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Evaluation of exogenous carbohydrase and protease enzymes supplementation to improve the nutritional value of soybean meal-based diets for hybrid striped bass

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ABSTRACT

Supplemental enzyme additives have the potential of improving digestibility of plant-based diets for farmed fish species as already demonstrated in terrestrial monogastric animals. The objective of this study was to investigate whether supplementation of commercial enzyme additives into plant-based diets could enhance digestibility and production performance of hybrid striped bass (HSB). Two additive enzymes, a carbohydrase that degrades dietary fiber and a protease that aids in the breakdown of protein were tested. In the 14-week digestibility trial, juvenile HSB were fed diets with or without supplemental enzyme and digestibility coefficients for dietary components were determined. In the subsequent eight-week growth trial, potential effects of supplemental carbohydrase on the production performance of HSB were assessed. In both trials, the quality of the rearing water (based on temperature, dissolved oxygen, pH, total ammonia nitrogen, and nitrite nitrogen) was maintained at adequate ranges for HSB. In the digestibility trial, supplementation of carbohydrase and protease additives to the plant-based diets did not affect dry matter, protein, and energy digestibility of HSB. However, it improved the digestibility of the fiber fraction (as neutral detergent fiber), which is largely indigestible to fish as they do not produce the specific enzymes. Since no improvements in protein digestibility were observed in the digestibility evaluation, only the carbohydrase additive was evaluated in the growth trial. Fish fed diets containing over 40% plant protein ingredients (soybean meal, distiller dried grains, and wheat gluten) displayed survival, final weight, and feed efficiency similar to those fed a control diet containing 30% fish meal. Nevertheless, no significant effects of the supplemental carbohydrase were found. In conclusion, supplemental carbohydrase may improve fiber digestibility in plant-based diets for HSB, but additional studies are needed to evaluate the applicability and cost benefit of enzyme additives in feed formulations for HSB and other farmed species.

RATIONALE

The use of exogenous enzymes in feeds for terrestrial livestock, notably poultry and swine, has been widely adopted by the industry given the positive effects of these additives in improving the nutritional value of plant-based feeds largely comprised of soybean meal (SBM) and corn. Inclusion of carbohydrase enzymes in feeds have shown to improve the digestibility of otherwise indigestible oligosaccharides and non-starch polysaccharides (fiber) present in plant-based protein and energy ingredients for monogastric animals. Likewise, the supplementation of protease enzymes to poultry and swine feeds has been shown to increase the availability of proteinogenic amino acids by enhancing protein digestibility. Such positive effects of enzyme additives translate into economic and environmental benefits by directly improving production efficiency, reducing pollution caused by unabsorbed feed nutrients, and by reducing the footprint of livestock production.

Although well established in the poultry and swine industries, the use of enzyme additives in aquaculture feeds is still incipient and information regarding their efficacy in aquatic animals is limited to only a few species. However, considering the increased use of plant-based ingredients in aquaculture feeds in recent decades and the similarities in digestive physiology between terrestrial monogastric animals and fish, the applicability of commercial enzyme additives in aquaculture feeds should be investigated. Therefore, the objective of this research was to assess the effects of commercial enzyme additives in SBM-based diets for hybrid striped bass (HSB) - one of the main food fish in the U.S. aquaculture industry. To this end, two feeding trials were conducted: a digestibility trial to access the effects of the additive enzymes on the digestibility of soybean meal (SBM)-based diets with or without distillers dried grains inclusion; and a subsequent growth trial to evaluate effects of supplemental enzyme on the production performance of HSB fed similar diets.

MATERIALS AND METHODS

Experimental fish

Two batches of hybrid striped bass (HSB) fingerlings were purchased from a commercial producer (Keo Fish Farm, 226 Main Street, Keo, AR) and transported to the Aquatic Animal Nutrition Laboratory (AANL) located in the Aquaculture Research Center, Kentucky State University, Frankfort, KY. Upon arrival, the fish were stocked in two 1500-L rectangular fiberglass tanks operating as a recirculating aquaculture system wherein they were grown to adequate size for the digestibility and growth trials. Before the beginning of either trial, the fish were fed a commercial feed (45% crude protein [CP] and 12% crude fat; AQUAXCEL®, Cargill® Inc. Minneapolis, MN, USA) thrice daily to apparent satiety using automatic belt feeders.

Experimental Diets and Test Ingredients

All dietary treatments in this project were planned following completely randomized experimental designs. All diets were formulated to contain 42% crude protein, 12% lipid, and different levels of enzyme additives. The enzyme additives evaluated in this project include a carbohydrase and a protease, both obtained from a commercial supplier (undisclosed). The manufacture recommended level (MRL) of each enzyme was use as the first supplementation level in the experimental diets.

