

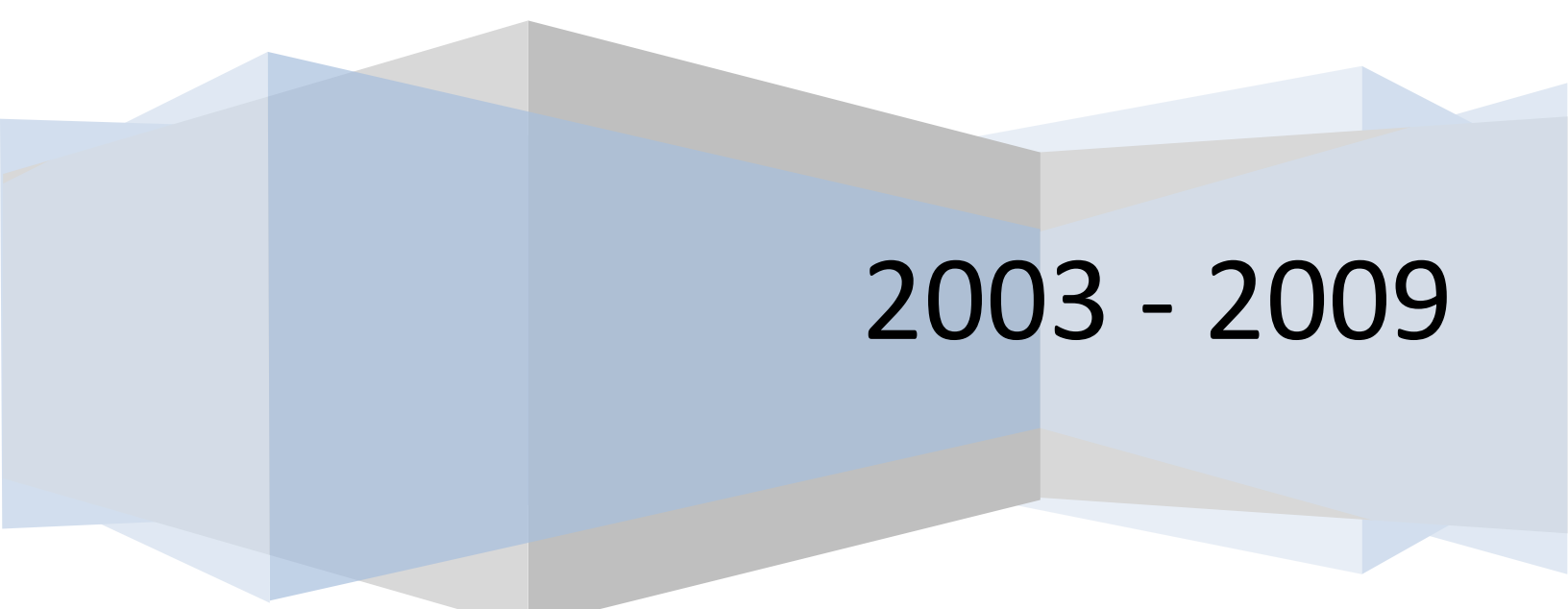


Institut de recherche
pour le développement

Nutrition and feeding of fish of aquaculture-interest in Indonesia

Final report

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A large, abstract graphic at the bottom of the page consists of several overlapping, semi-transparent geometric shapes in shades of blue and grey, creating a complex, layered effect. The shapes are arranged in a way that suggests depth and movement.

2003 - 2009

Introduction

General considerations

The aim of this report is to summarize the research action and results obtained during a 6-year assignment in Indonesia. A contextual review of the first objectives will firstly down according to the evolution initial needs and effective opportunities all over this assignment.

It is commonly assumed that feeding is the main cost for fish farming activity, but considering a fish species this importance will depend on human contribution in the control of breeding, the breeding system (intensive vs extensive system), and the phase of the rearing cycle. Regarding the total cost of production, the economical impact of any improvement in feeding technique or diet formulation will not be the same during the grow-out period than during the larval stage. If the bottleneck of breeding still relies to juvenile availability, the effectiveness of a better feeding practice during grow-out period will be more limited. For the record, if buying fingerling remained a long time the main cost as far this activity was still depending on the availability in young fish, the feeding represents now more than 50% of the cost for the production of Pangasius fish in Vietnam nowadays. These considerations must be kept in mind regarding the attempts and the perceptions of any improvement regarding feeding techniques and nutrition knowledge.

Feeding concept

Feeding refers to the act of giving food to animals (<http://en.wiktionary.org/>). It is often associated to the care to fish even feeding will concern an action related to food only. Research on fish feeding may concern the way feed is provide to the fish and the nature of the food which must be provided. Considering feeding techniques and their importance for the cost of production, feeding must be considered separately but not totally dissociated to the care as most of thrift will refer to the amount of feed effectively used for fish production. Feeding studies, which can also refer to feeding strategies, were developed within this scope.

Nutrition concept

Nutrition refers to the provision of the materials necessary, in the form of food, to support life (<http://en.wikipedia.org/>). Nutrition science investigates the metabolic and physiological responses of the body to diet. Regarding these definition, fish nutrition often refer primarily to identification and quantification of nutrient requirements for fish at the different steps of its development. These concern macronutrients, such as protein and essential amino-acid, during grow-out phases, or micronutrients supply, such specific form of fatty acid, during larval development. According to the amount of materials engaged in these two cases, the relative importance of one or other compounds will differ on economical and sustainable point of view. Nutrition studies concerned firstly macronutrients.

Considering these points and opportunities during this assignment, the feeding and nutrition studies carried out in Indonesia were structured on four work packages:

1. Feeding and nutrition during nursery stage,
2. Dietary protein supply during grow-out stage,



Feeding and nutrition during nursery stage

Regarding feeding and nutrition, fish farmers may be confronted to two successive problems during the nursery stage: to cover specific needs for fish larvae until they can accept compound diets, and then to optimize fingerlings production with compound diet. The first step corresponds to the pre weaning period, and the second step to the post weaning or pre growing period.

