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Evaluation of Plant and Animal Source Proteins for Replacement of Fish Meal in Practical Diets for the Largemouth Bass *Micropterus salmoides*

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Abstract

Two feeding trials were conducted with juvenile largemouth bass *Micropterus salmoides* to evaluate alternative plant and animal source proteins for their ability to replace fish meal in practical diets. The first trial was designed to identify the most promising candidates. The second trial was conducted to evaluate how much of the fish meal could be replaced by those candidates. In Study 1, feed-trained largemouth bass (3.1 ± 0.7 g) were randomly stocked into 18 114-L glass aquaria at 25 fish per aquarium. Fish were fed one of six experimental diets, each containing approximately 38% crude protein and 10% crude lipid, to apparent satiation twice daily. The control diet (CTL) contained 30% fish meal and 34.5% soybean meal. Diets 2–6 each contained 15% fish meal and at least 34.5% soybean meal with the remainder of the protein made up of either meat and bone meal (MBM), soybean meal (SBM), poultry by-product meal (PBM), a 50/50 mixture of blood meal and corn gluten meal (BM/CG), or 50/50 mixture of hydrolyzed feather meal and soybean meal (FM/SBM). There were three replicate aquaria per dietary treatment. After 12 wk, there was no significant difference ($P > 0.05$) among treatments in survival which averaged 92% overall. Only fish fed the PBM or BM/CG diets had average individual weights and feed conversion efficiencies that were not significantly different ($P > 0.05$) from the control diet (CTL). In Study 2, the formulation of the control diet (CTL) remained the same. Based on their performance in the first trial, PBM and BM/CG were chosen to now replace 75 or 100% of the fish meal. Fish were stocked at an average weight of 6.9 ± 1.7 g. After 11 wk, fish fed diets containing the BM/CG mixture at both levels were significantly smaller ($\underline{P} \leq 0.05$) than fish fed other diets and at 100% replacement survival was reduced. Fish fed diets containing poultry meal as the primary protein source performed as well as those fed the control diet (CTL). It appears that PBM can completely replace fish meal in diets for juvenile largemouth bass without adverse effects on growth, feed efficiency, or body composition.

The largemouth bass *Micropterus salmoides* is the largest member of the North American sunfish family *Centrarchidae*. Production in the U.S. likely exceeds 500,000 kg/yr and fish are marketed both for sportfish stocking and as a live food fish in ethnic Asian markets, where they are highly regarded (Tidwell et al. 2002). Largemouth bass are strict carnivores and most commercial production has historically relied on trout diets, which are expensive and contain high levels of fish meal (> 30%). This is mostly based on availability, rather than nutritional suitability; as most of the nutritional requirements of largemouth bass are not yet known (Tidwell et al. 1996). It is important to develop cost-effective largemouth bass diets for more profitable expansion of their production. Reducing the amount of fish meal in diet formulations, without reducing fish performance, could have a positive impact on the profitability of commercial bass production.

Fish meal is one of the most expensive ingredients of aquaculture diets. It is estimated that more than 50% of the variable costs of bass production are feed costs (Woods 1999), so profitability of production is significantly influenced by feed. Due to its relatively high cost, cost variability, and growing environmental concerns about harvesting wild fish to produce fish meal, it is desirable to replace fish meal with less expensive protein sources.

A number of animal and plant source proteins have been evaluated for fish meal replacement in diets for a number of different species. In freshwater omnivores, such as tilapia *Oreochromis* sp. and channel catfish *Ictalurus punctatus*, fish meal can be completely replaced by plant protein sources such as soybean meal (Lovell 1998). In carnivorous species, such as hybrid striped bass *Morone chrysops* X *M. saxatilis* and rainbow trout *Oncorhynchus mykiss*, diets based completely on plant-proteins have not been as successful (Webster et al. 1999). In general, animal protein sources perform better than plant protein sources in diets for carnivorous species (Hardy 1998). However, each of these plant and animal source proteins appears to have some deficiency or limitation when compared to fish meal. Combining plant and animal source proteins with complimentary amino acid profiles has shown promise (Webster et al. 1999). For example, corn gluten may be deficient in lysine while blood meal contains high levels of lysine, so their combination might improve fish performance more than feeding them singularly.

