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Effects of dietary protein level on second year growth and water quality for largemouth bass (*Micropterus salmoides*) raised in ponds

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Abstract

Juvenile largemouth bass (122.1 ± 2.6 g), trained to accept artificial diets, were stocked into nine 0.04 ha ponds at a density of 12350 fish ha^{-1} and fed one of three practical diets containing either 42, 44, or 47% protein (dry weight). Fish meal composed a constant percentage (53%) of the dietary protein in each diet. Fish were fed all they would consume in 30 min once daily for 12 months (May 1994–May 1995). At final harvest, fish fed the 47% protein diet had significantly higher ($P < 0.05$) individual weights (436 g), weight gain (313 g), total biomass (5330 kg ha^{-1}), and survival (99%), and significantly lower ($P < 0.05$) feed conversion ratios (2.0) than fish fed the 42% protein diet. For fish fed the diet containing 44% protein, these variables were not significantly different ($P > 0.05$) from fish fed either 42% or 47% protein. Averaged over the study period, total ammonia–nitrogen (TAN) concentrations were significantly higher ($P < 0.05$) in ponds in which bass were fed the 47% protein diet than in ponds in which bass were fed the other diets. Concentrations of TAN in ponds in which fish were fed 44% protein were significantly higher ($P < 0.05$) than those with fish fed 42% protein. These results suggest that largemouth bass are amenable to high density culture conditions but have relatively high dietary protein requirements.

Keywords: *Micropterus salmoides*; Feeding and nutrition-fish; Growth-fish

1. Introduction

Although considerable research effort has been devoted to the largemouth bass, most has been directed toward hatchery production for, and utilization in, sport fisheries and

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fisheries management (Simco et al., 1986). Little work has been done on the growout of largemouth bass to larger sizes (Brandt, 1991), elucidation of their nutritional requirements, or development of practical diets for intensive production (JSA, 1983). In the 1960s, Snow and associates conducted a series of studies on the culture of feed-trained largemouth bass utilizing the Oregon Moist Pellet (Snow, 1968a, Snow, 1968b; Snow and Maxwell, 1970). Later, Anderson et al. (1981) determined minimum protein requirements of Age 0 and Age 1 largemouth bass to be 39.9% and 40.8%, respectively.

In recent years, interest in the culture of Centrachidae species to sizes larger than those normally produced for sportfish stocking has increased (Brandt, 1991). Demand for large (> 100 g) largemouth bass has grown dramatically in the past few years and now far exceeds availability (JSA, 1983). This demand is based largely on increasing utilization in fee-fishing (JSA, 1983), managed trophy fisheries (Dupree and Huner, 1984), and as live food products in ethnic markets. In the United States, the Joint Subcommittee on Aquaculture listed determination of efficient growout procedures, evaluation of effects of water quality (metabolic wastes) under intensive culture conditions,' and development of species-specific, cost-efficient, diets utilizing practical feed ingredients as research priorities for largemouth bass aquaculture (JSA, 1983). If this information can be generated, there appears to be a favorable financial potential for commercial production of this species (JSA, 1983). The objective of the current study was to evaluate growth, body composition, and survival of second year largemouth bass fed practical diets containing different protein levels and their impacts on water quality under intensive pond culture conditions.

2. Materials and methods

Test diets contained either 42, 44, or 47% protein (Table 1) and were formulated to be isocaloric based on gross energy values of 5.64 kcal g^{-1} protein, 4.11 kcal g^{-1} carbohydrate, and 9.44 kcal g^{-1} fat (NRC, 1993). The percentage of dietary protein contributed by fish meal was maintained at approximately 53% in all three diets. Diets were extruded into floating pellets by a commercial feed mill (Integral Fish Foods, Inc. Grand Junction, CO) and stored frozen (−10°C) until fed. Diets were analyzed for crude protein, lipid, ash, and moisture (Table 1). Crude protein was determined using a LECO FP-228 nitrogen determinator (Sweeney and Rexroad, 1987), crude lipid by chloroform-ethanol extraction, ash was determined in a muffle furnace (600°C) for 24 h, and moisture by drying to constant weight (AOAC, 1990). Diets were also analyzed for amino acid composition (Table 1) and fatty acid composition (Table 2) by a commercial analytical laboratory (Woodson-Tenent Laboratories, Dayton, OH).

