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Growth and Body Composition of Juvenile Hybrid Bluegill *Lepomis cyanellus* × *L. macrochirus* Fed Practical Diets Containing Various Percentages of Protein

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Abstract

Growth, survival, and body composition were evaluated in two feeding trials using juvenile hybrid bluegill *Lepomis cyanellus* × *L. macrochirus*. In Experiment 1, hybrid bluegill (20 g) were stocked into 1.25-m^3 cages at a rate of 300 fish/cage and fed diets containing 35, 40, 44, or 48% protein for 12 wk. Fish meal comprised 32% of the dietary protein in all diets. Fish were fed all they could consume in 40 min. No significant differences ($P > 0.05$) in individual length, individual weight, specific growth rate (SGR), condition factor (K), and feed conversion ratio (FCR) were found among treatments and averaged 13.4 cm, 47.4 g, 1.02%/d, 1.96, and 4.06, respectively. Whole-body composition of hybrid bluegill indicated that fish fed a diet containing 35% protein had a significantly lower ($P < 0.05$) percentage protein (56.3%) and a higher ($P < 0.05$) percentage lipid (29.3%) compared to fish fed diets containing 40, 44, and 48% protein. In Experiment 2, 15 hybrid bluegill (15 g) were stocked into 110-L aquaria and fed one of four diets containing 28, 32, 36, or 38% protein for 10 wk. Fish were fed twice daily all they would consume in 20 min. Fish fed a diet containing 38% protein had higher ($P < 0.05$) percentage weight gain (265%) than fish fed diets containing 28% (203%) and 32% (219%) protein, but were not significantly different ($P > 0.05$) compared to fish fed a diet containing 36% protein (251%). Feed conversion ratio (FCR) of hybrid bluegill fed diets containing 36% and 38% protein (average 1.39) were significantly lower ($P < 0.05$) than fish fed a diet with 28% protein (1.73). Results from these studies indicate that hybrid bluegill can be fed a practical diet containing 35–36% protein (with fish meal comprising 32% of the protein). Further refinement of the diet formulation may allow producers to reduce diet and production costs.

Hybrid bluegill *Lepomis cyanellus* × *L. macrochirus* have become increasingly popular for stocking into recreational (fee-fishing) ponds in temperate regions of the United States (e.g., Kentucky). Hybrid bluegill are particularly desirable for marketing through fee-fishing operations that provide a market in areas where food-fish processing is not well established. However, hybrid bluegill have several attributes that make them suitable for commercial aquaculture production, including ready acceptance of prepared diets (Lewis and Heidinger 1971; Tidwell et al. 1992; Webster et al. 1992) and rapid growth (Brunson 1983). These traits may allow producers to grow hybrid bluegill to marketable size. Heidinger (1975) suggested that in regions having a short growing season (when air temperatures are higher than 21 C), hybrid bluegill may be more economical to culture than channel catfish.

Many producers do not feed prepared diets to hybrid bluegill, relying on natural food organisms within the pond. Since intensive production of fish may quickly deplete natural food items in a pond, use of prepared diets may allow hybrid bluegill to grow more rapidly and be stocked at higher densities. Because protein is the most expensive component of a diet, knowledge of the protein requirements of the fish is essential for the formulation of nutritious, economical diets.

Various studies have shown that the percentage of protein required for optimal growth varies with species (DeLong et al. 1958; Garling and Wilson 1976; Daniels

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and Robinson 1986; Kim et al. 1991; Nematipour et al. 1992) and fish size (Satia 1974). However, information regarding the nutritional requirements for hybrid bluegill is limited and may limit the culture potential. Tidwell et al. (1992) reported that growth of small (5 g) hybrid bluegill increased with increasing protein level when reared in aquaria and suggested that a diet containing at least 37% protein should be fed to small (<20 g) hybrid bluegill. Webster et al. (1992) reported that growth of small (3.5 g) hybrid bluegill raised in ponds and fed a diet containing 32% protein was similar to fish fed a diet containing 38% protein. These fish had access to natural food organisms which may have masked potential growth differences in fish fed the two diets. As fish grow, their protein requirement may be reduced (Lovell 1989). Information on the protein requirement of larger (>20 g) hybrid bluegill is needed for the formulation of production diets. The objectives of these studies were to determine growth and body composition of juvenile hybrid bluegill fed practical diets containing various percentages of protein.