The objectives of the present research were to evaluate the potential of meat and bone meal, soybean meal, poultry by-product meal, a 50/50 mixture of blood meal and corn gluten, and a 50/50 mixture of feather meal and soybean meal as potential candidates for fish-meal replacement (Study 1) in practical diets for largemouth bass; Study 2 was designed to determine what percentage of the total fish meal could be replaced by the most promising protein sources identified in Study 1.

Materials and Methods

Study 1

In the first experiment, six experimental diets containing different animal and plant protein sources were formulated to be isonitrogenous and isocaloric. The control diet (CTL) contained 30% fish meal and 34.5% soybean meal. Diets 2–6 each contained 15% fish meal and at least 34.5% soybean meal with the remainder of the protein made up of either: meat and bone meal (MBM), soybean meal (SBM), poultry by-product meal (PBM), a 50/50 mixture of blood meal and corn gluten meal (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM) (Table 1).

Table 1. *Study 1—Formulated composition of six diets containing different plant and animal protein sources. The control (CTL) was based on fish meal (30%). In the experimental diets 50% of the fish meal was replaced with either meat and bone meal (MBM), additional soybean meal (SBM), poultry by-product meal (PBM), a 50/50 mixture of blood meal and corn gluten (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM).*

	Diets					
Ingredient	CTL	MBM	SBM	PBM	BM/CG	SBM/FM
Fish Meal	30.0	15.0	15.0	15.0	15.0	15.0
Soybean Meal	34.5	34.5	34.5	34.5	34.5	34.5
Wheat	20.0	20.0	20.0	20.0	20.0	20.0
Added SBM	0.0	0.0	20.6	0.0	0.0	7.4
M&BM	0.0	18.5	0.0	0.0	0.0	0.0
Poultry Meal	0.0	0.0	0.0	16.8	0.0	0.0
Blood Meal	0.0	0.0	0.0	0.0	7.6	0.0
Corn Gluten	0.0	0.0	0.0	0.0	7.6	0.0
Feather Meal	0.0	0.0	0.0	0.0	0.0	7.4
Fish Oil	5.2	4.4	4.8	4.1	6.0	5.5
Cel-u-fil[1]	7.0	4.5	2.0	6.5	6.0	7.0
Others[2]	3.4	3.4	3.4	3.4	3.4	3.4

[1] *Cel-u-fil (non-nutritive bulk; United States Biochemical Corp., Cleveland, Ohio, USA.*

[2] *Others: choline chloride, 0.5; Rangen trace mineral mix F1, 0.3; vitamin C, 0.2; vitamin pre-mix Rangen No. 30, 0.4; dicalcium phosphate, 1.0, carboxymethylcellulose binder, 1.0.*

Table 2. *Study 2—Formulated composition of five diets containing different animal and plant protein sources. The control diet (CTL) was based on fish meal (30%). In the experimental diets, 75% or 100% of the fish meal was replaced with either poultry by-product meal (PBM-75 and PBM-100, respectively) or by a 50/50 mixture of blood meal and corn gluten (BM/CG-75 and BM/CG-100, respectively).*

	Diets				
Ingredient	CTL	PBM-75	PBM-100	BM/CG-75	BM/CG-100
Fish meal	30.0	7.5	0.0	7.5	0.0
Soybean meal	34.5	34.5	34.5	34.5	34.5
Wheat	20.0	20.0	20.0	20.0	20.0
Poultry meal	0.0	25.1	33.4	0.0	0.0
Blood meal	0.0	0.0	0.0	11.3	15.1
Corn gluten	0.0	0.0	0.0	11.3	15.1
Fish oil	5.2	3.5	2.9	6.4	6.8
Cel-u-fil[1]	5.9	5.1	4.8	4.6	4.1
Others[2]	3.4	3.4	3.4	3.4	3.4

[1]*Cel-u-fil (non-nutritive bulk; United States Biochemical Corp., Cleveland, Ohio, USA.*

[2]*Others: choline chloride, 0.5; Rangen trace mineral mix F1, 0.3; vitamin C, 0.2; vitamin pre-mix Rangen No. 30, 0.4; di-calcium phosphate, 1.0, carboxymethylcellulose binder, 1.0.*

Table 3. *Study 1—Analyzed nutrient composition (% as fed) of six experimental diets containing different plant and animal protein sources. The control (CTL) was based on fish meal (30%). In the experimental diets, 50% of the fish meal was replaced with either meat and bone meal (MBM), additional soybean meal (SBM), poultry by-product meal (PBM), and 50/50 mixture of blood meal and corn gluten (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM).*

