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Response of Largemouth Bass *Micropterus salmoides* to Dietary Supplementation of Lysine, Methionine, and Highly Unsaturated Fatty Acids

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Abstract.—A 12-wk feeding trial was conducted in aquaria with juvenile (36.0 ± 1.2 g) largemouth bass *Micropterus salmoides* to examine the effects of dietary supplementation of methionine, lysine, and long-chain polyunsaturated fatty acids (PUFA) on growth, feed conversion and body composition. Diets were formulated to increase dietary concentrations of methionine, lysine, and PUFAs to match levels found in whole body samples of largemouth bass. The control diet was formulated similar to diets previously tested for largemouth bass. Diets 2 and 3 were similar to the control diet but were supplemented with 2% lysine and 1% methionine, respectively. Diet 4 was formulated to increase PUFAs, especially docosahexaenoic acid (DHA) (22:6n-3), by replacing menhaden fish oil with squid oil. Fish were fed all they would consume in 10 min, twice daily. At harvest, there were no statistically significant differences ($P > 0.05$) in average individual weight or specific growth rate (SGR) among fish fed the four diets. The feed conversion ratio (FCR) of largemouth bass fed the diet supplemented with methionine (1.7) was significantly lower ($P \leq 0.05$) than fish fed the control diet (2.5). Fish fed the diet high in PUFA had significantly lower ($P \leq 0.05$) whole body lipid levels and significantly higher ($P \leq 0.05$) protein levels than fish fed the other three diets. These data indicate that the control diet in this study likely contained sufficient lysine, methionine and PUFA to meet the requirements of largemouth bass; however, additional methionine may improve feed conversion efficiency, and increased levels of PUFAs or other factors in squid oil may have a significant impact on body composition.

The largemouth bass *Micropterus salmoides* represents one of the most important freshwater fish in terms of sportfishing activities and expenditures. Although considerable research effort has been devoted toward their hatchery production and utilization in sport fisheries and fisheries management (Simco et al. 1986), relatively little research has been conducted on the culture of largemouth to sizes greater than 15 cm. In recent years, interest in culturing largemouth bass to sizes larger than those normally produced for sport fish stocking has increased (Brandt 1991). Demand for large (> 100 g) largemouth bass has grown dramatically and far exceeds availability (JSA 1983). This demand is based largely on increased utilization in fee-fishing operations (JSA 1983), managed trophy fisheries (Dupree and Huner 1984), and increased popularity as a live food product in ethnic markets. In the United States, the Joint Subcommittee on Aquaculture (JSA) listed the determination of efficient grow-out procedures, and development of cost-efficient diets utilizing practical feed ingredients, as research priorities for largemouth bass aquaculture (JSA 1983).

In the 1960s, a series of studies was conducted on the culture of feed-trained largemouth bass utilizing the Oregon Moist Pellet (Snow 1968a, 1968b; Snow and Maxwell 1970). Anderson et al. (1981), using purified diets in tanks, determined the minimum protein requirements of age 0 and age 1 largemouth bass to be 39.9% and 40.8%, respectively. Tidwell et al. (1996) suggested that largemouth bass fed practical diets appear to need higher protein levels (42–48%) than previously reported. Largemouth bass in commercial culture are often fed high protein (> 40%) salmonid diets, based primarily on diet availability rather than nutritional suitability. However, the specific nutritional requirements, particularly, essential amino acids and fatty acids may be different for largemouth bass than salmonids.

Fishes, like other animals, do not have an absolute requirement for protein, but re-

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quire a balanced mixture of essential amino acids (Lovell 1989). A deficiency of any of these may cause reduced growth and poor feed conversion. Requirement levels for individual amino acids differ among different species of fish (Wilson 1989). When Tidwell et al. (1996) compared the composition of experimental diets with whole body amino acid profiles, results indicated that largemouth bass might benefit from higher dietary levels of methionine and lysine.

