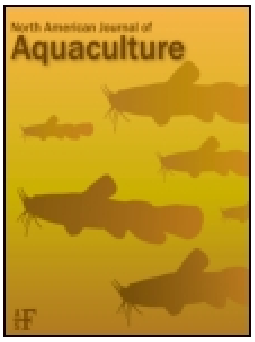

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ARTICLE

Effect of Taurine Supplementation on Growth Response and Body Composition of Largemouth Bass

Catherine A. Frederick, Shawn D. Coyle, Robert M. Durborow, Leigh Anne Bright, and James H. Tidwell*
Kentucky State University, Aquaculture Research Center, 103 Athletic Road, Frankfort, Kentucky 40601, USA

Abstract
Taurine is "conditionally essential" for some fish species, particularly when fish meal levels in their diets are reduced. Taurine supplementation has not yet been evaluated in the Largemouth Bass *Micropterus salmoides*. A 12-week feeding trial was conducted using a 2 × 2 factorial design with the main effects being fish meal inclusion (0% or 30%) or taurine supplementation (0% or 2%). The fish meal diets contained sardine fish meal while the nonfish meal diets used pork meal, which has a very low taurine content, as the animal source protein. The four experimental diets were formulated to contain 40% crude protein and 12% lipid. The feeding trial was conducted using juvenile Largemouth Bass (19.3 ± 3.9 g; mean ± SD) stocked at 25 fish/tank into twelve 230-L aquaria within a recirculating system. Fish were fed to apparent satiation twice daily. Dissolved oxygen, pH, and temperature were monitored daily, while alkalinity, total ammonia nitrogen, and nitrite-nitrogen were monitored three times weekly. Analysis of harvest data indicated a significant statistical interaction between fish meal inclusion and taurine supplementation only for feed conversion ratio (FCR). In diets containing no fish meal, the addition of taurine significantly increased the FCR (from 2.2 to 2.6). However, in fish fed diets containing fish meal, supplemental taurine significantly decreased the FCR (from 2.2 to 1.9). In terms of main effects, taurine supplementation did not significantly affect any of the measured growth or survival variables. Survival and growth in Largemouth Bass fed 0% fish meal were not significantly different from survival and growth in those fed 30% fish meal. Body composition variables were not significantly affected by either fish meal inclusion or taurine supplementation. These data support previous studies that found that by-products from terrestrial animal sources can successfully replace fish meal in diets for Largemouth Bass. These data also indicate that Largemouth Bass do not have a significant dietary requirement for taurine.

Fish meal has historically been the preferred protein source used in many aquaculture diets, but high costs and environmental concerns may limit its future use (Naylor et al. 2000). Alternative sources of proteins from animals and plants have been reviewed in Glencross et al. (2007) and Naylor et al. (2009). Currently, Largemouth Bass *Micropterus salmoides* are often fed a commercial salmonid diet based more on availability than species suitability (Tidwell et al. 2000). Efforts have been made to formulate a species-specific diet for Largemouth Bass using alternative ingredients (Tidwell et al. 2005; Sampaio-Oliveira and Cyrino 2008; Cochran et al. 2009). Significant decreases in growth and feed efficiency have been observed in Largemouth Bass fed diets with very high concentrations of soybean meal (Tidwell et al. 2005).

Thompson et al. (2008) reported that plant proteins are often associated with nutritional deficiencies due to incomplete amino acid profiles, reduced palatability, and various antinutritional factors. However, when plant and animal protein sources are properly combined to produce complementary amino acid profiles, growth and survival can significantly increase (Naylor et al. 2009). Experimental diets combining plant and animal proteins have successfully replaced fish meal in the diets of

*Corresponding author: james.tidwell@kysu.edu
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Largemouth Bass (Tidwell et al. 2005; Sampaio-Oliveira and Cyrino 2008).

Taurine deficiencies have recently been considered to be a cause for poor growth response in some fish fed diets free of fish meal (Goto et al. 2001; Yokoyama et al. 2001; Takagi et al. 2008). Fish meal is rich in taurine, while terrestrial animal meals contain lower concentrations and plant-based ingredients contain no taurine (Spitze et al. 2003). Consequently, as fish meal is replaced with other ingredients, taurine may become a limiting nutrient for some fish species. This suggests that taurine could be described as a "conditionally essential" amino acid (Kim et al. 2005; Zhang et al. 2006; Lunger et al. 2007).

