BIOTROPIA VOL. 15 NO. 2, 2008 : 110 - 118

EFFECT OF PHYTASE SUPPLEMENTATION IN SOYBEAN MEAL BASED DIET ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF GREEN CATFISH (*Hemibagrus nemurus*)

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ABSTRACT

This experiment was conducted to evaluate the effect of phytase supplemented to the diet on phosphorus (P) digestibility and growth performance of the green catfish *Hemibagrus nemurus*. Five kinds of experimental diets were used in this experiment, namely diets A, B, C, D and E. Diet A, as a control, was supplemented with inorganic P, without phytase supplementation. Diet B, C, D and E were supplemented with 0, 20, 40 and 60 mg phytase/100 g soybean meal (SBM), respectively, without inorganic P supplementation. Fifteen fish with initial body weight of 6.9 $\pm$ 0.2 g were stocked into each 60-l aquarium. Fish were fed on the diets for 60 days. Results indicated that P digestibility increased from 64.5% to 87.0% as phytase supplement increased from 0 mg in diet B to 60 mg phytase/100 g SBM in diet E. P digestibility in diet E was higher than that in diet A (77.6%). The daily growth rate and feed conversion ratio followed similar trend. P, Ca and Zn concentration in the whole body and bone of fish fed diet E were higher than the fish fed diet B, C and D, but were insignificant compared to the fish fed diet A. Nitrogen (N) and P loading by fish fed diet E were, respectively, 76% and 20% lower than those in fish fed the control diet. It is concluded that the inclusion of 60 mg phytase/100 g SBM in the diet of the green catfish could replace the utilization of inorganic P increase the digestibility of the diet thereby resulting in increased growth rate and reduced excretion of P and N into the waters.

Key words: *Hemibagrus nemurus,* phytase, soybean meal, phosphorus.

INTRODUCTION

The expansion of global aquaculture production increases the demand for aquaculture feeds. For carnivore species as green catfish *Hemibagrus nemurus*, protein can form up to 60% of the diet and fishmeal is the main source of dietary protein. Fishmeal is one of the most expensive and demanded ingredients and has become the main and most critical ingredient in aquafeed production. The increasing cost and demand of fishmeal has encouraged feed manufacturers to search for cheaper alternative protein source as plant protein.

Soybean meal (SBM) is a common plant protein source used as a substitute for

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fishmeal in the fish diet. It could replace 75% of fishmeal as a protein source in the diet of the green catfish (Pebriyadi 2004). A major obstacle in using SBM is their high phytic acid content. Phytic acid is a substance that contains unavailable phosphorus (P) to monogastric animals, including fish, because these animals lack phytase, which can hydrolyze phytic acid (Baruah *et al*. 2004). By using phytase supplement to the diet, the P digestibility can be improved. Li *et al*. (2004) reported that 250 units of phytase per kg diet could effectively replace di-calcium phosphate supplement in the diet of channel catfish without affecting growth, feed efficiency or bone phosphorus deposit. Masumoto *et al*. (2001) showed that 0.2 g of phytase supplement to the diet containing 67% SBM increased the growth rate and feed efficiency of the Japanese flounder *Paralicthys olivaceus*. Therefore, using phytase supplement in the fish diet could increase the bioavailability of phosphorus of SBM. Hence, replacing the inorganic P of the diet, resulting in lower fecal P loss. This experiment was conducted to evaluate the effect of phytase supplement to the diet on P digestibility and growth performance of the green catfish *Hemibagrus nemurus*.

MATERIALS AND METHODS

Experimental diets

Five experimental diets were used in this experiment, namely diets A, B, C, D and E. Diet A, as a control, was supplemented with inorganic P ($NaH_2PO_4.H_2O$, KH_2PO_4, $Ca_2(PO_4)_3$) only, without phytase supplement. Diet B, C, D and E were supplemented with 0, 20, 40 or 60 mg phytase (Natuphos® 5000)/100 g SBM, respectively, without inorganic P supplement (Table 1). The proximate composition of experimental diets is shown in Table 2.

