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Effects of *Ulva rigida* (C. Agardh) and *Chaetomorpha crassa* (C. Agardh) as Feed Supplements on Growth Performance and Body Composition of Nile tilapia *Oreochromis niloticus* (Linnaeus) Fingerlings

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Abstract
Seaweeds have received attention as suitable alternative protein sources for farmed fish since their protein content and production rate are high (Guroy, 2005). In a 12-week feeding trial, the effect of two seaweed supplements on growth performance and body composition of Nile tilapia fingerlings was investigated. The fish were fed to satiation with fish meal that was supplemented with various levels of seaweeds C. crassa and U. rigida (5%, 8%, 10%, 20% and 30%). A diet without seaweed served as a control. Determination of carcass nutritional composition was done at the beginning and end of experiment. Fish weight and length was taken for each treatment every two weeks. Water quality parameters were also monitored during the experimental period. The highest weight gain was observed in fish fed the 8% U. rigida and C. crassa diets while, the lowest weight gain was recorded in the 30% C. crassa diet. There were significant differences (p<0.05) in Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and protein efficiency (PE) among the treatments. The results suggested that both seaweeds U. rigida and C. crassa meals could be used in small percentages in tilapia diets.
Key words: *C. crassa, body composition, growth performance, Nile tilapia, seaweed suppliments, U. rigida.*

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I. Introduction

Traditionally, fish meal is the preferred dietary protein source for many farmed fish species and is appreciated for its amino acid balance, vitamin content, palatability and un-identified growth factors[1]. However, fish meal is an expensive feed ingredient and supplies often vary unpredictably because of overfishing. Hence, its increasing cost has restricted the use of fish meal as a protein source for fish diets. Therefore, suitable alternatives for fish meal in fish diets have to be identified to take care of the protein needs of farmed fish. *U. rigida* and *C. crassa* have been shown to contain all the essential amino acids (EAA) that account for 42.1- 48.4% of the total amino acids content[2]. Other reports have shown *U. rigida* to have a high protein content, similar to traditional high protein plant sources such as legumes and grains, especially soy and amaranth[3], thus justifying its direct use for the development of balanced diets for animal and fish nutrition.

The focus on seaweeds in animal nutrition and in fish feeds has not only been a result of their nutritional value but also because of the world food situation where traditional foods used in animal nutrition such as wheat, corn and other cereals are not enough to cater for the increasing human populations leave alone animal nutrition. Hence, in the light of the present and prospective world food situation, the more widespread use of seaweed meals merits further attention. Although seaweeds represent the world's third-largest aquaculture crop behind finfishes and mollusks[4], few of the macroalgae have been studied for their possible use as feed additives for fish. The use of seaweeds in animal nutrition has become more widespread by the establishment of processing plants to dry and grind the weed into a meal for use as an additive to stock feeds. These practices appear largely to be restricted to northern Europe especially Norway, Denmark, Iceland, Ireland, Scotland and France - and parts of North America, although reference can be found to such uses in Hong Kong and the islands of the Bering Sea and New Zealand[5]. Along the East Africa coastal region, there are hardly any reports or scientific research on the use of seaweeds in in fish feeds. Nevertheless, some wild stocks of seaweed species are harvested for use as fishing baits by artisanal fishermen while in Zanzibar two species *Eucheuma denticulatum* (N.L Burman) and *Kappaphycus alvareizi* (Doty) are cultivated and harvested for export to Denmark and USA where they are used for the extraction of carrageenan and other products.

O. niloticus is one of the tilapia species being propagated in Kenya owing to its desirable characteristics. Tilapia, are the most abundantly cultured species worldwide[6].This is because of their enormous adaptability to a wide range of physical and environmental conditions, ability to reproduce in captivity, relative resistance to handling stress and disease-causing agents compared to other cultured finfish species. Other qualities include good flesh quality, the ability to feed on a low trophic level and have excellent growth rate on a wide variety of natural and artificial diets. The objective of this study was therefore to examine and evaluate the growth performance, feed utilization, and body composition of hapa cultured *O. niloticus* when fed diets supplemented with various levels of macroalgae meals, namely *U. rigida* and *C. crassa*, so as to determine the optimal level of dietary seaweed meal supplementation.

