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Effect of seaweed supplemented diets on Nile tilapia, *Oreochromis niloticus* performance

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Abstract
In the present study the effects of the seaweed *Taonia atomaria* supplementation in diets for Nile tilapia were investigated. A 12-week feeding trial was carried out using triplicate groups of Nile tilapia, *Oreochromis niloticus* with 1.1±0.04 g initial weight. Four experimental diets were formulated and marked as D1 (control), D2, D3, and D4. There were significant differences (P<0.05) on growth performances among dietary treatments. The growth of the fish fed control diet showed higher FI compared to other treatments (P<0.05). Significant differences (P<0.05), were however shown in lipid and ash contents. Somatic indexes in terms of HSI and VSI differed significantly (P<0.05) by the inclusion of seaweed. No diet related histopathological changes were noted in intestine and/or liver samples from any dietary groups. Seaweed can be supplemented to diets for Nile tilapia up to 5% without any adverse effect or abnormalities of fish tissues.

Keywords: Nile tilapia, seaweed, performance, histology

1. Introduction
Using unconventional protein sources such as torula yeast [1], single cell proteins, [2] algae by-product [3] in fish diets have been studied to reduce fish meal protein in order to decrease the production cost and increased the aquaculture production. Many herbivorous fish can ingest algae as a natural food source. It has been suggested [4] that *Spirulina platensis* can be used as a sole source of dietary protein in common carp (*Cyprinus carpio*) as a replacement for fish meal protein. It has been reported that the inclusion of spirulina in diets improved fish growth [5, 6] due to its high protein content, suitable amino acid profiles, vitamins, and minerals [7]. Also, it has been concluded that using algae as a feed additive (5%) to red sea bream (*Pagrus major*) improved feed efficiency, protein efficiency ratio, and muscle protein deposition [8]. For instance, it has been indicated that algae had a positive effect on growth because of their high protein content and high productivity. Thus they found improvement in growth, protein deposition, feed utilization, physiological indicators, stress response, starvation tolerance, disease resistance, and carcass quality of cultured fish [9-11].
Seaweed has a potential attention for food additive and vegetable production. The protein content in seaweed presented as a possible component in fish diets [12]. Using seaweed in fish diets as an alternative ingredient in diets for different species has been studied [13-15]. In Egypt, there is a wide area of the coast occupied with seaweed represented as a good standard of cleanness of a number of resorts [14]. Furthermore, using algae as the protein source, feed attractant and source of antibacterial compounds will encourage increased aquaculture effectiveness in the production of human food [16]. Tilapia is considered as the suitable species for culture because of their high easiness stocking, tolerance to adverse environmental conditions, and their comparatively fast growth [17]. The present study is conducted to test the effect of different levels of seaweed, *Taonia atomaria* incorporated in the diets on Nile tilapia, *Oreochromis niloticus* performance. Two dry seasons and two rainy seasons. The main dry season lasts four months (December to March) and the short, two months (August-September). Regarding the long rainy season, it extends from April to July and the short rainy season, from October to November [10].

2. Materials and Methods
The present study was carried out in the fish laboratory at Faculty of Agriculture, Minufiya University during the period from May to August 2015.

2.1. The experimental fish

Nile tilapia, *Oreochromis niloticus* fingerlings were obtained from a local fish hatchery (Saft Khaled, Behera Governance, Egypt). Twelve 80-L glass aquaria were randomly stocked with15fish each with an initial average weight 1.1± 0.04 g. The aquaria were supplied with aerated and chlorine free fresh water. Each three groups of fish were fed one of the experimental diets three times per day at a feeding rate of 4% of body weight at the beginning and the feeding rates were adjusted according to fish periodical live body weights every two weeks for 12 weeks feeding. The aquaria were cleaned daily and two thirds of the water replaced before feeding.

