Development of formulated diets for snakehead (*Channa striata*): use of rice bran and feeding stimulants in fish meal/soybean meal diets

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Abstract- The development of the snakehead (*Channa striata*) aquaculture industry depends on the development of formulated feeds. The previously showed that soybean meal (SBM) could replace up to 40% of the fish meal (FM) in formulated feeds for snakehead as long as phytase and essential amino acids (EAA) were added. In the current study, we investigated the use of rice bran (RB) to replace FM and SBM in snakehead diets and a feeding attractant solution to increase the percentage of SBM that could be incorporated. In Experiment 1substituted 0% (control), 10, 20, and 30% of RB and found no differences in survival or growth among any of the treatments, except that the 10% treatment had higher growth than did the control. In Experiments 2substituted 0% (control), 50, 60, or 70% of FM with SBM (and added EAA and phytase) plus a feeding attractant solution. The study found that either 70% substitution was feasible (based on growth data), 60% was feasible (based on food conversion ratio data), and 50% was feasible (based on economic benefit).

Keywords: *Channa striata*, rice bran, feeding attractant

I. Introduction

Developing formulated feed for snakehead (*Channa striata*) aquaculture industry in Vietnam in order to replacement low-value fish, or small-size fish to reduce fishing pressure on the small fish. In a previous study (Hien et al., 2015) we demonstrated that soybean meal (SBM) can replace up to 30% of fish meal (FM) in formulated diets for *Channa striata* and *Channa micropeltes* if essential amino acids (EAA's) are added to make up the EAA deficiencies in SBM and that this value can be increased to 40% if phytase is added to the diets to break down the phytin in SBM.

Rice bran (RB) also is a rich source of protein (8.34-16.3%), oil, dietary fiber, and micronutrients (Hien et al., 2006). RB has been used in formulated feed for terrestrial animals and aquaculture species (Hertrampf and Piedad-Pascual, 2000). Moreover, RB is an available and abundant crop by-product in the Mekong Delta of Vietnam. To reduce the cost of feed for snakehead, diminish trash fish use, and reduce the environmental impact, RB is thought to be the best ingredient. Research has been conducted on utilization of RB in diets for Nile tilapia *Oreochromis niloticus* (Perschbacher and Lochmann, 1999; Liti, et al., 2006), silver barb *Puntius gonuonotus* (Mohanta et al., 2006), and *Streptocephalus proboscideus* (Ali and Dumont, 2002). Hien et al. (2006) reported defatted RB could be used in diets for tilapia *Oreochromis niloticus* and striped catfish *Pangasius hypophthalmus* with increased growth rate and reduced feeding cost. The popular forms of RB and cassava meal (CM) are dried full-fat rice bran, wet full-fat rice bran, defatted rice bran, and dry chopped CM **(**Hien et al., 2006).

Soybean meal (SM) is considered as the most nutritive plant ingredients widely used in fish feed and is potentially considered as the most pertinent protein source as an alternative to fish meal (Brown et al., 2008). Among plant protein ingredients, soybean meal has a well-balanced amino acid profile. Furthermore, SM has the advantage of being resistant to oxidation and spoilage and is naturally clean from organisms such as fungi, viruses, and bacteria that are harmful to fish (Swick et al., 1995). It has been observed in many fish species that anti-nutritional components in SM, such as trypsin inhibitor, antigens, lectins, saponins, and oligosaccharides, can harm digestibility of nutrients and performance of fish. Especially, SM contains approximately 15% of oligosaccharides (sucrose, raffinose, stachyose) that can have negative effects on nutrients utilization in fish. 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., 1984); and Dover sole, *Solea solea* (Mackie and

Mitchell, 1982). More recently, squid viscera meal has been used as a feeding stimulant for Japanese seabass, *Lateolabrax japonicus* (Mai et al., 2006), and yellow croaker, *Pseudosciaena crocea* (Li et al., 2009).

