

Effects of Protein Level on Growth and Body Composition of
Hybrid Sunfish (*Lepomis cyanellus* × *L. macrochirus*)
Reared in Ponds

CARL D. WEBSTER, JAMES H. TIDWELL, LAURA S. GOODGAME,
JULIA A. CLARK, AND DANIEL H. YANCEY

Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky 40601

ABSTRACT

Hybrid sunfish (*Lepomis cyanellus* × *L. macrochirus*) juveniles (3.4 g) were stocked into six 0.04 ha ponds at a rate of 12,350 fish/ha and fed twice daily 1 of 2 diets containing either 32% or 38% protein. After 100 days, no significant differences ($P > 0.05$) in individual fish weight, percentage survival, food conversion, or growth rate were found among treatments. Final individual fish weights were 49 and 43 g for fish fed diets containing 32% and 38% protein, respectively. No significant differences ($P > 0.05$) in percentage moisture and protein were found in fish fed either diet. However, percentage fat was significantly higher ($P < 0.05$) in fish fed a diet containing 32% protein (18.7%) compared to fish fed a diet containing 38% protein (12.6%). These data indicate that, when stocked at the rate of 12,350 fish/ha, hybrid sunfish can utilize a 32% protein diet with similar growth rates as fish fed a diet containing 38% protein.

INTRODUCTION

The pay-lake industry (fee-fishing ponds) is an important sector of the aquaculture industry in many states, including Kentucky (1). Pay lakes provide a source of income for the pond owner, a source of food and recreation for the public, and a market for producers of live fish. The hybrid sunfish (female green sunfish, *Lepomis cyanellus* × male bluegill, *L. macrochirus*) appears to have potential as a desirable fish for the pay-lake industry.

Growth of green sunfish × bluegill (GS × BG) hybrids has been reported to be higher than that of either parental stock (2). The GS × BG sunfish reach an acceptable catch-size in a shorter period of time than channel catfish, *Ictalurus punctatus*, a very popular pay-lake fish (1).

Increasing the growth rate of GS×BG sunfish by feeding prepared diets is desirable. Lewis and Heidinger (3) reported that, of the sunfish crosses evaluated, only the GS×BG sunfish is well suited to feeding prepared diets.

This is due primarily to the aggressive feeding response the hybrid exhibits (4). This response also increases the vulnerability to hook-and-line capture by fisherman. Approximately 66% of GS × BG sunfish stocked into a pond were captured by hook-and-line after 18 hours of angling (5). From a fisherman's viewpoint, a larger fish is desirable. Larger fish also are of benefit to the fish producer and pay-lake operator in that higher prices can be attained for their products.

Because protein is the most expensive component of a diet, knowledge about 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 optimum growth varies with species (6-8). However, information about the nutritional requirements for GS×BG sunfish is lacking. This impedes formulation of a nutritionally complete diet and limits the culture of the fish. Tidwell et al. (9) reported that growth of GS×BG sunfish reared

in aquaria increased with increasing protein levels. It is essential to know the minimum protein requirements for optimum growth in ponds. The objective of this study was to determine the growth, food conversion, survival, and body composition of fingerling GS×BG sunfish fed two protein levels (32% and 38%) reared in ponds.

MATERIALS AND METHODS

Diets.—Fish were fed 1 of 2 extruded diets formulated by a commercial feed mill (Delta Western, Indianola, Mississippi) to contain either 32% or 38% protein. Diets were analyzed for crude protein, fat, and moisture. Crude protein was determined using macro-Kjeldahl, crude fat was determined by the acid-hydrolysis method, and moisture was determined by placing 2 g of the diet in a drying oven (95°C) until constant weight (10). Chemical analysis of the diets showed the 32% protein diet had $33.1 \pm 0.07\%$ protein and $4.4 \pm 0.02\%$ fat, while the 38% protein diet had $37.8 \pm 0.02\%$ protein and $3.4 \pm 0.02\%$ fat. Diets were stored (-30°C) in plastic-lined bags until fish were fed.

