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Evaluation of Meat and Bone Meal in Practical Diets Fed to Juvenile Hybrid Striped Bass Morone chrysops x M. saxatilis

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ABSTRACT

We fed juvenile hybrid striped bass (Morone chrysops x M. saxatilis) one of eight diets to evaluate meat and bone meal as a source of crude protein and essential amino acids. Diets contained either 0, 15, 20, 25, 30, 35, 40 or 45% meat and bone meal substituted for an isonitrogenous amount of soybean meal and fish meal. All diets were fed for seven weeks, followed by a 2-week digestibility trial. Mean consumption, weight gain and feed conversion ratio were not significantly affected by addition of meat and bone meal into diets. Intraperitoneal and liver lipid concentrations were not significantly affected by meat and bone meal. Fillet proximate composition was not significantly different among treatments. protein, phosphorus and amino acid availabilities were significantly lower in fish fed 45% meat and bone meal compared to fish fed 30% and lower concentrations and generally lower in fish fed greater than 30% meat and bone meal. Based on these data, it appears meat and bone meal can be used as the primary source of crude protein and essential amino acids in practical growout diets for hybrid striped bass, comprising as much as 45% of the diet. Nutrient availability values were lower in fish fed greater than 30% meat and bone meal and may restrict usage in some applications.

Culture of hybrid striped bass (Morone chrysops x M. saxatilis) is one of the new aquacultural production industries in the US and one of the most rapidly growing. According to the first census of aquaculture in the US, culture of hybrid striped bass totaled \$28 million is sales in 1998, producing approximately 4.1 million kg of food size fish and 27 million juvenile fish. However, dietary formulations are not standardized and significant opportunities exist for evaluation of new feedstuffs.

Detailed nutritional research with the hybrid striped bass began in the late 1980's. To date, nutritional requirements for lysine (Griffin et al. 1992), arginine (Griffin et al. 1994a), methionine (Griffin et al. 1994b), threonine (Keembiyehetty and Gatlin 1997) choline (Griffin et al. 1994c), and phosphorus (Brown et al. 1993) have been quantified. Further, estimates of the remaining essential amino acid requirements are available (Brown, 1995). Given several of the critical nutritional requirements for this hybrid, practical diets were developed and are currently available. To date, only soy products have been evaluated as alternative sources of crude protein and essential amino acids (Brown et al. 1997). A low level of fish meal is considered important in practical diets as a flavor component (Brown et al., 1993; Webster et al., 1997).

The objective of this study was evaluation of meat and bone meal in practical diets fed to juvenile hybrid striped bass.

Materials and Methods

Meat and bone meal (33% beef, 29% pork and 38% poultry; 38% offal, 8% bone, 12% trimming and 42% deadstock) was supplied by Fats and Protein Research Foundation Inc. (Bloomington, Illinois, USA). Proximate analyses of the meat and bone meal is presented in Table 1. Soybean meal, corn grain and dicalcium phosphate were obtained from local sources (Cargill, Inc., Lafayette, Indiana, USA). Menhaden meal and oil, and reagent grade minerals were obtained from commercial suppliers (Omega Protein, Reedville, Virginia, USA and Sigma Chemical, St. Louis, Missouri, USA, respectively). Vitamins were acquired from U.S. Biochemical (Cleveland, Ohio, USA). L-ascorbyl 2-polyphosphate was obtained from Roche Inc. (Nutley, New Jersey, USA). Purified soybean lecithin, containing 90% phosphatidylcholine and 3% lysophosphatidylcholine, was provided by the American Lecithin Company (Oxford, Connecticut, USA). Vitamins (with the exception of ascorbic acid and choline chloride) and minerals were added to the diets as nutritionally complete premixes. Diets were mixed and pelleted as previously reported (Twibell et al., 2000).

Proximate composition and amino acid concentrations of meat and bone meal, soybean meal, corn grain and fish meal were analyzed prior to dietary formulation. Dietary ingredients were analyzed using the same methods described below for tissues. All diets were formulated to contain 44% crude protein and 10% lipid. Additionally, all diets met the established and predicted essential amino acid requirements for this hybrid (Brown 1995). Meat and bone meal was incorporated at either 0, 15, 20, 25, 30, 35, 40 or 45% of the dry diet, for an isonitrogenous amount of soybean meal and fish meal. Both ingredients had to be reduced in order to maintain EAA concentrations at the desired levels. Dietary formulations are in Table 2.

