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Growth Performance and Body Composition of Nile Tilapia *Oreochromis niloticus* Fed Diets Containing Graded Levels of Seaweed *Ulva lactuca*

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Three experimental diets, including fish meal replaced with different ratios (20%, 40% and 60%) of *Ulva lactuca* and a seaweed-free control diet were used to evaluate the effect of dietary seaweed incorporation on the growth performance, feed utilization and body composition of the Nile tilapia, *Oreochromis niloticus*. The experiment lasted for 12 weeks and the results of the growth indicators; final weight (FW), weight gain (WG), percentage of weight gain (WG%), daily growth rate (DGR) and specific growth rate (SGR) indicated no significant differences (P>0.05) between the fish on the control diet (C) and those on a diet with 20% *U. lactuca* (U20), despite the fact that an outstanding performance of fish growth was accomplished on the control diet. Furthermore, no significant differences among C and U20 for the feed conversion ratio (FCR) and protein efficiency ratio (PER) whereas the feed utilization efficiency, FCR and PER decreased significantly when the proportion of *U. lactuca* meal was increased to 40% and 60% (U40 and U60). Carcass and muscle composition showed a similarity between the various groups. Moisture, protein and ash content showed an increase in line with the increase in *U. lactuca* meal in the diets, but the reverse was observed for the lipid level. The present study observed that *U. lactuca* meal attained considerable performance for feeding the Nile tilapia at dietary inclusion levels up to 20% without undesirable effects on growth performance and feed utilization efficiency.

Keywords: Nile Tilapia, Growth, Seaweeds, FCR, PER

Introduction

The aquaculture sector is expanding and developing rapidly, and its production is necessary to maintain fish supply, especially with the decrease of production of fisheries as a result of unsustainable performance[1]. Feed expense constitutes the highest ratio of the semi-intensive and intensive aquaculture production systems, and according to Azaza *et al*.[2] feed costs range from 30% to 60% of the total variable expenses, depending on the intensity of the culture system. In aquaculture diets, protein is a vital dietary ingredient for the growth and good health of the farmed fish[3,4]. Aquaculture is highly dependent on fishmeal as a protein source for fish feeds[5] because it contains at least 50% crude protein as well as all the essential amino acids required by fish; it is also palatable for fish[6]. Furthermore, as the aquaculture industry expands an increasing demand for fishmeal expected and the pelagic fishes faced further endangering as they are the main source of fishmeal[5]. However, fishmeal is one of the most expensive ingredients in aquaculture diets[7]. In this context it is necessary to find alternative protein sources to reduce feed costs, and thus to make aquaculture sector as an attractive venture, feasible and sustainable[8]. The necessary criteria for an alternative protein source are that it should be less expensive and available in large amounts to meet demand. Furthermore, it should be palatable and contain the basic amino acid requirements of fish. Seaweed has deserved concern as appropriate alternative protein source for its high protein content and production rate. *Ulva* species are important seaweeds that have been used as a dietary ingredient in aquaculture due to their content of protein, vitamins, minerals, and pigments[9]. Improved diet utilization, growth rate, immune response and fish quality have been reported for farmed fish fed diets based on *Ulva* species[10-12].

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Boosted by their numerous advantages, such as fast growth, high fecundity, tolerance to high stocking density and consumer demand, Nile tilapia, *Oreochromis niloticus*, is a widely used farmed fish species globally[13]. The aim of the present study was to evaluate the feed utilization, growth rate and body composition of Nile tilapia fed diets containing different levels of seaweed, *Ulva lactuca*.

Materials and methods

Diet formulation

Ulva lactuca were collected from the near-shore waters of the Red Sea coast. Seaweed samples were carefully washed with fresh water followed by distilled water then sun dried for 48 h. A laboratory blender was used to grind the dried seaweeds and then subjected to chemical analysis as similar most of the dietary ingredients were subjected according to AOAC[14] to determine their chemical composition (Table 1). A seaweed-free control diet (C) and three experimental diets were formulated, replacing graded level of conventional fishmeal with *U. lactuca* meal (20% = U20); (40% = U40) and (60% = U60), and each with approximately 32% protein (Table 2). As described by Younis *et al.*[15], the ingredients of each diet were mixed together by using a laboratory mixer (Hobart Mixer, HL120). A suitable paste for extrusion was made by adding hot water (at around 50°C) to the mixture then the paste was extruded through a disc of numerous 2-mm holes. The diet formulation was finalized by drying the extrusion at 105°C in an oven (Labe Tech, LDO-250N).