Several nutritional diseases observed in juvenile Red Seabream *Pagrus major* and Yellowtail *Seriola quinqueradiata* are believed to be associated with taurine deficiencies (Takagi et al. 2006). Metabolic studies indicate that higher levels of dietary taurine increase amino acid retention and may increase the growth rates of certain fishes (Lunger et al. 2007). Taurine-enriched experimental diets have increased the growth, survival, and/or efficiency of feed utilization in Cobia *Rachycentron canadum* (Lunger et al. 2007; Salze et al. 2011), Rainbow Trout *Oncorhynchus mykiss* (Gaylord et al. 2006, 2007), Atlantic Salmon *Salmo salar* (Espe et al. 2012), Common Dentex *Dentex dentex* (Chatzifotis et al. 2008), Japanese Flounder *Paralichthys olivaceus* (Park et al. 2002; Kim et al. 2003, 2005), Red Seabream (Matsunari et al. 2008), European Sea Bass *Dicentrarchus labrax* (Martinez et al. 2004), Senegal Sole *Solea senegalensis* (Pinto et al. 2010), Yellowtail (Matsunari et al. 2005b, 2006; Takagi et al. 2008), Pacific Cod *Gadus macrocephalus* (Matsunari et al. 2005a), Florida Pompano *Trachinotus carolinus* (Rossi 2011; Rossi and Davis 2012), and white shrimp *Litopenaeus vannamei* (Yue et al. 2013). Although taurine requirements for fish are not well established, it appears that the dietary requirement of taurine is at least 0.5% of the diet for some species (Park et al. 2002; Kim et al. 2005, 2007; Matsunari et al. 2008), while optimal levels of taurine supplementation have been suggested to be 1.5–2.0% (Park et al. 2001).

Previous research indicates that taurine supplementation may benefit carnivorous species fed alternative protein sources. The nutritional value of taurine has been primarily evaluated in marine species, and the information available for freshwater species is largely limited. Although taurine requirements for Largemouth Bass are not yet established, taurine supplemented into diets containing animal protein sources are known to have improved the growth, condition, and feed efficiency in Florida Pompano when fish meal was replaced by poultry by-product meal (Rossi 2011). Largemouth Bass may also require taurine supplementation when fed a diet free of fish meal. The current study was conducted to determine whether supplemental taurine would improve the growth performance in Largemouth Bass fed diets in which fish meal was replaced by a pork by-product meal, an animal-source protein with a very low concentration of taurine.

TABLE 1. The formulation of four experimental diets (with 30% or 0% fish meal and 0% or 2% added taurine) fed to Largemouth Bass and their analyzed compositions as fed. Values for ingredients are given as percent of total feed weight.

	With fish meal; added taurine:		Without fish meal; added taurine:	
Ingredient	0%	2%	0%	2%
Menhaden fish meal	30	30	0	0
Soybean meal	40	40	40	40
Blood meal	0	0	4	4
Pork by-product meal	0	0	37	37
Corn meal	21.1	19.1	9.28	7.28
Fish oil[a]	6	6	6	6
Choline	0.30	0.30	0.30	0.30
Mineral mix	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Vitamin mix	0.50	0.50	0.50	0.50
Di-Cal-P	1.50	1.50	1.50	1.50
Taurine	0	2.0	0	2.0
Lysine	0	0	0.5	0.5
Methionine	0	0	0.32	0.32
Content as analyzed (%):				
Moisture	11.19	12.56	10.82	11.76
Protein	39.40	40.21	38.89	39.12
Ash	9.46	9.81	9.56	9.59
Lipids	9.80	9.32	9.56	9.59
Taurine	0.17	2.07	0.02	1.98

[a]Fish oil was added at 6% of the total feed weight after the extrusion and after drying in air for 24 h.

METHODS

A feeding trial was conducted using a 2 × 2 factorial treatment design with the main effects being fish meal inclusion (0% or 30% fish meal) and taurine supplementation (0% or 2%). The fish meal diets contained sardine fish meal while the non-fish meal diets used a pork by-product meal with low taurine content as the animal-source protein. The level for taurine was based on the maximum recommended by Park et al. (2001). The four diet formulations are described in Table 1.