	Diets					
Analyzed Composition	CTL	MBM	SBM	PBM	BM/CG	SBM/FM
Moisture	12.0	11.5	14.6	11.1	10.5	10.9
Protein	37.2	38.1	37.4	40.1	40.5	38.7
Ash	8.5	10.4	7.2	8.9	6.4	6.8
Lipid	7.7	7.4	6.8	8.1	7.2	7.8
NFE	28.4	27.5	30.5	25.2	30.5	29.7
Fiber	6.2	5.1	3.5	6.5	4.9	6.1
Energy (kcal/g diet)	33.2	32.9	33.3	33.4	34.8	34.4
E:P (kcal/g protein)	8.9	8.6	8.9	8.3	8.6	8.9

Table 4. *Study 2—Analyzed nutrient composition (% as fed) of five experimental diets containing different plant and animal protein sources. The control diet (CTL) was based on fish meal (30%). In the experimental diets, 75% or 100% of the fishmeal was replaced with either poultry by-product meal (PBM-75 and PBM-100, respectively) or by a 50/50 mixture of blood meal and corn gluten (BM/CG-75 and BM/CG-100, respectively).*

	Diets				
Analyzed Composition	CTL	PBM-75	PBM-100	BM/CG-75	BM/CG-100
Moisture (%)	7.3	7.0	8.8	8.7	7.2
Protein (%)	40.1	42.5	42.9	42.1	42.2
Ash (%)	9.1	8.8	9.1	5.8	4.9
Lipid (%)	8.2	8.2	8.0	8.5	8.1
NFE (%)	28.4	26.5	25.7	31.0	33.3
Fiber (%)	7.0	7.1	5.5	3.9	4.3
Energy (kcal/g diet)	34.8	35.0	34.6	36.9	37.5
E:P (kcal/g protein)	8.7	8.2	8.1	8.8	8.9

TABLE 5. *Study 1—Concentrations of essential amino acids and cystine (% protein) of six diets containing different plant and animal protein sources. The control (CTL) was based on fish meal (30%). In the experimental diets, 50% of the fish meal was replaced with either: meat and bone meal (MBM), additional soybean meal (SBM), poultry by-product meal (PBM), and 50/50 mixture of blood meal and corn gluten (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM).*

	Diets					
Amino Acid	CTL	MBM	SBM	PBM	BM/CG	SBM/FM
Arginine	6.5	6.6	6.9	6.9	5.8	6.8
Cystine	1.3	1.2	1.4	1.6	1.3	1.7
Histidine	2.7	2.5	2.7	2.4	3.0	2.4
Isoleucine	4.5	4.2	4.6	4.4	4.0	4.6
Leucine	7.6	7.3	7.6	7.5	9.1	7.7
Lysine	6.7	6.1	6.4	6.1	6.0	5.7
Methionine	2.0	1.6	1.6	1.6	1.7	1.4
Phenylalanine	4.7	4.4	4.8	4.6	5.0	4.8
Threonine	4.7	4.4	4.5	4.5	4.4	4.7
Valine	5.1	5.0	5.1	5.3	5.3	5.5
Met + Cys	3.3	2.8	3.0	3.2	3.0	3.1

Study 2

In the second experiment, the formulation of the control diet (CTL) remained essentially the same. Based on their performance in the first trial, PBM and a 50/50 mixture of BM and CGM were chosen to be evaluated for further reductions in fish meal concentrations. In this experiment, 75% or 100% of the fish meal in the CTL diet was replaced with the same amount of protein from either PBM (PBM-75 and PBM 100, respectively) or a 50/50 mixture of BM and CGM (BM/CG-75 and BM/CG-100, respectively) (Table 2).

Experimental Diets

All diet ingredients were obtained from a commercial feed manufacturer (Rangen Inc., Houston, Texas, USA). In both trials, experimental diets were individually manufactured on site at Kentucky State University. Dry ingredients were mixed in a Hobart mixer (Hobart Inc., Troy, Ohio, USA) for 30 min, and then mixed with water to obtain a 25% moisture level. Diets were then passed through a mechanical extruder with a 12-3/8-mm die (Hobart Inc., Troy, Ohio, USA) to form “spaghetti-like” strands and dried at 38 C for 12 h using a convection oven (Grieve Corporation, Round Lake, Illinois, USA). Once dried to ≤ 10% moisture, strands were chopped into pellets using a grinder (Glen Mills, Clifton, New Jersey, USA), and sieved to approximately 3-mm pellets using a #10 sieve (HACH, Loveland, Colorado, USA). Diets were stored frozen (-20 C) until fed.

