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Quantitative Dietary Threonine Requirement of Juvenile Striped Bass *Morone saxatilis*

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Abstract

A 10-wk feeding trial was conducted to quantify the dietary threonine requirement of juvenile striped bass *Morone saxatilis*. The basal diet was analyzed to contain 42% crude protein with a calculated digestible energy (DE) level of 3,200 kcal/kg. L-threonine was added to the basal diet to yield five dietary treatments of 0.61, 0.81, 1.00, 1.18, and 1.40% available threonine on a dry-matter basis. Weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and apparent nitrogen utilization (ANU) were significantly ($P < 0.05$) affected by dietary threonine concentrations. Least-squares regression analysis of weight gain, SGR, FCR, and ANU indicated dietary threonine requirements (±SE) of 1.00 ± 0.19, 0.91 ± 0.11, 1.06 ± 0.09 and 1.13 ± 0.22% of dry diet, respectively. From these results, the average recommended dietary threonine requirement of juvenile striped bass is 1.03% of dry diet, 2.45% of dietary protein or 3.22 mg/kcal DE.

Determining the essential amino acid requirements of cultured fish is of extreme importance due to the significant effects of these nutrients on muscle deposition, feed costs and nitrogen pollution. The complete quantitative essential amino acid requirements have been published for a limited number of cultured fish species. These include rainbow trout *Oncorhynchus mykiss* (Ogino 1980), Nile tilapia *Oreochromis niloticus* (Santiago and Lovell 1988), catla *Catla catla* (Ravi and Devaraj 1991), common carp *Cyprinus carpio* (Nose 1979), Japanese eel *Anguilla japonica* (NRC 1993), channel catfish *Ictalurus punctatus* (NRC 1993), chinook salmon *Oncorhynchus tshawytscha* (NRC 1993), coho salmon *Oncorhynchus kisutch* (Arai and Ogata 1993), chum salmon *Oncorhynchus keta* (Akiyama and Arai 1993), milkfish *Chanos chanos* (Borlongan and Coloso 1993), and white sturgeon *Acipenser transmontanus* (Ng and Hung 1995).

Amino acid requirements of the striped bass *Morone saxatilis* have been predicted using ratios of amino acids in the muscle as a model (Small and Soares 1998). The predicted dietary threonine requirement using this methodology was 1.1% of dry diet. The dietary threonine requirement for sunshine bass *M. chrysops* ♀ × *M. saxatilis* ♂ has been reported as 0.9% of dry diet (Keembiyehetty and Gatlin 1997). From these data and average amino acid concentrations in common feed ingredients, it can be concluded that threonine is the second or third limiting amino acid in practical feeds for the striped bass and its hybrids. Thus, the purpose of this study was to quantify and verify our previous dietary threonine requirement estimates for the striped bass via a traditional dose-response feeding trial.

Materials and Methods

Juvenile striped bass were obtained from Blackwater Fisheries Research and Development Center, Holt, Florida, USA as fry, and reared in circular tanks to the sizes indicated. Striped bass with an average weight of 2.7 g per fish were stocked in 19-L tanks at 10 fish per tank. For 2 wk prior to starting the experiment, all fish were fed a common commercial diet.

Fish were reared in tanks supplied with dechlorinated water in a flow-through sys-

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tem at a rate of two complete turnovers per day. Calcium chloride and sodium chloride (9 : 1), as a solution, were injected directly into the incoming water line at a continuous rate in order to achieve a minimum concentration of 36 ppm calcium in the rearing tanks. A photoperiod of 12L : 12D was maintained throughout both experiments. Water temperature was maintained at approximately 23 C and recorded four times daily via a central computerized monitoring system (REES Scientific, Trenton, New Jersey, USA). Various other conditions were monitored weekly throughout the experiments to insure safe levels of pH, dissolved oxygen, ammonia, chlorine, and Ca/hardness. The environmental conditions were maintained at safe levels as outlined by Nicholson et al. (1990).