Building on our previous results, we next wanted to increase the level of locally available plant products in diets for snakehead and to see if replacement of FM by SBM could be increased if soluble fish attractant. The objectives of this study were to: a) determine the optimal of RB in a diet containing fixed amounts of FM and SBM for optimum growth and cost-effectiveness for *C. striata*, and b) investigate the effects of supplementing dietary a feeding attractant solution on growth performance in fingerling snakehead fed diets containing more than 40% SBM.

II. MATERIALS AND METHODS

Two experiments were conducted: Experiment 1 was a study of the replacement of an FM/SBM mixture with an RB in diets for *C. striata*; Experiment 2 was a study of the effects of adding a feeding attractant solution to *C. striata* diets containing at least 50% SBM.

Experiment 1: Diets for Experiment 1 were formulated from the main ingredients FM, defatted SBM, CM, and dried RB to be isonitrogenous and isoenergetic and to contain 45% crude protein (CP) and 4.7 Kcal gross energy/g diet. The ratio of FM: SBM was 6:4. The experiment consisted of four treatments identified by the amount of RB: 0% RB (control), 10%, 20%, or 30% RB, as indicated in the composition of the experimental diets shown in Table 1. Experimental ingredients were homogeneously mixed and pelleted at 80°C without steam to produce feed with a diameter of 2 mm. The experimental feed was then sterilized at 105°C for 10 min and dried at 45°C for 24 h to reach 11–12% moisture.

Each treatment had three replicates with 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 27.0–28.0ºC, pH and dissolved oxygen, measured weekly, were 7.6–7.7 and 6.68–6.76 ppm, respectively. The experimental period was 8 wks. Fish were fed to satiation twice a day (8:00 and 14:00). Uneaten food was removed from the tank following feeding and the feed intake recorded based on the amount fed minus that recovered (on a dry weight basis following placement in a drying oven for at least 4 hours at 105ºC). Dead fish were collected and weighed.

Table 1. Composition and proximate analyses of four experimental diets (% of dry matter basis) used in Experiment 1

Ingredients	Diets			
	0% RB	10%RB	20%RB	30%RB
Fish meal[a]	34.5	33.6	32.8	31.9
Soybean meal[b]	33.6	32.7	31.9	31.0
Dried rice-bran[c]	0.00	10.0	20.0	30.0
Cassava meal[d]	21.4	14.2	7.12	0.00
Vitamin Premix[e]	1.00	1.00	1.00	1.00
Mineral Premix[f]	1.00	1.00	1.00	1.00
Fish oil[g]	5.63	4.47	3.32	2.17
Binder[h]	1.82	1.80	1.79	1.78
Lysine	0.40	0.42	0.44	0.46
Methionine	0.28	0.8	0.28	0.28
Threonine	0.41	0.41	0.40	0.39
Phytase	0.02	0.02	0.02	0.02
Total	100	100	100	100
Proximate composition (%)				
Dry matter	90.3	90.2	90.6	89.9
Crude protein	44.4	44.5	44.7	44.8
Crude lipid	9.27	9.59	9.75	9.33
Crude ash	10.1	10.5	10.2	10.6
Fibre	2.30	2.60	3.52	4.31
Nitrogen free extract	34.0	32.8	31.8	30.9
Gross energy (kcal/g)	4.81	4.79	4.78	4.71

Note:

[a]*FM: Kien Giang fishmeal was supplied by Minh Tam Co., Ltd (Vietnam). Moisture: 11.7%, crude protein: 65.1%, crude lipid: 7.94%, crude fiber: 0.55% and Ash:13.7%.*

[b]*SBM: Argentine Soybean meal was supplied by Quang Dung Co.,Ltd (Vietnam) Moisture: 8.78%, crude protein: 46,1%, crude lipid: 1,98%, crude fiber: 6.36% and Ash:6.20%.*

[c]*Dried rice bran was supplied by Cai Lan Oils & Fats Industries Company Ltd, Can Tho Branch, Cantho City, Vietnam. Moisture: 11.5%, crude protein12.7%, crude lipid: 14.3%, crude fiber: 5.00% and Ash: 7.94%.*