Pellet-trained juvenile largemouth bass (*Micropterus salmoides*) were stocked on 5 May 1995 into 12 0.04 ha ponds at a rate of 12350 fish ha^{-1} at an initial size (mean ± SE) of 122.1 ± 2.7 g. Fish were fed once daily all they would consume in a 30 min period. Each of the three treatments (diets) was replicated in three ponds. Ponds used in this study were approximately 1.5 m deep and were supplied with water from a reservoir filled by rain runoff. Water levels in the ponds were maintained at a constant depth by periodic additions. Water temperature and dissolved oxygen (DO) were

Table 1
Ingredients and chemical composition of diets, containing three levels of protein, fed to largemouth bass

Ingredient	Dietary protein (%)		
	42	44	47
Menhaden fish meal (67%)	30.0	35.0	40.0
Soybean meal (44%)	25.5	33.0	41.0
Corn meal	35.9	26.9	17.4
Menahaden oil	7.0	3.5	0.0
Dicalcium phosphate	0.4	0.4	0.4
Vitamin mix [a]	0.5	0.5	0.5
Mineral mix [b]	0.6	0.6	0.6
Chemical analysis			
Protein [c]	41.7 ± 0.3	44.1 ± 0.4	46.9 ± 0.1
Lipid [c]	8.7 ± 0.2	5.9 ± 0.1	3.7 ± 0.0
Moisture	6.4 ± 0.0	9.7 ± 0.1	11.6 ± 0.1
Ash	11.5 ± 0.0	11.1 ± 0.1	11.5 ± 0.1
Lysine [d]	6.11 ± 0.00	6.28 ± 0.01	6.33 ± 0.07
Methionine [d]	1.98 ± 0.05	2.02 ± 0.05	2.07 ± 0.05
Arginine [d]	6.61 ± 0.06	6.76 ± 0.02	6.56 ± 0.02
Energy (kcal g^{-1}) [e]	4.56 ± 0.01	4.32 ± 0.01	4.13 ± 0.01
Protein/energy (mg $kcal^{-1}$)	91.5 ± 0.4	102.1 ± 0.6	113.6 ± 0.4

[a] Vitamin mix supplied the following vitamins (IU or mg kg^{-1} of diet): vitamin A (as retinol palmitote), 6000 IU; vitamin C (as ascorbic acid), 780 mg vitamin D (as cholecalciferol), 2200 IU; vitamin E (as αtocopherol), 150 IU; vitamin K (as menadione), 10 mg; niacin, 200 mg; pantothenic acid, 60 mg; thiamin, 30 mg; riboflavin, 20 mg; pyridoxine, 20 mg; folic acid, 5 mg; B_{12}, 0.01 mg; biotin, 2 mg; choline, 2500 mg.
[b] Mineral mix supplied the following (mg kg^{-1} of diet): manganese, 180 mg; copper, 8 mg; cobalt, 1.5 mg; iron, 66 mg; zinc, 150 mg; iodine, 6 mg; selenium, 0.3 mg.
[c] Moisture-free basis.
[d] Percent of total amino acids.
[e] Gross energy values (NRC, 1993).

monitored in each pond twice daily (09.00 and 16.00 h) at a depth of 0.5 m using a YSI Model 57 oxygen meter (YSI, Yellow Springs, OH). Mechanical aeration was supplied if DO was predicted (by graph) to fall below 5 mg l^{-1} during the night. Ammonia,

Table 2
Concentrations of selected fatty acid (% of total fatty acids) in largemouth bass eggs, experimental diets, and white muscle from fish fed those diets