Experimental diets were extruded through a 2-mm die with a meat grinder (KRUPS North America, Peoria, Illinois, USA) and dried for 6 h in an oven at 60 C. Diets were determined to contain 42% crude protein by microKjeldahl analysis and calculated to contain 3200 kcal DE/kg using averages of published digestible energy (DE) values for rainbow trout, chinook salmon and channel catfish (NRC 1993). Menhaden fish meal and corn gluten meal, having a combined true digestibility of greater than 90% (Small et al. 1999), provided the source of intact protein in the basal diet (Table 1). Crystalline amino acids provided the remaining protein in the diets (Table 2). The essential amino acid premix was formulated to contain amino acid concentrations in a pattern similar to that found in striped bass muscle (Small and Soares 1998). A nonessential amino acid (NEAA) premix was added to the diet to maintain the proper balance of EAA : NEAA (Cowey 1995). The available threonine concentration in the basal diet was determined to be 0.61% of dry diet. L-threonine was substituted for cellulose in the basal diet to yield concentrations of 0.61, 0.81, 1.00, 1.18, and 1.40% threonine on a dry-matter basis. Diet amino acid concentrations were determined via acid hydrolysis followed by chromatographic separation with an amino acid analyzer (Commonwealth Biotechnologies Inc., Richmond, Virginia, USA).

TABLE 1. *Composition of the basal diet for determining the dietary threonine requirement of juvenile striped bass* Morone saxatilis.

Ingredient	% as fed
Menhaden fish meal[a]	15.0
Corn gluten meal	19.0
Dextrin	10.0
Corn starch	10.0
Menhaden fish oil[a]	11.0
Vitamin premix[b]	1.5
Mineral premix[c]	1.0
Choline chloride, 77%	0.5
Calcium phosphate	1.0
Cellulose	10.7
Non-essential amino acid premix[d]	10.0
Essential amino acid premix[e]	9.5
L-lysine-HCl	0.8

[a] Omega Protein, Reedville, Virginia, USA.

[b] Contains (as mg/kg diet unless otherwise noted): calcium ascorbate-2-monophosphate, 1,000; inositol, 396; niacin, 153; α-tocopheryl acetate, 45; calcium pantothenate, 50.4; riboflavin, 20.7; menadione sodium bisulfate, 9.9; thiamin, 12.6; pyridoxine-HCL, 12.6; cyanocobalamin (3,000 μg/g), 5.8; folic acid, 5.4; retinyl acetate, 3.9; biotin, 4.5; cholecalciferol, 5 μg/kg; ethoxyquin (anti-oxidant), 125.

[c] Contains (as mg/kg diet): KCL, 5,200; NaCl, 3,600; $MgSO_4$, 1,640; Fe citrate, 92; $MnSO_4$, 80; $ZnCO_3$, 100; $CuSO_4$, 3.2; KI, 0.4; Na_2SeO_3, 0.328.

[d] Contains (as g/kg premix): L-glycine, 700; L-serine, 100; L-alanine, 100; L-proline, 100.

[e] See Table 2.

The five dietary treatments were fed in duplicate to 10 tanks of juvenile striped bass for a period of 10 wk. Fish were fed twice daily Monday through Friday and once daily Saturday and Sunday. At the start of the experiment, fish were fed the respective diets at a level of 3% of body weight per day. This level was appropriately reduced over the course of the experiment in order to maintain feeding at levels close to satiation.

At the start of the experiment, eight fish were euthanized by overdose of tricaine methanesulfonate (MS-222) and stored at −20 C for subsequent protein analysis of

TABLE 2. *Analyzed essential amino acid composition (% of diet) of basal diet for determining the threonine requirement of juvenile striped bass* Morone saxatilis.

Amino acid	Provided by crystalline amino acids	Provided by menhaden fish meal	Provided by corn gluten meal	Total	Estimated dietary requirement[a]
Arginine	1.33	0.57	0.38	2.28	1.4
Histidine	0.57	0.22	0.25	1.04	0.6
Isoleucine	0.86	0.40	0.48	1.74	0.9
Leucine	1.81	0.67	1.94	4.42	1.9
Lysine	1.64	0.71	0.21	2.56	2.2
Methionine	0.95	0.26	0.31	1.52	1.0
Phenylalanine	1.62	0.36	0.75	2.73	1.7
Threonine	0	0.38	0.39	0.77	1.1
Tryptophan	0.38	0.10	0.08	0.56	0.3
Valine	0.95	0.48	0.59	2.02	1.0

[a] Small and Soares (1998).

the whole carcass. Fish were weighed every 2 wk during the 10-wk feeding trial. At the last sampling, all fish were again weighed, and six fish per treatment, three samples per replication (tank), were sacrificed and stored at −20 C for subsequent protein analysis. Whole-body protein concentrations were determined by microKjeldahl analysis for calculation of apparent nitrogen utilization.