Table 1. Ingredients composition of experimental diet (g/kg diet)

Ingredients	Treatments				
	A	B	C	D	E
Fish meal[1]	130	130	130	130	130
Soybean meal[1]	640	640	640	640	640
Fish oil	40	40	40	40	40
Soybean oil	44	44	44	44	44
Tapioca[1]	57	57	47,87	47,74	47,62
Vitamin mix	15	15	15	15	15
Choline chloride	5	5	5	5	5
L-Methionine	5	5	5	5	5
Taurin	6	6	6	6	6
Mineral mix[2]	58	0	0	0	0
P-free mineral mix[3]	0	58	58	58	58
Phytase	0	0	0.13	0.26	0.38
Citric acid	0	0	9	9	9
P content (%):					
Total P	1.46	0.55	0.55	0.56	0.58
Water-soluble P	0.59	0.19	0.45	0.48	0.53

Table 1. Continued

Ingredients	Treatments				
	A	B	C	D	E
Water-soluble P/total P ratio	40.29	34.87	82.79	86.06	91.13

[1] Crude protein (dry weight) concentration of: fish meal 70.39%, SBM 42.75%, tapioca meal 0.91%.

[2] The mineral mix had the following composition (g/kg dry diet): NaCl 0.5; $MgSO_4.7H_2O$ 7.5; $NaH_2PO_4H_2O$ 12.5; KH_2PO_4 16; $Ca2(PO_4)_3$ 14.49; Fe-citric 1.25; filler 3.60 and trace element mix (0.5 g) had the following composition: $ZnSO_4.7H_2O$ 17.65; $MnSO_4$ 8.1; $CuSO_4.5H_2O$ 1.55; $CoCl.6H_2O$ 0.05; KIO_3 0.15; and filler 30.5 (Takeuchi 1988).

[3] P-free mineral mix had the following composition (g/kg dry diet): NaCl 0.5; $MgSO_4.7H_2O$ 7.5; KCl 17.53; Fe-citric 1.25; $CaCl_2.2H_2O$ 13.34; filler 30.5 and trace element mix (0.5 g) had the following composition: $ZnSO_4.7H_2O$ 17.365; $MnSO_4$ 8.1; $CuSO_4.5H_2O$ 1.55; $CoCl.6H_2O$ 0.05; KIO_3 0.15; and filler 30.5.

Table 2. Proximate composition of experimental diets (% wet weight)

Proximate Composition	Treatments				
	A	B	C	D	E
Crude Protein	31.19	29.49	29.30	30.11	29.19
Crude Lipid	10.58	10.62	10.18	10.30	9.92
Ash	11.09	8.74	8.92	9.12	8.79
Water	14.01	18.98	20.38	17.04	20.23
Fiber	4.20	3.21	2.62	2.92	2.24
NFE[1]	28.93	28.96	28.60	30.51	30.87
Total Energy[2] (kcal/100g)	392.73	383.71	377.03	390.53	387.38
Energy/Protein (kcal/g protein)	12.59	13.01	12.87	12.97	13.27

[1] Nitrogen Free Extract.

[2] Total Energy (GE) was calculated based on equivalent values of: protein 5.6 Kcal/g, lipid 9.4 Kcal/g, and NFE 4.1 Kcal/g (Takeuchi, 1988).

For the pretreatment of SBM with phytase, 640 g of SBM was mixed with 2240 ml water, and then phytase was added to the SBM-water mixed at 20, 40, 60 mg/100g, respectively, in the diets C, D and E. The pH was adjusted to 5.5 with citric acid. The mixtures were incubated at 37 °C for 2 h (Matsumoto *et al.* 2001). Then, the mixtures were mixed with other ingredients and formed into granules. The pellets were stored at -20 °C until use.

Feeding trial

Green catfish juveniles were obtained from the Main Center for Freshwater Aquaculture Development, Sukabumi, West Java, Indonesia. Upon arrival at the Laboratory of Fish Nutrition, of the Bogor Agricultural University, the fish were acclimated to experimental conditions for 10 days and fish readily adjusted to the experimental diets. Thereafter, fifteen juveniles with an initial body weight of 6.9 $\pm$ 0.2 g were stocked into each 60-l aquarium. Each aquarium was part of a closed recirculation system. During

rearing period, each aquarium was supplied with continuous aeration and water was allowed to flow through at the rate of 200-300 ml/min. Every day, impurities in the water of each aquarium were removed and 50% of the water was renewed to maintain water quality. From each aquarium, water was flown to the physical and biological filters, then in the conditioning tank. Thereafter, the water was drained back to each aquarium by using pump. Dissolved oxygen contents were 4.18-5.13 mg/l, water temperatures 28 to 30 ºC, and pH 6.30 to 6.35. Each experimental diet was fed to the fish in three aquaria. The diets were randomly assigned to groups of fish in the aquarium. The fish were fed on the diets to satiation three times a day at 08.00, 13.00 and 18.00 hrs for 60 days.