[d]*Cassava meal was supplied by Gentraco Fed, Cantho province, Vietnam. Moisture: 13.3%, crude protein: 2.73%, crude lipid: 2.54%, crude fiber: 2.69% and Ash: 3.12%.*

[e]*Vitamin mix consisted (IU kg^{-1} or g kg^{-1}): vitamin A: 2.500.000 IU,): vitamin D3: 1.500.000 IU, vitamin E: 80g, vitamin B1: 800mg, vitamin B2: 2000mg, vitamin B6: 800mg, vitamin B12: 20mg, vitamin C: 8 g, vitamin K3: 1000mg, Choline: 200g, Niacin: 6,5g, Folic acid: 250mg, Biotin: 40mg, .*

[f]*Mineral mix consisted of $CuSO_4$: 10g, $ZnSO_4$: 20g, $MgSO_4$: 10g, $CoSO_4$: 1g, $FeSO_4$: 5g, $MnSO_4$ 5g, $CaHPO_4$: 1g.*

[g]*Fish oil was supplied by Vemedim Company Ltd, Cantho City, Vietnam.*

[h]*Binder (Carboxymethylcellulose) was (Chinese product) imported by Thanh My Company Ltd, Cantho city, Vietnam.*

Experiment 2 consisted of four treatments with increasing levels of SBM: 0% (FM), which was the control diet, 50%, 60%, or 70% (referred to as 50% SBM, 60% SBM, and 70% SBM, respectively). All SBM diets contained supplemental lysine and methionine to correct the essential amino acid (EAA) deficiencies associated with lowered levels of FM, as well as 0.02% phytase and 1.5% of a feeding attractant solution (Table 2). Thus, the four diet treatments are again referred to as FM, 50% SBM, 60% SBM, and 70% SBM. Each treatment again had three replicates with 30 fish per replicate.

Table 2. Composition and proximate analyses of nine experimental diets with solution feeding attractant addition in Experiment 3 (% of dry matter basis). Diet details are as explained in footnotes to Table 1.

Ingredients (%)	FM	50% SBM	60% SBM	70% SBM
Fish meal	56.2	27.7	22.2	16.7
Soybean meal	0.00	40.4	48.5	56.7
Rice bran	15.0	15.0	15.0	15.0
Cassava meal	24.2	7.86	4.64	1.40
Vitamin Premix	1.00	1.00	1.00	1.00
Mineral Premix	1.00	1.00	1.00	1.00
Fish oil	2.18	4.22	4.61	5.01
Binder	0.50	0.50	0.50	0.50
Lysine	0.00	0.52	0.60	0.69
Methionine	0.00	0.35	0.41	0.48
Phytase	0.00	0.02	0.02	0.02
Fish attractant solution	0.00	1.50	1.50	1.50
Total	100	100	100	100
Proximate composition (%)				
Dry matter	91.0	91.2	91.5	91.6
Crude protein	44.7	44.7	44.5	44.6
Crude lipid	8.96	8.15	8.38	8.26
Crude ash	10.0	9.13	8.74	8.27

Note: *Fish attractant solution was supplied by Trai Viet Co., Ltd (Vietnam) (consisting of 30% Crude Protein; 2.1% Omega-3 and 22.63% amino acid)*

In experiments 2, 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. *Channa striata* fingerlings (2.24–3.79g in initial weight) were randomly assigned to each experimental tank (500-L composite tank) and were fed to satiety. Water temperature, measured daily, ranged from 26.5-27.5ºC. pH and dissolved oxygen, measured weekly, varied from 7.2-7.5 and 5.0–7.6 ppm respectively.

At the end of each experiment, fish were weighed and counted to calculate survival rate (SR), daily weight gain (DWG), feed intake FI = feed consumed per fish per day / $(\text{initial weight} * \text{final weight})^{0.5}$, feed conversion ratio (FCR), protein efficiency ratio (PER), and economic conversion ratio (ECR), which is simply fed cost multiplied by FCR.

