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Growth response and carcass composition of rabbitfish, *Siganus canaliculatus* (Park) fed diets supplemented with dehydrated seaweed, *Enteromorpha* sp.

O. M. Yousif,[1]* M. F. Osman,[2] A. R. Anwahi,[3] M. A. Zarouni [3] and T. Cherian [3]

[1]Fish and Shrimp Farming Project-Abu Al Abyad, P.O. Box 372, Abu Dhabi, United Arab Emirates; [2]Department of Animal Production, Faculty of Agriculture, Ain Shams University, Egypt; [3]Marine Resources Research Center, Ministry of Agriculture & Fisheries, Umm Al Qaiwain, United Arab Emirates

Abstract: A trial to incorporate dehydrated *Enteromorpha* in the diet of rabbitfish fry averaging 0.25 ± 0.04 g was undertaken for a period of 12 weeks. Four isonitrogenous (39% crude protein) and isoenergetic (19 kJ/g) diets were fed to fish. The dehydrated *Enteromorpha* was incorporated in the diets at a rate of 0 (control), 10, 20 and 30%. A fifth group of fish was fed control diet and supplemented with known weight of fresh *Enteromorpha* placed in plastic baskets at the bottom of the rearing tanks. The survival rate, growth performance and feed utilization efficiency were observed to decrease with increasing inclusion levels of dehydrated algae ($p < 0.05$). The best results of all parameters were achieved by fish fed control diet and fresh *Enteromorpha* ($P < 0.05$). Carcass protein was not affected by the different treatments while lipid content was observed to increase in the group of fish supplemented with fresh *Enteromorpha* ($P < 0.05$). It is concluded that further studies are needed on the dehydration techniques of *Enteromorpha* as well as on the digestibility and assimilation of diets containing dehydrated algae.

Key words: *Enteromorpha, Siganus canaliculatus*, growth performance, feed utilization

*Author to whom correspondence should be addressed

إستجابة النمو ومحتوى الذبيحة لأسماك الصافي المغذاة بعلائق مضاف لها طحاب الشبا البحري

عمر محمد يوسف[١]، محمد فتحي عثمان[٢]، عبد الرزاق أنولحي[٣]، محمد عبد الرحيم الزرعوني[٣] و توماس شريان[٣]

[١] مشروع تربية الأسماك و الروبيان – أبو الأبيض – ص. ب. ٣٧٢ – أبوظبي – الإمارات العربية المتحدة
[٢] قسم الإنتاج الحيواني – كلية الزراعة – جامعة عين شمس – جمهورية مصر العربية
[٣] مركز أبحاث الأحياء البحرية – وزارة الزراعة و الثروة السمكية – أم القيوين – الإمارات العربية المتحدة

ملخص: أجريت تجربة لمدة ١٢ أسبوعاً لدمج طحاب الشبا المجفف في علائق صغار أسماك الصافي التي تـزن فـي المتوسط ٠،٢٥ ± ٠،٠٤ جرام حيث تمت تغذيتها بأربعة علائق متساوية النيتروجين (٣٩% بروتين خام) و متـساوية الطاقة (١٩ كيلو جول/الجرام). وقد تم دمج الشبا في العلائق الأربعة بمعدل صفر % (الكنترول)، ١٠ ، ٢٠ و ٣٠%. و هناك مجموعة خامسة تمت تغذيتها بعليقة الكنترول زائداً وزن معلوم من الشبا الطازج الذي تم وضعه فـي سـلال بلاستيكية على قاع الحوض. و قد تم ملاحظة أن معدل الحياتية وأداء النمو و كفاءة استخدام العلائق قد تدنت بزيـادة نسبة الشبا المجفف في العلائق. و من بين كل المعاملات فقد حققت المجموعة التي تمت تغذيتها بعليقة الكنترول زائـداً الشبا الطازج أفضل النتائج. و لم يتأثر بروتين الذبيحة بمختلف العلائق بينما تأثر محتوى الدهن حيث تلاحظ زيادته في المجموعة التي تمت تغذيتها بالكنترول زائداً الشبا الطازج. و قد خلصت التجربة إلى الحاجة إلى مزيد مـن الدراسـة حول تقنيات تجفيف طحاب الشبا و كذلك لدراسة هضم و تمثيل العلائق المحتوية على الشبا المجفف.