At the end of the feeding trial, the fish of each aquarium were bulk weighed from each aquarium. Growth of the fish (as measured by the percentage of daily growth rate), feed conversion ratio (FCR), protein retention (PR) were calculated as described previously (Huisman 1976; Steffens 1989; Takeuchi 1988). After the final weighing, five fish were randomly sampled from each aquarium for body proximate and mineral compositions analysis, and three fish were randomly sampled from each aquarium for analyses of bone mineral (P, Ca and Zn) composition. The proximate composition and mineral analyses were carried out according to the methods described by Takeuchi (1988).

Digestibility trial

The effect of phytase supplementation to the diet on the P digestibility was determined by the indirect method with 0.5% of chromic oxide (Cr_2O_3) as an inert reference substance. The green catfish with the same size as that for the feeding trial were used in this digestibility trial. Twelve fish were stocked into each 60-l aquarium. Triplicate groups of fish were fed on each experimental diet to satiation three times a day for 7 days prior to collection of feces. Feces were collected after 2 h of feeding by siphoning into a plastic sieve. After each collection, the samples from each aquarium were pooled, frozen at -20 ºC and stored for subsequent analyses. Feces collections were conducted for 21 days. Thereafter, the samples of feces were analyzed for chromium (Cr), P and protein content according to the methods described by Takeuchi (1988). Apparent digestibility of total nutrient, protein, and P were calculated as follows:

% digestibility = 1 – (% Cr in feed/ % Cr in feces) x (% ingredient in feces/ % ingredient in feed) X 100

P and Nitrogen (N) losses through feces were calculated as follows:
P in feces (g) = undigestible P (%) x P consumption during culture period
N in feces (g) = (undigestible N (%) x N consumption during culture period)/6.25

Statistical methods

The data were statistically analyzed for differences among the means by the one-way analysis of variance. The Duncan's test was used to compare treatment means using the statistical software SPSS 12 for windows. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Results indicated that P and protein digestibility increased from 64.5% to 87.0%, and 87.1% to 90.5%, respectively, as phytase supplement increased from 0 mg in diet B to 60 mg phytase/100 g SBM in diet E. P digestibility of diet E was higher than that of diet A (77.6%) (Table 3). P digestibility was correlated with the water-soluble P. The water-soluble P content in control diet was 0.59 %. As the phytase supplement increased, water-soluble P increases from 0.19 % in diet B to 0.53 % in diet E (Table 1). It could be observed that the lowest P loss through feces was found for diet E, and the lowest nitrogen (N) loss through feces for diet D. Diet E gave the second lowest N loss through feces. Level of N and P loading by fish fed diet E were 76% and 20%, respectively, lower than those in the fish fed control diet (A).

Table 3. Phosphorus (P) and protein digestibility of experimental diets

Parameters	Experiment				
	A	B	C	D	E
P digestibility (%)	77.6	64.5	70.4	73.3	87.0
P consumption (g)	10.4 ± 0.1^{d}	3.2 ± 0.1^{a}	3.2 ± 0.1^{a}	$3..4\pm0.1^{b}$	4.0 ± 0.0^{c}
P digested (g)	8.1 ± 0.1^{e}	2.0 ± 0.0^{a}	2.3 ± 0.0^{b}	2.5 ± 0.1^{c}	3.5 ± 0.1^{d}
P output via feces (g)	2.3 ± 0.0^{e}	1.1 ± 0.0^{d}	1.0 ± 0.0^{c}	0.9 ± 0.0^{b}	0.5 ± 0.0^{a}
Protein digestibilty (%)	89.1	87.1	85.1	89.7	90.5
Protein consumption (g)	257.4 ± 2.8^{d}	207.6 ± 3.5^{a}	215.6 ± 3.5^{b}	218.2 ± 4.1^{b}	251.8 ± 1.0^{c}
Nitrogen (N) digested (g)	36.7 ± 0.4^{c}	28.9 ± 0.5^{a}	29.4 ± 0.5^{a}	31.3 ± 0.6^{b}	36.5 ± 0.2^{c}
N output via feces (g)	4.5 ± 0.1^{d}	4.3 ± 0.1^{c}	5.1 ± 0.1^{e}	3.6 ± 0.1^{a}	3.8 ± 0.0^{b}

*) Values within the same row with different superscript letters are significantly different, *P<0.05*.

