TOPIC AREA
SUSTAINABLE FEED TECHNOLOGY

ALTERNATIVE FEEDS FOR FRESHWATER AQUACULTURE SPECIES

Sustainable Feed Technology/Study/ 07SFT01UC

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INTRODUCTION

Aquaculture is growing rapidly in Vietnam and has the potential to do the same in Cambodia. Production of pangasiid catfish in the Mekong Delta of Vietnam alone exceeded 1 million metric tons in 2008. While some of the food provided to these fish, especially at the larger commercial farms, is pelleted feed from commercial feed mills, many small farmers still use "trash fish" from the Mekong in preparing feed by hand at the farm. In Cambodia, catfish culture is still at the small-farm stage and trash fish comprise the basic feed for the industry (which is considerably smaller in Cambodia than in Vietnam).

As aquaculture expands in Vietnam and Cambodia, the fish called snakehead is becoming popular to culture because of its high value in the market. There are actually two species currently being cultured, *Channa striata*, the snakehead murrel, and *Channa micropeltes*, the giant snakehead. While culture of these is permitted (and growing) in Vietnam, it is prohibited in Cambodia (except for some experimental work) due to its dependence on small fish in the diet. Catfish culture has available commercial pellet diets, so getting farmers to switch from small fish to pellets is a socioeconomic issue. On the other hand, pelleted diets do not yet exist for snakehead in Vietnam and Cambodia.

Piscivorous (fish-eating) fish like snakehead typically require high levels of protein in the diet, reflecting the high protein in their natural diet. The usual source of that protein in pellet diets is fish meal (FM), an international commodity made from species like anchovy, herring, menhaden, capelin and so on. Because of the high price of fish meal, and to reduce the fishing pressure on the aforementioned species, fish nutritionists and aquaculturists worldwide are trying to replace fish meal with plant proteins in diets for fish.

The objective of the study is the development of cost-effective alternative feed for carnivorous freshwater species to replace or reduce the dependence on low value/ trash fish. The results of this study are intended to provide information on alternative diets for snakehead, especially those diets that incorporate local plant materials, in order to build a long-term sustainable industry. Through an economic analysis of costs of the diets (based on costs of fish meal and plant proteins vs. trash fish) and the risks of the unavailability of trash fish in the future, study results allow decisions to be made on the development of feed mills for local production for the snakehead industry.

To meet the objective, the first study, qualitative and quantitative assessment of the regular freshwater trashfish diet for snakehead food, was done. Then, a series of formulated feed experiments were conducted at the wet laboratory and hapas at the College of Aquaculture and Fisheries (CAF) of Cantho University (CTU) to develop formulated feed for snakehead culture. The experiments conducted were:

(i) Weaning methods with formulated feeds for snakehead *(Channa striata)* larave

(ii) Replacement of fishmeal with soybean meal (SM) with or without phytase and taurine in diets for *Channa striata*, and *Channa micropeltes*

(iii) Utilization of rice bran in snakehead *Channa striata* feed

(iv) Replacement of fishmeal with soybean meal with additions of soluble fish attractant or alpha-galactosidase in diets for *Channa striata*

(v) Replacement of freshwater trash fish by formulated feed in snakehead (*Channa striata*) and (*Channa micropeltes*) fingerling diets

(vi) Taste analysis of snakehead fed by different feeds

RESULTS

2.1 Study 1: Qualitative and quantitative assessment of the regular freshwater trashfish diet for snakehead food

The Mekong River Basin hosts one of the most diverse freshwater faunas in the world. There are 1,200 recorded fish species and the number will increase as new species are discovered and classified. Diversity is based on a wide range of permanent and seasonal habitats, which are a result of the Mekong Basin's complex geological history. Most fish species depend on different habitats at different stages of their life and at different seasons of the year. During the flood season, most Mekong species take advantage of the floodplains for feeding, breeding and rearing their young (Sverdrup-Jensen, 2002).
In the Mekong Delta, the fisheries encompass a range of different gears and methods targeting different species groups. In a multi-species fisheries environment such as the Mekong system, it is useful to distinguish different species groups based on different life history strategies. Preliminary calculations suggest a 20 percent increase in fish demand in the Lower Mekong Basin over the next 10 years. Fishing is likely to increase due to population growth and ease of access and this may result in an increase in overall catches. On the other hand, the increase will be accompanied by a continued decrease in many important commercial species in the catches (Sverdrup-Jensen, 2002). However, the

freshwater fishes are used not only for human consumption but also for aquaculture, especially the juvenile fishes caught in flood seasons. Therefore, a study of the freshwater trash fishes was carried out in order to determine the species composition, size frequency and distribution of the low-value fishes used for aquaculture.

2.1.1 Methodology

The study has been carried out monthly during the flood season from August to October 2008. Trash fish samples were collected at snakehead culture farms in Chau Doc, Thoai Son and Chau Thanh district, An Giang province, the Mekong Delta, South of Vietnam. The fish samples were identified by the scientific names and the species composition, length frequency, seasonal occurrence and distribution were determined.

The common species: *Cirrhinus lobatus, Trichogaster microlepis, Anabas testudineus, Mystus mysticetus and Esomus metallicus* were analyzed for chemical composition (moisture, crude protein-CP, crude lipid-CL and crude ash-CF) according to AOAC (2000). Loss on drying was used to determine moisture content, protein (N x 6.25) was determined by Kjeldahl method, lipid was determined by Soxhlet method, and ash was determined by combustion in a muffle furnace.

2.1.2 Results

Species composition

The sampling surveys were carried out at three sampling sites from August to October 2008. Night samples of the trash fish were collected and analyzed. Results showed that 33 species of freshwater fish had been identified and the most common species were *Cirrhinus lobatus* (19.55%), *Trichogaster microlepis* (12.55%), *Anabas testudineus* (10.06%), *Trichogaster trichopterus* (8.24%), *Puntioplites proctozysron* (7.27%), *Mystus mysticetus* (5.63%), *Puntius orphoides* (5.59%), *Esomus metallicus* (4.75%), *Labiobarbus leptocheilus* (3.27%) and *Oreochromis niloticus* (2.95) (Figure 1; Table 1). In total, the species belong to 20 families, of which the families Cyprinidae (24%), Bagridae (10%), Cobitidae (10%) and Osphronemidae (10%) are most heavily represented (Fig 1).

