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Summary of Phytase Studies for Channel Catfish

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Introduction

Pond-raised channel catfish require about 0.3% available phosphorus (P) for optimum growth and bone development (Robinson et al. 1996). Because catfish feeds for grow-out consist primarily of plant ingredients that contain low levels of biologically available P, commercial catfish feeds are typically supplemented with inorganic P sources to meet the requirement. A considerable portion of the P in plant ingredients is in the form of phytate, a bound form of P that has low availability to simple-stomach animals including catfish. Thus a substantial amount of dietary P is unabsorbed and may accumulate in pond sediments, which essentially regulates P concentrations in pond water (Gross et al.1998). High levels of soluble P in the water stimulate phytoplankton growth in the water that can result in wide fluctuation of dissolved oxygen concentrations and may contribute to off-flavor problems in catfish.

Phytase, an enzyme derived from the fungus *Aspergillus niger*, has been shown to be effective in improving bioavailability of phytate P in diets of several animals including poultry (Simons et al. 1990), pigs (Cromwell et al. 1993), rainbow trout (Cain and Garling 1995; Rodehutscord and Pfeffer 1995), and common carp (Schafer et al. 1995). Using phytase in catfish feeds may improve P utilization of plant feed ingredients, replace inorganic P supplements, and reduce the P load in pond sediments. This report summarizes results from two laboratory and two pond studies that have been conducted at the Thad Cochran National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi, and the Delta Western Research Center (NWRC) in Indianola, Mississippi, to examine the efficacy of fungal phytase as a replacement of inorganic P supplements in catfish feeds.

Methods

Laboratory Studies

The basal diets were formulated to contain 32% protein and prepared as sinking pellets (Table 1). Total and available P in the basal diet were 0.60% and 0.19% for Experiment 1 and 0.52% and 0.23% for Experiment 2. The fungal phytase (Natuphos®) derived from *Aspergillus niger* was supplied by BASF Corporation (Parsippany, New Jersey). Phytase was added to the basal diet at 0, 500, 1,000, 2,000, and 4,000 FTU/kg diet in Experiment 1 and at 0, 250, 500, and 750 FTU/kg diet in Experiment 2. One FTU (phytase unit) is defined as the amount of phytase that liberates 1 µmol of inorganic P from 0.0051 mol/L of sodium phytate per minute at pH 5.5 and 37°C. An additional diet containing 1% dicalcium phosphate (no phytase) was also included in Experiment 2. The ingredients used in the basal diets were similar to those used in commercial catfish feeds. All known nutrient requirements of catfish were satisfied except P. The proper amount of phytase was dissolved in 40 mL of distilled water and sprayed onto 1 kg of the basal diet as needed. The basal diet and the diet containing dicalcium phosphate were sprayed with 40 mL of distilled water to maintain an equal level of moisture. The

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Table 1. Ingredient composition of the basal diets (expressed as percent on an as-fed basis).

Ingredient	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Soybean meal (48%)[1]	55.6	53.5	44.5	40.7
Cottonseed meal (41%)[1]	–	–	10.0	–
Animal protein source[2]	–	1.0	1.0	1.0
Corn grain	18.1	33.4	27.8	30.3
Wheat middlings	22.0	9.0	15.0	26.3
Vitamin C[3]	0.1	0.1	0.03	0.03
C-free vitamin mix[4]	0.1	0.1	0.1	0.1
Trace mineral mix[4]	0.1	0.1	0.1	0.1
Carboxymethyl cellulose[5]	2.0	2.0	–	–
Catfish oil	2.0	0.75	1.5	1.5

[1]Values in parentheses represent percentage crude protein.
[2]Meat and bone/blood meal, catfish by-product meal, and poultry by-product meal was used as animal protein supplements in Experiments 2, 3, and 4, respectively.
[3]Vitamin C was supplied by L ascorbyl-2-polyphosphate (Hoffmann La Roche, Inc., Nutley, New Jersey) with 15% activity (Experiments 1 and 2) and 25% activity (Experiments 3 and 4).
[4]Catfish vitamin and trace mineral premixes were the same as described by Robinson and Li (1996).
[5]Pellet binding agent used to prepare sinking feeds for laboratory studies.

diets were kept at 4°C until used. Phytase activities of the diets were analyzed by the BASF Corporation according to procedures described by Simons et al. (1990), and results shown in Table 2. Each of the diets was randomly assigned to five aquaria.

