by different feeding habits, fish age, size, experimental conditions and the species of both fish and algae. The results suggest that *Ulva rigida* or *Cystoseira barbata* meals could be used in small percentages in the tilapia diets.

Use of the RNA/DNA ratio to assess growth:

Growth is mainly a result of the process of protein biosynthesis, in which RNA plays a vital role. The use of RNA/DNA ratio as an indicator of growth has been used in marine environment for assessing fish growth **(Chícharo & Chícharo, 2008)**. This indicator is based on the fact that DNA content in the cell remains quite constant, while the RNA content improves with increasing protein synthesis needed for growth. This might be considered as result of the fact that when the rate of protein synthesis increases, a corresponding growth rate becomes greater **(Srinivasa *et al.*, 2003).**

In general, the RNA/DNA ratios for fish larvae seem to range between 0.5 and 3. It may also be lower when fishes are young because under-performing (e.g., poor feeding) individuals, with low RNA/DNA values, are still contributing to sample estimates of condition **(Chícaro *et al.*, 1998)**. One of the goals of the present study was to investigate the connection between RNA/DNA ratio and the somatic growth of hybrid red tilapia fed on different levels of substitution of fish meal with macroalgae.

Noticeably, the effect of substitution of fish meal with macroalgae meal has not been comprehensively studied so far. The present results showed that the ratios of the groups fed on the red macroalgae *Laurencia obtusa* increased dramatically compared to other experimented diets. Analysis of variance (One-Way ANOVA) revealed that groups fed L5, L10 and L15 were highly significant ($p \leq 0.05$) compared to the control one. The highest RNA/DNA ratio was recorded in the L15 treatment in which fish were fed 15% *Laurencia* level of substitution, which also showed the highest SGR. This agrees with the results obtained by **Ayisi and Zhao (2016),** who reported that the greatest RNA/DNA ratio in *Oreochromis niloticus* was found in fish with the highest SGR. Moreover, **Naik and Murthy (2012)** mentioned that the maximum ratio of RNA/DNA was found in shrimps that had the greatest growth although that the differerence in their RNA/DNA was not significant. The findings are in agreement with what was reported by **Nunez *et al*. (2002)**, who found that larvae of shrimp with higher RNA/DNA ratio had higher dry weight, survival rate and organic biomass. Similarly, **Bastrop *et al.* (1992)** reported the same results of the relationship between SGR and RNA/DNA ratio in rainbow trout. Furthermore, **Frantzis *et al.* (1992)** evaluated somatic growth and RNA/DNA ratios of the sea urchins (*Paracentrotus lividus)* reared for 6 months and fed on 12 types of macrophytes. A positive relationship between growth rate and RNA/DNA ratio was recorded.

The RNA/DNA ratio in fish flesh is connected with feeding habits and growth rates **(Park *et al.,* 2017**; **Zehra & Khan, 2020**). This ratio is an accurate indicator of the nutritional state, where poor results occurred as a result of starvation, while high results are indicative of rapid growth and better nutritional condition **(Naik & Murthy, 2012**). Additionally, **Ali *et al.* (2006)** reported a positive relationship between the quantity of the synthesized protein per ribosome and RNA/DNA ratio which may explain the decline in RNA/DNA ratios when the protein synthesis process decreased.

CONCLUSION

From all the previous findings in the current study, marine macroalgae can be considered as an acceptable alternative to dietary FM protein in diets of the hybrid red tilapia at small levels of substitution. In addition, it was found that the most effective group is *Laurencia obtusa* which increased all growth parameters at levels of substitution up to 15% to fish meal compared with other macroalgae species and the control group, and in turn, improved the RNA/DNA ratio dramatically. This makes the *Laurencia sp*. a good alternative to fish meal with a lower cost of production.

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الملخص العربى

تأثير الاستبدال الغذائي الجزئي لمسحوق السمك بمستويات مختلفة من الطحالب البحرية على مؤشرات النمو وكذلك نسبة RNA/DNA للبلطي الأحمر

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أجريت تجربة لمدة 90 يومًا لدراسة تأثير الاستبدال الجزئي لمسحوق السمك بتركيزات مختلفة من مسحوق فائق النعومة من طحلب اليولفا لاكتوكا و اللورانسيا اوبتوسا وسيستوزيرا ميركا على أداء النمو والإستفادة من الأعلاف وكذلك نسبة RNA/DNA لأسماك البلطى الأحمر من سلالة فلوريدا. تم تقسيم إجمالي 600 سمكة من *Oreochromis sp.* بمتوسط وزن 5.67 ± 0.89 جم ومتوسط طول 6.4 ± 0.41 سم تم بشكل عشوائي في 30 حوضًا (20 سمكة / حوض زجاجى في معاملات ذات ثلاث مكررات). تم تجهيز عشرة أنظمة غذائية على النحو التالي: المجموعة الضابطة (C) تحتوى على مسحوق السمك كمصدر رئيسي للبروتين. فى المعاملات الأخرى ، تم استبدال مسحوق السمك جزئيًا (على أساس نسبة البروتين) بـ 5٪ و 10% و 15% من طحلب يولفا لاكتوكا فى المعاملات (U5) ، (U10)، (U15)، و 5%و 10% و 15% من طحلب لورانسيا اوبتوسا فى المعاملات (L5) ، (L10)، (L15) و 5% و 10%و 15% من طحلب السيستوزيرا ميركا فى المعاملات (C5) ، (C10)، (C15) .

أظهرت النتائج عدم وجود فروقا معنوية فى معدلات البقاء (SR) في جميع المعاملات ولكن أظهرت المجموعة الضابطة أفضل نسبة إعاشة كما ظهرت أقل نسبة بقاء فى المعاملة C15 .اما فيما يتعلق بمؤشرات النمو في حالة اليولفا لاكتوكا، كانت جميع مؤشرات النمو في أعلى قيم لها في U5 وانخفضت مع زيادة مستويات الإحلال. كانت نتائج النمو عندما تم استبدال مسحوق السمك جزئيًا بـ سيستوزيرا ميركا في أقصى قيم لها عند C15 ٪. وفي مجموعات اللورانسيا تحسن النمو تدريجياً مع زيادة التركيز ليصل إلى أعلى مستوى في نسبة الإحلال 15٪. علاوة على ذلك ، زادت نسبة RNA / DNA في معاملات اللورانسيا بشكل واضح عنها في المعاملات الأخرى.

بشكل عام ، تشير النتائج إلى أن الطحالب البحرية تعتبر بديلاً مقبولاً يمكن أن يحل محل مسحوق السمك كمصدر للبروتين الغذائي في علف البلطي الأحمر *Oreochromis* sp. والمجموعة الأكثر فاعلية لاستخدامها هي لورينسيا اوبتوسا التي أدت إلى أداء نمو أفضل بمستويات استبدال تصل إلى 15٪ مقارنة بالمجموعة الضابطة.