

# SUBSTITUTION OF FISHMEAL WITH SOYBEAN MEAL IN HUMPBACK GROUPER, *Cromileptes altivelis* JUVENILE DIETS SUPPLEMENTED WITH PHYTASE

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## ABSTRACT

Feeding experiments were conducted to evaluate the effects of replacing fishmeal with soybean meal in diet on growth of humpback grouper. Fifteen cages of 1x1x1.2m<sup>3</sup> each stocked with 16 humpback grouper juveniles (61.3±0.4 g/pc) were set up randomly in seawater. Fish were fed to satiation twice daily for 112 days. The control diet contained 61.9% fishmeal (63.34% crude protein). Four isonitrogenous (48% crude protein) and isocaloric (4.7 kcal/g feed) diets supplemented with commercial phytase "Rhonozyme-P" at 0.075% were formulated to contain different levels (8, 16, 24, and 32%) soybean meal (43.65% crude protein) as a partial replacement for fishmeal. These diets contained total phosphorus levels between 3.6-4.5 (±0.4)% and 0.7-1.5 (±0.04)% available phosphorus. Replacement of fishmeal with soybean meal (8 to 32% replacement) was not significantly different ( $p>0.05$ ) to the control diet on daily growth rate (DGR), food conversion ratio (FCR), protein efficiency ratio (PER), and daily food consumption (DFC). However, the dietary levels of soybean meal significantly affected ( $p<0.05$ ) whole body protein and phosphorus retention. These data suggest that addition of phytase in diets could improve protein and phosphorus availability and reduce nitrogen and phosphorus loading in the environment. Phytase can therefore play an important role in formulating eco-friendly feed for humpback grouper. Based on P loading, supplementation of phytase is able to reduce fishmeal and replace up to 24% with soybean meal.

**KEYWORDS:** replacement, ecofriendly, nutrient retention, fish low pollution

## INTRODUCTION

The expansion of global aquaculture production increases the demand for aquaculture feeds. For carnivorous species, such as barramundi, protein can form up to 60% of the diet (William *et al.*, 2003) and fishmeal is the main source of dietary protein. Fishmeal is readily recognized as the best source of dietary protein and n-3 fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential dietary requirement of tropical marine species (King, 2004). Fishmeal is also 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 manufacture to search for cheaper alternative protein sources such as plant proteins. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fishmeal. Substitution with other ingredients, especially from plant origin, is likely to compromise nutrient balance and sometime fails to match the energy concentration achieved using fishmeal. Plant protein sources contain high lipid and fiber and lack essential amino acids. Considering the increasing cost of fishmeal and doubt concerning its long-term availability, much research has been carried out to find alternative protein sources as fishmeal substitutes. Williams *et al.*, (2003) reported that for aquaculture to make a net contribution to human supplies, the present use of fishmeal in aquaculture diets must be substantially reduced.

From economics point of view, market availability and nutritional value, a prime candidate for replacing fishmeal (FM) in aquaculture diets is soybean meal (SBM). Although the protein content of SBM is less than that of FM, the essential amino acid profiles of processed SBM products compares well with that of FM when considered on a percentage of protein basis (NRC, 1993). Replacement of FM with SBM has tended to reduce fish growth because of the numerous antinutritional factors. However, Nyirenda *et al.*, (2000) reported that animal protein sources could totally be replaced by soybean meal in order to get similar growth rates in *Oreochromis karongae*.

SBM that originates from seed contains phosphorus primarily as the calcium-magnesium salt of phytic acid known as phytin. Phytin phosphorus is unavailable to animals with simple stomachs because of their lack of phytase (the enzyme required to liberate phosphorus from phytat) in the gastrointestinal tract. Phytic acid content in diets can degrade growth rate, FCR and protein retention, influencing thyroid function, increasing mortalities, triggering cataract formation at Zn low level and causing abnormalities of pyloric cecal structure (Richardson *et al.*, 1985 in Halver, 1989). Although diets based on soybean meal generally contain less phosphorus than diets containing animal feedstuffs, increasing the availability of phosphorus from soybean meal products is desirable to restrict the amount of supplemental phosphorus required in diets and also to limit the amount excreted by fish into the environment.

