Research Summary

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Project title: Evaluation of Feed Additives for Enhancing Soybean Meal Utilization in the Diet of Largemouth Bass (*Micropterus salmoides*): Effects on Production Performance, Gut Micromorphology, and Expression of Target-Genes

Largemouth bass (LMB), *Micropterus salmoides* L., is a carnivorous and highly prized sportfish whose production for food-fish markets in North America and Asia has taken off in recent years. In the United States (US), feeds for LMB typically contain relatively high concentrations of marine fish meal and fish oil, but recent nutrition studies have shown that LMB display a high tolerance to plant-protein ingredients in feeds. The optimization of cost-effective plant-based feeds therefore represents an important step forward in the expansion of LMB production in the US. The objective of this study was to assess the applicability of different dietary supplements in LMB feeds formulated to contain high levels of locally produced soybean meal.

Study setup

A feeding experiment was designed to evaluate the responses of largemouth bass (LMB) to the dietary supplementation of glycine, the prebiotic GroBiotic®-A (GBA), and a nucleotide mixture in low-fish meal, soybean meal (SBM)-based diets. The nucleotide mixture contained 5'-monophosphates of adenosine (5'-AMP), cytidine (5'-CMP), guanosine (5'-GMP), inosine (5'-IMP), and uridine (5'-UMP) in equal proportions. A total of six diets formulated to contain 42% crude protein (CP) and 12% lipid were evaluated in the study (Table 1). One of the diets (FM-C) contained 44% FM (as menhaden meal) and 0% SBM and was used as the overall control for the other five diets containing 4% FM and 50% SBM. One of the SBM-based diets (SBM-C) was not supplemented with any of the test ingredients and served as the control for the remaining diets (GLY, GBA, NCTDs, and COMB) that were supplemented either with glycine (2%), GBA (2%), the nucleotide mixture (0.15%), or all three supplements combined, respectively. The diets were produced in the Aquatic Animal Nutrition Laboratory of Kentucky State University following standard procedures and consisted of 3.2 mm sinking pellets. Finished diets were stored at – 20 ºC until used. Analyzed CP and lipid levels of the experimental diets were close to formulation levels and very similar across diets. The levels of lysine, methionine, and threonine - the three essential amino acids most likely limiting in SBM-based formulations - were similar across diets and in excess of requirement values for LMB and/or other freshwater, carnivorous teleost species.

The study followed a completely randomized experimental design. Groups of 20 feed-trained LMB (6.0 g/fish) were stocked in each of 24, 110-L glass aquaria operating as a recirculating aquaculture system with propeller-washed bead filter, biological filter, UV sterilization unit, and constant aeration provided by a central regenerative air blower through air diffusers in each aquarium. Each diet was randomly assigned to four aquaria and the fish were fed twice daily to apparent satiation for 10 weeks. A photoperiod of 12h was provided using fluorescent lighting controlled by a timer and water quality parameters were maintained within adequate ranges for LMB throughout the study.

Results

After a three-day conditioning period to the culture system and FM-C diet, active feeding was observed in all aquaria. Survival of LMB in the study averaged 98.8% and no treatment effects were observed ($P = 0.907$), indicating that all diets supported adequate health of the fish.

Treatment effects on the growth performance of LMB were observed. Final weight of the fish ranged from 48 to 61g and weight gain from 712 to 913% of initial weight. Within the SBM treatments, growth of LMB fed the GBA, NCTDs and COMB diets was outperformed by that of GLY-fed fish ($P < 0.05$) (Fig. 1A, 1B). While a similar ($P > 0.05$) growth performance of LMB was observed between GLY and SBM-C fed groups, GLY was the only dietary treatment that did not differ ($P > 0.05$) from FM-C, showing a positive effect of supplemental glycine in improving the growth performance of LMB when dietary FM is almost completely replaced by SBM.