Fatty acid	LMB eggs	Dietary Protein					
		42%		44%		47%	
		Feed	Flesh	Feed	Flesh	Feed	Flesh
18:3n−3	1.3 ± 0.1	1.6 ± 0.0	1.1 ± 0.3	1.7 ± 0.0	0.8 ± 0.2	2.0 ± 0.0	0.9 ± 0.3
20:4n−6	2.3 ± 0.1	0.6 ± 0.0	1.6 ± 0.5	0.6 ± 0.0	1.5 ± 0.3	0.5 ± 0.0	2.3 ± 0.9
20:5n−3	3.8 ± 0.0	5.2 ± 0.1	3.0 ± 0.5	5.3 ± 0.1	3.9 ± 0.8	4.1 ± 0.1	4.2 ± 0.5
22:6n−3	19.8 ± 0.4	4.2 ± 0.2	17.9 ± 6.2	4.6 ± 0.3	21.3 ± 6.0	4.7 ± 0.1	21.4 ± 1.8

nitrite, and pH were determined weekly (16.00 h) using a HACH DREL/2000 spectrophotometer (HACH, Loveland, CO).

At stocking, 25 randomly-sampled fish were individually weighed (g), measured (total length), sexed, and killed to determine gut weight, liver weight, gonad weight (ovary or testis), head weight, and dressed weight (with and without scales). After stocking, a sample of ≥ 50 fish were captured monthly, weighed, counted, and returned to the pond for determination of average individual weights. The measurements listed above for stocking data were also determined on six fish per pond at the 27 October 1994 sampling (177 days post stocking) and at final harvest (3 May 1995). Data reporting 'summer growth' include the period from the 5 May stocking to 27 October sampling. Data reported as 'winter growth' extend from the 27 October 1995 sampling until the 3 May 1995 final harvest. For summer growth, survival was assumed to be 100%.

Total number and weight of fish in each pond were determined at harvest. Fifty fish were then randomly sampled from each pond and individually weighed (g) and measured for total length (cm). Five fish from each pond were removed for dressout and organ weights as described previously. Whole bodies (including head, frame, and scales) of three fish at harvest, and fillets of three fish at stocking, sampled from each pond during fall sampling, and at a final harvest, were homogenized in a blender and analyzed for lipid, protein, ash, and moisture. Samples at stocking were pooled while those at the fall sampling and final harvest were analyzed separately. Analyses were as described previously for diets, except that lipid was by ether extraction (AOAC, 1990). At the fall sampling eggs and white muscle from three randomly selected fish were frozen with liquid nitrogen (-185°C) for fatty acid composition (Table 2).

Feed conversion ratio (FCR) was calculated from FCR = weight of feed fed (kg)/live weight gain (kg). Specific growth rate (SGR, % body weight per day) was calculated from $\mathrm{SGR} = [(\ln W_f - \ln W_i)/t] \times 100$, where W_f is final weight; W_i is initial weight; and t is time in days (Ricker, 1975). Condition factor (K) was calculated from $K = 100 \times W/L^3$, where W is weight (g); and L is total length (cm) (Weatherly and Gill, 1987.)

Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (Statistical Analysis Systems, 1988) to determine the effects of dietary protein level on growth, condition factor, feed conversion, survival, body composition, dressout weights, organ weights, and water quality variables. All percentage and ratio data were transformed to arc sin values prior to analysis (Zar, 1984).

