The Potential of Dehydrated *Enteromorpha*, *Prosopis cineraria* Linn. (Leguminosae) Pods Meal and Date Pits in Formulated Feed for Bluespot Grey Mullet Fry, *Valamugil seheli* (Forsskal)

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Abstract: *Valamugil seheli* fry were acclimatized to fresh water conditions and subjected to feeding trials with diets supplemented with three non-conventional carbohydrate sources. Dehydrated *Enteromorpha*, *Prosopis cineraria* pods and date pits meals, were used to substitute the wheat flour at 0, 10, 20 and 30%. Diets supplemented with date pits achieved the best ($P < 0.05$) survival rate. The best growth performance and feed utilization efficiency ($P < 0.05$) were recorded in fish fed the diets supplemented with dehydrated *Enteromorpha* at all levels. Inclusion of 30% *P. cineraria* pods meal produced the poorest results. The carcass protein contents were similar for all treatments. The moisture content was inversely related to fat content, irrespective of treatments. The ash content was high ($P < 0.05$) in the groups fed the control diet and that supplemented with 30% dehydrated *Enteromorpha*. Energy deposition was best ($P < 0.05$) in the fish fed diets containing 10 and 20% dehydrated *Enteromorpha*.

Keywords: *Valamugil seheli*, *Enteromorpha*, *Prosopis cineraria* pods, date pits, growth

Introduction

The problem of high cost of fish feed has created the necessity to look for the use of cheap indigenous feedstuffs. Many studies have been reported on the use of materials of plant origin as a potential dietary sources for fish (Wee and Wang 1987; Pouomogne *et al*. 1992; Yousif *et al*. 1994; Alhadhrami and Yousif, 1994; Yousif *et al*. 1996-a; Yousif *et al*. 2004). Besides, non-protein nutrients such as lipid and carbohydrates are now effectively used in reducing feed costs (Shiau and Peng 1993). Carbohydrates are the least expensive form of energy that could be used in fish diets (Nijhof and Bult 1993; Wilson 1994). Mullets (Mugilidae) which feed on the lowest trophic level, were found from the point of view of aquaculture to accept a wide range of artificial foodstuffs in their diets (Brusle 1981). The fish are commonly cultured in both freshwater and seawater. However it was reported that the metabolic rate of the fish was negatively affected by high salinity levels and that an improved growth performance was achieved in freshwater (Cardona 2000). On the contrary, Peterson *et al*. (1998) claimed that the peak growth of juvenile *Mugil* sp. occurred at water salinity of 17 ppt. *Prosopis cineraria*, locally known as Ghaf, is a vigorous and drought-tolerant leguminous tree. It is wide spread in the United Arab Emirates (UAE) and mainly used for afforestation purposes under irrigation and occasionally browsed by camels and goats. In many tropical regions the cylindrical-turgid pods of other *Prosopis* species are used as a replacement for the carbohydrate component in rations for egg laying hens and goats (Primo *et al.* 1986; da Silva 1986; Mahgoub *et al.* 2005). *Enteromorpha* is one of the most wide spread genera of the green algae. Like most dried seaweeds, its composition is predominantly carbohydrates (64.9%) with less proteins (19.5%), minerals (15.2%), mostly sodium and potassium, little lipid (0.3%) and vitamins (Nisizawa *et al.* 1987; Naidu *et al.* 1993). The algae grow along the coasts of UAE during the whole year in the littoral zones exposed to shallow water. Date pits, a by-product of date fruits, are abundantly produced in UAE but only a small scale of the annual production is mainly used by camel breeders as a low price ingredient for routine feeding (Ahmed *et al.* 1995). Many trials were conducted to investigate the potential of this by-product in poultry (Vandepopuliere *et al.* 1995) and fish diets (Yousif *et al.* 1996-a; Belal and Al-Owafeir 2004). This study was conducted to assess the performance of mullet fry, *Valamugil seheli*, fed diets containing varying levels of dehydrated *Enteromorpha*, *P. cineraria* pods meal and date pits meal.

Materials and Methods

Experimental diets

The fresh algae, was air-dried for two weeks and then finely milled. The meals of date pits and *P. cineraria* pods were further finely ground. Major ingredients of the experimental diets were individually sieved through a 1.25 mm sieve and were subjected to chemical analysis (Table 1).