2.2. Preparation of algae

Algae seaweed: the brown algae seaweed *Taonia atomaria* was obtained from Edifina Company. It was collected from Alexandria water (Mediterranean Sea). These algae were identified by specialized researchers in the National Research center. The algae were washed firstly in water to remove all mechanical impurities. The algae were spread in the sun several days to dry, then the clean dry algae was cut into small pieces. Algae bran was finely powdered using a mixer (Brawn AG, Frankfurt M) then the bran finally sieved by mesh screen size. The powder algae were packaged in polyethylene bags and maintain at room temperature till used. The values calculated on dry weigh basis.

2.3. The experimental diets

The formulated diets containing 28% crude protein and 2455 kcal/kg. All ingredients were first ground to a small particle size (approximately 250 mm) in a Wiley mill (Labx Company, Midland, ON, Canada). The diets compositions were presented in Table 1. Four experimental diets were formulated including the control as basal diet (D1) without any supplementation, followed by three diets supplemented with seaweed meal at 50, 100 and 150 g kg^{-1} (D2, D3, D4, respectively). Dry ingredients were thoroughly mixed prior to adding water to 40% moisture. Proximate composition of the experimental diet was determined according to AOAC methods [18]. The water quality parameters as water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter. pH was monitored twice weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH). During the 12-week feeding trial, the water-quality parameters averaged (± SD): water temperature, 28± 0.9 °C: dissolved oxygen, 6.5 ± 0.5 mg $^{-l;}$ pH, 7.5 ± 0.2.

Table 1: Composition and proximate analysis of the experimental diets.

Ingredients %	D1	D2	D3	D4
Soybean meal (SBM, 44% CP)	40	39	38	37
Fish meal (70% CP)	10	10	10	10
Wheat flour	10	10	10	10
Yellow corn	10	10	10	10
Wheat bran	20	16	12	8
Seaweed (18% CP)	-	5	10	15
Vegetable oil	4	4	4	4
Molasses	2	2	2	2
Vitamin and minerals permix[1]	2	2	2	2
Di calcium phosphate	2	2	2	2
Total	100	100	100	100
Chemical composition (% DM)				
Crude protein	28.1	28.3	28.3	28.4
Crude lipid	9.8	10.1	10.5	10.7
Ash	8.2	8.5	8.8	8.9

[1]Vitamins and minerals permix (mg or IU if mentioned kg die-1t): vitamin A, 8000 IU; vitamin D3, 4000 IU; vitamin E 50 IU; vitamin K3, 19IU;vitamin B2, 25mg; vitamin B3, 69mg; nicotinic acid, 125mg; thiamine, 10mg; folic acid, 7 mg; biotin, 7mg; vitamin B12,75mg; choline, 400mg and vitamin C, 200 mg. 300 mg I, 100mg Co, 100mg Si, 50000mg Zn, 70000mg Mn, 30000mg, Fe, 4000 Cu, and Ca Co_3 even 1 KG.

2.4. Analytic parameters

Before the beginning of the feeding trial, proximate compositions (%) of seaweed including moisture and ash were determined according to AOAC methods [18], Crude protein, crude lipid and crude fibre were determined by Kjeldahl, Soxlet and Fibre Tech Analyzer respectively. By the end of the feeding trial analyses of body composition as crude protein, moisture, and ash were performed by standard procedures [18]. Six fish from each tank were sampled for biochemical analysis. Fish were homogenized individually for whole body composition and frozen at-18 °C for proximate chemical analysis at the laboratory of the faculty of Agriculture at Minufiya University. Samples were analyzed as follows: dry matter after desiccation in an oven (105 °C for 24 h), crude protein (micro kjeldahl, N x 6.25), and crude lipid (ether extraction by soxlhet method).

All fish were counted and weighed by the end of the feeding trial to calculate percent weight gain (PWG; [BW – initial BW] × 100/initial BW), feed conversion ratio (FCR; dry feed consumed/WG), feed efficiency ratio (FER; WG/ dry feed consumed), protein efficiency ratio (PER; WG/protein intake), specific growth rate (SGR; [ln final BW – ln initial BW] × 100/days), and survival ([no. of fish at the end of the experiment/no. of fish at the beginning of the experiment] × 100).

Somatic indexes:

HSI = 100 × [liver weight (g)/fish weight (g)]

GSI= 100 × [gonad weight (g)/fish weight (g)]

VSI = 100 × [viscera weight (g)/fish weight (g)].