3. Results

3.1. Water quality

Overall means for total ammonia-nitrogen (TAN) (Fig. 1), were significantly different ($P \leq 0.05$) among ponds in which fish were fed the 42, 44, and 47% protein diets, averaging 0.62, 0.83, and 1.3 mg l^{-1}, respectively. Differences at individual sampling dates occurred primarily during periods of low water temperatures, especially December,

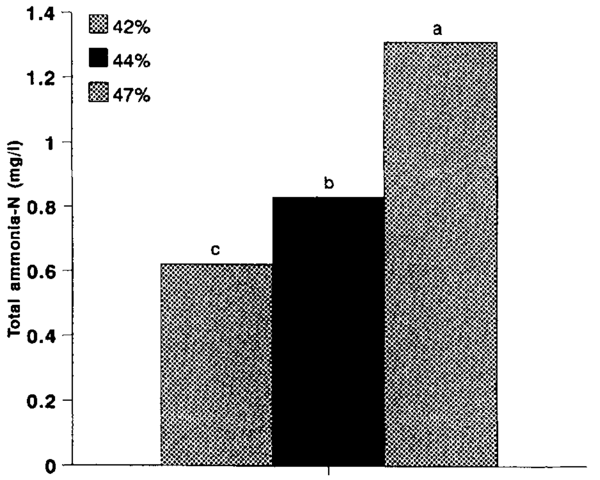

Fig. 1. Overall means of total ammonia-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each bar represents a mean of 52 weekly samples per pond in three replicate ponds per diet. Different letters above bars indicate significant difference among treatment means ($P < 0.05$).

January, and February (Fig. 2). Concentrations of TAN during those periods reflected the same relationships to dietary protein levels as did overall TAN means (47 > 44 > 42% diets).

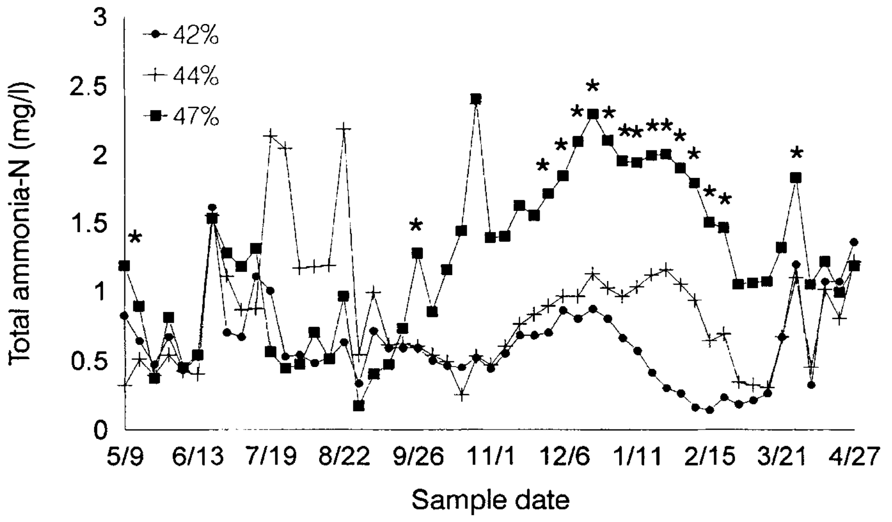

Fig. 2. Weekly means of total ammonia-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each point represents four weekly samples per pond in three replicate ponds per diet. An asterisk indicates a significant difference among treatments ($P < 0.05$).

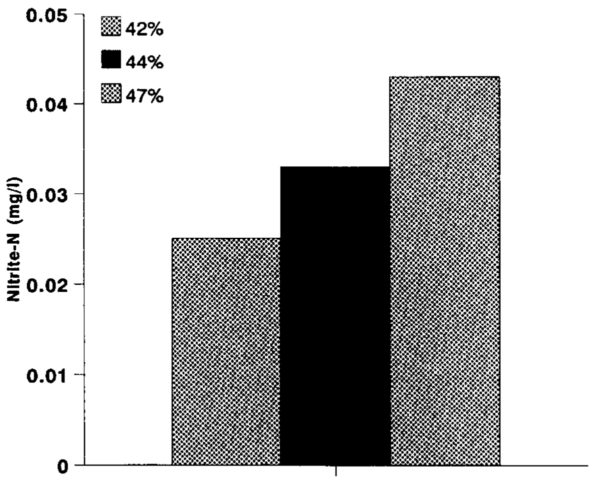

Fig. 3. Overall means of nitrite-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each bar represents a mean of 52 weekly samples per pond in three replicate ponds per diet. There were no significant difference among treatments ($P > 0.05$).

