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Evaluation of red seaweed *Gracilaria arcuata* as dietary ingredient in African catfish, *Clarias gariepinus*



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KEYWORDS

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Abstract The aim of this study was to evaluate the use of dried marine seaweed, *Gracilaria arcuata* for the first time as dietary ingredient in partial substitution of fishmeal on the growth performance, feed utilization and body composition of African catfish, *Clarias gariepinus*. Four experimental diets were formulated: D1 as a control group; D2; D3 and D4 which included 10%, 20% and 30% *G. arcuata* meal respectively. One hundred and eighty African catfish weighing 9.62 ± 0.42 g, (mean \pm SE) was divided into four groups corresponding to the different feeding regimes. The final body weight of the fishes showed significant differences ($P < 0.05$) between the control (D1); D2 and other treated groups D3 and D4, with weights of 66.98, 59.60, 47.34 and 30.73 g recorded for D1, D2, D3 and D4, respectively. Significant differences ($P < 0.05$) were also evident in weight gain, specific growth rate, and feed utilization between treatment and control groups. However, no significant differences ($P > 0.05$) were observed between the control group and fishes fed D2 for all previous parameters. Protein productive value, protein efficiency ratio, daily dry feed intake and total feed intake were also significantly lower in fish fed with a diet containing *G. arcuata* than in the control group and D2 which contains 10% of *G. arcuata*. Overall, the results of the experiment revealed that African catfish fed a diet with *G. arcuata* included in 20% and 30% levels showed poorer growth and feed utilization than the control group and D2. However, the study recommended that *C. gariepinus* can accept this ingredient up to 10% in their diets. More defined experiments therefore seem to be necessary in order to determine the maximum level of this marine seaweed in diets with amino acid supplementation for African catfish.

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1. Introduction

Fish feeding represents over 50% of operating costs in intensive aquaculture, with protein which is a highly expensive dietary source (Lovell, 2002). Therefore, uninterrupted efforts have been made over the past decades to find alternative protein

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sources to aquaculture nutrients, with special emphasis on terrestrial plants such as legumes and oilseeds (Glencross et al., 2007; Borquez et al., 2010). Many of these plants play an important role as a protein source for fish: soybean, peas, canola and lupin. Deficiency in some essential amino acids have been attributed to using a certain amount of plant protein sources which might contain anti-nutritional factors and result in palatability problems (Hardy, 1996; Drew et al., 2007).

Even though algae are the natural plant source for fish diets, the main plant source tested with success in fish feeds is still soybean. However, nowadays soybean has become an important source of biodiesel, thus, raising the world's requirements for this crop and consequently its price. This results in the presence of anti-nutritional factors and nutritional profiles that do not fully match the fish requirements, especially with respect to amino-acids and fatty acids (Francis et al., 2001; Geurden et al., 2005).

However, genus *Gracilaria* (Rhodophyta) has also been shown to be a good replacement applicant for intensive culture because of its ability to reach higher yields and economically valuable products (Buschmann et al., 1996). Al-Hafedh et al. (2012) have been improving ecologically integrated aquaculture technology, specifically a tank-based integrated technique for bio-remediation of effluents using the red alga, *Gracilaria arcuata*, and the green alga, *Ulva lactuca*, both of which are available in the Red Sea off the Jeddah coast of Saudi Arabia. Aquaculture entrepreneurs in Saudi Arabia may consider a possible reduction of feed concentrations in seawater effluent and a chance to diversify the materials of production in changing market status as offering the possibility for additional sources of income. This work is highly relevant to a developing aquaculture industry in Saudi Arabia (FAO, 2010) and to reducing the environmental dangers to an oligotrophic sea that has a high level of biodiversity (Khalil and Abdel-Rahman, 1997; Baars et al., 1998).