Juvenile hybrid striped bass (male <u>Morone chrysops</u> x female <u>M. saxatilis</u>) were obtained from a commercial producer (Keo Fish Farms, Keo, Arkansas, USA) and transported to the Purdue University Aquaculture Research Facility. Procedures used during transport, quarantine and

experimental period were approved by the Purdue Animal Care and Use Committee (PACUC No. 89-060-98, "Nutritional Studies with Aquatic Animals," Principal Investigator Qualification No. BRO-249).

The closed recirculating system used contained 32 individual 190-L aquaria. The experimental system was equipped with four submerged filtration tanks for solid material removal and a submerged biological filtration tank for denitrification of the water. Water was pumped through a sand filter to each aquarium at a rate of ~ 1 L/min. Water temperature was maintained at 28 ± 2 C throughout the experiments. The diurnal light:dark cycle of the aquaculture facility remained at 16 h light:8 h dark throughout the study.

Groups of 20 randomly chosen fish were stocked into each of 24 aquaria. Fish were acclimated to the experimental system and their respective diets for 2 wk prior to each experiment. Following the acclimation period, the number of fish in each tank was reduced to 15 so that the total weight of fish in each tank was 820 ± 5.0 g. Dietary treatments were randomly assigned to triplicate aquaria. All fish were fed to satiation. The experiment lasted 7 wk.

Water quality was monitored daily and was within acceptable limits throughout the study. Dissolved oxygen concentrations were not below 5.0 mg/L at any time. Ammonia-N and nitrite-N concentrations did not exceed 0.38 mg/L and 0.18 mg/L, respectively.

At the conclusion of the experiment, all fish were anesthetized (tricaine methanesulfonate, Argent Chemical, Redmond, Washington, USA) and weighed 24 h after the final feeding. Weight gain, feed efficiency (FE) and protein efficiency ratio (PER) were determined. No mortalities occurred during the experiment. All fish were then placed back into their respective aquaria and fed for an additional two weeks. Diets fed during this period were the same as those used during the 7-wk growth trial, but each diet contained 0.5% barium carbonate as an indicator of nutrient availability (Riche et al. 1995). At the end of the 2-wk digestibility trial, all fish were killed with a lethal dose of tricaine methanesulfonate and fecal samples collected from the posterior 10% of the gastrointestinal tract by dissection (Austreng 1978). Additionally, various tissue samples were collected.

Three randomly chosen fish were collected from each dietary replicate and frozen at -20 C for subsequent determination of carcass and fillet proximate composition. Whole bodies were sliced and dried in a forced air oven at 100 C for moisture determination. Dried samples were ground and used for determination of crude protein, lipid and ash. Crude protein was determined using a nitrogen analyzer (Perkin-Elmer 2410 Series II; Norwalk, Connecticut, USA). Ash was determined by incinerating samples in a muffle furnace at 550 C (AOAC 1990). Lipid was determined with chloroform-methanol extraction in a soxhlet extractor (Folch et al. 1957).

Muscle samples were dissected from three separate fish and stored at -20 C prior to proximate analysis by the methods described above. Visceral fat was dissected from three fish for calculation of intraperitoneal fat (IPF) ratio (IPF x 100/body weight), livers were removed and weighed, then stored at -20 C for subsequent lipid determinations.

Crude protein concentration of feed and fecal samples was analyzed by the method described previously. Barium and phosphorus in dried diets and fecal samples were wet ashed in an analytical microwave oven (CEM Corporation, Matthews, North Carolina, USA) and analyzed using inductively coupled plasma–atomic emission spectroscopy (Perkin-Elmer Plasma 400, Norwalk, Connecticut, USA). Essential amino acid concentrations in feed and fecal samples

were analyzed using high-performance liquid chromatography (HPLC) at the Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, Missouri, USA). The samples were hydrolyzed with 6 N HCl for 4 h at 145 C (Gehrke et al.1987), and the amino acids were determined by cation exchange chromatography in a Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Inc., San Ramon, California, USA). Methionine and cystine were oxidized with performic acid before hydrolysis (Moore 1963). Tryptophan was measured according to a colorimetric method after enzymatic hydrolysis by pronase (Spies and Chambers 1949; Holz 1972). Digestibility values were calculated using a standard formula (Maynard et al. 1979).