Twenty small catfish fingerlings were stocked into each of the five 110-L flow-through aquaria located at the NWAC. The aquaria were supplied with well water and continuous aeration. Water temperature was maintained at 30±1°C. Fish were fed twice daily (8 a.m. and 4 p.m.) to approximate satiation for 10 and 12 weeks for Experiment 1 and 2, respectively, based on percentage of fish biomass. Feed consumption was monitored at each feeding. Feeding rate was adjusted once or twice a week based on feeding activity of fish. Other management practices followed our standard protocol of aquarium studies.

At the end of the feeding period, all fish were counted and weighed to determine feed consumption, weight gain, feed conversion ratio (FCR), and survival. Ten fish from each aquarium were killed by overdose of tricaine methanesulfonate. Fecal material was collected from the rectal portion of the intestine (12 hours after last feeding). Trunk and caudal vertebrae from these fish were analyzed for bone ash (AOAC 2000) and P (Hach Company 1990). Analysis of variance and Fisher's protected least significant difference procedure (Steel et al. 1997) were used to evaluate the treatment effects. A significance level of $P \leq 0.05$ was used.

Pond Studies

Basal diets (Table 1) were formulated to contain 32% protein (Experiment 3) and 28% protein (Experiment 4) and meet all known nutritional requirements of catfish except for P. The basal diets were extruded floating pellets manufactured in an experimental feed mill at the DWRC. Total and available P in the basal diets were 0.63% and 0.27% for Experiment 3 and 0.60% and 0.25% for Experiment 4, respectively. Phytase was added to the basal diet at 250 and 500 FTU/kg diet in Experiment 3 and at 0 and 500 FTU/kg diet in Experiment 4. An additional diet containing 0.75% dicalcium phosphate (no phytase) was also included in Experiment 3. The proper amount of phytase was dissolved in 500 mL of distilled water and sprayed with a hand sprayer onto 25 kg of the basal diet while the feed pellets rotated in a concrete mixer to achieve adequate mixing. The basal diet and the diet containing dicalcium phosphate were sprayed with 2% distilled water to maintain an equal level of moisture as diets containing phytase.

Catfish fingerlings were stocked into 0.04-ha ponds at a rate of 18,525 fish per hectare in Experiment 3 and 17,290 fish per hectare in Experiment 4. Experiments 3 and 4 were conducted at the NWAC and DWRC, respectively. Four ponds were used for each treatment in Experiment 3, and five ponds were used in Experiment 4. Fish were fed the experimental diets once daily to satiation from May to October. Apparent satiation was achieved by allowing the fish to eat as much as they would consume within 20 minutes. Amounts of diet consumed by the fish in each pond were recorded daily to determine feed consumption per fish at the end of the study. The ponds were managed in a manner similar to commercial catfish farms. At the end of the feeding period, all fish were counted and weighed. Twenty fish from each pond were collected for bone ash and P analyses. Data collection and statistical analysis of the data were basically the same as described for laboratories studies.

Table 2. Phytase activity of experimental diets.

Fungal phytase	Dicalcium phosphate	Phytase by analysis[1,2]		
		Experiment 2[3]	Experiment 3[4]	Experiment 4[4]
FTU/kg	*%*	*FTU/kg*	*FTU/kg*	*FTU/kg*
0	0	BD[5]	BD	BD
250	0	345	238±54	–
500	0	618	584±151	495±27
750	0	793	–	–
0	0.75	–	BD	–
0	1.0	BD	–	–

[1]Phytase activity was not determined for experimental diets in Experiment 1.
[2]One FTU is defined as the amount of phytase that liberates 1 μmol of inorganic phosphorus from 0.0051 mol/L of sodium phytate per minute at pH 5.5 and 37°C.
[3]A single sample per diet was analyzed for phytase activity.
[4]Six batches of feed samples per diet were collected and analyzed during the feeding period. Values represent mean ± SD.
[5]Below detection limit.

Results and Discussion

Table 3. Mean feed consumption, weight gain, feed conversion ratio, survival, bone ash, and bone phosphorus of channel catfish fed diets containing different concentrations of fungal phytase for 70 days in aquaria in Experiment 1.[1]

Fungal phytase	Feed consumption per fish	Weight gain per fish[2]	Feed conversion ratio	Survival	Bone ash	Bone phosphorus	Fecal phosphorus[3]
FTU/kg	*g*	*g*	*feed/gain*	*%*	*%*	*%*	*%*
0	101.8 b	51.4 b	1.99 a	100.0	51.8 b	9.7 b	1.4
500	110.6 a	59.1 b	1.89 ab	100.0	57.7 ab	10.8 ab	1.4
1,000	113.1 a	63.4 a	1.79 b	100.0	55.1 ab	10.3 ab	0.9
2,000	111.9 a	59.2 a	1.90 ab	99.0	58.6 a	10.9 a	0.9
4,000	113.3 a	60.7 a	1.87 ab	100.0	56.2 ab	10.6 ab	0.5
Pooled SEM[4]	2.3	2.4	0.06	0.5	1.7	0.3	

