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ORIGINAL ARTICLE

Potential use of green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish, *Clarias gariepinus*

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Abstract This study aimed to evaluate the effects of diet containing the green macroalgae, *Ulva lactuca*, on the growth performance, feed utilization and body composition of African catfish *Clarias gariepinus*. Four experimental diets were formulated: D1 as a control group and D2, D3 and D4 which included 10%, 20% and 30% *U. lactuca* meal, respectively. 180 African catfish, weighing 9.59 ± 0.43 g, and with an average length of 11.26 ± 0.21, (mean ± SE) were divided into four groups corresponding to the different feeding regimes. The final body weight of the fish showed insignificant differences ($P > 0.05$) between the control and fish fed D2, whereas, there was a significant difference ($P < 0.05$) between these two diets compared with D3 and D4, with weights of 70.52, 60.92, 40.57 and 35.66 g recorded for D1, D2, D3 and D4, respectively. In the same trend significant differences were also evident in weight gain, specific growth rate and feed utilization. Fish fed with a diet containing 20% or 30% *U. lactuca* meal had poorer growth performance and feed utilization. Protein productive value, protein efficiency ratio, daily dry feed intake and total feed intake were also significantly lower in fish fed with D3 and D4 than in the control D1 and D2.

Overall, the results of the experiment revealed that African catfish fed a diet with *U. lactuca* included at 20% and 30% levels showed poorer growth and feed utilization than the control group and fish fed diets containing 10% of *U. lactuca*.

1. Introduction

Seaweeds are excellent dietary sources of vitamins, proteins, carbohydrates, trace minerals and other bioactive compounds (Kumar et al., 2008). In an effort to exploit the nutritional value of seaweeds fully, several studies have been conducted to find the biochemical and nutritional composition of various

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seaweeds collected from different parts of the world (Rupérez, 2002; McDermid and Stuercke, 2003; Ortiz et al., 2006; Marsham et al., 2007; Chakraborty and Santra, 2008; Matanjun et al., 2009).

Improving ecologically integrated aquaculture technology, specially a tank-based integrated technique for bioremediation 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 in feed concentrations in seawater effluent and a chance to diversify materials of production in changing market status as offering the possibility for additional sources of income (Al-Hafedh et al., 2012). This work is highly relevant for the developing aquaculture industry in Saudi Arabia (FAO, 2010) and reduction of 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) summarized the importance of algae as ingredients in fish feed. Since then, several studies have evaluated the incorporation of various seaweed species in aquafeeds *Ascophyllum nodosum*, (Nakagawa et al., 1997), *Porphyra* (Davies et al., 1997; Kalla et al., 2008; Khan et al., 2008; 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 have reported promising results for the use of seaweed as partial replacement of fishmeal or protein hydrolysate in aquafeeds. It is clear, however, that the effect of the inclusion of seaweed in aquafeeds seems to depend on the seaweed species, the level at which it is included in feed and the species of fish on whom the seaweed is tested as shown, for instance, by Khan et al. (2008) and Kalla et al. (2008) using *Porphyra* in black sea bream and Red Sea bream.

Ulva is a good source of protein, pigments, minerals and vitamins, and is especially rich in vitamin C (Ortiz et al., 2006; Garcia-Casal et al., 2007) and, in recent years, *Ulva* species have become important macroalgae, which have been investigated as a dietary ingredient for a wide range of fish species.

Even though the valuable effects of *Ulva* spp. are well known, to the authors' knowledge there is to date no literature concerning the use of *Ulva* meal in diets for the African catfish, *Clarias gariepinus*, particularly in terms of the impact of this macroalgae on growth performance and feed utilization.

The aims of this study are to determine the effect of replacing a proportion of conventional fish meal with *U. lactuca* meal on the growth performance, feed utilization and body carcass and muscle composition of the African catfish.

2. Materials and methods

2.1. Diet formulations

Green seaweed, *U. lactuca*, was freshly collected from the nearshore waters of the Red Sea coast at Jeddah, Saudi Arabia. Algal samples were thoroughly washed with sea water, tap water and distilled water, sundried for 48 h and fine-milled with a laboratory blender. The Ulva meal was then passed through a mesh sieve to produce raw Ulva 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 formulation of experimental diets (Table 2). All diets contained approximately 35% protein (Table 3). The diet without *U. lactuca* served as a control diet (D1), while the three other diets were formulated such that *U. lactuca* replaced a proportion of standard fish meal, respectively 10% for D2, 20% (D3) and 30% (D4). Table 2 presents the complete diet formulation and proximate 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 Sciences 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 fish, weighing 9.59 ± 0.43 g (mean ± SE), and with an average length of 11.26 ± 0.21 were divided into four groups. Each of these four groups were then divided into triplicate glass aquaria, each with a capacity of 80 l (100 × 50 × 40 cm), with 15 fish in each tank. The first group served as the control (D1), while each of the three other groups were fed one of the experimental diets (D2, D3 and D4) containing of 10%, 20% and 30% of Ulva meal, respectively. Fish were fed twice daily with a 35% crude protein diet at a rate of 3% of body weight, seven days a week for 10 weeks. Proximate composition of the moisture, protein, lipid, and ash in the body carcass and muscles of fish from each of the diets was determined according to AOAC (1995). The water temperature was maintained at 28 ± 1 °C by a thermostatically controlled immersion heater and pH, ammonia (NH_3), nitrite (NO_2), nitrate (NO_3) and dissolved oxygen were

