The Progressive Fish-Culturist 54:234–239, 1992

Growth, Feed Conversion, and Protein Utilization of Female Green Sunfish × Male Bluegill Hybrids Fed Isocaloric Diets with Different Protein Levels

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Abstract.—A 10-week feeding trial was conducted in aquaria to evaluate protein requirements of juvenile (4.7-g) hybrids of female green sunfish (*Lepomis cyanellus*) and male bluegills (*Lepomis macrochirus*). Three isocaloric diets containing 26, 31, or 37% protein were evaluated for effects on fish growth, feed conversion, protein utilization, and body composition. Fish fed the 37% protein diet had significantly higher ($P < 0.05$) body weights, percentage weight gains, and specific growth rates than fish fed other diets. There were no significant differences ($P > 0.05$) among diets in protein efficiency ratio (fish weight gained/protein weight fed). Fish fed the 26% protein diet had a significantly higher ($P < 0.05$) feed conversion (feed weight fed/fish weight gained) than fish fed the 37% protein diet. These results suggest that the use of higher-protein feeds (35% or greater), rather than the 32% protein catfish feed that is often fed to bluegill hybrids, may improve growth performance and production potential of this hybrid.

The hybrid produced by crossing female green sunfish (*Lepomis cyanellus*) with male bluegills (*L. macrochirus*), here denoted the GS × BG hybrid, has several characteristics that may make it desirable for production in temperate regions of the USA. These traits include rapid growth and efficient feed conversion during summer (Lewis and Heidinger 1971) and winter (Brunson and Robinette 1985), a sex ratio highly skewed towards males (Brunson 1983), and ready acceptance of prepared diets (Lewis and Heidinger 1971). The GS × BG hybrid reaches an acceptable catch size for recreational fishing in less time than channel catfish (*Ictalurus punctatus*) and it is easier to catch because of its aggressive nature (Brunson and Robinette 1986), making it desirable for marketing through fee-fishing ponds.

Presently, many producers do not feed prepared diets to hybrid bluegills, relying on natural production within ponds. Use of a prepared diet may allow the hybrids to grow more rapidly, thereby allowing increased stocking densities and faster development of fishable populations. Although GS × BG hybrids accept prepared diets, little information is available on their nutritional requirements. In practice, commercial channel catfish diets (32% crude protein) are often fed. However, this use is based more on diet availability than suitability. Because protein is the most expensive component in a prepared feed, it is of considerable importance to determine the lowest level that will support optimal growth and survival. The objective of this study was to assess the effect of prepared diets containing various protein levels (26%, 31%, and 37%) on growth, feed conversion, protein utilization, and body composition of GS × BG hybrids.

Methods

Experimental diets.—Three experimental diets were formulated to contain varying percentages (25, 30, or 35%) of crude protein (Table 1). When analyzed, the three diets actually contained 25.7, 31.2, and 37.2% crude protein, respectively. All diets were formulated to be isocaloric (4.0–4.2 kcal gross energy per gram of diet) based on physiological fuel values of 5.65 kcal/g protein, 4.4 kcal/g carbohydrate and 9.65 kcal/g fat (El-Sayed 1990).

Dry diet ingredients were first ground to a small particle size (approximately 250 μm) in a Wiley mill. Ingredients were thoroughly mixed and water was added to obtain a 30% moisture level. Diets were extruded into 1.6-mm-diameter pellets and dried (25°C) for 16 h in a drying oven. Cod liver oil was sprayed onto dried pellets, which were stored frozen (−15°C) until immediately before use.

Percentage protein of diets was determined with a LECO FP-228 nitrogen determinator (LECO Corp., St. Joseph, Michigan; Sweeney and Rexroad 1987), percentage fat was determined by ether extraction (AOAC 1990), and percentage moisture was determined by drying a 15-g sample at 95°C for 24 h (AOAC 1990). Diets were also analyzed for amino acid composition (lysine, cystine, and

TABLE 1.—Formulation and proximate composition of three experimental diets fed to female green sunfish × male bluegill hybrids in aquaria.