Mustafa and Nakagawa (1995) investigated the importance of algae as ingredients in fish feed. For aquafeeds, numerous studies determined the inclusion level of various seaweed species: *Ascophyllum nodosum* (Nakagawa et al., 1997), *Porphyra* (Davies et al., 1997; Kalla et al., 2008; Khan et al., 2008 and Soler-Vila et al., 2009), *Ulva* (Wassef et al., 2001), *Sargassum* spp. (Casas-Valdez et al., 2006), *Hizikia fusiformis* (Pham et al., 2006), *Gracilaria bursa-pastoris*, *Gracilaria cornea* and *Ulva rigida* (Valente et al., 2006), and *Padina arborescens*, *Sargassum siliquastrum* (Ma et al., 2005). Most of these investigations summarized encouraging results for the use of seaweed as a partial substitution of fishmeal in aquafeeds. It is also obvious that the effect of seaweed inclusion in aquafeeds appears to depend on species of seaweed, its incorporation level and also on the fish species where the seaweed is treated, as illustrated by Khan et al. (2008) and Kalla et al. (2008) using *Porphyra* in black sea bream and red sea bream.

Supplementations of macroalgae meals in diets enhance the growth, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality of many fishes (Ergun et al., 2009; Güroy et al., 2011, 2013). The annual global aquaculture production of marine algae was 14.5×10^6 tonnes (including brown, green and red seaweeds and miscellaneous aquatic plants) in 2007, (FAO, 2009). Even though, *G. arcuata* has valuable effects, according to the authors' knowledge there is no literature to date concerning the use of *G. arcuata* meal in diets. Also, there is no information about the impact of this

macroalgae on the growth performance and feed utilization of African catfish, *Clarias gariepinus*.

The aim of the present study was to evaluate the use of dried marine seaweed, *G. arcuata* for the first time as a dietary ingredient in partial substitution of fishmeal on the growth performance, feed utilization and body composition of African catfish.

2. Materials and methods

2.1. Diet formulations

Red marine seaweed, *G. arcuata*, was freshly collected from the near-shore waters of the Red Sea coast at Jeddah, Saudi Arabia. Algal samples were thoroughly washed with sea water, tap water and distilled water, sun dried for 48 h and fine-milled with a laboratory blender. The *Gracilaria* meal was then passed through a mesh sieve to produce a raw *Gracilaria* meal for proximate analysis (Table 1). Other dietary ingredients were purchased from a local feed company (Maram Feed Company, Riyadh, KSA). Proximate analysis of major dietary ingredients was performed prior to the formulation of experimental diets. All diets contained approximately 35% protein (Table 2). The diet without *G. arcuata* served as a control diet (D1), while the three other diets were formulated such that *G. arcuata* replaced a proportion of standard fish meal, respectively 10% for D2, 20% (D3) and 30% (D4). Table 2 represents the formulation of the diets and their proximate chemical composition.

Dietary ingredients were mixed in a food mixer (Legacy, USA) with water (at around 50 °C) to produce a 2 mm pellet. The moist pellets were then oven dried at 105 °C and stored frozen at -20 °C until use.

2.2. Experimental fish

African catfish *C. gariepinus* were collected from the fish seed hatchery at King Abdulaziz City for Science and Technology Mozahmiya, Riyadh, Saudi Arabia. Fish were acclimatized to laboratory conditions for two weeks prior to experiments.

2.3. Experimental design

One hundred and eighty acclimatized fishes, weighing 9.62 ± 0.42 g (mean \pm SE) with an average length of 11.84 ± 0.18 cm were divided into four groups. Each of these groups was then divided into triplicate glass aquaria, each with a capacity of 80 L (100 \times 50 \times 40 cm), with 15 fishes in each tank. The first group served as the control (D1), while each of the other three groups was fed on the experimental diets (D2, D3 and D4) containing about 10%, 20% and 30% of *G. arcuata* meal, respectively. Fish were fed twice daily with a 35% crude protein diet at a rate of 3% of body weight, five days a week for 10 weeks. Proximate content of the moisture, protein, lipid and ash in the body carcass and muscles of fish from each group were determined according to AOAC (1995). The water temperature was maintained at 28 ± 1 °C by thermostatically controlled immersion heaters. The other parameters of water quality were: pH 6.9–8.0, ammonia (NH₃) 0.08–0.21 mg/L, nitrite (NO₂) 0.17–0.36 mg/L, nitrate (NO₃) 4.28–5.71 mg/L, and dissolved oxygen 5.9–7.4 mg/L.