The optimization of production during nursery stage aims to reach a target number of seeds with a specific size rather than a total biomass. Pre weaning period is generally focused on optimization of survival rate to allow the highest number of fish through weaning process. On the other hand, the optimization of growth is expected during pre growing period to obtain the most homogenous group of fish of the right size.

Pre weaning studies dealt mainly on the rearing of the striped catfish, *Pangasianodon hypophthalmus* larvae, for the high level of species exploitation as consumption or as ornamental fish, and for its biology (longer weaning time than *Pangasius djambal*). Pre growing studies addressed a larger panel of fish species, including striped catfish, but also some ornamental fish as the clown loach, *Chromobotia macracanthus* or the bala shark, *Balantiocheilos melanopterus*.

These research activities were supported by the French Embassy from 2005 to 2008.

Pre weaning period

The weaning of the striped catfish is reputed to occur 12 to 14 days after hatching in common fish nursery. Even shorter delay can be expected, the period during which the striped catfish larvae must be fed on alive or natural food (artemia nauplii or chopped tubifex) is longer than for *P. djambal*. This quite close species weaned in 3 days or less. As several physiological processes may be engaged before the fish achieves its weaning, the possibility to follow this process over several days gives an advantage for the former species.

The way fish larva must be fed before weaning was one of the key of success to control the breeding of numerous marine species (Kolkovski *et al.*, 2004, Cahu and Zambonino Infante, 1997, Kolkovski, 2001). The delay before weaning is associated to achievement of the ontogeny of the digestive system which is more or less complete at hatching time according to the species. Several characters were then identified during development such as the appearance of pancreatic enzymes, the implementation of stomachic functions (Verreth and van Tongeren, 1989), or settlement of brush border enzymes (Cahu and Infante, 2007). These observations were comforted by the identification of particular requirements such as protein hydrolysate (Cahu *et al.*, 1999) or phospholipids (Cahu *et al.*, 2003) which allowed the development of specific compound diets for marine fish larvae.

On the striped catfish larvae, experiments have been performed in three directions: 1) to test suitability of the compound diet designed for marine fish larvae, 2) to define environmental rearing conditions for optimal development and repetitive results, and 3) to follow the appearance of pancreatic during the development.



Suitability of diet design for marine fish larvae

The utilization of the compound diet developed by IFREMER and INRA (Zambonino Infante *et al.*, 2000) for Atlantic sea bass larvae was tested. Growth and survival were strongly impaired when larvae fed compound diet. The ability of larvae to ingest inert diet was considered among the potential explanations of such poor results. A new test was designed to compare voluntary consumption of *artemia* and inert diet by the striped catfish larvae. Based on our observations, the ingestion of compound diet seemed partial but slightly lower compare to *artemia* nauplii in all case. Thus, more works should be carried out for the development of a compound diet suitable for the weaning of *Pangasius*, especially in order to improve consumption of inert diet.

Environment rearing conditions

The first attempts at experimentation on the striped catfish larvae were quickly confronted with large variation in growth and survival and then poor repeatability of the results. This point confirmed the previous observations made by professional in Indonesia and our Vietnamese colleagues.

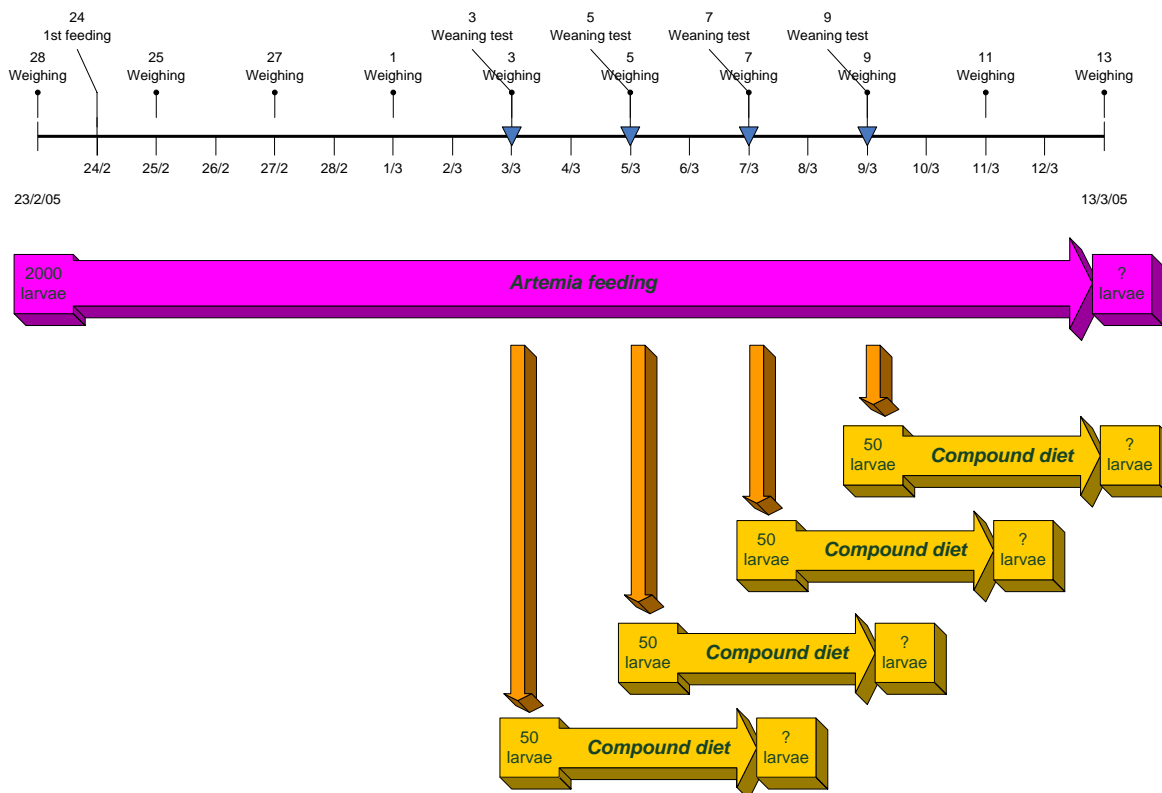


Figure 1 Scheme of protocol used to test influence of rearing conditions (stagnant or recycled water) on growth, survival rate and weaning aptitude by striped catfish larvae.