Feed was analyzed for the following composition: moisture, crude protein (CP), crude lipid (CL), crude fiber (CF), nitrogen-free extract (NFE), and gross energy, all 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).

Data were checked for normal distribution by One-Sample Kolmogorov-Smirnov test and homogeneity of variances by Levene's test. Data were analyzed using One-way analysis of variance (One-way ANOVA) test followed by a Duncan's Multiple Range Test. Differences in growth and feed efficiency between diet treatments were considered to be statistically significant when $p \leq 0.05$. The statistical tests were performed using the SPSS statistical package (ver. 16.0, SPSS Company, Chicago, IL, USA).

III. RESULTS AND DISCUSSION

In Experiment 1, daily weight gain of fish in treatment 10% RB (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 were not significantly different from those of fish in the control treatment and there was no significant difference among 10%, 20%, and 30% RB treatments in daily weight gain (Table 3). Survival rates of fingerlings were high (60.0 - 69.3%) and no significant differences were observed (Table 3). Feed intake fluctuated between 2.55 - 3.14% BW per day among treatments and showed significant differences, with the lowest FI in treatment 0% RB and other treatments; however, no significant differences were seen in FCR or PER (Table 3).

Table 3. Initial (Wi) and final (Wf) body weights (g), daily weight gain (DWG) (g.day^{-1}), survival rate (SR) (%), feed intake (FI) (%), feed conversion ratio (FCR), and protein efficiency ratio (PER) of *Channa striata* fed experimental diets (% of moisture matter basis) with different ratios of the replacement of Rice bran (mean±SE, n=3) in Experiment 1. Values in a row followed by the same letter are not significantly different (p<0.05).

Diets	RB 0%	RB 10%	RB 20%	RB 30%
SR	60.7±3.71	60.0±3.46	62.0±6.11	69.3±2.91
Wi	4.54±0.05	4.57±0.04	4.63±0.04	4.51±0.02
Wf	17.4±0.36^{a}	20.9±1.22^{b}	19.9±1.11^{ab}	19.4±0.70^{ab}
DWG	0.23±0.01^{a}	0.29±0.02^{b}	0.27±0.02^{ab}	0.27±0.01^{ab}
FI	2.55±0.03^{a}	3.04±0.09^{bc}	2.90±0.08^{b}	3.14±0.06^{c}
FCR	1.29±0.05	1.26±0.07	1.27±0.07	1.28±0.06
PER	1.75±0.07	1.79±0.10	1.77±0.10	1.75±0.08

These results extend those of our previous study (Hien et al., 2015) and further indicate the possibility of using formulated diets for rearing snakehead in captive conditions. The improvement in the growth performance of snakehead fingerlings fed the 10% RB diet compared to the result from the 0% RB diet may have been caused by the presence of micronutrients in RB. The CM content decreased along with the increase of RB in diets (Table 1). RB is abundant in trace minerals and vitamin B, especially vitamin B1 (thiamine), that are necessary for growth (Guillaume et al., 2001). Vitamin B1 plays a major role in carbohydrate metabolism (Hertrampf and Piedad-Pascual, 2000)**.** No significant difference in FI was observed between 10% RB and 30% RB treatments. However, the growth response of snakehead in 10% RB treatment was better, inducing the lowest FCR in this experiment. That result may be caused by the highest fiber content in 30% RB 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, although no significant differences in FCR and PER were observed among treatments. Suhenda et al. (2005) found in Asian catfish (4.9 g/fish in initial weight) fed 55.6% RB in the diet that FCR was low (1.3), and SGR and PER were high (4 % per day and 2.6, respectively). 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% crude protein (CP) (1.19%.day^{-1}) which were studied by Trieu et al. (2001). In summary, RB could be well utilized by snakehead fingerlings with levels from 10% to 30% without any differences in growth performance. Hence, RB could be used in formulated feed for snakehead fingerlings up to 30% to reduce feed cost.