Overall nitrite concentrations for the 12 month culture period (Fig. 3) reflected dietary protein levels (47 > 44 > 42%), though these differences were not statistically significant ($P > 0.05$). There were significant treatment differences ($P \leq 0.05$) in nitrite concentrations at some individual sampling dates (Fig. 4), primarily during periods of rapidly decreasing (November–December), and increasing (February–March) water

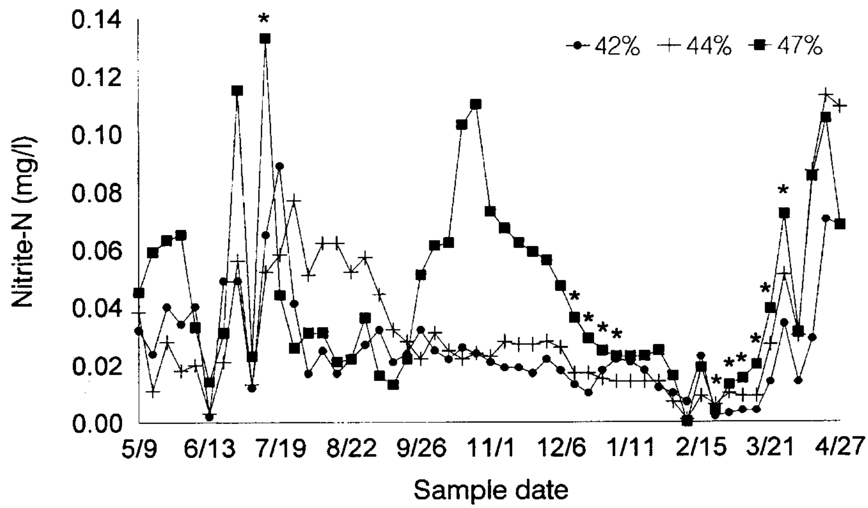

Fig. 4. Weekly means of nitrite-nitrogen (mg l^{-1}) in ponds in which juvenile largemouth bass were fed diets containing 42, 44, or 47% protein. Each point represents four weekly samples per pond in three replicate ponds per diet. An asterisk indicates a significant difference among treatments ($P < 0.05$).

Table 3
Individual weight, weight gain, percentage survival, feed conversion ratio (FCR), and unit production of largemouth bass fed diets containing three protein percentages [a]

Production variable	Dietary protein		
	42%	44%	47%
Harvest weight (g)	374 ± 19b	406 ± 23ab	436 ± 38a
Total individual gain (g)	313 ± 22b	331 ± 18ab	351 ± 25a
Survival (%)	86 ± 6b	92 ± 7ab	99 ± 1a
Total feed conversion ratio	2.6 ± 0.3a	2.3 ± 0.2ab	2.0 ± 0.2b
Gross yield (kg ha^{-1})	3972 ± 325b	4613 ± 469ab	5330 ± 505a
Summer individual gain (%)	162.5 ± 14.8b	157 ± 7.0b	201.7 ± 6.2a
Summer specific growth rate (%)	0.54 ± 0.04b	0.53 ± 0.02a	0.62 ± 0.01a
Summer feed conversion ratio	2.2 ± 0.1ab	2.4 ± 0.2a	1.9 ± 0.0b
Winter individual gain (%)	19.6 ± 11.0	28.5 ± 5.4	17.3 ± 5.1
Winter specific growth rate (%)	0.09 ± 0.6	0.12 ± 0.03	0.09 ± 0.03
Winter feed conversion ratio	4.2 ± 1.9	2.4 ± 0.4	3.9 ± 1.0

[a] Values are means of ± s.e. of three replicate ponds. Means in the same row with different letters are significantly different ($P < 0.05$).

temperatures, with ponds in which fish were fed the 47% protein feed having higher levels.