Grow-out.—Juvenile hybrid bluegill (female green sunfish, *Lepomis cyanellus* × male bluegill, *L. macrochirus*; average weight 3.4 ± 0.1 g) were stocked on 3 July 1991 in six 0.04-ha earthen ponds at the Aquaculture Research Center, Kentucky State University, at a rate of 12,350 fish/ha. Ponds were approximately 1.5 m deep and were supplied with water from a reservoir which was filled by rain runoff. Water levels in ponds were maintained at a constant depth by periodic additions.

Fish were fed a fixed amount (0.92 kg/day) of either a 32% or a 38% protein diet twice (0900 and 1530) daily for 100 days. One-half of the total amount was fed in each of the 2 feedings. Each treatment was replicated in 3 ponds. Diets were spread uniformly inside a 3.0-m diameter floating feeding ring in the pond and the immediate surrounding area to prevent a feeding "pecking order" from being established. Rings were made from 1-cm diameter plastic pipe and had a 0.58-mm plastic mesh skirt extending 20 cm below the water surface.

Dissolved oxygen (DO) and temperature of all ponds were monitored twice daily (0800 and 1430) by means of a YSI Model 57 oxygen

meter. When the DO level of any pond was predicted (graphically) to decline to below 4.0 mg/liter, aeration was provided. Total ammonia nitrogen (TAN) and nitrite were measured once weekly (at 1300) by means of a Hach DREL/5 spectrophotometer, and pH was measured once weekly (at 1300) using an electronic pH meter (Accumet 900, Fisher Scientific). Through the duration of the study, these water quality features were not significantly different ($P > 0.05$) among treatments, and means were (\pm SE): morning water temperature, $24.7 \pm 0.3^{\circ}\text{C}$; afternoon water temperature, $25.8 \pm 0.4^{\circ}\text{C}$; morning DO, 7.2 ± 0.3 mg/liter; afternoon DO, 10.4 ± 1.0 mg/liter; TAN, 0.25 ± 0.15 mg/liter; nitrite, 0.03 ± 0.02 mg/liter; pH, 8.85 ± 0.23 .

Harvest Data.—Fish were not fed 24 hours prior to harvest and were harvested by seine on 14 October 1991. Total number and weight of fish in each pond were determined at harvest. Fifty fish were randomly sampled from each pond and were individually weighed to the nearest gram and measured (total length) to the nearest 0.5 centimeter. Ten fish were randomly sampled for analysis of body composition. Whole fish were homogenized in a blender and analyzed for moisture, protein, and fat. protein was analyzed using a LECO FP-228 nitrogen determinator (11); fat was analyzed by ether extraction; and moisture was determined by drying in a convection oven (95°C) until constant weight (10).

Food conversion ratio (FCR) and specific growth rate (SGR) were calculated as follows: $\text{FCR} = \text{total diet fed (kg)}/\text{total wet weight gain (kg)}$; $\text{SGR (\%/day)} = (\ln W_t - \ln W_0)/T \times 100$, where 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.

Statistical Analysis.—Data were analyzed using the SAS ANOVA procedure (12) for significance. Differences between means were determined by Duncan's multiple range test. All percentage and ratio data were transformed to arcsine values prior to analysis (13).

RESULTS AND DISCUSSION

There were no significant differences ($P > 0.05$) in individual fish length, individual fish weight, survival, food conversion ratio (FCR), specific growth rate (SGR), and yield (kg/ha)

between GS×BG sunfish fed either a 32% or 38% protein diet (Table 1).

The lack of significant differences in weight gain and food conversion in GS × BG sunfish fed diets containing 32% and 38% protein suggests that the diets may be within optimal range for fish growth when fish are stocked at the low rate used in the present study. It is not known if a diet with a higher percentage of protein (i.e., 45%) would have significantly improved growth rates. Protein is the most expensive dietary component in finfish diets and is a primary concern in diet formulation. Feed producers desire to provide the minimum level of protein in a diet that will supply essential amino acids and nitrogen to support acceptable weight gain in fish.

Growth rates for GS × BG sunfish cultured in ponds are not currently available in the literature for comparison. The SGR reported in this study (2.6) is somewhat higher than other studies and other fishes. Tidwell et al. (9) reported that hybrid bluegill fed a diet containing 35% protein had an SGR of 1.98. Specific growth rates for other fish species have been reported at 2.1 for channel catfish, *Ictalurus punctatus* (14), 2.1 for chinook salmon, *Oncorhynchus tshawytscha* (15), 1.9 for blue catfish, *Ictalurus furcatus* (16), and 0.7 for rainbow trout, *Oncorhynchus mykiss* (17). This higher value may indicate that BG×GS hybrids have faster growth rates than more commonly reared species. Webster et al. (18) reported an SGR value of 1.8 for pond-reared channel catfish. Comparison of results of protein requirements from other studies is 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) (19).