Data were analyzed by analysis of variance mixed model procedures (SAS 1992). Nonlinear procedures were used to fit two regression lines to identify the threonine level corresponding to the joint-point of the lines with minimum variance. The least-squares regression model was adapted from the model described in the SAS System for Regression, Nonlinear Models, Fitting Splines with Unknown Knots (SAS 1991). The model used fit the slope of the first line, the dependent variable value for a second line with zero slope, and the dietary threonine level at the joint-point of the two lines.

Results and Discussion

Increasing levels of dietary threonine to approximately 1.00% of dry diet enhanced weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and apparent nitrogen utilization (ANU) (Table 3). Increasing the dietary threonine concentration

TABLE 3. *Mean growth performance of juvenile striped bass* Morone saxatilis *fed diets containing graded levels threonine for 10 wk.*

Dietary threonine (% dry diet)	Weight gain (g)	SGR[a] (%/d)	FCR[b]	ANU[c] (%)
0.61	3.8	1.37	2.41	21.5
0.81	4.9	1.64	1.90	27.8
1.00	6.4	1.83	1.66	26.3
1.18	5.9	1.80	1.53	30.4
1.40	5.4	1.70	1.61	28.0
Requirement estimate ± SEM[d]	1.00 ± 0.19	0.91 ± 0.11	1.06 ± 0.09	1.13 ± 0.22
PSE[e]	0.4	0.09	0.13	1.7

[a] Specific growth rate = [ln(final weight) − ln(initial weight)/70 d] × 100.
[b] Feed conversion ratio = [weight of feed consumed (g as fed)/wet weight gain] × 100.
[c] Apparent nitrogen utilization = [nitrogen deposition/nitrogen intake] × 100.
[d] Based on least-squares regression analysis.
[e] Pooled standard error.

beyond 1.00% of dry diet did not further enhance any of the variables tested, indicating the dietary threonine requirement for the striped bass was between 1.00 and 1.18% of dry diet. Least-squares regression analysis of weight gain, SGR, FCR, and ANU indicated dietary threonine requirements of 1.00 ± 0.19, 0.91 ± 0.11, 1.06 ± 0.09, and 1.13 ± 0.22% of dry diet, respectively. From these results, the computed average dietary threonine requirement for juvenile striped bass is 1.03% of dry diet, 2.45% of dietary protein or 3.22 mg/kcal DE.

The quantitative dietary threonine requirement of juvenile striped bass, expressed as % dry diet and % dietary protein, determined in the present study is within a broad range of threonine requirements reported for other species of fish, and correlates well to that published for the sunshine bass, 0.9% of dry diet (2.6% of dietary protein), by Keembiyehetty and Gatlin (1997). Dietary threonine requirements reported for other species of fish range from a low of 0.5% of diet (2.0% protein) for channel catfish (Wilson et al. 1978) to a high of 1.5% of diet (4.0% protein) reported for the common carp and Japanese eel (Nose 1979).

In an effort to rapidly estimate the quantitative amino acid requirements of striped bass, Small and Soares (1998) used fillet A/E ratios [(essential amino acid/total essential amino acid including cysteine and tyrosine) × 1,000] to design a dose-response feed trial in which each amino acid was graded proportionately. Mitchell (1950) was the first to demonstrate a high correlation between muscle or whole-body tissue A/E ratios and dietary amino acid requirement ratios in growing animals. This high degree of correlation also has been reported for several species of fish (Arai 1981; Ketola 1982; Ogata et al. 1983; Cowey and Tacon 1983; Wilson and Poe 1985; Borlongan and Coloso 1993; Ng and Hung 1995). Using this approach, Small and Soares (1998) determined a threonine requirement of 1.1% of dry diet for striped bass, closely approximating the 1.03% of dry diet reported in the present study. In addition, Small (1998) demonstrated a good agreement between the dietary lysine requirement of striped bass determined with traditional dose-response feeding trials and that determined with the fillet A/E ratio methodology, 2.07% and 2.2% of dry diet, respectively. These data further support the use of muscle tissue A/E ratios as a method for rapidly estimating the dietary essential amino acid requirements of cultured fish. From the data presented here, we calculate that practical diets formulated for striped bass should contain a minimum threonine concentration of 1.03% of dry diet (2.45% of dietary protein), which equates to 3.22 mg/kcal DE.

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Literature Cited

Akiyama, T. and S. Arai. 1993. Amino acid requirements of chum salmon fry and supplementation of amino acids to diet. Pages 35–48 *in* M. R. Collie and J. P. McVey, editors. Proceedings of the Twentieth U.S.–Japan Symposium on Aquaculture Nutrition. UJNR Department of Commerce, Newport, Oregon, USA.