Ingredient or measure	Diet (% protein) 26%	31%	37%
Formulation (% by weight)			
Soybean mean (44% protein)	30.0	45.0	55.0
Menhaden meal (66.7% protein)	10.0	10.0	10.0
Meat and bone meal (54.1% protein)	0.0	0.0	2.0
Corn	49.0	36.0	29.0
Premix[a]	1.0	1.0	1.0
Calcium phosphate	1.0	1.0	1.0
Cod liver oil (0.02% BHT added)	9.0	6.0	3.0
Chemical analysis (mean ± SD of two replicates), energy content, and cost			
Protein (%, moisture-free)	25.7 ± 0.5	31.2 ± 0.1	37.2 ± 0.4
Lipid (%, moisture-free)	7.2 ± 0.7	9.1 ± 0.8	10.9 ± 0.8
Moisture (%)	16.6 ± 0.2	13.01 ± 0.4	12.4 ± 1.2
Lysine (g/100 g)	7.86 ± 0.25	10.09 ± 0.54	11.84 ± 0.51
Cystine (g/100 g)	1.37 ± 0.08	1.85 ± 0.08	2.17 ± 0.04
Methionine (g/100 g)	2.92 ± 0.33	3.60 ± 0.88	3.90 ± 0.63
Energy (kcal/g)[b]	4.22	4.09	3.95
Protein/energy ratio (mg/kcal)	60.9	76.3	94.2
Relative ingredient cost[c]	1.000	1.027	1.057

[a] Premix supplied the following vitamins and minerals per kilogram of diet: retinal palmitate (vitamin A), 4,532 IU; cholecalciferol (D_3), 2,266 IU; alpha-tocopherol (E), 75 IU; menadione (K), 11 mg; cyanocobalamine (B_{12}), 11 mg; ascorbic acid (C), 778 mg; folic acid, 2.2 mg; riboflavin (B_2), 13.2 mg; pantothenic acid, 35.2 mg; niacin, 88.0 mg; choline chloride, 516 mg; thiamine (B_1), 11 mg; pyridoxine (B_6), 11 mg; Zn (as $ZnSO_4$), 173 mg; Fe (as $FeSO_4$), 60 mg; Cu (as $CuSO_4$), 7.5 mg; I (as $CaIO_3$), 3.75 mg; Co (as $CoSO_4$), 1.6 mg; Mn (as $MnSO_4$), 180 mg; Al (as $AlK[SO_4]_2$), 1.0 mg; Se (as Na_2SeO_3), 0.3 mg; K (as KCl), 3,474 mg; and Na (as Na_2PO_4), 1,932 mg.

[b] Gross energy based on physiological fuel values (El-Sayed 1990).

[c] Ingredient costs based on prices quoted in *Feedstuffs*, 1991, 63(37):36.

methionine) by a commercial analytical laboratory (Woodson-Tenent Laboratories, Dayton, Ohio).

Experimental system and animals.—The feeding trial was conducted in twelve 37.5-L acrylic aquaria. Each aquarium was continuously supplied with dechlorinated municipal water at a rate of 1.6 L/min. Black plastic covered the back and sides of all aquaria to minimize disturbances (Hale and Carlson 1972). Illumination was supplied by fluorescent ceiling lights on a 14 h:10 h light : dark cycle. Water temperature and dissolved oxygen were measured daily with a YSI Model 57 meter (YSI Industries, Yellow Springs, Ohio). Total ammonia and nitrite were measured with a DREL/5 spectrophotometer (Hach Co., Loveland, Colorado) and pH was measured with an electric pH meter (Omega Engineering, Inc., Stamford, Connecticut) once each week in all aquaria. No significant differences ($P > 0.05$) were found among treatments for any water quality variable, so means were pooled. Through the duration of the study, average (±SE) water quality variables were water temperature, 24.0 ± 0.1°C; dissolved oxygen, 8.4 ± 0.1 mg/L; total ammonia, 1.40 ± 0.16 mg/L; un-ionized ammonia, 0.007 ± 0.001 mg/L; nitrite, 0.03 ± 0.00 mg/L; and pH, 7.09 ± 0.23.

Juvenile F_1 GS × BG hybrids (mean individual weight, 4.7 ± 0.1 g) were obtained from a commercial supplier (Whiskers Catfish Farm, Bowling Green, Kentucky). Fish were randomly stocked into all aquaria at a rate of 10 fish/aquarium, giving four replicates per treatment (diet). For 1 week before treatments began, the hybrids were acclimated in aquaria while being fed the 31% protein diet as a conditioning feed. Fish were fed one of three diets containing 26, 31, or 37% protein twice daily (0800 and 1600 hours) to satiation for 10 weeks. Fish from each aquarium were weighed as a group at the beginning of the experiment and then weekly until the conclusion of the feeding trial. Total length of each fish was measured at the end of the experiment. At the start and conclusion of the feeding trial, several fish were sacrificed (20 fish total at stocking and 10 fish per aquarium at conclusion), homogenized in a blender, stored in polyethylene bags, and frozen (−15°C) for subsequent analyses of moisture, protein, lipid, and amino acids by methods previously described for diets.

