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Effect of dietary fish meal replacement by red algae, *Gracilaria arcuata*, on growth performance and body composition of Nile tilapia *Oreochromis niloticus*

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ABSTRACT

A 12-week long feeding experiment was initiated to evaluate the effect of dietary supplementation of red algae, *Gracilaria arcuata*, on the growth performance, feed utilization and body composition of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). The fish were fed with an algae-free control diet (C) and three experimental diets which replaced conventional fish meal with varying levels of dried *G. arcuata* (20%, 40% and 60%, represented as G20, G40 and G60, respectively). The growth parameters of final weight (FW), weight gain (WG), percentage of weight gain (WG%), daily growth rate (DGR) and specific growth rate (SGR) were significantly reduced ($P < 0.05$) at all levels of algae incorporation compared to the control diet. Moreover, the negative impact of *Gracilaria* meal on the growth performance of Nile tilapia increased as the proportion of algae in the diet increased, with fish on diet G20 exhibiting a significantly higher growth performance than the fish on either of the G40 and G60 diets. On the other hand, the feed utilization parameters feed conversion ratio (FCR) and protein efficiency ratio (PER) did not show significant differences between the fish in the control group and those on diet G20, although poorer FCR and PER outcomes were achieved in the case of fish on diet G60. The content of moisture, protein and ash in muscle and carcass increased as the proportion of *Gracilaria* meal in the diets increased, but the reverse was true for lipid level. These results indicate that incorporation of less than 20% red algae, *Gracilaria arcuata*, could be feasible in the diet of Nile tilapia and further studies are recommended to optimize the level of algae to improve growth performance.

1. Introduction

The aquaculture sector has expanded rapidly in recent year to maintain fish supply, especially given the decline in the capture fish industry due to unsustainable practices (FAO, 2006). Under intensive and semi-intensive aquaculture regimens, feed constitutes the largest proportion of the overall costs, often ranging from 30% to 60% of the total variable expenses, depending on the intensity of the culture operation (Azaza et al., 2009). Protein is the most important component in aquaculture feed; therefore the formulation of fish feed attempts to provide a certain level of protein that will supply essential amino acids for the growth and good health of the farmed fish (Khan et al., 2012; Naz and Javed, 2013). Fish meal is the primary protein source for aquaculture diets because of its high protein content, well balanced amino acid and fatty acid composition, high digestibility and palatability (Yildirim et al., 2014). Fish meal is the most expensive ingredient, however, and is highly desired by other livestock industries (Kristofersson and Anderson, 2006). With the predicted continuous growth of the aquaculture sector, the demand for fish meal will continue to increase, with a consequent negative impact on pelagic fishes since they are the major source of fish meal (Tacon and Metian, 2008; Luo et al., 2012). The use of less-expensive protein sources as partial or total replacements for fish meal is an international research priority, therefore.

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One approach to reduce the use of fish meal is to replace it with alternative, less expensive animal- or plant-protein ingredients. An alternative less-expensive protein source should be easy to obtain in sufficient quantities to meet demand. Additionally, it should meet the basic amino acid requirements of fish and should be sufficiently palatable to avoid rejection by fish. In this context, macro and micro algae have been used as dietary supplements to enhance the health and nutritional performance of a range of farmed fish species (Güroy et al., 2011). Many previous studies have evaluated the inclusion of various algae species in aquafeeds: *Ulva* (Wassef et al., 2001), *Padina arborescens*, *Sargassum siliquastrum* (Ma et al., 2005), *Sargassum* spp. (Casas-Valdez et al., 2006), *Gracilaria bursa-pastoris*, *Gracilaria cornea* and *Ulva rigida* (Valente et al., 2006), *Hizikia fusiformis* (Pham et al., 2006) and *Porphyra* (Kalla et al., 2008; Khan et al., 2008; Soler-Vila et al., 2009; Tsuyoshi et al., 2010). The results of the majority of these studies encourage the use of algae as a partial substitution of fishmeal. Among the different species of macroalgae, the red algae seems to be the most suitable source for animal nutrition because of their relatively high protein content and structurally diverse bioactive compounds with great pharmaceutical and biomedical potential (Fleurence, 2004).