In experiment 2, survival rate fluctuated between (47.8 – 58.9%) among treatments and showed significant differences, with the FM and the others. There were again no significant differences among the treatments in the final weight, daily weight gain, or FI (Table 4); however, 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 4).

Table 4. Initial (Wi) and final (Wf) body weights (g), daily weight gain (DWG) (g.day^{-1}), survival rate (SR) (%), feed intake (FI) (%), feed conversion ratio (FCR), protein efficiency ratio (PER) of *Channa striata* fed experimental diets (% of moisture matter basis)

with solution feeding attractant addition (mean±SE, n=3) in Experiment 3. Values in a row followed by the same letter are not significantly different (p<0.05).

Diets	FM	50% SBM	60% SBM	70% SBM
SR	53.3±1.92[ab]	58.9±2.94[b]	47.8±1.11[a]	57.8±2.94[ab]
Wi	2.28±0.03	2.27±0.03	2.24±0.01	2.24±0.02
Wf	8.53±1.11	8.32±0.50	8.67±0.87	8.33±1.00
DWG	0.15±0.03	0.15±0.01	0.16±0.02	0.15±0.02
FI	3.69±0.47	3.79±0.11	4.48±0.25	4.65±0.32
FCR	1.06±0.02[a]	1.06±0.05[a]	1.23±0.06[a]	1.37±0.09[b]
PER	2.11±0.04[b]	2.11±0.09[b]	1.83±0.09[ab]	1.65±0.12[a]

From an economic point of view, replacing up to 30% of FM and SBM by RB yielded economic benefits (Table 5). The cost for one kg fish weight gain was reduced by 6.88 % in treatment 30% RB compared to the control treatment. In Experiments 2, the cost for one kg fish weight gain decreased 9.91% and 10.5%, respectively, in the 50% SBM diet compared to the control diet; however, the replacements over 50% were not economically beneficial because of increasing FCR (Table 5).

Table 5. Feed costs for producing one kg weight gain by fish fed the experimental diets in the two experiments a) The different ratios of the replacement of Rice bran of the Experiment b) the replacement of Fish meal by Soybean meal with solution feeding attractant addition in the Experiment 2.

Diets	Cost/ Kg fish weight gain (USD)	The reduction compared to control (%) in the experiment
a) Experiment 1		
RB 0%	0.84	--
RB 10%	0.81	3.58
RB 20%	0.79	6.10
RB 30%	0.78	6.88
b) Experiment 2		
FM	0.70	--
50% SBM	0.62	10.5
60% SBM	0.70	-1.05
70% SBM	0.76	-9.41

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., 2015). The results of the current study show that addition of feeding attractant solution 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 and economic results). Experimental results showed that when adding odors, stimulating the feeding, the growth of fish in the 60-70% high protein replacement diet was still good, not different from the control treatment. Hydrolyzed fish protein is used as a protein supplement and food stimulant, leading to increased growth and feed efficiency of salmonids (Berge and Storebakken, 1996; Carvalho et al., 1997). This positive effect may be increased digestibility of the protein of hydrolyzed fish due to the enzyme processing of raw materials. Protein breakdown produces short-chain peptides and free amino acids. Free amino acids act as a food stimulant for some fish, increasing growth and feed utilization. Papatryphon and Soares (2000) reported the addition of 2% fish soluble to fish food, including the amount of striped bass fish *Morone saxatilis* has a similar effect very close to the effect of neutral, non-essential amino acids. However, the addition of 2% fish fluid to *Sciaenops ocellatus*, did not affect growth (Davis et al., 1995), but added 2% or 4% of fish fluid to soybean meal-based diets has increased the food intake and growth of the red drum. (McGoogan and Gatlin, 1997). Positive effects of feeding attractants were 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).

IV. CONCLUSION

In the snakehead diet can use 30% RB. For the addition of feeding stimulants, 70% SBM protein for fish meal protein was feasible (based on growth data), 60% was feasible (based on food conversion ratio data), or 50% was feasible (based on protein efficiency ratio data and economic benefit).

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