All data were analyzed as a completely randomized design using each aquarium as an experimental unit. Data were subjected to one-way ANOVA (Zar 1984). All data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, North Carolina, USA). Student-Newman-Keuls test separated mean values when significant differences were detected by ANOVA. Accepted level of significance was 0.05.

Results

Mean consumption of the diet containing 30% meat and bone meal (MBM) was significantly higher than mean consumption of the control diet or the diet containing 20% MBM (Table 3). No other significant differences were detected, but consumption of all diets containing MBM was higher than consumption of the control diet. Mean weight gain of fish fed the control diet was significantly lower than fish fed any level of MBM. Weight gains of fish fed the diets containing MBM were not significantly different. Feed conversion ratio of fish fed 30% MBM was significantly higher than in fish fed 20% MBM, but other values were not significantly different. Protein efficiency ratios were not significantly different among dietary treatments.

Mean intraperitoneal fat, hepatosomatic indices and liver lipid concentrations were not significantly affected by dietary additions of MBM (Table 4). Similarly, proximate composition of whole bodies (Table 5) and fillet samples from dietary treatments were not significantly different (Table 6).

Mean apparent crude protein digestibility in fish fed 45% MBM was significantly lower than in fish fed other dietary treatments (Table 7). No other significant differences were detected. Mean apparent phosphorus availability values were not significantly affected by addition of MBM to practical diets. Apparent availability of all amino acids was significantly lower in fish fed 45% MBM compared to fish fed other treatments (Table 8). No other significant differences were detected.

Discussion

Whole animal responses were not significantly affected by feeding MBM up to 45% of the dry diet. Those diets contained 27% soybean meal and 2.75% fish meal compared to the control diet that contained 54% soybean meal and 16% fish meal. Thus, it appears MBM can be used as the primary source of crude protein and essential amino acids in diets fed to hybrid striped bass. It is unclear if higher levels of MBM can be used or if MBM can serve as the sole source of crude protein and essential amino acids.

MBM has been identified as an appropriate ingredient in diets fed to Nile tilapia Oreochromis niloticus (Wu et al., 1999; El-Sayed, 1998), Indian carp Labeo rohita (Paul et al., 1997), African catfish Clarias batrachus (Rao et al. 1997), vellowtail (Shimeno et al., 1993), channel catfish Ictalurus punctatus (Mohsen and Lovell, 1990), milkfish Chanos chanos (Alava and Lim, 1988) and rainbow trout Oncorhynchus mykiss (Rehulka, 1985). However, most of these evaluations incorporated MBM at relatively low levels in the diet (6-12% of dry diet), or were complete replacement of fish meal with MBM (El-Sayed, 1998). Bureau et al. (2000) fed either 12 or 24% of the diet as MBM and reported no significant differences in weight gain, feed efficiency or retention of nitrogen, lipid or energy in rainbow trout fed either of 3 sources of MBM compared to fish fed a control diet. Kikuchi et al. (1997) evaluated MBM levels of 9, 18, 36 or 44% of the diet and determined that levels up to 18% were efficacious in diets fed to Japanese flounder Paralichthys olivaeceus. Robaina et al. (1997) determined that MBM could be safely used in diets for gilthead seabream Sparus aurata at levels of 20% of the dietary protein. Additionally, feed efficiency and protein efficiency ratio of fish fed 20% of the protein as MBM were higher than in fish fed the control diet. Tacon et al. (1983) determined that MBM could be used in place of 75% of the dietary crude protein in diets fed to Nile tilapia. Channel catfish consumed more feed, converted that food to tissue better, and gained more weight than catfish fed no MBM (Mohsen and Lovell, 1990). However, dietary concentrations of 5 and 10% of the dietary crude protein were the only levels evaluated. Thus, it appears the hybrid striped bass can utilize MBM as well as most other species tested to date.