كلمات مفتاحية: الشبا ، أسماك الصافي ، أداء النمو ، استخدام العلائق

Introduction

The potential of siganids for mariculture has been recognized for many years. This potential was attributed to their palatability, hardiness under culture conditions, ability to reproduce easily in captivity and to the fact that the fish are primarily first trophic level feeders and thus can be grown on cheap feeds (Ben-Tuvia *et al*., 1973; Lam, 1974; Von Westernhagen and Rosenthal, 1976; Tseng and Chan, 1982). In the coastal waters of the United Arab Emirates (UAE) two species are found, *Siganus canaliculatus*, which is the commoner species and one of the most favored food fish; and *S. javus*, which is less abundant and less valuable (MAF, 1984; Al-Ghais, 1993). The fish is herbivorous, feeding predominantly on filamentous green algae. Early studies of stomach contents and food preference revealed that among the many different algal species and vascular plants eaten, the presence of *Enteromorpha* was high and was the preferred species. This preference for *Enteromorpha* by siganids is not directly related to the calorific value of the algae but is related to the texture of its thalli which are crispy and thin (Von Westernhagen, 1973; 1974). *Enteromorpha* is one of the most widespread genera of the green algae with a long history of food use, both for humans and animals (Jeong *et al*., 1993). The alga is rich in minerals, carbohydrates and vitamins (Nisizawa *et al*., 1987; Naidu *et al*., 1993; Reviers and Leproux, 1993). The alga grows along the coasts of UAE throughout year in the littoral zones exposed to shallow water. The only use of this alga in UAE is that by fishermen as bait to catch siganids (Al-Ghais, 1993).

Efforts to reduce the cost of fish feed have concentrated on the use of low-cost by-products and plant materials (Yousif *et al*., 1994). The present study was conducted to evaluate the possibility of utilizing the dehydrated *Enteromorpha* as a dietary ingredient in the formulated feed for *S. canaliculatus* fry.

Materials and Methods

Experimental procedure and diets

Rabbitfish fry, *S. canaliculatus*, were collected by seine netting from Umm Al Qaiwain lagoon and stocked in a 40-t holding concrete tank at the Marine Resources Research Center. The fish were fed a 40% crude protein formulated diet *ad. libitum* twice daily. After two weeks of adaptation the fish fry averaging 0.25 ± 0.04 g (mean ± s.d.) were randomly distributed in 15 outdoor 1000-L circular fiberglass tanks. The tanks received a continuous flow of filtered sea water (40 ± 0.5 ‰) at a rate of 30 L/min. The fish were stocked at a rate of 200 fry per tank.

The fresh algae were collected from the seagrass area of Umm Al Qaiwain lagoon and immediately washed with running filtered seawater to remove the epiphytes and other foreign matter such as sand and shells. The algae were then air-dried in the sun for two weeks. After complete drying the algae were finely milled using a laboratory mill.

Proximate analysis of major feed ingredients was performed prior to formulation of the experimental diets (Table 1). A lyophilized extract of fresh algae was analyzed for amino acids composition (Table 2).

Table 1. Chemical composition (% DM basis) of major feed ingredients

	Fish meal	soybean meal	wheat flour	Dehydrated algae
Dry matter	89.84	93.27	89.34	91.65
Crude protein	72.89	55.28	14.34	11.02
Crude lipid	11.50	1.93	3.23	0.90
Crude fibre	0.38	7.16	0.62	8.97
Ash	8.88	8.29	0.97	18.34
NFE[1]	6.35	27.34	80.84	60.77

1 Nitrogen -free extract = 100- (crude protein +lipid + fibre + ash)

Table 2. Amino acid composition of fresh *Enteromorpha* (g 100g $^{-1}$ dry weight)

Essential		Non-essential	
Arginine	4.5	Alanine	12.5
Histidine	1.6	Aspartic acid	14.7
Isoleucine	5.0	Cysteine	3.0
Leucine	7.5	Glutamic acid	14.2
Lysine	4.6	Glycine	5.6
Methionine	3.1	Proline	5.0
Phenylalanine	3.3	Serine	6.0
Threonine	6.5	Tyrosine	2.2
Valine	7.8		

Four isonitrogenous (39% crude protein) and isocaloric (19 KJ/g) test diets were formulated (Table 3). The ingredients of each experimental diet were thoroughly mixed with water and oil in a laboratory feed mixer (Nakayasu Co., Japan) until a uniformly moist mixture was obtained. Pellets were made by passing the diet mixture through a laboratory feed pelletizer (Fuji Mzuho, Model 52-k, Japan) equipped with a 1-mm die. The pellets were then air-dried for 48 hours. The dry pellets of each experimental diet were packed in sealed plastic bags and stored in a freezer at -20°C until used. A fifth group of fish was fed a control diet with addition of a known weight of fresh *Enteromorpha* placed in plastic baskets at the bottom of the rearing tanks (treatment 4).

Each experimental diet was fed to triplicate tanks of fish fry for a period of 12 weeks. The fish were fed three times at a rate of 7% body weight per day. Fish from each tank were bulk weighed biweekly and the daily feed allowance was adjusted accordingly. At the start and end of the experiment, fish were retained from each treatment for subsequent proximate analysis.

