

Effects of Graded Levels of Carbohydrate on Growth and Survival of Largemouth Bass, *Micropterus salmoides*

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Abstract

Excessive carbohydrates (CHO) in diets for largemouth bass (LMB), *Micropterus salmoides*, are suspected of accumulating glycogen in hepatocytes, which may result in liver dysfunction. This study evaluated the effect of graded levels of dietary CHO on growth, survival, and liver histology of LMB. One hundred feed-trained advanced fingerling LMB (128.5 ± 21.5 g) were stocked into each of nine 3400-L polyethylene tanks. Tanks were randomly assigned one of three experimental diets containing different CHO levels (13, 19, or 25% of diet). The extruded diets were approximately isonitrogenous (42% crude protein) and isocaloric (3 kcal/g energy). There were three replicate tanks per dietary treatment. Bass were fed to apparent satiation twice daily for 148 d. Survival was significantly higher ($P \leq 0.05$) for fish fed the 13 and 19% CHO diets (89 and 90%, respectively) compared to those fed the 25% CHO diets (82%). Average harvest weight of fish fed the 13% CHO diet (380 g) was significantly greater ($P \leq 0.05$) than for fish fed other diets. Average harvest weight of fish fed the 19% CHO diet (347 g) was significantly greater ($P \leq 0.05$) than for fish fed the 25% CHO diet (310 g). Specific growth rates (%/d) were significantly higher ($P \leq 0.05$) in fish fed the 13 and 19% CHO diets than in fish fed 25% CHO diet. Feed conversion ratios for fish fed the 13 and 19% CHO diets (2.3 and 2.4, respectively) were both significantly lower ($P \leq 0.05$) than in fish fed the 25% CHO diet (3.6). There were no significant differences ($P > 0.05$) in condition factor, protein efficiency ratio, hepatosomatic index, or liver glycogen concentration among fish fed the different experimental diets. Overall, mean blood glucose levels in fish fed the 13 and 19% CHO diets (61.0 and 71.2 mg/dL, respectively) were significantly lower ($P \leq 0.05$) than in fish fed the 25% CHO diet (87 mg/dL). Histopathological examination of livers from fish fed the three diets was used to score the degree of vacuolization of hepatic tissues (0 = normal, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe). Regression of vacuolization scores on dietary CHO levels was statistically significant ($P \leq 0.05$) and indicated a direct positive relationship between liver vacuolization and dietary CHO level ($R^2 = 0.57$). These data indicate that LMB grow faster and use feeds more efficiently when CHO are maintained at <20% of diet. CHO levels >20% negatively impacted liver histology, but a liver tissue analyses did not document glycogen accumulation.

Largemouth bass (LMB), *Micropterus salmoides*, are considered one of the most sought-after sport fish in North America (Heidinger 2000) and have been cultured in the USA since the late 1800s. Since the 1960s, research has been conducted on methods to train this fish to accept prepared diets. LMB are carnivores by nature but are increasingly raised on pelleted feeds for food-fish production (Tidwell et al. 2005). Food-fish markets catering to ethnic populations exist in major metropolitan areas throughout the USA and Canada and require live fish ≥ 500 g. Competing demand for LMB sport fish stocking and

live food-fish markets has maintained wholesale prices of approximately US\$10/kg for live bass (Tidwell et al. 2002).

Dietary carbohydrates (CHO) are widely included in fish diets to enhance their physical quality, provide inexpensive energy, and allow for pellet expansion during extrusion (Webster and Lim 2002). However, there are instances of nutritional problems when excessive CHO are fed to carnivorous fish (Vielma et al. 2003). The ability to use dietary CHO varies considerably even among carnivorous species (NRC 1993). Serrano et al. (1992) reported that in red drum, *Sciaenops ocellatus*, fed high-CHO diets (>38%), growth rate was not reduced and there was no accumulation of glycogen in the liver.

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However, negative impacts of high dietary CHO levels have been reported in many carnivorous species such as yellowtail, *Seriola quinqueradiata* (Shimeno et al. 1979), and rainbow trout, *Onchorhynchus mykiss* (Hilton and Atkinson 1982). Other studies have found that CHO readily induced liver glycogen deposition in striped bass, *Morone saxatilis* (Millikin 1983; Berger and Halver 1987), even though striped bass appeared to use dietary CHO more efficiently than other carnivorous fish (Berger and Halver 1987). Optimal levels of dietary CHO for carnivorous fish have been reported to range from 7 to 20% (Furuichi and Yone 1982; Shimeno and Mommsen 1991; Hemre et al. 2002).