2.5. Histological analysis

Six fish per treatment were randomly selected and sacrificed. The viscera were dissected and preserved in 10% neutral buffered formalin (Thermo Fisher, Kalamazoo, MI) for 48 h. The following day, the viscera were washed with water several times and preserved in 75% ethyl alcohol for further processing. The liver and intestine were separately dissected and examined. Tissues were routinely processed and stained hematoxylin and eosin (H&E) stain for examination through the light electric microscope [19].

2.6. Statistical analysis
A one way ANOVA test was used to test the differences among dietary treatments. The percentage data of weight gain and specific growth rate were arcsine transformed before the ANOVA analysis. Differences were considered significant at the $P<0.05$. The differences among means were determined using Duncan ´s multiple range test [20].

3. Results
3.1. Growth performance
The growth performance and feed utilization of seaweed incorporated diets on Nile
Tilapia, *Oreochromis niloticus* are presented in Table 2. The final weight, total weight gain (g) and weight gain (%) were significantly ($P<0.05$) increased by increasing the seaweed level in the diets. Fish fed D4 have exhibited the highest value (13.3), and the lowest value (9.6) was obtained when fish fed D2. Also, the highest value (12.2) of total weight gain was observed with fish fed D4 and the lowest value (8.5) was obtained with fish fed D2. The results of weight gain % increased to (1111.3) when fish fed D4 and decreased to (777.6) when fish fed D2. The same trend was observed with specific growth rate (SGR%/fish/day); although the differences were not significant, the highest value (2.94) was observed when fish fed D4 whereas; the lowest value (2.57) was observed when fish fed D2. The lowest feed conversion value (0.93) was observed with fish fed D4 and the highest value (1.4) was observed with fish fed D2. The differences between treatments were not significant ($P>0.05$). The same trend was observed with FER values. Fish fed D4 obtained the highest value (1.1) and the lowest value was observed with fish fed D2 (0.73). The difference between treatments was not significant ($P>0.05$). PER values did not differ significantly ($P>0.05$) among all treatments. The highest value was obtained with fish fed D4 (4.2), followed by D3 (3.01), D2 (2.8) and then the control diet (2.8). Concerning somatic indexes, the differences were significant ($P<0.05$) among all treatments in terms of HSI and VSI. D4 realized the lowest value (1.32) of HSI and the highest value was obtained by using D1 and D2 (2.3). While, the highest value of VSI was obtained by using D3 (4.7) and the lowest value (3.3) was observed with the control diet, D1. However, GSI tended to increase from (1.56) when fish fed D2 to (1.9) when fish fed D4. The results were not significantly differed ($P>0.05$) among all treatments. The survival rate of all treatments was 100% during the experimental period.

Table 2: Growth performance and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (initial wt 1.1±0.04 g) fed seaweed meal as feed additive in the diets for 12 weeks. Values are mean ± SD of triplicate groups.

Values	0 g kg^{-1}	50 g kg^{-1}	100 g kg^{-1}	150 g kg^{-1}
FBW (g fish^{-1})	10.5 ±1.3 ^{ab}	9.6 ±2.3 ^{a}	10.0 ±0.6 ^{ab}	13.3 ±2.0 ^{b}
TWG (g fish^{-1})	9.4 ±1.3 ^{ab}	8.5 ±2.2 ^{a}	8.9 ±0.7 ^{ab}	12.2 ±2.1 ^{b}
WG (%) †	913.6 ±68.6 ^{a}	777.6 ±155.9 ^{a}	838.3 ±163.9 ^{a}	1111.3 ±285 ^{a}
SGR (% day^{-1}) ‡	2.76 ±0.1 ^{a}	2.57 ±0.2 ^{a}	2.65 ±0.2 ^{a}	2.94 ±0.3 ^{a}
FI (g fish^{-1})	12.6 ±0.7 ^{b}	11.5 ±1.1 ^{ab}	11.4 ±0.1 ^{ab}	11.2 ±0.3 ^{a}
FCR§	1.3 ±0.1 ^{b}	1.4 ±0.2 ^{b}	1.3 ±0.1 ^{b}	0.93 ±0.2 ^{a}
FER	0.75 ±0.1 ^{a}	0.73 ±0.1 ^{a}	0.78 ±0.1 ^{a}	1.1 ±0. 2 ^{b}
PI (g)	3.3 ±0.2 ^{b}	3.0 ±0.3 ^{ab}	2.9 ±0.02 ^{ab}	2.9 ±0.1 ^{a}
PER¶	2. 8 ±0.2 ^{a}	2.8 ±0.5 ^{a}	3.01 ±0.2 ^{a}	4.2 ±0.7 ^{b}