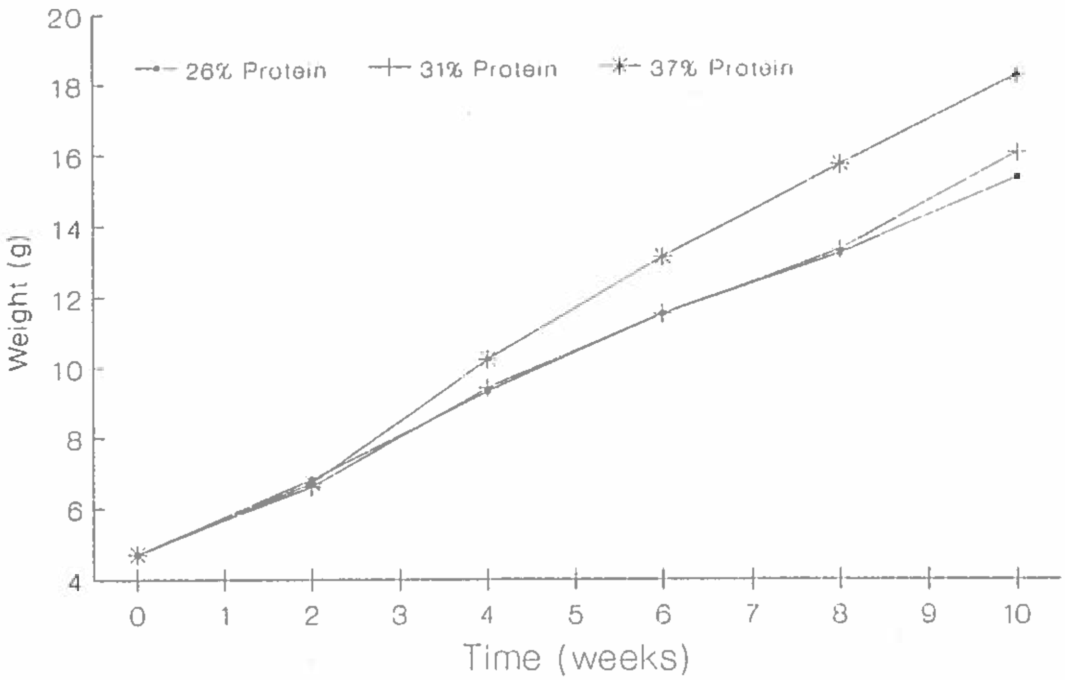

FIGURE 1.—Effects of dietary protein levels on growth of female green sunfish × male bluegill hybrids. Values are means of four replications.

Statistical analysis.—Growth performance and feed conversion were measured in terms of final individual fish weight (g), total length (mm), percentage weight gain, percentage survival, specific growth rate, feed conversion, and protein efficiency ratio. Growth responses were calculated as follows. Specific growth rate (%/d) = $100 \cdot (\log_e W_t - \log_e W_0)/ T$; W_t is the weight of fish at time t, W_0 is the weight of fish at time 0, and T is the culture period in days. Feed conversion = feed weight fed (g)/fish weight gained (g). Protein efficiency ratio = fish weight gained (g)/protein weight fed (g). Condition factor = $100 \cdot$(weight, g)/(total length, cm)^3. Feed consumption was determined once per week, the day after sampling, so body weight percentages are based on known body weight (g). Consumption for the duration of the experiment was taken as the mean of weekly determinations (Clark et al. 1990).

Data were subjected to analysis of variance (ANOVA) with the SAS ANOVA procedure (SAS Institute 1988). Duncan's multiple-range test was used to compare individual means. Percentage survival, percentage weight gain, specific growth rate, feed conversion, feed consumption, protein efficiency ratio, and carcass composition (percentages of moisture, protein, and lipid) were transformed to arcsine values prior to analysis (Zar 1984).

Results and Discussion

Fish fed the 37% protein diet were significantly heavier ($P < 0.05$) after 10 weeks than fish fed the 26% protein diet, but not significantly heavier than fish fed the 31% protein diet (Figure 1; Table 2). Percentage weight gain and specific growth rate were significantly higher for fish fed the 37% protein diet than for fish fed the 26% or 31% protein diets. There were no significant differences in final fish length, feed consumption, or protein efficiency ratio among treatments. Feed conversion was significantly higher for fish fed the 26% protein feed than for fish fed the 37% protein feed, but not significantly different from fish fed the 31% protein feed.