Nile tilapia, *Oreochromis niloticus*, has been termed the 'aquatic chicken due to its extraordinary production capabilities (Huecht, 2000). Specifically, its fast growth, high fecundity, resistance to disease, good-quality flesh and consumer demand, mean that Nile tilapia is the most widely used farmed fish species globally, with annual aquaculture production reaching 3.7 million metric tons (MT) in 2010 (FAO, 2010).

In this experiment, we partly replaced fish meal with dry red algae, *Gracilaria arcuata* to evaluate this material as a fish meal substitute and to assess its effect on the growth performance and body composition of Nile tilapia *Oreochromis niloticus* under laboratory feeding conditions.

2. Materials and methods

2.1. Diets formulation

Red alga, *Gracilaria arcuata*, was freshly collected from the near-shore waters of the Red Sea coast at Jeddah city, Saudi Arabia. Algae samples were thoroughly washed with freshwater and with distilled water and then sun dried for 48 h, before being fine-milled with a laboratory blender to produce raw *Gracilaria* meal in a dried powder form. Proximate chemical composition of the powdered algae and the major dietary ingredients were performed according to AOAC (1995), prior to the formulation of experimental diets (Table 1). An algae-free control diet (C) and three experimental diets, including varying levels of *Gracilaria* meal (G20 = 20%); (G40 = 40%) and (G60 = 60%) to make up a constant level of approximately 32% protein were formulated (Table 2). The pre-weighed dry ingredients were carefully mixed using a laboratory food mixer (Hobart Mixer, HL120) with the separate addition of the oil and vitamin/mineral premix. The mixtures were primed with hot water (at around 50 °C) to yield a suitable mash for extrusion. The diets were extruded through a series of 2-mm diet holes and then dried at 105 °C in a natural convection oven (LabeTech, LDO-250N).

2.2. Experimental fish

Nile tilapia, *O. niloticus* were collected from the fish seed hatchery at King Abdulaziz City for Science and Technology, Mozahmiya, Riyadh, Saudi Arabia, and acclimatised to laboratory conditions for two weeks while fed a commercial diet (32% crude protein).

2.3. Experimental design

Three replicate groups of 16 pre-weighed fish for each diet (C; G20; G40 and G60) were randomly stocked in 80-L glass aquariums (100 × 40 × 40) at a stocking density of 100 fish/m^3. Each aquarium was equipped with a biological filter containing a high porosity filter sponge which was washed thoroughly every three days. Dissolved oxygen was added with diffused air at the top of the filter. The water temperature was maintained at 28 °C ± 1 by means of thermostatically controlled aquatic heaters. The other water quality parameters that were monitored were pH 6.8–8.1, ammonia (NH_3) 0.09–0.20 mg/L, nitrite (NO_2) 0.19–0.33 mg/L, nitrate (NO_3) 4.36–5.69 mg/L, and dissolved oxygen 6.2–7.9 mg/L. The fish were fed twice daily with a ratio of 3% of fish weight for six days per week. The fish of each aquarium were weighed every two weeks at which point the water in the aquariums was exchanged. The experiment lasted for 12 weeks and at the end of the experimental period all fish were killed after their body weight and length had been recorded. The fish from each group were divided into two sub-groups, one for the proximate chemical composition of fish muscles and the second for whole body chemical composition according to AOAC (1995). Also, a sample of 24 fish was chosen randomly for proximate muscle and carcass analysis prior to the start of the experiment.

2.4. Growth parameters

Fish growth performance and feed conversion were determined in terms of weight gain (WG), percentage of weight gain (WG%), daily growth rate (DGR), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival (SR).

2.5. Statistical analysis

The collected data was subjected to statistical analysis using one-way Analysis of Variance (ANOVA). The average values (means ± standard deviation) were compared using Fisher's Least Significant Differences test (LSD-test) as described by Snedecor and Cochran (1989).