Samples of diets were submitted to a commercial laboratory (Eurofins Scientific, Inc., Woodson-Tenent Laboratories Division, Des Moines, Iowa, USA) for proximate analysis (Study 1, Table 3); (Study 2, Table 4) and amino acid analysis (Study 1, Table 5); (Study 2, Table 6). The difference in amino acid composition of the control diets between Study 1 and 2 is likely a result of variations in batches of diet ingredients. Moisture content of diets was determined by drying feeds at 135 C for 2 h, AOAC official method 930.15 (AOAC 1990). Lipid content was determined by ether extraction (gravimetric method), AOAC official method 954.02 (AOAC 1990). Protein content of diets was determined by combustion method, AOAC official method 990.03 (AOAC 1990). Fiber content of diets was determined by ceramic fiber filter method, AOAC official method 962.09 (AOAC 1990). Ash content was determined by AOAC official method 942.05 (AOAC 1990). Amino acid content of diets was determined by two methods: 1) performic acid oxidation with acid hydrolysis sodium metabisulfite method, AOAC official method 994.12, and 2) protein efficiency

Table 6. *Study 2—Concentrations of essential amino acids and cystine (% protein) of five diets containing different animal and plant source proteins. The control diet (CTL) was based on fish meal (30%). In the experimental diets, 75% or 100% of the fishmeal was replaced with either: poultry by-product meal (PBM-75 and PBM-100, respectively) or by a 50/50 mixture of blood meal and corn gluten (BM/CG-75 and BM/CG-100, respectively).*

	Diets				
Amino Acid	CTL	PBM-75	PBM-100	BM/CG-75	BM/CG-100
Argenine	5.1	5.6	5.5	5.1	5.4
Cystine	0.9	1.0	0.9	1.2	1.4
Histidine	2.0	1.9	1.8	2.8	3.3
Isoleucine	2.8	2.8	2.6	2.9	3.9
Leucine	5.9	6.0	5.6	9.1	11.4
Lysine	5.2	5.0	4.7	5.4	5.7
Methionine	1.5	1.4	1.3	1.4	1.5
Phenylalanine	3.5	3.6	3.4	4.9	5.7
Threonine	3.8	3.8	3.6	4.2	4.6
Valine	3.2	3.2	3.0	4.1	5.4
Met + Cys	2.4	2.4	2.3	2.6	2.9

calculation ratio, AOAC official method 982.30 (AOAC 1990).

Experimental System and Fish

In Study 1, feed-trained largemouth bass (3.1 ± 0.7 g), approximately 2 mo of age, were randomly stocked at 25 fish per aquarium into 18 114-L glass aquaria. In Study 2, initial fish weight was 6.9 ± 1.7 g for fish approximately 4 mo of age, stocked into 15 aquaria at 25 fish per aquarium. In both studies, fish were fed twice daily to apparent satiation. In Study 1, the duration of the experiment was 12 wk. For Study 2, the study period was 11 wk. Three replicate aquaria were randomly assigned to each dietary treatment. The experimental system was a pump driven recirculation system using a common biofilter (Aquaculture Systems Technology, Jefferson, Louisiana, USA) so that all treatments had similar water quality. Water temperature and dissolved oxygen were measured twice daily using a YSI 85 DO meter (YSI Company, Yellow Springs, Colorado, USA). Total ammonia, nitrite, pH and alkalinity were measured three times per week using a HACH DR/2500 spectrophotometer (HACH, Loveland, Colorado, USA).