Digestibility Trial

To evaluate the effects of the supplemental enzymes on the digestibility of the plant-protein based diets by HSB, five experimental diets were formulated to derive ~ 50% of the protein from SBM or a combination of SBM and distiller dried grains with solubles (DDGS) (Table 1). Two diets (SOY and DDGS) were formulated without enzyme supplementation and used as negative controls. Two additional diets (SOY+CP1X and DDGS+CP1X) were formulated to contain supplemental carbohydrase and protease at 1X MRL. A fifth diet (DDGS+CP2X) was formulated to evaluate the supplemental enzymes at 2X MRL in the formulation containing DDGS. Yttrium oxide was included in all diets at 1% as a digestibility indicator.

Growth Trial

In this evaluation, seven diets (Table 2) were designed following the same ingredient composition of the diets used in the digestibility trial. However, based on the lack of significant effects of the protease additive on protein digestibility revealed by the digestibility evaluation, only the

carbohydrase additive was tested in this growth trial. Three diets were formulated to contain SBM at 40%, including a negative control (SOY) and two diets supplemented with carbohydrase at 1X (SOY+C1X) or 2X MRL (SOY+C2X). An additional set of three diets (DDGS, DDGS+C1X, and DDGS+C2X) were formulated to contain DDGS at 7% replacing a portion of SBM (reduced to 36.9%) in the SOY diet. These diets also evaluated supplemental carbohydrase at 1X and 2X MRL using the DDGS diet as negative control. The seventh diet (FM-30) was formulated to contain 30% FM and without SBM and DDGS, and served as a standard reference.

Experimental Conditions

Digestibility Trial

Hybrid striped bass fingerlings were grown in the AANL until they attained ~100 g/fish for the trial. At the beginning of the evaluation, groups of 20 HSB were stocked into 20, 180-L polyethylene tanks operating as a recirculating aquaculture system. Fish in each tank (4 tanks/diet) were fed their randomly assigned diet to apparent satiation once daily, for fourteen weeks. Fecal samples were collected by stripping once a week until ~3.0 g of dried fecal matter/tank was obtained. Water quality was maintained within acceptable levels for HSB culture. Temperature (mean ± SD = 24.8 ± 0.8 °C), pH (7.8 ± 0.2), dissolved oxygen (6.1 ± 0.9 mg/L), and salinity (2.6 ± 0.8 g/L) were measured once daily while total ammonia-nitrogen (0.47 ± 0.1 mg/L) and nitrite-nitrogen (0.06 ± 0.03 mg/L) were measured once a week.

Growth Trial

The growth trial was conducted indoor under controlled conditions in the AANL. Once adequate size for the evaluations was attained, 20 HSB juveniles (~9.0 g/fish) were stocked in 110-L glass aquaria operating as a recirculating aquaculture system. Five replicate tanks were used for each experimental diet, and the fish were fed twice daily to apparent satiation for a total of eight weeks. Apparent satiation was judged arbitrarily based on the feeding activity of the fish at each feeding. Water quality was monitored as for the digestibility trial and parameters were maintained at similar ranges.

Data Acquisition

All chemical analyses of dietary ingredients, experimental diets, and fecal material were contracted out to the Agricultural Experiment Station Chemical Laboratories (ESCL) at the University of Missouri, Columbia, MO.

In the digestibility trial, fecal samples were collected by stripping once a week at ~5 h postprandial. Collected fecal material was dried in a convection oven at 65°C for 24 hours, pooled by tank into sample bags, and kept at -20°C pending chemical analyses. Dried fecal matter and subsamples of each diet were sent to ESCL for yttrium and proximate composition analyses. These data were used to calculate apparent digestibility coefficients (ADCs) of the diets for dry matter, crude protein, neutral detergent fiber, and gross energy.

Upon conclusion of the growth trial, HSB were sampled after fasting overnight. Fish from each aquarium were group weighed and counted for computation of production performance parameters.

Statistical Analyses

A one-way ANOVA was performed to detect significant ($P \leq 0.05$) differences among dietary treatments in the digestibility and growth trial. When significant differences were detected, the Tukey's HSD test was used to identify differences. All statistical analyses were performed using the Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Digestibility Trial

No significant effects of supplemental protease and carbohydrase on the digestibility of dry matter, protein, and gross energy by HSB were observed (Table 3). On the other hand, the ADC of non-starch polysaccharides (expressed as neutral detergent fiber, NDF) significantly increased with the supplementation of carbohydrase additive at 1X in the SOY and at 2X in the DDGS diet (Fig. 1), revealing a positive effect of the enzyme additive.