Ten experimental diets with equal levels of crude protein (400g kg^{-1}) and energy contents (17 kJg^{-1}) were formulated. The main carbohydrate source in the diets, the wheat floor, was replaced with equal proportions (0, 10, 20, and 30%) of the tested ingredients (Table 2). Ingredients of each experimental diet were blended in a laboratory mixer and then pelleted using a meat grinder with a 2-mm die. The diets were then oven dried at 55°C for 24 h and subsequently broken into crumble. Each diet was packed in a sealed bag and kept in a freezer at -20°C until used. All experimental diets were analysed for proximate composition (Table 2).

Experimental procedure

Wild mullet fry were collected from Umm Al Qaiwain Lagoon (40 ppt salinity) and kept in 1000-L outdoor circular fiberglass tanks at the Marine resources research Center, Umm Al Qaiwain, Ministry of Environment and Water, where they were gradually acclimatized to the freshwater within a period of 20 days. During this period the fish were fed *ad. Libitum* twice daily with a 40% crude protein formulated diet. The acclimated fish, averaging 0.6 g, were then transported in freshwater to the Aquaculture Laboratory of UAE University where they were kept in a 1000-l indoor fiberglass tanks and fed *ad Libitum* three times a day with the control diet (diet 1). After two weeks of adaptation, the fish fry averaging 0.61 ± 0.001 (mean ± SD) were randomly distributed in 30 indoor 15-L fiberglass tanks at rate of 1 fish L^{-1}. The adaptation and rearing indoor tanks were part of complete recirculating systems in the Aquaculture Laboratory. Both systems were provided with aeration, thermostatically controlled heating elements set at 27 ± 1°C and mechanical and biological filteration. Mean environmental conditions (± SD) of rearing tanks recorded during the experimental period were: water exchange rate, 3.5 ± 0.2 L min^{-1}; photoperiod, 12:12 h light: h dark; dissolved oxygen, 6.8 ± 0.4 mg

L^{-1}; pH, 8.12 ± 0.2; NH_4-N, 0.25 ± 0.02 mg L^{-1}; NO_2-N, 0.006 ± 0.002 mg L^{-1}; total hardness, 161.7 ± 3.7 mg $CaCO_3$ L^{-1}; and total alkalinity, 170.1 ± 4.02 mg L^{-1}. Each experimental diet was alloted to triplicate sets of 15 fish each. Fish in all treatments were fed daily at 5% body weight for a period of 7 weeks. Daily rations were offered three times at 08:00, 13:00 and 18:00 h. The daily feed allowance was adjusted biweekly after weighing the fish. At the start and end of the experiment fish from each treatment were retained for carcass analysis.

Analytical methods

Feed ingredients, experimental feeds and carcasses were analysed for proximate composition following AOAC standard procedures (2000). Fish growth performance and feed utilization efficiency were evaluated in terms of survival (%), weight gain (%), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV, %) and energy utilization (EU, %) and were computed as follows (Yousif *et al.* 1996-b):

Survival rate (%) = 100 [(final no. of fish – initial no. of fish) / initial no. of fish]

Weight gain (%) = 100 [(final body wt – initial body wt) / initial body wt]

SGR (% day^{-1}) = 100 [(log_e final body wt – log_e initial body wt) / time, days]

FCR = feed intake (g) / fish wt gain (g)

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

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

Statistical methods

Statistical analysis of data were analyzed by two-way ANOVA using the MSTAT 4 package (Nissen 1987). Duncan's multiple range test was used to evaluate specific differences between treatment means at $P < 0.05$.