Sampling and Statistical Analysis

At the conclusion of each experiment, all fish in each aquarium were removed, weighed and measured for total length. Three randomly selected fish from each aquarium were homogenized in a blender and frozen for subsequent analysis of whole body proximate composition (percent protein, lipid, ash, and moisture) in a commercial laboratory (Woodson-Tenant Laboratories Inc., Dayton, Ohio, USA). Growth performance parameters were calculated as follows: specific growth rate (SGR, % body wt/d) was calculated from 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. Apparent protein utilization APU = [(final body protein - initial body protein) X 100] / total protein fed. Feed conversion ratio was calculated as FCR = total diet fed (g) / total wet weight gain (g). Hepatosomatic index was calculated as HSI = [weight of liver (g) / weight of whole body (g)] x 100. Protein efficiency ratio was calculated as PER = (final body weight – initial body weight) / protein fed. Treatments were statistically compared by ANOVA ($P \leq 0.05$) using Statistix version 7.0 (Statistix Analytical Software 2000). If significant differences were found among treatments,

TABLE 7. *Study 1—Mean (± SD) of average final weight, specific growth rate (SGR), survival, feed conversion ratio (FCR), amount of diet fed, apparent protein utilization (APU), protein efficiency ratio (PER), and hepatosomatic index (HSI) of juvenile largemouth bass fed diets containing different protein sources. The control (CTL) was based on fish meal (30%). In the experimental diets 50% of the fish meal was replaced with either: meat and bone meal (MBM), additional soybean meal (SBM), poultry by-product meal (PBM), a 50/50 mixture of blood meal and corn gluten (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM). Significant differences (*P *< 0.05) are indicated by different letters within rows.*

	Diets					
Variable	CTL	MBM	SBM	PBM	BM/CG	SBM/FM
Ave final wt (g)	44.7 ± 3.4 a	38.5 ± 1.6 bc	30.8 ± 3.3 d	42.7 ± 2.7 ab	42.7 ± 0.6 ab	37.5 ± 1.8c
SGR (%/day)	3.3 ± 0.1 a	3.1 ± 0.1 bc	2.8 ± 0.1 d	3.2 ± 0.1 ab	3.2 ± 0.0 ab	3.1 ± 0.1 c
Survival (%)	96.0 ± 4.0 a	92.0 ± 10.6 a	94.7 ± 6.1 a	96.0 ± 0.0 a	89.3 ± 9.2 a	90.7 ± 2.3 a
FCR	1.5 ± 0.0 d	1.7 ± 0.1 c	2.0 ± 0.1 a	1.5 ± 0.0 d	1.6 ± 0.1 d	1.9 ± 0.1 b
Total feed intake (kg)	1.5 ± 0.1 a	1.4 ± 0.1 a	1.3 ± 0.1 a	1.5 ± 0.1 a	1.4 ± 0.1 a	1.4 ± 0.1 a
APU (%)	30.6 ± 1.5 a	27.3 ± 0.9b	23.8 ± 0.9 d	27.7 ± 0.9b	26.9 ± 2.5 bc	24.2 ± 2.1 cd
PER	1.8 ± 0.1 a	1.7 ± 0.2 ab	1.4 ± 0.1 c	1.7 ± 0.1 ab	1.8 ± 0.2 a	1.6 ± 0.1 bc
HSI (%)	1.7 ± 0.3 a	1.4 ± 0.1 c	1.7 ± 0.0 ab	1.4 ± 0.0 c	1.4 ± 0.2 c	1.5 ± 0.1 bc

TABLE 8. *Study 1—Means of whole body proximate composition of juvenile largemouth bass fed six diets containing different plant and animal protein sources. The control (CTL) was based on fish meal (30%). In the experimental diets, 50% of the fish meal was replaced with either: meat and bone meal (MBM), additional soybean meal (SBM), poultry by-product meal (PBM), a 50/50 mixture of blood meal and corn gluten (BM/CG), or a 50/50 mixture of soybean meal and feather meal (SBM/FM). Values represent means of three replications per treatment. There were no significant differences (*P *> 0.05) among treatments.*

	Diets					
Analysis	CTL	MBM	SBM	PBM	BM/CG	SBM/FM
Moisture (%)	71.3 ± 0.8	71.3 ± 1.0	73.4 ± 1.9	70.6 ± 1.3	71.1 ± 1.4	71.9 ± 0.6
Lipid (%)	7.3 ± 0.7	6.6 ± 0.8	5.4 ± 1.0	7.2 ± 1.1	6.8 ± 0.6	6.5 ± 0.7
Protein (%)	17.2 ± 0.6	17.6 ± 0.1	17.7 ± 0.7	17.3 ± 0.3	17.0 ± 0.6	17.4 ± 0.5
Fiber (%)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.20 ± 0.1
Ash (%)	3.7 ± 0.4	4.0 ± 0.2	3.7 ± 0.5	3.8 ± 0.5	3.0 ± 0.6	3.6 ± 0.3

treatment means were separated using Fisher's Least Significant Difference method (Steel and Torrie 1980). All percentage and ratio data were arc sin transformed prior to analysis (Zar 1984). However, data are presented untransformed to facilitate comparisons.

