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Response of Atlantic Croaker Fingerlings to Practical Diet Formulations with Varying Protein and Energy Contents

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Abstract

Atlantic croaker *Micropogonias undulatus* is a demersal marine species that is heavily utilized as both a bait and food fish, and which may have commercial culture potential as a bait species. This research sought to initiate the development of practical diet formulations for juvenile growout and to evaluate the response of this species to controlled culture conditions. Eight practical diets were formulated to evaluate the effects of: 1) increasing lipid levels in a 45% protein diet; 2) dietary protein levels of 45, 40, 35 and 30%; and 3) progressive reduction in dietary energy content of diets with 30% protein. Each diet was offered to four replicate groups of fish having a mean initial weight of 3.8 g over a 7-wk growth trial. At the conclusion of the trial, final mean weights ranged from 17.0 g (410% weight gain) to 8.1 g (210% weight gain) with 100% survival for all dietary treatments. Weight gain and feed efficiency increased with protein content of the diet, with fish fed the 45% protein diet performing the best. Weight gain of fish maintained on the 45% protein diet decreased, but not significantly, as the lipid content of the diet was increased from 8 to 12 or 16%. Feed efficiencies for fish maintained on the diet containing 8% lipid were significantly higher than that of fish maintained on the diet containing 16% lipid. In low protein diets, the replacement of wheat starch with indigestible fiber did not significantly influence growth or feed utilization, indicating that high levels of digestible carbohydrate were not well utilized as an energy source by the fish. The observed results would indicate that growth is optimized on a high protein diet with moderate lipid levels.

Atlantic croaker *Micropogonias undulatus* belongs to the family Sciaenidae. This demersal sciaenid is most abundant from Chesapeake Bay to Florida and along the northwestern Gulf of Mexico (Ross 1988). This species is abundant in the bay systems year-round and is heavily utilized as both a bait and food fish. The southeastern U.S. commercial croaker fishery reported an average yield of approximately 5,000 metric tons of food fish during 1988–1990 and supported a sport fishery of about equal catch (NOAA 1991). The commercial ground fishery between Mobile Bay, Alabama, and Trinity Shoals, Louisiana, was reported in 1976 to land about 50,000 tons annually with Atlantic croaker representing about 70% of the landings (Gutherz 1976). Moreover, the Atlantic croaker is one of the primary species making up the bulk of the offshore bycatch of finish caught in the shrimp fishery (NOAA 1991) for which landings have been estimated at more than 200,000 tons (Gutherz 1976). With continuing increases in fishing pressure, habitat destruction, and environmental pollution, the long term health of this fishery may be compromised. The development of culture techniques for this and other species will allow an alternative to harvesting wild stocks, diversify aquaculture, and expand our understanding of this species. However, to successfully culture a species, considerable information must be obtained on major life stages, environmental requirements, and nutritional needs.

Although there are few studies on the culture of Atlantic croaker, this species has been extensively used in laboratory-based toxicology and reproductive physiology studies (Thomas 1990, 1994). Techniques for the cryopreservation of spermatozoa

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(Gwo et al. 1991; Gwo and Arnold 1992) and the use of hormones to induce spawning (Middaugh and Yoakum 1974) have also been published. Additionally, results from a preliminary growout trial indicated that bait fish (mean wt 24 g, total length 122 mm) can be mass produced after 140 d of culture. These fish were readily accepted by fishermen and commercial bait stands which generally retail bait fish for $0.25 each. Consequently, the development of a juvenile production facility is technically feasible; however, there is little information on the nutritional requirements or the growth rates of juveniles under controlled conditions. This research sought to initiate the development of practical diet formulations for this species and to evaluate the growth potential under controlled conditions.