Seasonal occurrence and length frequency distribution

The results showed seasonal occurrence of the most common species as follows:

+ In August: The most abundant species were *Cirrhinus lobatus* (50.4%); *Anabas testudineus* (11.1%); *Mystus mysticetus* (8.7%) and *Barbonymus gonionotus* (7.4%)

+ In September: The most abundant species were *Pangasius bocourti* (22.3%); *Puntius orphoides* (19.2%); *Helicophagus waandersii* (11%); *Ompok bimaculatus* (8.4%) and *Brachirus panoides* (7.9%)

+ In October: The most abundant species were *Puntioplites proctozysron* (47.5%); *Oreochromis niloticus* (25.3%) and *Labeo chrysophekadion* (10.3%).

The results also indicated that many commercial species were exploited at very small size or in the juvenile stage (Table 1) such as *Anabas testudineus*, Cá Rô Đồng (TL_{min}= 13mm); *Puntius orphoides,* Cá Đỏ Mang (TL_{min}= 17 mm). The length frequency analysis showed that *Cirrhinus lobatus* was exploited mostly from 60 to75 mm in total length, while the other species were exploited with a larger range of total lengths.

Chemical composition

Chemical composition of the common species is shown in Table 2. Crude protein, crude lipid and crude ash of *Anabas testudineus* are the highest values reported, whereas those of *Trichogaster microlepis* are lowest for both protein and lipid.

To sum up, thirty-three species of freshwater fish have been used as "trash fish" or low-value fish for aquaculture in An Giang province, and the most abundant and common species is *Cirrhinus lobatus*. Chemical composition of some common species fluctuated from 14.3 - 16.5% protein, 1.97-8.39% lipid, and 2.48-4.67% ash. Many of those fishes are commercial species and some of them are target species for aquaculture in Vietnam, such as *Anabas testudineus*, *Pangasius bocourti, Oreochromis niloticus.* Therefore, those fish stocks should be assessed and the inland fishery should be managed properly, especially in flood season.

2.2 Study 2: Weaning methods using formulated feeds for snakehead *(Channa striata)* larvae

First feeding is one of the critical periods in fish larval rearing. Zooplankton such as *Brachionus, Moina* and *Daphnia* are frequently used as food resources in freshwater larviculture and for ornamental fish. They contain a broad spectrum of digestive enzymes such as proteinase, peptidase, amylase, lipase and even cellulase that can serve as exo-enzymes in the gut of the fish larvae (Lavens and Sorgeloos 1996). The quantity and quality of food given, including the types of food used in each of the developmental stages, can also be critical in larval rearing and most importantly can affect economic aspects. Larval rearing has been successful for freshwater and marine fish larvae using brine shrimp *Artemia* sp (Léger et al., 1986), for walking catfish *Clarias macroceplalus* using *Moina* (Fermin et al., 1991), and for European catfish *Silurus glanis* using *Tubifex* worms (Ronyai and Ruttkay, 1990). It was also reported that some catfish (*Clarias gariepinus* and *Heterobranchus longifilis*) can be reared exclusively on formulated diet (Appelbaum et al., 1988). However, it often resulted in lower growth and survival rate than live foods or trash fish. So the present study aims at comparing growth performance and survival rate of *Channa striata* larvae when weaning from live feed to formulated diets.

2.2.1 Methodology

After absorbing the yolk on day 3 after hatch, larvae were fed with *Moina*. The experimental treatments were initiated when the larvae were 10 days after hatch (dah). We tested three ages of larvae to begin weaning: 10, 17 and 24 dah. Larvae were fed ad libitum. For 10-dah treatments, the weaning procedure consisted of 10% or 20% of *Moina* biomass replaced daily by formulated diet until fish were feeding exclusively on formulated diet (i.e., the procedure took 10 days or 5 days, respectively, for the 10% and 20% treatments). Similarly, for the 17-dah and 24-dah treaments, trash fish biomass was replaced by formulated diet at a rate of 10% or 20% per day for each treatment. The control treatment was fed with *Moina* replaced by 20% per day trashfish within 5 consecutive days and then trashfish was fed to the end of the experiment (Table 3). Formulated feed contained 50% crude protein and 4.7 $kcal.g^{-1}$.

Larvae were stocked in 50-L tanks with stocking density of 5 fish/L. The fish were fed to satiation by hand twice daily. The remaining feed and faeces were siphoned out before feeding. Daily records were kept on mortality, food consumption and water quality, such as temperature, pH and dissolved oxygen. Larvae were weighed and measured at biweekly intervals. The water was maintained at 28±2°C. At the beginning and the end of the experiment, samples of fish were sacrificed for proximate analysis.

At the end of the experiment, final body weight (FBW, mg) and wet weight gain (WWG, mg) were determined. Differences among treatments were determined by one-way ANOVA with means separated using Duncan's Multiple Range test at $p = 0.05$ using SPSS 13.0.

2.2.2 Results and Discussion

The growth of larvae in treatment 10day-20FF (3.21 g) differed significantly from other treatments (Table 4), but growth in other treatments was not significantly different. The survival rates of larvae in treatments 10day-10FF and 10day-20FF (15.23 and 2.33%) were significantly lower than other treatments and were significantly different from each other (Table 4). The total biomass gain was highest in the control treatment but not significantly different from the 17- and 24-dah at replacement treatments of 10% per day. Hence, *Channa striata* larvae can be fed with trash fish for 17 days and then be weaned to formulated diet. This could greatly reduce the amount of trash fish used and therefore protect freshwater fishes, which are usually fed to snakehead.