Results and Discussion

Study 1

Over the 12-wk feeding period, water quality variables averaged (± SD) as follows: water temperature, 25.6 ± 1.0 C; dissolved oxygen, 6.3 ± 0.6 mg/L; total ammonia-N, 0.5 ± 0.2 mg/L; un-ionized ammonia-N, 0.02 ± 0.01 mg/L; nitrite-N, 7.8 ± 0.3 mg/L; pH, 7.8 ± 0.3; alkalinity, 87.3 ± 22.7 mg/L, and represented conditions well suited for largemouth bass growth and health (Tidwell et al. 2003).

After 12 wk, there was no significant difference in survival among fish fed the six diets, which averaged 92% overall (Table 7). Whole body proximate composition did not differ among fish fed any of the six diets (Table 8). Final average weight, SGR, survival, PER, and FCR of largemouth bass juveniles fed diets in which 50% of the fish meal had been replaced by poultry by-product meal (PBM) or a 50/50 mixture of blood meal and corn gluten (BM/CG) were not significantly different than those fed the control diet. APU and HSI of fish fed the PBM and BM/CG diets were significantly lower than those fed the CTL diet.

Fish fed diets in which 50% of the fish meal was replaced by soybean meal (SBM) had significantly lower final weights, SGR, APU, PER, and higher FCR than other treatments. SGR and FCR in fish fed diets in which 50% of the fish meal was replaced by MBM or a 50/50 mixture of SBM/FM were significantly reduced compared to those fish fed the CTL diet, but were significantly better than those of fish fed the SBM diet. Fish fed the CTL diet had a significantly higher APU than fish fed any other experimental diet. The APU for fish fed the MBM diet was significantly lower than those fed the SBM diet but was not significantly different from those fed the SBM/FM diet. The PER of fish fed the MBM diet was greater than those fed the SBM diet but not significantly different from the CTL. Fish fed the SBM/FM diet had significantly lower PER than those fed the CTL, but not significantly different than those fed the SBM diet. The hepatosomatic index of fish fed the MBM, PBM, BM/CG, and SBM/FM diets was significantly lower than those fed the CTL diet. The HSI of fish fed the SBM diet was not significantly different from fish fed either the CTL or SBM/FM.

Decreased growth as fish meal was replaced by soybean meal has been reported in a number of species including rainbow trout (Yamomoto and Akiyoma 1991) and gray mullet *Mugil cephalus* (El-Sayed 1994). Although some salmonids find soybean meal highly unpalatable (Hardy 1998), the reduced performance of largemouth bass fed the SBM diet does not appear to be due to palatability problems. The amount of SBM diet consumed was not significantly different from the control diet or other experimental diets. Comparison of the amino acid profile with the CTL diet (Table 5) does not indicate differences of sufficient magnitude to indicate problems. The methionine level of the SBM diet is slightly lower than the CTL, as is the combined methionine and cystine level. However, these differences are not large, and levels in the SBM are very similar to those in the PBM or BM/CG diets, which performed well. Since these results indicate that adverse effects of SBM-based diets do not seem to be caused by palatability or amino acid imbalances, it is likely that anti-nutritional factors are involved (Hertrampf and Piedad-Pascual 2000). Anti-nutritional factors associated with soybean meal are due to the presence of trypsin inhibitors which decrease protein digestibility (Hertrampf and Piedad-Pascual 2000). In sunshine bass, Webster et al. (1999) found that when soybean meal was used as a primary protein source, 15% fish meal was required in the diet formulation for optimal growth. These results indicate that fishmeal levels may need to be maintained at levels > 15% when high levels of soybean meal (> 30%) are used in largemouth bass diets.

The SBM/FM diet was included based on the premise that combinations of plant and animal proteins might be complementary. However, the lysine concentration was lower than those of the other diets (Table 5), and below the 6% of protein recommended by Coyle et al. (2000). Also, the methionine and cystine concentration was relatively low (2.3% of protein). Requirements for largemouth bass are not currently known, but the requirements for other species range from 2.3% in channel catfish *Ictalurus punctatus* to 4.0% in chinook salmon *Oncorhychus tschawytscha* (Lovell 1998).

