North American Journal of Aquaculture 69:000–000, 2007

DOI: 10.1577/A06-040.1

[Article]

Effects of Different Types of Dietary Lipids on Growth and Fatty Acid Composition of Largemouth Bass

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Abstract.—The effects of feeding diets supplemented with oils of varying sources and fatty acid compositions on growth, survival, and biochemical composition of juvenile largemouth bass *Micropterus salmoides* were evaluated under controlled conditions in aquaria for 12 weeks. Feed-trained juvenile largemouth bass (15.7 ± 0.6 g) were stocked into eighteen 114-L glass aquaria at 25 fish/tank and were fed one of five experimental diets (3 replicate aquaria/diet). All diets were formulated to be approximately isocaloric (4,200 kcal gross energy per kilogram) and isonitrogenous (40% crude protein), containing protein primarily from solvent-extracted fish meal and soybean meal. Each diet was supplemented with 5% oil (by weight) using lipids from various sources and with different fatty acid compositions. These included fish oil, corn oil, sunflower oil (high oleic), linseed oil, and a fungal oil (high arachidonic acid). Fish were fed twice daily to apparent satiation. At the end of the study period, there were no significant differences ($P > 0.05$) between treatment groups in terms of survival (98%), weight gain (595%), specific growth rate (2.2% per day), feed conversion ratio (1.5), percent protein deposited (28%), or hepatosomatic index (2.3). Whole-body proximate composition was not significantly affected ($P > 0.05$) by source of added lipid, but whole-body fatty acid composition showed large differences and primarily reflected the fatty acid compositions of added oils. Largemouth bass may be able to use diets containing vegetable- and animal-source lipids, which are less expensive than the previously recommended fish oil.

In prepared diets for fishes, lipids represent a concentrated, cost-effective energy source. However, lipids also have other important nutritional functions, including structural roles in cell membranes, as components in hormones, as precursors for prostaglandins and other eicosanoids, and as sources for essential fatty acids (EFAs). Essential fatty acids are unsaturated fatty acids that must be provided preformed in the diet (Bell et al. 1986). In general, warmwater fishes require polyunsaturated n-6 fatty acids or a mixture of n-3 and n-6 fatty acids, while coldwater species require n-3 forms (Webster and Lim 2002). Metabolically active forms of n-6 and n-3 fatty acids consist of 20 or more carbons (highly unsaturated fatty acids [HUFAs]). For the n-6 family, the metabolically active form is primarily arachidonic acid (AA; 20:4[n-6], where 20 is the number of carbon atoms, 4 is the number of double bonds, and 6 is the position of the first double bond from the methyl end); for the n-3 family, the active forms are eicosapentaenoic acid (EPA; 20:5[n-3]) and docosahexaenoic acid (DHA; 22:6[n-3]). In freshwater fishes, EFA requirements can usually be met by supplying the shorter-chain precursors: linolenic acid (LNA; 18:3[n-3]), linoleic acid (LA; 18:2[n-6]) or both, although better growth performance can be often achieved by supplying the "bioactive" HUFA forms preformed in the diet (Kanazawa 1985). In all marine species studied to date, conversion of LNA to EPA and DHA and of LA to AA is so limited that DHA, EPA, and AA are considered EFAs (Sargent et al. 1999, 2002).

The largemouth bass *Micropterus salmoides* is a large freshwater predator that shows promise as an aquaculture species. While feed costs account for an extremely high proportion of production costs (Woods 1999), studies conducted on largemouth bass nutrition to date are limited in number (see review by Tidwell et al. 2002). More recent work has demonstrated that much, if not all, of the expensive fish meal component in the diet can be replaced by less-expensive protein sources, such as poultry by-product meal (Tidwell et al. 2005). These low fish meal diets were supplemented with fish oil to fulfill anticipated EFA requirements. Earlier studies implied that largemouth bass have unusually high DHA requirements for a freshwater, warmwater species (Tidwell et al. 1996). Research by Coyle et al. (2000) did not confirm this but did find that high dietary concentrations of HUFAs decreased lipid deposition in largemouth bass. The present study was designed to evaluate the effects of feeding diets supplemented with oils from different sources and that possess different fatty acid compositions on largemouth bass growth, survival, and chemical composition.