Materials and Methods

Atlantic croaker eggs were obtained from broodstock maintained by Texas Parks and Wildlife, GCCA/Central Power and Light Marine Development Center. The eggs were hatched and the larvae reared under controlled culture conditions similar to those used for red drum (Holt et al. 1990). After 78 d of culture, juveniles were hand-graded to a uniform size and stocked into a semi-closed recirculation system consisting of 32 culture chambers (105-L), sand filter, biological filter and circulation pump. Fish were acclimated to experimental conditions for 5 d during which they were offered a commercial trout feed (Rangen Inc., Buhl, Idaho, USA). Ten fish (mean weight 3.8 g/fish) were stocked into each tank and diets randomly assigned to four replicate tanks per treatment. Ten fish from the remaining population were randomly collected and frozen at −70 C for subsequent proximate analysis. Each diet was fed twice daily to the fish on a dry-matter basis over the 7-wk feeding trial. To minimize overfeeding, while maintaining a level approaching satiation, the initial feeding rate of 8% of body weight per day was progressively reduce to 4.5% of body weight per day. Feeding rates were fixed for all treatments during a given week and were reduced at the end of each week based on growth, weekly feed efficiency values, and observations of feeding. Daily feed rations were adjusted weekly based on weekly feeding rate and weekly weights of the fish. Photoperiod was set for a 12:12 h light dark cycle. The fish were counted and weighed, and the culture chambers were scrubbed on a weekly basis. System maintenance such as siphoning of settled solids was conducted as needed. Salinity, temperature, and dissolved oxygen were measured daily; total ammonia-nitrogen, nitrite-nitrogen, and pH were measured twice weekly using photometric methods (Spotte 1979). Water quality parameters (mean ± standard deviation) were as follows: temperature, 28.6 ± 1.2 C; salinity, 33.2 ± 1.7 ppt; dissolved oxygen, 5.8 ± 0.7 ppm; total ammonia-nitrogen, 0.07 ± 0.07 ppm; nitrite-nitrogen, 0.07 ± 0.07 ppm; pH, 8.0 ± 0.18. At the conclusion of the feeding trial, weight gain, survival and feed utilization (based on offered feed) were evaluated.

Eight practical diets (Table 1) were formulated to evaluate the effects of: 1) increasing lipid levels in a 45% protein diet (diets 1–3); 2) dietary protein levels of 45, 40, 35 and 30% (diet 1, 4, 5 and 6, respectively); and 3) progressive reduction in energy:protein ratio of 30% protein feed (diets 6-8). Feed was prepared during the acclimation period. Coarse ingredients were ground with a laboratory hammer-type mill with a #40 mesh screen (1.02-mm diameter hole). Dry ingredients and oil were mixed in a food mixer (Hobart Corp., Troy, Ohio, USA) for 20 min. Hot water was then blended into the mash to attain a consistency appropriate for pelleting. Each feed was extruded through a 3-mm die in a meat grinder, and the pellets were dried to a moisture content of less than 10%. Protein content was confirmed by micro-Kjeldahl analysis (Ma and Zuazago 1942). Diets were stored in a refrigerator and were crum-

TABLE 1. *Composition of experimental diets (g/100 g dry weight).*

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Menhaden fish meal[a]	30.0	30.0	30.0	26.7	23.3	26.6	26.6	26.6
Soybean meal[b]	51.1	51.1	51.1	45.4	39.7	25.1	25.1	25.1
Wheat starch[c]	9.4	5.4	1.4	19.4	29.5	41.8	26.8	11.8
Menhaden fish oil[d]	4.0	8.0	12.0	3.0	2.0	1.0	1.0	1.0
Soy lecithin[e]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fish solubles[h]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineral premix[f]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[g]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin C[h]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium phosphate, monobasic, monohydrate[i]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Alphacellulose	—	—	—	—	—	—	15.0	30.0
Formulated to contain:								
Protein	45	45	45	40	35	30	30	30
(analyzed value)	(43.6)	(43.6)	(43.4)	(39.6)	(34.1)	(29.4)	(29.9)	(30.0)
Lipid	8.0	12.0	16.0	6.5	5.1	4.2	4.2	4.2
Energy (Kcal/100 g diet)[j]	402	422	442	397	392	387	327	267
Energy : protein ratio (Kcal/g protein)	9.0	9.4	9.8	9.9	11.2	12.9	10.9	8.9