Experimental fish

Fingerlings of Nile tilapia, *O. niloticus*, were obtained from a governmental project for fish breeding and acclimatized to laboratory conditions for two weeks prior to actual start of the experiment. Also, a random sample of 24 fish was chosen for initial chemical analysis of muscle and carcass.

Experimental design

Body weight and length of one hundred and ninety two fish were recorded before distributing them randomly in twelve glass aquariums(100x40x40) with sixteen fish for each. Three replicate of aquariums were used for each experimental diet (C; U20; U40 and U60). The aquariums were fitted with biological filters to pull the wastes and to offer diffused air and were equipped also with thermostatically controlled aquatic heaters to maintain the water temperature at 28°C±1. The water qualities such as dissolved oxygen, pH, ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) were monitored and kept within the suitable ranges according to Younis *et al.*[15]. The fish were fed twice a day with a ratio of 3% of fish weight for six days per week. The water of the aquariums was changed every two weeks where the fish were weighed, and their length was recorded. After 12 weeks, the fish of all treatments were euthanized and then divided into two groups, one for determining of the chemical composition of fish muscles and the

Table 2 — The experimental diets: formulation and chemical composition (on dry weight basis)

	Diets (g/kg)			
Ingredients	C	U20	U40	U60
Fish meal	350	280	210	140
U. lactuca	00	70	140	210
Soybean meal	120	240	350	470
Wheat meal	200	180	160	100
Wheat bran	150	100	60	20
Yellow corn	130	80	30	10
Corn oil	30	30	30	30
Vitamin mix	10	10	10	10
Mineral mix	10	10	10	10
Proximate composition (%)				
CP (%)	32.74	32.73	32.45	32.18
CL (%)	5.44	5.04	4.67	4.37
Ash (%)	10.33	10.96	11.59	12.22
NFE (%)	51.49	51.27	51.29	51.23

C: Control; U20: 20% *U.Lactuca*; U40: 40% *U.Lactuca*; U60: 60% *U.Lactuca.*

CP: crude protein; CL: crude lipid; NFE: (Nitrogen-free extracts) = dry matter (100) – (crude protein + crude lipid +ash).

Table 1 — Chemical composition of the dried dietary ingredients

Proximate composition	Ingredients					
	Ulva lactuca	Fish meal	Soybean meal	Wheat meal	Wheat bran	Yellow corn
CP (%)	11.5	61.41	44.24	14.36	15.03	9.45
CL (%)	6.08	12.33	2.91	1.40	5.25	3.10
Ash (%)	24.29	18.64	6.50	1.80	4.79	3.40
NFE (%)	58.13	7.62	46.35	82.44	74.93	84.05

CP: crude protein; CL: crude lipid; NFE: (Nitrogen-free extracts) = dry matter (100) – (crude protein + crude lipid +ash).

second for carcass chemical composition according to AOAC[14].

Growth indicators and Feed utilization

Growth indicators and feed conversion, in terms of survival (SR), weight gain (WG), percentage of weight gain WG (%), daily growth rate (DGR), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and protein efficiency ratio (PER) were determined using the following equations:

SR = (final fish number / initial fish number) ×100
WG = final weight (g) - initial weight (g)
WG (%) = 100 × [final weight (g) - initial weight (g)] / initial weight (g)
DGR = weight gain (g) / experimental period
SGR = 100× [Ln (final weight) - Ln (initial weight)] / experimental period
K = [body weight (g) / body length^3 (cm^3)] ×100
FCR = feed intake (g) / body weight gain (g)
PER = weight gain (g) / protein intake (g)

Statistical analysis

One-way analysis of variance (ANOVA) was used to statistical analysis of the collected data (WG, WG%, DGR, SGR, FCR and PER) and body composition of fish (moisture, protein, lipid and ash). Fisher's Least Significant Differences test (LSD-test) was applied to compare the average values (means ± standard deviation) and the significant differences were defined at $p < 0.05$.