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

Parameters	Ingredients					
	Ulva lactuca	Fish meal	Soybean meal	Wheat meal	Wheat bran	Yellow corn
Moisture	10.45	8.89	6.84	11.20	7.00	10.6
Protein	11.50	61.41	44.24	14.36	15.03	9.45
Lipid	6.08	12.33	2.91	1.40	5.25	3.10
Ash	24.29	18.64	6.50	1.80	4.79	3.40

Table 2 Formulation of experimental diets (g/kg dry weight) containing *Ulva lactuca* fed to African catfish *Clarias gariepinus*.

Ingredients	Diets			
	D1 (control)	D2 (10% Ulva)	D3 (20% Ulva)	D4 (30% Ulva)
Fish meal	450.00	405.00	360.00	320.00
Ulva meal	00	45.0	90.00	130.00
Soybean meal	50.00	140.00	220.00	290.00
Wheat meal	180.00	150.00	100.00	100.00
Wheat bran	100.00	100.00	100.00	80.00
Yellow corn	170.00	110.00	80.00	30.00
Corn oil	30.00	30.00	30.00	30.00
Vitamin mix	10.00	10.00	10.00	10.00
Mineral mix	10.00	10.00	10.00	10.00
Total	1000	1000	1000	1000

Table 3 Proximate composition for experimental diets (% as fed) containing *Ulva lactuca* fed to African catfish *Clarias gariepinus*.

Parameters	Diets			
	D1	D2	D3	D4
Moisture	6.24	7.34	6.68	7.22
Protein	35.14	35.31	35.60	35.55
Lipid	10.82	8.83	8.63	8.56
Ash	13.28	15.35	15.48	16.20

monitored and remained at acceptable levels throughout the experimental period.

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

The growth performance and feed utilization data for the African catfish fed the four diets are set out in Table 4. There was a significant difference ($P < 0.05$) between the final average body weights between fish fed the control diet D1 and the other groups. Fish fed the fishmeal based control diet (D1) and D2 demonstrated the highest mean final body weight 70.52 and 60.92 g, respectively compared with the other two diets D3 and D4 containing 20% and 30% of Ulva these fish weighed, 40.57 and 35.66 g, respectively. There was also a significant difference ($P < 0.05$) in the final length observed between fish fed on the control group D2 and on the other two diets, with results ranging between 19.90 and 17.11 cm (Table 4). The specific growth rate (SGR%) values further supported this trend, with SGR reducing from 2.73 for the fish fed on the control diet to 2.69, 2.11 and 1.91 for the fish fed on the other three diets (Table 4) with also, insignificant differences ($P > 0.05$) between D1 and D2. The condition factors (K) ranged between 0.92 and 0.674, although no mortality was observed during the experimental period and the overall health of the fish appeared normal.

3.2. Feed consumption and feed utilization

The diets, D1 and D2 were well accepted by the African catfish, while diets containing 20% and 30% replacement of Ulva appeared less palatable to the fish. Mean daily feed intake ranged between 0.978 and 0.661 g/fish/day with a significant difference ($P < 0.05$) between control and other groups. There

Table 4 Weight increase, feed consumption and nutritive utilization of African catfish *Clarias gariepinus* fed experimental diets containing *Ulva lactuca*.