Hepatic lesions have long been reported in LMB fed commercial dry diets (Ashley 1974). Goodwin et al. (2000) reported a case study of LMB mortality during live shipment. Histological examinations indicated glycogen accumulation and massive necrosis of the liver. A subsequent aquarium trial compared LMB fed an extruded trout diet (35% CHO), a steelhead trout diet (27% CHO), and a custom diet (21% CHO). Weight gain was lowest in fish fed the extruded trout diet (35% CHO), and liver glycogen was significantly higher in fish fed diets with >21% CHO. However, these were closed-formulation commercial diets that varied in several other important factors in addition to CHO content.

These studies appear to indicate that LMB may not metabolize CHO well, presumably because of their piscivorous nature. Excess CHO may be stored as glycogen in the liver, causing loss of liver function and reducing their ability to handle stress. Because this problem appears to be primarily manifested in market-size fish, it is important that experiments be conducted with larger fish than practical in aquaria-scale trials. The objective of this study was to evaluate growth, survival, body composition, and liver histology of relatively large LMB when fed graded levels of dietary CHO in otherwise similar formulations.

Materials and Methods

Test System

One hundred feed-trained advanced fingerling LMB (128.5 ± 21.5 g) were stocked into

each of nine 3400-L polyethylene round tanks (Polytank, Inc., Litchfield, MN, USA). Tanks were enclosed in a greenhouse covered with shade cloth to reduce ambient light levels by 60% (Tidwell et al. 2003). Tanks were randomly assigned one of three experimental diets (Table 1) containing CHO levels of 25, 19, and 13% of the diet with three replicate tanks per treatment. The 25% CHO diet was most similar to the analyzed composition of a steelhead trout commercial diet (Silver Cup, Murray, UT, USA) that is most widely used as a feed for LMB in commercial production in the region. All diets contained 30% fish meal and maintained similar protein to energy ratios by varying levels of soybean meal and/or added oil. Because soybean meal contains over 30% CHO, to attain very low CHO levels, it had to be reduced and could not be held fixed in all diets. Protein levels were maintained by adding poultry by-product meal. A study by Tidwell et al. (2005) demonstrated that soybean meal and poultry by-product meal are both well used by LMB. Diets were extruded into floating pellets by Integral Fish Foods Inc. (Grand Junction, CO, USA). Full flotation was not achieved in the 13% CHO diet, which was considered a slow sink. Samples of the diets were submitted to a commercial laboratory (Eurofins Scientific, Des Moines, IA, USA) for proximate analysis. During the first week, all fish were fed the diet containing 25% CHO as a conditioning diet and then switched to their assigned diets according to treatment. Fish were fed to apparent satiation twice daily.

Water Quality

Temperature and dissolved oxygen were monitored daily using a YSI 550 DO meter (YSI Company, Yellow Springs, CO, USA). Water quality analyses were conducted three times a week for concentrations of total ammonia-nitrogen and nitrite-nitrogen using a DR/2500 spectrophotometer (HACH Company, Loveland, CO, USA) and pH measured with a YSI 60 electronic pH meter (YSI Company). Alkalinity and hardness were determined once a month by digital titration (HACH Company), and tanks were siphoned three times a week to remove accumulated solids. Un-ionized

ammonia was calculated based on total ammonia–nitrogen, water temperature, and pH according to Boyd (1979).

Samples

Every 4 wk, a minimum of 18 fish were sampled from each tank to determine average body weight. Fish in the sample were bulk weighed, counted, and returned to the tank. Two sampled fish per tank were also tested for blood glucose by anesthetizing the fish with Finquel™ (Argent Chemical Laboratories, Redmond, WA, USA). The caudal fin was cut and blood was tested for glucose levels using a Solartek™ blood glucose meter and Solartek test strips (Kroger Co., Bedford, MA, USA). Blood glucose sampling was conducted approximately 6 h after the morning feeding.

Harvest

After 148 d, all fish were harvested, bulk weighed, and counted. Individual weights (g) and total length (cm) of all fish from each tank were taken. Fifteen fish per tank were sent for disease evaluation and to ensure that any changes were because of dietary treatments and not disease-causing organisms. Samples from livers were plated for bacterial growth, and an API 20 NE diagnostic test (identification for nonfastidious, nonenteric gram-negative rods) (Biomérieux, Durham, NC, USA) was run on isolated colonies of cultured bacteria. Livers of these fish were visually examined and observations were recorded. Fifteen additional fish from each tank were anesthetized and processed for dress-out analysis. An additional three fish per tank were randomly selected for chemical analysis (proximate) of white muscle, gut, and liver. Samples of the three fish were combined and homogenized before being submitted for chemical analysis. The gut sample included both the intestines and the surrounding fat. This species does not have a distinct visceral fat pad (fat is interspersed among the pyloric caecae). This allowed the best measurement of visceral fat deposition. Sections of livers (from same three fish per tank) were also preserved in formalin for subsequent histological analysis.