Concentrations of P, Ca and Zn in the whole body and bone of the fish fed diet E were higher than those for the groups of fish fed on the diets supplemented with 0-40 mg phytase/100 g SBM, but were not significantly different from those of the fish fed diet A (Table 4).

Table 4. P, Ca and Zn content in green catfish (% dry weight) fed experimental diets

Parameters	Experiment				
	A	B	C	D	E
Whole body					
P	3.18 ± 0.28^{b}	2.08 ± 0.27^{a}	2.34 ± 0.39^{a}	2.97 ± 0.19^{b}	3.20 ± 0.12^{b}
Ca	3.26 ± 0.06^{c}	1.92 ± 0.30^{a}	2.12 ± 0.07^{a}	2.90 ± 0.20^{b}	3.30 ± 0.09^{c}
Zn	0.012 ± 0.001^{b}	0.009 ± 0.001^{a}	0.012 ± 0.000^{b}	0.012 ± 0.001^{b}	0.013 ± 0.002^{b}

Table 4. Continued

Parameters	Experiment				
	A	B	C	D	E
Bone					
P	21.41± 1.12^{c}	13.44 ± 0.26^{a}	16.65 ± 0.77^{b}	16.99 ± 1.24^{b}	21.46 ± 0.43^{c}
Ca	33.33 ± 0.11^{d}	21.44 ± 1.15^{a}	24.42 ± 0.76^{b}	26.04 ± 1.15^{c}	33.60 ± 1.66^{d}
Zn	0.032 ± 0.002^{b}	0.021 ± 0.002^{a}	0.023 ± 0.002^{a}	0.024 ± 0.003^{a}	0.032 ± 0.001^{b}

*) Values within the same row with different superscript letters are significantly different, *P<0.05*.

The mean body weight gain of the fish fed diet E was higher than that of the fish fed with the other diets. The daily growth rate and feed conversion ration (FCR) followed similar trend, while the fish fed diet B had the lowest daily growth rate and FCR (Table 5). The protein retention had the similar trend with the protein digestibility, which increased from 36.8% from 0 mg phytase/100 g SBM in the diet B to 44.4% from 60 mg phytase/100 g SBM in the diet E.

Table 5. Initial body weight (Wo), final body weight (Wt), protein retention (PR), daily growth rate (DGR), feed consumption (FC), feed conversion ratio (FCR) and survival rate (SR) of green catfish

Parameters	Treatments				
	A	B	C	D	E
Wo (g)	7.0±0.01	7.0±0.02	6.9±0.12	6.8±0.09	6.8±0.05
Wt (g)	43.8±0.8	31.9±1.1	35.2± 0.9	40.2±0.4	47.7±0.4
DGR (%)	3.1±0.0^{d}	2.6±0.1^{a}	2.8±0.1^{b}	3.0±0.0^{c}	3.3±0.0^{e}
PR (%)	39.5±0.2^{b}	36.8±0.4^{a}	40.1±1.4^{b}	41.6±1.4^{b}	44.4±1.9^{c}
FC (g)	709.7±7.6^{e}	570.4±9.7^{a}	585.9±9.5^{b}	601.1±11.2^{c}	688.1±2.7^{d}
FCR	1.3±0.0^{c}	1.5±0.1^{e}	1.4±0.0^{d}	1,2±0.0^{b}	1.1±0.0^{a}
SR (%)	100±0.0	100 ± 0.0	100±0.0	100±0.0	100±0.0

*) Values within a row with different superscript letters are significantly different, *P<0.05*.

Increasing levels of phytase level from 0 mg to 60 mg/100g of SBM resulted in increasing levels of water-soluble P, thereby increasing water-soluble P/total P ratios in the diets from 34.87% to 91.13%. Diet E produced the highest P and protein digestibility, followed by diet A (control diet) (Table 3). It means that phytase released P from the insositol ring of phytate to be water-soluble P (Baruah *et al.* 2004). The water-soluble P was digested in the intestine of fish, and improved the absorption and utilization of P. Phytase also released some protein and amino acids from the phytic acid, hence the absorption and utilization of protein also increased. The same results were found for other fish species, such as the Atlantic salmon *Salmo salar* (Sajjadi and Carter 2004), striped bass *Morone saxatilis* (Papatryphon and Shoares 2001), rainbow trout *Onchorhynchus mykiss* (Cheng and Hardy 2004), Korean rockfish *Sebastes schlegeli* (Yoo *et al.* 2005), and tilapia *Oreochromis niloticus* (Liebert and Portz 2005). On the other hand, the fish fed on the diet B had the lowest P digestibility. It appears that the concentration of phytase in diet B could not enough release all P from phytic acid in the SBM.