Results and Discussion

Growth performance

The results of the growth indicators (FW, WG, WG%, DGR, SGR, K) are shown in Table 3. No significant differences were recorded (P>0.05) between the fish of diet C and those of diet U20, despite the fact that the highest growth rate was obtained in fish on diet C, where the FW was (42.30±2.154), WG (29.31±0.894), WG% (225.6±0.932), DGR (0.349±0.061) and SGR (1.405±0.156). In contrast, there were significant differences (P<0.05) between the growth performance for fish on diet C and U20 compared to that of groups U40 and U60. The U60 diet produced the lowest growth indicators, FW (34.52±2.871), WG (21.40±1.022), WG% (163.1±1.156), DGR (0.255±0.034), SGR (1.152±0.096), followed by fish on diet U40 with no significant differences between them. The condition factor (K) of fish on all the treatments was not significantly different (P>0.05). No mortality was recorded for fish receiving any of the experimental diets. Results show that *U. lactuca* meal could be successfully integrated into tilapia diets replacing up to 20% of conventional fish meal without a negative impact on growth performance and feed utilization efficiency, as evidenced by the fact that there was no significant differences in the growth rate and feed utilization of the fish in the control diet and those on diet U20. Both the growth indicators (FW, WG, WG%, DGR and SGR) and the feed utilization parameters (FCR and PER) did decrease significantly when the incorporation level of *U. lactuca* meal was raised to 40% and 60%. However, the growth performance and feed utilization in this study were in agreement with the results of Azaza *et al*.[11] who fed Nile tilapia with formulated practical diets containing 10%, 20% and 30% of *Ulva* meal (*Ulva rigida*) and concluded that, *Ulva* meal can

Table 3 — Growth performance and feed utilization indicators of *Oreochromis niloticus* fed the experimental diets

Parameter	C	U20	U40	U60
IW (g/fish)	12.99±0.153	13.07±0.309	13.15±0.300	13.12±0.235
FW (g/fish)	42.30±2.154^a	41.72±2.055^a	35.63±2.372^b	34.52±2.871^b
WG (g/fish)	29.31±1.118^a	28.65±0.894^a	22.48±0.677^b	21.40±1.022^b
WG %	225.6±0.932^a	219.2±1.031^a	171.0±1.246^b	163.1±1.156^b
DGR (g/day)	0.349±0.061^a	0.341±0.048^a	0.268±0.028^b	0.255±0.034^b
SGR (%/day)	1.405±0.156^a	1.382±0.114^a	1.187±0.082^b	1.152±0.096^b
K (%)	1.676±0.066	1.708±0.030	1.719±0.036	1.649±0.035
FCR	1.779±0.248^a	1.977±0.240^{ab}	2.256±0.195^{ab}	2.545±0.324^b
PER	1.933±0.261^a	1.717±0.233^{ab}	1.446±0.144^{ab}	1.406±0.288^b
SR (%)	100±00	100±00	100±00	100±00

Values in the table represent mean ±SE.
Values in each row with the same superscript or without superscripts are not significantly different from each other (P >0.05).
C: Control; U20: 20% *U.Lactuca*; U40: 40% *U.Lactuca*; U60: 60% *U.Lactuca*
IW: initial weight; FW: final weight; WG: weight gain; WG %: weight gain percentage; DGR: daily growth rate;
SGR: specific growth rate; K: condition factor; FCR: feed conversion ratio; PER: protein efficiency ratio; SR: Survival.

composed a proportion up to 20% of the tilapia diet without adverse impact on fish growth and feed utilization efficiency while beyond this level (20%), a decline of the fish growth was recorded. A potential reason for the declined growth in this study at higher levels of *U. lactuca* meal (U40 and U60) could be the high Nitrogen-free extracts content, such as the fibre in the *Ulva* meal, and the possible effects of this on the digestibility of protein and dry matter. Diet with high levels of fibre content leads to rapid passage of food through the alimentary canal, result in reduce the time available for digestion, thus maybe negatively affecting nutrient absorption of the diet. Fibre could possibly act as physical barriers between digestive enzymes in the intestine and nutrients such as starch, protein and lipids thus making them less beneficial. Therefore, high fibre content could be a limiting factor for use of *U. lactuca* meal at high inclusion levels in fish feeds as established in the current study for both the U40 and U60 diets. This explanation was in line with Ortiz *et al.*[16] reporting that *U. lactuca* contains about 60% fibre and this might reduce its value in aqua feeds. Azaza *et al*.[11] suggested the same reason as one of his explanations for the growth reduction of *O. niloticus* at high levels of dietary *Ulva* meal (30%). They also concluded that anti-nutrients such as saponins, tannins, and phytic acid which are present in the vegetative tissues of several plants might cause a growth reduction of *O. niloticus* at higher levels of *Ulva* meal (30%).