[1]Means within each column followed by different letters were different at the 0.05 probability level by Fisher's protected least significant difference procedure.
[2]Mean initial weight was 6.5 g per fish.
[3]Statistical analysis was not conducted for feces phosphorus because a single pooled sample per treatment was analyzed.
[4]SEM = standard error of mean.

Experiment 1

Catfish fed diets containing 500 FTU of phytase per kilogram or more consumed more feed and gained more weight than fish fed a basal diet without phytase (Table 3). Feed conversion ratio (FCR) did not differ among treatments except for fish fed the diet containing 1,000 FTU of phytase per kilogram as compared with fish fed the basal diet. Bone ash and P concentrations were higher for fish fed the diet containing 2,000 FTU phytase than fish fed the basal diet. There was a trend indicating that as dietary phytase levels increased, fecal P levels decreased. However, because a single pooled sample per diet was analyzed for fecal P, no statistical comparisons were made. Results from this experiment suggest that fungal phytase is effective in improving bioavailability of phytate P in catfish diets as has been observed for other animals and fish (Simons et al. 1990; Cain and Garling 1995; Schafer et al. 1995).

Experiment 2

Fish fed diets containing 250 FTU of phytase per kilogram or more consumed more feed, gained more weight, and converted feed more efficiently than fish fed a basal diet without phytase (Table 4). Bone ash and P concentrations were higher and fecal P level was lower in fish fed phytase diets than those fed the basal diet. Fish fed the diet containing 1% dicalcium phosphate without phytase consumed the same amount of feed as compared with the fish fed the phytase diets but had intermediate weight gain and FCR as compared with fish fed the basal diet and phytase diets. Bone ash for fish fed the dicalcium phosphate diet was lower than fish fed the diet containing 750 FTU of phytase per kilogram. Fish fed the dicalcium phosphate diet had a lower bone P level and higher fecal P than those fed the phytase diets. Results from this experiment further confirm that fungal phytase could effectively increase utilization of phytate P by catfish as evidenced by an increase in bone P and a decrease in fecal P. A dietary phytase level of 250 FTU per kilogram could totally replace inorganic P supplement in catfish diets without affecting growth, feed efficiency, and bone ash and P concentrations.

Table 4. Mean feed consumption, weight gain, feed conversion ratio, survival, bone ash, and bone phosphorus of channel catfish fed diets containing different concentrations of fungal phytase for 84 days in aquaria in Experiment 2.[1]

Fungal phytase	Dicalcium phosphate	Feed consumption per fish	Weight gain per fish[2]	Feed conversion ratio	Survival	Bone ash	Bone phosphorus	Fecal phosphorus
FTU/kg	*%*	*g*	*g*	*feed/gain*	*%*	*%*	*%*	*%*
0	0	117.1 b	46.5 b	2.53 a	98.0	42.0 c	7.4 c	0.78 a
250	0	133.9 a	65.0 b	2.07 bc	97.0	53.6 ab	9.6 a	0.28 b
500	0	131.7 a	65.6 a	2.01 c	96.7	53.9 ab	9.8 a	0.22 b
750	0	131.0 a	61.1 a	2.19 bc	97.0	54.2 a	9.9 a	0.12 b
0	1.0	128.5 a	55.2 ab	2.35 ab	93.8	51.7 b	9.1 b	0.96 a
Pooled SEM[3]		3.3	3.3	0.09	1.8	0.8	0.15	0.09

[1]Means within each column followed by different letters were different at the 0.05 probability level by Fisher's protected least significant difference procedure.
[2]Mean initial weight was 6.8 g per fish.
[3]SEM = standard error of mean.