Percentage whole-body lipid was significantly higher for fish fed the 37% protein diet than for fish fed the 31% protein diet, but not significantly different from that of fish fed the 26% protein diet (Table 2). The reasons for decreased lipid levels in fish fed a diet containing 31% protein are not known. Reinitz (1987) found that neither whole-body fat nor moisture was significantly correlated with either dietary protein or metabolizable energy for rainbow trout (*Oncorhynchus mykiss*). Also, low within-treatment variation probably helped make relatively small absolute differences in lipid levels statistically significant. No signifi-

TABLE 2.—Individual fish weights and lengths, weight gains and specific growth rates, feed consumption and feed conversion, protein efficiency ratio, condition factor, survival, protein gain, lipid gain, and carcass composition of female green sunfish × male bluegill hybrids fed diets with different protein levels in aquaria for 70 d. Values are means ± SE of four replicates. Means along a row without a letter in common are significantly different ($P < 0.05$).

Variable	Diet (% protein) 26%	31%	37%
Initial body weight (g)	4.7 ± 1.2 z	4.7 ± 1.1 z	4.7 ± 0.5 z
Final body weight (g)	15.3 ± 0.3 z	16.0 ± 2.5 zy	18.7 ± 1.8 y
Final body length (cm)	9.1 ± 0.1 z	9.2 ± 0.3 z	9.2 ± 0.7 z
Weight gain (%)	225.8 ± 10.1 z	237.4 ± 48.3 z	299.6 ± 35.1 y
Specific growth rate (%/d)	1.69 ± 0.04 z	1.72 ± 0.22 z	1.98 ± 0.13 y
Feed consumption (% body weight/d)[a]	5.3 ± 0.4 z	4.8 ± 1.0 z	4.8 ± 0.4 z
Feed conversion	2.6 ± 0.2 y	2.3 ± 0.4 zy	1.9 ± 0.2 z
Protein efficiency ratio	1.6 ± 0.1 z	1.5 ± 0.2 z	1.5 ± 0.2 z
Relative feed cost[b]	2.600	2.362	2.008
Condition factor	2.02 ± 0.07 z	2.04 ± 0.21 z	2.49 ± 0.75 z
Survival (%)	97.5 ± 5.0 z	100.0 ± 0.0 z	97.5 ± 5.0 z
Protein gain (g)	17.3 ± 1.2 z	19.2 ± 3.8 zy	23.1 ± 3.1 y
Lipid gain (g)	6.8 ± 1.1 zy	6.6 ± 1.8 z	9.2 ± 1.7 y
Carcass composition at end of treatment (% of wet weight)			
Moisture (%)	71.5 ± 0.5 z	71.8 ± 0.5 z	71.4 ± 0.4 z
Protein (%)	16.9 ± 0.2 z	17.2 ± 0.3 z	17.1 ± 0.3 z
Lipid (%)	6.6 ± 0.8 zy	5.8 ± 0.5 z	6.8 ± 0.4 y
Ash (%)	4.8 ± 0.2 z	4.9 ± 0.2 z	4.6 ± 0.2 z

[a] Feed consumption was based on 1-d satiation totals for each tank the day following weekly weighing.
[b] Relative feed cost (per unit fish weight produced) = (relative ingredient cost, Table 1) × (feed conversion).

cant differences in moisture or protein were found among fish fed the three diets. Because a difference was found in body composition (lipid), composition of gain was also analyzed (Lovell 1989). Fish fed the 37% protein diet had significantly higher protein gain than fish fed the 26% protein diet and significantly higher lipid gain than fish fed 31% protein feed.

Growth rates of GS × BG hybrids are not currently available in the literature for comparison. However, the specific growth rate for hybrid bluegills fed 37% protein feed (1.98) compares favorably with those of other species: channel catfish, 2.09 (Webster et al. 1991); chinook salmon (*Oncorhynchus tshawytscha*), 2.13 (Fowler 1990); blue catfish (*Ictalurus furcatus*), 1.92 (Webster et al. 1992). Comparison of protein requirements from other studies is complicated by different experimental conditions including species, size, and age of fish; stocking density; protein source; nonprotein constituents of diet; and variations in abiotic factors such as water temperature (Jauncey and Ross 1982). Optimum dietary protein levels for fingerlings and subadults of other species have been reported as 30–35% of diet for channel catfish (Page and Andrews 1973; NRC 1983), 31–38% for common carp (*Cyprinus carpio*; NRC 1983), and 25–30% for *Tilapia* species (Balarin and Haller 1982). In the present study, maximum growth and feed conversion for GS × BG hybrids were obtained with a diet containing 37% crude protein. The protein-to-energy ratio for this diet was approximately 94 mg protein/kcal. This agrees with results for channel catfish when based on physiological fuel values (88 mg protein/kcal; Garling and Wilson 1976).