Table 1 Chemical composition of dried ingredients used in diets formulation fed to African catfish *Clarias gariepinus*.

Ingredients Parameters (%)	<i>Gracilaria arcuata</i>	Fish meal	Soybean meal	Wheat meal	Wheat bran	Yellow corn
Moisture	3.98	8.89	6.84	11.20	7.00	10.6
Protein	13.50	61.41	44.24	14.36	15.03	9.45
Lipid	6.97	12.33	2.91	1.40		3.10
Ash	31.90	18.64	6.50	1.80	4.79	3.40

Table 2 Formulation and proximate composition for experimental diets (g/100 g dry weight) containing *Gracilaria arcuata* fed to African catfish *Clarias gariepinus*.

Diets	D1	D2	D3	D4
Ingredients	(control)	(10% Ulva)	(20% Ulva)	(30% Ulva)
Fish meal	45.00	40.50	36.00	31.50
Gracilaria meal	00	4.50	9.00	13.50
Soybean meal	5.00	13.00	20.00	28.00
Wheat meal	18.00	15.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	8.00
Yellow corn	17.00	12.00	10.00	4.00
Corn oil	3.00	3.00	3.00	3.00
Vitamin mix	1.00	1.00	1.00	1.00
Mineral mix	1.00	1.00	1.00	1.00
Total	100	100	100	100
<i>Proximate composition (% dry matter)</i>				
Moisture	6.24	5.84	6.01	5.59
Protein	35.14	35.06	35.07	35.44
Lipid	10.82	10.20	9.96	8.70
Ash	13.28	12.19	16.85	18.25

2.4. Statistical analysis

The statistical analysis of the data was done using the one-way analysis of variance (ANOVA) technique. The means were separated by Fisher's LSD test and compared using Duncan's Multiple Range Test (DMRT) as described by [Snedecor and Cochran \(1989\)](#). Significant differences were defined at $P < 0.05$.

3. Results

3.1. Growth performance and feed utilization

Averages of initial weights (IW); final weights (FW); weight gain (WG), specific growth rate (SGR) and survival rate of African catfish as affected with *G. arcuata* level are presented in [Table 3](#). Initial weights ranged between 8.85 g and 10.42 and final weights were found to be 66.98 ± 1.83 ; 59.60 ± 2.22 ; 47.34 ± 3.17 and 30.73 ± 2.46 g (mean \pm SE) for the control (D1); D2; D3 and D4, respectively with nonsignificant differences ($P > 0.05$) between control group and fishes fed diets containing 10% replacing fish meal by *G. arcuata*, whereas, there is a significant difference ($P < 0.05$) between D1 and D2 in comparison with D3 and D4. Results revealed that feed intake decreased significantly ($P < 0.05$) with increase of the *G. arcuata* level

supplemented compared to the control group and D2. Averages of total weight gain were 56.56 ± 1.14 ; 50.67 ± 1.11 ; 37.07 ± 1.15 and 21.88 ± 1.16 g, for diets D1, D2, D3 and D4, respectively. The statistical analysis of WG showed that the total weight gain of African catfish decreased significantly ($P < 0.05$) with higher levels of *G. arcuata*, D3 and D4 compared to D1 and D2. As presented in [Table 3](#), averages of SGR during the whole experimental period for the D1; D2; D3 and D4 groups were found to be 2.66 ± 0.08 ; 2.71 ± 0.05 ; 2.18 ± 0.09 and $1.78 \pm 0.08\%$, respectively. As presented in the same Table, survival rate was found to be 100% for all experimental groups.

Results of feed utilization including feed conversion ratio (FCR); protein efficiency ratio (PER); protein productive value (PPV) and hepatosomatic index (HSI) were illustrated in [Table 3](#). Averages of FCR for the control; D2; D3 and D4 were found to be 0.74 ± 0.07 ; 0.78 ± 0.06 ; 1.02 ± 0.07 and 1.30 ± 0.07 , respectively. Statistically, results indicated that FCR of African catfish increased significantly ($P < 0.05$) with an increase of *G. arcuata* supplementation level. The same trend was observed for PER which decreased significantly ($P < 0.05$) with an increase of *G. arcuata* level compared to the control group and D2. On the other hand, PPV values of control and D2 showed a significant decrease ($P < 0.05$) compared to the D3 and D4. HSI was significantly low for the fish fed on D4 compared with other treatments ([Table 3](#)).