Table 1
Chemical composition of the dried dietary ingredients.

Proximate composition (%)	Ingredients					
	Gracilaria arcuata	Fish meal	Soybean meal	Wheat meal	Wheat bran	Yellow corn
Protein	13.00	61.41	44.24	14.36	15.03	9.45
Lipid	6.97	12.33	2.91	1.40	5.25	3.10
Ash	34.24	18.64	6.50	1.80	4.79	3.40
NFE[a]	45.79	7.62	46.35	82.44	74.93	84.05

[a] NFE (Nitrogen-free extracts) = dry matter (100) – (crude protein + crude lipid + ash).

Table 2
Formulation and chemical composition (on dry weight basis) of the experimental diets.

Ingredients	Control (C)	20% *G. arcuata* (G20)	40% *G. arcuata* (G40)	60% *G. arcuata* (G60)
Experimental diets (g/kg)				
Fish meal	350	280	210	140
G. arcuata	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 (%)				
Protein	32.74	32.35	32.64	32.23
Lipid	5.44	5.08	4.83	4.58
Ash	10.33	12.06	13.79	15.52
NFE[a]	51.49	50.51	48.74	47.67

[a] NFE (Nitrogen-free extracts) = dry matter (100) – (crude protein + crude lipid + ash).

3. Results

3.1. Growth performance

The growth parameters (FW, WG, WG%, DGR, SGR, and K) are shown in Table 3. A significant difference ($P < 0.05$) was noted between the fish on diet C compared to all other treatments, with the best growth performance being recorded for the fish of diet C where the FW was (42.30 ± 2.154), WG (29.31 ± 1.118), WG% (225.6 ± 0.932), DGR (0.349 ± 0.061) and SGR (1.405 ± 0.156). This was followed by the growth performance for the fish of diet G20 where the FW was (36.13 ± 3.207), WG (23.12 ± 0.681), WG% (177.7 ± 0.893), DGR (0.275 ± 0.038) and SGR (1.216 ± 0.095). In contrast, the G60 diet produced the poorest growth parameters: FW (29.78 ± 1.529), WG (16.61 ± 0.942), WG% (126.1 ± 0.917), DGR (0.198 ± 0.018), SGR (0.971 ± 0.061) with no significant difference ($P > 0.05$) compared to the growth performance for the fish of diet G40, whereas the fish growth indicators for the two diets were significantly decreased in comparison with those for diet C and G20. The condition factor (K) was not significantly different for any of the treatments ($P > 0.05$). Following the 12-week growth experiment, no mortality was recorded in any of the experimental diets.

3.2. Feed utilization

The results of feed utilization in the form of the feed conversion ratio (FCR) and the protein efficiency ratio (PER) are also represented in Table 3. The best value for the FCR (1.779 ± 0.248) and the highest PER (1.933 ± 0.261) were recorded for diet C with no significant difference ($P > 0.05$) compared to the results for diet G20. Meanwhile, the FCR and PER for both diet G40 and G60 were significantly reduced ($P < 0.05$) in comparison to those of both diet C and G20. The results for diet G60 showed the poorest FCR (3.493 ± 0.639) and the lowest PER (1.169 ± 0.337).

3.3. Body composition

The body composition (as a percentage based on the fresh weight) is shown in Table 4. The moisture content of fish muscle showed significant difference ($P < 0.05$) between the fish taking any of the *Gracilaria* meal diets compared to the fish from the con-

Table 3
Growth performance and feed utilization parameters of *O. niloticus* fed the experimental diets.