Meat and bone meal has been reported to be an excellent protein source in several carnivores, such as yellowtail *Serida quinqueradiata* (Shimeno et al. 1993). However, in this study, SGR and FCR of bass fed diets in which 50% of the fish meal was replaced with MBM were significantly reduced compared to the CTL diet. This is in agreement with Webster et al. (1999) who reported that complete replacement of FM with MBM in sunshine bass diets caused reduction of growth. Kureshy et al. (2000) reported that the digestibility of MBM varies and is generally lower than fish meal. They reported that diets with high levels of MBM were relatively unpalatable to red drum *Sciaenops ocellatus*. For largemouth bass, palatability does not appear to be a problem as the amount of MBM diet consumed was not significantly different from other diets. Although amino acid content of the diets does not appear to be a problem, actual availability of the amino acids may be the issue. McGoogan and Reigh (1996) reported that in red

Table 9. *Study 2—Means (± SD) of average final weight, specific growth rate (SGR), survival, feed conversion ratio (FCR), amount of diet fed, apparent protein utilization (APU), protein efficiency ratio (PER), and hepatosomatic index (HSI) for juvenile largemouth bass fed diets containing different levels of animal and plant protein sources. The control diet (CTL) was based on fish meal (30%). In the experimental diets, 75% or 100% of the fishmeal was replaced with either: poultry by-product meal (PBM-75 and PBM-100, respectively) or by a 50/50 mixture of blood meal and corn gluten (BM/CG-75 and BM/CG-100, respectively). Values represent means of three replications per treatment. Significant differences (*P *< 0.05) are indicated by different letters within rows.*

	Diets				
Variable	CTL	PBM-75	PBM-100	BM/CG-75	BM/CG-100
Ave harvest weight (g)	57.3 ± 0.7 a	57.6 ± 1.7 a	57.6 ± 1.3 a	52.3 ± 1.1 b	41.2 ± 2.45 c
SGR	2.7 ± 0.0 a	2.8 ± 0.0 a	2.8 ± 0.0 a	2.6 ± 0.0 b	2.3 ± 0.1 c
Survival	96.0 ± 0.0 a	86.7 ± 12.9 a	86.7 ± 8.3 a	86.7 ± 12.2 a	30.7 ± 8.3 b
FCR	1.6 ± 0.1 b	1.6 ± 0.2 b	1.7 ± 0.1 b	1.9 ± 0.2 b	6.5 ± 2.2 a
Total feed intake (kg))	1.9 ± 0.1 a	1.7 ± 0.1 a	1.8 ± 0.1 a	1.8 ± 0.1 a	0.9 ± 0.1 b
APU	25.2 ± 1.3 a	24.2 ± 2.6 a	22.1 ± 2.2 ab	19.6 ± 2.6 b	5.3 ± 2.6 c
PER	1.7 ± 0.1 bc	1.8 ± 0.1 b	1.6 ± 0.0 bc	1.5 ± 0.2 c	2.3 ± 0.2 a
HSI	1.6 ± 0.1 a	1.7 ± 0.1 a	1.5 ± 0.2 a	1.8 ± 0.2 a	1.9 ± 0.3 a

Table 10. *Study 2—Means (± SD) of whole-body tissues analyzed for moisture, lipid, protein, and ash (wet-weight basis) of juvenile largemouth bass[1] fed diets containing different levels of animal and plant protein sources. The control diet (CTL) was based on fish meal (30%). In the experimental diets, 75% or 100% of the fishmeal was replaced with either: poultry by-product meal (PBM-75 and PBM-100, respectively) or by a 50/50 mixture of blood meal and corn gluten (BM/CG-75 and BM/CG-100, respectively). Values represent means of three replications per treatment. There were no significant differences (*P *> 0.05) among treatments.*

	Diets				
Analysis	CTL	PBM-75	PBM-100	BM/CG-75	BM/CG-100
Moisture (%)	73.8 ± 0.7	74.1 ± 0.5	73.8 ± 0.2	73.2 ± 0.9	74.1 ± 0.7
Lipid (%)	6.8 ± 0.0	6.8 ± 0.4	7.2 ± 0.4	7.1 ± 0.2	7.0 ± 0.6
Protein (%)	16.1 ± 0.5	16.6 ± 0.6	16.5 ± 0.3	16.1 ± 0.3	15.8 ± 0.1
Ash (%)	3.5 ± 0.4	3.7 ± 0.7	3.2 ± 0.5	3.0 ± 0.3	3.2 ± 0.8

drum, the apparent digestibility coefficient for crude protein of meat and bone meal was 23% lower than for menhaden fish meal. Allan et al. (2000) reported that in silver perch *Bidyanus bidyanus*, meat and bone meals (lamb and beef) had lower nitrogen and lysine availability coefficients than any other animal meals tested (e.g., fish meal, blood meal, poultry offal meal, and feather meal).