II. Materials and Methods

The experiment was performed at government certified fish farm in Makueni County in Kenya. Fingerlings of *O. niloticus* weighing on average 1.0 ± 0.2 g were obtained from the fish farm. Five replicate earthen ponds of the same size, shape and supplied by the same continuously flowing source of water were used in this experiment. Each pond had eight 1 m^3 fish net-cages (hapas) that were each stocked with 30 randomly selected fish fingerlings of approximately the same weight and length. A sample of 5 juveniles from each hapa were weighed at the beginning of the study, while 10 were processed for determination of the initial body composition of crude proteins, lipids, ash, fibre and carbohydrates. Each hapa had 20 juveniles at the onset of the experiment. The fish were fed on the seaweed supplemented experimental diets to apparent satiation twice daily at 10:00 hrs and 16:00 hrs for three months. They were weighed and total length measured every 2 weeks from the beginning of the study to the end, during which the net cages were also cleaned of epiphytes. The feeding trial experiment was carried out for 12 weeks. Water quality parameters including temperature, dissolved oxygen, and temperature were monitored weekly using a thermometer, dissolved oxygen meter (YSI model 57) and pH meter (model Corning 345) respectively.

Processing of Experimental Diets of Seaweeds *U. rigida* and *C. crassa*

Seaweeds *C. crassa* and *U. rigida* were freshly obtained from the intertidal zone at Diani at the beginning of the experiment. Enough quantities were collected on the basis of estimations of amounts required to run the experiment to the end, to allow consistency in terms of nutritional components which vary with seasons and sites. The seaweeds were cleaned of epiphytes, epifauna, pebbles and other molluscan shells in seawater and washed at least five times in running fresh water, gently blotted dry and fresh gross weights recorded to an accuracy of 0.1 g. The samples were dried at 70°C (in hot air oven) to a constant weight, then powdered, sifted through a piece of muslin to get fine powder and stored in a desiccator for inclusion in the diets. A commercial feed (fishmeal) with predetermined nutritional composition was supplemented with varying levels in percentage of seaweed species *U. rigida* and *C. crassa*. The seaweed diets were formulated according to[7] using pre-weighed dry ingredients of seaweeds and commercial fishmeal. The nutritional composition including total protein, crude fiber, total lipids, moisture and ash in the experimental diets and fish were analyzed at the beginning of the experiment according to standard AOAC (Association of Analytical Chemists) methods. The values are as shown in Table 1, 2 and 3. The algae free diet acted as the control.

The following were the percentages of the seaweed ingredients used:

(a)An algae free control diet b) 5% *U. rigida* (Ur5) c) 8% (Ur8) (d) 10% (Ur10) (e) 20% (Ur20) (f) 30% (Ur30).

The second experimental diet was formulated as:

(a) An algae free control diet (b) 5% *C. crassa* (Cc5) (c) 8% (Cc8) (d) 10% (Cc10) (e) 20% (Cc20) (f) 30% (Cc30).

At the end of the experiment fish body composition in terms of proteins, total lipids, moisture, ash, crude fiber were determined while fish performance based on weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), food conversion rate (FCR), protein utilized per kg of growth (PU) and protein efficiency ratio (PER) were calculated as follows:

- WG (%) = [(final weight (g) – initial weight (g)/initial weight (g) x 100
- DWG (g) = Mean final body weight (g) – mean initial body weight (g) /number
- FCR = feed intake (g) / weight gain (g)
- SGR (% day^{-1}) = 100 x [(In final fish weight) - (in initial fish weight)]/experimental days
- Protein Efficiency ratio = Weight gain (g)/crude protein intake
- Protein utilized kg-1 growth (g) = (dietary protein consumption (g)/weight gain (g) x 1000.

Data Analysis

To determine differences between treatments the non-parametric tests such as Kruskal-Wallis ANOVA and Mann-Whitney U test were applied. The level of significance in all cases was set at $p<0.05$.

