# Alternative Feeds and Processing for Freshwater Aquaculture Species, Part II

## USE OF SOY PRODUCTS IN SNAKEHEAD DIET

Enhanced Trade and Investment for Global Fishery Markets/Experiment/13SFT03UC

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### ABSTRACT

Soybean meal-based formulated feeds have recently become available for snakehead culture in Vietnam. The first part of this study was conducted to determine the appropriate replacement of fish meal (FM) protein by soy protein concentrate (SPC) in snakehead (*Channa striata*) diet. The study included five iso-nitrogenous (45%crude protein) and iso-caloric (19KJ/g) practical diets that were formulated to replace 0% (control), 40%, 60%, 80% and 100% of protein FM by protein SPC (100% FM, 40% SPC, 60% SPC, 80% SPC, and 100% SPC, respectively). Moreover, a digestibility experiment was also followed with the same formulated diet with addition of 1% of chromium oxide. Results showed that fish fed 100% FM and 40% SPC diets had significantly better growth rate (weight gain and daily weight gain) and survival rate compared to other treatments. Feed intake, feed conversion ratio, protein efficacy ratio, and net protein utilization, trypsin and chymotrypsin activities of experimental fish fed 100% FM and 40% SPC diets was significantly higher than those fed other diets. The apparent digestibility coefficient (ADC) of diet (ADC diet), ADC protein and ADC lipid of fish fed diet 40% SPC and 100% FM treatment was significantly higher than those of other treatments. The feed production cost in diet 100% FM and 40% SPC was much lower compared to other treatments. The increase level of SPC in diet above 40% significantly affected on the fish growth, economic efficiency, digestibility, and trypsin and chymotrypsin activities though fish chemical composition unlikely affected.

The second objective of this study was to evaluate the effectiveness on growth performance and immune responses of mannan oligosaccharide (MO) supplementation in both soybean meal (SBM) and soy protein concentrate (SPC) formulated feeds for snakehead (Channa striata). The experiment included three feed groups, one using fishmeal (FM) as the only protein source, the second replacing 40% of the FM with soybean meal (SBM), and the third replacing 40% of FM with SPC. Each feed group was then divided into three feed treatments which added 0%, 0.2%, and 0.4% MO. The diets were 44.3-45% protein and 19KJ/g energy. Eighty snakehead fingerlings (7.05 ± 0.08 g/fish) were assigned randomly to each of twenty-seven 500-L composite tanks with continuous aeration and 30% daily water exchange. MO supplementation of diets based solely on FM versus diets in which 40% of FM had been replaced by either SBM or SPC, both final weight (Wf) and weight gain (Wg) were significantly affected by diet and MO supplementation, as well as the interaction between the two. In general (with some exceptions), growth performance of fish was significantly better when they were fed SPC than when they were fed SBM or FM, and MO supplementation generally improved growth of the fish. FCR, PER, and survival of fish in this experiment was significantly affected by diet, but only survival was significantly affected by MO supplementation and in no case were the interactions significant. FCR was significantly improved

(i.e. lower) when fish were fed the SPC diet compared to the SBM diet, but neither was significantly different from fish fed the FM diet. PER for fish fed the FM and SPC diets was significantly greater than that for fish fed the SBM diet.
Survival of fish fed SPC diet was significantly lower than that of fish fed the FM and SBM diets, but supplementation with MO, especially at the level of 0.2%, significantly improved survival. Red blood cell (RBC) counts were not significantly affected by either diet, MO supplementation, or the interaction of the two, but white blood cell (WBC) counts were significantly affected by both diet and MO supplementation (although not the interaction). Fish fed the SPC diet had significantly higher WBC counts than fish fed the FM diet, but neither group was significantly different from fish fed the SBM diet. MO supplementation at both 0.2% and 0.4% levels significantly increased WBC counts compared to the unsupplemented diets. Immunoglobulin (Ig) levels were significantly increased by MO supplementation and the interaction of MO and diet, but diet did not affect Ig levels. At the end of the feeding trial but prior to the bacterial challenge, lysozyme levels were significantly affected by diet, MO supplementation and the interaction between the two. For each diet, the greater the level of MO supplementation, the greater the level of lysozymes is. Reduction of fish production costs per kg fish produced, compared to fish fed the FM unsupplemented diet as the standard, ranged from 8.7 – 15.1% for the various other diets tested. Following a 15-d bacterial challenge with Aeromonas hydrophila, fish lysozyme levels were significantly increased by MO supplementation and the interaction between MO and diet, but not by the diets themselves. Again, the greater the level of MO supplementation, the greater the lysozyme level is. After the 15-day challenge, cumulative mortality was lower for fish given MO supplementation than it was for fish fed the unsupplemented diets. In general, fish fed the SBM diet, supplemented or not, had lower cumulative mortality than fish fed the FM or SPC diets.

Women in An Giang province were trained to feed formulated feeds with MO to snakehead and they carried out demonstration projects to show that snakehead can be reared in hapas or tanks to increase household income by US$200–400.

## INTRODUCTION

Snakehead is popularly cultured in the Mekong Delta of Vietnam in a variety of farming systerns such as ponds, hapas, cages, and lined tanks (Sinh and Chung, 2009). Over the past few years, snakehead culture increased rapidly; in 2010, total production of snakehead in the Mekong Delta was about 40,000 tons (Sinh et al. 2011). However, most snakehead farmers at that time cultured by the traditional method, using fishmeal and trash fish, meaning high production costs. According to Sinh and Pomeroy (2010) to harvest 30,000 tons of cultured snakehead fish, about 50,000 tons of small freshwater fish and 75,000 tons of small marine fish respectively are required per year. Wild fish supplies are reduced due to overfishing, so finding other sources of protein, especially plants, to replace fish meal (FM) is necessary and urgent. Feed costs account for 80% of the total snakehead production cost (Hien et al. 2011). Snakehead is a carnivorous species with high protein requirement, up to 40% (Samantary and Mohanty, 1997; Be and Hien, 2010). In aquaculture feed, FM is the main protein source due to high digestibility, vitamin, mineral, and high unsaturated fatty acid which is essential for aquatic animals. However, FM production is being reduced and prices have increased, so alternative protein sources for aquatic animals are important. Soybean meal (SBM) is becoming more widely used. Research on the replacement of FM by SBM has been conducted on many species, e.g. cobia (Rachycentron canadum) (Chou et al. 2004), Korean rockfish (Sebastes schlegeli) (Lim et al. 2004), Asian seabass (Lates calcarifer) (Tantikitti et al. 2005), Atlantic cod (Walker et al. 2010), snakehead (Channa striata) (Be and Hien, 2010), giant snakehead (Hien et al. 2010), and knife fish (Dan et al. 2013). However, SBM contains anti-nutritional factors like trypsin inhibitor, hemagglutinins, phytate, soyantigens, and lacking of methionine and cysteine (O'Keefe and Newman, 2011).

Many soybean products are used in aquafeed, e.g. SBM, soy protein concentrate (SPC), and fermented soybean. FM can be replaced by SBM up to 30% in diets for knife fish (Dan et al. 2013), but only at 20% inclusion in diets for spotted rose snapper (Lutjanus guttatus) (Silva-Carrillo et al. 2012). SPC (65- 67% crude protein) has had anti-nutritional factors removed by alcohol extraction (Dersjant-Li, 2002). FM can be replaced by SPC from 40 - 100% in diets for rainbow trout (Médale et al. 1998), juvenile cobia (Salze et al. 2010), and Atlantic cod (Walker et al. 2010). Moreover, the supply of soy products is more stable and economical than the supply of FM (Hertrampf and Piedad-Pascual, 2000). Since Hien et al. (2014, 2015, submitted, in preparation) found that replacing 40% of FM by SBM does not affect the survival and growth rate of snakehead (C. striata) use of commercial feeds containing SBM in snakehead farming has increased rapidly. The first aim of this study was to determine the appropriate replacement level of FM by SPC in diets for C. striata fingerlings.

Ward et al. (2016) showed that SBM-based diets likely contain immunostimulants for summer flounder (*Paralichthys dentatus*), whereas SPC-based diets do not. Furthermore, Ward (2014) and Ward et al. (in preparation) showed that the immunostimulants were most likely the oligosaccharides stachyose and raffinose. We had wanted determine whether those compounds would stimulate immune response in snakehead as well. Unfortunately, purified forms of those substances are prohibitively expensive for commercial usage in snakehead diets. We therefore chose to test commercial immunostimulant products (see below).

Intensive farming of snakehead often leads to disease development, with pathogens such as bacteria (*Aeromonas hydrophila* and *A. sobria*) and fungi (*Aphanomyces invandans*) causing economic losses. In intensive systems, the fish are sometimes cultured at stocking densities up to 120-160 fish/$m^2$ (Sinh et al. 2011) and fish easily get stressed and develop infectious diseases, especially epizootic ulcerative syndrome (EUS) (Miles et al. 2001). Additionally, Duc et al. (2012) demonstrated that parasites, fungi, and bacteria infected cultured snakehead in An Giang and Dong Thap provinces.