Materials and Methods

Experiment 1

Juvenile hybrid bluegill (female *Lepomis macrochirus* X male *L. cyanallus*; average weight of 20.0 ± 1.0 g) were stocked on 16 July 1993 into 12 1.25-m^3 floating cages moored over the deepest area (4 m) of a 1.0-ha pond (average depth = 2.0 m) located at the Agricultural Research Farm, Kentucky State University, Frankfort, Kentucky, USA. Each cage had a wooden frame with a removable lid and was constructed of 10-mm polyethylene mesh. An 8.0-cm panel of polyethylene mesh (0.2 mm) was installed around the top of the inside of each cage to prevent loss of the floating diet. Cages were anchored to the dock with a minimum distance of 2 m between cages.

Three-hundred juveniles were hand-counted and randomly stocked into each randomly assigned cage. Fish were fed one of four extruded (floating) diets formulated to contain either 32, 36, 40, or 44% protein for 12 wk. Actual protein levels were 35, 40, 44, and 48% protein (Table 1). Diets were extruded by a commercial feed mill (Integral Fish Foods, Inc., Grand Junction, Colorado, USA) into 3/16-inch pellets. Fish meal was added at a fixed percentage (32%) of the total protein of the diet. Fish were fed twice daily for the first 6 wk of the study (0800 and 1900 h). As water temperature increased during the summer, fish did not feed actively during the morning feeding. Subsequently, fish were fed only once daily (1900 h). During each feeding period, fish were fed all they could consume in 40 min. Due to the lower level of starch (corn) in the diet containing 48% protein (diet 4), this diet did not float. Fish fed this diet were fed in small increments so diet pellets would be consumed before sinking past the fish. There were three replications of each treatment (diets). However, fish from one cage receiving the diet containing 40% protein (diet 2) escaped, and this treatment had only two replications.

Diets were analyzed for crude protein, fat, ash, fiber, and moisture (Table 1). Protein was determined using the Kjeldahl method, fat was determined by the acid-hydrolysis method; ash was determined by placing 10 g of sample into a muffle furnace (600 C); crude fiber was determined using method 962.09 (AOAC 1990); and moisture was determined by placing a 10-g sample in a drying oven (95 C) until constant weight (AOAC 1990). Diets were analyzed for amino acid composition (Table 1) by a commercial analytical laboratory (Woodson-Tenent Laboratories, Dayton, Ohio, USA). Since digestible energy values for the ingredients have not been determined for hybrid bluegill, available energy was calculated using physiological fuel values of 4.0, 4.0, and 9.0 kcal/g for carbohydrate (NFE), protein, and lipid, respectively (Garling and Wilson 1976).

Temperature and dissolved oxygen (DO)

TABLE 1. *Formulation and proximate composition of four practical diets containing various percentages of protein and fed to hybrid bluegill reared in cages (experiment 1). Fish meal was added to comprise a constant percentage (32%) of the dietary protein.*

	Diet no.			
	1	2	3	4
Ingredient				
Menhaden fish meal	15.00	17.00	19.00	21.00
Soybean meal (48%)	41.00	49.00	57.50	63.50
Corn meal	36.95	27.45	17.65	10.15
Dicalcium phosphate	1.00	1.00	0.80	0.80
Vitamin mix[a]	1.00	1.00	1.00	1.00
Mineral mix[b]	1.00	1.00	1.00	1.00
Cod liver oil[c]	4.00	3.50	3.00	2.50
Ascorbic acid	0.05	0.05	0.05	0.05
Chemical analysis				
Moisture (%)	9.31 ± 0.03	9.63 ± 0.09	9.25 ± 0.02	8.69 ± 0.12
Protein (%)[d]	35.2 ± 0.02	40.2 ± 0.33	44.2 ± 0.68	47.7 ± 0.20
Lipid (%)[d]	7.76 ± 0.01	7.16 ± 0.32	6.67 ± 0.05	6.03 ± 0.32
Fiber (%)[d]	1.9 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.1 ± 0.1
Ash (%)[d]	6.18 ± 0.09	7.48 ± 0.09	7.72 ± 0.06	8.33 ± 0.01
NFE[e]	48.96 ± 0.09	43.16 ± 0.36	39.31 ± 0.53	35.84 ± 0.26
Energy (kcal/g)[f]	4.07 ± 0.04	3.98 ± 0.02	3.94 ± 0.03	3.88 ± 0.03
P:E[g]	86.5	101.0	112.2	122.9
Lysine[h]	1.84	2.33	2.50	2.75
Methionine[h]	0.59	0.72	0.73	0.74

[a] Vitamin mix supplied the following vitamins (mg or IU/kg of diet): vitamin A, 6,000 IU; vitamin D, 2,200 IU; vitamin E, 150 IU; vitamin K, 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.