Processing characteristics of hybrid striped bass fed MBM were not significantly affected. There were no significant increases in visceral lipid deposits or liver weight that would decrease dressout percentage. Further, liver health does not appear to have been impacted as liver lipid concentrations were not significantly altered as dietary MBM increased in the diet. Carcass and fillet proximate composition were not significantly affected by dietary treatments; thus, the nutritional benefits of consuming hybrid striped bass appear to have been maintained. Effects on fatty acid profile should be evaluated. As the dietary concentration of MBM reached 45% of the diet, several nutrient availability values decreased significantly. The reason for this decrease is unclear and it did not impart a significant impact on whole-animal production characteristics. The decreases in nutrient availability may simply be a situation in which the intake of those nutrients measured was in excess of the animals' needs and absorption mechanisms from the gastrointestinal tract were overwhelmed. Additional research into this situation may prove useful. However, it is clear that hybrid striped bass can efficiently use MBM as a source of nutrients.

Brown et al. (1985) reported the apparent crude protein digestibility (ACPD) of MBM was 82% in channel catfish, while Cruz (1975) reported ACPD of 72%. Cho et al. (1982) reported ACPD values for rainbow trout of 82%. Hajen et al. (1993) reported ACPD value of 85% for chinook salmon Oncorhynchus tshawytscha fed MBM. True amino acid availability from MBM has been reported for channel catfish (Wilson et al., 1981). Those values ranged 76-88%. Apparent phosphorus availability values for feedstuffs of animal origin range from 20 to over 80%, with most around 40 to 50% (Riche and Brown 1996). Values determined in this study were for a combination of ingredients, but are within the range of previously reported nutrient availability values for fishes.

Apparent crude protein digestibility and amino acid availability values were lower in fish fed 35 and 45% MBM than in fish fed other concentrations, but similar in fish fed 40% and

concentrations below 35%. We suspect that analytical variability associated with small sample size contributed to the higher values in fish fed 40% MBM. While MBM concentrations up to 45% did not adversely affect whole animal responses, nitrogen digestibility decreased. In those situations where nitrogen discharges are limited, MBM concentrations below 35% might be appropriate.

Production of hybrid striped bass is one of the newest and most rapidly growing aquaculture industries in the US. Based on these data, MBM can serve as the primary source of crude protein and essential amino acids in diets fed to this hybrid. Based on the cost of ingredients, a change from current formulations to those containing high levels of meat and bone meal could reduce feed costs by approximately 20% opening a new market for MBM and helping this new aquaculture industry grow.

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Table 1. Proximate composition of the meat and bone meal fed to hybrid striped bass. Crude protein, ash, and fat are expressed on an as-is basis. Values are means of duplicate evaluations.

Crude Protein	52.4%
Ash	22.6%
Fat	11.0%
Moisture	3.4%

Table 2. Composition of diets fed to hybrid striped bass for evaluation of meat and bone meal.

	Conce	entration o	of meat a	nd bone r	<u>neal (%, c</u>	dry matte	<u>r basis)</u>	
Ingredient	0	15	20	25	30	35	40	45
Soybean meal	54.34	41.76	39.35	36.93	34.52	32.10	29.69	27.28
Fish meal, menhaden	16.00	15.01	12.96	10.92	8.88	6.84	4.79	2.75
Meat and bone meal	0.00	15.00	20.00	25.00	30.00	35.00	40.00	45.00
Corn grain	20.30	20.30	20.30	20.30	20.30	20.30	20.30	20.30
Fish oil, menhaden	6.78	5.08	4.68	4.28	3.88	3.48	3.07	2.67
Dicalcium phosphate	1.31	1.57	1.40	1.24	1.07	0.90	0.73	0.57
Ascorbic acid ^a	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Vitamin premix ^b	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Mineral premix ^b	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

^a Ascorbic acid supplied as L-ascorbyl 2-polyphosphate (Roche Inc., Nutley, NJ).

Table 3. Mean feed consumption (g/replicate), weight gain (% increase), feed conversion ratio (FCR) and protein efficiency ratio (PER) of hybrid striped bass fed varying amounts of meat and hone meal

_	varying amounts	of meat and bone mea	11.			
	Concentration	Feed consumption	Weight gain	FCR ^a	PER ^b	
	0	1372.9b	109a	1.4ab	1.7	
	15	1594.4ab	140b	1.3ab	1.8	
	20	1444.7b	136b	1.2b	2.0	
	25	1608.0ab	139b	1.3ab	1.8	
	30	1825.2a	136b	1.5a	1.6	
	35	1633.7ab	137b	1.4ab	1.8	
	40	1639.5ab	140b	1.3ab	1.8	
	45	1619.2ab	138b	1.3ab	1.9	
	Pooled SEM	74.5	6.3	0.05	0.1	

^b Vitamin and mineral premixes were the same as reported by Twibell et al. (2000).