Means with different letters are significantly different at ($P<0.05$).
†WG (%) = 100 × (final body weight – initial body weight)/initial body weight
‡SGR (%/day) = 100 × (Ln final weight – Ln initial weight)/Time (days)
§FCR = Total feed consumed (g fish^{-1})/weight gain (g fish^{-1})
¶Protein efficiency ratio (PER) = weight gain (g fish^{-1})/protein intake (g)

3.2. Body composition
The analysis of fish body composition is presented in Table 3. A slight difference between treatments was observed in terms of moisture and protein contents of body composition (%) but non-significant. The highest value (68.2) of moisture was observed with fish fed D4 and the lowest value (65.1) was obtained with fish fed D1. Also, the same trend was observed with the protein content. The highest value (55.6) was observed with fish fed D4 and the lowest value (50.2) was obtained with fish fed D1. There were significant differences among all treatments ($P<0.05$) in lipid content (%), and the lowest value (14.1) was observed with fish fed D1. While, the highest value (19.7) was obtained with fish fed D3. The ash content (%) of fish body differed significantly among all treatments. The highest value (12.3) was observed with fish fed D4; however, the lowest value (9.3) was obtained with fish fed D1.

Table 3: Body composition of Nile tilapia *Oreochromis niloticus* juvenile (initial wt 1.1±0.04 g) fed seaweed supplemented diets for 12 weeks.

Treatments	Moisture	Protein	Lipid	Ash
Initial *	70.8	43.4	14.3	9.1
D1, Control	65.1±0.7^{a}	50.2±1.2 ^{a}	14.1±0.0 ^{a}	9.3±0.2^{a}
D2, 5% diet	66.3±0.4 ^{b}	55.2±0.0^{b}	16.3±0.1 ^{b}	11.2±0.1 ^{b}
D3, 10% diet	67.9±0.4^{b}	54.5±1.4 ^{b}	19.7±0.2 ^{d}	11.3±0.2^{b}
D4, 15% diet	68.2±0.6 ^{b}	55.6±0.0 ^{b}	19.1±0.1 ^{c}	12.3±0.1^{c}

Values are mean ± S D of triplicate groups within a column. Means with different letters are significantly different at ($P<0.05$).

Table 4: Hepatosomatic, visecra and gonado somatic indexes of Nile tilapia *Oreochromis niloticus* juvenile fed seaweed supplemented diets for 12 weeks. Values are mean ± SD of triplicate groups.

Treatments	HSI	VSI	GSI
D1	2.3±0.2^{c}	3.3±0.2^{a}	1.9± 0.5^{a}
D2	2.3±0.1^{c}	4.1±0.5^{c}	1.57±0.6 ^{a}
D3	1.69±0.2^{b}	4.7±0.2^{bc}	1.6± 0.6 ^{a}
D4	1.32±0.1^{a}	3.8±0.4^{ab}	1.9±1.2 ^{a}

Means with different letters are significantly different at ($P<0.05$).

3.3. Histological analysis

No histopathological changes were noted in the liver and/or intestine samples in the present study (Fig. 1 and 2). No diet-related histopathological changes were noted in the intestine samples from any of the dietary group (Fig. 2). In fish fed seaweed supplemented diet, the intestinal mucosa was not affected and displayed typical features of the Control diet group. The simple mucosal folds and the lamina propria of the posterior intestine appeared normal. However, the absorptive vacuolization of cells was increased, and lamina propria was only moderately enlarged (Fig. 2). No basophilic granulocytes were observed in the lamina propria of intestinal mucosa, thus no inflammation occurred. Significant differences were observed in the incidence of lipid droplets in the hepatocytes of the fish liver when fed seaweed supplemented diet (Fig. 1).