Feed utilization

Although the feed conversion ratio (FCR) and protein efficiency ratio (PER) varied in response to the variations of diets, no significant differences (P>0.05) were observed between the treatments except that the FCR and PER of diet C showed significant differences (P<0.05) compared to those of diet U60 (Table 3). The best value of FCR (1.779±0.248) and highest PER (1.933±0.261) were noticed for diet C, conversely, the results of diet U60 showed the poorest FCR (2.545±0.324) and lowest PER (1.406±0.288).

Growth rate and feed utilization in the this study were also in accordance with other previous studies that have used seaweeds as dietary ingredient in fish feed, with El-Tawil[17] who reported that final body weight, weight gain and specific growth rate of red tilapia (*Oreochromis* sp.) increased significantly when increasing levels of seaweeds (*Ulva* sp.) in the diet up to 15% compared to the seaweed free diet. On the other hand, a diet of 20% seaweed did not show significant difference with the control diet whereas a diet of 25% seaweed showed the poorest growth performance. Meanwhile, the best feed conversion ratios (FCR) were observed in fish fed diet with 20% *Ulva* sp. followed by diets with 15% and 10 % *Ulva* sp. There are slight differences between the findings of El-Tawil[17] and the results of the present study, however, which can probably be attributed to the difference in the fish species, as well as the type of seaweed. These factors, along with the difference in the initial weight of the experimental fish, might also explain the differences with the results of Güroy *et al*.[10] (2007) who revealed that Nile Tilapia fed on diets with 15% additive *Ulva* meal (*U. rigida*) exhibited the lowest weight gain and no significant differences in the growth indicators and feed conversion ratio were noticed between the control and the other additive *Ulva* meals. Also, Valente *et al*.[18] suggested that the incorporation level of *U. rigida* up to 10% in diets for sea bass juveniles was very promising, since no negative impact on growth performance or nutrient utilization were observed.

Body composition

The results of muscle composition are presented in Table 4. The muscle moisture content exhibited a significant difference (P<0.05) between the treatments, with the highest moisture content being recorded in the fish on diet U60 (80.470 ±0.155), while the lowest moisture was observed in the fish on diet C (79.817 ±0.120). The highest muscle protein was obtained in the fish on diet U60 (17.761 ±0.593) with no significant differences (P>0.05) compared to the other experimental treatments. The highest muscle content of the lipid was noticed in the fish on the diet C (0.765 ±0.079) but this was significantly different only to the lipid levels in fish on diet U60 (0.517±0.043). The muscle content of ash did not show a significant difference between the treatments, although the ash content did vary with the variations in the experimental diets, with the ash content of fish taking diet U60 being the highest (1.216 ±0.054) while diet C showed the poorest level of ash (1.086 ±0.123) in the fish muscle. The results of carcass (whole body) composition (as a percentage based on the fresh weight) are also shown in Table 4. Altogether, carcass composition was very similar to the results of muscle composition, with the fish on

Table 4 — Body composition of *Oreochromis niloticus* fed the experimental diets (based on the wet weight)

Proximate composition (%)			Muscle composition		
	Initial	C	U20	U40	U60
Moisture	81.03	79.817±0.120^{b}	80.050±0.199^{ab}	80.253±0.092^{ab}	80.470±0.155^{a}
Protein	15.43	17.325±0.213^{a}	17.490±0.044^{a}	17.621±0.232^{a}	17.761±0.593^{a}
Lipid	0.96	0.765±0.079 ^{a}	0.646±0.084^{ab}	0.645±0.043^{ab}	0.517±0.043^{b}
Ash	2.04	1.086 ±0.123^{a}	1.118 ±0.041^{a}	1.123 ±0.021^{a}	1.216 ±0.054^{a}
			Carcass composition		
Moisture	79.95	78.273±0.099^{b}	78.773±0.434^{b}	79.080±0.384^{b}	80.977±0.618^{a}
Protein	12.27	2.614 ±0.023^{c}	13.915 ±0.052^{b}	13.925 ±0.001^{b}	14.490±0.074^{a}
Lipid	2.43	3.182±0.254^{a}	2.653 ±0.047^{a}	2.059±0.113^{b}	1.481±0.209^{c}
Ash	3.36	4.036±0.054^{b}	4.097 ±0.109^{ab}	4.293±0.055^{a}	4.343 ±0.083^{a}