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The availability of phosphorus in SBM is between 29 and 54% (Lovell, 1989).

Phosphorus (P) is an essential element in fish diet for skeletal growth, cellular structure, protein, lipid, and energy metabolism. Soybean meal contain more than 60% P in the form of phytat, a molecule of inositol phosphate. Only 10-30% P in soybean meal is available as inorganic P such as dicalcium phosphate ( $\text{CaHPO}_4$ ), which must be enhanced in diet to fulfill requirements. The combination of inorganic P addition in the diet and phytat-P content in feed ingredients (in an unavailable form) will result in the released high concentrations of free P to the environment and cause eutrophication, algal blooms, fish death, smell, scum, increased turbidity, and sedimentation. For that reason, phytat-P in feed ingredients have to be converted to inorganic P by the enzyme phytase before becoming available to fish.

Felix & Selvaraj (2004) reported that phytase is able to release the phosphorus bound in phytat and this permits feed manufacturers to reduce the fishmeal and lower the cost of feed production. Improved phosphorus utilization can also help reduce the discharge of nutrients into the environment. Phytase can therefore play an important role in formulating eco-friendly aquafeeds. The use of the enzyme is able to reduce fishmeal inclusion by around 5% in most aquafeeds with potential for more as techniques are refined. This may help to reduce the demand for fishmeal from the aquaculture in coming years. The purpose of this study was to evaluate the feasibility of replacing some fishmeal with soybean meal supplemented with phytase in diets of humpback grouper, *C. altivelis*.

## MATERIALS AND METHODS

### Experimental Cages

Feeding experiments were conducted at floating net cages facilities of the Research Institute for Coastal Aquaculture (RICA) in Awarange Bay, Barru Regency, South Sulawesi for 16 weeks from June to September 2003. Fifteen sea cages of  $1 \times 1 \times 1.2 \text{ m}^3$  were hung at one wooden raft and randomly allocated to the different treatments in such a way that each treatment was three replicated.

### Experimental Design and Diets

A complete random design with three replicates (15 experimental units) was used to investigate the feasibility of replacing some fishmeal with soybean meal supplemented with phytase in diets of humpback grouper, *C. altivelis* during 16 weeks growth assay. Before the diets were formulated, all ingredients were analyzed for approximate composition (Table 1), using standard methods for crude protein, crude lipid, crude fiber and ash content (AOAC 1995). The percentage

of crude protein in the diets was determined by semimicro-Kjeldahl analysis, the percentage lipid was determined by the acid hydrolysis method, and crude fiber and moisture determined by drying  $100^\circ\text{C}$  until constant weight.

Five experimental diets were formulated to contain various percentages of soybean meal (SBM) as a partial replacement for fishmeal. The formulation of the diets used in this experiment is given in Table 2. The nutritional composition of experimental diets is presented in Table 3. All diets were formulated to be isonitrogenous (48% crude protein) and isocaloric (4.7 kcal/g feed). The control diet contained 61.9% fishmeal (63.34% crude protein) and 0% SBM. The experimental diets were formulated to contain soybean meal (43.65% crude protein) at different levels (8, 16, 24, and 32%) as partial replacements for fishmeal and supplemented with commercial phytase "Rhonozyme-P" at 0.075%. These diets contained total phosphorus levels between 3.6-4.5 ( $\pm 0.4$ )% and 0.7-1.5 ( $\pm 0.04$ )% available phosphorus.