Growth Trial

Performance metrics of HSB are presented in Table 4. Survival was high (≥ 98%) and unaffected by diet. No significant differences in growth and feed efficiency (FER) were observed among SOY and DDGS groups, regardless of carbohydrase supplementation. Fish fed SOY+C1X and DDGS displayed significantly lower FER compared to FM-35-fed fish.

CONCLUSIONS

All soy-based diets evaluated performed extremely well by supporting growth and feed efficiency similar to the FM-30 diet, despite containing only 5% fish meal. This corroborates previous studies showing that HSB accept and grow well on soy-based diets.

We did not find positive effects of either supplemental enzyme on protein digestibility in this study, whereas an increase in NDF digestibility was observed. Interestingly, a positive effect of carbohydrase supplementation in the diet containing 7% DDGS was only found when the dose of the enzyme was doubled (2X MRL), indicating that levels higher than the MRL should be considered in practical formulations for HSB. In the growth trial, no significant effects of the supplemental carbohydrase on the production performance of HSB were found, indicating that diets had similar nutritional value.

Based on the current findings, digestibility of non-starch polysaccharides by HSB might improve when carbohydrase enzymes are supplemented in diets. However, supplementation of protease to these formulations appears ineffective. More studies should be conducted to evaluate supplemental carbohydrases and proteases in plant-based diets for farmed fish.

Table 1. Ingredient composition (%, dry matter basis) of the experimental diets evaluated in the Digestibility Trial.

Diet	1	2	3	4	5
Treatment	SOY	SOY+CP1X	DDGS	DDGS+CP1X	DDGS+CP2X
Carbohydrase		0.01		0.01	0.02
Protease		0.02		0.02	0.04
Fish meal (sardine)	5.00	5.00	5.00	5.00	5.00
Poultry by product meal	15.00	15.00	15.00	15.00	15.00
DDGS			7.00	7.00	7.00
Soybean meal (conventional)	40.00	40.00	36.68	36.68	36.68
Corn protein concentrate	3.50	3.50	3.50	3.50	3.50
Wheat gluten	3.50	3.50	3.50	3.50	3.50
Wheat flour	14.21	14.19	11.19	11.16	11.13
CMC 1.5% + GeoBond 0.5%	2.00	2.00	2.00	2.00	2.00
Fish oil (Sardine)	5.00	5.00	5.00	5.00	5.00
Vegetable oil	3.22	3.22	2.57	2.57	2.57
Vitamin premix	0.60	0.60	0.60	0.60	0.60
Stay C (35% vitamin C)	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.20	0.20	0.20	0.20	0.20
KSU-1 mineral premix	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.20	2.20	2.20	2.20	2.20
Others	2.27	3.27	3.27	3.27	3.27

FM = fish meal; DDGS = Distiller dried grains w/solubles; CMC = carboxymethyl cellulose; blanks denote no inclusion of an ingredient.

Table 2. Ingredient composition (%, dry matter basis) of the experimental diets evaluated in the Growth Trial.

Diet	1	2	3	4	5	6	7
Treatment	SOY	SOY+C1X	SOY+C2X	DDGS	DDGS+C1X	DDGS+C2X	FM-30
Carbohydrase		0.01	0.02		0.01	0.02	
Fish meal (sardine)	5.00	5.00	5.00	5.00	5.00	5.00	30.00
Poultry by product meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DDGS				7.00	7.00	7.00	
Soybean meal (conventional)	40.00	40.00	40.00	36.85	36.85	36.85	
Corn protein concentrate	3.10	3.10	3.10	3.10	3.10	3.10	3.10
Wheat gluten	3.10	3.10	3.10	3.10	3.10	3.10	3.10
Wheat flour	16.62	16.61	16.60	13.33	13.32	13.31	34.33
CMC (1.5%) + GeoBond (0.5%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil (Sardine)	5.00	5.00	5.00	5.00	5.00	5.00	2.00
Vegetable oil	2.22	2.22	2.22	1.66	1.66	1.66	3.35
Vitamin premix	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Stay C (35% vitamin C)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20
KSU-1 mineral premix	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	0.60
Others	2.87	2.87	2.87	2.87	2.87	2.87	3.42

FM = fish meal; DDGS = distiller dried grains w/solubles; CMC = carboxymethyl cellulose; blanks denote no inclusion of an ingredient.