III. RESULTS

Nutritional Composition of Nile Tilapia Fingerlings Fed on the Experimental Diets

Following the 12-week growth experiment, survival rate ranged from 68.75% to 80% for all the *U. rigida* and *C. crassa* supplemented diets as shown in Table 4 and 5. There were no significant differences in survival rate among the treatments. The nutritional composition at the end of the experiment showed that the percentage of dry matter, fibre and ash did not differ signicantly (P>0.05) in the fish carcass from that determined at the beginning of the experiments. However, protein and total lipids changed significantly (P<0.05) in fish fed all the experimental diets. Final crude protein content of the fish at the end of all treatments increased significantly (P < 0.05) compared to the initial measurements; however, there were no significant differences (P > 0.05) in the final crude protein content between those supplemented with *U. rigida* and those supplemented with *C. crassa.* The lipid content of the fish at the end of all treatments was significantly much higher than at the onset (P < 0.05). Carcass lipid levels tended to increase with increasing levels of *U. rigida* and *C. crassa* meals. A significant (P < 0.05) drop in ash content was marked in all fish at the end of the experiment compared to initial measurements, though among the experimental treatments the ash content was not significantly different (P > 0.05).

Table 4: Body composition of *O. niloticus* (L) fed the experimental diet supplemented with *U. rigida*.

	Initial	C	Ur5	Ur8	Ur10	Ur20	Ur30
Moisture%	71.3±0.4	70.7±0.8	70.9±0.1	72.3±0.3	71.4±0.2	70.1±0.2	71.4±0.7
Protein%	18.9±1.5	18.8±0.9	19.3±0.9	19.3±0.2	19.2±0.9	17.8±1.2	17.2±1.3
Lipid%	3.7±0.3	8.6±0.8	8.7±0.6	12.4±0.4	12.6±0.3	14.7±1.3	14.5±0.2
Ash%	3.7±0.5	3.5±0.1	3.1±0.3	3.7±0.1	3.5±0.5	3.6±0.3	3.6±0.4
Fibre%	3.2±0.2	3.1±0.1	3.3±0.1	3.4±0.6	3.3±0.7	3.4±0.1	3.4±0.2

Table 5: Body composition of *O. niloticus* (L) fed the experimental diet supplemented with *C. crassa.*

	Initial	C	Cc5	Cc8	Cc10	Cc20	Cc30
Moisture%	71.3±0.4	70.7±0.8	67.5±0.1	63.8±0.3	64±0.5	65.3±0.5	68.5±0.3
Protein%	18.9±1.5	17.8±0.9	19.0±0.2	19.2±0.5	18.8±0.2	16.3±0.6	16.2±1.2
Lipid%	3.6±0.3	8.6±0.8	9.9±0.2	11.7±0.4	11.8±0.1	10.9±0.3	9.3±0.9
Ash%	3.8±0.5	3.5±0.1	3.6±0.1	3.7±0.2	3.9±0.4	3.7±0.5	3.5±0.3
Fibre%	3.1±0.2	3.1±0.1	3.8±0.9	4.2±0.3	4.1±0.3	3.8±0.4	3.5±0.1

Weight Gain (WG)

The results of growth performance and feed utilization are summarized in Table 6 for *U. rigida* supplemented diets and Table 7 for *C. crassa* supplemented diets. The trend in weight from all the treatments is as shown in Figure 1. The highest weight gain was observed in the fingerlings fed with 8% *U. rigida* and *C. crassa* supplementation levels with significant differences (p<0.05) in weight gain between Ur8 level, Ur20 and Ur30. There was no significant difference in weight gain in the fish fed the Ur5, Ur10, Cc5 and Cc10 when compared to the control group. However, significant differences (p<0.05) were recorded between the control group and Cc30 while the same scenario was noted between Cc8 group and Cc30. The fingerlings fed with Ur20, Ur30, Cc20 and Cc30 produced the poorest final fish weight.