A first set of experiments was realized to compare traditional methods for rearing of striped catfish larvae to utilization of a water-recycled system. Growth and survival were followed on fish placed in stagnant or recycled water. All the fish were fed on *artemia* nauplii. Weaning tests were performed switching from *artemia* to a compound diet at 8, 10, 12 or 14 days after hatching (DAH). A scheme for typical protocol is summarized by figure 1. The survival rates were significantly lower for fish reared in

stagnant water, even they displayed higher growth. The fish reared in recycled water showed also a better preparation to the weaning which could occur since the 8th DAH. These experiments were realized at the BBAT Mandiangin.

An experimental water-recycled system (see Experimental and laboratories facilities) was then built in LRBIHAT Depok to continue study on larval development and rearing with the transfer of the team to this new place. However, growth and survival rates were still highly variable whatever the apparently better control in the quality of water and the limited number of supplier for newly hatched fish to minimize intrinsic of variability due to the quality of the fish. Consecutively, a set of test were performed to improve reliability and allow definition of standard rearing conditions. Several factors were suspected to be engaged: water quality (drinking or well water), feeding frequency, or sanitary status (with or without antibiotic or formalin prophylaxis). These experiments were performed first in 20-L aquariums or then in 500-mL plastic basket for an easier checking.

In all tests, survival rates remained low and irregular. However some trends could be noted:

- larval growth were higher in recycled than stagnant water (Figure 2A);
- growth were not affected for fish fed six times rather than seven when omitting the last meal of the day (Figure 2B);
- survival rates and growth were significantly higher in drinking water (Aqua® Danone) compare to stagnant well water, in aquarium but not in basket;
- growth was not improve by utilization water-recycled system in aquarium (Figure 2C);
- antibiotic treatment did not improve growth in aquarium, but survival rates were significantly higher with both prophylactic treatment (antibiotic or formalin) in plastic basket .

Considering all these observations, it can be stated that water-cycled system is more suitable than stagnant water to rear larva in small volume (tenths liters) and prophylactic treatment may be recommended. Regarding the utilization of drinking water as standard medium, aquarium and basket experiments differs by the packaging and then the origin of the Aqua® water. This difference may be involved to justify the disparity of the results as water quality may not be strictly equivalent. Thus, special care should be done regarding the use of this kind of water as even bottle from the same brand may not be strictly equivalent over the time or the place of buying (Aqua® is taken from different spring over Indonesian territory).



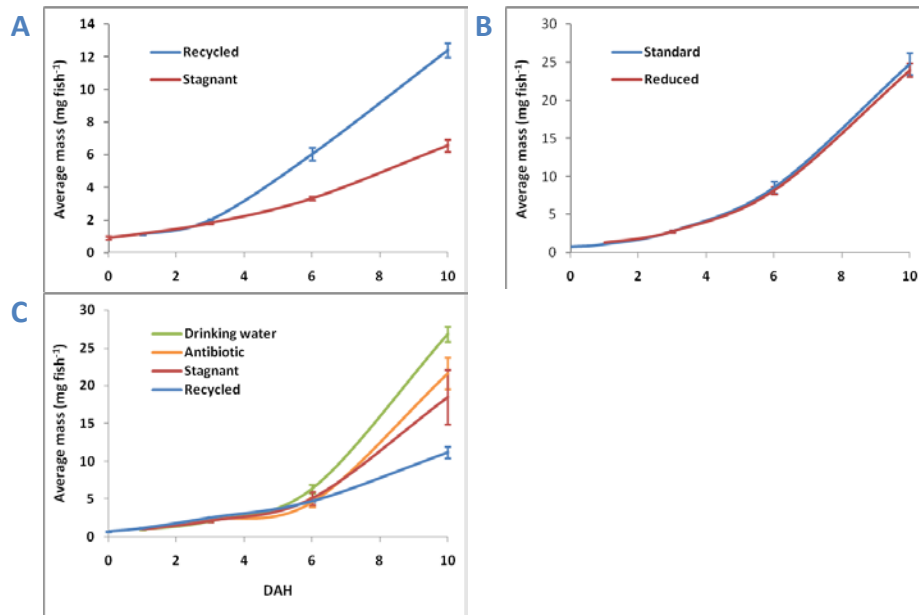


Figure 2 Comparison of growth of *P. hypophthalmus* larvae in different conditions: A, reared in stagnant or recycled well water; B, reared in recycled water and fed with a standard or a reduced number of meals (see text); C, reared in stagnant well water with or without antibiotic, in stagnant drinking water, or in recycled well water. DAH, day after hatching. Bars indicate standard error of means.

Pancreatic enzymes

Besides the difficulty encountered for a standardization of larval rearing, the activity of major digestive enzymes was determined on larvae. Amylase, trypsin and chymotrypsin-like activities were measured in an extract obtained from the whole larvae for fish taken at 0 and 1 DAH, or from the intestinal segment at 3, 6 and 10 DAH (Figure 3). The 0, 1, 3, 6 and 10 DAH serial was retained for a better coverage of an apparently “exponential” chronology of the development during the early life of the fish.

