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## Optimization of Feed Formulation for Mature Female Striped Bass

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**Abstract.**—To optimize the essential amino acid (EAA) profile for mature striped bass *Morone saxatilis*, diets with graded levels of three essential amino acids—lysine (Lys), threonine (Thr), and total sulfur amino acids (Tsaa) methionine and cysteine—were fed to 4-year-old striped bass females. The relative concentrations of these EAAs were maintained in ratios similar to those determined from the analysis of striped bass skeletal muscle, (i.e., 2.2:1.1:1.0 [Lys:Thr:Tsaa]). All other dietary EAAs were maintained above the requirement levels published for juvenile striped bass. Three diets with graded levels of Lys, Thr, and Tsaa were fed to mature female striped bass for 11 weeks. Statistical analysis of weight gain, specific growth weight (percent per day), and feed conversion ratio (weight of feed fed/weight gained) indicated significant differences ( $\alpha = 0.05$ ) among treatments. However, there were no significant differences in whole-body proximate composition, egg size, or gonadosomatic index among fish fed the three dietary treatments. The results of this experiment suggest that the dietary amino acid profile required for optimal growth and feed efficiency in mature female striped bass is similar to the requirement profile for juvenile striped bass.

### Introduction

Domestication of striped bass *Morone saxatilis* is of great significance to the striped bass aquaculture industry (Woods et al. 1999). In the United States, production of hybrid striped bass, a cross between striped bass and white bass *M. chrysops*, increased nearly 10-fold over the past 10 years to 8.4 million pounds in 1997 (SBGA 1998). To identify and rank future research priorities for improving the striped bass aquaculture industry, the Striped Bass Growers Association ranked nutrition and feeding as second in importance after year-round fingerling availability (SBGA 1998). To make significant progress toward closing the life cycle of striped bass, nutrition that facilitates adequate growth and reproduction in captivity is essential.

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Perhaps the most researched dietary consequence concerning broodfish nutrition is the lipid fraction of the diet. Altering the lipid composition of broodfish diets has been shown to affect egg quality and viability in gilthead seabream *Sparus aurata* and the European seabass *Dicentrarchus labrax* (also known as the European bass *Morone larax*) (Harel et al. 1994; Carrillo et al. 1995). Eggs from captive striped bass fed commercial salmonid diets, when compared with those obtained from wild striped bass of the same geographic strain, had significantly lower total lipid, eicosapentaenoic, docosahexaenoic, as well as n-3 highly unsaturated fatty acids (where “n” designates the position of the first double bond from the methyl end) (Harrell and Woods 1995).

Proteins and amino acids have received only a limited study in broodfish nutrition. Amino acids play a vital role in virtually all metabolic processes and are essential components of the many biosynthetic activities necessary during the early stages of embryogenesis (Metcoff 1986). However, little is known concerning the amino acid requirements of reproductively mature fish.

The essential amino acid requirements of juvenile striped bass have been determined using ratios of amino acids in skeletal muscle as a model (Small and Soares 1998) and verified in individual dose–response feeding trials (Small and Soares 1999, in press). However, whether mature striped bass require dietary amino acids in the same pattern as juveniles is unknown. The objective of this experiment was to estimate the optimal balance of amino acids required by mature female striped bass.

### Methods

**Diets.**—Experimental diets, as formulated, contained graded levels of lysine (Lys), threonine (Thr), and total sulfur amino acids (Tsaa; Table 1). The ratio of Lys: Thr: Tsaa was maintained at 2.2: 1.1:1.0, a ratio similar to the requirement levels determined for juvenile striped bass (Small and Soares 1998). As the concentrations of Lys, Thr, and Tsaa were uniformly increased in the diet, so

TABLE 1.—Composition of diets for estimating the optimal dietary amino acid profile for mature female striped bass.

Ingredient	Dietary percentages		
	Diet 1	Diet 2	Diet 3
Herring fish meal	25.0	29.6	30.0
Menhaden fish solubles <sup>a</sup>	2.0	2.0	3.0
Corn gluten meal	16.0	15.0	16.0
Wheat middlings	30.0	30.0	30.0
Blood meal	0	0	2.0
Wheat flour	10.0	5.0	6.3
Menhaden fish oil <sup>a</sup>	2.3	2.0	0
Squid oil	7.8	7.0	7.0
Lignin sulfonate	2.0	2.0	2.0
Cellulose	2.0	4.1	0.3
Mineral premix <sup>b</sup>	1.2	1.2	1.2
Vitamin premix <sup>c</sup>	1.0	1.0	1.0
Choline chloride, 77%	0.2	0.2	0.2
Lysine HCl	0.5	0.9	1.0
Amino acids			
Lysine	1.83	2.28	2.53
Threonine	0.90	1.12	1.24
Total sulfur amino acids <sup>d</sup>	0.84	1.05	1.16

<sup>a</sup> Omega Protein Corp., Reedville, Virginia.