Averages of moisture, crude protein, lipid and ash calculated as percentage of wet weight for carcass and muscles were presented in [Table 4](#). Results of carcass moisture contents ranged between (72.97% for D3 and 75.55% for D4). Results of carcass protein content recorded the highest values (17.53%; 16.81%) for D3 and D4, respectively with significant differences compared to D1 and D2. On the contrary, the highest carcass lipid was observed for D1 (5.51%) then, D2 (4.67%) with significant differences in comparison with D3 and D4. In contrast, ash content in carcass increased at the higher inclusion levels of *G. arcuata* in the diets. Proximate chemical composition of the muscles showed that the highest protein (17.86%) and lipid (2.76%) were recorded for fish of D2 whereas, the highest muscle ash (1.22%) was observed for fish of D4 with significant difference compared to the other treatments.

4. Discussion

The potential use of macroalgae in fish feeds depends on the costs involved in their production, harvesting and processing prior to their inclusion in fish diets. The current study observed that replacement of fish meal with *G. arcuata* up to 10% in the diet of African catfish did not influence growth performance or feed efficiency. These were in agreement with data obtained by

Table 3 Weight gain, feed consumption and nutritive utilization of African catfish *Clarias gariepinus* fed experimental diets containing *Gracilaria arcuata*.

	D1	D2	D3	D4
Initial weight (g)	10.42 ± 0.654	8.92 ± 0.77	10.27 ± 0.72	8.85 ± 0.54
Initial length (cm)	12.20 ± 0.251	11.65 ± 0.33	12.07 ± 0.26	11.45 ± 0.24
Final weight (g)	66.98 ± 1.83 ^a	59.60 ± 2.22 ^a	47.34 ± 3.17 ^b	30.73 ± 2.46 ^c
Final length (cm)	20.34 ± 0.28 ^a	19.71 ± 0.62 ^a	19.99 ± 0.72 ^a	17.59 ± 0.68 ^b
Weight gain (g)	56.56 ± 1.14 ^a	50.67 ± 1.11 ^a	37.07 ± 1.15 ^b	21.88 ± 1.16 ^c
Condition factor (K) ¹	0.80 ± 0.03 ^a	0.78 ± 0.06 ^a	0.59 ± 0.03 ^b	0.56 ± 0.04 ^b
SGR (%) ²	2.66 ± 0.08 ^a	2.71 ± 0.05 ^a	2.18 ± 0.09 ^b	1.78 ± 0.08 ^c
Feed intake (g/fish)	41.59 ± 1.26 ^a	39.32 ± 1.12 ^a	34.82 ± 1.07 ^b	28.38 ± 1.03 ^c
Daily feed intake (g/fish/day)	0.83 ± 0.07 ^a	0.79 ± 0.05 ^a	0.70 ± 0.03 ^b	0.57 ± 0.06 ^c
FCR ³	0.74 ± 0.07 ^c	0.78 ± 0.06 ^{bc}	1.02 ± 0.07 ^b	1.30 ± 0.07 ^a
PER ⁴	3.87 ± 0.09 ^a	3.68 ± 0.07 ^a	2.79 ± 0.07 ^b	2.20 ± 0.06 ^c
PPV (%) ⁵	15.89 ± 1.08 ^b	16.30 ± 1.22 ^b	32.78 ± 1.24 ^a	32.98 ± 1.36 ^a
HSI ⁶	1.54 ± 0.06 ^a	1.25 ± 0.05 ^{ab}	1.37 ± 0.06 ^{ab}	0.97 ± 0.06 ^c
Survival rate %	100	100	100	100

Values in the same row with the same superscript (a–c) are not significantly different ($P > 0.05$).

¹ Condition factor (k) = body weight (g)/body length (cm³) × 100.

² SGR: [Ln final body weight (g) – Ln initial body weight (g)]/experimental period (days) × 100.

³ FCR: feed intake (g)/body weight gain (g).