Successful supplementation with immunostimulants to diets for aquatic animals can increase the resistance to pathogens. Some of these immunostimulants derive from *Saccharomyces cerevisiae*, a fraction of the cell wall of which is rich in mannan oligosaccharides (MO). MO is effectively used as an immunostimulant in many species. For example, in striped catfish (*Pangasianodon hypophthalmus*) addition of 0.12% MO (Actigen, Alltech, USA) significantly increased the survival rate, lysozymes, and leukocytes during bacterial challenges with *Edwardsiella ictaluri* (Hung et al. 2008). Supplementation of 0.2% MO in the diets for salmon (*Oncorhynchus mykiss*), led to an absolute growth rate 5.59% higher than that of the control and survival rates of fish tended to rise (Sara et al. 2011). In tilapia (*Oreochromis noloticus*), adding 0.2% MO significantly improved the FCR, lysozyme, bactericidal activity compared to control and reduced by 20% the cost of feed to produce one kilogram of fish (Ahmad et al. 2013).

The second aim of this study was therefore to determine whether MO supplementation in diets containing 40% replacement of FM with SBM or SPC improves the growth and immune response of snakehead (*C. striata*). The study included both feeding trials and bacterial challenge experiments. If MO does improve growth and immune response, then a suitable concentration of MO might be used to improve commercial snakehead farming and increase economic efficiency.

Finally, scenarios of climate change and/or damming of the Mekong River could greatly impact the availability of fish-based products for inclusion in diets for snakehead. Soy-based products would still be available however. Furthermore, from the international commodity perspective, FM is both very expensive and subject to variable availability that causes price spikes. SPC is less expensive and not subject to such variability. For these reasons, development of snakehead diets with maximum inclusion of SPC and added immunostimulants should represent a more sustainable future for snakehead aquaculture. Women make up more than 50% of the population in the Lower Mekong Basin (LMB). Our previous

studies showed that male labor was dominant in fish farming practices (78.4% of farmers), but the participation of women in farming snakehead species was high (21.6% of farmers) in comparison with other cultured fish species in Vietnam (often less than 10%) (AquaFish-CRSP project, 2010). In flooding season, the changes in the hydrological regime (water levels, duration of flooding, timing of flooding) affects aquaculture in the LMB. From 2010, Can Tho University has developed small-scale aquaculture for flooding areas, especially small-scale farming of snakeheads in hapas and plastic linned tanks. These models were judged to be very effective for flooding seasons and women participants (more than 70% women participants). However, small-scale farmers of snakeheads still use small fish for feeding. So, developing the small-scale farming of snakeheads using formulated feed is very necessary for environmental control and aquatic resources management. On-farm trials in An Giang province aimed to apply the optimal formulated feed for snakehead culture from the CTU trials under actual farm conditions with owners.

## OBJECTIVE

To continue the development of cost-effective alternative feeds for carnivorous freshwater species for small-scale farming of snakeheads by women during the flooding season; and to improve the processing activities for added value of cultured snakehead products, particularly for women.

## MATERIALS AND METHODS

**Replacement of FM by SPC, growth experiment.** The study included five iso-nitrogenous (45% protein) and iso-caloric (19 KJ/g) practical diets that were formulated to replace 0% (control), 40%, 60%, 80%, and 100% of protein FM by protein SPC (100% FM, 40% SPC, 60% SPC, 80% SPC, and 100% SPC, respectively) (Table 1). Experimental fish (10.0 ± 0.5g in initial weight) were transferred to CTU from a nursery in An Giang province and acclimated in tanks (four $m^3$) for two weeks. Fish were then randomly assigned at stocking density of 30 fish/tank to 15 composite tanks (500L/tank) with aeration supplied. Each treatment was triplicated. Water was exchanged every two days at a rate of 50%. Experimental period was 42 days. Fish were fed twice a day (8:00 and 16:00) to satiation. Amount of feed was recorded very day and excess feed was removed and recorded. Dead fish were collected and weighed. Temperature, pH and dissolved oxygen were measured twice a day by YSI 556 (USA); $NO_2^-$ and $NH_3$ were recorded every week by test kit SERA (Germany). Temperature was 28.6 – 31.2°C (morning and afternoon). Dissolved oxygen was above 5 mg/L. pH was 7.70 – 8.05. $NO_2^-$ ranged from 0.63 – 0.70 mg/L and $NH_3$ was below 0.1 mg/L.

Initial fish weight (Wi) and final fish weight (Wf) were determined before and after the experiment. We determined Survival Rate (SR, %), Daily Weight Gain (DWG; g/d), Feed Intake (FI; %/fish/d), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Net Protein Utilization (NPU) as follows (where t = time in days):

SR = (number of initial fish/number of fish at the end of experiment) x 100

$DWG = (W_f - W_i)/t$

$FI = \text{consumed feed}/(W_f \times W_i)^{0,5}/t$

FCR = amount of consumed feed in dry matter (g)/ weight gain (g)

$PER = (W_t - W_o)/$ protein intake

NPU = (protein intake-protein waste)*100/protein intake

Initial fish (six fish/tank), final fish (six fish/tank) and feed (100 g) were collected, minced and stored at -20°C until analysis. Chemical composition of fish and feed were analyzed following methods of AOAC (2000). Fish at the end of experiment (final fish, three fish/tank) were collected after one day of starvation for digestive enzyme analysis (trypsin and chymotrypsin) following methods described by Tseng et al. (1982) and Worthing (1982).

**Replacement of FM by SPC, digestibility experiment.** Experimental feeds were formulated in the same composition as the growth experiment, except chromic oxide ($Cr_2O_3$) was added to all diets at a level of 1%. The experimental system for feces collection (Figure 1) consisted of a series of 250-L composite tanks with aeration. Fish (10 g) were stocked at 20 fish/tank and fed twice a day (8:00 and 16:00) to satiation for the first ten days. Excess feed and dead fish were removed. Feces collection began after 10 days of feeding and continued for 20 days, when the amount of feces (about 10 g dry matter) was sufficient for analysis. For feces collection, fish were fed once at 8:00 and the remaining feed was removed after two hours, tanks were cleaned, and feces collection chambers were installed. Feces were collected overnight in a collection bottle stored in the chamber on ice. Feces were collected every day, dried at 60°C and stored at -20°C until analysis. Temperature, pH and dissolved oxygen of water were measured twice a day by YSI 556 (USA); $NO_2^-$ and $NH_3$ were recorded every week by test kit SERA (Germany). Temperature was 27.0 – 29.3°C (morning and afternoon), dissolved oxygen was above five mg/L, pH was 7.60 – 8.20, $NO^{2-}$ ranged from 0.12 – 0.17 mg/L and $NH_3$ was below 0.1 mg/L.

Feces and experimental feed were analyzed for crude protein, crude lipid, moisture and ash following AOAC (2000). Chromic oxide ($Cr_2O_3$ was analyzed following the method of Furukawa and Tsukahara (1966). Calculations included:

Apparent Digestibility Coefficient of diet (ADCdiet) $ADC_{diet} = 1 - \frac{\%A}{\%B}$

Apparent Digestibility Coefficient of nutrient in diet (ADCNu-Diet)

$ADC_{Nu-Diet} = 1 - \frac{\%A}{\%B} \times \frac{\%B'}{\%A'}$ where A is % $Cr_2O_3$ in feed, B is % $Cr_2O_3$ in feces, A' is % nutrient in feed, and B' is % nutrient in feces.

**Feeding and bacterial challenge trials with mannan oligosaccharides. A** feeding trial was conducted to evaluate survival, growth, feed efficiency, and immune response parameters. The experiment included three feed groups, one using only FM as the protein source and the second and third using SBM and SPC, respectively, to replace 40% of the FM. Each feed group was subdivided into three feed treatments to which were added either 0.0% (control), 0.2%, and 0.4% MO (Alltech, USA). Thus, the experiment consisted of nine treatments with three replicates. The nine experimental diets were formulated to be 45% protein, 9% lipid, and 4.2 Kcal/g energy (Table 2). All ingredients were mixed mechanically with water for 30 minutes and the resulting dough was then passed through an extruder to obtain pellets of 2-mm diameter. The diets were dried in direct sunlight for six hours, then allowed to cool at room temperature for 0.5 hours, and finally stored in airtight plastic bags until use. Proximate composition of the diets was analyzed using AOAC (2000).