Parameters	Diets			
	C	G20	G40	G60
IW (g)	12.99 ± 0.153	13.01 ± 0.273	13.06 ± 0.373	13.17 ± 0.420
FW (g)	42.30 ± 2.154^{a}	36.13 ± 3.207^{b}	30.76 ± 1.696^{c}	29.78 ± 1.529^{c}
WG (g)	29.31 ± 1.118^{a}	23.12 ± 0.681^{b}	17.70 ± 0.908^{c}	16.61 ± 0.942^{c}
WG%	225.6 ± 0.932^{a}	177.7 ± 0.893^{b}	135.5 ± 0.968^{c}	126.1 ± 0.917^{c}
DGR (g/day)	0.349 ± 0.061^{a}	0.275 ± 0.038^{b}	0.211 ± 0.020^{c}	0.198 ± 0.018^{c}
SGR (%/day)	1.405 ± 0.156^{a}	1.216 ± 0.095^{b}	1.020 ± 0.068^{c}	0.971 ± 0.061^{c}
K	1.676 ± 0.066^{a}	1.668 ± 0.032^{a}	1.665 ± 0.036^{a}	1.608 ± 0.014^{a}
FCR	1.779 ± 0.248^{b}	2.276 ± 0.292^{b}	3.092 ± 0.588^{a}	3.493 ± 0.639^{a}
PER	1.933 ± 0.261^{a}	1.487 ± 0.180^{ab}	1.295 ± 0.316^{b}	1.169 ± 0.337^{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 are not significantly different from each other ($P > 0.05$).
IW: Initial weight.
FW: Final weight.
WG = (final weight (g) – initial weight (g).
WG% = [(final weight (g) – initial weight (g))/initial weight (g)] × 100.
DGR = weight gain (g)/experimental period (days).
SGR = [Ln final weight (g) – Ln initial weight (g)]/experimental period (days) × 100.
k = body weight (g)/body length (cm^3) × 100.
FCR = feed intake (g)/body weight gain (g).
PER = weight gain (g)/protein intake (g).
SR = 100 × final fish number/initial fish number.

Table 4
Body composition of *O. niloticus* fed the experimental diets.

Proximate composition	Initial	C	G20	G40	G60
Muscle composition (% of wet weight)					
Moisture	81.03	79.817 ± 0.120[b]	80.350 ± 0.176[a]	80.430 ± 0.081[a]	80.580 ± 0.106[a]
Protein	15.43	17.325 ± 0.213[a]	17.367 ± 0.355[a]	17.523 ± 0.080[a]	17.631 ± 0.010[a]
Lipid	0.96	0.765 ± 0.079[a]	0.461 ± 0.028[b]	0.458 ± 0.052[b]	0.401 ± 0.002[b]
Ash	2.04	1.086 ± 0.123[a]	1.160 ± 0.085[a]	1.234 ± 0.075[a]	1.389 ± 0.080[a]
Carcass composition (% of wet weight)					
Moisture	79.95	78.273 ± 0.099[b]	79.893 ± 0.312[a]	80.353 ± 0.334[a]	80.487 ± 0.417[a]
Protein	12.27	12.614 ± 0.023[b]	13.150 ± 0.072[a]	13.513 ± 0.053[a]	13.519 ± 0.267[a]
Lipid	2.43	3.182 ± 0.254[a]	1.936 ± 0.111[b]	1.520 ± 0.128[c]	1.433 ± 0.196[c]
Ash	3.36	4.036 ± 0.054[b]	4.274 ± 0.041[bc]	4.610 ± 0.087[ac]	4.893 ± 0.101[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).

trol group, which recorded the lowest muscle moisture content (79.817 ± 0.120), while the highest moisture content was observed for the fish on diet G60 (80.580 ± 0.160), although this was not significantly different (P > 0.05) from that for fish on diets G20 and G40. The muscle protein for fish on any of the *Gracilaria* meal diets was higher than the content for fish on diet C, although the differences between these treatments was not significant (P > 0.05). The highest muscle protein levels were measured in the fish on diet G60 (17.731 ± 0.010), while the lowest values were recorded for the fish on diet C (17.325 ± 0.213). The highest muscle lipid level was observed in the fish on the control diet (0.765 ± 0.079) which was significantly more (P < 0.05) than the lipid content of the muscles of fish on any of the *Gracilaria* meal diets. In contrast, the fish on diet G60 showed the lowest level of lipid content G60 (0.401 ± 0.002). Although the muscle content of ash varied with the different experimental diets, no significant difference (P > 0.05) was noticed between the treatments. The ash content for fish on diet G60 was the highest (1.389 ± 0.080) while the fish on diet C recorded the lowest level of muscle ash (1.086 ± 0.123).