The study by Tidwell et al. (1996) also suggested that largemouth bass may require relatively high levels of polyunsaturated fatty acids (PUFA) for a warmwater, freshwater species. Levels of DHA (22:6n-3) in the lipids of the bass egg and muscle tissues were > 290% higher than levels measured in the diets, possibly indicating relative importance. Largemouth bass may be able to synthesize DHA from other n-3 precursors similar to other freshwater fishes. However, dietary supplementation of DHA may be beneficial if biosynthesis is not efficient or sufficient to supply the metabolic demand under maximum production situations.

This study was not designed as a requirements study but was intended to facilitate a rapid assessment of primary nutritional constraints by evaluating the response of fish to diets supplemented to match body concentrations of the most important nutrients (Tidwell et al. 1996). The specific objectives of this research were to evaluate the response of largemouth bass to dietary supplementation of lysine, methionine, and polyunsaturated fatty acids under controlled conditions. These data are intended to provide expedient improvements in diet formulations until specific requirements for individual nutrients can be determined.

Materials and Methods

Experimental System and Animals

Approximately 1,500 5-cm largemouth bass were obtained from the Kentucky Department of Fish and Wildlife, Frankfort, Kentucky, USA. These fingerlings were held in flow-through tanks and trained to accept prepared diets using established procedures (Brandt 1991). Fish were then overwintered in a 0.02-ha pond at 10,000 fish/ha prior to utilization in the growth trial. Fifteen feed trained fish (36.0 ± 0.5 g) were randomly stocked into each of 20 110-L glass aquaria on 6 March 1998. During a 1-wk conditioning period, fish in all tanks were fed the control diet. After 1 wk, densities were reduced from 15 to 10 fish of uniform size. From that point, fish were fed one of four randomly assigned experimental diets with five replicate tanks per dietary treatment. Fish were fed all they would consume for 10 min twice daily (0830 and 1530) for a period of 12 wk. All fish from each aquaria were weighed at the beginning of the experiment and again every 3 wk until the conclusion of the feeding trial. At the conclusion of the study, all fish from each aquarium were individually counted and weighed.

Water was recirculated through a common biological and mechanical filter system so that all replicates shared similar water quality. The recirculating system was a 1,000-L vertical flow bead filter (Bubble Bead Filter, Aquatic Ecosystems Inc., Apopka, Florida, USA). Continuous aeration was provided by a blower and air stones. The water exchange rate for the total system was approximately 5% of volume per day. Each aquarium was supplied with water at a flow rate of approximately 5.0 L/min and cleaned by siphon daily. Black plastic was used to cover the back and sides of all aquaria to minimize disturbances. Continuous illumination was supplied with fluorescent ceiling lights.

Water temperature and dissolved oxygen were measured daily using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, Ohio, USA). Total ammonia-nitrogen, nitrite-nitrogen, and total alkalinity were measured three times weekly using a DREL 2000 spectrophotometer (Hach Company, Loveland, Colorado, USA); pH was measured three times weekly using an

TABLE 1. *Ingredient composition and percentage moisture, protein and lipid of a control diet (CTL) and experimental diets supplemented with 2% lysine (LYS), 1% methionine (METH), and polyunsaturated fatty acids (PUFA), fed to juvenile largemouth bass.*

	CTL	LYS	METH	PUFA
Ingredient (%)				
Menhaden meal	35.0	33.0	34.0	35.0
Soybean meal	35.0	35.0	35.0	35.0
Corn	14.5	14.5	14.5	14.5
Wheat flour	10.0	10.0	10.0	10.0
Menhaden oil	1.5	1.5	1.5	—
Corn oil	2.5	2.5	2.5	—
Squid oil	—	—	—	4.0
Trout premix	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.4	0.4	0.4	0.4
Mineral premix	0.6	0.6	0.6	0.6
Lysine	—	2.0	—	—
Methionine	—	—	1.0	—
Analyzed Composition				
Moisture (%)	23.4 ± 0.7	24.1 ± 0.5	24.9 ± 1.6	24.5 ± 0.4
Protein (%)[a]	47.1 ± 0.1	46.8 ± 1.0	46.5 ± 0.5	46.2 ± 0.2
Lipid (%)[a]	9.8 ± 0.2	9.6 ± 0.3	9.8 ± 0.1	9.9 ± 0.1
Energy[b]	4.22	4.17	4.13	4.15
P/E[c]	116.84	117.22	118.12	118.93

[a] Moisture free basis.
[b] Gross energy in kcal/g of diet.
[c] P/E = protein to energy ratio in mg protein/kcal.

electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA). Total alkalinity was maintained above 50 mg/L by weekly additions of sodium bicarbonate.