[b] Mineral mix supplied the following minerals (mg/kg 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] Cod liver oil had 0.02% BHT added as anti-oxidant.

[d] Moisture-free basis.

[e] NFE (nitrogen free extract) = 100 − (%protein + %lipid + %ash + %fiber).

[f] Available energy based upon 4 kcal/g, 4 kcal/g, and 9 kcal/g for protein, carbohydrate, and lipid, respectively.

[g] Protein to energy ratio (mg/kcal).

[h] Amino acids expressed as g amino acid/100 g of diet. Values are of two replications.

were monitored twice daily (0800 and 1530 h) outside the cages, at a depth of 0.75 m, using a YSI Model 57 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). If the DO was graphically predicted to decline below 4.0 mg/L during the night, aeration was provided using a 5-hp electric paddlewheel (S&N Sprayer Company, Inc., Greenwood, Mississippi, USA). Weekly measurements of pH were recorded using an electronic pH meter (pH Pen, Fisher Scientific, Cincinnati, Ohio, USA). Total ammonia$^-$ nitrogen, nitrite, and alkalinity were measured weekly using a DR-5 spectrophotometer (Hach Co., Loveland, Colorado, USA).

During the first week of the study, fish were diagnosed with an infection of *Columnaris flexibactor*. All fish were treated so as to maintain a concentration of approximately 2 mg/L of potassium permanganate for 30 min. Thereafter, every 2 wk during the study, fish were treated in an identical manner.

Fish were harvested on 18 October 1993 and were not fed 16 hours prior to harvest. Total number and weight of fish in each cage were determined at harvest. Twentyfive fish were randomly sampled from each cage and individually weighed (g) and measured for total length (cm). Five fish were randomly sampled from each cage for analysis of body composition (percentage moisture, protein, and fat). All five fish from each cage were homogenized together in a blender and analyzed for protein using the Kjeldahl method, for fat using the ether extraction method, and for moisture by drying a 10⁻ g sample (95 C) until constant weight (AOAC 1990). Five fish were randomly sampled from each cage, homogenized separately in a blender, and analyzed for amino acid composition.

Food conversion ratio (FCR) and specific growth rate (SGR) were calculated as follows: FCR = total diet fed (kg)/total wet weight gain (kg); SGR = $((\ln W_t - \ln W_i)/T) \times 100$, where W_t is the average individual weight of fish at time t, W_i is the average individual weight of fish at time 0, and T is the culture period in days. Condition factor (K) was calculated using the formula: $K = 100 \times (\text{weight})/(\text{total length})^3$.

Data were analyzed using the SAS General Linear Models procedure (Statistical Analysis Systems 1988) for significant differences among treatment means. Means were analyzed by Duncan's multiple range test. All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Significance was tested at the $P = 0.05$ level.

Experiment 2

The feeding trial was conducted in 16 110-L glass aquaria. Water was recirculated through biological and mechanical filters. The recirculating system consisted of a 3,375-L vertical screen filter system utilizing high-density polyester screens (Red Ewald, Inc., Karnes City, Texas, USA) and a drum filter system comprised of plastic "bio-balls" and fibrous polyester material. These two filter systems removed particulate material and provided substrate for *Nitrosomonas* and *Nitrobacter* bacteria. Continuous aeration was provided by a blower and air stones. Water replacement rate for the system was approximately 2% of total volume per day. Chloride levels were maintained at approximately 200 mg/L, by addition of food grade NaCl, to minimize any potential adverse effects of nitrite to fish health. Each aquarium was supplied with water at a rate of 5.0 L/min and cleaned daily to remove uneaten feed and feces. Black plastic covered the back and sides of all aquaria to minimize disturbances resulting when personnel were present in the laboratory. Continuous illumination was supplied by fluorescent ceiling lights.