Quantitative amino acid requirements have not been established for the GS × BG hybrid. The essential amino acid requirements of certain fish have been shown to correlate well with the essential amino acid pattern of the whole-body tissue of that fish (Cowey and Tacon 1983; Wilson and Poe 1985). The whole-body amino acid composition of the GS × BG hybrid is presented in Table 3, along with those of rainbow trout, coho salmon (*Oncorhynchus kisutch*) and channel catfish. Only slight differences appear to exist in the essentiality of amino acids in different fishes, methionine and possibly tryptophan being exceptions (Wilson 1989). Methionine and tryptophan ratios were higher for the GS × BG hybrid than for rainbow trout or channel catfish. Tryptophan content was very similar to that of coho salmon, but methionine content was slightly lower.

The cystine, methionine, and lysine values for feeds (Table 1) can be compared with levels in hybrid body tissues (Table 3). The lysine level of the 26% protein feed (7.86 g/100 g) was below the

TABLE 3.—Whole-body amino acid compositions (g/100 g amino acids) of rainbow trout, coho salmon, channel catfish, and female green sunfish × male bluegill hybrids.

Amino acid	Rainbow trout[a]	Coho salmon[a]	Channel catfish[a]	Hybrid bluegill[b]
Alanine	6.57	6.08	6.31	6.67 ± 0.29
Arginine	6.41	5.99	6.67	5.92 ± 0.01
Aspartic acid	9.94	9.96	9.74	11.57 ± 0.24
Cystine	0.80	1.23	0.86	0.92 ± 0.06
Glutamic acid	14.22	15.25	14.39	14.94 ± 0.29
Glycine	7.76	7.31	8.14	7.95 ± 0.28
Histidine	2.96	2.99	2.17	2.08 ± 0.04
Isoleucine	4.34	3.70	4.29	4.28 ± 0.17
Leucine	7.59	7.49	7.50	7.34 ± 0.24
Lysine	8.49	8.64	8.51	8.27 ± 0.07
Methionine	2.88	3.53	2.92	3.10 ± 0.23
Phenylalanine	4.38	4.14	4.14	4.08 ± 0.05
Proline	4.89	4.76	6.02	5.13 ± 0.47
Serine	4.66	4.76	4.89	4.14 ± 0.04
Threonine	4.76	5.11	4.41	4.61 ± 0.04
Tryptophan	0.93	1.40	0.78	1.43 ± 0.37
Tyrosine	3.38	3.44	3.28	2.94 ± 0.24
Valine	5.09	4.32	5.15	4.76 ± 0.16

[a] Data from Wilson (1989).
[b] Mean ± SD of two replicates.

whole-body value (8.27) for the fish, indicating the 26% protein diet could be limiting in lysine. However, lysine levels in this diet meet requirements established for channel catfish (NRC 1983). Methionine in the 26% protein diet (2.92 g/100 g) was below the level found in fish tissues (3.10). However, if cystine can partially replace methionine, as it does in some fish (Lovell 1989), methionine may not be limiting. In the 31 and 37% protein diets, levels of lysine and methionine were higher than levels found in the whole body. If the minimum requirements for lysine and the sulfur amino acids (methionine and cystine) are met, the requirements for the other essential amino acids should also be met (Lovell 1989).

In the present study, feeding a diet containing 37% protein to GS × BG hybrids resulted in significantly improved growth rates without a significant lowering of protein efficiency ratios compared with feeding diets containing 26 or 31% protein. A decrease in protein efficiency has been demonstrated when protein levels in feeds exceed requirements (Mazid et al. 1979; Siddiqui et al. 1988). Relative feed costs for the 26, 31, and 37% protein diets were 2.600, 2.362, and 2.008 (1.00: 0.90:0.77), respectively (Table 2). Thus, the higher monetary cost of the 37% crude protein diet was more than offset by increased growth.

Acknowledgments

We thank Woodson-Tenent Laboratories for performing amino acid analyses, Robert Harrod and Eddie Reed, Jr., for technical assistance, and Sandy Hall for typing the manuscript. This research was supported by a U.S. Department of Agriculture, Cooperative State Research Service, grant to Kentucky State University under agreement KYX-80-91-04A.

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