Lavens and Sorgeloos (1996) reported that many small fish larvae produce insufficient enzymes for digesting non-living diets. Enzymes present in their live prey carried out digestion (autolysis) in the fish larvae. Formulated diets often lead to poor growth and survival rates. Use of a commercial trout starter diet resulted in good survival rates with value of 12% in *Clarias gariepinus* (Fermin and Bolivar, 1991), 32% in *Heterobranchus longifilis* (Kerdchuen and Legendre, 1994) and 67.5% in *Pangasius bocourti* (Hung *et al.,* 1999).

In fishes, cannibalism is usually associated with heterogeneous size variation, lack of food, high density, lack of refuge area and light condition. Among these variables, size variation and unsuitable food are considered the primary causes of cannibalism. Cannibalism was reported in most larval rearing especially in artificial diet treatments. For example, the cannibalism in *Clarias gariepinus* larval rearing contributed more than natural mortality (Hecht and Appelbaum, 1987). In this study, *Channa striata* displayed a high cannibalism in dry diet treatments. Low survival rate may be related to snakehead behavior. Conversely, *Pangasius bocourti* larvae had a low cannibalism even in the artificial feeding treatment (Hung et al., 1999). In summary, weaning snakehead on formulated feed can begin at 17 dah with relacement of 10% per day.

2.3 Study 3: Replacement of fishmeal with soybean with or without phytase and taurine in diets for *Channa striata* and *Channa micropeltes*

Soybean meal is the most common fish meal-replacement source in aquafeeds, having the best amino acid profile among plant protein sources (NRC, 1993). Nevertheless, the use of soy protein in feeds presents a number of challenges associated with low methionine and cystine content, lower protein digestibility, indigestible oligosaccharides, low phosphorus availability, antinutritional factors and poor palatability (Hertrampf and Piedad-Pascual, 2000). Soybean meal contains phytic acid (NRC, 1993), which is the major compound for phosphorus storage (over 70%) in the plant seeds and can not be digested and absorbed by mono-gastric animals including fish (Jackson et al.,1996). Many fish nutritionists have tried to supplement phytase, myo-inositol-hexaphosphate phosphohydrolase enzyme, to liberate free phosphorus from phytic acid. Phytase is produced either by microorganisms or present in some plant ingredients. Therefore, phytase supplementation is advantageous when significant portions of plant protein meals such as soybean meal are used in fish feeds. Research on phytase supplementation was carried out with positive results in several species, including channel catfish (Jackson et al. 1996), striped bass (Hughes and Soares, 1998), Atlantic salmon (Sajjadi and Carter 2004), rainbow trout (Cheng and Hardy 2004), and *Labeo rohita* (Baruah et al. 2007). Taurine derives from methionine via cysteine and is not considered to be among the ten indispensable amino acids, nor is it incorporated into protein; however, it has several physiological roles and is relatively abundant in fish meal. The ability of fish to synthesize taurine is species dependent and possibly affected by the stage of development. The beneficial effects of dietary taurine supplementation have been demonstrated in many species. In sea bass fry, *Dicentrarchus labrax,* a taurine supplemented diet caused an increase in growth rate when fish meal and soybean meal were the primary sources of protein (Martinez et al., 2004). The main goal of this study is to find out the appropriate soybean meal level to replace fish meal with and without phytase or taurine supplementation in snakehead (C*hanna striata*) and giant snakehead (*Channa micropeltes*) diets. In addition, essential amino acids (EAA) were added to all the diets containing soybean, in order to eliminate any EAA deficiencies compared to the control diet.

2.3.1 Methodology

Experimental fish

Before starting the experiments, all the fish were reared in 2000-L round tanks and were fed with trash fish combined with pellet diets for 4 weeks. Replacement of trash fish by pellet feed was applied gradually at a rate 10% per day until 100% of trash fish was substituted by pellet feed.

Experimental design

In each of experiments 1 and 2, nine diet treatments were set up randomly into 27 experimental tanks (500-L composite tank) with three replicates for each treatment. Thirty *Channa striata* fingerlings (4.7g in initial weight) were assigned to each tank and were fed to satiety.

In experiment 3, five diet treatments were set up randomly into 15 experimental tanks (500-L composite tank) with three replicates for each treatment. *Channa micropeltes* fingerlings (4.70–4.82g in initial weight) were randomly distributed into the 15 tanks with 25 individuals per tank. At the beginning of each experiment, 20 fingerlings from the stock tank were sacrificed for assessment of their initial proximate body composition. In addition, at the end of the experiment, all fish from each replicate were collected for the final proximate body composition analysis.

During the trial, the fish were fed by hand three times a day at 8:00, 12:00 and 17:00 hours. The amount of feed consumed was adjusted on a daily basis and recorded. Total fish weight in each aquarium was determined every 4 weeks and dead fish were recorded and weighed for calculating feed conversion ratio (FCR). Water temperature, measured daily, ranged from 27.0–28.5^{o}C. pH and dissolved oxygen, measured weekly, varied from 7.0–7.2 and 5.0–7.6 ppm, respectively.

Experiment 1: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa striata *diets

Nine practical diets were formulated to replace 0%, 20%, 30%, 40% and 50% of fish meal by soybean meal without phytase supplemention (FM, SM 20%, SM 30%, SM 40% and SM 50%, respectively); and 20%, 30%, 40% and 50% with phytase addition (SM-P 20%, SM-P 30%, SM-P 40% and SM-P 50%, respectively) on a protein equivalent basis in the diet. In addition, the EAA's lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Composition of the experimental diets is shown in Table 5.

Experiment 2: Replacing fish meal by soybean meal with EAA and taurine additions in* Channa striata *diets

Nine practical diets were formulated to replace 0%, 20%, 30%, 40% and 50% of fish meal by soybean meal without taurine addition (FM, SM 20%, SM 30%, SM 40% and SM 50%, respectively); and 20%, 30%, 40% and 50% with taurine supplemention (SM-T 20%, SM-T 30%, SM-T 40% and SM-T 50%, respectively) on a protein equivalent basis in the diet. In addition, the EAA's lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Composition of the experimental diets is shown in Table 6.