Results and Discussion

The growth performance and feed utilization efficiency of *V. seheli* fry in terms of percent survival, per cent weight gain, per cent/day specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), percent protein productive value (PPV) and percent energy utilization (EU) are depicted in Table 3. The survival was variable between treatments. The best ($P < 0.05$) survival rate was achieved by the group fed diet 9 (20% date pits). This was followed by the groups fed diets supplemented with 30% and 10% date pits as well as the group that received diet 3 (20% *Enteromorpha*). The poorest ($P < 0.05$) survival rate was observed in the groups fed diets supplemented with 30% *Enteromorpha* and 10% *P. cineraria* pods meal. The best ($P < 0.05$) growth performance in terms of percentage weight gain and SGR (%/day) was achieved by diets 2 and 3 (10% and 20% dehydrated *Enteromorpha*, respectively). These were closely followed by the diets 5 and 8 (10% *P. cineraria* pods meal and date pits, respectively). The lowest growth performance was observed in the group fed diet 7 (30% *P. cineraria* pods meal). The inclusion of 10 and 20% dehydrated *Enteromorpha* produced significantly better ($P < 0.05$) feed utilization efficiency in terms of FCR, PER, PPV (%) and EU (%). Diet 7 containing 30% *P. cineraria* pods meal resulted in the lowest ($P < 0.05$) feed utilization efficiency. The carcass composition of experimental fish before and after the experiment is shown in Table 4. The body protein content was similar ($P < 0.05$) for all groups. The crude lipid content was highest ($P < 0.05$) in the fish fed diet 3 containing 20% dehydrated *Enteromorpha*. The lowest ($P < 0.05$) value was attained by the group fed diet 7 (30% *P. cineraria* pods meal). The rest of the groups gave similar ($P < 0.05$) lipid contents. Generally, higher percentage of body lipid associated with lower percentage of body moisture was observed in all treatments. The highest ($P < 0.05$) ash content was recorded in the groups fed diet 1 (control) and diet 4 (30% dehydrated *Enteromorpha*). The lowest ($P < 0.05$) ash content was recorded in the group fed 20% dehydrated *Enteromorpha.* Other groups gave similar ($P < 0.05$) results of body ash. Energy deposition was best ($P < 0.05$) in the fish fed diets containing 10 and 20% dehydrated *Enteromorpha* (diets 2 and 3, respectively). All other groups gave similar ($P < 0.05$) values. The growth performance and feed utilization efficiency of fish fed diets supplemented with 10 and 20% dehydrated *Enteromorpha* was superior ($P < 0.05$) to all other groups tested in this study. The inclusion of the algae beyond 20% resulted in reduced growth performance and feed utilization efficiency of the fish. The same trend was observed on the rabbitfish *Siganus canaliculatus* fed dehydrated *Enteromorpha* (Yousif *et al*. 2004) which was partially attributed to the high ash content in the algae (23.34%) resulting from sand and shells adhering to the algae. However, this should not be a problem for the mullets which in nature thrive mainly on plant detritus and filamentous algae such as *Enteromorpha* (Brusle 1981). It could also be hypothesized that at high inclusion of the algae (30%), the anti-nutritional factors such as lectins (haemagglutinins) which was reported to occur in the algae (Naidu et al. 1993) might have impaired the nutrients absorption leading to growth depression. The poor growth of fish fed diets supplemented with different levels of *P. cineraria* pods meal could be attributed to the presence of some anti-nutritional substances. Probably like other legumenous plants the plant contains toxic substances such as tannin and memosine. Tannins are polyphenols that are able to bind and precipitate protein leading to reduction in nutrient digestibility (Cheeke and Shull 1985; Vaithiyanathan *et al.* 2006). Mimosine is a toxic non-protein amino acid that is present in the leaves, seeds and other parts of the leguminous trees and was reported to cause certain adverse effects on growth of fish and other farmed animals (Leong and Wang 1987; Santiago *et al*. 1988). The occurrence of anti-nutritional factors in different parts of *P. cineraria* is of interest and worth future investigation. The inclusion of date pits in the diets at all levels did not positively influence the growth and feed utilization efficiency of the fish. Yousif *et al.* (1996-a) attributed the poor growth of blue tilapia, *Oreochromis aureus*, fed date pits to their high fiber content. Ahmed *et al.* (1995) reported that the depitting process and storage conditions in UAE, which usually takes place on the farm, support the growth of toxigenic Aspergilli and aflatoxin production. Saad *et al.* (1989) tested the milk of camels fed with date pits in UAE and a range of 0.25-0.8 ng ml^{-1} of aflatoxin M was found. In other incidents, a level of 5-50 ng ml^{-1} aflatoxin M was reported in camels fed with soaked date pits (Osman and Abdul-Gadir 1991). This suggest that the date pits meal used in this study might have been contaminated with some mycotoxins which have somehow affected the growth of the experimentaldate pits to their high fiber content. Ahmed *et al.* (1995) reported that the depitting process and storage conditions in UAE, which usually takes place on the farm, support the growth of toxigenic Aspergilli and aflatoxin production. 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Table (1): Proximate composition (%DM basis) of feed ingredients

	Dry matter	Crude protein	Crude fat	Crude fibre	Ash	NFE [a]	Fructose	Glucose	Sucrose
E. flexuosa	91.65	11.02	0.90	8.97	23.34	55.77			
P. cineraria pods	90.13	15.13	0.80	16.22	8.19	59.66	5.9	<0.2	17.83
Date pits	95.35	6.08	7.97	18.40	2.85	64.70			
Fish meal	96.65	71.03	7.04	0.21	16.23	5.49			
Soybean meal	91.77	43.55	2.48	4.50	7.95	41.52			
Wheat flour	87.73	12.75	0.67	1.47	1.36	83.85			