Prior to the experiment, fingerlings were transferred from a hatchery in An Giang province to CTU, acclimated in a 2,000-L circular tank, and fed on the control (FM) diet. The average initial weight per fish was 7.05 g for each experiment. To start the experiment, fingerlings were randomly distributed into 27 composite tanks (500-L capacity, filled with 300 L of water) at a stocking density of 80 fish/tank. Each experimental tank was provided with continuous aeration and flow through water supply with 30% water exchange/day. Fish were fed two times/day (9:00 am, 3:00 pm) to satiation. The amount of consumed feed and uneaten feed in each tank was recorded daily (the amount of uneaten feed was siphoned out after 30 minutes, dried and weighed). At the end of the experiment, all of the fish in each tank were counted and weighed for calculation of growth rate and survival rate. Any fish mortality was recorded daily and dead fish were removed and weighed immediately. The experimental period was eight weeks. Temperature ranged from 27.5-30.1ºC, dissolved oxygen from 5.22 to 5.42 mg/L, pH from 7.53 to 8.01, $NO_2^-$ from 0.62 to 0.69 mg/L and $NH_3$ <0.1 mg/L. Therefore, the water quality parameters in all treatments were a suitable range for the normal growth and development of fish.

After eight weeks, three fish from each tank were randomly collected and blood withdrawn for analysis of erythrocytes, leukocytes, lysozymes, and total immunoglobulin (Ig). The remainder of the fish were then tranferred to the bacterial challenge experiment. Data on FCR and PER were calculated as indicated above for the earlier feeding experiment. Red blood cells were counted by the usual method using the Neubauer chamber and Natt – Hedrick solution (Natt and Hedrick, 1952). White blood cells were counted on lame that was stained by Wright's & Giemsa solution (Hang et al. 2013). Lysozyme was analyzed by the method of Ellis et al. (1990). Total Ig was analyzed by the method of Siwicki and Anderson (1993), modified by Milla et al. (2010).

A bacterial challenge was conducted after the growth trial to determine the snakehead's immune response to *Aeromonas hydrophila*. Fish from each growth trial treatment were divided into two groups. Thus, the experiment consisted of 18 treatments: 9 treatments (3 from the FM group, 3 from the SB group, and 3 from the SPC group) were injected with 0.2 ml physiological saline (0.85%) and the remaining nine treatments (three from the FM group, three from the SB group, and three from the SPC group) were injected with $2.32x10^5$ CFU/fish of bacterial strain CL1403 *Aeromonas hydrophila* based on the lethal dose determined by Duc et al. (2013). Each treatment was triplicated. Experimental fish had average weights of 49.87-50.73 g and were randomly assigned to 100-L plastic tanks at a density of 15 individuals/tank. The experimental period was 15 days, during which time fish were fed their respective diets and dead fish were recorded daily. For moribund fish, clinical signs were observed by gross inspection, and the lesions were sampled directly for bacteria. Re-isolation and re-identification of bacteria were carried out according to methods of Barrow and Feltham (1993). Water exchange was 20%/day. After 15 days, three fish were randomly collected to withdraw blood and analyze lysozyme as feeding trial.

**On-farm trials with women farmers.** Women snakehead farmers in An Giang province participated in a training course on snakehead culture using pelleted feed led by CTU researchers. Six of the women who operated small-scale snakehead farms were selected for demonstration projects using formulated feed. Three farms culture snakehead in hapas (24 $m^2$) and the others use plastic-lined tanks (15 $m^2$). Formulated feed was provided based on previous results with added 0.2% MO. Snakehead fingerlings were stocked at 80 fish/$m^2$ in hapas and 100 fish/$m^2$ in tanks. Culture period was 5-7 months, until snakehead reached market size. Information on total cost (fingerlings, feed, chemical and etc.) and income were recorded and calculated for economic benefits. Calculation methods were as described above for the laboratory studies. Dissolved oxygen, pH, nitrite, and ammonia were monitored during the rearing trial and were considered suitable for snakehead survival and growth.

**Statistical analysis.** Results of the first growth experiment and the digestibility study were compared by one-way ANOVA followed by Duncan's multiple range test (SPSS 16.0, USA) at significant level of 95%. Results of the second feeding trial and the bacterial challenge experiment were analyzed by two-way ANOVA followed by Duncan's multiple range test.t. at significant level of 95%.

## RESULTS

**Replacement of FM by SPC, growth experiment.** Survival rate did not differ significantly between treatments 100% FM and 40% SPC ($p>0.05$), which were both significantly greater than the other treatments ($p<0.05$) (Figure 2). Fish fed 100% FM and 40% SPC diets did not show significant differences in growth, but both grew significantly more than fish fed 60% SPC and 80% SPC diets ($p>0.05$), which in turn did not differ, and the smallest growth performance was found in fish fed 100% SPC diets (Table 3). There was no significant difference in the fish growth performance between and also in between 100% FM and 40% SPC diets. Feed intake (FI), feed conversion ratio (FCR), protein efficacy ratio (PER) and net protein utilization (NPU) of experimental fish fed 100% FM and 40% SPC diets was significantly higher than those fed other treatments ($p<0.05$; Table 4). Fish were classified into four size groups such as 10 – 20g, 20 – 40g, 40 – 60g and 60 – 75g. The fish size distribution was highly varied

between treatments (Figure 3). Fish fed 60% SPC, 80% SPC and 100% SPC diets were mostly in sizes of 10 – 20g and 20 – 40g. However, in treatments 100% FM and 40% SPC, harvested fish were mostly in sizes of 40 – 60 g and 60 – 75g, whereas only 2 – 4% were in the size range of 10 – 20g.

Chemical composition of experimental fish between treatments showed little variation (Table 5). Fish moisture in fish of treatment 100% SPC was significantly lower than that of 100% FM treatment ($p<0.05$). Fish protein content showed no significant differences between treatments ($p>0.05$). Lipid content of fish fed 100% FM (3.45%) was significantly higher than those of fish fed 60%, 80 and 100% SPC. Ash content of fish fed the 40% SPC diet was significantly lower than that of fish in all other treatments except 80% SPC, and ash content of fish fed the 60% SPC diet was significantly higher than that of all other treatments except 100% FM. Trypsin and chymotrypsin activities in fish fed diet 100% FM and 40% SPC, which did not differ, were significantly higher than those of fish fed 60, 80 and 100% SPC ($p<0.05$) (Fig. 4).

Feed production cost in diets 100% FM and 40% SPC was much lower compared to other treatments (Table 6). Although feed costs were quite similar between diets during replacement of FM by SPC, the significantly lower FCR in treatments 100% FM and 40% SPC appears to have lowered feed production cost.

**Replacement of FM by SPC, digestibility experiment.** The apparent digestibility coefficient of diet ($ADC_{diet}$) of fish fed diet 40% SPC was significantly higher than those of other treatments while ADC diet of 100% FM treatment was significantly higher than those of 60% SPC and 80% SPC treatments ($p<0.05$) (Table 7). No data are available for fish fed the 100% SPC diet due to failure of feces collection caused by limited feed consumption of these fish. $ADC_{protein}$ and $ADC_{lipid}$ in treatments fed 100% FM and 40% SPC were significantly higher than those of 60% SPC and 80% SPC treatments ($p<0.05$).

**Feeding and bacterial challenge trials with mannan oligosaccharides**. In the feeding trial testing MO supplementation of diets based solely on FM versus diets in which 40% of FM had been replaced by either SBM or SPC, both final weight (Wf) and weight gain (Wg) were significantly affected by diet and MO supplementation, as well as the interaction between the two (Table 8). In general (with some exceptions), growth performance of fish was significantly better when they were fed SPC than when they were fed SBM or FM, and MO supplementation generally improved growth of the fish (Table 8). FCR, PER, and survival of fish in this experiment was significantly affected by diet, but only survival was significantly affected by MO supplementation and in no case were the interactions significant (Table 9). FCR was significantly improved (i.e., lower) when fish were fed the SPC diet compared to the SB diet, but neither was significantly different from fish fed the FM diet (Table 9). PER for fish fed the FM and SPC diets was significantly greater than that for fish fed the SBM diet (Table 9). Survival of fish fed SPC diet was significantly lower than that of fish fed the FM and SBM diets, but supplementation with MO, especially at the level of 0.2%, significantly improved survival (Table 9). Red blood cell (RBC) counts were not significantly affected by either diet, MO supplementation, or the interaction of the two, but white blood cell (WBC) counts were significantly affected by both diet and MO supplementation (although not the interaction) (Table 10). Fish fed the SPC diet had significantly higher WBC counts than fish fed the FM diet, but neither group was significantly different from fish fed the SBM diet (Table 10). MO supplementation at both 0.2% and 0.4% levels significantly increased WBC counts compared to the unsupplemented diets (Table 10). Immunoglobulin (Ig) levels were significantly increased by MO supplementation and the interaction of MO and diet, but diet did not affect Ig levels (Table 11). At the end of the feeding trial but prior to the bacterial challenge (i.e. "pre-challenge"), lysozyme levels were significantly affected by diet, MO supplementation and the interaction between the two (Table 11). For each diet, the greater the level of MO supplementation, the greater the level of lysozymes is (Table 11). Reduction of fish production costs per kg fish produced, compared to fish fed the FM unsupplemented diet as the standard, ranged from 8.7 – 15.1% for the various other diets tested (Table 12).