Table 3. Apparent digestibility coefficients of the experimental diets (Digestibility Trial).

Diet (treatment)	Dry matter	Protein	GE
1 (SOY)	0.48	0.72	0.65
2 (SOY+CP1X)	0.49	0.71	0.67
3 (DDGS)	0.45	0.69	0.67
4 (DDGS+CP1X)	0.45	0.68	0.65
5 (DDGS+CP2X)	0.46	0.69	0.69
PSE	0.009	0.007	0.007
ANOVA (Pr > F)	0.619	0.125	0.226

NDF = neutral detergent fiber; GE = gross energy.

Table 4. Production performance metrics of hybrid striped bass fed the experimental diets for eight weeks (Growth Trial).

Diet (Treatment)	IW g	FW g	FER	Survival %
1 (SOY)	9.4	44.9^{ab}	0.74 ^{ab}	100
2 (SOY+C1X)	9.1	43.3^{ab}	0.73 ^{ab}	98
3 (SOY+C2X)	9.2	44.2^{ab}	0.72^{b}	98
4 (DDGS)	9.3	45.4^{ab}	0.73 ^{ab}	100
5 (DDGS+C1X)	9.3	43.1^{ab}	0.73 ^{ab}	99
6 (DDGS+C2X)	9.2	47.0^{a}	0.75 ^{ab}	100
7 (FM-35)	9.3	39.3^{b}	0.79^{a}	100
PSE	0.03	0.64	0.01	0.3
ANOVA (Pr > F)	0.311	0.036	0.053	0.189

IW = initial weight; FW = final weight; WG = weight gain; FI = feed intake; FER = feed efficiency ratio; HSI = hepatosomatic index.

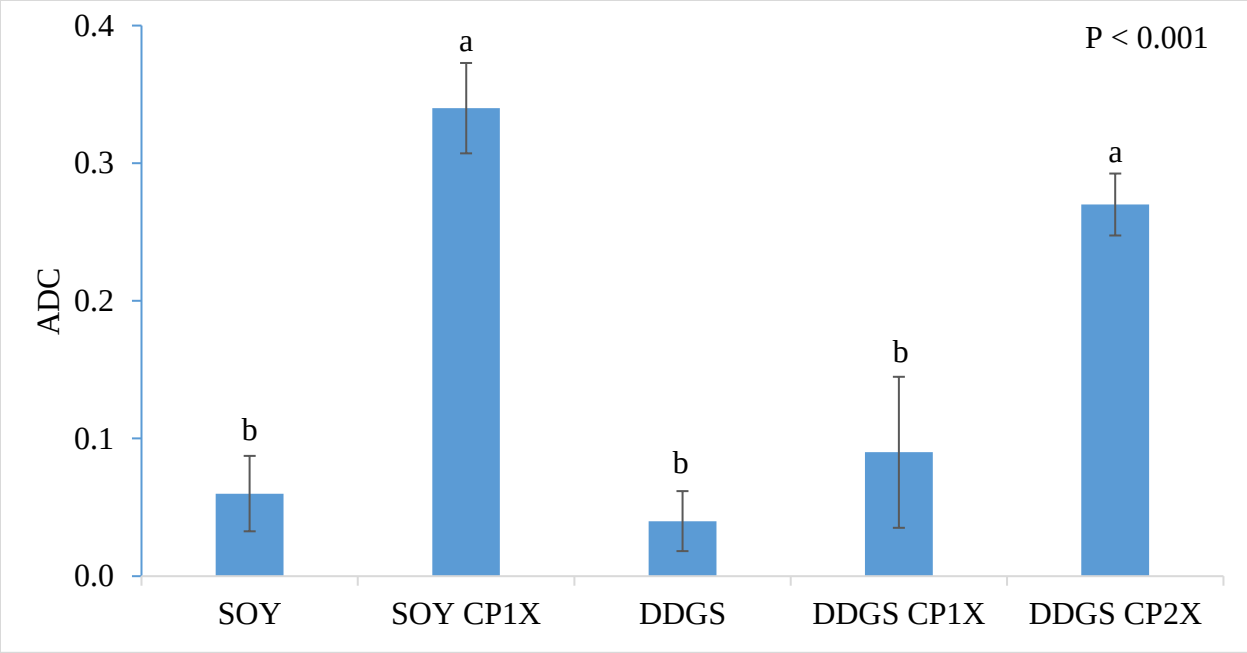

Figure 1. Apparent digestibility coefficient (ADC) of neutral detergent fiber of the experimental diets by hybrid striped bass (Digestibility Trial). Means ± SE.