Water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, Ohio, USA). Total ammonia-nitrogen and nitrite were measured every other day using a DREL 2000 spectrophotometer (Hach Co., Loveland, Colorado, USA). Total alkalinity and chloride were monitored twice weekly using the titration method of the DREL 2000; pH was monitored three times weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA).

Juvenile hybrid bluegill were obtained from a commercial supplier (Van Winkle's Fish Hatchery, Birdseye, Indiana, USA) and had an average weight (±SE) of 14.6 ± 5.9 g. Fifteen fish were randomly stocked into each aquarium with four replications per treatment. Fish were not weighed for the duration of the feeding trial. All fish were fed twice daily (0730 and 1600 h) all they could consume in 20 min for 10 wks. Fish were fed one of four floating diets containing 28, 32, 36, and 40% protein. Diets were extruded by a commercial feed mill (Integral Fish Foods, Inc., Grand Junction, Colorado, USA) into 3/16-inch pellets for use in the study. Diets were analyzed by a commercial laboratory (Woodson-Tenent Labs, Dayton, Ohio, USA; Table 2) as previously described. Due to differences in composition of ingredients, diets actually contained 28, 32, 36, and 38% protein. Data

TABLE 2. *Formulation and proximate composition of four practical diets containing various percentages of protein and fed to hybrid bluegill in aquaria (experiment 2).*

	Diet (% protein)			
	1 (28%)	2 (32%)	3 (36%)	4 (38%)
Ingredient				
Menhaden fish meal	18.00	22.00	28.00	35.00
Soybean meal	6.85	13.35	14.85	13.85
Corn meal	39.00	36.00	20.00	13.00
Wheat meal	28.00	20.50	29.00	30.00
Hydrolyzed feather meal	3.50	3.50	3.50	3.50
Monocalcium phosphate	0.75	0.75	0.75	0.75
Vitamin and mineral mix[a]	0.75	0.75	0.75	0.75
Menhaden oil[b]	3.00	3.00	3.00	3.00
Choline chloride	0.05	0.05	0.05	0.05
Ascorbic acid	0.10	0.10	0.10	0.10
Chemical analysis				
Moisture (%)	7.6 ± 0.2	9.5 ± 0.2	8.1 ± 0.1	8.5 ± 0.0
Protein (%)[c]	27.9 ± 0.1	32.0 ± 0.2	36.6 ± 0.2	37.8 ± 0.1
Lipid (%)[c]	11.1 ± 0.1	11.4 ± 0.2	11.7 ± 0.1	11.7 ± 0.2
Fiber (%)[c]	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.0	1.5 ± 0.0
Ash (%)[c]	6.93 ± 0.05	8.12 ± 0.02	9.41 ± 0.00	9.97 ± 0.01
NFE[d]	52.40 ± 0.16	46.72 ± 0.02	40.64 ± 0.16	38.980 ± 0.29
Energy[e]	4.21 ± 0.01	4.17 ± 0.02	4.10 ± 0.04	4.13 ± 0.01
P:E[f]	66.3	76.7	89.3	91.5
Lysine[g]	1.24	1.45	1.72	1.72
Methionine[g]	0.48	0.55	0.72	0.74

[a] Vitamin and mineral mix supplied the following vitamins and minerals (mg or IU/kg of diet): vitamin A, 6,000 IU; vitamin D, 2,200 IU; vitamin E, 150 IU; vitamin K, 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; manganese, 180 mg; copper, 8 mg; cobalt, 1.5 mg; iron, 66 mg; zinc, 150 mg; iodine, 6 mg; selenium, 0.3 mg.

[b] Cod liver oil had 0.02% BHT added as anti-oxidant.

[c] Moisture-free basis.

[d] NFE (nitrogen free extract) = 100 − (%protein + %lipid + %ash + %fiber).

[e] Available energy based upon 4 kcal/g, 4 kcal/g, and 9 kcal/g for protein, carbohydrate, and lipid, respectively.

[f] Protein to energy ratio (mg/kcal).

[g] Amino acids expressed as g amino acid/100 g of diet.

were collected and analyzed as previously described. Due to a sampling error, body composition analysis at the conclusion of the study could not be conducted.