A positive effect of supplemental glycine was also observed on the feed efficiency (FE) of LMB. Although no improvements in FE were observed within SBM-based treatments in response to the test ingredients ($P>0.05$) (Fig. 1C), no statistically significant differences were found among GLY and FM-C fed groups. The substantial improvement in FE with the additional inclusion of glycine in the SBM-based diet appears to have driven the growth rate of LMB fed the GLY diet to a level similar to the FM-C fed groups.

Whole-body proximate composition of LMB was unaffected by dietary treatment ($P>0.05$). Significantly lower activities of alkaline phosphatase alanine aminotransferase in plasma, and lower MDA concentration in liver were observed in FM-C fed groups, suggesting adverse effects of high dietary SBM on bone and amino acid metabolism, liver integrity and hepatic lipid peroxidation. Histomorphological and pathological analyses of LMB intestine at the end of the feeding trial revealed mild enteritis and transient changes in goblet cell densities across intestinal segments. Although unexpected, inflammatory responses were also found in FM-C groups.

Conclusions

Although some of the dietary additives evaluated in this study negatively affected the performance of LMB, good production values were obtained for most SBM-based diets evaluated, particularly the glycine supplemented diet (GLY). The mild adverse effects on the micromorphology of LMB intestine supplied new findings regarding the relative high tolerance of the species to dietary SBM.

In this study, FM was reduced from 44 to 4% in the diet of LMB with the inclusion of SBM at 50% and only small adjustments were necessary in the formulations. Considering the wide differences in prices of these ingredients, a substantial reduction in feed costs can be assumed with the removal of FM. This, allied with the overall high survival and FE supported by the best-performing SBM-based diets evaluated, can represent higher profit margins for LMB producers and increased utilization of SBM in feeds. Nevertheless, more studies investigating the effects of high inclusion rates of SBM in feeds on the mineral and amino acid metabolism, as well as lipid peroxidation and overall health of LMB are recommended.

Table 1. Formulation and analyzed composition of the experimental diets.

	FM-C	SBM-C	GLY	GBA	NCTDs	COMB
Ingredients			%, dry matter basis			
Glycine	0.0	0.0	2.0	0.0	0.0	2.0
GroBiotic®-A	0.0	0.0	0.0	2.0	0.0	2.0
Nucleotides	0.0	0.0	0.0	0.0	0.15	0.15
Menhaden fish meal	44.0	4.0	4.0	4.0	4.0	4.0
Poultry by-product meal	8.0	8.0	8.0	8.0	8.0	8.0
Soybean meal	0.0	50.0	50.0	50.0	50.0	50.0
Wheat flour	29.0	13.8	13.8	12.4	13.9	12.2
Menhaden oil	1.4	4.6	4.6	4.5	4.5	4.5
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix	0.6	0.6	0.6	0.6	0.6	0.6
Mineral premix	2.0	2.0	2.0	2.0	2.0	2.0
Aspartate	0.0	2.5	0.5	2.0	2.4	0.0
Others	10.0	9.5	9.5	9.5	9.5	9.6
Composition						
Crude protein	42.6	40.7	41.7	40.5	40.5	41.8
Crude fat	12.7	12.3	12.4	12.4	12.1	12.4
Crude fiber	2.2	2.1	2.1	2.4	2.5	2.7
Ash	14.1	9.8	9.9	9.9	9.8	9.7
Lysine	2.8	2.4	2.5	2.5	2.4	2.5
Methionine	1.0	0.9	0.9	0.9	0.9	0.9
Threonine	1.6	1.4	1.4	1.4	1.4	1.4

FM = fish meal; SBM = soybean meal; Gly = glycine; GBA = GroBiotic®-A; NCTDs = nucleotides; COMB = combination (Gly+GBA+NCTDs).