3.2. Production

At harvest, largemouth bass fed the 47% protein diet had significantly higher ($P \leq 0.05$) average weights (436 g), gains (351 g), survival (99%), and total biomass

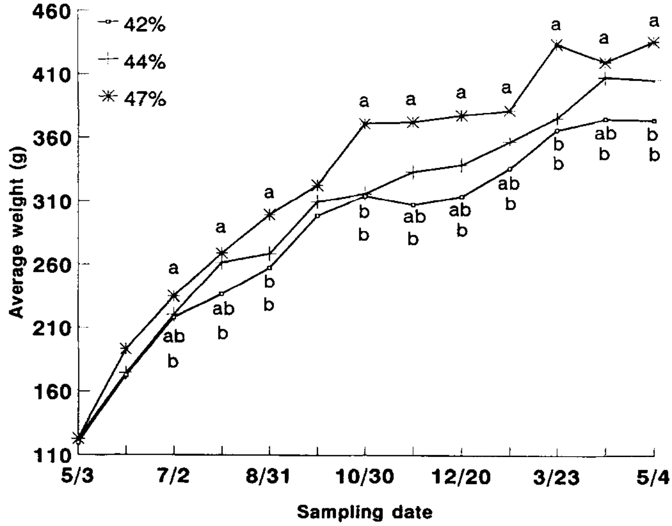

Fig. 5. Mean sample weights of largemouth bass fed diets containing 42, 44, 47% protein. Each point represents three replicate ponds. Significant differences among sample weights ($P < 0.05$) are indicated by different letters.

(4252 kg ha^{-1}), and significantly lower ($P \leq 0.05$) feed conversion ratios (FCR; 2.0), than bass fed the 42% protein diet (Table 3). Bass fed the 44% protein diet were not significantly different ($P > 0.05$) from those fed 42 or 47% protein diets in any of these variables. The patterns of weight gain in fish fed the three diets over the 12 month period are presented in Fig. 5. Significant differences in average weights developed by two months post-stocking. Bass fed 47% protein gained approximately 202% during the summer period and 17% during the winter (Table 3). There were no significant differences ($P > 0.05$) between fish in the three treatments in winter individual gains, winter SGR, or winter FCR.

Dressout percentages, head, fillet, and gut weights (as a percentage of body weight) did not differ significantly ($P > 0.05$) among fish fed the three diets and averaged 61.2, 27.4, 37.5, and 11.6%. Proximate compositions of whole bodies and fillets did not differ significantly ($P > 0.05$) among bass fed the three diets. Moisture, lipid, protein, and ash averaged 71.2, 6.0, 18.9, and 3.8% in the whole body and 76.9, 1.3, 20.6, and 1.1% in the fillet.

4. Discussion

The bass were active feeders, especially in early summer (June) and slowed later in the summer (Fig. 6). Feeding continued until temperatures dropped below approximately 8°C. In the spring, bass began to feed actively again as temperatures rose above 8°C. This response is similar to those for the hybrid bluegill (*Lepomis cyanellus* × *L. macrochirus*) (Tidwell et al., 1994).

Major differences in total ammonia-nitrogen and nitrite-nitrogen concentrations did

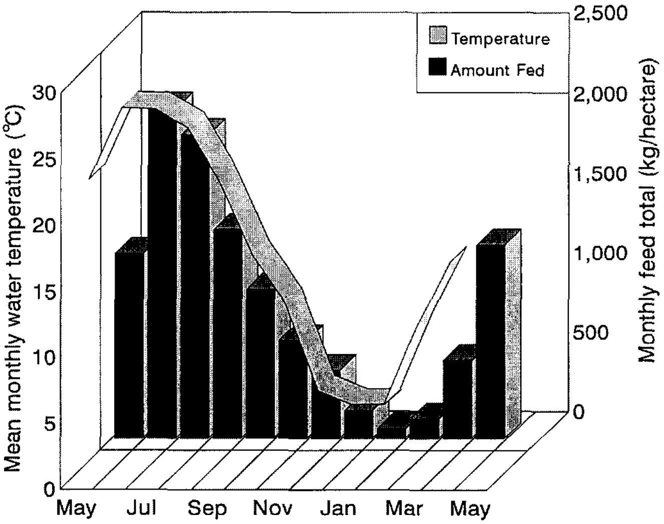

Fig. 6. Overall monthly feed totals per pond (y_1) for all diets at different water temperatures (y_2) over a one year culture period.