Results

Experiment 1

Average monthly morning water temperatures (+SE) ranged from 27.4 ± 0.7 (for July) to 14.4 ± 1.1 (for October); whereas average monthly afternoon water temperatures ranged from 29.9 ± 0.9 (for July) to 14.8 ± 1.2 (for October). Morning DO levels averaged 8.2 ± 1.5, 7.0 ± 2.0, 8.6 ± 1.3, and 11.3 ± 0.7 mg/L for July, August, September and October, respectively; whereas afternoon values were 12.6 ± 4.6, 10.11 ± 2.52, 10.3 ± 1.4, and 12.2 ± 0.9 mg/L for those respective months. Total ammonia-nitrogen averaged 0.4 ± 0.1 mg/L, nitrite averaged 0.01 ± 0.01 mg/L, and pH averaged 8.7 ± 0.1 during the study.

There were no significant differences ($P > 0.05$) in individual length, individual weight, SGR, K, or FCR among treatments and these averaged 13.4 cm, 47.4 g, 1.02%/d, 1.96, and 4.06, respectively (Table 3).

TABLE 3. *Individual length, individual weight, yield, percentage survival, specific growth rate (SGR), condition factor (K), and feed conversion ratio (FCR) of juvenile hybrid bluegill fed practical diets containing various percentages of protein (Experiment 1). Values are means (±SE) of three replications, except diet 2 which had only 2 replications. Means followed by different superscripts are significantly different* (P < *0.05).*

	Diet (% protein)			
Variable	1 (35%)	2 (40%)	3 (44%)	4 (48%)
Indiv. length (cm)	13.4 ± 0.2^{a}	13.5 ± 0.1^{a}	13.6 ± 0.3^{a}	13.2 ± 0.2^{a}
Indiv. wt. (g)	47.9 ± 1.9^{a}	47.4 ± 1.9^{a}	50.7 ± 3.8^{a}	43.4 ± 1.4^{a}
Yield (kg/cage)	10.2 ± 0.4^{a}	8.3 ± 0.5^{bc}	9.5 ± 0.4^{b}	7.5 ± 0.4^{c}
Survival (%)	82.9 ± 1.1^{a}	66.8 ± 1.5^{c}	74.8 ± 2.4^{b}	66.1 ± 2.5^{c}
SGR	1.03 ± 0.04^{a}	1.03 ± 0.05^{a}	1.09 ± 0.09^{a}	0.91 ± 0.04^{a}
K	1.99 ± 0.03^{a}	1.93 ± 0.04^{a}	2.02 ± 0.25^{a}	1.91 ± 0.09^{a}
FCR	3.54 ± 0.26^{a}	4.44 ± 0.53^{a}	3.87 ± 0.29^{a}	4.39 ± 0.51^{a}

Fish fed a diet containing 35% protein had significantly higher ($P < 0.05$) survival (83%) and total harvest weight (10.2 kg/cage) than fish fed diets containing 40, 44, and 48% protein (Table 3). Fish fed diets containing 40% protein had a higher ($P < 0.05$) percentage survival (75%) compared to fish fed diets containing 40% and 48% protein.

Analysis of body composition of hybrid bluegill indicated that there was no significant difference ($P > 0.05$) in percentage moisture among treatments (Table 4). Fish fed a diet containing 35% protein had a significantly lower ($P < 0.05$) percentage protein (56.3%) compared to fish fed diets containing 40, 44, and 48% protein. Fish fed a diet containing 35% protein had a significantly higher ($P < 0.05$) percentage whole-body lipid (29.3%) compared to fish fed the other three diets (Table 4). Fish fed diets containing 40% and 44% protein had significantly higher ($P < 0.05$) percentage lipid (22.3% and 23.5%, respectively) than fish fed a diet containing 48% protein (17.4%). Hybrid bluegill fed a diet containing 48% protein had a significantly higher ($P < 0.05$) ash level (14.4%) than fish fed diets containing 35% and 44% protein; however, fish fed a diet containing 40% protein had whole-body ash levels similar ($P > 0.05$) to fish fed all other diets.

Whole-body amino acid analysis of hybrid bluegill indicated that there were no significant differences ($P > 0.05$) in levels of any amino acid among fish fed the four diets (Table 5). Glutamic acid comprised the highest percentage of the amino acid composition of hybrid bluegill (with an average of 13.99%) while cystine comprised the lowest percentage of the total amino acid pool (with an average of 0.87%).