Table 3. Formulation and chemical composition of the experimental diets

Ingredients (% as fed)	**Diet**			
	Control	**1**	**2**	**3**
Fish meal	27.0	27.0	27.0	27.0
Soybean meal	22.0	22.0	22.0	22.0
Wheat flour	45.0	35.0	25.0	15.0
Dehydrated algae	-	10.0	20.0	30.0
Sunflower oil	4.0	4.0	4.0	4.0
vitamin-mineral mix[1]	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	1.0	1.0	1.0	1.0
Proximate analysis (%DM)				
Dry matter	88.01	86.94	85.88	85.00
Crude protein	39.40	39.58	39. 41	39.81
Ether extract	9.31	9.48	8.93	7.36
crude fibre	3.81	3.76	3.16	3.03
Ash	7.08	11.53	15.96	20.39
NFE	40.40	35.65	32.54	29.41
GE (kJ/g diet)[2]	19.91	19.21	18.41	17.37

[1] vitamin-mineral mix: shrimp tonic, JV Marine East, Co. Ltd., Taiwan (A 30,000,000 IU, D_3 12,000,000 IU, B 1250 mg, B_2 12,000 mg, B_6 1250 mg, B_{12} 4250 mg, E 12,500 mg, K 5000mg, C 50,000 mg, Nicotinmide 30,000 mg, CaO panthotecat 15,000 mg, Folic acid 1500 mg, Niacinamide 20,000 mg, Insitol 112,500 mg, Lecithin 25,000 mg and Cholin 37,500 mg)

[2] Gross energy calculated based on the conversion factors: protein 23.51 kJ/g, fat 39.75 kJ/g and carbohydrates (as NFE) 17.20 kJ/g (Yousif *et al*.,1996).

Analytical Methods

Analysis for chemical composition of feed ingredients, experimental diets and fish carcass were made according to the standard methods of AOAC (1984).

Survival rate, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy utilization (EU) were calculated as follows:

Survival rate (%) = 100 [(final number of fish) – (initial number of fish) / (initial number of fish)]

Wt. Gain (%) = 100 [(final body wt – initial body wt.) /(initial wt.)]

SGR (%/day) = 100 [(log final body wt. – log initial body wt.) / (time, days)]

FCR = [feed fed (dry wt., g)] / [fish wt. gain (fresh wt., g)]

PER = [fish wt. gain (fresh wt., g)] / [protein fed (g)]

PPV (%) = 100 [(final body protein, g) –(initial body protein, g) / (protein intake, g)]

EU (%) =100 [(final body energy, kJ) –(initial body energy, kJ) / (energy intake, kJ)]

Statistical Analysis

All values are reported as means ± SEM. Performance and carcass composition means were compared using the MSTAT 4 package (Nissen, 1987). Duncan's multiple range test of the same program was used to test for differences among treatment means at $P < 0.05$.

Results and Discussion

The comparison of growth response and feed performance of *S. canaliculatus* fed the experimental diets is shown in Table 4. Survival, weight gain, specific growth rate, feed conversion ratios, protein efficiency ratio, protein productive value and energy utilization of fish fed the control diet supplemented with fresh algae were significantly better (P<0.05) than those of fish fed diets including various levels of dehydrated algae. All these parameters were observed to decrease with increasing inclusion levels of the dehydrated algae (P<0.05). The poorest performance (P<0.05) was observed in the group of fish receiving diet supplemented with 30% dehydrated algae. The carcass composition (Table 5) showed no significant differences (P<0.05) in the body moisture and protein content among all treatments. The lipid and energy contents were higher (P<0.05) in the fish fed the control diet and fresh *Enteromorpha* whilst the values of other treatments decreased with increasing the level of inclusion of dehydrated algae. The lowest (P<0.05) ash content was produced by fish fed the control diet supplemented with fresh *Enteromorpha* sp.

When comparing the chemical composition of local *Enteromorpha* sp. used in this study with those from other countries (Nisizawa *et al*., 1987; Naidu *et al*., 1993), it is found that local algae have a lower protein content and high carbohydrate level. The lipid and fibre contents do not differ from those reported elsewhere. The amino acid composition indicated that the green algae used in this study are poor in histidine, tyrosine, methionine and cystine and rich in aspartic acid, glutamic acid and alanine. This tendency was reported by other workers for Enteromorpha. (Amano and Noda, 1992). Nevertheless, these values fall within the recommended ranges for finfishes (NRC, 1983; De Silva and Anderson, 1995).