### Fish and Culture Condition

Juvenile humpback grouper (*Cromileptes altivelis*) were obtained from a commercial backyard hatchery in Gondol, Bali and had an average weight of  $7 \pm 0.2 \text{ g}$ . After arrival the fish were placed in sea cages of  $2 \times 2 \times 2.2 \text{ m}^3$  with stocking densities of 500 fish per cage for acclimation. The duration of acclimation was 2 months. During acclimation the fish were fed a commercial feed twice daily at 07.00 and 16.00 hours to satiation. After acclimation, fish were selected to the uniform in weight with an initial mean ( $\pm \text{sd}$ ) start weight of  $61 \pm 0.4 \text{ g}$ . Sixteen fish were randomly distributed to floating net cages with 3 replication per treatment. Sea cages were suspended from a floating raft in seawater approximately 10 m depth and 1 m daily tidal fluctuation. Before the treatment diets, 10 fish were sacrificed for carcass analysis to determine nutritional composition of the initial fish. During the experiment, fish were fed by hand as much related feed as they would consume in 30 min at 07.00 and 16.00. Feeding was carefully monitored to minimize any food wastage. The feeding trial lasted for 16 weeks. Fish were weighed individually at 4-weekly intervals. The individual fish in each cage were weighed using a top loading balance with a precision of 1 g. At the end of the experiment, four representative fish from each cage were randomly sampled and frozen for determination of the whole body composition according to standard methods (AOAC 1995).

### Laboratory Analysis

#### Chemical analysis

Representative samples of each feed were randomly taken from the feed and ground. Material that passed through a 1 mm sieve used for analysis.



Table 1. Composition (%) of the local feed ingredients used

Ingredients	Crude protein	Total lipid	Crude fiber	Ash	NFE	Total P
Fish meal	63.34	10.08	1.23	18.45	6.90	4.16
Soybean meal	43.65	3.06	5.00	1.63	39.48	1.0
Mysid meal	64.35	4.31	2.31	14.36	14.67	2.13
Wheat meal	9.10	1.68	0.21	1.17	87.83	nd
Rice brand	5.21	7.98	11.95	9.13	65.77	nd
Wheat gluten	71.21	3.43	1.44	8.41	22.66	0.39

NFE: Nitrogen Free Extract

Nd : no data

Table 2. Composition of ingredients of test diets (g/kg diet)

Feed ingredients	Soybean meal levels in diets (g/kg diet)				
	SBM0	SBM8	SBM16	SBM24	SBM32
Fish meal	615.8	557.9	500.0	442.1	384.2
Mysid meal	70.0	70.0	70.0	70.0	70.0
Wheat gluten	70.0	70.0	70.0	70.0	70.0
Rice brand	80.0	60.0	50.0	25.0	0.0
Wheat flour	50.0	50.0	50.0	50.0	50.0
Soybean meal	0.0	80.0	160.0	240.0	320.0
Fish oil	24.0	30.0	36.0	42.0	48.0
Soybean oil	30.0	27.0	24.0	21.0	18.0
Cellulose	21.2	15.3	0.4	0.15	0.05
Vitamin mix <sup>a)</sup>	24.0	24.0	24.0	24.0	24.0
Mineral mix <sup>b)</sup>	15.0	15.0	15.0	15.0	15.0
Phytase	0.0	0.75	0.75	0.75	0.75
Total	100.0	100.0	100.0	100.0	100.0

<sup>a)</sup> At 2.4 g/ kg inclusion level, provided in 1 kg of final diet: retinol, 432 mg; cholecalciferol, 7.125 mg;  $\alpha$ -tocopherol, 169.92 mg; menadione, 300 mg; thiamin, 240 mg; riboflavin, 600 mg; pyridoxin, 240 mg; cyanocobalamin, 2.8 mg; ascorbic acid, 3600 mg; folic acid, 120 mg; nicotinic acid, 1440 mg; D-pantothenic acid, 1200 mg; biotin, 3 mg; and D/L-methionine, 1200 mg

<sup>b)</sup> Mineral mixture (value are in mg/100 g diet): NaHPO<sub>4</sub>, 618; KH<sub>2</sub>PO<sub>4</sub>, 415; CaCO<sub>3</sub>, 282; FeCl<sub>3</sub> 4H<sub>2</sub>O, 166; ZnSO<sub>4</sub>, 10; MnSO<sub>4</sub>, 6.75; CuSO<sub>4</sub>, 2; KI, 0.15; CoSO<sub>4</sub> 7H<sub>2</sub>O, 0.1