Growth data reported in the present study may be confounded by the availability of natural foods present in the pond. Stocking density in the present study was lower than intensively-stocked channel catfish ponds. Research should be conducted on the optimum stocking rates for GS × BG sunfish. With fewer fish present in ponds, natural foods in the ponds may have been utilized as food items. The high FCR value (3.7) may indicate that the prepared diets were not optimally consumed. Juvenile bluegill could feed on zooplankton and

TABLE 1. Average yield, individual fish weight, individual fish length, survival, food conversion ratio (FCR), and specific growth rate (SGR) for hybrid bluegill fed diets containing either 32% or 38% protein.¹

	Protein (%)	
	32	38
Yield (kg/ha)	522.5 ± 5.58 ^a	505.3 ± 27.8 ^a
Indiv. fish weight (g)	49.1 ± 1.6 ^a	43.5 ± 2.0 ^a
Indiv. fish length (cm)	13.0 ± 0.1 ^a	12.4 ± 0.2 ^a
Survival (%)	94.87 ± 1.54 ^a	92.00 ± 3.11 ^a
FCR	3.72 ± 0.04 ^a	3.87 ± 0.20 ^a
SGR	2.67 ± 0.03 ^a	2.55 ± 0.05 ^a

¹ Values are means ± SE of three replications. Means within a row that have the same superscript are not significantly different ($P > 0.05$).

benthic organisms to supplement the diet (20). The high FCR may also indicate that the fixed amount of diet fed per day was too high. However, in conducting feeding studies, diet should not be limiting and feeding to excess is preferable to underfeeding (19).

Whole-body composition analysis indicates that diet did not affect percentage body protein in GS × BG sunfish (Table 2). No significant differences ($P > 0.05$) in percentage moisture and protein were found between treatments. Percentage protein averaged 63.3%. Percentage fat of fish fed a diet containing 32% protein was significantly higher (18.7%) than fish fed a diet containing 38% protein (12.6%) ($P < 0.05$). The level of digestible energy in a diet affects the amount of food consumed by fish and the ratio of energy to protein in the diet will influence conversion efficiency of the diet (21). A high ratio may increase fat deposition in fish, whereas a low ratio will cause protein to be used as an energy source. In the present study, the higher percentage of fat in fish fed a diet containing 32% protein may indicate that this diet had a higher energy-to-protein ratio for GS × BG sunfish than the diet containing 38% protein. This would lead to the increase in percentage fat reported in this study.

Formulation of a nutritious diet for GS × BG sunfish will allow producers to feed the most economical diet possible, while allowing for optimal growth. The present study indicates that a 32% protein diet appears to be suitable for rearing hybrid bluegill juveniles in ponds when stocked at 12,350 fish/ha. Protein requirements may change if fish are stocked at higher rates. Research into feeding diets with various protein levels and higher stocking rates

TABLE 2. Whole-body composition (percentage moisture, protein, and fat) of juvenile hybrid bluegill at stocking and fed diets containing either 32 or 38% protein.¹

	At stocking	Diet (% Protein)	
		32	38
Moisture (%)	77.05 ± 0.25	73.64 ± 0.26 ^a	73.99 ± 0.40 ^a
Protein (%) ²	66.74 ± 0.52	63.33 ± 1.28 ^a	63.93 ± 3.21 ^a
Fat (%) ²	7.09 ± 0.30	18.72 ± 1.48 ^a	12.62 ± 1.33 ^b

¹ Values are means ± SE for three replications. Means in the same row with different superscripts were significantly different ($P < 0.05$).

² Dry-weight basis.

should be conducted to more fully elucidate protein requirements of GS × BG sunfish.

ACKNOWLEDGMENTS

We thank Eddie Reed, Steven Mims, and Wendell Harris for their technical assistance, and Paul Weston for use of his laboratory. We also thank Sandra Hall for typing this manuscript. This study was partially funded by a USDA/CSRS grant to Kentucky State University under agreement KYX-80-91-04A.