	D1	D2	D3	D4
Initial weight (g)	10.42 ± 0.654	9.28 ± 0.604	9.25 ± 0.479	9.40 ± 0.344
Initial length (cm)	12.20 ± 0.251	11.03 ± 0.256	11.08 ± 0.184	10.72 ± 0.112
Final weight (g)	70.52 ± 2.37^{a}	60.92 ± 3.10^{a}	40.57 ± 3.43^{b}	35.66 ± 3.19^{b}
Final length (cm)	19.90 ± 0.479^{a}	18.76 ± 0.728^{a}	18.19 ± 0.586^{b}	17.11 ± 0.494^{b}
Weight gain (g)	60.10 ± 0.79^{a}	51.64 ± 0.815^{a}	31.32 ± 1.24^{b}	26.26 ± 1.05^{b}
Condition factor (K)[1]	0.895 ± 0.004^{a}	0.922 ± 0.005^{a}	0.674 ± 0.004^{b}	0.712 ± 0.003^{b}
SGR (%)[2]	2.73 ± 0.035^{a}	2.69 ± 0.018^{a}	2.11 ± 0.012^{b}	1.91 ± 0.027^{b}
Feed intake (g/fish)	68.45 ± 0.77^{a}	62.61 ± 0.58^{b}	49.77 ± 0.57^{c}	46.28 ± 0.66^{c}
Daily feed intake (g/fish/day)	0.978 ± 0.008^{a}	0.894 ± 0.007^{b}	0.711 ± 0.009^{c}	0.661 ± 0.005^{c}
FCR[3]	1.14 ± 0.015^{b}	1.21 ± 0.055^{b}	1.59 ± 0.031^{a}	1.76 ± 0.053^{a}
PER[4]	2.50 ± 0.023^{a}	2.34 ± 0.025^{a}	1.77 ± 0.027^{b}	1.60 ± 0.018^{b}
PPV (%)[5]	26.28 ± 0.52^{a}	24.56 ± 0.46^{b}	24.44 ± 0.77^{b}	23.83 ± 0.72^{b}
HSI[6]	1.25 ± 0.098^{a}	1.48 ± 0.256^{a}	1.46 ± 0.094^{a}	1.30 ± 0.100^{a}

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 (cm^3) × 100.

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

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

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

[5] PPV (%) = (% final body protein – % initial body protein)/total protein intake (g) × 100.

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

Table 5 Body composition of African catfish fed graded levels of *Ulva lactuca* (mean ± SE).

	Initial	D1	D2	D3	D4
Carcass					
Moisture	80.16	71.99 ± 0.67[b]	73.45 ± 1.13[a]	73.380 ± 0.34	74.738 ± 0.30[a]
Protein	11.53	17.85 ± 0.36[a]	16.96 ± 0.63[a]	15.86 ± 0.24[b]	15.45 ± 0.25[b]
Lipid	1.93	5.76 ± 0.58[a]	5.12 ± 0.51[a]	4.803 ± 0.517[a]	4.390 ± 0.40[a]
Ash	3.37	4.03 ± 0.08[a]	4.05 ± 0.10[a]	4.02 ± 0.06[a]	4.03 ± 0.03[a]
Muscles					
Moisture	ND	77.16 ± 0.97[a]	77.69 ± 0.42[a]	77.75 ± 0.21[a]	77.06 ± 0.09[a]
Protein	ND	17.21 ± 0.22[b]	17.60 ± 0.20[ab]	17.59 ± 0.04[ab]	17.96 ± 0.04[a]
Lipid	ND	2.21 ± 0.30[a]	2.65 ± 0.10[a]	2.30 ± 0.11[a]	2.47 ± 0.10[a]
Ash	ND	0.97 ± 0.04[b]	1.02 ± 0.03[b]	1.14 ± 0.01[a]	1.18 ± 0.06[a]

Values in the same row with the same superscript are not significantly different ($P > 0.05$).
ND: not detected.

was a noticeable effect of the dietary inclusion of alternative protein sources on feed intake (Table 4). Feed intake for catfish fed on the control diet containing the highest amount of fishmeal and D2 containing 10% of Ulva meal were significantly better than those observed for fish fed D3 and D4 containing 20% and 30% Ulva. FCR values also differed significantly ($P < 0.05$) between the control group and D2 (1.14 and 1.21) when compared to fish fed on D3 and D4 (1.59 and 1.76). The protein efficiency ratio (PER) was noticeably different between treatments and supported the same trend, with the fish fed the control diet displaying a superior PER (2.50) and fish receiving the different levels of Ulva exhibiting a PER of 2.34, 1.77 and 1.60, respectively. Protein productive values (PPV%) values also showed a decrease when fishmeal was replaced by the *U. lactuca* meal source. These values were 26.28 for fish fed control diets and 24.56, 24.44 and 23.83 for D2, D3 and D4, respectively (Table 4).

3.3. Fish body composition

Table 5 presents the initial and final carcass composition of the fish fed the experimental diets. The final carcass composition showed little significant variation in proximate composition as a result of the diet formulations. Fish fed the fishmeal based control diet and the diets based on different levels of Ulva did not yield any variations in their carcass lipid and ash content ($P > 0.05$) whereas, there was a significant difference ($P < 0.05$) between the control group and D2 compared with D3 and D4 in moisture and protein contents. Also, there was a significant increase in percentage of protein in muscle composition from 17.21% for D1 to 17.96 for D4. Also, ash content in muscles showed slight differences among groups (Table 5).