Experiment 3: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa micropeltes *diets

Five basal diets were formulated to be isonitrogenous, 44% crude protein, and isocaloric, 4.5 Kcal / g diet in gross energy. The control diet (FM) was made with FM as the main protein source. In diets 2 - 5, SM was substituted for an isonitrogenous amount of FM control diet, to replace 20% (diet 2 - SM 20), 30% (diet 3 - SM 30), 40% (diet 4 - SM 40) and 50% (diet 5 - SM 50) of the FM crude protein (Table 7). In addition, the EAA's lysine, threonine and methionine were added to the SM diets to eliminate deficiencies caused by substitution of fish meal. Also, phytase enzyme (Ronozyme, dry powder) was added in diets 2 to 5 at 0.02%/ kg feed.

The experimental diets were made in a laboratory pellet mill by blending all of the dry ingredients. The extruding temperature did not exceed 40^{0}C. After extruding, all diets were dried at 45°C within 48h and stored at 4°C prior to use.

Data calculation

At the end of each period, fish were weighed and counted to calculate survival rate (SR), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and economic conversion ratio (ECR)

SR (%) = (Numbers of fish at the end of experiment / numbers of initial fish) x 100

WG = Final body weight – Initial body weight

DWG = (Final body weight – Initial body weight) / Experiment time

FI = (Feed intake/no. fish)/ No. days

FCR = Feed intake / Weight gain

PER = (Final body weight – Initial body weight) / Protein intake

ECR = Feed cost x FCR

Chemical analysis

Feed was analyzed for chemical composition (moisture, crude protein-CP, crude lipid-CL, crude fiber-CF, nitrogen free extracts-NFE and gross energy) according to AOAC (2000). Loss on drying was used to determine moisture content; protein (N x 6.25) was determined by Kjeldahl method; lipid was determined by Soxhlet method; crude fiber was determined by acid and base hydrolysis; and gross energy was determined by bomb calorimetry. Carbohydrate-NFE equals 100-(CP+CL+CF). Moisture, crude protein, crude lipid, crude ash and nitrogen free extracts were determined in fish collected at the beginning and end of each experiment.

2.3.2 Results and Discussion

Experiment 1: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa striata *diets

The fish growth showed that the final weight, weight gain, daily weight gain of fish fed FM, SM 20%, SM 30%, SM-P 20%, SM-P 30% and SM-P 40% were not significantly different (Table 8). In contrast, the growth performance of fish fed SM 40%, SM 50% and SM-P 50% were significantly lower than those fed the control (FM) feed. There was no significant difference in survival rate among diets (Table 8).

FCR in fish fed the SM 50% was significantly higher than that of fish fed all the diets with less than 40% SM replacement; all the other diets were not significantly different from each other (Table 9). Similarly, PER in fish fed the SM 50% diet was significantly lower than that of fish fed FM, SM 20%, SM – P 20% and SM 30%; all the other diets were not significantly different from each other (Table 9).

From an economic point of view, it can be seen that replacing FM by SM up to 40% in protein content achieved economical benefit; however, the greatest gains were seen in the

SM – P 20% and SM – P 30% diets (Table 10). Thus, replacement of fish meal with SM, especially with phytase added, appears to be an economically benficial strategy.

Experiment 2: Replacing fish meal by soybean meal with EAA and taurine additions in* Channa striata *diets

In this experiment, the final weight and daily weight gain of fish fed FM, SM 20%, SM 30%, SM-T 20%, SM-T 30% and SM-T 40% were not significantly different; in contrast, the growth performance of SM 40%, SM 50% and SM-T 50% were significantly lower than control (FM) feed. There was no significant difference in survival rate of fish among diets (Table 11).

Feed intake (FI) data indicated significant differences only between fish fed the SM 50% diet and those fed the FM, SM 20%, and SM – T 20% diets: feed conversion ratio (FCR) data indicated significant differences between both of the SM 50% diets and all the diets up to 30% SM, with the exception that the SM – T 50% diet did not differ from the SM 30% diet; and protein efficiency ratio (PER) data indicated differences between the SM 50% diets and the FM diet, as well as between the SM – T 50% diet and the SM – T 20% diet (Table 12).

From an economic point of view, it can be seen that replacing fishmeal by soy meal up to 30% in protein content achieved economical benefit (Table 13). Cost for one kg fish weight gain was reduced by a maximum of 6.90 % in treatment SM-T 30% compared to the control treatment.

Experiment 3: Replacing fish meal by soybean meal with EAA and phytase additions in* Channa micropeltes *diets

Fish in the FM treatment showed significantly higher final weight and daily weight gain (0.38±0.05 $g.day^{-1}$) than those in the SM 50 treatment (0.28±0.01 $g.day^{-1}$). Survival rate of fish among treatments was not significantly different (Table 14).
Significant differences in FCR and PER were seen only between the FM and SM 50 treatments (Table 15). Thus, soybean meal can replace up to 40% of fish meal in terms of protein with phytase supplementation in diets for *C. micropeltes*.

From an economic point of view, it can be seen that replacing FM by SM up to 40% in protein content achieved economical benefit (Table 16). Cost for one kg fish weight gain was reduced 9.59% in treatment SM 40 compared to control (FM) treatment. The substitution can be applied in practical terms because it did not affect growth rate and mortality of fish.

The study concluded that with phytase supplement of 0.02% in diet, FM could be replaced by SM up to 40% in terms of protein which not only achieved growth performances, feed utilizations and survival rates of giant snakehead juveniles, but also succeeded from economic point of view.

There was no significant difference in survival rate among treatments *(P>0.05)*. Daily weight gain of fish showed a downward trend when replacement fish meal with soybean meal level from FM to 50% SM without phytase or taurine supplemention. However, the growth was improved with phytase or taurine supplemention, namely, this growth performance was good at SM-P 40% or SM-T 40% while it reached SM 30% without phytase or taurine additions. It is clear that soybean meal can replace up to 40% of fish meal diets for juvenile snakehead if phytase or taurine are added. However, taurine was disadvantagious in cost for one kg fish weight gain because feed conversion ratios in taurine diets were not optimal. Thus, taurine should not be used in snakehead diets. Many previous studies that used taurine in marine fish diets achieved positive results. In the results of Experiment 3 optimal results for both survival rate and economic efficiency were achieved with replacement up to 50%, but growth performance and efficiency ratio results indicated that the replacement level should only be up to 40%.