^a Feed conversion ratio = g dry feed fed/g wet weight gain.
^b Protein efficiency ratio = g wet weight gain/g protein fed.

Table 4. Mean intraperitoneal fat (IPF), hepatosomatic index (HSI), and liver lipid concentrations (% dry matter) of hybrid striped bass fed graded additions of meat and bone meal.

Concentration	$\mathrm{IPF}^{\mathrm{a}}$	HSI ^b	Liver lipid	
0	2.02	0.96	54.28	
15	1.63	0.94	49.97	
20	1.85	0.92	53.15	
25	1.74	0.91	53.54	
30	1.92	1.00	50.98	
35	1.40	1.26	50.02	
40	1.67	0.95	56.01	
45	1.29	0.91	57.31	
Pooled SEM	0.2	0.1	3.4	

^a Intraperitoneal fat = weight of lipid removed from viscera/wet weight of fish.

^b Hepatosomatic index = wet weight of liver/wet weight of fish.

Table 5. Mean proximate composition of whole hybrid striped bass fed graded additions of meat and bone meal. Crude protein, lipid and ash concentrations expressed as a percentage of dry matter.

Concentration	Moisture	Crude protein	Lipid	Ash
0	63.4	57.5	33.3	13.9
15	67.8	54.7	28.1	14.4
20	68.0	58.3	28.9	13.9
25	66.5	55.3	28.5	13.7
30	67.6	56.4	28.3	13.6
35	66.7	57.1	28.6	13.9
40	64.6	57.0	29.1	14.2
45	66.2	58.5	27.6	14.4
Pooled SEM	2.2	1.4	1.3	0.8

Table 6. Mean proximate composition of fillets from hybrid striped bass fed graded additions of meat and bone meal. Crude protein, lipid and ash concentrations expressed as a percentage of dry matter.

Concentration	Moisture	Crude Protein	Lipid	Ash
0	77.0	82.1	9.8	7.6
15	76.6	80.2	14.3	7.2
20	76.3	80.3	12.5	7.6
25	76.6	81.0	12.4	7.4
30	76.7	80.4	14.4	7.3
35	76.7	80.3	14.0	7.4
40	76.8	80.9	12.7	7.3
45	76.7	81.9	10.9	7.6
Pooled SEM	0.2	1.1	1.6	0.2

Table 7. Mean apparent crude protein digestibility and phosphorus availability of hybrid striped bass fed graded additions of meat and bone meal. Values in the same column with different letters are significantly different.

Concentration	Crude Protein	Phosphorus	
0	81.0a	43.0	
15	84.3a	37.5	
20	84.3a	36.1	
25	80.6a	40.9	
30	80.8a	44.4	
35	74.0a	38.9	
40	81.2a	40.0	
45	68.6b	34.9	
Pooled SEM	2.6	4.4	

same column with different superscripts were significantly different. Table 8. Mean apparent amino acid availability from hybrid striped bass fed graded additions of meat and bone meal. Values in the

Concentration	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
0	90.2a	81.7b	85.5a	88.6a	85.7a	89.4a	87.1a	82.5ab	73.2a	84.2a
15	91.3a	87.9a	83.2a	85.0ab	88.8a	85.3a	87.6a	80.7ab	77.1a	81.7a
20	91.7a	89.7a	85.5a	87.2ab	89.4a	85.4a	88.8a	81.5ab	79.0a	84.7a
25	89.4a	85.2ab	81.4a	83.0b	84.5a	83.6a	84.8a	78.0b	73.5a	80.3a
30	90.2a	85.3ab	81.2a	84.1ab	87.5a	83.9a	85.6a	79.6ab	73.4a	82.1a
35	74.9b	70.3c	62.4b	66.4c	61.1b	65.6b	68.2b	60.8c	48.0b	63.0b
40	92.4a	90.0a	86.2a	88.6a	90.4a	90.2a	89.9a	85.1a	80.8a	86.0a
45	70.8c	64.2d	65.0b	61.1d	63.5b	58.1c	69.5b	54.3d	48.0b	59.8b
Pooled SEM	1.0	1.3	1.5	1.2	1.9	1.8	1.6	1.5	2.2	1.5