Arai, S. 1981. A purified test diet for coho salmon, *Oncorhynchus kisutch*, fry. Bulletin of the Japanese Society of Scientific Fisheries 47:547–550.

Arai, S. and H. Ogata. 1993. Quantitative amino acid requirements of fingerling coho salmon. Pages 19–28 *in* M. R. Collie and J. P. McVey, editors. Proceedings of the Twentieth U.S.–Japan Symposium on Aquaculture Nutrition. UJNR Department of Commerce, Newport, Oregon, USA.

Borlongan, I. G. and R. M. Coloso. 1993. Requirements of juvenile milkfish (*Chanos chanos* Forsskal) for essential amino acids. Journal of Nutrition 123:125–132.

Cowey, C. B. 1995. Intermediary metabolism in fish with reference to output of end products of nitrogen and phosphorus. Water Science and Technology 10:21–28.

Cowey, C. B. and A. G. J. Tacon. 1983. Fish nutrition-relevance to invertebrates. Pages 13–30 *in* G. D. Pruder, C. J. Langdon, and D. E. Conklin, editors. Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Division of Continuing Education, Baton Rouge, Louisiana, USA.

Keembiyehetty, C. N. and D. B Gatlin, III. 1997. Dietary threonine requirement of juvenile hybrid striped bass, *Morone chrysops* × *M. saxatilis.* Aquaculture Nutrition 3:217–221.

Ketola, H. G. 1982. Amino acid nutrition of fishes: requirements and supplementation of diets. Comparative Biochemistry and Physiology 73B:17–24.

Mitchell, H. H. 1950. Some species and age differences in amino acid requirements. Pages 1–32 *in* A. A. Albanese, editor. Protein and amino acid requirements of mammals. Academic Press, New York, New York, USA.

Ng, W. K. and S. S. O. Hung. 1995. Estimating the ideal dietary essential amino acid pattern for growth of white sturgeon, *Acipenser transmontanus* (Richardson). Aquaculture Nutrition 1:85–94.

Nicholson, L. C., L. C. Woods, III, and J. G. Woiwode. 1990. Intensive culture techniques for the striped bass and its hybrids. Pages 141–157 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division, American Fisheries Society, Maryland, USA.

Nose, T. 1979. Summary report on the requirements of essential amino acids for carp. Pages 145–156 *in* J. E. Halver and K. Tiews, editors. Finfish nutrition and fishfeed technology. Heenemann, Berlin, Germany.

NRC (National Research Council). 1993. Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Ogata, H., S. Arai, and T. Nose. 1983. Growth responses of cherry salmon, *Oncorhynchus masou,* and amigo salmon, *O. rhodurus,* fry fed purified casein diets supplemented with amino acids. Bulletin of the Japanese Society of Scientific Fisheries 49:1381–1385.

Ogino, C. 1980. Requirements of carp and rainbow trout for essential amino acids. Bulletin of the Japanese Society of Scientific Fisheries 46:171–174.

Ravi, J. and K. V. Devaraj. 1991. Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). Aquaculture 96:281–291.

Santiago, C. B. and R. T. Lovell. 1988. Amino acid requirements for growth of Nile tilapia. Journal of Nutrition 118:1540–1546.

SAS Institute, Inc. 1991. SAS system for regression, 2nd edition. Cary, North Carolina, USA.

SAS Institute, Inc. 1992. Technical report P-229, SAS/STAT software: changes and enhancements, release 6.07. Cary, North Carolina, USA.

Small, B. C. 1998. A study of dietary amino acid requirements, availability, and carbohydrate utilization for striped bass (*Morone saxatilis*). Doctoral dissertation. University of Maryland, College Park, Maryland, USA.

Small, B. C. and J. H. Soares, Jr. 1998. Estimating the quantitative essential amino acid requirements of striped bass, *Morone saxatilis,* using fillet A/E ratios. Aquaculture Nutrition 4:225–232.

Small, B. C., R. E. Austic, and J. H. Soares, Jr. 1999. Amino acid availability of four practical feed ingredients fed to striped bass *Morone saxatilis.* Journal of the World Aquaculture Society 30:58–64.

Wilson, R. P. and W. E. Poe. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus.* Comparative Biochemistry and Physiology 80B:385–388.

Wilson, R. P., O. W. Allen, Jr., E. H. Robinson, and W. E. Poe. 1978. Tryptophan and threonine requirements of fingerling channel catfish. Journal of Nutrition 108:1595–1599.