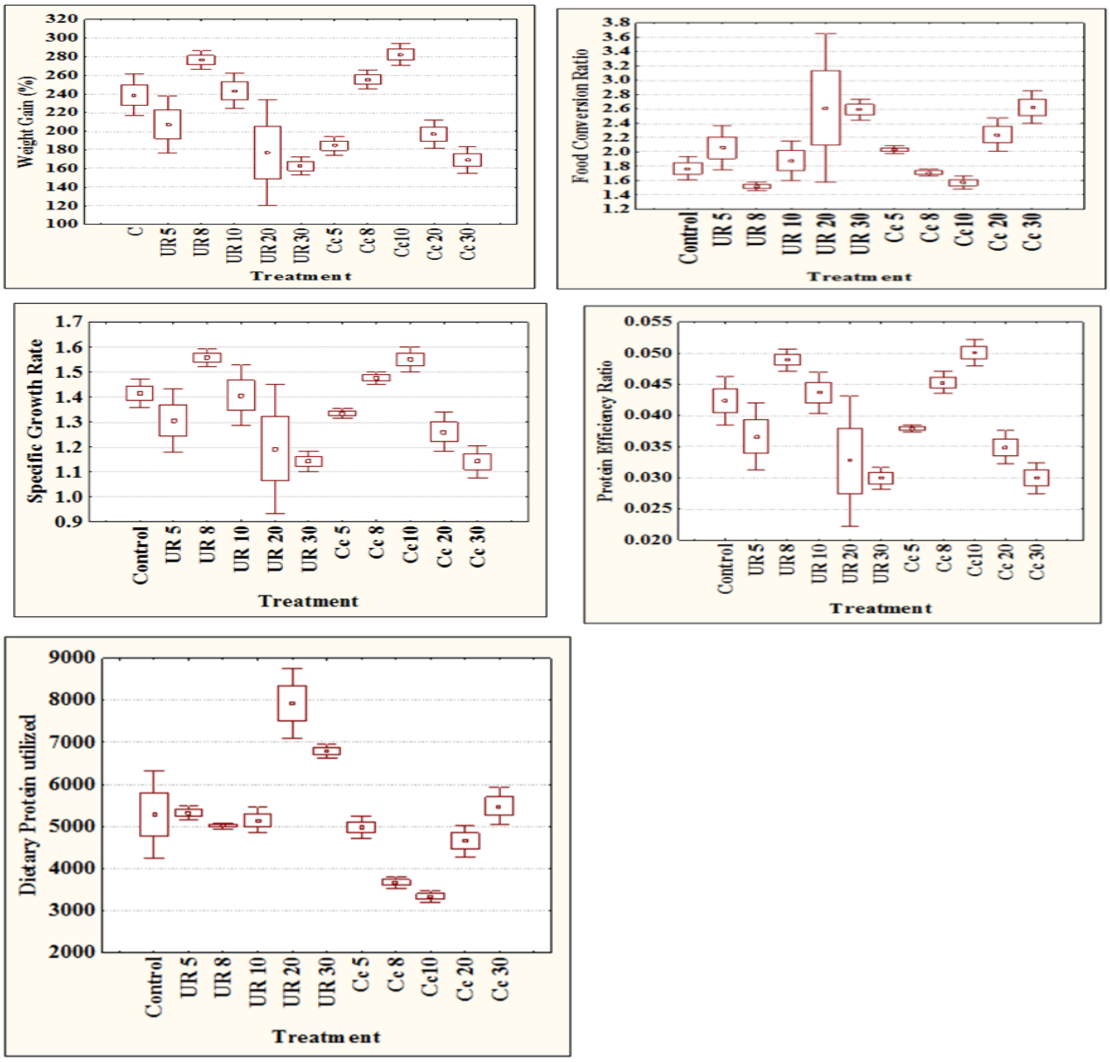

Figure 1: Weight gain (%) (Mean ± SE), Food conversion ratio (Mean ± SE), Specific growth rate, Protein Efficiency Ratio and Dietary protein per kg of Growth (Mean ± SE) in Nile tilapia fingerlings fed with *U. rigida* and *C. crassa* supplemented diets (5%, 8%, 10%, 20% and 30%) for 12 weeks.