Figure captions

Fig.1. Sections of Liver in Nile tilapia fed different diets (A, basal diet) without any supplementation, followed by three diets supplemented with seaweed at 50, 100, 150 g kg^{-1} (B, C, D, respectively). Liver structure of fish fed control or diet supplemented with 50 g kg^{-1} showing normal histological structure (A, and B). In fish from seaweed diets (C and D) group hepatocytes contained larger lipid deposits (white spaces marked by arrows) than those in fish fed the control diet, and the nuclei of the hepatocytes were pushed to the cell wall. Scale bars= 40 µm; H&E staining.

Fig.2. intestine histology of Nile tilapia fed different diets (A, basal diet) without any supplementation, followed by three diets supplemented with seaweed at 5, 10, 15% diet (B, C, D, respectively) showing normal histological structure. (H&E staining); scale bars = 40 µm.

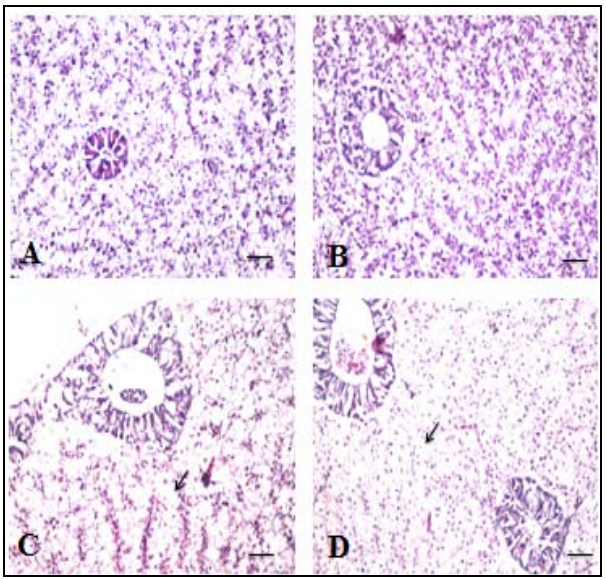

Fig 1: Histopathological changes in liver of Nile tilapia fed different diets (A, Control) without any supplementation, followed by three diets supplemented with seaweed at 50, 100 and 150 g kg^{-1} (B, C, D, respectively). There was no histopathological alteration in the liver structure of fish fed control (A) and fish fed diet supplemented with seaweed at 50 g kg^{-1} (B). Hepatocytes contained larger lipid deposits (white spaces marked by arrows) than those in fish fed the control diet, and the nuclei of the hepatocytes were pushed to the cell wall in liver of fish fed diets with increased level of seaweed 100 and 150 g kg^{-1} (C, D) (H&E; staining) scale bars = 40 µm.