The carcass (whole body) composition in general followed a similar pattern as for the muscle composition, with the carcass moisture at its lowest level (78.273 ± 0.099) for the fish on diet C, with a significant difference (P < 0.05) compared to all the *Gracilaria* meal diets. The carcass moisture for the fish on diet G60 showed the highest level (80.487 ± 0.417) but was not significantly different (P > 0.05) from the findings for the fish on either diet G20 or G40. The results of the carcass protein content exhibited the poorest value (12.614 ± 0.023) for the fish on diet C with a significant difference (P < 0.05) compared to all the *Gracilaria* meal diets. The highest carcass protein was noted for the fish on diet G60 (13.519 ± 0.267) but was not significantly different (P > 0.05) to the carcass protein content for the fish on diets G20 and G40. A significant difference (P < 0.05) was observed, however, in the carcass lipid content results, with the highest content being achieved for the fish on diet C (3.182 ± 0.254) and the lowest for the fish on diet G60 (13.519 ± 0.267). Here, the fish on diet G20 showed a significantly higher amount of carcass lipid (1.936 ± 0.111) than those on diet G40 and G60. The carcass ash also content showed variations between the treatments, with the fish on diet G60 being recorded to have the highest ash content (4.893 ± 0.101), with this decreasing at levels close to statistical significance in the other treatments. The fish on diet C showed the lowest ash content (4.036 ± 0.054).

4. Discussion

The results of this study showed that *Gracilaria* meal had a significant effect on the growth performance of Nile tilapia, with FW, WG, WG%, DGR and SGR parameters all decreasing significantly (P < 0.05) at all incorporation levels (20%, 40% and 60%) of *Gracilaria* meal in the diet compared to the *Gracilaria*-free diet. Moreover, the negative impact of *Gracilaria* meal on the growth indicators of Nile tilapia increased as the proportion of algae in the diet increased, with the fish on diet G20 exhibiting a significantly higher growth performance than the fish on either diet G40 or G60. On the other hand, the feed utilization parameters (FCR and PER) did not show a significant difference between the fish in the control group and those in the G20 group, whereas the poorest FCR and PER were achieved in case of fish on diet G60 which also were not significantly different (P > 0.05) to fish on diet G40.

These results showed that *Gracilaria* meal could be incorporated into tilapia diets instead of fish meal by less than 20%. Similar results were reported by Xuan et al. (2013), who concluded that the growth performance in terms of weight gain and feed efficiency ratio of juvenile black sea bream, *Acanthopagrus schlegelii*, and receiving *Gracilaria lemaneiformis* based diets did not decrease even at the inclusion level of 15%. When the *Gracilaria* meal inclusion level reached 20%, significantly poor growth performance was observed in the fish. Also, the growth performance and feed utilization in this experiment were generally in accordance with the findings of Valente et al. (2006) who concluded that inclusion of *Gracilaria bursa-pastoris* at up to 10% of the meal, can be considered as very promising ingredient in the diet of sea bass juveniles, since no negative consequences were observed in respect to growth performance, nutrient utilization or body composition.

A possible reason for the reduced growth seen in this study with the inclusion of *Gracilaria* meal in the diets of Nile tilapia could be due to the high Nitrogen-free extracts content (45.79% of dry matter), compared with (7.62%) in fishmeal and its possible effects on the digestibility of protein and dry matter; moreover the protein content of fish meal was about five times that of *Gracilaria* meal (61.41%: 13% of dry matter) respectively. This explanation follows that of Xu et al. (2011) who reported that dried *Gracilaria lemaneiformis* contained a high level of Nitrogen-free extracts content (61.3% of dry matter), compared with the very low level in fishmeal (3.7%), while the protein content of *G. lemaneiformis* meal was 19.2% compared to 70.1% for the fish meal, in addition to lower levels of some essential amino acids such as methionine or tyrosine in *Gracilaria* meal. This may be illustrated in the poor growth of the rabbit fish *Siganus canaliculatus* fed a diet with 33% *G. lemaneiformis* meal.