Table 6: Growth, feed, and nutrient utilization of *O. niloticus* fingerlings fed the experimental diet 1 (supplemented with *U. rigida)*.

Diet	C	Ur5%	Ur8	Ur10	Ur20	Ur30
Survival %	72.5 ±2.89	76.25±4.79	76.25±4.15	71.25±8.5	76.25±4.8	68.75±6.3
Initial mean weight (g)	1.05±0.03	1.04±0.02	1.03±0.02	1.08±0.14	1.0±0.11	1.01±0.01
Final mean weight (g)	3.44±0.25	3.11±0.29	3.79±0.101	3.52±0.24	2.77±0.49	2.64±0.1
Weight gain (%)	239±37.04	207.5±30.7	276.5±9.9	227.15±33.8	177±48.92	160.89±9.01
Daily Weight Gain (g)	0.028±0.003	0.025±0.004	0.033±0.001	0.03±0.002	0.021±0.007	0.019±0.001
Feed conversion ratio	1.77±0.16	2.06±0.30	1.52±0.06	1.88±0.28	2.62±0.90	2.59±0.15
Specific Growth Rate %	1.41±0.15	1.31±0.13	1.56±0.04	1.41±0.12	1.19±0.25	1.14±0.04
Protein Efficiency Ratio	0.042±0.004	0.037±0.005	0.049±0.0004	0.04±0.003	0.033±0.002	0.030±0.002
Dietary protein utilized per kg of growth (g)	5280.97±102 6.87	5322.37±177.83	5014.50±70.0 9	5151.78±305. 91	7928.84±840. 96	6795.53±170.85

Table 7: Growth, feed, and nutrient utilization of *O. niloticus* fingerlings fed the experimental diet 2 (supplemented with *C. crassa)*.

Diet	C	Cc5	Cc8	Cc10	Cc20	Cc30
Survival %	72.5±2.89	77.5±2.89	80±4.08	76.25±4.79	77.5±6.45	80±4.08
Initial mean weight (g)	1.05±0.03	1.04±0.03	1.04±0.02	1.06±0.03	1.05±0.03	1.05±0.01
Final mean weight (g)	3.44±0.25	2.88±0.07	3.60±0.11	3.88±0.09	3.02±0.13	2.74±0.14
Weight gain (%)	239±37.04	184.5±9.98	255.5±9.98	244.5±31.76	197±15.19	169±14
Daily Weight Gain (g)	0.028±0.003	0.022±0.001	0.030±0.001	0.033±0.001	0.023±0.002	0.020±0.002
Feed conversion ratio	1.77±0.16	1.96±0.03	1.71±0.05	1.57±0.08	2.24±0.23	2.62±0.23
Specific Growth Rate%	1.41±0.15	1.13±0.02	1.48±0.02	1.55±0.05	1.26±0.08	1.14±0.06
Protein Efficiency Ratio	0.042±0.004	0.038±0.0005	0.045±0.001	0.050±0.002	0.035±0.003	0.030±0.002
Dietary protein utilized per kg of growth (g)	5280.97±1026.87	4980.90±264.06	3363.68±142.90	3331.71±137.68	4651.23±377.19	5483.55±450.20

Food Conversion Ratio (FCR)

FCR for all fingerlings in the experiment ranged from 1.52 to 2.62. The results show significant differences in FCR among the five dietary supplementation levels ($p<0.05$) of the two seaweed diets. The lowest FCR was recorded in the fish fed the Ur8 diet and Cc10 while the highest was recorded in the fish fed the Ur20, Ur30, Cc20 and Cc30 diets. A significant difference was noted between fish diet Ur8 and Ur30 ($p<0.05$) (Figure. 1). There were more significant differences in FCR among the *C. crassa* supplemented treatments compared to that recorded among the *U. rigida* diets. In this case, a significant difference was noted between the control and Cc20 as well as the Cc30 diet ($p<0.05$). The FCR in the Ur5, Ur10, Cc5 and Cc10 were relatively similar to that exhibited by the control group.