To prepare the test diets, dry ingredients were mixed together for 1 h using a Hobart mixer (model A200, Hobart, Troy, Ohio). After mixing dry ingredients, sufficient tap water was added to obtain 35% moisture, and the resultant blend was then mixed for an additional 10 min. Diets were passed through a grinder with a 4.5-mm die twice to form pellet strands, and then air-dried for 24 h. Diet strands were broken up and sieved (4-mm-opening mesh) using a U.S. standard testing sieve (Fisher Scientific, Pittsburgh, Pennsylvania). Fish oil was added at 6% (by dry weight) to all diets until pellets were uniformly

TABLE 2. Amino acid composition of four experimental Largemouth Bass diets formulated to be isonitrogenous with 0% and 30% fish meal and supplemented with or without 2% taurine.

	With fish meal; added taurine:		Without fish meal; added taurine:	
Amino acid	0%	2%	0%	2%
Threonine	1.59	1.54	1.4	1.31
Serine	2.01	1.93	1.84	1.76
Glutamic acid	6.35	6.07	5.73	5.47
Proline	2.08	1.99	2.51	2.37
Glycine	2.56	2.48	3.41	3.29
Alanine	2.19	2.14	2.40	2.26
Valine	1.92	1.84	1.87	1.77
Isoleucine	1.67	1.64	1.30	1.23
Leucine	3.01	2.84	2.88	2.70
Tyrosine	1.20	1.20	1.08	1.06
Phenylalanine	1.85	1.77	1.82	1.74
Total lysine	2.65	2.60	2.73	2.51
Histidine	0.96	0.94	0.96	0.93
Arginine	2.76	2.68	2.73	2.62
Taurine	0.17	2.07	0.02	1.98

coated, and then the pellets were allowed to dry for another 24 h. Diets were stored at −19°C in plastic containers until used for feeding. A subsample of each diet was submitted for proximate analysis, amino acid composition, and taurine content (Eurofins Scientific, Des Moines, Iowa) (Tables 1 and 2, respectively).

Juvenile Largemouth Bass were obtained from a local producer (Robert Mayer, Cox's Creek, Kentucky). Fish (19.3 ± 3.9 g; mean ± SE) were randomly stocked at a rate of 25 fish per aquarium into twelve 230-L glass aquaria within a recirculating system at the Aquaculture Research Center, Kentucky State University, Frankfort. Five additional fish were frozen for baseline proximate composition analyses and stored at −4°C in a freezer until all samples were ready for shipment. Initial weights (g) and lengths (cm) were based on 100 randomly sampled fish from the same population. Each of the four diets was randomly assigned to three replicate aquaria. Fish were fed twice daily (0900 and 1600 hours) to apparent satiation for 12 weeks. The amount of diet fed was recorded daily. Mortalities were monitored daily with replacements only made during the first week.

Water temperature was maintained between 22°C and 24°C by the use of an immersion heater, and continuous aeration was provided to maintain dissolved oxygen (DO) concentrations. Dissolved oxygen, pH, and temperature were monitored daily using a YSI 85 DO meter (YSI, Yellow Springs, Ohio). Levels of alkalinity, total ammonia nitrogen (TAN), and nitrite-nitrogen (NO_2-N) were monitored three times weekly using a spectrophotometer (HACH Odyssey DR 2500, HACH, Loveland, Colorado). Sodium bicarbonate was added to the recirculating system as needed to maintain alkalinity near 100 mg/L. All tanks were siphoned daily to remove feces and uneaten feed.

After 12 weeks, fish from individual aquaria were harvested, bulk-weighed, counted, and then individually weighed and measured (TL). Six fish from each tank were randomly selected and anesthetized with clove oil. The gut, muscle, liver, and fish weights were recorded from these fish. Livers from three fish were preserved in 10% formalin for 1 week and were then submitted to Dr. Lester Khoo at Mississippi State University, Mississippi State, for histological analysis. The remaining three fish from each tank were submitted for whole-body proximate analysis (Eurofins Scientific). Fish muscle was chemically analyzed for moisture, protein, ash, and crude fat content (see Table 4).

From each tank, 14 remaining fish were subjected to an low-oxygen stress challenge within 48 h after the feed trial. Half of the water was drained from each tank and air stones were removed until the concentration of DO was reduced to 0.6 mg/L. Fish were then held at 0.6 mg/L of DO for a total of 30 min. After the 30-min exposure, water flow and aeration were restored. The initial and final total ammonia concentrations as well as temperature and pH were determined for each tank. After the low-oxygen challenge, fish were fed for an additional 2 weeks following the same protocol as the feed trial. Mortalities were monitored twice daily and recorded throughout the period. Our plans were to examine any mortalities for pathogenic bacteria and to see whether mortality or disease occurrence correlated with the diets.