Histopathology

Slides were prepared by embedding liver tissue in paraffin (Leica Instruments; GmbH, Heidelberg, West Germany) and sectioning under a microtome (Microm D-6900; GmbH); tissues were then stained with a fixed counterstain (Fisher Scientific, Fairlawn, NJ, USA) method using hematoxylin and eosin yellow 0.25% w/v. Prepared liver slides were examined under a microscope by a certified fish pathologist, and the degree of vacuolization in examined liver slides was given a numerical score (0 = normal, 1 = slight, 2 = mild, 3 = moderate, or 4 = severe). Glycogen analysis of liver tissues was carried out at the University of Arkansas at Pine Bluff using the method of Hassid and Abraham (1957).

Statistical Analysis

Effects of dietary CHO level on growth, survival, and body composition were compared by ANOVA using Statistix 8.0 (Analytical Software, Tallahassee, FL, USA). If ANOVA indicated significant differences among treatments, means were separated using the LSD test ($P \leq 0.05$) (Steel and Torrie 1980). Feed conversion ratio (FCR) was calculated as total diet fed (g)/total weight gain (g) of the fish. Hepatosomatic index (HSI) was calculated as the weight of the liver (g)/weight of the fish (g). Condition factor was calculated as $K = [\text{individual harvest weight (g)/average length}^3 \text{ (cm)}] \times 100$. Specific growth rate (SGR, % body weight/d) was calculated from $\text{SGR} = [(\ln W_f - \ln W_i) / t] \times 100$, where W_f = final weight (g), W_i = initial weight (g), and t = time in days. Protein efficiency ratio (PER) was calculated as $\text{PER} = \text{weight gain (g)/amount of protein fed (g)}$. Regression analysis was used to test for a significant relationship between vacuolization score of hepatic tissue and dietary CHO levels (Snedecor and Cochran 1980).

Results

During the 21-wk study period, overall water quality values averaged (\pm SD): dissolved oxygen, 6.0 ± 0.4 mg/L; temperature, 22.2 ± 0.4 C; pH, 7.2 ± 0.3 ; un-ionized ammonia–nitrogen, 0.014 ± 0.007 mg/L; total ammonia–nitrogen, 0.94 ± 0.13 mg/L; nitrite–nitrogen, $0.74 \pm$

0.28 mg/L; alkalinity, 93.6 ± 14 mg/L; and total hardness, 156 ± 14.4 mg/L. Water quality parameters were within ranges suitable for health and growth of LMB (Tidwell et al. 2003). At harvest, survival (Table 2) was not significantly different ($P > 0.05$) in fish fed the 13 and 19% CHO diets (90 and 89%, respectively) but both were significantly greater ($P \leq 0.05$) than survival of fish fed the 25% CHO diet (82%). Average harvest weight (Table 2) was significantly greater ($P \leq 0.05$) in fish fed the 13% CHO diet (380 g) than in fish fed the 19% CHO diet (347 g), which was significantly larger ($P \leq 0.05$) than those fed the 25% CHO diet (310 g). SGRs (%/d) were not significantly different ($P > 0.05$) in fish fed the 13 and 19% CHO diets but both were significantly greater ($P \leq 0.05$) than in fish fed the 25% CHO diet. FCRs were also not significantly different ($P > 0.05$) in fish fed the 13 (2.3) and 19% CHO (2.4) diets but were both significantly lower ($P \leq 0.05$) than in fish fed the 25% CHO diet (3.6). Production (g/L) (which combines weight

gain and survival) was not significantly different ($P > 0.05$) in LMB fed the 13 (11.1 g/L) and 19% CHO (10.3 g/L) diets; however, both were significantly ($P \leq 0.05$) greater than fish fed the 25% CHO diet (8.4 g/L).