Following the 15-d bacterial challenge with *A. hydrophila*, fish lysozyme levels (i.e. "post-challenge") were significantly increased by MO supplementation and the interaction between MO and diet, but not by the diets themselves (Table 11). Again, the greater the level of MO supplementation, the greater the lysozyme level (Table 11). After the 15-day challenge, cumulative mortality was lower for fish given MO supplementation than it was for fish fed the unsupplemented diets (Figure 5). In general, fish fed the SBM diet, supplemented or not, had lower cumulative mortality than fish fed the FM or SPC diets (Figure 5).

**On-farm trials.** Women who completed the training course are shown in Figure 6. Fingerling snakehead had been trained to feed on pellets at the hatchery before stocking into tanks and hapas (Figures 7-9). Diets used during the trial are indicated in Table 13. Fish survival rate was relatively high in the on-farm trial, ranged from 72.4 to 76.2% in tank rearing and 62.5 to 80.0% in hapa rearing (Table 14). In both tanks and hapas, fish growth was similar, with FCR in tank models ranging from 1.15 to 1.34 and in hapas 1.00 to 1.34 (Table 15). In hapa farm 1 the FCR was 1.0 during a shorter culture period, four months compared to six months in the other farms. The early harvest at this farm was determined by the woman owner who saw the high price in the market during the rearing period. In tank farm three, lower fish survival led to lower production and yield (Table 15). Production in tanks was lower than in the hapas but its profit was higher (Tables 15 and 16). Farmers reared snakehead in tanks selected big size fish for selling in the time when the market price was high. Discussions with farmers indicated that they saw less disease during the rearing period compared to rearing snakehead with trash fish.

## DISCUSSION

Our results show that FM can be replaced by SPC at levels up to 40% and that MO supplementation improves growth, physiological variables, and survival in a bacterial challenge. Salze et al. (2010) concluded that replacement of herring meal by SPC up to 75% did not effect on cobia survival. In Atlantic cod, replacement of FM by SPC up to 50% did not affect growth and survival (Walker et al. 2014). SPC can be used up to 40% in diet for different fish species e.g. Japanese flounder (Deng et al. 2006), gilthead seabream (Kissil et al. 2000), Atlantic salmon (Restie et al. 1998). FM can be replaced by SBM up to 30% in diet for carnivorous fish like snakehead (Be and Hien, 2010), red tail catfish (Lam et al. 2012), and knife fish (Dan et al. 2013). FM replacement by SB can be done at 20% inclusion in diet for spotted rose snapper, but inclusion at 40 and 60% resulted in lower growth and reduced protein and lipid utilization (Silva-Carrillo et al. 2012). For Diplodus puntazzo, 60% FM could be replaced by SB (Hernandez et al. 2007) and replacement of 40% of FM by SBM did not affect growth of Oncorhynchus mykiss (Jalili et al. 2013). In our experiment the significantly lower fish growth found in treatments 60% SPC, 80% SPC, and 100% SPC can be explained by limited feed intake, suggesting that SPC levels above 40% reduced palatability of the feed.

Our findings on the chemical composition of the fish agreed with previous studies in which replacement of FM by SPC did not affect on fish protein content (Cheng et al. 2003; Dan et al. 2013), but did lead to decreased fish lipid content (Dan et al. 2013; Tantikiti et al. 2015). Trypsin and chymotrypsin inhibitors limit the use of SB in diets for carnivorous fish (Baeverfjord and Krogdagl, 1996; O'Keefe and Newman, 2011). Eshel et al. (1993) reported that trypsin contributed to 40 – 50% protein digestibility process in carnivorous fish. Replacing FM by SBM in diets for rainbow trout, Krogdahl et al. (1994) concluded that trypsin activity was reduced due to proteinase inhibitors and reduced enzyme activities were observed with increase levels of SBM in diet. Hart et al. (2010) also concluded that SBM levels above 40% reduced digestibility due to the presence of trypsin inhibitor. Protein and lipid digestibility of SPC in snakehead in our study was relatively high and similar to those for sharp snout sea bream (Hernandez et al. 2007) and rainbow trout (Mambrini et al. 1999). Protein and lipid digestibility were reduced when SBM levels were increased in diets for gilthead sea bream (Venou et al. 2006) and rainbow trout (Mambrini et al. 1999).

Staykvo et al. (2007) demonstrated that growth of rainbow trout given MO was 10% higher than that of fish fed control diets. Sara et al. (2011) showed that growth rate increased by 5.23 % when feed of rainbow trout was supplemented with 0.2% MO. FCR and PER of Diplodus puntazzo were not affected when up to 0.8% MO was added to feed in which 40% SB flour replaced by FM. MO significantly reduced FCR and increased PER in O. mykiss (Staykov et al. 2007), Sparus aurata (Gultepe et al. 2011) and Oreochromis niloticus (Ahmad et al. 2013). However, in our experiment, MO did not affect FCR or PER, a result also seen in Dicentrarchus labrax (Torrecillas et al. 2007), Ictalurus punctatus (Perterson et al. 2010), Piaratus mesopotamicus (Sado et al. 2013) and Channa striata (Talpur et al. 2014).

Increase in number of WBC in fish may serve as protection against pathogenic infection. According to Huong and Tu (2010), leukocytes greatly change under physiological and nutritional conditions of fish and the number of leukocytes can reflect the health status of fish. Lysozyme plays an important role in the innate immune response, it widely distributed in vertebrate and invertebrate (Magnadóttir et al. 2005). Lysozyme is an enzyme that is effective against gram-positive bacteria (Ellis, 1999) and has the ability to resolve gram-negative bacteria, parasites and fungi (Dalmo et al. 1997; Saurabh and Sahoo, 2008). Serum and mucus lysozyme are an important part of non-specific immune system (Kiczka, 1994; Bayne and Gerwick, 2001). Lysozymes kill gram-negative bacteria after the outer cell wall of bacteria is disrupted (Ahmad et al. 2014). Ahmad et al. (2014) indicated that adding 0.2% MO significantly increased lysozyme and bactericidal activity of fish. Talpur et al. (2014) demonstrated that adding 0.2% MO significantly increased lysozyme of C. striata). Oanh and Phuong (2007) stated that globulin antibodies in the blood serum of animals have are able to link to specific antigens. Jalili et al. (2013) demonstrated no difference in lysozyme and Ig compare to the control when plant-based protein (mainly from SBM) replaced 40, 70 or 100% of FM in diets for O. mykiss. Talpur et al. (2014) showed that Bio-Mos at 0.2% in feed for 8 weeks increased Ig and lysozyme but did not statistically improve survival in a bacterial challenge with A. hydrophila. IgM in serum of experimental fish increased as probiotics (yeast cell wall) were added to the feed of Sparus aurata (Cuesta et al. 2004) and Salmo couhensis (Can et al. 2012).

MO supplementation appears to improve survival in bacterial challenges for many species. Torrecillas et al. (2007) fed seabass Dicentrarchus labrax MO at 0.4% of diet to improve survival and Ahmad et al. (2014) demonstrated that the mortality of tilapia (Oreochromis niloticus) is 0% when fed diets supplemented 0.05; 0.1, 0.2% MO. Samrongpan et al. (2008) showed that mortality of tilapia (Oreochromis niloticus) fingerlings decreased with MO supplementation and saw 0% mortality at the level of 0.4 and 0.6%. Addition of 0.2 MO in diet for C. striata significantly reduced mortality in a challenge with A. hydrophila (Talpur et al. 2014).

## CONCLUSIONS

The replacement of fishmeal by soy protein concentrate can be done at level of 40% in diet for snakehead. The increase level of SPC in diet above 40% significantly affected on the fish growth, economic efficiency, digestibility, and trypsin and chymotrypsin activities though fish chemical composition unlikely affected. Follow up study should focus on supplementation of other nutrients which can enhance the use of SPC in diet for snake head. Incorporation of MO from 0.2% to 0.4% level in snakehead diets yields better growth performance results and higher immune response. In short, FM based diets with 0.2 MO could be used for snakehead farming, which has more chance to gain revenue. Moreover, SPC based diets with 0.2 MO should be used not only to reduce fish caught to produce fishmeal but also to ensure the sustainable development. This test should be carried out in ponds or lined tanks. Women are now trained for, and active in, rearing snakehead in their spare time in ponds and hapas, increasing income for their households by 200-400 USD. They confidently feed fish with commercial pellets with less disease occurrence, likely due to addition of mannan oligosaccharide to the diets.