[a] Nitrogen-free extract = 100 - (crude protein + crude fat + crude fibre + ash)

Table (2): Ingredient composition and proximate composition of the experimental diets

	Diet									
	1 (control)	2	3	4	5	6	7	8	9	10
Ingredients (g kg^{-1} dry weight)										
Fish meal	471.7	476.4	481.0	485.8	466.1	460.5	454.8	472.9	474.1	475.3
Soybean meal	32.1	39.8	47.5	55.1	35.8	39.5	43.2	34.1	36.1	38.1
Wheat flour	400.0	300.0	200.0	100.0	300.0	200.0	100.0	300.0	200.0	100.0
Enteromorpha	-	10.0	20.0	30.0	-	-	-	-	-	-
P cineraria	-	-	-	-	100.0	200.0	300.0	-	-	-
Date pits	-	-	-	-	-	-	-	100.0	200.0	300.0
Corn oil	38.1	38.5	35.6	29.1	42.7	47.9	52.8	47.4	46.1	55.4
Alpha cellulose	28.1	15.3	5.9	-	25.4	22.1	19.2	15.6	13.7	1.2
Vit.-mineral mix [1]	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Nutrient content (g kg^{-1} dry weight)										
Dry matter	936.7	956.2	964.7	951.0	946.3	939.7	954.4	965.4	972.7	970.2
Crude protein	413.0	404.4	413.7	424.2	417.5	397.5	399.8	413.4	420.0	420.3
Crude fat	40.1	65.7	33.8	51.9	35.8	32.1	43.7	57.9	73.4	76.5
Crude fibre	30.1	36.7	62.0	78.5	40.6	48.3	59.3	43.0	55.9	78.4
Ash	107.0	103.7	109.2	108.0	108.7	118.9	126.7	125.3	139.3	164.2

NFE	409.8	389.5	381.3	337.4	397.4	403.2	370.5	360.4	311.4	260.6
GE (kJ g^{-1})	18.35	18.82	17.63	17.83	18.08	17.57	17.51	18.22	18.15	17.40
P/E ratio (mg CP kJ^{-1})	22.51	21.49	23.47	23.79	23.09	22.62	22.83	22.69	23.14	24.16

Shrimp tonic, Jv Marine Ent. Co. Ltd, Taiwan (ingredients kg^{-1} supplement): A, 30 000 000 IU; D_3, 12 000 000 IU; B, 1250.0 mg; B_2, 12 000.0 mg; B_6, 1250.0 mg; B_{12}, 4250.0 mg; E, 12 500.0 mg; K, 5000.0 mg; C, 50 000.0 mg; Nicotinamide, 30 000.0 mg; CaO panthotecat, 15 000.0 mg; Folic acid, 1500.0 mg; Niacinamide, 20 000.0 mg; Insitol, 112 500.0 mg; Lecithin, 25 000.0 mg; Cholin, 37 500.0 mg.

Table (3): Performance of *V. seheli* fry fed the experimental diets for 49 days [a]

	Diet										
	1 (cont.)	2	3	4	5	6	7	8	9	10	± SE
Initial wt (g)	0.61	0.61	0.61	0.61	0.62	0.61	0.62	0.61	0.62	0.62	0.001
Final wt (g)	1.40^{ab}	1.40^{a}	1.52^{a}	1.26^{abc}	1.33^{ab}	1.23^{abc}	0.99^{c}	1.27^{ab}	1.25^{abc}	1.13^{bc}	0.034
Survival (%)	75.56^{bc}	75.56^{bc}	91.11^{ab}	68.89^{c}	69.89^{c}	77.78^{abc}	84.44^{abc}	91.11^{ab}	95.56^{a}	93.33^{ab}	2.290
weight gain (%)	128.48^{ab}	132.10^{a}	146.68^{a}	104.52^{abc}	114.88^{ab}	100.96^{abc}	60.12^{c}	107.86^{ab}	103.08^{abc}	83.45^{bc}	5.599
SGR (%/day)	1.68^{a}	1.77^{a}	1.83^{a}	1.45^{ab}	1.54^{ab}	1.42^{ab}	0.96^{c}	1.49^{ab}	1.44^{ab}	1.23^{bc}	0.057
Feed intake (mg/day)	67.31^{a}	66.37^{a}	68.21^{a}	60.63^{ab}	63.51^{ab}	61.63^{ab}	55.42^{b}	63.13^{ab}	62.15^{ab}	57.26^{b}	0.976
FCR	3.64^{bc}	3.53^{c}	3.26^{c}	4.04^{bc}	3.90^{bc}	4.23^{bc}	6.40^{a}	4.03^{bc}	4.14^{bc}	4.91^{b}	0.186
PER	0.67^{abc}	0.71^{ab}	0.75^{a}	0.59^{abcd}	0.56^{bcd}	0.52^{cd}	0.35^{e}	0.53^{cd}	0.51^{cde}	0.44^{de}	0.025
PPV (%)	11.79^{ab}	12.42^{a}	12.41^{a}	10.77^{abc}	9.27^{abc}	8.70^{bcd}	5.90^{d}	9.37^{abc}	8.49^{cd}	8.16^{cd}	0.446
EU (%)	13.66^{abc}	14.67^{ab}	16.67^{a}	12.00^{bc}	13.00^{abc}	12.00^{bc}	7.00^{d}	12.16^{bc}	12.12^{bc}	10.29^{cd}	0.541