Average individual gain was significantly different ($P \leq 0.05$) among fish fed all three diets, with fish fed 13% CHO (296 g) significantly greater ($P \leq 0.05$) than fish fed 19% CHO (270 g), which gained more than those fed 25% CHO (241 g). Total feed fed (kg) was significantly higher ($P \leq 0.05$) in fish fed the 13% CHO diet (0.43 kg) than in bass fed the 19 (0.38 kg) and 25% (0.37 kg) CHO diets, respectively, which were not significantly different ($P > 0.05$) from each other. There were no statistically significant differences ($P > 0.05$) among fish fed the three diets in terms of condition factor (K) (9.5 ± 1.3), PER (1.5 ± 0.3), or HSI (1.7 ± 0.4). Overall, blood glucose levels were not significantly different ($P > 0.05$) in fish fed the 13 and 19% CHO diets (61 and

TABLE 1. The percentage composition of three diets formulated to be isonitrogenous (42% protein) and isocaloric (10% lipid) with varying levels of carbohydrate (25, 19, and 13%) and analyzed composition of the diets.

Ingredient	Dietary carbohydrate levels		
	25%	19%	13%
Fish meal	30.0	30.0	30.0
Soybean meal	33.5	15.5	0.00
Poultry meal	0.00	15.3	28.0
Rice mids	7.80	31.9	30.0
Wheat	19.50	0.00	1.00
Fish oil	4.80	2.90	6.60
Choline	0.50	0.50	0.50
Mineral mix	0.30	0.30	0.30
Vitamin C	0.20	0.20	0.20
Vitamin mix	0.40	0.40	0.40
Dicalcium phosphate	1.00	1.00	1.00
Binder (CMC)	2.00	2.00	2.00
	Analyzed composition (dry matter basis)		
Protein	42.3 ± 0.7	41.9 ± 0.6	42.2 ± 0.1
Fat	10.9 ± 0.4	12.6 ± 0.3	14.8 ± 0.2
Fiber	1.5 ± 0.3	5.1 ± 0.4	6.7 ± 0.2
Ash	13.2 ± 0.1	14.8 ± 0.2	15.9 ± 0.0
Moisture	6.9 ± 0.4	6.9 ± 0.9	7.6 ± 0.2
NFE	25.2 ± 0.1	18.7 ± 0.2	12.9 ± 0.1
Energy ¹ (kcal/g)	3.07	3.01	2.99
P/E (mg/kcal)	89.0	92.3	86.8

CMC = carboxymethylcellulose; NFE = nitrogen-free extract; P/E = protein to energy ratio in mg protein/kcal.

¹ Gross energy values of 4.0, 4.0, and 9.0 kcal/g for carbohydrate, protein, and lipid, respectively (Garling and Wilson 1977; Nematipour and Gatlin 1993; Webster et al. 1995).

TABLE 2. Mean (\pm SD) of survival, final individual weight, feed conversion ratio (FCR), hepatosomatic index (HSI), blood glucose readings, liver glycogen, specific growth rate (SGR) (%), production, condition factor (K), individual gain, total feed intake, and protein efficiency ratio (PER) of juvenile largemouth bass fed three extruded diets formulated to contain three levels of carbohydrate (25, 19, and 13%). Values are means of three replications per diet.¹

Variable	Dietary carbohydrate level		
	25%	19%	13%
Survival (%)	82.0 \pm 4.6 ^b	90.0 \pm 2.0 ^a	89.0 \pm 2.6 ^a
Final individual weight (g)	310.1 \pm 25.3 ^c	347.1 \pm 7.1 ^b	380.1 \pm 8.6 ^a
Individual gain (g)	241.4 \pm 19.7 ^c	270.2 \pm 5.5 ^b	295.9 \pm 6.7 ^a
SGR (%/d)	0.59 \pm 0.05 ^b	0.67 \pm 0.014 ^a	0.74 \pm 0.015 ^a
FCR	3.6 \pm 0.6 ^a	2.4 \pm 0.2 ^b	2.3 \pm 0.3 ^b
Total diet intake (kg)	0.37 \pm 0.02 ^b	0.38 \pm 0.02 ^b	0.43 \pm 0.02 ^a
Production (g/L)	8.4 \pm 1.0 ^b	10.3 \pm 0.2 ^a	11.1 \pm 0.6 ^a
Fillet (%)	41.9 \pm 4.9 ^a	37.3 \pm 2.9 ^a	42.3 \pm 6.2 ^a
Whole dress (%)	67.5 \pm 6.9 ^a	59.0 \pm 5.3 ^a	67.5 \pm 10.2 ^a
HSI	2.1 \pm 0.4 ^a	1.5 \pm 0.5 ^a	1.2 \pm 0.1 ^a
Blood glucose (mg/dL)	86.9 \pm 12.9 ^a	71.2 \pm 9.3 ^b	61.0 \pm 6.0 ^b
K factor	9.2 \pm 1.6 ^a	10.4 \pm 2.0 ^a	10.1 \pm 0.5 ^a
PER	1.6 \pm 0.4 ^a	1.5 \pm 0.2 ^a	1.2 \pm 0.1 ^a

¹ Significant differences ($P \leq 0.05$) are indicated by different superscript letters within rows.