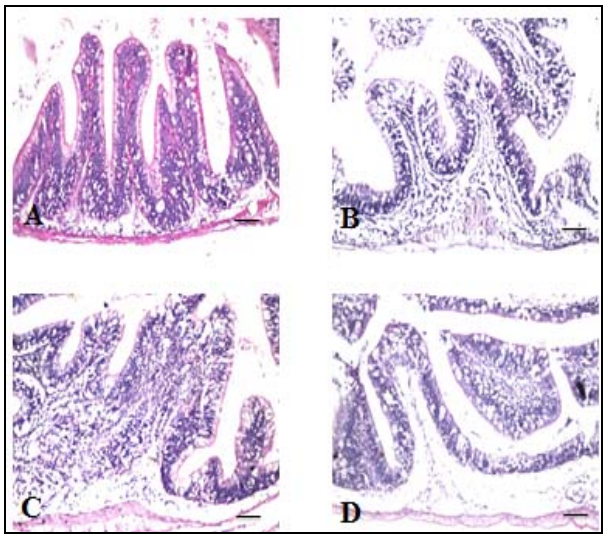

Fig 2: Intestine histology of Nile tilapia fed different diets (A, control) without any supplementation, followed by three diets supplemented with seaweed at 50, 100 and 150 g kg^{-1} diet (B, C, D, respectively) for 12 weeks. (A) The intestine of fish fed control diet showed few inflammatory cells infiltration in the mucosal layer. Diets supplemented with seaweed (B, C, D) at different levels exhibit diffuse goblet cells formation was observed in the lining mucosal epithelium associated with inflammatory cells infiltration in the underlying lamina propria. (H&E staining); scale bars = 40 µm.

4. Discussion

Fish fed seaweed supplemented diets with increasing level showed increase in growth performance of Nile tilapia during the experimental period in terms of the final weight, total weight gain (g) and weight gain (%) were significantly (P<0.05, Table 2). These results differed with [21] who found reduction, in weight gain of Nile tilapia (*O. niloticus*) fingerlings fed brown seaweed (*A. nodosum*) at level of 3%. Also, it has been observed a decrease in juvenile grey mullet (*Chelon labrosus*) fed red seaweed (*Porphyra purpurea*) at 0, 16.5, and 33% [12]. Moreover, [22, 23] found no effects on gilthead seabream (*Sparus aurata*) performance and Senegalese sole (*Solea senegalensis*), respectively when fish were fed diets supplemented with seaweed. On contrast, higher growth performance in Red tilapia (*Oreochromis sp*.) fed diets up to 15% level of seaweed [24]. Also, it has been reported enhanced growth in Rainbow trout (*Oncorhynchus mykiss*) fed diets with up to 10% of seaweed supplementation [25]. Concerning the aquatic macrophytes, [15] recommended lower levels of inclusion (25%) to obtain good growth of Tilapia. The lowest feed conversion value (0.93) was observed with fish fed D4 and the highest value (1.4) was observed with fish fed D2. The differences between treatments were not significant (P>0.05). The decrease of fish growth and feed utilization when fed diets supplemented with seaweed at levels higher than 5% could be explained by the presence of anti-nutritional factors such as saponin, tannins and phytic acid which occurred in several plants [26]. Seaweed supplemented diets did not affect the body composition of Nile tilapia. No significant differences in body composition, in terms of moisture and protein were observed in the present work. The highest values of moisture and protein were obtained with fish fed D4, while the lowest values were observed with fish fed D1 respectively. Moreover, the significant differences were observed in lipid and ash contents of fish body composition, and the highest value of lipid

content was obtained with fish fed D3; whereas the lowest value was observed with fish fed D1. Furthermore, fish fed D4 gave the highest value of ash content and fish fed D1 gave the lowest value. These results are similar to [27]. Our results are in contrast with [28] who resulted that adding seaweed to Nile tilapia diets caused a reduction in body lipid and an improvement in protein of the carcass. The inclusion of Ulva meal in diets improved lipid metabolism, especially lipolysis [29] Dietary algae speed up the absorption of ascorbic acid which motivates lipolysis and depresses lipogenesis [30]. Regarding to somatic indexes, which are used to determine the nutritional status of fish. There were significant differences in somatic indexes such as HSI, and VSI of fish fed seaweed supplemented diets but GSI did not differ significantly (P>0.05). These results are in contrast with [27] who found reduction in HSI with fish fed on Ulva supplemented diets and it was explained by the decreasing of fat deposition in liver, which obviously affected its weight. The same author reported no effect of Ulva supplemented diets on GSI. Our results of GSI are in agreement with the same author. No histopathological changes were noted in the liver, or intestine in the present study (Fig. 1 and 2). The present results are in accordance with [3]. Feeding seaweed supplemented diets did not affect the digestive tract system functions in Nile tilapia.

5. Conclusion

Our findings suggest that seaweed *Taonia atomaria* supplemented diets did not affect the performance of Nile tilapia. It can be supplemented to diets for Nile tilapia up to 5% without any adverse effect. Further studies will be focus on digestibility coefficients of seaweed to evaluate its nutritive value.

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