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Received May 31, 2006; accepted August 30, 2006
Published online Month 00, 2007

TABLE 1.—Formulation and analyzed composition (%) of experimental diets containing lipids from different sources that were fed to juvenile largemouth bass in aquaria for 12 weeks.

Ingredient or component	Source of added lipid				
	Anchovy fish oil	Corn oil	Sunflower oil[a]	Linseed oil	Fungal extract oil
Fish meal (lipid extracted)	30.0	30.0	30.0	30.0	30.0
Soybean meal	34.5	34.5	34.5	34.5	34.5
Wheat flour	26.0	26.0	26.0	26.0	26.0
Lipid	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Mineral mix[b]	0.4	0.4	0.4	0.4	0.4
Vitamin C	0.2	0.2	0.2	0.2	0.2
Vitamin mix[c]	0.4	0.4	0.4	0.4	0.4
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	2.0	2.0	2.0	2.0	2.0
Analyzed composition (as fed)					
Moisture	14.9	9.0	7.8	7.8	10.9
Protein	38.1	40.5	40.5	40.7	40.1
Lipid	7.2	7.9	8.1	7.0	7.5
Fiber	1.8	1.9	1.9	1.9	2.1
Ash	10.4	10.9	10.9	11.2	10.6

[a] High-oleic sunflower oil.
[b] Rangen trace mineral mix F1.
[c] Rangen vitamin pre-mix number 30.

Methods

Experimental diets.—Diets were formulated to be isocaloric (4,200 kcal of gross energy per kilogram) and isonitrogenous (40% crude protein) calculated on gross energy values of 5.64 kcal/g protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g fat (NRC 1993), which met protein and energy recommendations for largemouth bass (Bright et al. 2005). All diets used lipid-extracted fish meal as the primary protein source at a fixed rate of 30% of the total formulation (Table 1). The fish meal was solvent extracted with ethyl alcohol (ETOH; 95%) in six consecutive baths: baths 1–4 at 2:1 ETOH : fish meal, bath 5 at 3:1, and bath 6 at 4:1 (Thompson et al. 2003). Each diet was supplemented with 5% added lipid from different sources. Fatty acid compositions of the various oils are given in Table 2. Lipid types evaluated included anchovy fish oil (high in EPA and DHA; Rangen, Inc., Buhl, Idaho), corn oil (high in LA; The Kroger Company, Cincinnati, Ohio), high-oleic sunflower oil (oleic acid; 18:1[n-9]; Martek Biosciences Corporation, Columbia, Maryland), linseed oil (high in LNA; Double S Liquid Feed Services, Inc., Danville, Illinois), and a fungal extract oil (high in AA; Martek).

To prepare the diets, practical ingredients were ground into small particle sizes (approximately 250 μm) in a Wiley mill. Ingredients were thoroughly mixed, and water was added to obtain a 25% moisture level. Diets were then passed through a mincer with a die, made into 2-mm-diameter strands, and air dried (24°C) for 2 h. Pellets were broken up and sieved to a pellet size appropriate for the size of fish (Piper et al. 1982). These semi-moist, soft pellets were stored frozen (−20°C) until fed to the fish. Two samples of each diet were submitted to a commercial laboratory (Eurofins Scientific, Inc., Des Moines, Iowa) for proximate analysis (Table 1) using standard methods (AOAC 1990). Two samples of each lipid source were stored under liquid nitrogen until fatty acid analysis (Table 2). Two samples of finished diets were also analyzed for fatty acids (Table 3) by the commercial laboratory according to official methods (AOCS 2005).

Fish and experimental system.—Feed-trained largemouth bass fingerlings were purchased from a commercial producer (Mayer's Fish Farm, Bardstown, Kentucky), graded to a similar size (15.7 ± 0.6 g), and randomly stocked into eighteen 114-L glass aquaria at 25 fish/aquarium. Fish were fed the fish oil diet as a conditioning diet for 1 week while being acclimated to the system, then were switched to the experimental diets. There were three replicate tanks per experimental diet. Five fish were taken before stocking for baseline whole-body proximate analyses. For 12 weeks, fish were fed one of the six experimental diets twice daily (0800 and 1600 hours) over a 30-min period to apparent satiation. The amount of diet fed to fish in each aquarium was recorded at the end of each week.