⁴ PER: body weight gain (g)/protein intake (g).

⁵ PPV (%): retained protein (g)/total protein intake (g) × 100.

⁶ HSI: liver weight (g)/fish weight (g) × 100.

Table 4 Body composition of African catfish fed graded levels of *Gracilaria arcuata* (% wet basis).

	Initial	D1	D2	D3	D4
<i>Carcass</i>					
Moisture	80.16	72.99 ± 0.67 ^b	73.78 ± 0.68 ^{ab}	72.97 ± 1.08 ^b	75.55 ± 0.21 ^a
Protein	13.53	15.85 ± 0.36 ^b	15.78 ± 0.22 ^b	17.53 ± 0.75 ^a	16.81 ± 0.38 ^{ab}
Lipid	1.93	5.51 ± 0.58 ^a	4.67 ± 0.33 ^a	2.92 ± 0.33 ^b	3.13 ± 0.084 ^b
Ash	3.37	4.03 ± 0.08 ^{ab}	3.80 ± 0.09 ^b	4.24 ± 0.15 ^a	4.30 ± 0.03 ^a
<i>Muscles</i>					
Moisture		77.16 ± 0.78 ^{ab}	76.79 ± 0.24 ^b	78.48 ± 0.17 ^a	78.25 ± 0.35 ^{ab}
Protein		17.21 ± 0.22 ^b	17.86 ± 0.02 ^a	17.31 ± 0.04 ^{ab}	17.71 ± 0.11 ^{ab}
Lipid		2.21 ± 0.30 ^{ab}	2.76 ± 0.13 ^a	2.27 ± 0.01 ^{ab}	1.91 ± 0.12 ^b
Ash		0.97 ± 0.006 ^c	1.17 ± 0.032 ^a	1.05 ± 0.012 ^b	1.22 ± 0.006 ^a

Values in the same row with the same superscript (a–c) are not significantly different ($P > 0.05$).

Values expressed as mean ± SE ($n = 3$).

Valente et al. (2006) who found that the increasing incorporation of both *G. bursa-pastoris* and *U. rigida*, from 5% to 10%, did not show any differences in growth performance and feed utilization of European sea bass. Mustafa et al. (1995) found that the incorporation of three different seaweeds (*A. nodosum*, *Porphyra yezoensis* and *Ulva pertusa*) upto 5% increased body weight, feed utilization and retained protein in the muscle of red sea bream fingerlings (*Pagrus major*). The final body weight of catfish fed D2 was slightly reduced but with no significant difference when compared to D1 while fish fed on D3 and D4 exhibited a significant depression in their body weights. These were in agreement with data obtained by Soler-Vila et al. (2009) who reported that the final body weights of rainbow trout fed on red alga – *Porphyra dioica* 5, and 10% diets showed nonsignificant differences while 15% recorded a significant depression in final weight from

the control. Similarly Appler (1985) found similar growth performances and protein utilization efficiencies in *Oreochromis niloticus* and *Tilapia zillii* when fed diets containing 5% of freshwater algae, *Hydrodictyon reticulatum*, but reduced growth performances with high dietary inclusion levels. Nonetheless, Davies et al. (1997) demonstrated that the use of the macroalgae *Porphyra purpurea* at high inclusion levels (16% and 33%), as an ingredient for mullet (*Chelon labrosus*) diets showed depression in growth performance and feed utilization efficiency. These data were in accordance with our results where the substitution of *G. arcuata* in African catfish diets more than 10% (D3 and D4) displayed a reduction in growth rate and feed efficiency. In contrast, data obtained by El-Sayed (1994) demonstrated that the use of a microalgae meal, *Spirulina*, as protein source for silver sea bream (*Rhabdosargus sarba*) fingerlings could successfully substitute

up to 75% of the fish meal protein without any adverse effects on growth performance and feed utilization efficiency. These results revealed that the attraction of fish to algae diets seems to be species-specific and might be indicative of a general beneficial effect of low level supplementation of seaweeds in fish diets. Valente et al. (2006) concluded that the relative low nutritive value of seaweeds could explain their depression effect on overall growth performance at high inclusion levels, and could also indicate that the lowest performance of fish fed the *G. cornea* at a level of 10% in diet of European sea bass. Wahbeh (1997) analyzed the nutritional quality of several algae and detected some amino acid deficiencies (*U. lactuca*, for example, lacked cysteine) and high ratios of *n*-6 to *n*-3 PUFAs. The author suggested that a mixture of several algae species could provide fish with an adequate supply of all essential amino acids and fatty acid composition that would result in increased growth performances.