Table 3. Nutritional composition of the diets

Analyze	Soybean meal levels in diets (%)				
	SBM0	SBM8	SBM16	SBM24	SBM32
Crude protein (%)	48.56	48.88	48.31	47.75	47.20
Crude lipid (%)	12.93	11.65	11.76	11.81	11.81
Crude fiber (%)	2.89	2.85	2.61	2.81	2.73
Ash (%)	15.27	13.88	14.40	14.32	14.92
Nitrogen Free Extract (%)	20.35	22.74	22.95	23.31	23.34
Total P (%)	4.3	3.6	4.3	4.5	4.2
P available (%)	0.07	0.04	0.15	0.11	0.07
Gross Energy (kcal/kg feed) <sup>1)</sup>	4795.8	4791.2	4776.9	4766.0	4736.2

<sup>1)</sup> Calculated from the determined protein, lipid and non-cellulose carbohydrate of the diet using gross energy conversion coefficients of 5.64, 9.44 and 4.11 kcal/g, respectively (NRC, 1993).

For determination of the body composition of the initial fish, 10 fish from the fish stock were combined for chemical analysis. For determination of the body composition of the fish at the end of the experiment, two fish from each sea cages were combined for chemical analysis. Fish sampled for pooled chemical analyses were frozen and then chopped into small pieces that could be easily minced through a 3 mm

die plate fitted to a meat grinder. A uniform sample of the whole fish body was dried in an oven at 60°C overnight and then ground and sieved through a 1 mm mesh size. A representative sample of feed or whole fish body was analyzed for approximate composition using standard methods for crude protein, total lipid, crude fiber and ash content (AOAC 1995). The percentage of crude protein was determined by micro-



Kjeldahl analysis with distillation into 4% boric acid and titration with sulphuric acid using methyl red indicator for end point determination. Percentage lipid was determined gravimetrically following a chloroform:methanol extraction of the sample, and moisture was determined by drying at 105°C until constant weight.

### Assessment of growth and feed utilization

Biological and nutritional parameters measured were absolute growth (g/fish), survival (%), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention value (PRV). Biological response parameters were calculated as follows:

Weight Gain =  $\{(\text{final weight} - \text{initial weight}) / \text{initial weight}\} \times 100$

Daily Growth Rate (%/d) =  $(\sqrt[t]{\frac{Wt}{Wo}} - 1) \times 100$

Where:

Wt is final weight (g/fish), Wo is initial weight (g/fish), t is rearing periode (day)

Specific Growth Rate =  $100 * (\text{Log}_e \text{ final weight (g)} - \text{Log}_e \text{ initial weight (g)}) / \text{number of days}$

Food Conversion Ratio =  $\text{total dry feed fed (g)} / \text{increase in biomass of fish (g)}$

Daily food consumption (%/d) =  $\{ \text{Food consumed} / ((\text{initial weight} + \text{final weight}) / 2) \times \text{rearing periode} \} \times 100$

Protein Efficiency Ratio =  $\text{increase in biomass of fish (g)} / \text{protein fed (g dry matter basis)}$

Protein Retention =  $100 * \{ \text{protein deposition (g)} / \text{protein intake (g)} \}$

Phosphorus Retention =  $100 * \{ \text{phosphorus deposition (g)} / \text{phosphorus intake (g)} \}$

Nitrogen loading =  $\text{N intake (g)} - \text{N deposit (g)}$

Phosphorus loading =  $\text{P intake (g)} - \text{P deposit (g)}$

### Statistical analysis

Response data were analyzed for statistical significance ( $P < 0.05$ ) by ANOVA in a complete randomized design. Significant differences among means were identified using the Least Significant Different (LSD) procedure. All percentage and ratio data were transformed to arcsine value prior to analysis. Homogeneity of variances between samples was tested using the Bartlett test, normality data was tested using the Lillifors test and additivity was tested using the Tukey test. The significance level of the tests was taken as 0.05. Statistical analysis was performed using MINITAB ver.6.0.