Values in the table represent mean ±SE

Values in each row with the same superscript are not significantly different from each other (P >0.05)

C: Control; U20: 20% U. Lactuca; U40: 40% U. Lactuca; U60: 60% U. Lactuca; CP: crude protein; CL: crude lipid

diet U60 recording the highest moisture content (80.977 ±0.618) with a significant difference ($P<0.05$) compared to all other treatments, while the lowest moisture content was obtained in the fish on diet C (78.273 ±0.099), with no significant difference ($P>0.05$) with the moisture levels in fish on either diet U20 or diet U40. Carcass protein content diversity between the nutrition trials compared to fish on the diet U60 which recorded the highest protein content (14.490 ±0.074), but this decreased significantly in all other trials, with the fish on diet C showing the poorest protein content (12.614 ±0.023). No significant difference was observed in the carcass protein content between the fish on diet U20 and U40. Carcass lipid level was significantly influenced by the variation in the diets. Lipid level was the highest for the fish of the control group (3.182 ±0.254) without a significant difference ($P>0.05$) with the lipid level of fish of diet U20 (2.653 ±0.047). A significant decrease ($P<0.05$) in the lipid content was observed for fish on diet U40 (2.059 ±0.113) and U60 (1.481 ±0.209), however, compared to the previous two groups, with the latter having the lowest carcass lipid content. The fish on diet U60 showed the highest ash content (4.343 ±0.083) without significant difference with the ash content for fish on diet U40 (4.293 ±0.055), but fish in diet C did show a significantly lower ($P<0.05$) ash content (4.036 ±0.054) compared to those on either diet U40 or U60. The analysis of the body composition of fish is the determining of their content of water, protein, fat and ash. Knowledge of body composition of fish is good index to its physiological condition and health status[19]and good condition and health are fundamental for maximize their utilization[20]. The current study has displayed changes in the body composition of Nile tilapia which appear to be related to the variation of the diets. Altogether, the muscle composition results were very similar to those of the carcass composition. The moisture, protein and ash content increased as the ratio of *Ulva* meal in the diets increased, with the reverse being true for the lipid levels in the fish. With respect to the body composition of red tilapia reported[17], the results are generally consistent with the findings of the current study, where protein content increasing gradually with increasing dietary supplementation of *U. lactuca* meal. Güroy *et al*.[10] reported a marked decrease in Nile tilapia body lipid levels when *Ulva* meal integration was increased, and this is accordance with the results of the present study.The results of the current study showed significant increase in carcass moisture and ash at higher levels of *U. lactuca* meal (U40 and U60) and this is in accordance with the outcomes reported by Azaza *et al*.[11], who stated that the level of carcass moisture of Nile tilapia was significantly higher in fish fed with diets of *U. rigida* up to 20% (U2) and 30% (U3) compared to those fed diets of U0 (control) and U1 (10%), while being inversely related to the body lipid. The variations of fish composition with the supplementation level of *Ulva* meal were not significant in the case of muscle content of protein and ash. These observations were generally consistent with the results of Valente *et al*.[18] who reported that the partial substitution of fish meal by *Gracilaria bursa-pastoris* and *U. rigida* up to 10%, and by *Gracilaria cornea* at 5% did not influence on the carcass composition of sea bass, whereas fish fed 10% *G. cornea* showed higher ash content.

Conclusion

Based on the outcomes of the current study, it was reported that, seaweed *U. lactuca* has large chance as an alternative ingredient in diets for Nile tilapia at dietary inclusion levels up to 20% without inverse impacts on the growth and feed utilization efficiency. However, further research is needed, to evaluate the efficiency of this seaweed in longer term feeding trials so as to determine the optimum dietary inclusion level without compromising the growth performance and body composition of fish. More investigations should be performed to evaluate the cost of the seaweed per kg, in order to estimate its cost effectiveness at a commercial scale in comparison to fish meal.

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