Experimental Diets

The control diet (CTL, Table 1) was based on the 47% protein diet previously evaluated by Tidwell et al. (1996). The three experimental diets were based on the control diet but modified to raise dietary concentrations of lysine, methionine and DHA, respectively, to concentrations reported for fillet of largemouth bass reported by Tidwell et al. (1996) (Table 2). Diet 2 (LYS) received 2% supplemental crystal-

TABLE 2. *Calculated concentrations of nutrients, as percent of diet (or as percent of dietary protein), provided by a control diet (CTL) and experimental diets supplemented with 2% lysine (LYS), 1% methionine (METH), and polyunsaturated fatty acids (PUFA).*

	Methionine[c]	Lysine	PUFA
CTL	0.9 (1.9)	2.8 (6.0)	0.96
LYS	0.9 (1.9)	4.8 (10.3)	0.96
METH	1.9 (4.0)	2.8 (6.0)	0.96
PUFA	0.9 (1.9)	2.8 (6.0)	1.94
Largemouth bass fillet[a]	2.2	4.9	2.10
Channel catfish requirement[b]	0.6	1.5	-not required-
Rainbow trout requirement[b]	1.0	1.9	-not required-
Common carp requirement[b]	1.2	2.2	-not required-

[c] In the absence of dietary cystine.

Concentrations of these nutrients in largemouth bass fillet (Tidwell et al. 1996)[a] and known nutritional requirements (as percent of diet) of commonly cultured fish species (Wilson 1989)[b] are also presented for comparative purposes.

line L-lysine (Sigma Chemical Co., St. Louis, Missouri, USA), Diet 3 (METH) received 1% supplemental crystalline L-methionine (Sigma Chemical Co., St. Louis, Missouri, USA). In Diet 4 (PUFA) PUFA concentration in the diet was increased 1.5% by addition of 4% squid oil (Table 1). The squid oil used had a guaranteed analysis of 17.4% DHA and 33.6% highly unsaturated fatty acids by volume (Argent Chemical Laboratories, Redmond, Washington, USA). Diets were formulated to be isocaloric and isonitrogenous based on gross energy values of 5.64 kcal/g protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g fat (NRC 1993).

To prepare the diets, practical ingredients were ground into small particle sizes (approximately 250 μm) in a Wiley mill. Ingredients were mixed thoroughly and water was added to obtain a 25% moisture level. Diets were then passed through a mincer with a die and made into 2-mm diameter strands and air dried (24 C) for 2 h. Pellets were broken up and sieved to an appropriate pellet size for size of fish (Piper et al. 1982). These semi-moist, soft pellets were stored frozen (−20 C) until fed.

At stocking, 15 fish were randomly selected, killed by decapitation, homogenized in a blender, stored in polyethylene bags, and frozen for subsequent proximate analysis. At harvest, three fish per aquarium were prepared for analysis as described above. Fish samples and a duplicate sample of each diet were analyzed for crude protein, lipid, and moisture by a commercial analytical laboratory (Woodson-Tenent Laboratories, Dayton, Ohio, USA).

Data Analysis Procedures

Growth performance values were calculated as follows: Feed conversion ratio (FCR) was calculated from FCR = total diet fed (g)/total wet weight gain (g). Specific growth rate (SGR, % body wt/d) was calculated from $SGR = [(\ln W_f - \ln W_i)/t] \times 100$, where W_f = final weight, W_i = initial weight, and t = time in days (Ricker 1975). Percent protein deposited (PPD) was calculated from PPD = final body protein − initial body protein × 100/total protein fed.