Water quality.—Water quality was maintained by recirculation through a common biofilter (Bubble Bead Filtration Systems, Inc., Marion, Texas; Model BBF-10) so that all aquaria shared similar water chemistry and quality. Dissolved oxygen and temperature were

TABLE 2.—Fatty acid composition (percentage of total fatty acids) of supplemental lipids (from different sources) used in experimental diets for largemouth bass.

	Lipid type				
Fatty acid	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
14:0	5.9	0.0	0.0	0.0	0.8
16:0	18.9	10.6	3.2	4.9	12.8
(palmitoleic acid) 16:1(n-7)	9.2	0.1	0.1	0.1	0.2
18:0	4.2	2.0	3.6	3.6	10.1
(oleic acid) 18:1(n-9)	20.5	28.2	81.2	18.7	10.7
(linoleic acid) 18:2(n-6)	7.6	56.4	9.6	16.0	7.6
(linolenic acid) 18:3(n-3)	1.0	1.4	0.2	55.8	3.7
(arachidonic acid) 20:4(n-6)	1.9	0.0	0.0	0.0	43.0
(eicosapentaenoic acid) 20:5(n-3)	14.4	0.0	0.0	0.0	0.0
(docosapentaenoic acid) 22:5(n-3)	2.0	0.0	0.0	0.0	0.0
(docosahexaenoic acid) 22:6(n-3)	4.2	0.0	0.0	0.0	1.7
Other	12.2	1.3	2.1	0.9	11.1
Saturates	30.3	13.4	8.4	8.9	26.8
Monenes	31.3	28.6	81.6	19.0	11.7
Dienes	8.6	56.4	9.6	16.0	8.1
PUFA[a]	36.6	57.8	9.7	71.9	60.7
HUFA[b]	25.3	1.1	1.9	0.7	52.7
n-3	22.7	1.4	0.2	55.8	5.4
n-6	9.9	56.4	9.6	16.1	54.7
n-3 or n-6	2.3	0.0	0.0	3.5	0.1

[a] PUFA = polyunsaturated fatty acid.
[b] HUFA = highly unsaturated fatty acid.

monitored twice daily with a dissolved oxygen meter (YSI, Inc., Yellow Springs, Ohio; Model 85). Levels of total ammonia nitrogen (TAN), un-ionized ammonia nitrogen, nitrite-nitrogen, and pH were monitored three times per week with a spectrophotometer (HACH Company, Loveland, Colorado; Model Odyssey DR 2500). Alkalinity and hardness were monitored three times per week by titration (HACH digital titrator). For the duration of the study, water quality variables averaged 24.9 ± 3.3°C for water temperature; 94.6 ± 23.1 mg/L for alkalinity; 146.0 ± 19.0 mg/L for water hardness; 0.7 ± 0.4 mg/L for TAN; 0.03 ± 0.50 mg/L

TABLE 3.—Fatty acid composition (percentage of total fatty acids) of finished diets as fed to largemouth bass.

	Lipid type				
Fatty acid	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
14:0	4.9	0.8	0.8	0.9	1.3
16:0	20.2	14.1	9.1	11.4	16.2
(palmitoleic acid) 16:1(n-7)	7.4	1.1	1.1	1.3	1.0
18:0	4.6	3.0	4.1	4.5	9.0
(oleic acid) 18:1(n-9)	19.5	24.9	61.3	20.0	12.7
(linoleic acid) 18:2(n-6)	16.5	49.7	17.5	21.9	16.0
(linolenic acid) 18:3(n-3)	2.1	2.3	1.4	35.2	3.9
(arachidonic acid) 20:4(n-6)	1.6	0.2	0.2	0.2	28.9
(eicosapentaenoic acid) 20:5(n-3)	11.0	1.1	0.9	1.0	1.0
(docosapentaenoic acid) 22:5(n-3)	1.6	0.2	0.2	0.2	0.2
(docosahexaenoic acid) 22:6(n-3)	3.9	1.0	0.9	1.0	1.0
Other	8.3	1.8	2.7	2.6	9.0
Saturates	31.0	18.9	15.4	18.8	29.6
Monenes	28.2	26.7	63.1	22.3	14.9
Dienes	17.2	49.7	17.5	21.9	16.4
PUFA[a]	40.7	54.6	21.4	59.7	54.4
HUFA[b]	20.3	3.9	4.2	3.8	38.7
n-3	19.4	4.7	3.7	37.7	6.3
n-6	18.2	49.9	17.7	22.1	47.8
n-3 or n-6	1.1	0.1	0.2	1.7	0.1

[a] PUFA = polyunsaturated fatty acid.
[b] HUFA = highly unsaturated fatty acid.