The present study demonstrated that SM with phytase supplements could replace dietary FM protein up to 40% without negative effects on growth performances, feed utilizations and survival of *Channa striata* and *Channa micropeltes* fingerlings. Many studies reported that the supplementation of phytase to P-inadequate diets has been shown to enhance growth performance. Soltan *et al*. (2008) studied the maximum replacement levels of fish meal (FM) by a plant protein mixture (cottonseed, sunflower, canola, sesame and linseed meals) in diets for Nile tilapia. They found that fishmeal can be replaced up to 45% with growth rate not differing significantly from that of fish fed control diet. The incorporation of plant protein mixture in diets did not significantly affect whole-body dry matter and crude protein of fish. From an economic view, it reduced feed costs/kg diet and feed costs/kg weight gain by 11.40 and 6.74%, respectively. An increase of weight gain has been reported in channel catfish fed phytase supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson et al., 1996). The supplemental effect of phytase on growth performance in fish cannot simply be compared because it could be different depending on fish species and rearing conditions, and more specifically on dietary composition in each feeding study (Cao et al., 2007). Generally, growth improvements were observed in the studies that used diets entirely or almost entirely based on plant protein sources. However, growth performance responses to phytase supplementation were somewhat inconsistent (Cao et al., 2007).

The present study showed that fish meal protein in *Channa striata* and *Channa micropeltes* fingerlings diets can be replaced by soybean meal protein up to 40% with phytase supplements in which growth performances, feed utilizations and survival of two species are not affected.

2.4 Study 4: Utilization of rice bran in snakehead *Channa striata* feed

Introduction

Rice bran also is a rich source of protein (8.34-16.3%), oil, dietary fiber, and micro nutrients (Hien et al., 2006). Rice bran has been used in formulated feed for terrestrial animals and aquaculture species. Moreover, rice bran is an available and abundant crop by-product in Mekong Delta. In order to reduce the cost of feed for snakehead, diminish trash fish use and reduce the environmental impact, rice bran is thought to be the best ingredient. The goal of this study is to find out the appropriate level of rice bran in formulated feed that can achieve the optimum growth and cost-effectiveness. There has been some research on utilization of rice bran as feed of some species such as Nile tilapia *Oreochromis niloticus* (Perschbacher and Lochmann, 1999; Liti et al., 2006), silver barb *Puntius gonuonotus* (Mohanta et al., 2006), *Streptocephalus proboscideus* (Ali and Dumont et al., 2002), sub - adult mud crab *Scylla paramamosain*. Hien et al., (2006) reported defatted rice bran could be used in diets for tilapia *Oreochromis niloticus* and striped catfish *Pangasius hypophthalmus* with increased growth rate and reduced feeding cost.

2.4.1 Methodology

Feed was formulated from main ingredients such as Kien Giang fish meal, defatted soybean meal, cassava meal, and dried rice-bran. Experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.7 Kcal gross energy/g diet. The ratio of protein fish meal and protein soybean meal level was 6:4. Diet 0% RB was considered as control treatment. The other treatments contained 10% , 20%, or 30% RB, as indicated in the composition of the experimental diets shown in Table17. The experiment consisted of four treatments with three replicates per treatment and 50 fish per replicate. Snakehead fingerlings (4.51-4.63g in initial weight) were assigned randomly to each 500-L composite tank. Water temperature, measured daily, ranged from 27.0–28.0^{o}C, pH and dissolved oxygen, measured weekly, were 7.6–7.7 and 6.68–6.76 ppm, respectively. The experiment lasted eight weeks.

Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments**.**

2.3.2 Results and Discussion

Daily weight gain of fish in treatment 10% rice bran (0.29±0.02 g.day^{-1}) was significantly higher than that of fish in the control treatment that had no rice bran in the diet; moreover, final weight and daily weight gain of fish in treatment 20% and 30% rice bran in diet was not significantly different from that of fish in the control treatment.and there was no significant difference among RB 10%, 20%, 30% treatments in daily weight gain (Table 18). Survival rates of fingerlings were high and ranged from 60 to 69.3% and there was no significant difference observed (Table 18). This study obviously indicates the possibility of using formulated diets for rearing snakehead in captive conditions. According to Trieu *et al.,* (2001), survival rates of snakehead fingerlings during 4 weeks in tank conditions ranged from 60.2 to 100%. The growth performance of fish in this experiment was better than that of snakehead fingerlings (initial weigh 5.22g) fed with 50% CP (1.19%.day^{-1}) which were studied by Trieu *et al.,* (2001).

Feed intake fluctuated between 227 and 293 mg fish^{-1}day^{-1} among treatments and showed significant idfferences, with the lowest FI in treatment RB 0% and the highest FI in RB 10% treatment; however, no significant differences were seen in FCR or PER (Table 19).

The improvement in growth performance of snakehead fingerling fed rice bran diets when compared to the result from 0% RB diets may have been caused by the presence of micronutrients in rice bran. Data shown in table 17 indicate the cassava meal content decreased along with the increase of rice bran in diets. Rice bran is abundant in trace minerals and vitamin B, especially vitamin B1 (thiamine) that are neccesary for growth.

Vitamin B1 plays a major role in carbohydrate metabolism (Jean *et al*, 2001). There was no significant difference in FI observed in RB 10% and RB 30% treatment. However, the growth response of snakehead in RB 10% treatment was better, inducing an FCR in this treament lower than the FCR in RB 30% and the lowest FCR in this experiment. That result may be caused by the highest fibre content in RB 30% diet (4.31%) whereas the cassava meal (which plays a role as a good binder for the diet) was absent, reducing the stability of the pellet and raising waste feed,alhough there was no significant difference in FCR and PER observed among treatments. The lower the FCR is in cultivation, the more efficient the feed utilization is. Ningrum (2005) found in Asian catfish (4.9 g/fish in initial weight) fed 55.6% rice bran in diet that FCR was low (1.3), and SGR and PER were high (4 % per day and 2.6, respectively)

From an economic point of view, it can be seen that replacing fishmeal and soybean meal by rice bran up to 30% in protein content achieved economical benefit (Table 20). Cost for one kg fish weight gain was reduced 6.88 % in treatment RB 30% compared to the control treatment.