## QUANTIFIABLE ANTICIPATED BENEFITS

The project has developed and refined a formulated, pelleted snakehead feed that reduces the use of small-scale fish (SSF) and FM without decreasing growth performance and marketability. Results of the research were disseminated directly to feed manufacturers and more than ten aquaculture fish feed manufacturers in the Mekong Delta now make pellet diets containing a mixture of fish meal and soybean meal. In 2015, more than 90% of snakehead farmers (who produce 99% of the total production of snakehead) in 13 provinces the southern region of Vietnam, including the Mekong Delta, were using these diets instead of SSF, thereby reducing fishing pressure on the SSF in the Lower Mekong Delta. In An Giang, Dong Thap and Tra Vinh provinces, mainly snakehead culture provinces, about 2500 farmers now use pelleted feed.

This investigation supported research activities of one female PhD student, theses of four Master students (two female and two male), and dissertations of 10 undergraduate students (six female and four male). Nine faculty members (six female and three male) in CTU and three local staff members (two female and one male) participated in this project. Eighty-seven women were trained in small-scale snakehead culture with pelleted feed. Five-hundred copies of a handbook of snakehead (*C. striata*) culture using pelleted feed (in Vietnamese) was published and delivered to farmers.

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## TABLES AND FIGURES

**Table 1.** Formulation and chemical composition (% dry matter) of diets based completely on fish meal (FM) versus diets in which FM was replaced at varying levels with soy protein concentrate (SPC).

| Ingredients (%) | 100%FM | 40%SPC | 60%SPC | 80%SPC | 100%SPC |
|---|---|---|---|---|---|
| Kien Giang fish meal | 60.7 | 36.2 | 23.9 | 11.6 | 0.00 |
| Soy protein concentrate (SPC) | 0.00 | 24.1 | 36.1 | 48.2 | 59.6 |
| Rice bran | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Wheat flour | 23.8 | 20.7 | 19.1 | 17.6 | 16.1 |
| Premix and Vitamin C[1] | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Oil | 2.69 | 3.38 | 3.72 | 4.07 | 4.39 |
| Carboxymethylcellulose | 0.82 | 3.40 | 4.70 | 5.99 | 7.22 |
| Lysine | 0.00 | 0.06 | 0.36 | 0.11 | 0.13 |
| Methionine | 0.00 | 0.24 | 0.08 | 0.47 | 0.59 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Chemical composition (% in dry matter) | | | | | |
| Crude protein | 44.40 | 43.22 | 43.22 | 44.65 | 44.47 |
| Crude lipid | 8.28 | 8.53 | 8.61 | 8.48 | 8.44 |
| NFE | 29.0 | 29.04 | 29.04 | 29.04 | 29.04 |
| Ash | 16.2 | 12.77 | 11.15 | 9.46 | 8.35 |
| Fibre | 2.05 | 6.39 | 7.96 | 8.35 | 9.68 |
| Energy (KJ/g) | 18.9 | 18.7 | 18.7 | 19.0 | 19.0 |

[1]Mineral mixture and vitamin (unit/Kg) (Vemedim. Can Tho. Vietnam): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6 g; Vitamin $B_1$. 800 mg; Vitamin $B_2$. 800mg; Vitamin $B_{12}$. 2mg; Calcium D. Panthotenate. 2g; Folic acid. 160mg; Vitamin C. 15 g; Cholin Chloride. 100 g; Ferous ($Fe^{2+}$). 1 g; Zinc ($Zn^{2+}$). 3 g; Manganese ($Mn^{2+}$). 2g; Copper ($Cu^{2+}$). 100mg; Iodine ($I^-$). 20mg; Cobalt ($Co^{2+}$). 10mg. Kien Giang fishmeal were obtained from local provider (Minh Tam company. Can Tho. Viet Nam). Soy protein concentrate (SPC) was provided from Yihai (Fangchenggang) soybeans industries Co. Ltd. Oil including vegetable oil (Simply. Vietnam) and squid oil (Vemedim. Vietnam) was supplied at ratio of 1:1. Carboxylmethyl cellulose (CMC) was provided by Xilong Chemical Industry Incorporated Co.. Ltd (China).

**Table 2.** Ingredients and proximate chemical composition of experimental diets primarily made of fish meal (FM). soybean meal (SBM) or soy protein concentrate (SPC) with or without supplementation with mannan oligosaccharides (MO).

| **Ingredients (%)** | **FM** | **FM 0.2MO** | **FM 0.4MO** | **SBM** | **SBM 0.2MO** | **SBM 0.4MO** | **SPC** | **SPC 0.2MO** | **SPC 0.4MO** |
|---|---|---|---|---|---|---|---|---|---|
| Kien Giang fishmeal | 60.7 | 60.7 | 60.7 | 35.8 | 35.8 | 35.8 | 36.2 | 36.2 | 36.2 |
| Defatted soybean meal | - | - | - | 33.4 | 33.4 | 33.4 | - | - | - |
| SPC | - | - | - | - | - | - | 24.07 | 24.07 | 24.07 |
| Cassava | 23.8 | 23.6 | 23.4 | 8.26 | 8.06 | 7.86 | 20.68 | 20.48 | 20.28 |
| Rice bran | 10.0 | 10.0 | 10.0 | 15.0 | 15.0 | 15.0 | 10.0 | 10.0 | 10.0 |
| Premix mineral and vitamins | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Oil | 2.69 | 2.69 | 2.69 | 3.08 | 3.08 | 3.08 | 3.38 | 3.38 | 3.38 |
| Carboxymethyl cellulose | 0.82 | 0.82 | 0.82 | 0.40 | 0.40 | 0.40 | 3.40 | 3.40 | 3.40 |
| Lysine | - | - | - | 0.40 | 0.40 | 0.40 | 0.06 | 0.06 | 0.06 |
| Methionine | - | - | - | 0.28 | 0.28 | 0.28 | 0.24 | 0.24 | 0.24 |
| Fish solution | - | - | - | 1.50 | 1.50 | 1.50 | - | - | - |
| Phytase | - | - | - | 0.02 | 0.02 | 0.02 | - | - | - |
| Mannan oligosaccharides | 0 | 0.20 | 0.40 | 0 | 0.20 | 0.40 | 0 | 0.20 | 0.40 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **The chemical composition of feed (%)** | | | | | | | | | |
| Crude protein | 44.5 | 44.3 | 44.8 | 45.0 | 44.3 | 44.3 | 45.5 | 44.7 | 44.2 |
| Crude lipid | 8.56 | 8.62 | 8.69 | 8.91 | 8.45 | 8.76 | 8.76 | 8.83 | 8.90 |
| Ash | 15.4 | 15.2 | 15.3 | 12.5 | 12.3 | 12.5 | 12.18 | 12.18 | 12.17 |
| Fiber | 1.55 | 1.61 | 1.50 | 2.15 | 2.16 | 2.15 | 4.79 | 4.75 | 4.81 |
| NFE | 29.99 | 30.27 | 29.71 | 31.4.8 | 32.7 | 32.2.9 | 28.81 | 29.58 | 29.95 |
| Energy (KJ/g) | 19.17 | 19.20 | 19.25 | 19.69 | 19.58 | 19.61 | 19.27 | 19.25 | 19.22 |

Premix mineral and vitamin (unit/Kg): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6g; Vitamin $B_1$. 800mg; Vitamin $B_2$. 800mg; Vitamin $B_{12}$. 2mg; Calcium D. Pantothenate. 2g; Folic acid. 160mg; Vitamin C. 15g; Choline Chloride. 100g; Iron ($Fe^{2+}$). 1g; Zinc ($Zn^{2+}$). 3g; Manganese ($Mn^{2+}$). 2g; Copper ($Cu^{2+}$). 100mg; Iodine ($I^-$). 20mg; Cobalt ($Co^{2+}$). 10mg. Mannan oligosaccharides were the products of Alltech. USA. Fishmeal was from Kien Giang. SPC was a product of Taiwan. Cassava and rice bran were local products. CMC. methionine and lysine were products of Evonik

**Table 3.** Initial weight (Wi). final weight (Wf). weight gain (WG) and daily weight gain (DWG) of *Channa striata* fed diets in which the indicated percentage levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

| Treatment | Wi (g) | Wf (g) | WG (g) | DWG (g/day) |
|---|---|---|---|---|
| 100% FM | 10.0±0.13$^{a}$ | 44.4±1.42$^{a}$ | 34.8±1.55$^{a}$ | 0.83±0.04$^{a}$ |
| 40% SPC | 9.87±0.12$^{a}$ | 42.7±2.44$^{a}$ | 33.1±2.27$^{a}$ | 0.79±0.05$^{a}$ |
| 60% SPC | 10.0±0.18$^{a}$ | 19.3±0.98$^{b}$ | 9.50±1.07$^{b}$ | 0.23±0.03$^{b}$ |
| 80% SPC | 10.0±0.07$^{a}$ | 18.6±0.54$^{b}$ | 8.93±0.64$^{b}$ | 0.21±0.02$^{b}$ |
| 100% SPC | 9.98±0.20$^{a}$ | 13.4±0.26$^{c}$ | 3.73±0.07$^{c}$ | 0.09±0.00$^{c}$ |