Specific Growth rate (SGR)

The highest SGR was realised in the fish fed the Ur8 diet while the lowest was recorded in the fish fed the Ur20 and Ur30 diets (Figure 1). The results showed significant differences in SGR among the five *C. crassa* supplimented diets ($p<0.05$).

Protein Efficiency Ratio (PER)

There were significance differences in PER among the five treatments ($p<0.05$) in the two seaweed supplemented diets. The differences were noted between the control and Ur30, the Ur5 and Ur8 as well as Ur8, Ur20, Ur30 ($p<0.05$). Ur8 registered the highest PER while the lowest was registered in Ur30 (Fig 1). In the *C. crassa* supplemented diets, the highest PER was observed in Cc8 while the lowest was recorded in Cc30. There were significant differences PER noted between the control diet group and those of Ur10, Ur20 and Ur30 ($p<0.05$) (Figure 1).

Dietary Protein Utilized per kg of Growth

The results show the dietary protein utilized per kg of growth had significant differences among the different treatments. It was noticed that significantly high protein ($p<0.05$) was utilized per kg of growth in the groups fed with the highest algae meal concentration. The lowest protein utilized per kg was noted in the fish fed the Ur8 diet while the highest protein utilized per kg of growth was noted among the fish the fed the Ur20 diet. The amount of protein per kg of growth utilized in Ur5 and Ur10 was relatively similar the fish fed the control diet. The results further show significant differences in protein utilized per kg of growth among the five *C. crassa* supplemented diets. The least protein utilized per kg of growth was noted in fish fed the Cc8 and Cc10 supplemented diets while the highest was noted in the Cc30 diet.

IV. Discussion

The results indicate that the survival rate of the fingerlings was more than 72% for the duration of the experiment in both diets. The observed fish loss was probably not due to inclusion of seaweed meals, but could have been due to handling during weighing and the daily routine management of the experimental system. Overall, the significant differences on the effects of different diets to the fingerling growth variables could be an indication of the differences in food digestibility and feed utilization among the treatments. As pertains to growth performance, the fish weight gain tended to increase up to the 8% seaweed supplementation level, and then decreased with an increase in seaweed meal concentrations towards the 30% level. This tendency was observed in both *U. rigida* and *C. crassa* supplemented meals. This observation could be attributable to the variations in protein levels, fibre and probably lipids among the treatments as shown in nutritional composition analysis that showed 8% and 10% diets had particularly higher crude protein and lipid contents in comparison to the rest of the treatments. Further studies on feed digestibility and utilization can isolate the exact components that lead to these findings.

These results are comparable to the findings of [8] on the utilization of filamentous green algae *Clodophora glomerata* as a protein source in feeds for tilapia (*O. niloticus*) fingerlings. The researchers reported that the highest growth rates were observed in the fish that were fed the diet containing the least macroalgae meal at 7% inclusion. The findings in the current study were also similar to previous studies of black sea bream (*Acanthopagrus schlegeli*)[9], tilapia (*O. niloticus*)[10] and snakehead (*Channa striatus*)[11] who noted higher growth rates in lower seaweed inclusions. On the contrary[12] indicated that the best weight gain in striped mullet (*Mugil cephalus*) was obtained with a diet including 20% *Ulva* meal, whereas [13] observed no effect of *Ulva* inclusion on fish growth in the gilthead sea bream, *Sparus aurata*. These findings point to the fact that the effect of any given seaweed species in supplementation diets depends on the fish species involved.

The results indicate that crude lipids changed significantly in fish given the seaweed supplemented diets. The final crude lipid was significantly much higher than at the onset of the experiment. These results are in agreement with those of other researchers who have studied the nutritional value of different seaweeds (*Undaria penatifida* and *Ascophyllum nodosum*) as dietary supplement for red sea bream (Pagrusmajor)[14]and [15]. These studies suggested that seaweed inclusion contributes to the lipid deposition to the muscle and mobilize the lipid to energy. [15] also suggested that the use of macroalgae as a feed additive for fish accelerates the assimilation of ascorbic acid, improving the physiological conditions related to nutrition, and the metabolism of lipids altogether. Further explanations to this phenomenon indicate that seaweeds such as *U. rigida* are known to change fat deposition and mobilization patterns in sea bream apparently resulting in a more efficient use of fat deposits [16] which probably could be the case in the current study. Further studies could shed further light on the exact effects of *U. rigida* in lipid metabolism in *O. niloticus*.