71 mg/dL, respectively), with both significantly lower ($P \leq 0.05$) than in fish fed the 25% CHO diet (87 mg/dL) (Table 2).

There were no significant differences ($P > 0.05$) in proximate composition of liver tissue or white muscle tissue among fish fed the

TABLE 3. Mean \pm SE chemical composition of gut, liver, and white muscle from juvenile largemouth bass fed three diets with different levels of carbohydrate (25, 19, and 13%).¹

Analysis	Dietary carbohydrate level		
	25%	19%	13%
Gut tissue			
Protein	9.7 \pm 1.2 ^a	8.8 \pm 0.2 ^a	8.5 \pm 1.2 ^a
Fat	23.7 \pm 5.4 ^b	31.8 \pm 2.0 ^a	33.9 \pm 2.9 ^a
Fiber	0.2 \pm 0.2 ^a	0.1 \pm 0.1 ^a	0.2 \pm 0.1 ^a
Ash	0.8 \pm 0.0 ^a	0.7 \pm 0.1 ^a	0.8 \pm 0.0 ^a
Moisture	59.9 \pm 3.6 ^a	58.2 \pm 3.6 ^a	65.2 \pm 5.5 ^a
NFE	5.7 \pm 1.9 ^a	1.4 \pm 1.7 ^b	1.2 \pm 1.3 ^b
Liver tissue			
Protein	12.9 \pm 1.7 ^a	11.2 \pm 2.7 ^a	13.7 \pm 0.2 ^a
Fat	5.1 \pm 1.8 ^a	6.9 \pm 4.4 ^a	3.7 \pm 1.0 ^a
Fiber	0.4 \pm 0.1 ^a	0.6 \pm 0.3 ^a	0.3 \pm 0.1 ^a
Ash	1.0 \pm 0.0 ^a	1.0 \pm 0.1 ^a	1.1 \pm 0.1 ^a
Moisture	74.8 \pm 2.0 ^a	72.5 \pm 3.8 ^a	74.3 \pm 1.8 ^a
NFE	5.7 \pm 5.1 ^a	7.8 \pm 4.3 ^a	6.9 \pm 0.7 ^a
Glycogen (%)	32.2 \pm 3.7 ^a	30.3 \pm 2.5 ^a	27.3 \pm 2.2 ^a
White muscle tissue			
Protein	20.1 \pm 0.8 ^a	19.9 \pm 0.4 ^a	17.2 \pm 3.4 ^a
Fat	5.0 \pm 0.8 ^a	6.1 \pm 2.6 ^a	7.0 \pm 2.5 ^a
Fiber	0.1 \pm 0.1 ^a	0.3 \pm 0.1 ^a	0.1 \pm 0.1 ^a
Ash	1.09 \pm 0.05 ^a	1.13 \pm 0.08 ^a	1.07 \pm 0.03 ^a
Moisture	74.8 \pm 1.2 ^a	73.8 \pm 2.5 ^a	71.8 \pm 2.0 ^a
NFE ²	0.00 \pm 0.00 ^a	0.02 \pm 0.03 ^a	3.15 \pm 2.73 ^a

¹ Significant differences ($P \leq 0.05$) are indicated by different superscript letters within rows.

² NFE = nitrogen-free extract.

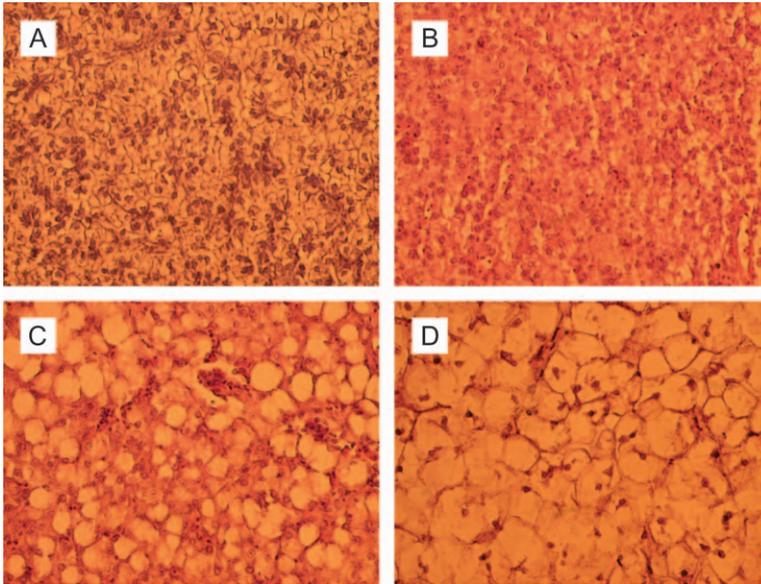


FIGURE 1. Cross sections of liver (stored in buffered formalin and stained with hematoxylin and eosin) of largemouth bass including (A) baseline fish at stocking, (B) fed a low (13%)-carbohydrate diet, (C) a medium (19%) carbohydrate diet, or (D) a high (25%)-carbohydrate diet for 148 d.