Experiment 2

Over the duration of the study, water-quality parameters averaged (±SD): water temperature, 25.8 ± 0.5 C; DO, 6.5 ± 0.4 mg/L; total ammonia-nitrogen, 0.08 ± 0.08

TABLE 4. *Body composition (dry-matter basis) of hybrid bluegill fed practical diets containing various percentages of protein (Experiment 1). Values are means (±SE) of three replications, except for diet 2 which had two. Means in a row having the same superscript are not significantly different* (P > *0.05).*

	Diet (% protein)			
	1 (35%)	2 (40%)	3 (44%)	4 (48%)
Moisture	71.6 ± 0.8^{a}	73.1 ± 0.2^{a}	72.4 ± 0.3^{a}	73.3 ± 1.0^{a}
Protein	56.3 ± 1.2^{b}	61.0 ± 0.4^{a}	60.7 ± 0.7^{a}	64.6 ± 1.8^{a}
Fat	29.3 ± 1.2^{a}	22.3 ± 0.3^{b}	23.5 ± 0.7^{b}	17.4 ± 1.3^{c}
Ash	10.6 ± 0.7^{b}	12.4 ± 0.3^{ab}	11.3 ± 1.0^{b}	14.4 ± 0.1^{a}

TABLE 5. *Whole-body amino acid composition (g/100 g amino acid) of hybrid bluegill fed practical diets containing various percentages of protein in cages (Experiment 1). Values are means (±SE) of three replications, except for diet 2 which had two replicates. There were no significant differences (P > 0.05) in any amino acid level among treatments.*

	Diet (% protein)			
	1 (35%)	2 (40%)	3 (44%)	4 (48%)
Alanine	7.26 ± 0.15	7.37 ± 0.24	6.97 ± 0.05	7.30 ± 0.01
Arginine	6.53 ± 0.12	6.61 ± 0.04	6.53 ± 0.06	6.54 ± 0.19
Aspartic acid	10.47 ± 0.18	10.43 ± 0.13	10.51 ± 0.19	10.32 ± 0.09
Cystine	0.88 ± 0.04	0.86 ± 0.06	0.85 ± 0.04	0.89 ± 0.02
Glutamic acid	13.82 ± 0.17	14.00 ± 0.19	14.06 ± 0.17	14.09 ± 0.32
Glycine	8.62 ± 0.14	8.37 ± 0.47	8.08 ± 0.17	8.25 ± 0.14
Histidine	2.94 ± 0.07	3.04 ± 0.04	3.00 ± 0.02	3.05 ± 0.02
Isoleucine	3.69 ± 0.03	3.86 ± 0.02	3.90 ± 0.10	3.91 ± 0.02
Leucine	7.44 ± 0.04	7.32 ± 0.09	7.53 ± 0.10	7.43 ± 0.11
Lysine	8.17 ± 0.10	8.15 ± 0.04	8.22 ± 0.07	8.27 ± 0.07
Methionine	3.01 ± 0.11	3.06 ± 0.15	2.93 ± 0.10	3.01 ± 0.11
Phenylalanine	4.16 ± 0.06	4.11 ± 0.13	4.11 ± 0.06	4.23 ± 0.08
Proline	6.02 ± 0.35	5.68 ± 0.27	6.14 ± 0.20	5.48 ± 0.15
Serine	4.67 ± 0.02	4.79 ± 0.04	4.73 ± 0.04	4.77 ± 0.06
Threonine	4.82 ± 0.05	4.82 ± 0.00	4.76 ± 0.02	4.87 ± 0.05
Tyrosine	3.06 ± 0.04	3.07 ± 0.00	3.12 ± 0.07	3.14 ± 0.06
Valine	4.43 ± 0.08	4.50 ± 0.12	4.53 ± 0.08	4.48 ± 0.10

mg/L; nitrite, 0.037 ± 0.027 mg/L; total alkalinity, 134 ± 27 mg/L; chlorides, 153 ± 26 mg/L; pH, 8.5 ± 0.2.