TABLE 4.—Final weight, feed conversion ratio (FCR), survival, weight gain, specific growth rate (SGR), percent protein deposited (PPD), condition factor (*K*), and hepatosomatic index (HSI) for largemouth bass fed experimental diets formulated with lipids of varying sources and fatty acid profiles. Values are means (±SE) of three replicates. An ANOVA indicated no significant differences among treatments ($P > 0.05$).

	Lipid type				
Variable	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
Average weight (g)	94.2 ± 9.7	91.8 ± 1.2	87.8 ± 2.8	93.1 ± 2.3	94.3 ± 4.0
FCR (%)	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1
Survival (%)	96.0 ± 6.9	100.0 ± 0.0	97.3 ± 4.6	100.0 ± 0.0	92.0 ± 0.0
Weight gain (%)	599 ± 62	584 ± 8	558 ± 18	592 ± 15	600 ± 26
SGR (% per day)	2.2 ± 0.1	2.2 ± 0.0	2.1 ± 0.0	2.2 ± 0.0	2.2 ± 0.1
PPD	28.6 ± 0.8	28.2 ± 1.7	26.8 ± 5.3	27.4 ± 0.5	27.6 ± 2.4
K	3.3 ± 0.6	3.3 ± 0.1	3.3 ± 0.1	3.6 ± 0.6	3.7 ± 0.8
HSI	2.3 ± 0.0	2.2 ± 0.2	2.0 ± 0.3	2.3 ± 0.3	2.3 ± 0.1

for un-ionized ammonia; 0.1 ± 0.1 mg/L for nitrite; and 7.7 ± 0.4 for pH.

Harvest.—After 12 weeks, fish from each individual aquarium were harvested, bulk weighed, and counted and then were individually weighed and measured (total length). Nine of these fish were randomly selected, and liver weights were also recorded. Six fish from each tank were randomly selected, anesthetized with quinaldine sulfonate (Sure-Life Laboratories, ?1 Inc., Seguin, Texas; Tranquil), and either individually homogenized and submitted for whole-body proximate analysis (three fish) or stored under liquid nitrogen until fatty acid analysis (three fish) by Eurofins Scientific, Inc., according to procedures described previously.

Statistical analysis.—We calculated several growth performance parameters with the following equations. Specific growth rate (SGR; percent body weight per day) was calculated as

$$\text{SGR} = [\log_e W_f - \log_e(W_i/t)] \times 100,$$

where W_f = final weight (g), W_i = initial weight (g), and t = time in days. Feed conversion ratio (FCR) was calculated as dry feed intake (g) divided by total wet weight gain (g). We determined percent protein deposited (PPD) as 100 times the final body protein minus initial body protein divided by total protein fed. Condition factor (*K*) was calculated as 100 times the harvest weight divided by harvest length cubed. For each individual, a hepatosomatic index (HSI) was calculated as 100 times the liver weight (g) divided by whole-body weight (g).

The term polyunsaturated fatty acid (PUFA) was used to designate all fatty acids with two double bonds or more, and HUFA was used to designate a subsample of PUFA with 20 or more carbons, as described by Brett and Müller-Navarra (1997). Data were analyzed with analysis of variance (ANOVA) using Statistix software to determine treatment effects of the different diets on growth performance and body composition. If ANOVA indicated significant differences ($P \leq 0.05$), the least-significant-difference test was used to separate means (Steel and Torrie 1980). All percentage and ratio data were transformed to arcsine values before analysis (Zar 1984). Untransformed data are presented to facilitate interpretation.

Results

At the end of 12 weeks, there was no significant difference ($P > 0.05$) in survival among fish fed the five diets (Table 4); treatment means ranged from 92% to 100%. There were also no significant differences ($P > 0.05$) in the different measures of growth among fish fed the five diets. Weight gain averaged 595.1 ± 39.9%, and SGR averaged 2.2 ± 0.1% per day. Measures of feed utilization efficiency (i.e., FCR and PPD) were not significantly different ($P > 0.05$) among treatments, averaging 1.54 ± 0.1% and 28.0 ± 2.3%, respectively. Whole-body proximate composition did not differ significantly ($P > 0.05$) among fish fed the different diets. Proximate component averages were 71.1 ± 1.2% for moisture, 17.3 ± 0.8% for protein, 7.0 ± 0.7% for lipid, and 4.0 ± 0.5% for ash (Table 5). The HSI was also not influenced by diet, averaging 2.3 ± 0.2 overall (Table 4).