Data on fish growth, survival, and feed conversion ratio (FCR) were each analyzed as a 2 × 2 factorial (main effects of protein source and taurine supplementation) and tested for significant interactions ($P \leq 0.05$) between main effects using Statistix 10.0 software (Analytical Software, Tallahassee, Florida).

Growth performance values were calculated as follows: condition factor (K) was calculated as $100 \times W/L^3$, where W is weight (g) and L is total length (cm). Average harvest weight was calculated as total weight gain (g) / number harvested. Average individual gain (%) was calculated as average harvest weight (g) / average stock weight (g) × 100. Hepatosomatic index (HSI) and feed conversion ratio (FCR) were calculated using equations found in Gaylord et al. (2006).

RESULTS

Overall mean ± SE values for measured water quality variables were: water temperature, 25.2 ± 0.2°C; DO, 7.9 ± 0.1 mg/L; TAN, 0.08 ± 0.01 mg/L; NO_2-N, 0.04 ± 0.01 mg/L; total alkalinity, 90.1 ± mg/L; and pH, 8.1 ± 0.0.

Main Effect Interactions

There were no significant interactions ($P > 0.05$) between protein sources and taurine supplementation on any of the measured production variables except FCR. The significant interaction ($P \leq 0.05$) between fish meal and taurine supplementation

TABLE 3. Main-effect mean values for survival, growth, weight gain, *K*, and HSI of juvenile Largemouth Bass fed diets containing 0% or 30% fish meal (FM) with 0% or 2% supplemental taurine (TAU). *P*-values represent the significance level of the main effects of FM and TAU and the interaction of these two factors given.

	With fish meal; added taurine:		Without fish meal; added taurine:		*P*-value		
Variable	0%	2%	0%	2%	FM	TAU	FM × TAU
Survival (%)	97.3	97.3	92.0	89.3	0.737	0.737	0.120
Average weight (g)	70.5	69.0	67.8	72.2	0.319	0.616	0.938
Weight gain (%)	343.6	337.6	322.6	354.8	0.071	0.191	0.838
Weight gain (g)	50.0	48.6	46.7	51.8	0.218	0.463	0.997
K	1.4	1.4	1.4	1.4	0.069	0.420	0.558
HSI	5.5	7.0	6.3	5.2	0.230	0.667	0.334

TABLE 4. Mean values (% of total) of whole-body moisture, protein, ash, and lipids of juvenile Largemouth Bass fed diets containing 0% or 30% fish meal with 0% or 2% supplemental taurine. *P*-values represent the significance level of the main effects of FM and TAU and the interaction of these two factors given.

	With fish meal; added taurine:		Without fish meal; added taurine:		*P*-value		
Analyzed composition	0%	2%	0%	2%	FM	TAU	FM × TAU
Moisture	69.4	68.6	68.0	68.6	0.344	0.869	0.329
Protein	17.4	17.5	17.8	17.4	0.239	0.385	0.305
Ash	4.6	5.8	4.1	3.8	0.315	0.508	0.112
Lipid	9.0	7.8	10.0	9.0	0.889	0.266	0.265

was based on differential responses of fish to taurine supplementation for diets containing fish meal versus those that did not. In diets containing no fish meal, the addition of taurine significantly decreased ($P \leq 0.05$) the FCR (2.2 versus 2.6, respectively). However, in fish fed diets that did contain fish meal, supplemental taurine significantly decreased ($P \leq 0.05$) the FCR (2.2 versus 1.9, respectively). For variables without significant interactions ($P > 0.05$), main effects (protein source and taurine supplementation) were addressed separately (Dowdy and Wearden 1983).

Effects of Fish Meal or Taurine Supplementation

Effect of fish meal.—The presence or absence of fish meal had no impact ($P > 0.05$) on Largemouth Bass in terms of survival, average harvest weight, weight gain, *K*, or HSI (Table 3). The presence or absence of fish meal did not significantly affect ($P > 0.05$) the whole-body proximate composition of harvested fish.

Effect of taurine.—Inclusion of 2% supplemental taurine had no significant impact ($P > 0.05$) on any of the measured growth variables. Taurine supplementation also had no significant effect ($P > 0.05$) on the whole-body proximate composition of the fish (Table 4).