[a] Special Select TM, Zapata Haynie Corporation, Hammond, Louisiana, USA.
[b] Solvent extracted, Jupe Mills, Inc., West, Texas, USA.
[c] United States Biochemical Corporation, Cleveland, Ohio, USA.
[d] Zapata Haynie Corporation, Reedville, Virginia, USA.
[e] Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, Indiana, USA.
[f] g/kg premix: Cobalt chloride 0.004; Cupric sulfate pentahydrate 0.250; Ferrous sulfate 4; Magnesium sulfate heptahydrate 28.398; Manganous sulfate monohydrate 0.650; Potassium iodide 0.067; Sodium selenite 0.010; Zinc sulfate heptahydrate 13.193; Filler 53.428.
[g] g/100 g premix: Thiamin HCL 0.5; Riboflavin 3; Pyroxidine HCL 1; Dl Ca-Pantothenate 5; Nicotinic acid 5; Biotin 0.05; Folic acid 0.18; Vitamin B12 0.002; Choline chloride 100; Inositol 5; Menadione 2; Vitamin A acetate (20,000 IU/g) 5; Vitamin D3 (400,000 IU/g) 0.002; dL-alpha-tocopherol acetate (250 IU/g) 8; Alpha-cellulose 865.266.
[h] Stay C, L-Ascorbyl-2-Polyphosphate, Hoffman-LaRouche, Inc., Nutley, New Jersey, USA.
[i] Spectrum Chemical Mfg. Corp., Gardendale, California, USA.
[j] Energy values are estimated based on 4 kcal/g for protein and carbohydrate sources and 9 kcal/g for lipid sources.

bled and sieved to the desired size before use.

At the conclusion of the growth trial, four fish were randomly selected from each tank, homogenized and frozen for subsequent whole-body analyses. Samples from the start of the experiment were analyzed with samples obtained at the conclusion of the growth trial. Dry matter was determined by drying to constant weight at 100 C. Protein content was determined by the micro-Kjeldahl method. Percent lipid was determined by the method described by Folch et al. (1957). All analyses were conducted with duplicate subsamples from each tank. Feed efficiency (FE) and protein conversion efficiencies (PCE) were determined for each feed type. Feed efficiency was calculated as (wet weight gain/dry weight feed offered) × 100. Protein conversion efficiency (PCE) was calculated as (protein gain/protein offered) × 100.

All data were subjected to one-way analysis of variance (ANOVA) to determine significant ($P < 0.05$) differences among treatment means, and the Student-Neuman Keuls' multiple-range test (Steel and Torrie 1980) was used to distinguish significant

TABLE 2. *Growth response and body composition of Atlantic croaker fed isonitrogenous (45% protein) diets containing three levels of lipid. Means of four replicates. Numbers within the same column with different superscripts are significantly different* ($P < 0.05$).

Diet	Lipid	Weight gain (g)	FE (%)[1]	PCE (%)[2]	Whole body analyses (%) Dry matter	Protein[3]	Lipid[3]
1[4]	8.0	17.0^{z}	82.1^{z}	33.3^{z}	30.7^{z}	56.4^{z}	23.3^{z}
2	12.0	13.2^{z}	69.9^{yz}	27.5^{z}	29.7^{z}	56.6^{z}	25.6^{z}
3	16.0	12.1^{z}	63.0^{y}	23.6^{z}	30.7^{z}	52.3^{y}	27.8^{z}
PSE[5]		1.53	4.32	2.42	0.63	1.05	2.18

[1] Feed efficiency (FE) = wet weight gain/dry weight of feed offered × 100.
[2] Protein conversion efficiency (PCE) = protein gain/protein offered × 100.
[3] Expressed on a dry weight basis.
[4] Means of three replicates.
[5] Pooled standard error.

differences between treatment means. Statistical analyses were conducted using the SAS® System for Windows™ (v6.1, SAS Institute Inc., Cary, North Carolina).

Results

No notable problems with food acceptance or adaptation to the experimental conditions and protocol were observed after transferring juveniles to the research system. There were no mortalities observed during the initial acclimation period or the 7-wk growth trial.

The response of Atlantic croaker juveniles to diets containing 45% protein with three levels of lipids are presented in Table 2. Of the parameters measured, significant differences among treatment means were observed only for FE and protein content of whole body samples. There were no significant differences in weight gain data, which ranged from 17.0 to 12.1 g. However, plots of the growth data indicated diverging patterns, and there was a negative correlation (pearson correlation coefficient = 0.053) of weight gain to dietary lipid content. The FE for fish offered the diet containing 8% lipid was 82.1% which was significantly higher than that of fish fed the diet containing 16% lipid (63.0%). Fish fed the diet containing 12% lipid had FE of 69.9% which was not significantly different from the other two treatments. The PCE for fish maintained on the various diets ranged from 33.3% for fish offered the diet containing 8% lipid to 23.6% for the diet containing 16% lipid. There were no significant differences in dry matter content of whole body samples which ranged from 30.7% to 29.7%. Whole body protein content was significantly lower for fish fed diets containing 16% lipid as compared to diets containing 8% and 12% lipid. Although, there were no significant differences in lipid content of whole body, the lipid level increased from 23.3% in fish fed the 8% lipid diet to 27.8% for fish fed the diet containing 16% lipid.