The growth performance and feed utilization in the present study were also in agreement with other previous studies that have used algal species as diet ingredients in fish feed. Davies et al. (1997) concluded that the use of the red algae, *Porphyra purpurea*, as a diet ingredient for mullet (*Chelon labrosus*) at high inclusion levels (16% and 33%), inhibited growth performance and feed utilization efficiency. Also, Yousif et al. (2004) used *Enteromorpha* sp. as a dietary ingredient for *Siganus canaliculatus* with inclusion levels of 10%, 20%, 30% and noticed a decrease in the growth performance and feed utilization efficiency as the algae inclusion level increased. Our results, and the results of the previous studies, indicated that the maximum suitable algae inclusion levels in fish diets may depend on the feeding habits of the fish, and the species of algae, since the results of Stadtlander et al. (2013) showed improvements in the growth and feed utilization of Nile tilapia when 15% of fish meal was replaced with red algae Nori, *Porphyra yezoensis*, while Soler-Vila et al. (2009) mentioned that feeding rainbow trout, *Oncorhynchus mykiss*, with different levels of the red algae *Porphyra dioica* resulted in slightly inferior performance of the fish compared to control fed fish but that this algae could be effectively included in diets at up to 10%, without significant negative effects on weight gain and growth performance.

Proximate body composition means the determination of the water, protein, fat and ash content of the fish (Love, 1980) and this is considered as a good indicator of its physiological condition and health (Saliu et al., 2007), moreover it is essential in order to maximize their utilization (Silva and Chamul, 2000). The present study has shown changes in the chemical composition of Nile tilapia which appear to be related to the variation of the diets. Altogether, the carcass composition closely followed the results of the muscle composition, with the muscle and carcass content of moisture, protein and ash all showing an increase as the level of inclusion of *Gracilaria* meal in the diets increased, with the reverse being true for the lipid level. The results of moisture and protein contents in this study were generally in agreement with Davies et al. (1997) who reported a significant increase in the moisture content and a slightly increase of crude protein in the carcass of thick-lipped grey mullet, *Chelon labrosus*, with the addition of *Porphyra purpurea* algae in the diet at levels of 16% and 33%. Also, red alga, *P. dioica*, at levels of 5%, 10% and 15% resulted in an increase in carcass protein content in rainbow trout, *O. mykiss*, for all three experimental diets, and was significantly higher for the diet with 10% alga inclusion (Soler-Vila et al., 2009).

FAO (1999) stated that the moisture and lipid content of fish fillets are inversely related and this was clearly evidenced in the current study. The results of fish lipid content reported in this study were generally in accordance with the findings of Valente et al. (2006) who concluded that the lipid content of European sea bass, *Dicentrarchus labrax*, juveniles fed on a *Gracilaria cornea* based diet decreased with 10% *Gracilaria* inclusion compared to both control and 5% *Gracilaria* diets. The lipid content in the body and liver of black sea bream, *A. schlegelii*, has meanwhile been shown to be greatly suppressed when the fish received a *G. lemaneiformis* based diet at a level of 20% (Xuan et al., 2013) and this agreed with the results of the current study.

In conclusion, it has been shown that the red algae, *Gracilaria arcuata*, could be incorporated into Nile tilapia diets instead of fish meal by less than 20%. Future studies are needed, however, to optimize the level of *Gracilaria* meal in diets of Nile tilapia to improve growth performance. It would also be interesting to assess the cost per kg of the algae, in comparison to fish meal, to assess whether low inclusion levels could be cost effective at a commercial scale.

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