Data were analyzed by analysis of variance (ANOVA) using Statistix version 4.1 (Analytical Software, Tallahassee, Florida, USA) to determine treatment effects on growth, condition factor, feed conversion, survival, body composition, and water quality variables. If ANOVA indicated significant treatment effects the Least Significant Difference test (LSD) was used to determine differences among means ($P \leq 0.05$). All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Data are presented in the untransformed form to facilitate interpretation.

Results

There were no significant differences in water quality variables among treatments either weekly or overall. Overall means for water quality variables were: temperature, 23.9 ± 1.3 C; dissolved oxygen, 7.4 ± 0.8 mg/L; pH, 8.5 ± 0.5; total ammonia-nitrogen, 0.72 ± 0.42 mg/L; and nitrite-nitrogen, 0.14 ± 0.13 mg/L. These values represent suitable conditions for bass culture.

Average weight at harvest, percentage weight gain, and specific growth rate (SGR) were not significantly different among fish fed any of the four diets and averaged 80.30 g, 122.9%, and 0.97% day, respectively (Table 3). Fish fed METH demonstrated significantly lower feed conversion ratios (FCR) (1.70) than fish fed CTL (2.15); however, the FCR of fish fed LYS and PUFA were not significantly different from fish fed the other diets (Table 3). Compared to CTL, percentage survival was significantly higher in fish fed diets METH and PUFA. Survival in fish fed the LYS diet was not significantly different from fish fed other diets (Table 3).

Analysis of body composition of largemouth bass indicated that there was no significant difference in percentage moisture among fish fed the four diets (Table 4);

TABLE 3. *Individual weight, weight gain, survival, feed conversion ratio (FCR), and specific growth rate (SGR) of juvenile largemouth bass fed a control diet (CTL) or one of three experimental diets supplemented with 2% lysine (LYS), 1% methionine (METH), or polyunsaturated fatty acids (PUFA). Values are means ± SE of five replications. Means within a row followed by different letters are significantly different* ($P < 0.05$).

	CTL	LYS	METH	PUFA
Weight (g)	78.8 ± 5.6 a	78.5 ± 2.3 a	83.9 ± 3.5 a	80.0 ± 7.3 a
Weight gain (%)	118.9 ± 17.8 a	118.3 ± 7.2 a	130.5 ± 8.7 a	123.8 ± 19.9 a
Survival (%)	94.0 ± 4.9 b	98.0 ± 4.0 ab	100.0 ± 0.0 a	100.0 ± 0.0 a
FCR[1]	2.15 ± 0.39 a	1.99 ± 0.14 ab	1.70 ± 0.12 b	1.91 ± 0.37 ab
SGR[2]	0.94 ± 0.10 a	0.94 ± 0.04 a	1.01 ± 0.05 a	0.97 ± 0.10 a

[1] FCR = feed conversion ratio: total diet fed (g)/total wet weight gain (g).
[2] SGR = specific growth rate (%/d): $[(\ln W_f - \ln W_i)/t] \times 100$, where W_f = final weight, W_i = initial weight, and t = time in days.

however, fish fed the PUFA diet had significantly higher protein levels and significantly lower lipid levels than fish fed all other diets (Table 4). Percent protein deposited was not significantly different among treatments (Table 4).

Discussion

Fish fed actively on all diets through the duration of the study except for 1–2 d following sampling dates. Largemouth bass fed the different diets demonstrated similar growth patterns through the duration of the study indicating that palatability or diet acceptance did not differ. Careful feeding procedures were maintained to ensure that all fish were fed to apparent satiation. Based on the observations of this study, interim sampling of largemouth bass should be minimized or eliminated, and patient feeding is required to ensure all individuals receive feed.