**Table 4.** Feed intake (FI). feed conversion ratio (FCR). Protein efficacy ratio (PER) and net protein utilization (NPU) of fish fed diets in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

| Treatment | FI (%/fish/day) | FCR | PER (%) | NPU (%) |
|---|---|---|---|---|
| 100% FM | 3.36±0.13$^{a}$ | 0.84±0.10c | 2.76±0.34$^{a}$ | 46.9±4.25$^{a}$ |
| 40% SPC | 3.12±0.32$^{a}$ | 0.83±0.05$^{c}$ | 3.17±0.82$^{a}$ | 50.2±1.70$^{a}$ |
| 60% SPC | 1.96±0.14$^{b}$ | 1.20±0.03$^{b}$ | 1.93±0.05$^{b}$ | 21.3±1.78$^{b}$ |
| 80% SPC | 1.84±0.10$^{b}$ | 1.19±0.03$^{b}$ | 1.89±0.05$^{b}$ | 19.4±1.17$^{b}$ |
| 100% SPC | 1.31±0.08$^{c}$ | 1.70±0.09$^{a}$ | 1.32±0.07$^{b}$ | 12.6±2.83$^{c}$ |

**Table 5.** Chemical compositions of fish prior to (Initial fish) and after (Final fish) an experiment in which fish were fed diets in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

| Treatment | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|
| Initial fish | 75.2 | 16.2 | 2.71 | 1.31 |
| Final fish | | | | |
| 100% FM | 72.0±0.81$^{b}$ | 18.2±0.20$^{a}$ | 3.45±0.27$^{a}$ | 5.15±0.24$^{ab}$ |
| 40% SPC | 73.2±0.45$^{ab}$ | 17.9±0.44 $^{a}$ | 3.23±0.06$^{ab}$ | 4.48±0.22$^{c}$ |
| 60% SPC | 73.4±1.31$^{ab}$ | 17.7±0.71 $^{a}$ | 2.26±0.25$^{d}$ | 5.62±0.05$^{a}$ |
| 80% SPC | 73.6±1.01$^{ab}$ | 17.4±0.61 $^{a}$ | 2.81±0.51$^{bc}$ | 4.89±0.45$^{bc}$ |
| 100% SPC | 73.8±0.32$^{a}$ | 17.5±0.46 $^{a}$ | 2.47±0.12$^{cd}$ | 5.07±0.12$^{b}$ |

**Table 6.** Comparision of feed cost (FC) and fish production cost (FPC) among treatments in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC).

| Treatments | FC (USD/kg feed) | FPC (USD/kg fish gain) |
|---|---|---|
| 100% FM | 1.131 | 0.950 |
| 40% SPC | 1.096 | 0.910 |
| 60% SPC | 1.079 | 1.294 |
| 80% SPC | 1.061 | 1.263 |
| 100% SPC | 1.045 | 1.776 |

**Table 7.** Apparent digestibility coefficient (ADC) of diets ($ADC_{diet}$). $ADC_{protein}$ and $ADC_{lipid}$ in which varying levels of fish meal (FM) were replaced by soy protein concentrate (SPC). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different.

| Treatments | $ADC_{diet}$ (%) | $ADC_{protein}$ (%) | $ADC_{lipid}$ (%) |
|---|---|---|---|
| 100% FM | $78.1\pm0.26^{b}$ | $89.9\pm0.24^{a}$ | $92.9\pm1.08^{a}$ |
| 40% SPC | $78.9\pm0.08^{a}$ | $89.9\pm0.16^{a}$ | $92.2\pm1.41^{a}$ |
| 60% SPC | $77.4\pm0.13^{c}$ | $84.0\pm0.11^{b}$ | $82.1\pm0.20^{b}$ |
| 80% SPC | $77.5\pm0.29^{c}$ | $82.0\pm0.29^{c}$ | $76.5\pm0.50^{c}$ |

**Table 8.** Growth performance of *Channa striata* fed for 8 weeks on diets supplemented with mannan oligosaccharides and a control diet. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p>0.05$).

| **Treatment** | **Wi (g)** | **Wf (g)** | **WG (g)** |
|---|---|---|---|
| FM | $7.00\pm0.14^{a}$ | $47.7\pm0.6^{d}$ | $40.7\pm0.5^{f}$ |
| FM 0.2MO | $7.05\pm0.03^{a}$ | $52.5\pm1.2^{b}$ | $45.5\pm1.2^{b}$ |
| FM 0.4MO | $7.01\pm0.05^{a}$ | $52.1\pm0.2^{b}$ | $45.0\pm0.2^{b}$ |
| SBM | $7.08\pm0.11^{a}$ | $50.5\pm2.1^{bc}$ | $43.4\pm2.0^{bd}$ |
| SBM 0.2MO | $7.08\pm0.10^{a}$ | $57.7\pm1.7^{a}$ | $50.6\pm1.6^{a}$ |
| SBM 0.4MO | $7.05\pm0.06^{a}$ | $57.4\pm1.5^{a}$ | $50.3\pm1.5^{a}$ |
| SPC | $7.23\pm0.36^{a}$ | $49.4\pm0.6^{cd}$ | $42.2\pm0.4^{cf}$ |
| SPC 0.2MO | $7.00\pm0.03^{a}$ | $51.3\pm1.2^{bc}$ | $44.3\pm1.3^{bc}$ |
| SPC 0.4MO | $7.06\pm0.05^{a}$ | $48.9\pm1.8^{cd}$ | $41.9\pm1.8^{df}$ |
| *P values* | | | |
| *Diets* | - | *0.000* | *0.000* |
| *MO* | - | *0.000* | *0.000* |
| *Diets*MO* | - | *0.003* | *0.003* |

**Table 9.** Feed convertion ratio (FCR). protein efficiency ratio (PER)and survival rate (SR) of *Channa striata* fed for 8 weeks on different protein diets and supplemented with different mannan oligosaccharide levels. Values (mean ±

SD) in a column followed by the same superscript letter are not significantly different ($p$>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

| Treatment | Growth parameters | | |
|---|---|---|---|
| | **FCR** | **PER (%)** | **SR (%)** |
| **Diet sources** | | | |
| FM | 0.97±0.09$^{ab}$ | 2.56±0.28$^{a}$ | 76.2±3.3$^{a}$ |
| SBM | 1.05±0.08$^{a}$ | 2.20±0.24$^{b}$ | 78.1±3.9$^{a}$ |
| SPC | 0.93±0.09$^{b}$ | 2.67±0.24$^{a}$ | 66.6±5.3$^{b}$ |
| **MOS levels (%)** | | | |
| 0 | 1.03±0.20$^{a}$ | 2.35±0.57$^{b}$ | 67.9±12.4$^{b}$ |
| 0.20 | 0.94±0.23$^{a}$ | 2.53±0.66$^{a}$ | 78.8±0.2$^{a}$ |
| 0.40 | 0.98±0.35$^{a}$ | 2.54±0.52$^{a}$ | 74.2±9.9$^{ab}$ |
| *P values* | | | |
| *Diets* | *0.037* | *0.004* | *0.000* |
| *MO* | *0.167* | *0.260* | *0.000* |
| *Diets*MO* | *0.843* | *0.800* | *0.217* |

**Table 10.** Total red blood cell and white blood cell in serum of *Channa striata* fed different protein diets and different mannan oligosaccharide levels at the end of the growth trial. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p$>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

| Treatment | Blood parameters | |
|---|---|---|
| | **RBC ($10^6$ cells/mm$^3$)** | **WBC ($10^3$ cells/mm$^3$)** |
| **Diet sources** | | |
| FM | 2.17±0.18$^{a}$ | 60.9±5.9$^{b}$ |
| SBM | 2.18±0.33$^{a}$ | 66.3±7.1$^{ab}$ |
| SPC | 2.20±0.17$^{a}$ | 74.6±3.4$^{a}$ |
| **MOS levels (%)** | | |
| 0 | 2.17±0.87$^{a}$ | 58.6±2.7$^{b}$ |
| 0.20 | 2.26±0.66$^{a}$ | 71.8±1.8$^{a}$ |
| 0.40 | 2.14±0.52$^{a}$ | 71.4±19.8$^{a}$ |
| *P values* | | |
| *Diets* | *0.973* | *0.001* |
| *MO* | *0.567* | *0.000* |
| *Diets*MO* | *0.273* | *0.081* |