Final individual weight and percentage weight gain of hybrid bluegill fed a diet containing 38% protein (diet 4) were significantly ($P < 0.05$) higher (53.4 g and 265%, respectively) than fish fed diets containing 28% and 32% protein (average of 45.4 g and 211%, respectively), but were not different from fish fed a diet containing 36% protein (Table 6). There was no significant difference ($P > 0.05$) in percentage survival among treatments. Specific growth rate (SGR) of fish fed a diet containing 38% protein was significantly ($P < 0.05$) higher (1.85%/d) than that for fish fed a diet containing 28% protein (1.58%/d); however, there were no significant differences in SGRs of fish fed diets containing 28–36% protein. Fish fed a diet containing 28% protein had significantly higher feed conversion ratio (FCR, 1.73) than did fish fed diets containing 36% and 38% protein (average of 1.39), but was not different than fish fed a diet containing 32% protein (1.65).

Discussion

Since protein is the most expensive dietary component in finfish diets, producers desire to provide the minimum level of protein in a diet that will supply essential amino acids and nitrogen to support acceptable growth in fish. The lack of significant differences in weight gain and FCR in hybrid bluegill fed diets containing between 35% and 48% protein suggest that the diets used in Experiment 1 are suitable for use for feeding hybrid bluegill raised from 20 g to 50 g. Results from Experiment 2 also indicate that a diet with 36% protein is suitable to raise hybrid bluegill from 15 g to 50 g. However, a diet with between 28% to 32% protein does not appear to allow for optimal growth. While fish in Experiment 1 had to be treated for disease, data from Experiment 2 seem to support results from the first feeding study. Thus, despite disease and treatment of the infection, growth results observed in the first study appear to be valid.

Webster et al. (1992) reported that hybrid

TABLE 6. *Final individual weight, percentage weight gain, percentage survival, specific growth rate (SGR), and feed conversion ratio (FCR) of juvenile hybrid bluegill fed practical diets containing various percentages of protein (Experiment 2). Values are means (±SE) of four replications. Means followed by different superscripts are significantly different* ($P < 0.05$).

	Diet (% protein)			
	1 (28%)	2 (32%)	3 (36%)	4 (38%)
Final weight (g)	44.22 ± 1.48^{c}	46.50 ± 3.60^{bc}	51.25 ± 1.57^{ab}	53.35 ± 0.33^{a}
Weight gain (%)	202.9 ± 10.1^{c}	218.5 ± 24.6^{bc}	251.0 ± 10.7^{ab}	265.4 ± 2.3^{a}
Survival (%)	98.33 ± 1.67^{a}	96.67 ± 1.92^{a}	100.0 ± 0.00^{a}	100 ± 0.0^{a}
SGR	1.58 ± 0.05^{b}	1.64 ± 0.12^{ab}	1.79 ± 0.04^{ab}	1.85 ± 0.01^{a}
FCR	1.73 ± 0.10^{a}	1.65 ± 0.15^{ab}	1.40 ± 0.02^{b}	1.38 ± 0.04^{b}

bluegill reared in ponds had similar growth rates when fed diets containing either 32% or 38% protein. However, in that study, only two protein levels were fed, and smaller fish (3.5 g) were stocked at low densities (12,350 fish/ha) in ponds where fish could utilize natural food organisms present. Hybrid bluegill in the present studies were reared in cages and aquaria, and had limited, if any, access to natural food organisms. Hybrid bluegill do not appear to consume zooplankton, but can utilize benthic organisms from the taxa Oligochaeta, Chironomidae, and Planorbiidae effectively when raised in ponds (Brunson and Robinette 1982); however, these organisms would not be present in abundance when fish are raised in cages off the pond bottom. Brunson and Robinette (1986) stated that hybrid bluegill stocked in ponds fed on benthic invertebrates, insects, and small fish. While some insects and small fish may have entered the cage area, it seems unlikely that the caged fish could derive much of their diet from live foods. In Experiment 2, conducted in aquaria, there would be no chance of supplemental nutrient sources.

The average SGR reported in Experiment 1 (1.01) was lower than that reported in Experiment 2, which had SGRs averaging 1.76 for fish fed diets containing between 32% to 38% protein. This may be due to the use of different stocking densities between the two feeding studies, possible diet differences, or due to the more uniform growth of fish in aquaria (Experiment 2) compared to fish raised in cages (Experiment 1). Tidwell et al. (1992) reported that hybrid bluegill fed a diet containing 37% protein had an SGR of 1.98. Webster et al. (1992) reported an SGR value of 2.6. However, those two studies used much smaller (3–5 g) fish. Use of larger fish (>20 g) in the present studies may have resulted in slightly lower SGR values. When larger fish (40–60 g) were stocked in ponds during a summer growing season, an SGR of 0.37 was reported (Tidwell et al. 1994).