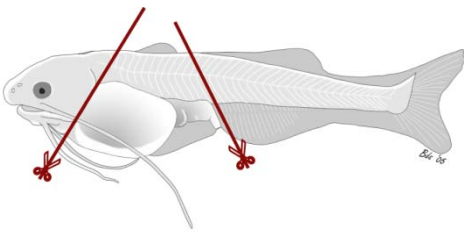


Figure 3 Preparation of intestinal segment from *P. hypophthalmus* larvae. Head and tail part are removed by cuts following the red lines.

As different methods were used for the preparation extract, results were expressed as activity per larva or per unit of body mass (Figure 4). Almost undetectable during the first day of life, the activity increase with growth for three enzymes. The chymotrypsin-like activity per body mass unit was maximal on the 3rd DAH and decrease afterwards, while amylase activity is still rising and trypsin-like nearly stable.



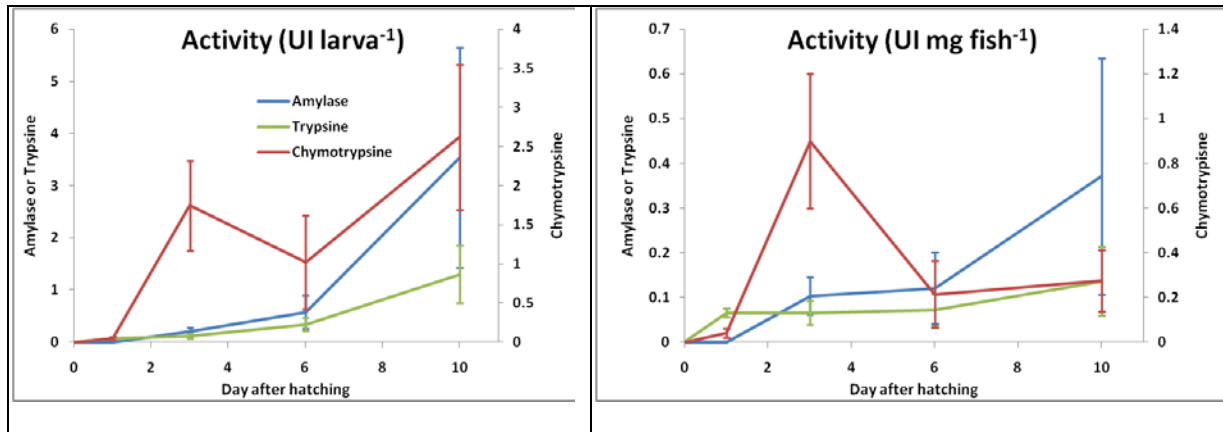


Figure 4 Amylase, trypsin- and chymotrypsin-like activity in *P. hypophthalmus* larva according to age. Bars indicate standard deviation.

Apart from the sharp increase in chymotrypsin-like activity, the results obtained in Depok agree quite well with those obtained on previous sample. Nevertheless, more analyses are still requested to confirm these observations as they can be related only to the specific fish condition. For the younger stage, 0 and 1 day after hatching, the low value may result from difficulty to measure enzyme activities. This can be the result of the very low amount of material available for each sample, the higher dilution of target enzyme by others body source of proteins, or also the higher fat content in the sample resulting from the remain of yolk sac. These constraints may be overcome by the development of molecular biology tools allowing a measure with higher sensibility.

Characterization of digestive enzyme

Molecular tools may be used to follow the ontogeny of digestive system in place of the measurement of enzyme activity during the first days of life. These tools are particularly effective when measurement of the target enzymes activities become difficult with small sample size or contamination by other products interacting directly with the enzymes. Enzyme-specific probes must be developed to follow the implementation of enzyme expression. Probes corresponding to the *P. hypophthalmus* trypsinogen were proposed on the basis of sequence of cDNA isolated from hepato-pancreas and analogy with cDNA for other trypsinogens. The cDNA sequence corresponds to a polypeptide of 242 amino-acid with a molecular weight of 26,361 Da (Uniprot access number Q7T1R8). Considering a putative activation site after the first lysine, trypsin may correspond to a 222-amino-acid protein with a molecular mass of 24,172 Da.

These results had to be confirmed in a second step by the characterization of *P. hypophthalmus* trypsin based on the purified enzyme. In addition, fish processing leads to large amount of waste mainly gut and viscera. This waste can be a valuable source of enzyme such as protease. Definition of purification procedure as well as characterization of enzymes is needed to develop a potential valorization of these wastes.

Chromatography equipment was purchased and installed in Depok in the attempt to purify trypsin from viscera of striped catfish. The equipment was completed by a set of prepacked columns with a large variety of media: ion exchange (anion and cation), affinity (SBTI and Benzamidine), hydrophobic



interaction chromatography, and gel filtration. The better isolation of trypsin leading to almost purified enzyme were obtained after affinity chromatography on Benzamidine Sepharose® gel. Recovery was as high as 40% with a purification factor of 9. Unfortunately, the purification could not be considered as totally achieved. If only one band was shown after SDS-PAGE analysis, the zymogram still displayed two bands (Figure 5). All tentative to improve the quality of the preparation (anion or cation exchange, HIC, and gel filtration) remained unsuccessful. Thus, the characterization of striped catfish trypsin could not be achieved. Several factors may explain this situation. The molecular mass determined on semi-purified product was lower than the expected one. The recovery dropped severely while trying any alternative procedures to improve purification. All these observations can indicate that hydrolysis or autolysis occurred during the treatment. This can be avoided if sample were maintained continuously at low temperature (4°C). Unfortunately, the lack of suitable cold chain facility did not allow to confirm this hypothesis.