In summary, rice-bran could be well utilized by snakehead fingerlings with levels from 10% to 30% without any differences in growth performance and carcass composition. Hence, rice bran could be used in home-made formulated feed for snakehead fingerlings up to 30% to reduce feed cost.

2.5. Replacement of fishmeal with soybean with soluble fish attractant or alpha-galactosidase in diets for *Channa striata*

Soybean meal can replace up to 30% of fish meal in the diet of snakehead without addition of phytase or 40% of fish meal with the addition of phytase (Hien et al., 2009). To improve utilization efficiency of plant protein for snakehead, replacement of fish meal by soybean meal was conducted at higher levels of 50, 60 and 70% with soluble fish attractant or alpha-galactosidase added. Alpha-galactosidase is added to some animal feeds to improve utilization efficiency of plant protein. Feeding attractants such as betaine, squid viscera meal, and L-amino acids (L-alanine, L-glutamic acid, L-arginine) have been used to increase the palatability of plant protein diets for fish. Mackie and Mitchell (1985) summarized the results of various studies using dietary feeding attractants, and reported the positive effect of mixtures of dietary free amino acids as feeding stimulants in rainbow trout, *O. mykiss* (Adron and Mackie, 1978); European eel, *Anguilla anguilla* (Mackie and Mitchell, 1983); Japanese eel, *A. japonicus* (Takeda et al., 1983); sea bass, *Dicentrarchus labrax* (Mackie, 1982); and red seabream, *Chrysophyrys major* (glycine betaine plus L-amino acids; Goh and Tamura, 1980). Therefore, the present study investigated the effects of supplementing dietary α-galactosidase and a feeding attractant solution on the nutriient digestibility and growth performance in fingerling snakehead fed diets containing more than 40% SM.

2.5.1 Methodology

Diets were formulated to contain different levels of soybean meal from 50% to 70% for protein fish meal with α-galactosidase (Experiment 1) (Table 21) and feeding attractant solution (Experiment 2) supplementation (Table 22), compared to control treatment (fish meal-FM). All of the experimental diets were formulated to be isonitrogenous and isoenergetic to contain 45% crude protein (CP) and 4.5 kcal gross energy/g of diet. Rice bran and cassava meal were also used as sources of plant protein in the diets. Methionine, lysine and phytase were all also added to the diets, based on results of our previous experiments.

In each of experiments 1 and 2, four diet treatments were set up randomly into 12 experimental tanks (500L composite tank) with three replicates per treatment. Thirty *Channa striata* fingerlings (2.24–3.79g in initial weight) were assigned to each tank and were fed to satiety. Water temperature, measured daily, ranged from 26.5-27.5^{o}C. pH and dissolved oxygen, measured weekly, varied 7.2-7.5 and 5.0–7.6 ppm respectively. Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments.

2.5.2 Results and Discussion

In experiment 1, there were no significant differences among the experimental treatments in final weight, daily weight gain, or survival rate (Table 23), as well as in FI (Table 25); However, there was a significant difference in FCR between the 70% SBM diet and all the other diets, as well as signficant differences in PER between the 60% and 70% SBM diets and the FM and 50% SBM diets (Table 25). We found in previous studies that SBM can replace up to 30% of FM in the snakehead diet without addition of phytase or 40% of FM with the addition of phytase (Hien et al., 2009). The results of this study showed that addition of α-galactosidase to snakehead diets could allow the replacement of FM with SBM to be as high as 70% (based on growth results), 60% (based on FCR results), or 50% (based on PER results. The decrease in PER with increased inclusion of plant protein was seen previously in Atlantic cod (*Gadus morhua L.*) (Hansen et al., 2007).

In experiment 2, there were again no significant differences among the treatments in final weight, daily weight gain, survival rate (Table 24) or FI (Table 26); howe ver, there were significant differences in FCR between the SBM 70% treatment and all the other diets, as well as in PER between the SBM 60% and 70% diets and the FM and SBM 50% diets (Table 26). Positive effects of feeding attractants was previously shown in *Epinephelus malabricus* by adding 1% of squid viscera meal for using a blend of rendered animal protein ingredients to replace fish meal in practical diets (Wang et al., 2008).

Considering economic efficiency, cost for one kg fish weight gain decreased 9.91% and 10.5% in 50% SBM diet compare to control diet in Experiments 1 and 2, respectively; however, the replacements over 50% were not economically efficienct because of increasing FCR (Table 27 and Table 28).

In summary, SBM can replace up to 60% of FM in the snakehead diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant. However, considering economic efficiency, SBM can only replace up to 50% of FM in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant.

2.6 Replacing freshwater trash fish by formulated feed in snakehead (*Channa striata*) and (*Channa micropeltes*) fingerling diets

Snakehead culture mainly relies on trash fish supplied and feeding cost is the biggest cost to the farmer, so many problems have been observed. The most important of these problems are poor quality of trash fish and variable nutritional composition because of inappropriate storage. Risk of disease introduction, environmental pollution and high feed conversion in snakehead rearing contributed more concerns. Moreover, the growing competition between human and aquaculture usage of low value fish (trash fish) led to increasing its price to the farmer (Rachmansyah et al., 2009). For those reasons, it is necessary to develop cost-effective and high-performing compounded feeds that would allow less reliance on trash fish and would have lower environmental impacts. It was recognized that snakehead previously fed on trash fish would not readily take a dry feed and thus development of an appropriate feed acceptable to the fish was an important aspect of our feed development work. This study, therefore, was designed to determine the percentage of trash fish that could be replaced by formulated feed for optimum growth and survival of snakehead. The cost of feed was also calculated.