The fatty acid composition of the added lipids (Table 2) had a large impact on the fatty acid composition of whole-body samples (Table 6). Fish fed the high-oleic (sunflower oil) diet contained significantly higher ($P < 0.05$) concentrations of 18:1(n-9) and total monenes than fish fed other diets. Largemouth bass fed the high-LA (corn oil) diet contained significantly higher ($P < 0.05$) body concentrations of 18:2(n-6) in the body as well as significantly higher levels of dienes and total n-6 fatty acids than fish fed other diets. Linolenic acid

TABLE 5.—Proximate composition (%) of whole-body tissue of largemouth bass fed experimental diets formulated with lipids of varying sources and fatty acid compositions. Values are means (±SE) of three replicates. An ANOVA indicated no significant differences among treatments ($P > 0.05$).

	Lipid type				
Variable	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
Moisture	71.1 ± 0.5	70.0 ± 1.8	71.1 ± 0.9	67.0 ± 0.4	71.8 ± 0.7
Protein	16.9 ± 0.6	17.4 ± 0.8	17.4 ± 2.0	17.6 ± 0.2	17.3 ± 0.3
Lipid	6.7 ± 0.6	7.8 ± 0.2	7.3 ± 0.3	7.8 ± 0.4	6.2 ± 0.3
Ash	4.4 ± 0.7	4.1 ± 0.7	3.8 ± 0.2	4.1 ± 0.2	3.5 ± 0.3

was significantly higher ($P < 0.05$) in fish fed the high-LNA diet (linseed oil). Fish fed this diet also had significantly higher ($P < 0.05$) levels of total n-3 fatty acids than fish fed the other diets. Fish fed the linseed oil diet had a total concentration of PUFAs (32%) that was significantly higher ($P < 0.05$) than that of fish fed other diets except the high-AA diet (supplemented with fungal oil), from which they were not significantly different ($P > 0.05$; 31%). Fish fed the AA diet had significantly higher ($P < 0.05$) levels of whole-body AA and 18:0 than fish fed the other diets.

Discussion

There has been very little work on the lipid metabolism of largemouth bass or the suitability of lipids other than fish oil for diet formulation. These data indicate that lipids from other sources can perform as well as fish oil in diets for juvenile largemouth bass. This differs from studies with marine species, which require relatively high levels of dietary HUFAs. Mourente et al. (2005) found that rapeseed, linseed, and olive oils could substitute for only 60% of the fish oil in the diet without compromising growth rates in European sea bass *Dicentrarchus labrax*. Similarly, only 50% of fish oils were replaceable by vegetable oils without growth reductions or increased mortality in Atlantic salmon *Salmo salar* (Storebakken 2002).

Although this study was not designed to determine fatty acid requirements (fish were relatively large and not previously depleted of fatty acid stores), some inferences can be made. Fish growth and survival were not significantly reduced with the corn oil diet, which contained only 0.1% LNA and 0.4% total n-3 fatty acids (Table 7). Satoh et al. (1989) found that channel

TABLE 6.—Fatty acid composition (percentage of total) of whole-body samples of largemouth bass fed experimental diets formulated with lipids of varying sources and fatty acid compositions. Means (±SE) within a row followed by different letters are significantly different ($P \leq 0.05$).