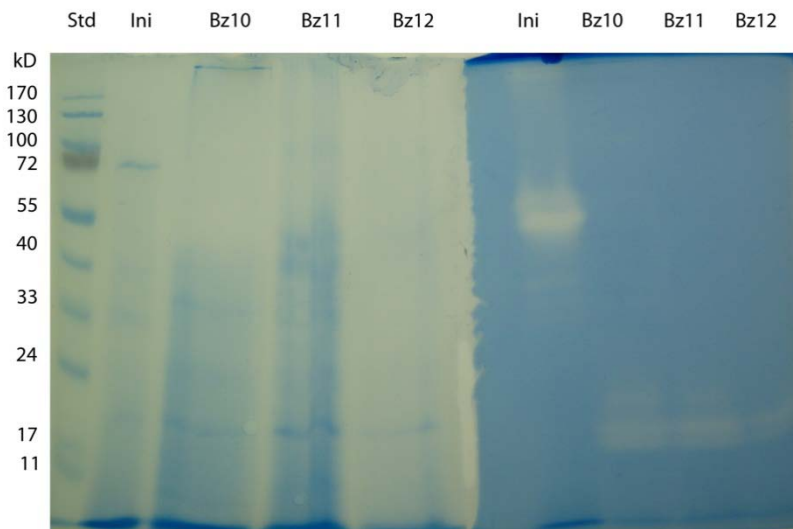


Figure 5 SDS-PAGE (left) and zymogram analysis (right) of *P. hypophthalmus* viscera extract before (ini) and after benzamidine affinity chromatography (Bz 10 to Bz 12).

Pre growing period

Beside the specific feeding requirements of fish larvae during their early life, young weaned fish are usually grow out using various kind of live or compound feeds (Xavier Andrillon, "Report on feeding practices by Indonesian fish farmer in Kalimantan Selatan and Java Barat", 2005, in French). With compound diets, fish are usually fed *in excess* on shrimp crumbles while there was no clear evidence on adequacy of these diets for the target species. As the price of these feeds is very expensive, production cost can also be optimized by a better knowledge on the suitable amount of food according to fish size. This work was carried out mainly on two species, the clown loach and the striped catfish.

All experiments conducted on the clown loach shown that feed efficiency remained very low with a compound diet. Several causes might be engaged: low growth rate of the species, poor acceptability of pellet diet, and/or stress level of the fish in the rearing system. Comparison between live and compound diet confirmed that fish grow better on live food even the growth rate remained limited. In all tests, the growth was better if fish were not disturbed by weekly or biweekly sampling. However, this was not

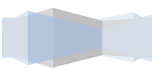
related to the condition of illumination in the rearing system. Comparing three types of live food, small maggot and bloodworm were proven better than earthworm. This could be related to a necessary slicing of earthworm to offer particles food small enough for young fish. Eating behavior was also not improved by association of clown loach with Bala shark specimens even these species display better behavior on pellet diet. Considering all these points, it is recommended to use live food which must not to be sliced before distribution, such as small maggot, tubifex or bloodworm.

Experiments with the striped catfish were performed both at the LORIBIHAT Depok and then BBBAT Sukabumi to overcome supply difficulty in young fish encountered at the beginning of the work in Depok. A second water-recycled system was realized in Sukabumi for this purpose. Experiments on feeding rate were performed in both places, while feeding frequency was tested at the BBBAT Jambi, and pellet size at LORIBIHAT only. Feeding rate and feeding frequency tests were based on utilization of either commercial or experimental diet.

Five feeding rates were tested, 25, 50, 100, 150 and 200 g kg⁻¹ day⁻¹. The results were consistent between LORIBIHAT and BBBAT stations. The better performances (SGR, FCR¹ and survival rate values) were obtained for feeding rate 150 g kg⁻¹ day⁻¹. Five feeding frequencies were tested 2, 3, 4, 5 and 6 meals day⁻¹. Considering the high heterogeneity of fish, no difference in growth and survival rate was observed between the feeding frequencies. This can be related also to rearing system where uneaten feed may remain between each meal.

Three pellet sizes – small (212- 425 μ), medium (425-710 μ) and large (710- 1000 μ) – were tested on newly weaned fish for three weeks. Fish displayed better growth using medium range pellet during the first two weeks after weaning (P=0.0076). However, increase of crumble size according to age did not alter growth or survival rate over the first three weeks. It can thus be recommended to feed fish only on the medium size range of pellet (425- 710 μ) during the first three-week of feeding with compound diet.

¹ In this experiment, FCR were calculated on the basis of average individual surviving fish between each sampling. Thus very low FCR may be encountered due to conjunction effect of necrophagy on dead fish and occurrence of mortality at the beginning of feeding period.