2.6.1 Methodology

Based on the results of our previous experiments, we developed a feed formulation for snakehead with FM, SBM, rice bran and cassava meal, with addition of the EAA's methionine, threonine, and lysine and the the addition of phytase and fish solution feeding attractant (Table 29). We then set up the experimental replacement of trash/low-value fish diets with this 45%-protein formulated feed (FF) (Table 29) at levels of 0 (control), 25, 50 75, or 100%. The five treatments each had three replicates with 50 fish per replicate and were conducted in hapas. Before the startof the experiment, snakehead fingerlings had been acclimated to formulated feed for 30 days.

The fish (4.7g in initial average weight) were assigned randomly to each hapa. The experiment lasted eight weeks, during which fish were fed twice/day to satiation at 9:00 and 16:00 hours. Total fish weight in each hapa was determined every 4 weeks and dead fish were recorded and weighed for calculating feed conversion ratio (FCR). After feeding, the remaining feed was weighed daily. Water temperature, measured daily, ranged from 29.5-30.5^{o}C. pH and dissolved oxygen, measured weekly, varied from 5.2-5.5 and 6.0–7.4 ppm, respectively.

Methods for data calculation, chemical analysis and statistical analysis were identical to those used in the phytase experiments**.**

2.6.2 Results

Experiment 1: Replacing freshwater trash fish by formulated feed in* Channa striata *fingerling diets

Survival rate of fish in the 100% FF treatment (complete replacement of trash fish by formulated feed) was 73.3%, significantly lower than all other treatments, which had very high survival rates, 95.3 to 92.7% (Table 30). There was a trend of reducing growth response with increased replacement by FF and fish fed diets consisting of 0% FF, 25% FF and 50 % FF exhibited significantly higher final weight and daily weight gain than did fish in the 75% FF and 100% FF treatments (Table 30).

FI and FCR were significantly reduced at each increasing level of FF replacement, but no significant differences were seen in PER (Table 31). It is likely that the significant reductions in FI and FCR were due to different moisture levels in the diets that the fish received, since trash fish is 72.7 % moisture, whereas formulated feed is 9.38% moisture.

According to the economic analysis, feed cost per kilogram weight gain decreased with increasing formulated feed in diets (Table 32), with a reduction up to 35.5% using 100% FF compared to the trash fish diet (0% FF). The aforementioned growth performance and survival rate of snakehead were not significantly different when 50% of trash fish was replaced by formulated feed. At this replacement proportion, the feed cost was reduced considerably by 22.1% compared to the diet containing 100% trash fish.

Experiment 2: Replacing freshwater trash fish by formulated feed in* Channa micropeltes *fingerlings diets

In this experiment, fish in the 0% FF treatment (i.e., fed only trash fish) had significantly higher survival than all of the other treatments (Table 33). Final weight and daily weight gain were significantly higher in the control, 25 FF and 50 FF treatments than were those in the 75 FF and 100 FF treatments.

In terms of feeding, FI differed significantly among all treatments, except that the 75 FF and 100 FF treatments did not differ significantly; FCR differed significantly among all treatments, except that the 50 FF and 75 FF treatments did not differ significantly; and PER in the 0 FF treatment was signifcantly higher than that of all other treatments, which did not differ significantly from each other (Table 34).

The economic analysis showed that cost per kg of fish weight gain was reduced the more the percentage of trash fish was replaced, except for the 25 FF treatment (Table 35).

Snakehead is a carnivorous species. In this study, although they were weaned from trash fish to formulated feed, live food was still their favorite feed. The diet containing no trash fish reduced attraction of fish to feed. Simply providing formulated feeds led to cannibalism in this species in a previous study (Qin et al., 1996). Utilization of commercial pellet feed nowadays is more popular, especially for carnivorous fish in order to reduce the dependence on trash fish, feeding cost and environmental impact. Several studies on replacing of trash fish by formulated feed in several species achieved better growth rate and more profit, such as tiger grouper, *Epinephelus fuscoguttatus* (Rachmansyah et al., 2009); Japanese sea bass, *Lateolabrax japonicus* and red drum, *Sciaenops ocellata* (Cremer et al., 2001); and sea bass, *Lates calcarifer* (Aquacop et al., 1989). Cremer et al. (2001) replaced trash fish with formulated diets in cage culture of red drum (*Sciaenops ocellata*) (172g/fish in initial weight) and Japanese sea bass *Lateolabrax japonicus*) (74g/fish) and concluded that fish consuming formulated diet (43% crude protein, 12% lipid) with 35% soybean meal showed better growth and less feeding cost than fish fed trash fish.

Replacing trash fish by formulated feed brought more benefits in feeding cost and less dependence in trash fish supply in Singapore (Aquacop et al., 1989). Sea bass (*Lates* *calcarifer*) could use pellet feed (45% crude protein) in fingerling stages but required high levels of fishmeal quality. Grouper (*Epinephelus fuscoguttatus*), a carnivorous species which requires a high protein content (445-50%) in diet, fed a mixed diet of formulated feed and trash fish with the ratio 1:1, performed insignificantly differently from fish fed trash fish completely (Rachmansyah *et al.,* 2009).

To sum up, if only economic efficiency were considered, replacing fresh water trash fish by formulated feed in two species diets up to 100% is possible. If both growth performance and feed conversion ratio were considered, the replacement should stop at 50%. Thus, depending on the farmer's situation, they should choose the optimal solution for replacing fresh water trash fish by formulated feed in snakehead culture. The data provided in this study help to enable them to make that choice.

2.7 Taste analysis of snakehead fed by different feeds

Snakehead is a good quality aquaculture species which is highly prized by consumers. We have conducted several experiments to improve pellet feed for culture of this fish. One urgent question is whether improved feed affects snakehead product quality, so it was necessary to conduct an experiment for snakehead sensory analysis with fish fed by different feeds for experimental treatments.