Table 4 shows that not only P availability in the diet could be improved by phytase supplementation, but also the other minerals as Ca and Zn which could be available to the fish. Accumulation of Ca, P and Zn in the body and bone of fish also increased as the phytase levels of the diets increased from 0 to 60 mg/100 g SBM. Other experiments also showed that phytase supplemented diet increased Ca, P and Mn contents of the African catfish *Clarias gariepinus* (Nwanna *et al*. 2005), striped bass *Morone saxatilis* (Hughes and Soares 1998), and Atlantic salmon *Salmo salar* (Sajjadi and Carter 2004).

It is known that phytic acid is the major P storage compound in plant seeds, including SBM. Phytic acid is also a strong chelator of important minerals such as Ca, Mg, K, Fe, Cu, Zn and forms poorly soluble complexes. Apart from minerals, phytic acid also forms complexes with protein and amino acids. The digestibility of these complexes by fish are very limited due to the lack of intestinal phytase (Pointillart *et al*.1987). The supplementation of phytase in this experiment could release those minerals and protein from phytic acid, resulted in increasing their (minerals and protein) digestibility for fish (Table 1 & 3), hence the absorption and utilization of minerals and protein also increased (Table 4 & 5). On the other hand, minerals also play a role in many processes of protein, lipid and carbohydrate metabolisms. Increasing the availability of some minerals in the diet by phytase supplementation also appears to improve the protein synthesis, which can be seen from the protein retention data (Table5). The highest protein retention was found in the groups of fish fed on diet E, while the fish fed on diet B had the lowest protein retention. The increased protein retention also improved the protein deposit in the body, hence increasing the daily growth rate (Table 5). In this experiment, the fish fed on diet E had the highest protein retention and daily growth rate. This indicates that phytase could increase the nutrient (protein) digestibility leading to higher protein retention, resulting in the increase of the growth rate and reduce feed conversion ratio. The same conclusions were also indicated by other authors that the phytase supplementation to SBM improved the weight and growth rate of juvenile Korean rockfish *Sebates schlegeli* (Yoo e*t al.* 2005), tilapia *Oreochromis niloticus* (Liebert and Ports 2005; Furuya *et al.* 2001), rainbow trout *Oncorhynchus mykiss* (Vandenberg *et al.* 2003), and *Pangasius pangasius* (Debnath *et al.* 2005). Nwanna *et al*. (2005) also found that supplementation of phytase to a diet containing SBM gave better feed conversion than that for diet without phytase supplementation for the African catfish *Clarias gariepinus*, and the striped bass *Morone saxatilis* (Hughes and Soares 1998).

As earlier explained some of the diet consumed by fish was undigested, and will be excreted through feces. Increasing phytase concentration in the diet from 0 mg to 60 mg/100g SBM reduced the levels of P and N excretion through feces. This indicated that phytase was able to release nutrient (protein) and minerals of phytate, resulting in the improvement of fish growth and also reduced P and N excretion through feces. Hughes and Soares (1998) concluded that phytase in a diet containing high level of plant protein could reduce the P excretion of striped bass *Morone saxatilis*, seabass *Dicentrarchus labrax* (Teles *et al*. 1998), Japanese flounder *Paralichthys olivaceus* (Masumoto *et al*. 2001), rainbow trout *Oncorhynchus mykiss* (Vielma *et al*. 2002), Atlantic salmon *Salmo salar* (Sajjadi and Carter 2004), tilapia *Oreochromis niloticus* (Furuya *et al*. 2001; Liebert and Portz 2005), and the African catfish *Clarias gariepinus* (Van Weerd *et al*. 1999; Nwanna *et al*. 2005), thereby reducing the loading of P waste into the environment.

CONCLUSIONS

The supplement of 60 mg phytase/100 g SBM in the diet improved P digestibility and the growth of the green catfish *Hemibagrus nemurus,* hence reducing the loading of P and N waste to the environment.

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