[a] Figures in the same row having the same superscript are not significantly different ($P < 0.05$)

Table (4): Carcass composition (%wet weight) of experimental fish fry at the start and end of the experiment [a]

Diet	Initial	1 (cont.)	2	3	4	5	6	7	8	9	10	± SE
Moisture	71.20	66.48^{b}	66.55^{b}	65.68^{b}	66.76^{ab}	66.60^{b}	66.70^{b}	68.98^{a}	67.64^{ab}	66.22^{b}	67.90^{ab}	0.242
Crude protein	15.11	16.51*	16.47	15.94	16.73	15.87	16.00	15.84	16.56	15.92	16.63	0.118
Crude lipid	7.25	8.69^{b}	9.74^{ab}	11.19^{a}	9.52^{ab}	9.77^{ab}	8.98^{ab}	8.27^{b}	9.10^{ab}	9.49^{ab}	9.26^{ab}	0.220
Ash	4.66	5.06^{a}	5.00^{ab}	4.36^{b}	5.19^{a}	5.04^{ab}	4.83^{ab}	4.97^{ab}	5.01^{ab}	5.03^{ab}	4.82^{ab}	0.070
Energy deposition (kJ/g)	6.54	7.67^{ab}	8.33^{a}	8.41^{a}	7.72^{ab}	7.78^{ab}	7.64^{ab}	7.04^{b}	7.68^{ab}	7.80^{ab}	7.53^{ab}	0.097

[a] Figures in the same row having the same superscript are not significantly different ($P < 0.05$)
[*] Means in this row are not significantly different ($P < 0.05$)

إمكانية إستخدام دقيق طحلب الشبا ، دقيق قرنات أشجار الغاف و دقيق نوى التمر في الأعلاف المصنعة لصغار أسماك البياح العربي

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المستخلص

تم أقلمت صغار أسماك العربي على ظروف الماء العذب و من ثم تمت تجربة تغذيتها بثلاثة أعلاف مضاف لها مصادر كربوهيدراتية غير تقليدية هي دقيق طحلب الشبا المجفف ، دقيق قرنات أشجار الغاف و دقيق نوى التمر. و قد تم إضافة هذه المصادر بدلاً عن دقيق القمح بنسب صفر ، 10 ، 20 و 30%. و قد تحققت أحسن نسبة بقاء ($P < 0.05$) في المجموعة التي تمت تغذيتها بالأعلاف المضاف لها دقيق نوى التمر و قد تم تسجيل أحسن نمو و أحسن كفاءة لإستخدام العلف ($P < 0.05$) في الأسماك التي تمت تغذيتها على الأعلاف المضاف لها طحلب الشبا المجفف بمختلف النسب بينما حققت الأسماك التي تمت تغذيتها بالعلف المضاف له دقيق قرنات الغاف بنسبة 30% أضعف النتائج. محتوى البروتين في الذبيحة كان متشابهاُ في كل المعاملات. محتوى الرطوبة كان متناسباً عكسياً مع محتوي الدهن في الذبيحة في كل المعاملات. محتوى الرماد كان عالياً ($P < 0.05$) في المجموعة التي تمت تغذيتها بالعلف المضاف له طحلب الشبا بنسبة 30%. و حققت الأسماك التي تمت تغذيتها بالأعلاف المضاف لها طحلب الشبا المجفف بنسب 10 و 20% أحسن مستوى تخزين للطاقة ($P < 0.05$).