Table 4. Performance of *S. canaliculatus* fry fed the experimental diets

	Diet					
	Control	**1**	**2**	**3**	**4***	**±SEM**
Initial weight (g)	0.25[a]	0.25[a]	0.26[a]	0.25[a]	0.25[a]	0.040
Final weight (g)	2.82[b]	2.83[b]	2.15[bc]	1.50[c]	4.61[a]	0.294
Survival (%)	27.00[b]	31.00[b]	29.00[b]	16.00[c]	52.33[a]	4.069
Weight gain (%)	1022.62[b]	1034.52[b]	724.49[bc]	498.67[c]	1741.51[a]	118.485
SGR (%/day)	2.87[b]	2.87[b]	2.50[bc]	2.10[c]	3.47[a]	0.131
Feed intake (mg/day)	151.85[a]	141.54[a]	119.96[b]	99.03[c]	162.50[a]	6.617
FCR	4.29[b]	4.05[b]	4.64[b]	6.06[a]	2.61[c]	0.357
PER	0.80[b]	0.85[b]	0.79[b]	0.63[b]	1.32[a]	0.071
PPV (%)	10.09[b]	12.33[b]	10.25[b]	8.84[b]	18.26[a]	1.022
EU(%)	5.09[b]	6.77[b]	5.66[b]	4.47[b]	9.91[a]	0.598

Means in the same row having the same superscript are not significantly different (P< 0.05)

* Treatment 4: fish fed control diet in addition to fresh algae

Table 5. Carcass composition of *S. canaliculatus* fry fed the experimental diets

		Diet					
	Initial	**Control**	**1**	**2**	**3**	**4**	**±SEM**
Dry matter	19.50	21.65*	23.91	22.70	22.05	23.07	0.334
Crude protein	51.81	57.42*	58.66	57.54	60.61	58.06	0.529
Crude lipid	7.57	15.50[bc]	17.11[b]	13.06[c]	8.03[d]	21.55[a]	1.260
Ash	35.38	18.61[b]	18.23[bc]	20.28[ab]	21.88[a]	15.95[c]	0.600
Energy content (kJ/g)	15.26	19.74[bc]	20.66[b]	18.79[cd]	17.52[d]	22.29[a]	0.471

Means in the same row having the same superscript are not significantly different ($p < 0.05$)

*Means in this row are not significantly different ($p < 0.05$)

Ash content was observed to increase with increasing levels of dehydrated algae in the diets. This was probably due to the presence of sand grains and shells or sea salts resulting from the inadequate washing of the algae. Jauncey and Ross (1982) recommended a level of less than 10% in the diets for tilapia. Ash *per se* is not known to be harmful to cultured organisms. However, ash content, such as silica, affects the digestibility of the diets (De Silva and Anderson, 1995). Since siganids usually naturally thrive on algae, the ingestion by *S. canaliculatus* of high ash levels contained in the experimental diets might not be the direct cause of growth retardation. A possible cause could be the drying process of the algae followed in this study. The sun-drying technique might have affected the nutritional properties of the algae as well as its palatability. The presence of high anti-nutritional factors in the dehydrated algae might have also affected the growth of the fish. Naidu *et al*. (1993) attributed the growth retardation of rats fed seaweed meal, among which was *Enteromorpha* sp., to the presence of lectins (haemagglutinins). Lectins are proteins which bind to specific receptor sites in the intestinal epithelial cells resulting in impairment of nutrient absorption (Naidu *et al*., 1993; De Silva and Anderson, 1995)

In the present study, it has been observed that, the fish body color became dark before death and the fish were observed to swim near the water surface, then completely cease feeding. These symptoms are characteristic of streptococcal infection described by Foo *et al*. (1985) in diseased *S. canaliculatus.* This best survival in the treatment receiving supplement of fresh *Enteromorpha* was probably due to the antibacterial activity found in the algae. This activity has been widely reported for *Enteromorpha* spp. and other marine algae (Ballantine *et al*., 1987; Kumar and Rengasamy, 2000). On the other hand this improved survival rate could be attributed to the qualitative lipid composition of the fresh algae. *Enteromorpha*, like the other marine green algae, usually contain highly polyunsaturated C_{16} and C_{18} fatty acids (Akinin *et al*., 1992; Jones and Harwood, 1993; Khotimchenko, 1993). The supplementation of dietary unsaturated fatty acid in the diet of marine fish larvae have been reported to improve the survival rate (NRC, 1983; Dhert *et al*., 1991; Izquierdo, 1996).

In conclusion, the present study shows that the dehydrated *Enteromorpha* incorporated in the diets of *S. canaliculatus* produced poor growth response and feed utilization efficiency. However, further studies on the digestibility and assimilation of diets containing dehydrated algae by *S. canaliculatus* are recommended. Also, further studies are needed to determine the proper techniques of processing the algae. Cooking and vacuum drying of seaweeds has been reported to increase digestibility of the plant (Naidu *et al*., 1993).

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