In the present study, two diets were readily accepted by fish, indicating that *Gracilaria* based diet is causing changes in the palatability to the experimental fish. Also, there were no significant difference effects in terms of weight gain for D1 and D2. In general, dietary inclusion of many species of seaweeds, such as *Cystoseira barbata* (Azaza et al., 2008), *U. lactuca* (Güroy et al., 2007), *U. rigida* (Valente et al., 2006) and *G. cornea* (Wassef et al., 2005) at level of 10% resulted in decreased growth performance and feed utilization of the experimental fish. However, in the current study, the growth performance in terms of weight gain, FCR of the catfish receiving 10% of *G. arcuata* based diets did not decrease. Similar data were obtained from different species such as red sea bream and black sea bream (Kalla et al., 2008). It might be explained that these fishes have the potential ability to ingest the algae (Nakagawa, 1997). It was also, indicated that the maximum suitable algae inclusion levels in fish diets may depend on the feeding habits of the fish and species of algae. In our results, high inclusion levels of *G. arcuata* up to 20% and 30% in the diets of African catfish achieved poor growth performance, feed intake and feed utilization. These were in agreement with the data obtained by Xuan et al. (2013) who demonstrated that when the *Gracilaria lemaneiformis* inclusion level reached 20% in diets for black sea bream, significantly poor growth performance of the fish were observed. This may be attributed to plants having soluble non-starch polysaccharide (NSPs) forms functional networks which bind water or minerals, exchange cations and adsorb organic compounds (Brinker, 2009). In the present study, the most likely reasons for any anti-nutritive effects may be that the algal soluble NSPs are generally viscous in nature, leading to the increased viscosity of the diet and the intestinal digestion. This stress will have an effect on protein and lipid digestibility, to be reduced as observed in a previous study in yellow sea bream and consequently the growth performance (Francis et al., 2001).

The hepatosomatic index (HSI), of diet 4 was significantly low compared to D1, D2 and D3. This might be related to *G. arcuata* meal supplement which effectively activated the lipid metabolism including accumulation and mobilization. It is generally accepted that if these indices are lower than normal values, it indicates a change of energy from the organ or tissue growth to combat stressors. These indices may vary naturally with food availability, state of sexual maturation and life history (Barton et al., 2002). These results were in accordance with the findings of Xuan et al. (2013) who illustrated that

supplementation of macro-algae, *G. lemaneiformis* in diets for the black sea bream at inclusion level of 20% significantly decrease the HSI. Furthermore Soler-Vila et al. (2009) found that HSI of rainbow trout declined significantly at supplementation level of 15% dietary red alga *P. dioica* compared to the control diet.

Carcass analysis of the treatments showed that the increasing level of *Gracilaria* inclusion in feed resulted in higher carcass protein content than the control and D2. These data were in agreement with Davies et al. (1997) who reported an increase of crude protein in the carcass of thick-lipped grey mullet with dietary inclusion level of 16% and 33% of seaweed *P. purpurea*.

Results of the current study also showed that muscles and carcass lipid reduced when inclusion level of *Gracilaria* increased. These were generally in accordance with the results obtained by Valente et al. (2006) who reported that lipid content of European sea bass (*Dicentrarchus labrax*) juveniles fed on *G. cornea* decreased at the inclusion level of 10% compared to the control and 5% of *Gracilaria* diets.

In conclusion, this study clearly showed that marine macroalgae *G. arcuata* up to 10% inclusion level have great potential as alternative ingredients in diets for African catfish with no adverse effects on growth performance and feed utilization efficiency. More comprehensive studies required to determine the effect of such products in long term feeding trials and evaluate the maximum dietary inclusion levels of *G. arcuata* in catfish diets.

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