**Table 11.** Immunoglobulin (Ig). pre-challenge lysozyme and post-challenge lysozyme levels of *Channa striata* fed supplemented with and without mannan oligosaccharides. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different ($p$>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and iteraction effects.

| Treatment | Ig (mg/ml) | Pre-challenge lysozyme (µg/ml) | Post-challenge lysozyme (µg/ml) |
|---|---|---|---|
| FM | 9.09±0.85$^{c}$ | 263±6$^{df}$ | 459±3$^{df}$ |
| FM 0.2MO | 10.10±0.57$^{bc}$ | 276±13$^{d}$ | 503±11$^{bc}$ |
| FM 0.4MO | 12.90±0.83$^{a}$ | 346±15$^{b}$ | 536±27$^{a}$ |
| SBM | 9.08±0.49$^{c}$ | 248±23$^{f}$ | 479±6$^{cd}$ |
| SBM 0.2MO | 9.42±0.50$^{c}$ | 283±3$^{d}$ | 485±7$^{c}$ |
| SBM 0.4MO | 12.00±0.69$^{a}$ | 308±14$^{c}$ | 529±24$^{a}$ |
| SPC | 8.75±1.36$^{c}$ | 271±14$^{df}$ | 443±4$^{f}$ |
| SPC 0.2MO | 11.60±0.69$^{ab}$ | 323±12$^{bc}$ | 524±7$^{ab}$ |
| SPC 0.4MO | 10.20±1.27$^{bc}$ | 371±11$^{a}$ | 487±3$^{c}$ |
| *P values* | | | |
| *Diets* | *0.383* | *0.000* | *0.069* |
| *MO* | *0.000* | *0.000* | *0.000* |
| *Diets*MO* | *0.000* | *0.023* | *0.003* |

**Table 12.** Comparision of feed costs (FC). fish production costs (FPC). and reduction in fish production cost compared to control among a diet based completely on fish meal (FM) versus diets in which 40% of FM was replaced by soybean meal (SBM) or soy protein concentrate (SPC). with or without supplementation with mannan oligosaccharides (MO) (based on February. 2015 prices).

| Treatments | FC (USD/kg feed) | FPC (USD/kg fish gain) | Reduction in fish production cost (%) |
|---|---|---|---|
| FM | 1.063 | 1.117 | 0.0 |
| FM 0.2MO | 1.072 | 0.964 | 13.4 |
| FM 0.4MO | 1.085 | 1.018 | 8.7 |
| SBM | 0.933 | 1.013 | 8.9 |
| SBM 0.2MO | 0.946 | 0.955 | 14.4 |
| SBM 0.4MO | 0.955 | 1.009 | 9.1 |
| SPC | 1.027 | 0.978 | 12.3 |
| SPC 0.2MO | 1.040 | 0.946 | 15.1 |
| SPC 0.4MO | 1.049 | 0.987 | 11.4 |

**Table 13.** Feed formulas used for snakehead culture. During the first 2 months, fish were fed CTU - CRSP 1 (44% CP). In the third and fourth months, fish were fed CTU- CRSP 2 (41% CP) and CTU- CRSP 3 (38% CP) were used for the last months.

| **Ingredients** | **CTU - CRSP 1** | **CTU – CRSP 2** | **CTU – CRSP 3** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Fish meal (Kien Giang) | 34.5 | 31.8 | 29.1 |
| Soybean meal | 32.0 | 29.5 | 27.1 |
| Rice bran | 20.0 | 20.0 | 20.0 |
| Cassava | 6.35 | 11.3 | 16.3 |
| Vitamin premix | 1.00 | 1.00 | 1.00 |
| Mineral premix | 1.00 | 1.00 | 1.00 |
| Fish oil | 2.45 | 2.70 | 2.94 |
| Binder | 0.40 | 0.40 | 0.40 |
| Lysine | 0.20 | 0.21 | 0.23 |
| Methionine | 0.19 | 0.21 | 0.23 |
| Phytase | 0.02 | 0.02 | 0.02 |
| Fish fluid | 1.5 | 1.5 | 1.5 |
| MO | 0.2 | 0.2 | 0.2 |
| Total | 100 | 100 | 100 |

**Table 14.** Survival rate (SR). initial fish weight (Wi) and final fish weight Wf (g). daily weight gain (DWG) (g/day) and feed conversion ratio (FCR) of *C. striata* reared in plastic lined tanks and hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

| Models | SR (%) | Wi (g) | Wf (g) | WG (g) | DWG (g/day) | FCR |
|---|---|---|---|---|---|---|
| Tank | | | | | | |
| Farm 1 | 74.1 | 4.80 | 450 | 445 | 2.42 | 1.15 |
| Farm 2 | 76.2 | 4.70 | 350 | 345 | 1.88 | 1.25 |
| Farm 3 | 72.4 | 5.00 | 350 | 345 | 1.88 | 1.34 |
| Mean | 74.2±0.02 | 4.83±0.15 | 383±57.7 | 379+57.8 | 2.06±0.31 | 1.25±0.1 |
| Hapa | | | | | | |
| Farm 1 | 72.0 | 11.5 | 250 | 239 | 1.99 | 1.00 |
| Farm 2 | 80.0 | 11.2 | 350 | 339 | 1.84 | 1.29 |
| Farm 3 | 62.5 | 4.60 | 400 | 395 | 2.15 | 1.34 |
| Mean | 71.5±0.01 | 9.10±3.9 | 333±76.4 | 324±79.4 | 1.99±0.15 | 1.21±0.2 |

**Table 15.** Production and yield of *C. striata* reared in plastic-lined tanks and in hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

| Models | Production (kg) | Yield (kg/m$^2$) |
|---|---|---|
| Tank | | |
| Farm 1 | 500 | 33.0 |
| Farm 2 | 400 | 26.3 |
| Farm 3 | 380 | 25.0 |
| Mean | 427±64 | 28.1±4.29 |
| Hapa | | |
| Farm 1 | 360 | 14.3 |
| Farm 2 | 560 | 22.6 |
| Farm 3 | 500 | 20.6 |
| Mean | 473±102 | 19.2±4.31 |

**Table 16.** Financial efficacy analysis of *C. striata* reared in plastic-lined tanks and in hapas at three farms each during the on-farm trials. Mean indicates mean value plus or minus standard deviation.

| Models | Cost per kg fish (USD | Total Investing cost (USD) | Selling price USD/kg fish | Total income (USD) | Profit (USD) |
|---|---|---|---|---|---|
| Tank | | | | | |
| Farm 1 | 1.19 | 594 | 2.00 | 1000 | 406 |
| Farm 2 | 1.32 | 530 | 1.70 | 682 | 152 |
| Farm 3 | 1.36 | 516 | 1.67 | 636 | 120 |
| Mean | 1.29±0.09 | 548±41.6 | 1.79±0.18 | 773±198 | 226±157 |
| Hapa | | | | | |
| Farm 1 | 1.05 | 378 | 1.73 | 623 | 245 |
| Farm 2 | 1.24 | 697 | 1.61 | 901 | 205 |
| Farm 3 | 1.30 | 649 | 1.55 | 773 | 124 |
| Mean | 1.20±0.13 | 575±172 | 1.63±0.09 | 766±139 | 191±61.6 |


**Figure 1.** Experimental system for feces collection in digestibility experiment.

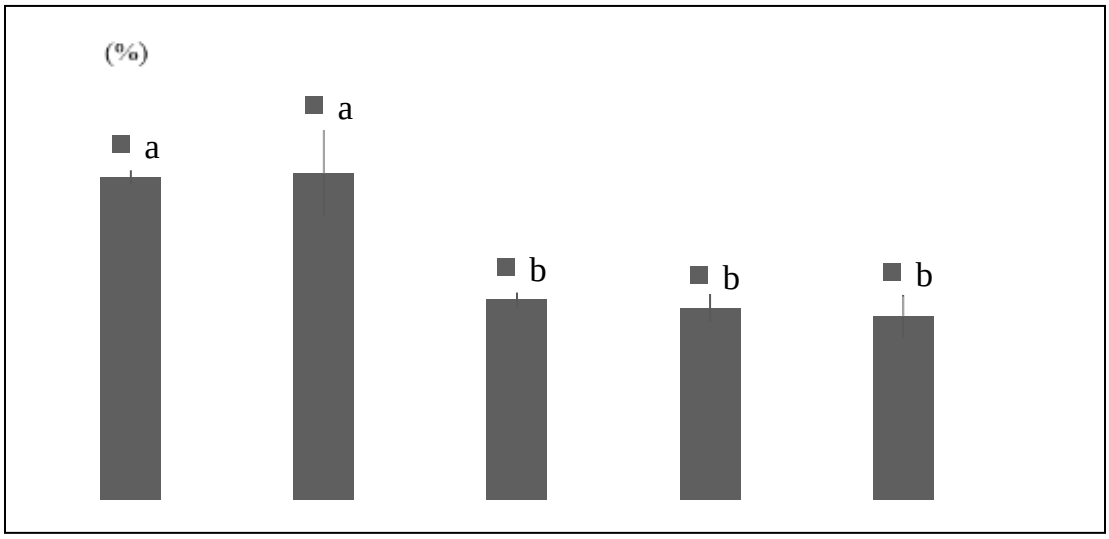


**Figure 2.** Survival of fish in treatments where fish meal (FM) was replaced by varying levels of soy protein concentrate (SPC). The different letters above the error bars indicated the significant difference between treatments ($p$<0.05).