different diets (Table 3). Proximate analysis of gut tissue indicated that fish fed the 25% CHO diet had significantly lower lipid levels ($P \leq 0.05$) and higher nitrogen-free extract levels (23.7 and 5.7, respectively) than in fish fed other diets (Table 3). There was no significant difference ($P > 0.05$) in moisture, protein, ash, or fiber of the gut among fish fed any of the three diets. Dress-out percentages in terms of whole dressed and fillet weights (as a percentage of total body weight) did not differ significantly ($P > 0.05$) among fish fed the three diets (Table 2).

Histopathological examination of livers from fish fed the three diets was used to score the degree of vacuolization of hepatic tissues (0 = normal, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe). Regression of vacuolization scores on dietary CHO levels was statistically significant ($P \leq 0.05$) and indicated a direct and positive relationship between vacuolization score and dietary CHO level ($R^2 = 0.57$) (Figure 1).

Discussion

In this study, growth performance of LMB was reduced approximately 8–9% as dietary CHO

level increased from 13 to 19%. As CHO level increased to 25%, growth was reduced 18% compared to fish fed the 13% CHO diet. This same increase in dietary CHO also increased FCR by 57%. Reduced growth and feed conversion efficiency have been reported in a number of carnivorous species fed high levels of dietary CHO, although the ability to use dietary CHO varies considerably, even among carnivorous species (NRC 1983). Poor utilization of CHO as energy source could potentially contribute to results seen here, as feed energy levels would be functionally reduced if CHO were not well digested by the bass. However, Portz and Cyrino (2004) reported that CHO were “highly digestible” by LMB. Another alternative explanation of the results could be a negative impact of soybean meal on palatability of high-CHO diets, which also contain the highest levels of soybean meal (33.5%). Soybean meal has also been shown to have a negative impact on the liver (Robaina et al. 1995). However, in a previous study, Tidwell et al. (2005) used diets containing much higher levels of soybean meal (55%) with no reductions in feed consumption and survivals $>95\%$.

Most carnivorous fish have dietary CHO optima below 20% (Wilson 1994). In some carnivorous species such as yellowtail and rainbow trout, CHO levels >20% have been shown to negatively impact growth and liver structure (Shimeno et al. 1979; Hilton and Atkinson 1982). Rainbow trout have been reported to digest CHO better than other species such as Atlantic salmon, *Salmo salar* (Krogdahl et al. 2005), and turbot, *Psetta maxima* (Burel et al. 2000). Sunshine bass, *Morone chrysops* × *M. saxatilis*, have been shown to effectively use diets with 25% dietary CHO (Webster et al. 1995), while other carnivorous species such as the red drum can be fed even higher levels of CHO (>38%) without adverse effects (Serrano et al. 1992). Japanese flounder, *Paralichthys olivaceus*, were discovered to use CHO poorly as an energy source with growth being significantly reduced when fed diets with 26% CHO and <40% crude protein (Kikuchi and Takeuchi 2002). Increases in CHO levels above 10% were reported to result in reduced feed utilization efficiency in cod, *Gadus morhua*, and salmon, both obligate carnivores (Hemre et al. 1993; Helland and Grisdale-Helland 1998).

The tolerance of carnivorous fishes to dietary CHO has been shown to be influenced by age in some species. Aksnes et al. (1996) reported increased growth and feed efficiency in juvenile halibut, *Hippoglossus hippoglossus*, when dietary CHO was decreased from 27 to 3% of diet. However, Hamre et al. (2003) found no difference in growth of larger halibut fed different CHO levels, suggesting that sensitivity to dietary CHO may be greater in juveniles than in larger fish. The current study evaluated CHO tolerance of 1-yr-old LMB during second-year growth and determined that the lowest CHO level evaluated (13%) yielded the best growth. It is unknown if further decreases would be preferable or if utilization efficiencies would be different for smaller LMB juveniles. This should be evaluated.