Lysine supplementation did not improve growth rate or feed conversion of largemouth bass in this study. Lysine is generally the most limiting amino acid in practical diets for fish and its deficiency will usually produce reduced growth and poor feed conversion (Wilson 1991). This suggests that the control diet contained sufficient lysine (approx. 2.8% of diet; 6% of protein) to meet the requirement of juvenile largemouth bass. These data also suggest that largemouth bass have a lysine requirement similar to more commonly cultured species (Table 2).

Fish fed the methionine-supplemented diet demonstrated a significant improvement in feed conversion efficiency compared to fish fed the control diet. While growth is obviously important in fish production, it can sometimes be less important economically than feed conversion (Lovell 1989).

Reduced feed conversion efficiency can be an indicator of amino acid deficiency

TABLE 4. *Percentage moisture, protein, and lipid of whole body samples of largemouth bass fed and a control diet (CTL) or experimental diets supplemented with 2% lysine (LYS), 1% methionine (METH), or poly-unsaturated fatty acids (PUFA). Values are means (± SE) of five replicate aquaria. Means in the same row followed by different letters are significantly different* ($P < 0.05$).

Whole body	CTL	LYS	METH	PUFA
Moisture (%)	72.4 ± 1.5 a	72.8 ± 0.5 a	72.4 ± 0.1 a	73.5 ± 0.5 a
Protein (%)[1]	62.9 ± 2.9 b	63.8 ± 2.8 b	61.4 ± 1.9 b	67.1 ± 1.1 a
Lipid (%)[1]	20.2 ± 2.3 a	20.0 ± 2.4 a	21.5 ± 1.1 a	17.3 ± 1.3 b
PPD[2]	23.31 ± 6.50 a	25.73 ± 3.11 a	30.48 ± 1.56 a	29.78 ± 6.91 a

[1] Moisture-free basis.
[2] PPD = percent protein deposited: final body protein − initial body protein × 100/total protein fed.

(Wilson 1991). However, since feed conversion was not improved compared to fish fed diets supplemented with lysine and PUFA (which contained methionine levels similar to controls), it is doubtful that these data represent a methionine deficiency. Additional research is needed to quantitatively determine the methionine requirement for largemouth bass and the effects of increased levels on feed conversion efficiencies.

Essential fatty acids are those that are needed preformed in the diet for optimum growth and survival (Bell et al. 1986). Marine fish are known to require dietary PUFA such as EPA or DHA due to a limited ability to metabolically alter shorter-chain fatty acids. Poly-unsaturated fatty acids are also known to be important physiologically in freshwater fish (Ackman and Kean-Howie 1995); though, EPA and DHA are generally not considered nutritionally "essential" for freshwater fish. However, it has also been suggested that elongation and desaturation of PUFA from shorter chain precursors is energetically expensive (De Silva et al. 1997); therefore, if these nutrients are supplied in the diet preformed, this may reduce the metabolic energy expenditure and improve growth rate or diet efficiency.

In the current study, growth rate and feed conversion were not improved by supplemental EPA and DHA provided by addition of squid oil in the diet. These results suggest that diets containing ≥ 1% EPA and DHA (the amount contained in the control diet in this study) are sufficient for adequate growth and survival of juvenile largemouth bass.

The significant impact of high PUFA concentrations on the body composition of juvenile largemouth bass is not currently understood. No previously published reports of lower total lipid content and/or higher protein content in the whole-body proximate composition of fish fed high PUFA levels or squid oil were found. Fernandez-Palacios et al. (1997) found no difference in total body lipid of broodstock gilthead sea bream *Sparus aurata* fed diets supplemented with squid oil. However, nutrition studies with rats have shown that diets high in PUFA can reduce the intestinal uptake of certain fatty acids by approximately 50% (Thomson et al. 1989). In the present study, it is unclear why whole-body proximate composition was affected by addition of squid oil. This potentially represents a new role for PUFA or other squid oil components on lipid metabolism and storage which should be investigated further.

It appears that the control diet formulation in this study contains sufficient lysine, methionine and PUFA concentrations for juvenile largemouth bass. Further research is needed to determine quantitative amino acid requirements and optimal protein to energy ratios for this species to allow development of more cost-effective production diets.

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