The results also indicate that at the 5%, 8% and 10% seaweed supplementation level the crude of the fish carcass protein was relatively higher than the control diet, however, the level decreased significantly with the 20% and 30% inclusion at the end of the experiment. This compares well with the findings by [16] in gray mullet, where protein content increased significantly with increasing *Ulva* sp. level up to 28% in the fish diet. The results also indicate an increasing trend in crude fibre from the initial value, to the end of the experiment among the treatments. However, the content was not significantly different among the treatments. Crude ash content remained the same among the treatments with no significant differences between the treatments and the control diet. This is contrary to the findings of [17] who found that with the inclusion of 8% kelp meal, ash and fibre content increased significantly with respect to the control diet from 6.6% to 9.3% and 0.99% to 1.4% in rainbow trout. Furthermore, [18] found that ash content of Nile tilapia were not significantly affected by the dietary supplementation of *Ulva* and *Cystoseira* meal, while their protein content decreased in comparison to the protein levels at the experiments onset.

Food conversion ratio has been described as the amount of feed it takes to grow a kilogram of fish [19]. This means that when a feed has a low FCR, it takes less feed to produce one kilogram of fish than it would if the FCR were higher. A low FCR is a good indication of a high quality feed. Food conversion ratio in this experiment ranged from 1.52 to 2.62. The lowest FCR was observed in the 8% and 10% supplemented diets. This was an indication that the two levels presented a better quality feed in comparison to the other levels of seaweed inclusion and that the 8% supplementation level could be the optimum supplementation level in both *U. rigida* and *C. crassa* for *O. niloticus* in this study. In a similar experimental set up with rainbow trout [20] recorded a higher FCR in the control diet, in comparison to the 1.3%, 3% and 6% supplementation levels, although the differences in the FCR among the three treatments were not significant. On the other hand, [19] has shown that food conversion ratio in different fish depends largely on the species, age, environmental conditions and the quality of feed. Hence, comparison of FCR in the current study with other studies is only possible if the fish are of the same species, age and the same type of feed. On this basis, other studies have shown that different fish species in diverse culture conditions and food quality may show variations in FCR. [21]noted higher FCR value for Nile tilapia fed on rice polish (5.27), followed by fish meal (3.026) and sunflower meal (3.021).

Specific growth rate (SGR) in fish is an indication of the increase in cell mass per unit time. In this study, the SGR ranged from 0.84 and 1.60 among the treatments. The highest SGR was recorded in fish fed the 8% and 10% supplemented diets while the lowest was observed in fish fed the 30% supplemented diet. The nutritional composition analysis of the 8% and 10% inclusion levels showed higher crude protein and lipid content that may have resulted to the increased growth of fish in these treatments in comparison to the rest of the treatments. In a similar experimental set up with rainbow trout, [20] recorded a SGR of 1.42 in the control diet which was significantly higher than the SGR in the other treatments.

Protein efficiency ratio (PER) is based on the weight gain of a test subject divided by the intake of a particular protein during the test period. In this study, fish fed the 8% and 10% seaweed supplemented diets had a higher PER compared to the other treatments. This is an indication that the individuals utilized less protein for every gram of body mass attained. [10] reported that 9% of dietary *Ulva* supplementation improved protein efficiency in Nile tilapia when compared to supplementation with 18% *Ulva* meal. Besides, the 8% and 10% seaweed inclusion levels had low protein utilized per kilogram of growth in comparison to the higher inclusion levels of 20% and 30%.

V. Conclusion

In conclusion, the 8% to 10% seaweed supplementation levels of *U. rigida* and *C. crassa* enhanced the growth rate, protein efficiency ratio and protein utilized per kilogram of growth and could be the optimum supplementation level for *O. niloticus*.

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