	Lipid type				
Fatty acid	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
14:0	4.1 ± 0.0 z	1.4 ± 0.1 x	1.6 ± 0.1 yx	1.4 ± 0.0 x	1.8 ± 0.1 y
16:0	21.3 ± 0.5 z	17.5 ± 0.2 x	14.4 ± 0.5 v	15.3 ± 0.3 w	19.0 ± 0.2 y
(palmitoleic acid) 16:1(n-7)	8.9 ± 0.1 z	3.8 ± 0.2 x	4.4 ± 0.4 y	3.9 ± 0.1 x	4.3 ± 0.2 y
18:0	3.7 ± 0.1 y	3.2 ± 0.1 x	3.0 ± 0.0 x	3.6 ± 0.1 y	5.1 ± 0.2 z
(oleic acid) 18:1(n-9)	28.3 ± 1.2 x	30.5 ± 0.6 y	56.1 ± 0.7 z	26.9 ± 0.5 w	24.1 ± 0.7 v
(linoleic acid) 18:2(n-6)	11.8 ± 0.4 x	34.2 ± 0.6 z	11.9 ± 0.4 x	15.2 ± 0.4 y	11.8 ± 0.1 x
(linolenic acid) 18:3(n-3)	1.8 ± 0.0 x	1.5 ± 0.0 xw	1.1 ± 0.1 w	22.6 ± 0.5 z	2.8 ± 0.2 y
(arachidonic acid) 20:4(n-6)	1.3 ± 0.1 y	0.5 ± 0.0 x	0.5 ± 0.0 x	1.1 ± 0.1 y	14.0 ± 0.5 z
(eicosapentaenoic acid) 20:5(n-3)	2.8 ± 0.4 z	0.5 ± 0.0 x	0.6 ± 0.1 x	0.9 ± 0.1 y	0.6 ± 0.0 x
(docosapentaenoic acid) 22:5(n-3)	3.9 ± 0. 1 y	0.7 ± 0.0 x	0.7 ± 0.1 x	1.0 ± 0.1 x	5.2 ± 0.2 z
(docosahexaenoic acid) 22:6(n-3)	6.4 ± 0.5 z	1.8 ± 0.1 x	1.9 ± 0.1 x	3.0 ± 0.3 y	1.8 ± 0.1 x
Other	5.9 ± 0.2 y	4.5 ± 0.1 w	4.0 ± 0.1 v	5.3 ± 0.1 x	9.6 ± 0.2 z
Saturates	29.6 ± 0.4 z	22.5 ± 0.3 x	19.6 ± 0.6 v	20.8 ± 0.4 w	27.1 ± 0.3 y
Monenes	38.6 ± 1.2 y	35.7 ± 0.5 x	62.8 ± 0.5 z	31.7 ± 0.5 w	29.5 ± 0.6 v
Dienes	12.6 ± 0.4 x	35.5 ± 0.6 z	12.3 ± 0.4 x	15.7 ± 0.4 y	12.5 ± 0.1 x
PUFA[a]	31.0 ± 1.7 x	41.7 ± 0.3 y	17.5 ± 0.4 w	47.4 ± 0.8 z	43.2 ± 0.8 y
HUFA[b]	17.4 ± 1.2 y	7.1 ± 0.1 w	4.0 ± 0.1 w	5.3 ± 0.1 x	29.9 ± 0.5 z
n-3	15.6 ± 1.1 x	4.8 ± 0.2 v	4.4 ± 0.3 v	28.5 ± 0.5 z	10.6 ± 0.4 w
n-6	13.6 ± 0.5 w	35.5± 0.5 z	12.6 ± 0.4 v	18.2 ± 0.4 x	31.8 ± 0.4 y
n-3 or n-6	1.1 ± 0.0 y	0.1 ± 0.0 w	0.4 ± 0.0 x	1.6 ± 0.0 z	0.3 ± 0.0 x

[a] PUFA = polyunsaturated fatty acid.
[b] HUFA = highly unsaturated fatty acid.

TABLE 7.—Fatty acid composition (percentage of total diet) of largemouth bassexperimental diets formulated with lipids of varying sources and fatty acid compositions.

Fatty acid	Lipid type				
	Anchovy fish oil	Corn oil	Sunflower oil	Linseed oil	Fungal extract oil
(linoleic acid) 18:2(n-6)	1.2	3.9	1.4	1.5	1.2
(linolenic acid) 18:3(n-3)	0.2	0.1	0.1	2.5	0.3
(arachidonic acid) 20:4(n-6)	0.1	0.0	0.0	0.0	2.2
(eicosapentaenoic acid) 20:5(n-3)	0.8	0.1	0.1	0.1	0.1
(docosapentaenoic acid) 22:5(n-3)	0.1	0.0	0.0	0.0	0.0
(docosahexaenoic acid) 22:6(n-3)	0.5	0.1	0.1	0.1	0.1
PUFA[a]	2.9	4.3	1.7	4.2	4.1
HUFA[b]	1.5	0.3	0.3	0.3	2.9
Total n-3	1.2	0.4	0.3	2.6	0.5
Total n-6	1.3	4.0	1.4	1.5	3.6
n-3 or n-6	1.1	0.1	0.2	1.7	0.1

[a] PUFA = polyunsaturated fatty acid.
[b] HUFA = highly unsaturated fatty acid.

catfish *Ictalurus punctatus* required n-3 fatty acids in the range of 1–2% dietary LNA or 0.50–0.75% n-3 HUFAs to satisfy their n-3 EFA requirement. However, this study was designed to evaluate the utilization of alternative lipid sources, not define fatty acid requirements, so definitive statements on largemouth bass EFA requirements cannot be made.