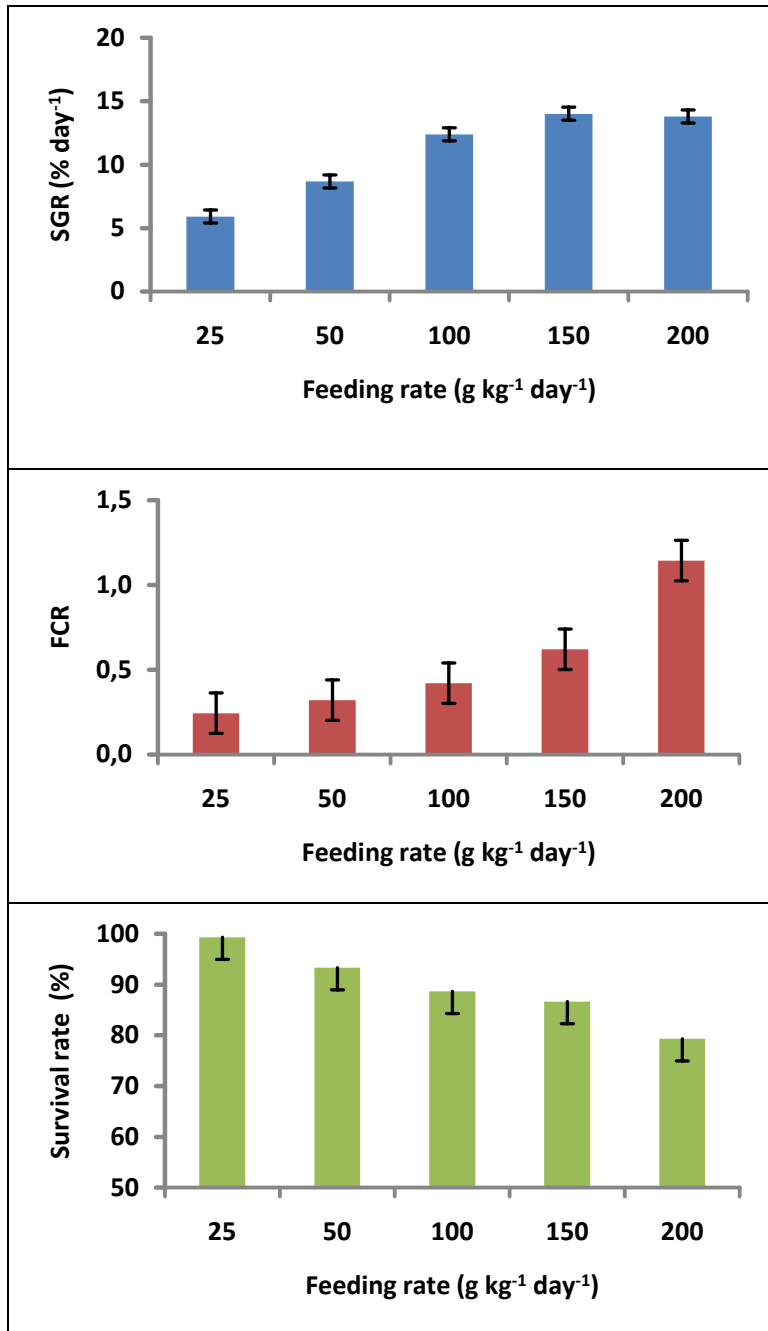
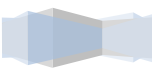


Figure 6 Specific growth rate (SGR), food conversion ratio (FCR) and survival rate of newly weaned *Pangasianodon hypophthalmus* according to feeding rate over 20 days.

Dietary protein supply during grow-out stage

As for the other animal species, survival and growth of fish depend upon the quantity and the quality of food. The nature and then the quality of protein supply is a decisive factor. Among the descriptors of food quality, the protein content is largely used. The concept of daily protein allowance, g protein per kg biomass per day, was developed for inter-specific comparisons to take into account the quantities of food really dispensed (Cowey & Luquet 1983). In addition, several indexes were proposed to define the



quality of protein supply on the basis of experimental data, among them they are the biological value, the protein efficiency, the protein net utilisation, and the protein retention also called Terroine's factor (Mambrini & Guillaume 1999). Other indices, like the chemical index of Block and Mitchell (Block and Mitchell, 1946), allow the evaluation of the quality of dietary protein on the basis of their composition in indispensable amino acids (IAA) compares to a reference protein. Thus regarding protein quality assessment, the fitness of dietary protein source to IAA requirement should be considered.

As for protein requirement, several expressions were proposed to express these requirements. Expressions using proportion in the diet show the higher variability which can be related to various causes, such as difference of growth between species or between experiments (Cowey, 1995). Besides, IAA requirements expressed in percentage of total protein are rather comparable among species (Mambrini and Guillaume, 2001). Considering the nature of the feedstuffs generally available, lysine is probably the first limiting factor for warm water fish (Wilson, 2002). More over for the channel catfish, *Ictalurus punctatus*, experimental determination for lysine requirement allow a good estimation of requirements for all essential amino acids on the basis of amino-acid profile in muscle (Wilson, 2003).

Assuming that excess for a specific amino-acid can be catabolised², it can be expected that deficiency for an IAA may be compensated by higher protein supply. All experiments were then realized using this assumption by feeding fish with different quality of protein at various levels of proteins supplies.

Plant protein

Usually, fish require feed with high protein content even protein efficiency and corresponding amount protein consumed per unit of biomass is lower than for other vertebrates. Thus, fish farming is usually reputed as a great consumer for feedstuff with high protein content such as fishmeal. Many papers deal with the problem of limits for fishmeal production (Naylor *et al.*, 2000, Tacon *et al.*, 1996) and the quest for a sustainable development of aquaculture encourages researcher to find alternative protein source to fish meal. Plant proteins, mainly those issued from proteaginous seeds, were regarded as good alternative protein source as their production is renewable. Protein substitution by plant protein sources were then tested on two pangasiid species, *Pangasius djambal* and *Pangasianodon hypophthalmus*, using soybean, black-eyed pea or mungbean meal.

On the basis of protein requirements previously defined by Hung (1999) for *P. djambal* and *P. hypophthalmus*, experiments were designed to test the effect of dietary protein substitution by plant source with suboptimal (80% of requirement), optimal (100%) and excessive protein (120%) supplies (Figure 7). Protein substitution level were usually compared with same protein level, however, the substitution is efficient only if it is proven that adding the substituted matter improve the results or adversely that expected results will be worst without it. A diet with suboptimal protein supply (diet D) was included in experimental design to test this point. In addition, excessive protein supplies (diet C) was tested to check if the lower performances expected with plant protein can be compensated by higher

² This theory is the basis amino-acid oxidation studies for determination of IAA requirement. Moreover, "the lack of control by dietary protein levels on amino acid oxidation" (Kaushik and Seiliez, 2010) may suggest that IAA deficiency may be compensated by higher protein supply.



protein level (see above). For all protein supply, diets without protein substitution (diets A, C and E) were added for control.