LITERATURE CITED

- Lopinot, A. C. 1972. Pond fish and fishing in Illinois. Illinois Department of Conservation, Fisheries Bulletin 5, Springfield, Illinois.
- Cremer, M. C., S. D. Mims, and G. M. Sullivan. 1984. Pay lakes as a marketing alternative for Kentucky fish producers. Research Bulletin No. 8, Kentucky State University, Frankfort, Kentucky.
- Lewis, W. M. and R. C. Heidinger. 1971. Supplemental feeding of hybrid sunfish populations. Trans. Am. Fish. Soc. 100:619-623.
- Lewis, W. M. and R. C. Heidinger. 1978. Use of hybrid sunfishes in the management of small impoundments. Pp. 104-108. In G. D. Novinger and J. C. Dillard (eds.) New approaches to the management of small impoundments. North Central Division, American Fisheries Society, Special Publication 5, Bethesda, Maryland.
- Brunson, M. W. and H. R. Robinette. 1986. Evaluation of male bluegill × female green sunfish hybrids for stocking Mississippi farm ponds. N. Am. J. Fish. Manage. 6:156-167.
- DeLong, D. C., J. E. Halver, and E. T. Mertz. 1958. Nutrition of salmonid fishes. VI. Protein requirements of chinook salmon at two water temperatures. J. Nutr. 65: 589-599.
- Satia, B. P. 1974. Quantitative protein requirements of rainbow trout. Prog. Fish-Cult. 36:80-85.
- Prather, E. E. and R. T. Lovell. 1973. Response of intensively fed channel catfish to diets containing various protein-energy ratios. Proc. Ann. Conf. Southeast. Assoc. Game and Fish Comm. 27:455-458.
- Tidwell, J. H., C. D. Webster, and J. A. Clark. In press. Growth, feed conversion, and protein utilization of female green sunfish (*Lepomis cyanellus*) × male bluegill (*L. macrochirus*) hybrids fed isocaloric diets with different protein levels. Prog. Fish-Cult.
- AOAC. 1990. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed. AOAC, Arlington, Va.
- Sweeney, R. A. and P. R. Rexroad. 1987. Comparison of LECO FP-228 'nitrogen determinator' with AOAC copper catalyst Kjeldahl method for crude protein. J. Assoc. Off. Anal. Chem. 70:1028-1030.
- Statistical Analysis Systems. 1988. SAS/STAT user's guide. Release 6.03 Edition. SAS Institute, Cary, N.C.
- Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.
- Webster, C. D., J. H. Tidwell, and D. H. Yancey. 1991. Evaluation of distillers grain with solubles as a protein source in diets for channel catfish. Aquaculture 96: 179-190.
- Fowler, L. G. 1990. Feather meal as a dietary protein source during parr smolt transformation in fall chinook salmon. Aquaculture 89:301-314.
- Webster, C. D., J. H. Tidwell, and D. H. Yancey. 1992. Effect of partially or totally replacing fish meal with soybean meal on growth in blue catfish (*Ictalurus furcatus*). Aquaculture 103:141-152.
- Tidwell, J. H., C. D. Webster, and R. S. Knaub. 1991. Seasonal production of rainbow trout, *Oncorhynchus mykiss*, in ponds using different feeding practices. Aquacult. Fish. Manage. 22:335-341.
- Webster, C. D., J. H. Tidwell, J. A. Clark, and D. H. Yancey. 1992. Effects of feeding diets containing 34 or 38% protein at two feeding frequencies on growth and body composition of channel catfish. J. Appl. Aquacult. 1: 67-80.
- Jauncey, K. and B. Ross. 1982. A guide to Tilapia feeds and feeding. Institute for Aquaculture, Univ. of Stirling, United Kingdom.
- Brunson, M. W. and H. R. Robinette. 1982. Supplemental winter feeding of hybrid sunfish in Mississippi. Proc. Ann. Conf. Southeast. Fish Wildl. Agencies 36:157-161.
- Reis, L. M., E. M. Reutebuch, and R. T. Lovell. 1989. Protein-to-energy ratios in production diets and growth, feed conversion and body composition of channel catfish, *Ictalurus punctatus*. Aquaculture 77:21-27.