### RESULTS

All fish accepted the experimental diets and the survival rate during 16 weeks feeding trial was between 86 and 94%. Fish mortality during the

experiment was caused by parasite, *Benedenia* sp infection even though the fish were immersed in freshwater for 5-10 minutes every 4 weeks. Humpback grouper juveniles fed diets with different soybean meal levels showed 100-129% weight gain during 16 weeks rearing period (Table 5). The weight gained was statistically not significantly different ( $P > 0.05$ ) for all test diets. The same response also happened in other biological variables such as daily growth rate, survival rate, daily feed consumption, food conversion ratio, protein efficiency ratio, and fat retention. The DGR was lower for fish fed the control diet than for the other four diets, but not significantly different ( $p > 0.05$ ). Weight gain, DGR, DFC, PER, protein retention, phosphorus retention tend to increase as the proportion of soybean meal in the diets increase, while lipid retention in the whole body of the fish decrease as dietary soybean meal increase, but there was no significant difference ( $p > 0.05$ ) (Table 5). The proximate analysis of crude protein and total lipid for fish carcasses (whole body) sampled at termination of the feeding trial correlated well with the protein retention and lipid retention (Table 6). These data suggest that humpback grouper juvenile can be fed diets containing up to 32% SBM without adverse effects on growth performance parameters.

Protein retention and phosphorous retention in the SBM18 and SBM24 treatments were significantly higher than for other treatments ( $p < 0.05$ ). There was no significant difference in N loading, but replacement of fishmeal with soybean meal caused reduction of N load. Partial replacement of dietary fishmeal by soybean meal also significantly affected the P loading (Table 6). The lowest P load was observed in the diets containing SBM8 and SBM24. The highest P loading was found for the control diet and for SBM32; 5.53 gP/kg fish produced and 5.44 gP/kg fish produced, respectively. The quadratic model analysis based on P load and P retention showed that the minimum of P load occurred at 16.27% and 16.31% replacement of fishmeal by soybean meal respectively. These data indicated that supplementation of phytase in test diets could increase phosphorus availability and reduce P nutrient release in the environment.

### DISCUSSION

Substituting fishmeal with less expensive protein sources reduced growth in grass carp (Dabrowski & Kozak 1979), carp (Viola *et al.*, 1982), rainbow trout (Hilton & Slinger 1986), tilapia (Jackson *et al.*, 1982, Shiao *et al.*, 1987), Chinook salmon (Fowler 1991), blue catfish (Webster *et al.*, 1992), red drum (Reigh & Ellis 1992), sleepy grouper, *Epinephelus suilus* (Ahmad *et al.*, 1992); and Australian snapper (Quartararo *et al.*, 1998). On the other hand, Cremer & Jian (1999) found that replacement of fishmeal with dehulled, a high protein soybean meal, resulted in no reduction in pompano fish growth and was a



Table 5. Biological response of humpback grouper fed with differences soybean levels in the diet