not occur during the high feeding rates of summer, but during the cooler water temperatures, and lower feeding rates, of fall and winter (September–March). This is likely due to reduced assimilation of ammonia by phytoplankton during the winter period (Tucker and Robinson, 1990) and reduced rates of bacterial nitrification of nitrite to nitrate at cool temperatures (Tucker and Robinson, 1990). Ammonia and nitrite data during these periods largely reflect differences in nitrogen input from the three diets, with ponds in which fish were fed the 47% protein diet having higher levels of ammonia and nitrite than ponds with fish fed 44% protein, which were higher than those in which fish were fed the 42% protein diet.

Protein requirements of largemouth bass, fed practical diets under production conditions, appear to be higher than requirements established by Anderson et al. (1981) (39–40%) using semi-purified diets under controlled conditions. It is possible that even higher protein levels in this study could have been advantageous. However, effects on water quality must be considered. A recent study by Brecka et al. (1995) found that tiger muskellunge (*Esox masquinongy* × *E. luscius*) fed their highest protein level (45%) exhibited the fastest growth, and stated that fish may have responded to even higher protein levels. In the present study, fish fed the 47% protein diet were larger than fish in other treatments at every post-stocking sampling throughout the 12 month study period. Differences in fish fed the 42 and 44% protein diets were less pronounced. Survival of largemouth was good (> 85%) in all treatments, but was improved by increased dietary protein.

Largemouth bass growth slowed during the winter months, but winter gains (October–March) averaged approximately 22% over all three diets. When compared with another centrachid, winter gains were similar to results for small (40 g) hybrid bluegill (28%) (Tidwell et al., 1992), and higher than winter gains of larger (100 g) hybrid bluegill (17%) (Tidwell et al., 1994). Total weight gains over the 12 month period (257%) were higher than those reported for hybrid bluegill (167%) stocked at the same density for a similar culture period (Tidwell et al., 1994). Feed conversion ratios were superior to those for hybrid bluegill, averaging 2.0 for largemouth bass, while hybrid bluegill averaged 5.7 (Tidwell et al., 1994).

Dressout and fillet percentages for bass (61% and 38%) were similar to those reported for channel catfish (59% and 40%) (Ammerman, 1985). Body composition data were similar to those reported for channel catfish of similar sizes except that bass fillets contained lower lipid levels and higher protein levels. Levels of DHA (22:6n − 3) in the lipids of bass egg and muscle tissues were > 290% than levels in the diets (Table 2), possibly indicating relative importance. Body protein of largemouth bass did not increase with increased dietary protein as reported for tiger muskellunge (Brecka et al., 1995) and walleye (*Stizostedion vitreum*) (Barrows et al., 1988), nor did body lipid decrease as reported for channel catfish (Reis et al., 1989); striped bass (*Morone saxatilis*) (Millikin, 1982); walleye (Barrows et al., 1988); or tiger muskellunge (Brecka et al., 1995). Fish readily utilize dietary protein as an energy source. However, inexpensive lipids can sometimes be added as a source of energy, sparing the expensive protein necessary for growth. In this study there were no significant differences in body compositions of fish fed the three diets, indicating it may be possible to add some additional lipids for protein sparing without negatively impacting body composition.

In summary, largemouth bass handled, survived, and grew well at what would be considered commercial densities. Bass responded well to relatively high protein levels, even at large sizes. The ability of dietary lipid to spare protein for growth should be evaluated as a potential method to reduce dietary protein levels and feed costs for largemouth bass culture.

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