4. Discussion

The present study reports the first use of macroalgae *U. lactuca* in African catfish diets. The results show that the growth performance of fish tended to reduce as the amount of *U. lactuca* in the diet was increased.

Previous investigations have used many types of seaweed as feed for other fish species, Azaza et al. (2008), Güroy et al. (2007), Valente et al. (2006) and Wassef et al. (2005). Generally, these have found that the inclusion of different seaweeds (*Cystoseira barbata*, *U. lactuca*, *U. rigida* and *G. cornea*) at a high level leads to poorer growth and feed utilization compared to fish fed a control diet. These are, therefore, in agreement with our data, which recorded a decline in all growth performance and feed utilization parameters such as final body weight, SGR, FCR, and PER for diets containing 20% and 30% of the *U. lactuca* diets compared with fish fed the control and D2 diets. These results indicate that the levels of *U. lactuca* added to feed in this study were ideal for African catfish up to 10% of *U. lactuca*.

Carnivorous fish, such as African catfish, would tend to prefer diets with animal ingredients rather than plant feedstuff, and such a conclusion is in accordance with our results showing a decrease in feed intake by fish at the levels 20% and 30% of *U. lactuca* in their diets was increased (Table 4). The growth reduction noted in this research might also be attributed to the effects of various anti-nutrients (e.g., saponins, tannins, phytic acid) which can reduce the palatability of diets (Francis et al., 2001). Azaza et al. (2008) reported that the inclusion of *U. rigida* up to a level of 10% of the meal produced diets containing a certain amount of anti-nutrients, for example saponins (1.13%), tannins (0.16%) and phytic acid (0.47%). Saponins could diminish the palatability of a diet by their bitterness and interference with the absorption of dietary lipids and bile salts (Guillaume and Choubert, 2001). These compounds with anti-nutritional characteristics may, therefore, inhibit growth performance and feed utilization when fish are fed high levels of *Ulva*. Thiessen et al. (2004) illustrates the reduction in growth when using seaweed in fish diets, arguing that most plant ingredients contain a certain amount of fibre and that this may have a negative effect on both their nutritional value and palatability. Ortiz et al. (2006), meanwhile, reported that *U. lactuca* contains about 60% fibre this might reduce its value in aqua feeds.

Azaza et al. (2008), therefore, has suggested that a possible reason for diminished growth at higher levels of Ulva meal (30%) could be due to the high fibre content and its possible effects on the digestibility of protein and dry matter. It is possible that fibre structures reduce the accessibility of intestinal enzymes to food nutrients such as starch, protein and lipids and thus act as physical barriers between nutrients and digestive enzymes in the intestine (Potty, 1996), thereby making the enzymes less active. The inclusion of plant protein in diets for fish has been reported to reduce daily feed intake (Davies et al., 1997). This is in agreement with the present study in which decreased feed intake was observed for all diets except D1 and D2 this might be another reason for the reduced growth performance and feed utilization. Our data, however, are in accordance with results obtained by Soler-Vila et al. (2009), who reported that increasing the amount of the red alga, *Porphyra dioica*, in rainbow trout diets up to a 10% level led to an increase in voluntary feed intake. The inclusion of *P. dioica* in the diets, therefore, increased the polysaccharide fibre content which may have reduced the available dietary energy.

Analysis of the carcass of African catfish fed *U. lactuca* did not show any variations between treatments for lipid and ash, however there were significant variations in carcass moisture and protein. Carcass ash and lipid content did not exhibit significant differences between diets (Table 5). These results also showed little increase in protein muscle with increasing amounts of Ulva, especially D4 which contain 30% Ulva, in contrast, Soler-Vila et al. (2009) found that muscle protein deposition in the rainbow trout was influenced by a 15% level of *Porphyra* in the diet, which means that a level of up to 10% would not have an effect on muscle protein deposition. Menghe et al. (2009), meanwhile, recommended that feeding dried algae up to 2% of the diet did not affect the body composition or feed utilization of channel catfish. Altogether, combining these previous studies with our own suggests a need for further work to determine the optimum substitution levels more than 10% of *U. lactuca* in the diets of African catfish.

In conclusion, the results of this study showed that feeding experimental diets containing 20% and 30% *U. lactuca* to African catfish resulted in poor growth and feed utilization, and that further investigations are required to evaluate the optimum dietary inclusion level of more than 10% of these green macroalgae in African catfish diets with adding amino acids or fatty acids to enhance palatability, growth performance and feed utilization for *U. lactuca*.

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