Variables	Test diets				
	SBM0	SBM8	SBM16	SBM24	SBM32
Initial weight (g/ind.)	61.17	61.53	61.20	61.07	61.40
Final weight (g/ind.)	122.47	141.10	132.00	133.17	131.13
Weight gain (%)	100.30 <sup>a</sup>	129.30 <sup>a</sup>	115.76 <sup>a</sup>	118.07 <sup>a</sup>	113.53 <sup>a</sup>
Survival rate (%)	85.60 <sup>a</sup>	94.00 <sup>a</sup>	85.67 <sup>a</sup>	91.67 <sup>a</sup>	94.00 <sup>a</sup>
Daily growth rate (%/day)	0.58 <sup>a</sup> (0.08)	0.69 <sup>a</sup> (0.01)	0.64 <sup>a</sup> (0.07)	0.65 <sup>a</sup> (0.04)	0.63 <sup>a</sup> (0.03)
Daily feed consumption (%/day)	1.05 <sup>a</sup> (0.03)	1.05 <sup>a</sup> (0.04)	1.07 <sup>a</sup> (0.09)	1.10 <sup>a</sup> (0.05)	1.17 <sup>a</sup> (0.09)
Feed conversion ratio	2.19 <sup>a</sup> (0.53)	1.67 <sup>a</sup> (0.04)	1.96 <sup>a</sup> (0.13)	1.88 <sup>a</sup> (0.03)	2.03 <sup>a</sup> (0.15)
Protein efficiency ratio	1.05 <sup>a</sup> (0.24)	1.32 <sup>a</sup> (0.03)	1.13 <sup>a</sup> (0.07)	1.20 <sup>a</sup> (0.02)	1.13 <sup>a</sup> (0.08)
Protein retention (%)	22.40 <sup>a</sup> (3.16)	25.03 <sup>ab</sup> (2.20)	29.91 <sup>bc</sup> (2.09)	28.75 <sup>c</sup> (0.66)	21.05 <sup>a</sup> (2.22)
Phosphorus retention (%)	37.79 <sup>ab</sup> (8.97)	43.00 <sup>b</sup> (7.06)	61.86 <sup>c</sup> (2.87)	61.31 <sup>c</sup> (2.21)	31.15 <sup>a</sup> (6.91)
Lipid retention (%)	33.40 <sup>a</sup> (1.00)	29.64 <sup>a</sup> (7.64)	27.80 <sup>a</sup> (12.84)	20.16 <sup>a</sup> (2.99)	21.07 <sup>a</sup> (3.42)

<sup>a)</sup> Data are presented as means with the standard deviation in brackets

<sup>b)</sup> Means in the same row followed by similar superscript are not significantly different (P>0.05)

<sup>c)</sup>

Table 6. Whole body crude protein, crude lipid, and phosphorus of humpback grouper fed diets containing different levels of soybean meal

Soybean meal levels in diets (%)	Crude protein (%)	Crude lipid (%)	Total P (%)
Initial	52.81	8.65	2.81
Final			
0 (SBM0)	58.06	16.82	4.3
8 (SBM8)	57.12	13.45	3.6
16 (SBM16)	59.56	12.27	4.3
24 (SBM24)	60.44	10.24	4.5
32 (SBM32)	58.56	12.42	4.2

significant cost saving for feed. Japanese flounder can effectively grow on diets containing an equal amount of fishmeal and soybean meal without adverse effects (Kikuchi, 1999). Substitution of fishmeal with soybean meal in diets of juvenile cobia *Rachycentron canadum* (initial mean weight, 32 g) showed that up to 40% of fishmeal protein can be replaced by soybean meal protein without causing reduction in growth and protein utilization, but the growth optimum occurred at 16.9% replacement of fishmeal protein by soybean meal protein (Chou *et al.*, 2004). Emperor snapper (*Lutjanus seabaes*) could utilize a diet containing up to 35% soybean meal (Cremer *et al.*, [www.soyaQua.org](http://www.soyaQua.org)). Kaushik, *et al.*, (1995) reported that as much 33-100% substitution of fishmeal by soy protein concentrate, in fish diet of rainbow trout, *Oncorhynchus mykiss*, did not negatively effect nutrient utilization, but up to 50% substitution of soybean meal caused reduction in growth. Hybrid tilapia, *Oreochromis niloticus* x *O. aureus* fed diet content fishmeal and soybean meal of 10% and 44.2% respectively, did not show differences in growth performance, FCR & PER (Wu *et al.*, 2004).

The lack of significant differences in growth performance variables was possibly explained by the supplementation of phytase in four the experimental diets playing an essential role in increasing the availability of nutrient from soybean meal. Phosphorus availability at SBM16 and SBM24 were higher than the other test diets and the control diet and showed a 214.7% and 157% increase respectively over the control diet (Table 7). Supplementation of phytase in test diets was also able to improve utilization of both N and P nutrients so that nutrient loading to environment could be minimized and even lower than in the fish fed the control diet. Phytase enhancement in test diets has certain capacities in improving the availability of nutrient in diets according to the amount of phytase added and the proportion of soybean meal in the feed formulation.