2.7.1 Methodology

The experiment was carried out with 18 hapas (1m x 1m x 2m) in an experimental pond for two fish species (*Chana striata* and *Chana micropeltes*), which were transferred from An Giang province, Vietnam. Before starting the experiment, snakehead fingerlings had been acclimated to formulated feed for 30 days. The fish ranging from 4-5 $g.fish^{-1}$ were assigned randomly to each hapa, fifty fish per hapa in stocking density. Every treatment was triplicated and the experimental period was 16 weeks. During the trial duration, fish were fed twice/day to satiation at 9:00 and 16:00 hours.

The experiment was conducted with 3 treatments: freshwater trash fish and two formulated feeds containing 45% crude protein that had been developed from our previous studies. The first formulated feed was based on fish meal only and the other was based on a combination of fish meal and plant proteins (soy bean meal and rice bran) (Table 36).

At the end of the experiment, all fish were killed, filleted and washed, then they were steamed for 8 minutes. First, these fish were used to determine the difference in the quality of fish fillet between the control and experimental groups by a triangle test (2 controls and 1 sample) with three replacements per test.

There were three groups of control-samples for both *C. micropeltes* and *C. striata*: trashfish-fishmeal (TF-FM), trashfish-plant protein (soybean and rice bran) (TF-PP), fishmeal-plant protein (FM-PP).

If less than 8 out of 12 subjects detected the odd sample correctly, we determined that there was no significant difference and therefore no need to conduct a sensory test. A pair test was run if there was any difference in any sensory attributes for texture or taste even if they were minor – called a 'descriptive pair test'. On the other hand, if 9 out of 12 people detected the odd sample correctly, there was a significant difference at $P<0.01$ or 8 out of 12 $P<0.05$. In this case, it was necessary to do a comprehensive pair test on appearance, texture and taste.

A pair test is hedonic and scored on an intensity scale (1-9 points) on appearance such as liking (1, least like – 5, o.k. – 9, like very much), whiteness (1, dark – 5,medium – 9, very white), and structural integrity (uniformity: 1, very irregular – 5, medium – 9, very uniform); taste, for example liking (1, least like – 5, o.k. – 9, like very much); snakehead-like taste (1, very little – 5, o.k. – 9, very much)' presence of objectionable taste (yes or no) and presence of objectionable odor (yes or no); texture, for instance, liking (1, least like – 5, o.k. – 9, like very much); firmness (1, very soft – 5, medium – 9, very firm); moistness (1, very dry – 5, medium – 9, very moist); chewiness (1, mushy – 5, medium – 9, very chewy); and flakiness(1, least or rubbery – 5, medium – 9, very flaky).

Statistical analysis
Mean values of results in different treatments were compared by paired sample t-test using SPSS 13.0 software. Treatment effects were considered with the significance level at $P< 0.05$.

2.7.2 Results
The result showed that there was no significant difference between paired samples in triangle tests (less than 8 out of 12 people detected the odd sample correctly) (Table 37). These samples were then subjected to "descriptive" pair tests, with the result that the quality of fish fillet samples did not significantly differ. In appearance, both *C.micropeltes* and *C.striata* were approximately 5 to 6 on the scale (Table 38 and Table 39), meaning that the fish fillet was passable or fairly likable for liking; medium or rather white for whiteness; medium or relatively uniform for structural integrity. In taste, the fish fillet was snakehead-like taste, and without the presence of objectionable taste and odor. In texture examination, for liking, the score was from 4 to 6, from not rather like – passable – fairly like. About firmness, the result was 3-5, soft – relatively soft – medium fish fillet. The fillet moistness was rather dry and medium (not dry and not moist). The fillet chewiness and flakiness was fairly mushy and relatively rubbery or medium (not mushy and not chewy; not rubbery and not flaky).

In summary, *C. micropeltes* and *C. striata* fillet quality was fairly liked and did not significantly differ between samples in triangle tests. The desciptive pair tests gave the same result as triangle tests and there was no significant difference between samples. So, these diets do not affect the quality of fish fillet for both *C. micropellets* and *C. striata.*

CONCLUSION

- Thirty-three species of freshwater fish were identified as being used as "trash fish" or low-value fish for snakehead culture and the most abundant and common species is *Cirrhinus lobatus*. Chemical composition of some common species fluctuated from 14.3 to 16.5, 1.97-8.39, 2.48-4.67 in crude protein, crude lipid and crude ash, respectively. Most of those fishes are commercial species and some of them are target species for aquaculture in Vietnam, such as *Anabas testudineus* and *Trichogaster trichopterus*. Therefore, those fish stocks should be assessed and the inland fishery should be managed properly, especially in flood season.

- Weaning onto formulated feed for snakehead larvae can begin by 17 days after hatch with relacement ratio of 10%.day^{-1}
- Up to 40% of fish meal in *Channa striata* and *Channa micropeltes* fingerling diets can be replaced by soybean meal with phytase supplements with no significant loss of growth performance, feed utilization or survival of the two species.
- Rice-bran could be well utilized by snakehead fingerlings with levels from 10% to 30% without any differences in growth performance and carcass composition. Hence, rice bran could be used in home-made formulated feed for snakehead fingerlings up to 30% to reduce feed cost.
- Soybean meal can replace up to 60% of fish meal in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant. However, considering economic efficiency, protein soybean meal only can replace up to 50% of protein fish meal in the diet with the addition of phytase and alpha-galactosidase or fish solution feeding attractant.
- Considering economic efficiency, replacing fresh water trash fish by formulated feed in two species diets up to 100% is possible. If both growth performance and feed efficiency ratio were of interest, the replacement should stop at 50%. Thus, depending on a farmer's situation, they should choose their own optimal solution for replacing fresh water trash fish by formulated feed in snakehead culture.
- *C. micropeltes* and *C. striata* fillet quality in a taste test was fairly liked and did not significantly differ between samples. In descriptive pair tests, there was no significant difference between samples. Thus, formulated feed (fish meal or plant protein) did not significantly affect the quality of fish fillet in both *C. micropeltes* and *C. striata* compared to a diet of trash fish.

ACKNOWLEDGMENT

The authors would like to thank Angiang Rural Development and Agriculture departments for data collection and snakehead fingerling nursing sites.

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