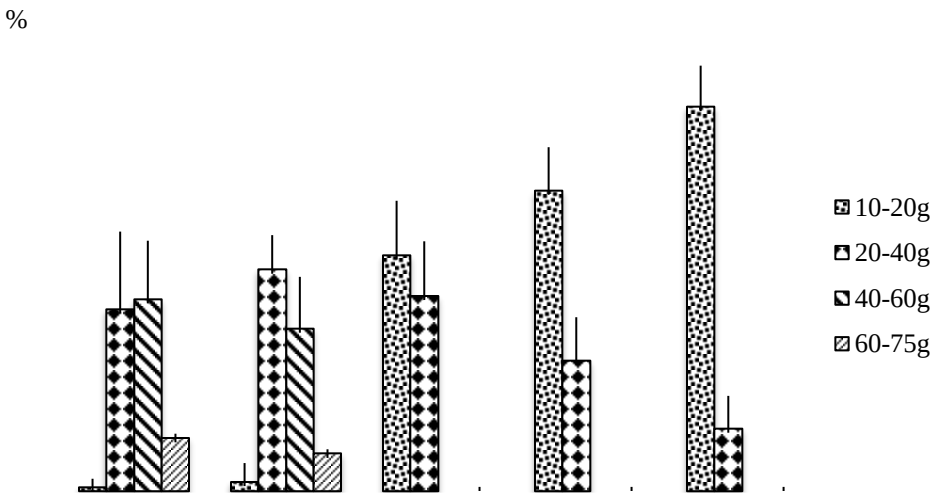


**Figure 3.** Size distribution of fish fed diets in which varying degrees of fish meal (FM) were replaced by soy protein concentrate (SPC).

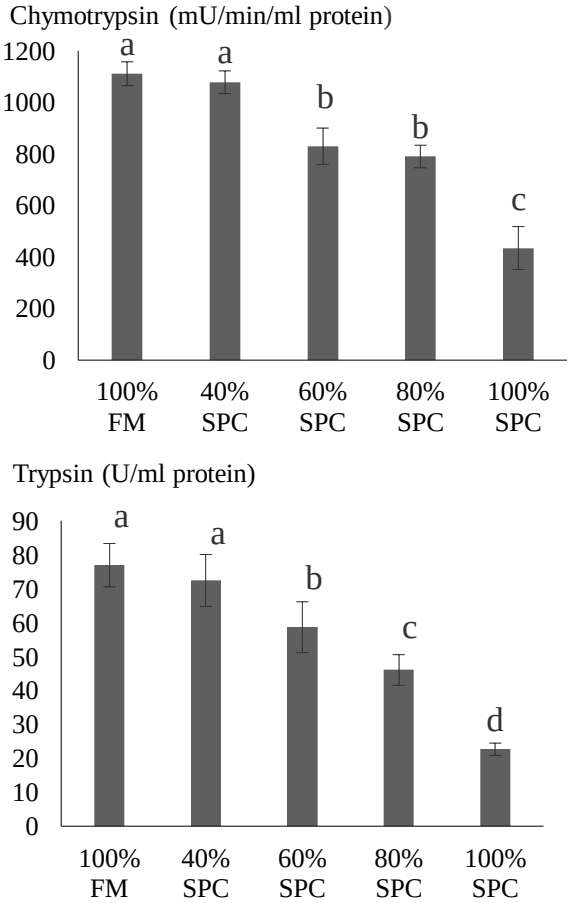


**Figure 4.** Trypsin and chymotrypsin activities of fish fed diets in which varying amounts of fish meal (FM) were replaced by soy protein concentrate (SPC). The different letters above the error bars indicated the significant difference between treatments ($p<0.05$).

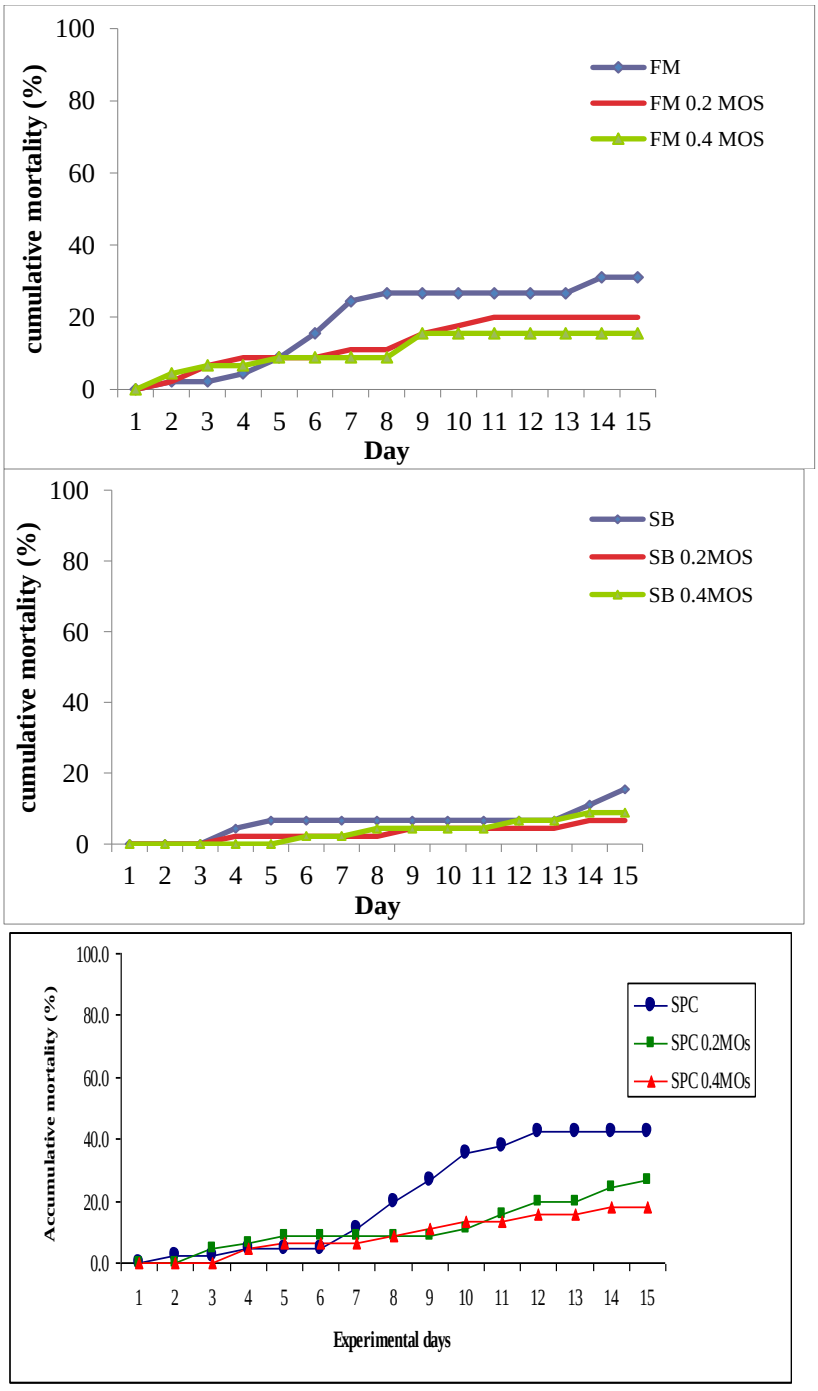


**Figure 5.** Cumulative mortalities of *Channa striata* during 15-d bacterial challenges with *Aeromonas hydrophila* following a feeding trial in which they had been fed fish meal (FM) diet (top panel). a diet in which soybean meal (SBM) replaced 40% of FM (middle panel) or a diet in which soy protein concentrate (SPC) diet replaced 40% of FM (bottom panel) with or without mannan oligosaccharides (MO) supplementation at 0.2% or 0.4% of the diet (as indicated).


**Figure 6.** Training course on snakehead aquaculture for women in An Giang province.


**Figure 7.** Releasing fingerlings at a farm for the on-farm trials and project feed bag.

**Figure 8.** Snakehead culture in a plastic-lined tank during the on-farm trials.


**Figure 9.** Snakehead culture in a hapa placed in a pond during the on-farm trials