The response of Atlantic croaker juveniles to diets containing various levels of protein is presented in Table 3. Of the parameters measured, significant difference among treatment means was observed for FE, weight gain and whole-body dry matter. Final weight gain of the fish maintained on the various diets ranged from 17.0 to 11.0 g for fish maintained on the high and low protein diets, respectively. Weight gain of fish maintained on diets containing 45% protein was significantly higher than that for fish fed diets containing 30 or 35% protein, but was not significantly different from that of fish fed the 40% protein diet. Feed efficiencies were also significantly greater for fish maintained on diets containing 45% protein than fish maintained on diets con-

TABLE 3. *Growth response and body composition of Atlantic croaker fed diets containing four levels of dietary protein. Menas of four replicates. Numbers within the same column with different superscripts are significantly different* ($P < 0.05$).

Diet	Protein	Weight gain (g)	FE (%)[1]	PCE (%)[2]	Whole body analyses (%) Dry matter	Protein[3]	Lipid[3]
1[4]	45	17.0^{z}	82.0^{z}	33.3^{z}	30.7^{z}	56.4^{z}	23.3^{z}
4	40	13.6^{yz}	76.3^{yz}	29.9^{z}	29.0^{z}	53.5^{z}	20.3^{z}
5	35	11.8^{y}	66.5^{y}	31.2^{z}	28.9^{z}	55.1^{z}	22.2^{z}
6	30	11.0^{y}	64.0^{y}	33.6^{z}	28.1^{y}	55.2^{z}	20.5^{z}
PSE[5]		1.32	3.58	1.93	0.70	1.05	1.57

[1] Feed efficiency (FE) = wet weight gain/dry weight of feed offered × 100.
[2] Protein conversion efficiency (PCE) = protein gain/protein offered × 100.
[3] Expressed on a dry weight basis.
[4] Means of three replicates.
[5] Pooled standard error.

taining 30% or 35% protein. The dry matter content of fish fed the diet containing 30% protein was significantly lower than values determined for fish fed the other diets. There were no significant differences or notable trends in the PCE values or whole body lipid and protein content among fish fed the various diets.

The response of the fish to diets containing reduced levels of wheat starch are presented in Table 4. Of the parameters measured, there were no significant differences among treatment means. Final weight gain of the fish ranged from 8.1 g for diets supplemented with 15% indigestible fiber (diet 7) to 11.0 g for fish fed the 30% protein diet without added fiber (diet 6). Feed efficiency and PCE values ranged from 54.6% to 64.0% and 28.9% to 33.6% for fish offered diets containing 15% supplemental fiber and diets without added fiber, respectively. There were no significant differences or notable trends in the proximate composition data for whole body samples for fish maintained on these diets.

Discussion

This research provided initial data to evaluate varying levels of dietary lipid, protein and carbohydrates for Atlantic croaker juveniles. In practical diets, lipids are generally optimized towards providing a source of energy to help spare protein and hence increase the efficiency of protein retention. In the present experiment, three levels (8, 12 and 16 g/100 g diet) of dietary lipid

TABLE 4. *Growth response and body composition of Atlantic croaker to the replacement of wheat starch with undigestible fiber in a low protein (30%) diet. Means of four replicates. Numbers within the same column with different superscripts are significantly different* ($P < 0.05$).

Diet	E:P ratio (Kcal/g protein)	Weight gain (g)	FE (%)[1]	PCE (%)[2]	Whole body analyses (%) Dry matter	Protein[3]	Lipid[3]
6	12.9	11.0^{z}	64.0^{z}	33.6^{z}	28.1^{z}	55.2^{z}	20.5^{z}
7	10.9	8.1^{z}	54.6^{z}	28.9^{z}	27.6^{z}	57.0^{z}	21.2^{z}
8	8.9	9.2^{z}	60.9^{z}	31.4^{z}	27.2^{z}	56.9^{z}	20.0^{z}
PSE[4]		0.90	3.17	1.92	0.25	1.12	1.97

[1] Feed efficiency (FE) = wet weight gain/dry weight of feed offered × 100.
[2] Protein conversion efficiency (PCE) = protein gain/protein offered × 100.
[3] Expressed on a dry weight basis.
[4] Pooled standard error.