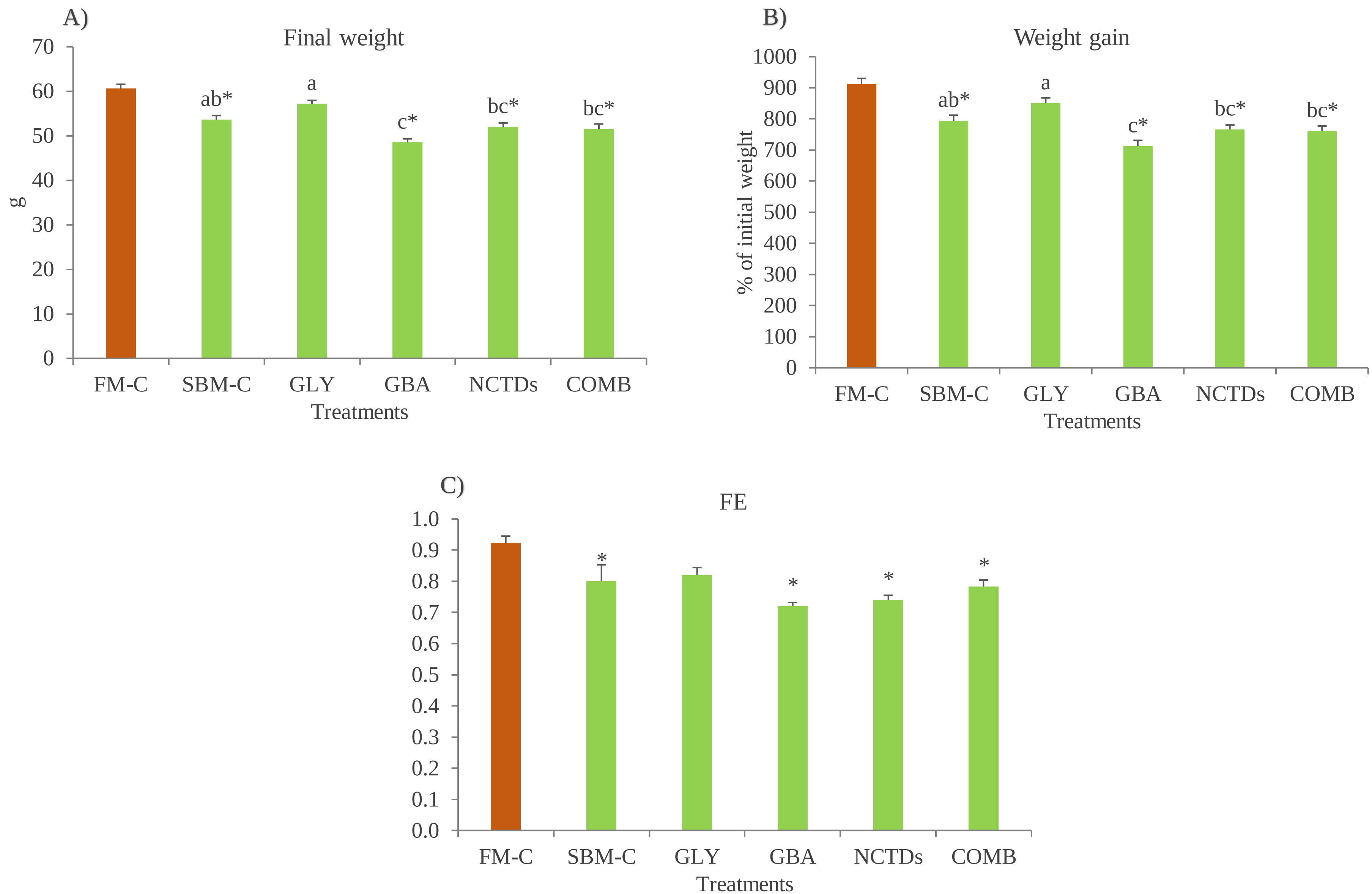

Figure 1. Responses of largemouth bass after feeding the experimental diets for 10 weeks: A) final weight; B) weight gain; C) feed efficiency (FE). When detected, statistically significant ($P < 0.05$) differences among SBM-based treatments (SBM-C, GLY, GBA, NCTDs and COMB), or between a SBM-based and the fishmeal control (FM-C) treatment, are indicated by different letters (Tukey HSD) or a * (Dunnett's test) above chart columns, respectively.