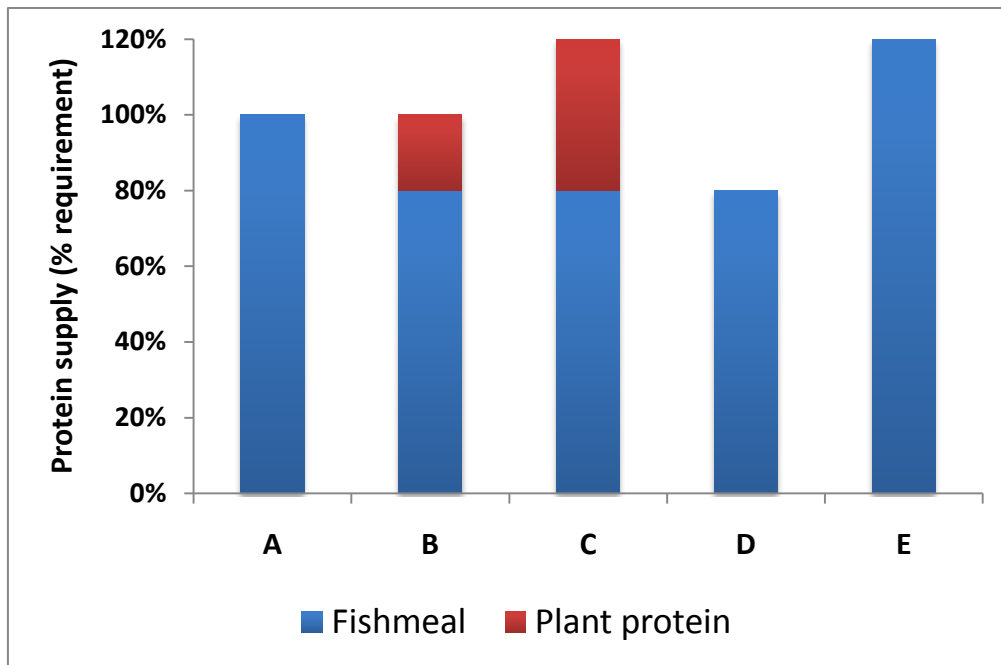


Figure 7 Definition diets used to test substitution of dietary protein by alternative plant protein source. A control diet (A) corresponding to 100% protein requirement, $15 \text{ g kg}^{-1} \text{ day}^{-1}$ (Hung, 1999), is compared to diet with 20% protein substitution with optimal (100%, diet B) or excessive (120%, C). Two diets without dietary protein substitution were include in the design with suboptimal (80%, D) and excessive (120%, E) protein supply. See text for explanation.

This experimental tested on the two species of pangasiid gave almost the same results. Figure 8 shows results obtained with striped catfish juveniles (initial mass, 2.7 g) using black eyed as alternative protein source. Growth rate increased with protein supply whatever dietary protein substitution level, while no effect of protein substitution was observed for the same protein supply. These observations suggested that the $15 \text{ g kg}^{-1} \text{ day}^{-1}$ reference for daily protein requirement was not appropriate. Moreover, the plot of SGR according to the average amount of protein offered per fish show strong positive correlation whatever the type of diet. This last point strongly suggested that protein requirement must be re-evaluated before going further for this species.



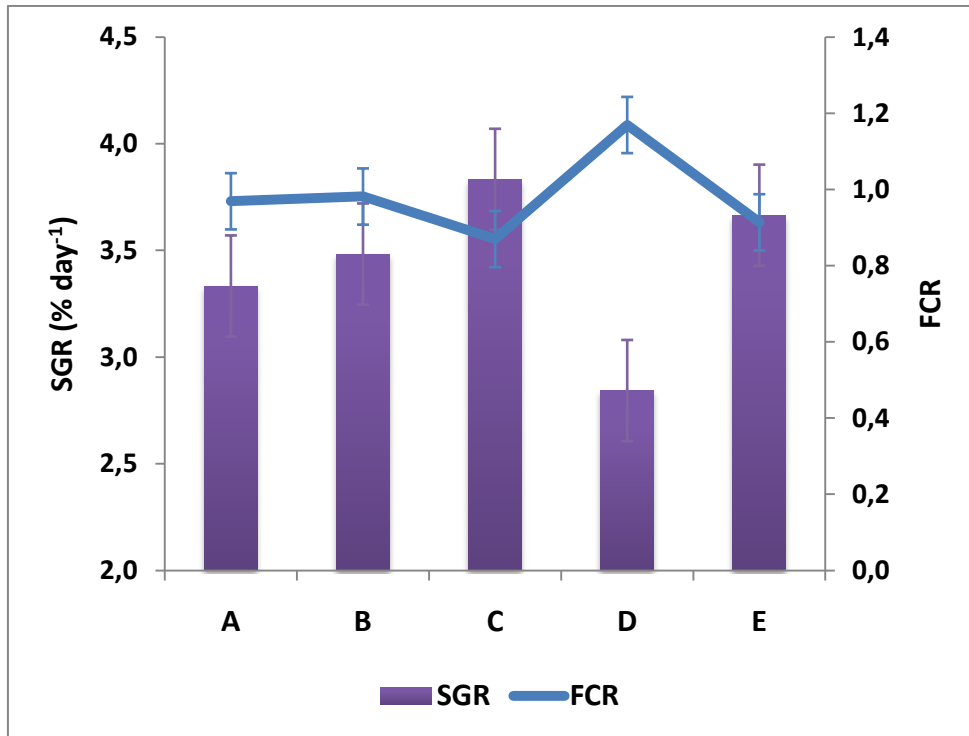
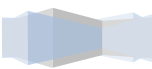


Figure 8 Specific growth rate (SGR) and food conversion ratio (FCR) of striped catfish juvenile, *P. hypophthalmus*, fed with (diet B & C) or without (diet A, C, E) dietary protein substitution by black eyed pea at three levels of protein supply: 80% (diet D), 100 % (diet A & B) and 120% (diet C & E) of daily protein requirement, 15 g kg⁻¹ day⁻¹ (Hung, 1999), over 35 days.