<sup>b</sup> Contains (as mg/kg of diet): KCl, 5,200; NaCl, 3,600; MgSO<sub>4</sub>, 1,640; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, 92; MnSO<sub>4</sub>, 80; ZnCO<sub>3</sub>, 100; CuSO<sub>4</sub>, 3.2; KI, 0.4; Na<sub>2</sub>SeO<sub>3</sub>, 0.328.

<sup>c</sup> Contains (as mg/kg of diet, unless otherwise noted) choline chloride, 3,465; calcium ascorbate 2-monophosphate, 1,000; inositol, 396; niacin, 153;  $\alpha$ -tocopheryl acetate, 45; calcium pantothenate, 50.4; riboflavin, 20.7; menadione sodium bisulfate, 9.9; thiamin, 12.6; pyridoxine-HCl, 12.6; cyanocobalamin (3,00  $\mu$ g/g), 5.8; folic acid, 5.4; retinyl acetate, 3.9; biotin, 4.5; cholecalciferol, 5  $\mu$ g/kg; ethoxyquin (antioxidant), 125.

<sup>d</sup> Methionine plus cysteine.

were the other essential amino acids, at levels equal to or slightly higher than the ratio defined by the profile of amino acids in striped bass filets (Small and Soares 1998). Diets were maintained isoenergetic by compensating for increases in amino nitrogen with decreases in lipids and carbohydrates. Crude protein in the diets, as fed, ranged from 34% to 39%. Diets were extruded through a 1.27-cm die with a laboratory extruder (C. W. Brabender Instruments, Inc., Hackensack, New Jersey) after the addition of 20% moisture and were then allowed to air dry to approximately 10% moisture.

**Husbandry and sampling procedures.**—Four year-old, F<sub>2</sub> generation, Chesapeake Bay-strain female striped bass were reared to maturity at the University of Maryland, Department of Animal and Avian Sciences' Crane Aquaculture Facility. Thirty-six fish, averaging 2.1 kg/fish, were maintained in nine 2,500-L circular tanks (four fish per tank). Each experimental tank was supplied with recirculated water (4‰ salinity and 22  $\pm$  1°C). For 1 month before starting the experiment, all fish

were fed a common commercial diet. Each fish was tagged with a passive integrated transponder (Destron/IDI, Boulder, Colorado) for individual identification.

After 4 weeks of acclimation, all bass were anesthetized in a 70-mg/L solution of Quinaldine (Eastman Kodak, Rochester, New York) and individually weighed (grams). Fish were similarly weighed at 4, 8, and 11 weeks. Throughout the growth trial, the three dietary treatments (90, 100, and 110% of juvenile striped bass amino acid requirements) were fed by hand twice daily to satiation to triplicate tanks, and the total feed intake was recorded. At the conclusion of the experiment, three fish per treatment were euthanized by overdose of tricaine methanesulfonate (MS-222); after determining their egg size and gonadosomatic index (GSI), they were stored at -20°C for subsequent proximate analysis of whole carcass.

**Analytical procedures.**—Samples for chemical analysis were first weighed and dried to obtain total moisture and then finely ground. Finished diets were analyzed for amino acid concentrations. Samples were hydrolyzed in duplicate in 6 N HCl at 110°C for 24 h before chromatographic separation using a Dionex D-600 amino acid analyzer (Dionex, Smyrna, Georgia). For methionine and cysteine, samples were first treated with performic acid to oxidize methionine and cystine to methionine sulfone and cysteic acid before the hydrolysis (Schram et al. 1954; Moore and Stein 1963). Proximate analysis for protein, moisture, and ether extract in finished diets and fish tissue were determined by micro-Kjeldahl analysis; standard methods were used for proximate analysis (AOAC 1984).

Data were analyzed by analysis of variance (ANOVA) mixed-model procedures (SAS Institute 1992). Pairwise contrasts were used to identify significant differences at the 5% level between the means of the dependent variable for different amino acid levels.