In summary, the data from Study 1 indicated that poultry by-product meal and a mixture of blood meal and corn gluten are the most promising protein sources evaluated for fish meal replacement in largemouth bass diets. These ingredients were further evaluated in Study 2 to determine maximum levels of fish meal replacement by either of these ingredients.

Study 2

Over the 11-wk feeding period, water quality variables averaged (± SD) as follows: water temperature, 26.3 ± 0.6 C; dissolved oxygen, 6.2 ± 0.7 mg/L; total ammonia-N, 0.65 ± 0.29 mg/L; un-ionized ammonia-N, 0.04 ± 0.03 mg/L; nitrite-N, 0.10 ± 0.06 mg/L; pH 8.0 ± 0.2; alkalinity, 100 ± 33 mg/L, and represented conditions well suited for largemouth bass growth and health (Tidwell et al. 2003).

After 11 wk, final weights of fish fed diets containing the BM/CG mixture at 75% or

100% were significantly lower than fish fed the CTL or PBM diets (PM-75 and PM-100) (Table 9). Even though average final weight was significantly decreased in fish fed the BM/CG-75 diet, survival and FCR were not significantly different than those of the CTL. The average final weight of fish fed BM/CG-75 was only 9% less than fish fed the control diet (CTL). The APU of fish fed BM/CG-75 was significantly lower than in fish fed the CTL, while the PER was not significantly different. Comparison of amino acid profiles of diets indicates no significant difference between the BM/CG-75 and the CTL diet. There was also no significant impact on body composition of fish fed the different diets (Table 10).

When fish meal was completely replaced by BM/CG, average final weight, APU, PER, and survival were significantly decreased compared to all other diets, while FCR was significantly increased. This appears to be largely related to reduced palatability. Feed consumption of fish fed the BM/CG-100 diet was significantly lower than in those fed the other diets, which were not significantly different from each other. It has been shown that blood meal negatively affects diet palatability for some fish species (Lovell 1998). In fish fed the BM/CG-100 diet, some fish refused to accept the diet and eventually became susceptible to cannibalism by those individuals that would accept the diet and became physically larger. In this study, feed consumption was not negatively influenced by blood meal inclusion at a rate of 11% of the diet (BM/CG-75). However, when blood meal was included at 15% of the diet (BM/CG-100), feed consumption was decreased by 50%. These data agree with Hertrampf and Piedad-Pascual (2000) who recommended a maximum inclusion rate of 10% for blood meal.

Fish fed diets in which 75% or 100% of the fish meal was replaced by PBM (PBM-75 and PBM-100) were not significantly different from fish fed the CTL diet in terms of average final weight, SGR, survival, FCR, APU, PER, or HSI (Table 7). While lysine and methionine + cystine levels were slightly lower in PBM than CTL diet, the difference did not appear to be of sufficient magnitude to impact survival or growth. Previous studies have found that PBM could replace 40% of the fish meal in diets for African catfish *Clarias gariepinus* (Abdel-Warith et al. 2001), 67% of fish meal for red drum (Kureshy et al. 2000), or 100% replacement in diets for channel catfish (Li et al. 2002) and sunshine bass (Webster et al. 1999).

Conclusion

These results indicate that among the animal and plant source proteins evaluated, poultry by-product meal and a mixture of blood meal and corn gluten show the best potential for substituting for fish meal in diets for largemouth bass. The blood meal/corn gluten mixture appears to be able to satisfactorily substitute for 50% of the fish meal. When substituting the mixture for 75% of the fish meal, diet palatability remains acceptable but growth was significantly reduced compared to CTL diet. However, at 100% substitution (30% of the total diet at a ratio of 1:1 blood meal/corn gluten mixture), both palatability of diet and growth of fish were significantly decreased. Poultry by-product meal appears to be able to completely replace fish meal in largemouth bass diets with no reduction in feed acceptance or fish performance.

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