Specific growth rates for hybrid bluegill are similar to some other species: between 1.7–2.0 for channel catfish *Ictalurus punctatus* (Li and Lovell 1992; Webster et al. 1993); 1.5 for blue catfish *I. furcatus* (Grant and Robinette 1992); 2.1 for chinook salmon *Oncorhynchus tshawytscha* (Fowler 1990); 1.2–2.6 for sockeye salmon *O. nerka* (Brett et al. 1969); 3.1 for red tilapia *Oreochromis urolepos hornorum* × *O. mossambicus* (Clark et al. 1990); and 0.8 for hybrid striped bass *Morone saxatilis* × *M. chrysops* (Woods et al. 1985). While comparison of results from other studies may be complicated by different experimental conditions including species, size and age of the fish used, stocking density, protein quality, and variations in abiotic factors (e.g., water temperature) (Jauncey and Ross 1982), results from the present studies indicate that hybrid bluegill do have growth rates similar to other important aquaculture species and may be a good candidate for culture. However, further production/feeding studies should be conducted to determine growth rates for larger fish.

Feed conversion ratios reported in Experiment 1 were similar to those reported from previous research on hybrid bluegill (Webster et al. 1992; Tidwell and Webster 1993; Tidwell et al. 1994). However, FCRs reported in Experiment 2 were much lower indicating efficient conversion of diet to fish weight. The higher FCRs from feeding caged fish may be due to some fish not being able to get to the diet since hybrid bluegill are territorial. Some fish may have been prevented by others in the cage from getting to the surface to consume the diet. Since fish used in the present studies were fed all they could consume during a given time period, overfeeding should not have been a problem. In Experiment 1, diet 4 (48% protein) sank; however, it is felt that careful feeding practices were followed and that there should not have been much diet, if any, wasted. In conducting feeding studies, diet should not be limiting, and feeding to excess is preferable to underfeeding (Jauncey and Ross 1982).

Whole-body composition analysis indicated that diet affected body protein, lipid, and ash levels. Hybrid bluegill fed a diet containing 35% protein (Experiment 1) had a lower percentage of protein and a higher lipid level than fish fed isocaloric diets containing 40, 44, and 48% protein. The level of digestible energy in a diet affects the amount of food consumed by fish and the ratio of protein to energy in the diet will influence conversion efficiency of the diet. Increased lipid levels in the fillet of fish may be undesirable for consumers; however, increased lipid levels may be advantageous if fish are to be stocked into a paylake where lipid stores may assist fish during winter months when food may be less prevalent or fish are not as active, when stocking densities may be high, or when fish are not normally fed.

Quantitative amino acid requirements have not been established for hybrid bluegill. The essential amino acid requirements of some fish have been shown to correlate well with the essential amino acid ratio pattern of whole-body tissues or muscle tissue (Cowey and Tacon 1983; Wilson and Poe 1985). The whole-body amino acid composition of the hybrid bluegill from Experiment 1 in the present study indicates that only slight differences appear to exist in the essentiality of amino acids in different species when compared to rainbow trout *Oncorhynchus mykiss*, coho Salmon *O. kisutch*, and channel catfish (Wilson 1989). Hybrid bluegill from Experiment 1 had similar whole-body amino acid compositions as reported in Tidwell et al. (1992). Lysine and methionine levels of the diets fed to hybrid bluegill exceeded the requirements established for channel catfish (NRC 1993), indicating that the diets used in Experiment 1 should not have been deficient in amino acid for hybrid bluegill.

Formulation of a nutritious diet for hybrid bluegill will allow producers to feed the most economical diet possible, while allowing for optimal growth. The present studies indicate that a diet containing between 35% to 36% protein appears to be suitable for rearing juvenile hybrid bluegill when fish meal comprises approximately 32% of the protein. Diets with less than 35% protein do not appear to give as high a growth rate for hybrid bluegill. Protein requirements may be lower if fish are grown in ponds where natural food organisms may play a more important role in supplying supplemental nutrients or if grown to a larger size when protein requirement may differ and possibly be reduced compared to the smaller fish used in these studies. Further evaluation of diets containing lower levels of protein for hybrid bluegill cultured in ponds and diets with reduced percentages of fish meal should be conducted.

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