### Results and Discussion

Striped bass fed diet 1 (Lys, Thr and Taa) at 90% of the requirement levels reported for juvenile striped bass; Small and Soares 1998) for a period of 11 weeks demonstrated significantly ( $P < 0.05$ ) reduced weight gain, specific growth rate (logarithmic growth per unit time), and feed conversion ratios (weight of food fed/weight gained) (Table 2). No significant differences ( $P > 0.05$ ) were found between fish fed diets 2 and 3 (100% and 110% of the dietary requirement levels of Lys, Thr,

TABLE 2.—Performance, carcass composition, egg size, and gonadosomatic index of reproductively mature female striped bass fed diets containing increasing levels of amino acids for 11 weeks. Means ( $N = 3$ ) within rows followed by the same letter are not statistically different ( $P < 0.05$ ).

	Diet			Pooled SE
	1	2	3	
Weight gain (g)	195 z	281 zy	354 y	36
SGR (%/d) <sup>a</sup>	0.16 z	0.21 y	0.23 y	0.02
FCR <sup>b</sup>	3.92 z	2.56 y	2.33 y	0.37
Carcass composition				
Moisture (%)	62.2	63.5	64.2	0.9
Protein (%)	19.3	19.2	19.0	0.3
Lipid (%)	15.9	15.4	14.6	0.6
Reproductive effort				
Egg size (mm)	799	805	825	105
GSI <sup>c</sup>	6.07	6.17	6.01	1.93

<sup>a</sup> Specific growth rate (SGR) =  $[\log_e(\text{final weight}) - \log_e(\text{initial weight})/77 \text{ d}] \times 100$ .

<sup>b</sup> Feed conversion ratio (FCR) =  $[\text{weight of feed consumed (g as fed)}/\text{wet weight gain}] \times 100$ .

<sup>c</sup> Gonadosomatic index (GSI) =  $[\text{gonad weight (g)}/\text{body weight (g)}] \times 100$ .

and T<sub>50</sub> for juvenile striped bass). Although increased growth and feed utilization were significant, no significant differences were found for carcass proximate composition, egg size, or GSI among the treatments.

Fish, like other animals, eat to satisfy their requirements for energy (Page and Andrews 1973). Therefore, it is expected that, as fish age and metabolic energy demands decrease, there will be a corresponding decrease in consumption as a percentage of biomass. Although the daily quantitative amino acid requirements may decrease, the dietary concentration relative to dietary energy required by mature fish would probably be similar to that required by juvenile fish, assuming little or no effect from gamete production. In this study, the gonads accounted for approximately 6% of fish biomass. Skeletal muscle, on the other hand, accounts for 50–60% of the mass of the fish (Houlihan et al. 1986).

It has been suggested that the EAA requirements of fish are related to the amino acid pattern in skeletal muscle protein because muscle is the major product in immature, growing fish (Cowey and Luquet 1983). This reflects the concept that the amino acid requirements of a growing animal might be determined by the amino acid composition of the protein formed during growth (Mitchell 1950). Various investigators have utilized this concept to formulate test diets for new aquaculture species (Arai 1981; Ketola 1982; Ogata et al. 1983).

Quantification of the EAA requirements for juvenile striped bass was first reported as a function of their muscle amino acid profile (Small and Soares 1998) and has since been verified with age-0

striped bass through dose–response trials with individual amino acids (Small and Soares 1999, in press). Mitchell's (1950) concept that an animal's amino acid requirements might be determined by the amino acid composition of the protein formed applies to the growing animal. How this concept applies to a reproductively active yet still growing fish is unknown. It has been demonstrated in Nile tilapia *Oreochromis niloticus* that dietary protein level influences the amount of protein and, thus, total amino acid pool in the eggs; however, dietary protein level had no effect on gonadosomatic index, relative fecundity, or egg size (Gunasekera et al. 1996, 1997). This agrees with the striped bass data presented here. Similar observations indicating no effect of dietary protein on egg size have been reported for red sea bream *Pagrus major* and European sea bass (Watanabe et al. 1984; Cerda et al. 1994).

The present study provides some indication of the minimal dietary amino acid levels that are required to sustain growth and gamete production in mature striped bass females and suggests that the pattern of amino acids required is similar to that of juvenile striped bass. This does not imply that these diets meet all the nutrient requirements necessary for production of high-quality eggs and larvae from striped bass. Further research is necessary to define the optimal dietary nutrient requirements of striped bass broodstock.

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