were evaluated in a high protein diet. In general, Atlantic croaker juveniles responded negatively to increasing lipid content of the diet (Table 2). Although differences were not significant, there was a general trend of decreasing final weight of the fish and PCE values, as well as rising lipid content of the fish which corresponded to increases in lipid content of the diet. These marginal responses corresponded to significant reductions in FE values and changes in the protein content of the fish. Although, this response should be evaluated with further research, the response indicates that Atlantic croaker juveniles perform best at moderate levels of lipids and that supplementation of high levels of lipid to the diet may effect growth and body composition. A similar response has been reported for other warm water marine species such as the red drum (Williams and Robinson 1988) and Florida pompano *Trachinotus carolinus* (Williams et al. 1985). Consequently, it is recommended that warm water fish feeds, which generally contain 8% or less lipid, should be preferentially utilized over cold water feeds which generally contain significantly higher levels of lipid.

In practical diet formulations for fish, protein constitutes one of the major nutrients and ingredient costs. To minimize feed costs, it is important to optimize dietary protein levels and utilization by the fish. In the present experiment, Atlantic croaker juveniles responded negatively to reductions in dietary protein and/or to increases in the E:P ratio (Table 3). Significant reductions in weight gain and FE values were observed as protein content was reduced from 45 to 35 g/100 g diet and E:P ratio was increased from 9 to 11 kcal/g protein. Since there were no significant differences in PCE values or protein and lipid content of the fish, it would appear that the energy was not limiting and that excess levels of energy did not influence proximate composition of the fish. The observed depressions in growth rates are probably due to reductions in protein intake, resulting from fixed feeding rates and reductions in protein levels of the feed. These results are consistent with those found for the red drum by Daniels and Robinson (1986) who reported increased FE with increasing dietary protein, and Serrano et al. (1992) who reported an increase in FE with increasing protein and lipid levels.

The utilization of low protein diets has several problems. These diets contain high levels of carbohydrates, which are generally considered poorly utilized by fish (Wilson 1994) and hence add to the quantity of undigested material entering the culture system. Additionally, low protein feeds generally have high E:P ratios which may reduce feed consumption, resulting in a reduction in nutrient intake and subsequently growth. Reducing the energy content of low protein practical diets could minimize adverse affects on growth rates. Consequently, the final component of this research evaluated the replacement of the carbohydrate source in the low protein feed with indigestible fiber resulting in a reduction of the E:P ratio from 12.9 to 8.9 kcal/g protein.

Under the reported conditions, the addition of 15 or 30 g alpha-cellulose/100 g diet as a replacement of wheat starch did not affect growth, feed conversion values or proximate composition of the fish. This result could be due to a variety of factors, such as differences in consumption or digestion of the test diets, which are beyond the scope of this study. However, results imply that high levels of carbohydrate were not well utilized as an energy source by the fish and that dietary E:P ratio above 8.9 kcal/g may not be required in low protein feeds. Consequently, the optimal E:P ratio for this species is probably similar to the 8–9 kcal/g protein that has been reported for other species (National Research Council 1983).

Of the diets tested, the best response occurred with fish fed the 45% protein diet containing 8% lipid, resulting in a mean final weight gain of 20.8 g (weight gain

17.0). Red drum juveniles maintained under similar laboratory conditions and diets have reached a final weight of approximately 40 g in the same period of time, indicating that juveniles of this species may not grow as fast as red drum.

Conclusion

Based on the observed results, Atlantic croaker juveniles adapted quickly to the culture conditions and practical diet formulations. Juveniles responded positively to increasing levels of dietary protein and negatively to increasing lipid content of the 45% protein diet, indicating that growth rates will be maximized on high protein diets with moderate lipid levels. The response of fish on the high protein diet to increasing energy levels and those of fish maintained on the low protein diet to reductions in energy indicate that the optimal dietary E:P ratio of approximately 9 kcal energy/g protein is required. The response to dietary manipulations was similar to those found with red drum, a closely related species, for which considerable nutritional information is available. Consequently, nutritional data from the red drum should be utilized as a model until nutritional requirements specific to this species can be determined. Based on the positive results of this study, this species adapts well to culture conditions and should be considered a suitable candidate for commercial culture as bait or possibly a food fish species. Based on these results, commercial feed formulations for warm water fish species such as the hybrid striped bass or red drum, which generally contain high levels of protein and moderate levels of lipid, are recommended. Further studies are suggested to define culture requirements, production costs and the nutritional requirements of this species.

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