Dicalcium phosphate	Fungal phytase	Diet consumption per fish	Weight gain per fish[2]	Feed conversion ratio	Survival	Bone ash	Bone phosphorus
%	*FTU/kg*	*g*	*g*	*feed/gain*	%	%	%
0	250	654	440	1.48	82.5	55.5	9.53
0	500	640	440	1.46	88.0	56.8	9.90
0.75	0	636	454	1.41	80.8	56.6	9.79
Pooled SEM[3]		20.1	17.5	0.04	4.0	0.41	0.13

Table 5. Mean feed consumption, weight gain, feed conversion ratio, survival, bone ash, and bone phosphorus of channel catfish fed diets containing dicalcium phosphate or microbial phytase for 128 days.[1]

[1]Means within each column did not differ at the 0.05 probability level by ANOVA.
[2]Mean initial weight was 23.4 g per fish.
[3]SEM = standard error of mean.

Experiment 3

No differences were observed in feed consumption, weight gain, FCR, survival, and bone ash and P concentrations among pond-raised catfish fed diets containing 250 and 500 FTU of phytase per kilogram or a diet containing 0.75% dicalcium phosphate (Table 5). Results from this pond experiment support the findings from the laboratory studies that demonstrated that phytase could totally replace inorganic P supplements in catfish feeds without adverse effects.

Experiment 4

Although data from previous studies indicate that phytase can be used to replace dicalcium phosphate in catfish diets, another study is needed because cottonseed meal — a protein source that is high in phosphorus — was included in the diets used in Experiment 3 since it was a common ingredient in commercial catfish feeds. However, the availability of cottonseed meal to the catfish industry has become sporadic primarily because whole cottonseeds are being fed to livestock and thus are not being processed into meal. Experiment 4 was conducted to evaluate the efficacy of phytase supplementation in diets without cottonseed meal for pond-raised channel catfish. Catfish fed a diet containing 500 FTU of phytase per kilogram had higher feed consumption, weight gain, and bone ash and P concentrations than fish fed a basal diet without phytase (Table 6). There were no differences in FCR and survival between treatments. Results from this study demonstrate that fungal phytase could be used in diets containing soybean meal and wheat middlings as primary sources of P without adverse effects on fish growth and bone P deposition.

Table 6. Mean feed consumption, weight gain, feed conversion ratio, survival, bone ash, and bone phosphorus of channel catfish fed diets containing 0 or 500 FTU of fungal phytase per kilogram for 134 days in ponds in Experiment 4.[1]

Dietary phytase	Feed consumption per fish	Weight gain per fish[2]	Feed conversion ratio	Survival	Bone ash	Bone phosphorus
FTU/kg	*g*	*g*	*feed/gain*	%	%	%
0	752 b	404 b	1.87	96.0	52.5 b	8.63 b
500	814 a	466 a	1.75	96.9	55.1 a	9.15 a
Pooled SEM[3]	17.9	15.0	0.04	1.75	0.18	0.08

[1]Means within each column did not differ at the 0.05 probability level by ANOVA.
[2]Mean initial weight was 69 g per fish.
[3]Standard error of mean.

Summary

Results from these studies indicate that a dietary phytase concentration of 250 FTU per kilogram applied post-pelleting can effectively replace the dicalcium phosphate supplement in catfish diets without affecting growth, feed efficiency, or bone P deposition. Since there is a chance that losses of enzyme activity may occur during and after phytase application, we recommend that a dietary phytase level of 500 FTU per kilogram be used in the diet to ensure that an adequate amount of P is available to catfish. In formulating diets utilizing phytase, it is important that the diet be formulated to contain an adequate level of total P so that sufficient P can be released by phytase that will be available to the fish. Based on results from these studies, a total P level of 0.60% should be adequate. Among the most commonly used plant ingredients, cottonseed meal and wheat middlings are good sources of P; therefore, these and other ingredients high in P should be included to ensure sufficient total P levels in the feed.

One possible benefit of using phytase to replace inorganic P supplements in catfish feeds is to reduce the dietary P load in the pond since phytase improves utilization of phytate P inherent in the feed ingredients and reduces fecal P concentrations. One would expect that minimizing the amount of P going into the pond water would lower the P level and thus reduce phytoplankton growth. However, the use of phytase in catfish feeds did not influence total P, soluble reactive P, or chlorophyll-a concentrations in pond water (Craig Tucker, personal communication). It should be noted that these pond studies were conducted for only one growing season. Since the uptake of P by pond sediments is a major factor controlling P levels in the pond water feeding phytase diets over a long term may be beneficial in catfish ponds by reducing the P load in pond sediments. Based on current prices of phytase and inorganic P sources, it is economical to use phytase in catfish diets. Since phytase will not withstand the high temperature associated with manufacturing extruded catfish feed, it must be applied post extrusion. However, effective and economical application methods that result in uniform and consistent phytase levels in finished feeds need to be developed before its use in commercial catfish feeds.

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