A number of studies have reported the successful use of enzymes to combat anti-nutritional factors in plant proteins for fish feeds. Phytase enriched diets have been shown to have a higher feed intake, growth, and better food conversion efficiency than



Table 7. Assessment of nutrient loading from humpback grouper fed diets containing different levels of soybean meal

Soybean meal levels in diets (%)	Nutrient loading (g/kg fish produced)	
	N	P
0 (SBM0)	123.34 <sup>a</sup> ± 35.31	5.53 <sup>a</sup> ± 1.96
8 (SBM8)	91.14 <sup>a</sup> ± 4.04	3.20 <sup>b</sup> ± 0.47
16 (SBM16)	99.09 <sup>a</sup> ± 6.82	3.01 <sup>b</sup> ± 0.42
24 (SBM24)	94.86 <sup>a</sup> ± 2.24	3.03 <sup>b</sup> ± 0.16
32 (SBM32)	112.64 <sup>a</sup> ± 10.97	5.44 <sup>a</sup> ± 0.30

<sup>a)</sup> Data are presented as means with the standard deviation in brackets

<sup>b)</sup> Means in the same column followed by similar superscript are not significantly different ( $P > 0.05$ )

control diets in channel catfish, as well as reduced phosphorus load in their faecal matter (Jackson *et al.*, 1996). Trout fed with phytase-incorporated soybean based diets have been reported to show a 22% improvement over control fish, as phosphorus availability increased from 46% to over 70% (Forster *et al.*, 1999). Microbial phytase added to diets containing a higher proportion of plant protein have been shown to improve phosphorus and protein digestibility in Atlantic salmon (Carter & Hauler 1998). Sugiura *et al.*, (2001) found that supplementation of phytase in fish diet can improve the absorption of crude protein and various minerals from diets of soybean meal with low ash content, but absorption was lower in diets content both soybean meal and fishmeal with high ash. Phosphorus digestibility will increase as phytase level in diet increases. Phosphorus excretion of fish fed a diet with low ash content with supplementation of phytase was reduced by between 95-98% compared to the phosphorous excretion of fish fed a commercial diet.

Masumoto *et al.*, (2001), reported the effects of phytase on phosphorus bioavailability in soybean meal-based diets for Japanese flounder, *Paralichthys olivaceus*. They found that high phosphorus absorption in fish fed diets supplemented with phytase compared to fish fed a control diet. Phosphorus retention in the whole body of the fish and in plasma P concentration were significantly higher than in fish fed a diet high in phytase content compared to the fish fed a control diet. This study indicated that supplementation of phytase in soybean meal can improve phosphorus bound by phytase Japanese flounder. Supplementation of phytase in diet of striped bass (*Morone saxatilis*) did not effects dry matter or protein digestibility of feed ingredients like isolated soy protein, soybean meal, corn gluten, and wheat middling, but phytase could increase digestibility of phosphorus by around 23% for all feed ingredients (Papatryphon & Soares, 2001). Li & Robinson (1997) found that supplementation of 250 units of microbial phytase per kg feed can improve biological phytat-P available and replace inorganic phosphorous supplements in channel catfish, *Ictalurus punctatus*. The results may indicate that phosphorus digestibility was significantly improved by supplementation of phytase in diets.

Our results showed that addition of phytase to the fish diet can improve nutrient availability in feed ingredients and reduce nutrient load to the environment. This result confirms those of previous studies in channel catfish (Li & Robinson, 1997; Yan *et al.*, 2002); rainbow trout (Vielma *et al.*, 2000; Sugiura *et al.*, 2001); Japanese flounder (Masumoto *et al.*, 2001); and striped bass (Papatryphon & Soares, 2001). This experiment has provided a useful practical formulation for humpback grouper diet. Phytase can therefore play an important role in formulating eco-friendly feeds for humpback grouper. Based on P loading, supplementation of phytase was able to reduce fishmeal and replaced it by up to 24% with soybean meal.

The potential for increased use of soybean meal in aquaculture diets is substantial due to its nutritional value and cost-effectiveness compared to other protein feedstuffs. Increasing the availability of nutrients from soybean meal can possibly be enhanced by enzyme addition.

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