Blood glucose levels increased as the level of dietary CHO in the diet increased. These findings agree with reports that carnivorous fish fed CHO in excess of 10% digestible CHO are less able to regulate blood glucose (Shimeno 1991; Brauge et al. 1994; Wilson 1994). Other

measures to evaluate the impact of dietary CHO on LMB were less definitive. In the current study, HSI and glycogen content of the liver were not impacted by dietary CHO level. This differs from Brauge et al. (1994) who reported that in both Atlantic salmon and rainbow trout, liver size and glycogen content increased when fish were fed diets with dietary CHO levels >10%. In Atlantic halibut juveniles, as dietary CHO increased from 0 to 15%, HSIs increased three- to fourfold and liver glycogen concentration increased from 1 to 16% (Hamre et al. 2003). In the present study, while liver size and chemical composition did not demonstrate a clear relationship with dietary CHO, liver histology did. Liver vacuolization scores did demonstrate a direct and positive relationship to dietary CHO level as reported by Goodwin et al. (2000). Decreased survival of fish fed the 25% CHO diet could likely represent a reduced ability to handle stress in fish fed excessive CHO levels because of reduced liver function.

Conclusion

Dietary CHO levels below 19% increased growth and survival of LMB. Higher levels had a negative impact on liver histology, although the relationship between dietary CHO and chemical compositions of the liver was not clear. Based on these data, it would appear that LMB should be fed diets containing <19% CHO. However, this represents a problem in terms of feed manufacture and feed management as ≥20% CHO is needed to produce a floating pellet (Lovell 1989). Future studies should evaluate species-specific diets under practical pond conditions and the acceptability of sinking pellets for LMB production.

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Literature Cited

- Aksnes, A., T. Hjertnes, and J. Opstvedt. 1996. Effect of dietary protein level on growth and carcass composition in Atlantic halibut (*Hippoglossus hippoglossus*, L.). *Aquaculture* 145:225–233.
- Ashley, K. R. 1974. Commercial diets for largemouth bass. Master's thesis. Colorado State University, Fort Collins, Colorado, USA.
- Berger, A. and J. E. Halver. 1987. Effect of dietary protein, lipid and carbohydrate content on the growth, feed efficiency and carcass composition of striped bass, *Morone saxatilis* (Walbaum), fingerlings. *Aquaculture and Fisheries Management* 18:345–356.
- Boyd, C. E. 1979. Water quality in warmwater fish ponds. Auburn University, Agricultural Experiment Station, Auburn, Alabama, USA.
- Brauge, C., F. Medale, and G. Corraze. 1994. Effects of dietary carbohydrate on growth, body composition and glycaemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. *Aquaculture* 123:109–120.
- Burel, C., T. Boujard, F. Tulli, and S. J. Kaushik. 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Aquaculture* 188:285–298.
- Furuichi, M. and Y. Yone. 1982. Availability of carbohydrate in nutrition of carp and red sea bream. *Bulletin of the Japanese Society of Scientific Fisheries* 48:945–948.
- Garling, D. L. and R. P. Wilson. 1977. Effect of dietary carbohydrate to lipid ratio on growth and body composition of fingerling channel catfish. *Progressive Fish-Culturist* 39:43–47.
- Goodwin, A. E., D. M. Tieman, R. T. Lochmann, and A. J. Mitchell. 2000. Massive hepatic necrosis and nodular regeneration in largemouth bass fed feeds high in available carbohydrate. *Journal of the World Aquaculture Society* 33(4):466–477.
- Hamre, K., A. Øfsti, T. Næss, R. Nortvedt, and J. C. Holm. 2003. Macronutrient composition of formulated diets for Atlantic halibut (*Hippoglossus hippoglossus*, L.) juveniles. *Aquaculture* 227:233–244.
- Hassid, W. Z. and S. Abraham. 1957. Chemical procedure for analysis of polysaccharides. Pages 34–37 in S.P. Colowick and N. O. Kaplan, editors. *Methods of enzymology*, volume 3. Academic Press, New York, New York, USA.
- Heidinger, R. C. 2000. A white paper on the status and needs of largemouth bass culture in the north central region. North Central Regional Aquaculture Center, East Lansing, Michigan, USA.
- Helland, B. and S. J. Grisdale-Helland. 1998. Growth, feed utilization and body composition of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in the ratio between macronutrients. *Aquaculture* 166:49–56.
- Hemre, G. I., Ø. Lie, and A. Sundby. 1993. Dietary carbohydrate utilization in cod (*Gadus morhua*): metabolic responses to feeding and fasting. *Fish Physiology and Biochemistry* 10:455–463.
- Hemre, G. I., T. P. Mommsen, and A. Krogdahl. 2002. Carbohydrates in fish nutrition: effects of growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition* 8:175–194.
- Hilton, J. W. and J. L. Atkinson. 1982. Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets. *British Journal of Nutrition* 47:597–607.
- Kikuchi, K. and T. Takeuchi. 2002. Japanese flounder, *Paralichthys olivareas*. Pages 113–120 in C. D. Webster and C. E. Lim, editors. *Nutrient requirements and feeding of finfish for aquaculture*. CABI Publishing, New York, New York, USA.
- Krogdahl, A., G. I. Hemre, and T. P. Mommsen. 2005. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquaculture Nutrition* 11:103–122.
- Lovell, R. T. 1989. *Nutrition and feeding of fish*. Van Nostrand Reinhold, New York, New York, USA.
- Millikin, M. R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Transactions of the American Fisheries Society* 112:185–193.
- Nematipour, G. R. and D. M. Gatlin. 1993. Requirement of hybrid striped bass for dietary (n-3) highly unsaturated fatty acids. *Journal of Nutrition* 123:744–753.
- NRC (National Research Council). 1993. *Nutrient requirements of fish*. National Academy Press, Washington, D.C., USA.
- Portz, L. and J. E. P. Cyrino. 2004. Digestibility of nutrients and amino acids of different protein sources in practical diets by largemouth bass, *Micropterus salmoides* (Lacepede, 1802). *Aquaculture Research* 35:312–320.
- Robaina, L., M. S. Izquierdo, F. J. Moyano, J. Socorro, M. Vergara, D. Montero, and H. Fernandez-Palacios. 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. *Aquaculture* 130:219–235.
- Serrano, J. A., G. R. Nematipour, and D. M. Gatlin, III. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. *Aquaculture* 101:283–291.
- Shimeno, S. 1991. Yellowtail, *Seriola quinqueradiata*. Pages 181–192 in R. P. Wilson, editor. *Handbook of nutrient requirements of finfish*. CRC Press, Boca Raton, Florida, USA.