Generally, coldwater fishes have a higher requirement for n-3 PUFAs, whereas warmwater fishes tend to have a greater requirement for n-6 fatty acids. In this study, all experimental diets contained more than 1% of n-6 fatty acids (Table 7). Common carp *Cyprinus carpio* are omnivorous fish that require both n-6 and n-3 fatty acids (at approximately 1% each) for best growth and feed efficiency (Takeuchi and Wantanabe 1977). Several studies have reported that various tilapias *Oreochromis* spp. require both n-3 and n-6 HUFAs for maximum growth (Stickney and Wurts 1986; Stickney and Hardy 1989; Chou and Shiau 1999). Growth performance in tilapia increased as the percentage of LA increased (Stickney et al. 1985) and as the percentage of fish oil increased to 10% of the diet (Stickney and Wurts 1986), suggesting that the specific fatty acid demands of tilapia are greater than those of largemouth bass.

In this study, fatty acid composition of the diet had no effect on the proximate composition of the test fish. This differs from the results reported by Coyle et al. (2000), who found that largemouth bass fed diets high in PUFAs (especially DHA) had lower whole-body lipid levels and higher protein levels than fish fed other diets. Earlier data indicating high DHA concentrations in largemouth bass eggs (Tidwell et al. 1996) could potentially indicate an important role for this fatty acid in reproduction and embryo development. Similarly, Ramachandran Nair and Gopakumar (1981) reported unusually high levels of DHA in eggs of Mozambique tilapia *O. mossambicus*, suggesting an important role in reproduction and embryo development.

As expected, the concentrations of individual fatty acids in the whole body were strongly influenced by fatty acid compositions of the added lipids and finished diets. Largemouth bass fed the diet supplemented with fish oil had the highest concentration of EPA and DHA. This differs from results reported on the ayu *Plecoglossus altivelis*, for which tissue levels of DHA remained relatively fixed despite dietary differences (Jeong et al. 2002). The body composition of fish fed the other diets also strongly mirrored the fatty acid composition of the added lipids.

Based on the lack of differences in survival, average weight, or feed conversion efficiencies, it would appear that the largemouth bass requirement for EFAs may be relatively low. However, this study was of limited duration and fish were not depleted of fatty acid stores before initiation of treatments. Before definitive conclusions can be reached, strict fatty acid requirement studies will need to be conducted.

In this study, alternative lipids appeared to be well utilized. These data agree with the recent findings of Subhadra et al. (2006), who reported no differences in weight gain, feed intake, FCR, or protein efficiency ratio of largemouth bass fed diets supplemented with 10% lipids from canola, chicken *Gallus gallus domesticus*, menhaden *Brevoortia* spp., or a mix of chicken and fish oils.

Conclusions

As a freshwater, warmwater species, the largemouth bass apparently does not have extremely high requirements for HUFAs of either the n-3 or n-6 families. However, this finding would need to be confirmed by

true fatty acid requirement studies using purified diets. For practical diets, the fish meal component would probably provide sufficient amounts of these fatty acids, and supplemental lipids could be in the form of cheaper vegetable oils, catfish oil, or a vegetable–fish oil mixture for production diets. However, diets to be used for broodfish should probably be evaluated further relative to the potential role of specific fatty acids on reproduction.

Acknowledgments

This research was supported by a U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service grant to Kentucky State University under agreement KYX-80-91-04A. Funding was also provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as Kentucky State University's Program of Distinction. We thank Karla Johnson for typing the manuscript and Harold Benson for administrative support. We also thank William Stilwell and Russell Neal for technical assistance, daily system maintenance, and assistance in making diets. We are grateful to David Yasharian, Nicholas Skudlarek, and Jason Danaher for technical assistance.

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