Stress challenge.—All but three fish survived the stress challenge test during the 2-week trial. The three mortalities were found in the same aquaria and had been fed a diet containing 0% fish meal and 0% taurine. A presumptive disease diagnosis was conducted on dead fish but no causative agent was identified.

Liver histology.—Liver histology analysis determined that experimental fish from all treatments had similar microscopic changes. There appeared to be no significant histopathological differences among Largemouth Bass related to the four experimental diets.

DISCUSSION

In the current study, Largemouth Bass fed diets containing as little as 0.02% taurine showed no changes in growth, survival, or body composition compared with those in which diets were supplemented with 2% added taurine. In previous studies, Largemouth Bass had been successfully raised on diets free of fish meal but containing other animal- and plant-based proteins (Tidwell et al. 2005). As a strict carnivore, it was thought that the Largemouth Bass might require taurine supplementation when protein sources had very low concentrations of dietary taurine. Since Largemouth Bass showed no response to taurine supplementation in terms of growth, survival, or body composition, results from this study indicate that Largemouth Bass do not appear to have a dietary requirement for taurine.

While a number of marine predators have demonstrated a dietary requirement for taurine, taurine supplementation studies of juvenile Atlantic Salmon (Espe et al. 2012) and juvenile Common Carp *Cyprinus carpio* (Kim et al. 2008) indicated that those species do not require added dietary taurine for good growth. However, newly hatched Nile Tilapia *Oreochromis niloticus* did show improvements in growth with increasing levels of supplemental taurine (G. S. Goncalves, M. J. P. Ribeiro, R. M. Vidotti, and F. R. Sussel, abstract presented at the World Aquaculture Society meeting, 2011). Taurine supplementation in the diets of Channel Catfish *Ictalurus punctatus* and juvenile Common Carp did not improve growth but did increase the osmoregulatory response in stressed individuals (Buentello and Gatlin 2002; Zhang et al. 2006).

Other studies have recently proposed that a taurine deficiency may be a cause for poor body condition and reduced growth response in some species only when fish are fed diets free of fish meal (Goto et al. 2001; Yokoyama et al. 2001; Takagi et al. 2008). If a species has a relatively high requirement, taurine may become “conditionally essential” if fish are fed diets formulated with ingredients lacking adequate concentrations of taurine (Kim et al. 2005; Zhang et al. 2006; Lunger et al. 2007). Rainbow Trout fed diets supplemented with taurine showed significant increases in growth performance and feed efficiency compared with those fed nonenriched diets (Gaylord et al. 2006). However, El-Sayed (2013) noted that the diets used by Gaylord et al. (2006, 2007) were deficient in methionine and cystine. In fish, methionine is a necessary precursor for the de novo synthesis of taurine, which is biosynthesized through a series of enzymatic pathways in the liver (Goto et al. 2001, 2003; Park et al. 2002). Under the conditions of the Gaylord et al. (2006) study, taurine likely became conditionally essential (El-Sayed 2013) based on a lack of precursors available for conversion into taurine.

The ability of Largemouth Bass to possibly biosynthesize taurine is supported by studies on their close centrarchid relative, the Bluegill *Lepomis macrochirus* (Goto et al. 2004). There is speculation that herbivores and omnivores have a greater ability to synthesize taurine than do their carnivorous counterparts (Spitze et al. 2003; Gaylord et al. 2006). Lunger et al. (2007) noted that herbivorous fish likely evolved the necessary mechanisms for taurine synthesis because of the lack of dietary taurine in plant-based diets. El-Sayed (2013) challenged this concept, stating that most freshwater fish, including carnivores, have the ability to synthesize taurine and do not require exogenous sources of taurine. It is possible that Largemouth Bass have the capability of meeting dietary requirements through endogenous synthesis.

Results from the current study suggest that Largemouth Bass do not require supplemental taurine when using proteins from animal sources, even at very low taurine concentrations. It may be justified to evaluate the role of taurine in Largemouth Bass if all-plant diets for Largemouth Bass are developed. When using practical feed ingredients, Largemouth Bass growth was not significantly decreased when the bass were fed diets that replaced fish meal with pork meal with or without taurine supplementation. These data appear to indicate that Largemouth Bass either have no, or a very low, requirement for taurine or have the ability to endogenously synthesis it.

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