- Shimeno, M. A. and T. P. Mommsen.** 1991. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of Coho salmon, *Oncorhynchus kisutch*. *General Comparative Endocrinology* 81:473–483.
- Shimeno, S., H. Hosokwa, and M. Takeda.** 1979. The importance of carbohydrate in the diet of a carnivorous fish. Pages 127–143 in J. E. Halver and K. Tiews, editors. *Finfish nutrition and fish feed technology*, volume 1. Heeneman GmbH, Berlin, West Germany.
- Snedecor, G. W. and W. G. Cochran.** 1980. *Statistical methods*. Iowa State University Press, Ames, Iowa, USA.
- Steel, R. G. D. and J. H. Torrie.** 1980. *Principles and procedures of statistics: a biometrical approach*, 2nd edition. McGraw-Hill Book Company, New York, New York, USA.
- Tidwell, J. H., S. D. Coyle, and C. D. Webster.** 2002. Centrarchids: largemouth bass, *Micropterus salmoides*. Pages 374–380 in C. D. Webster and C. E. Lim, editors. *Nutrient requirements and feeding of finfish for aquaculture*. CABI Publishing, New York, New York, USA.
- Tidwell, J. H., S. D. Coyle, L. A. Bright, A. VanArnum, and D. Yasharian.** 2003. Effect of water temperature on growth, survival, and biochemical composition of largemouth bass *Micropterus salmoides*. *Journal of the World Aquaculture Society* 34:175–183.
- Tidwell, J. H., S. D. Coyle, L. A. Bright, and D. Yasharian.** 2005. Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass, *Micropterus salmoides*. *Journal of the World Aquaculture Society* 36:454–463.
- Vielma, J., J. Koskela, K. Ruohonen, I. Jokinen, and J. Kettunen.** 2003. Optimal diet composition for European whitefish (*Coregonus lavaretus*): carbohydrate stress and immune parameter responses. *Aquaculture* 225:3–16.
- Webster, C. D. and L. Lim.** 2002. Introduction to fish nutrition. Pages 1–27 in C. D. Webster and C. E. Lim, editors. *Nutrient requirements and feeding of fish for aquaculture*. CABI Publishing, New York, New York, USA.
- Webster, C. D., L. G. Tiu, J. H. Tidwell, P. Van Wyke, and R. D. Howerton.** 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* × *M. saxatilis*) reared in cages. *Aquaculture* 131:291–301.
- Wilson, R. P.** 1994. Utilization of dietary carbohydrate by fish. *Aquaculture* 124:67–80.