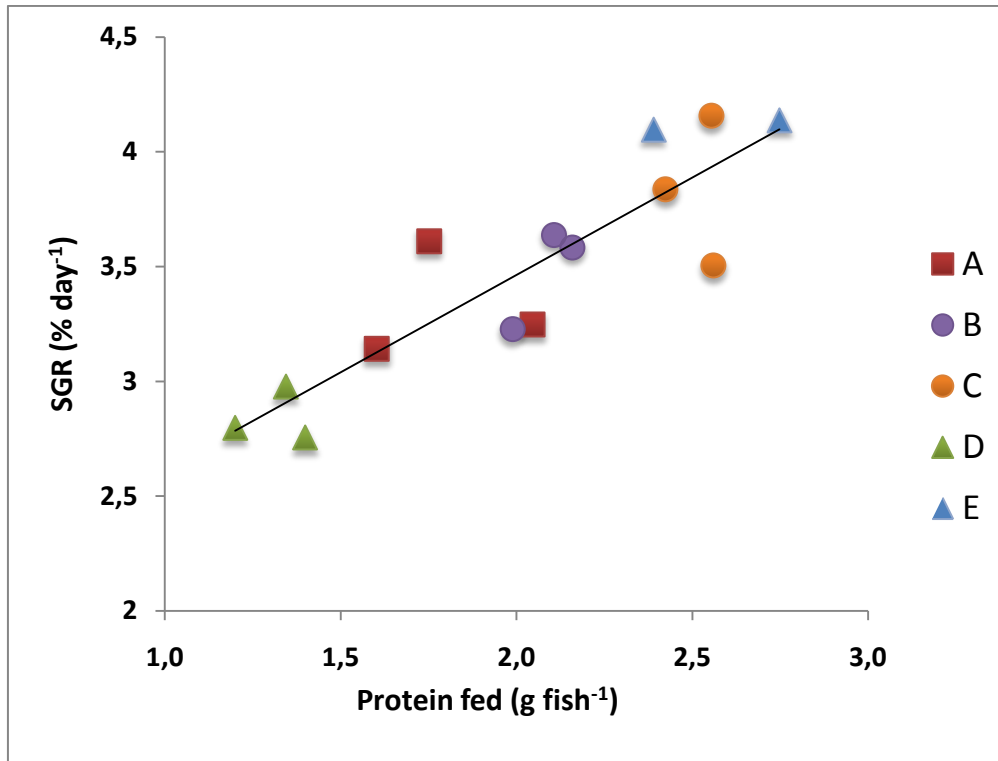


Figure 9 Specific growth rate (SGR) of striped catfish juvenile, *P. hypophthalmus*, fed with (diet B & C) or without (diet A, C, E) dietary protein substitution by black eyed pea at three levels of protein supply: 80% (diet D), 100 % (diet A & B) and 120% (diet C & E) of daily requirement, according to the amount of dietary protein provided over 35 days

Revision of protein requirements

New sets of experiment were designed for the re-evaluation of protein requirements with the striped catfish as target species. The some methodological points associated to feeding rate and protein energy ratio interaction was used.

In earlier 80's, scientist agree to use daily protein supply – i.e. g protein per kg fish per day – rather diet protein content – i.e. g protein per kg diet – for the definition of protein requirement in fish (Cowey and Luquet, 1983). Gatlin et al. (1986) determine optimal feeding rate – associated with the higher mass gain – for channel catfish fed on two diets, 25 % or 35 % protein, with the same protein to energy ratio, 21 mg CP kJ-1. They showed that the maximal expected growth can be the same for each diet, and further that optimal feeding rates for each diet correspond to the same daily protein supply. According to this, a farmer must increase the feeding rate to maintain the same level of production using diet with a lower protein content. Thus feeding rate should be considered regarding any change in the quality of feed as comparable growth may be obtained with different protein content fed *ad libitum*.

Ng (2003) compares two methods to increase protein supply for the determination the protein requirement of a tropical catfish. In the first method, the increase resulted from the variation of protein level in the feed, while variation of feeding rate using a single type of diet was used in the second one. Estimations of protein requirements, as daily protein supply, were very similar with both methods. Considering these observations, the variation of feeding rate could be a valuable tool for an estimation



protein requirement with a single diet. However, comparing the protein requirement proposed for *Pangasius bocourti* and *P. hypophthalmus* (Hung, 1999), the optimal daily protein supply was higher when fish were fed on pellet with high level of protein content and high protein-energy ratio (Hung *et al.*, 2004). Therefore, the determination of protein requirement based on protein supply rather than dietary protein content must depend on the protein-energy ratio (PE ratio, in mg kJ^{-1} , milligram crude protein per kilojoules gross energy) of the feed. This may also limit the compensation of protein supply by an increase of feeding level when dietary protein content is too low.

Two experiments were realized in Depok 1) to determine optimal feeding rate – and then dietary protein supply – for fish fed on diet with two different PE ratios, 19.2 and 23.6 mg kJ^{-1} ; and 2) to compare growth and feed efficiency of fish receiving the same dietary protein using diet with PE ratio ranging from 17 to 28 mg kJ^{-1} .

Protein requirement and determination of optimal feeding level with two dietary PE ratios

Optimal feeding rate differed significantly for fish fed with the lower dietary PE ratio, 19.2 mg kJ^{-1} (Figure 10). As protein content in 19.2 mg kJ^{-1} was lower than in 23.6 mg kJ^{-1} diet, this observation suggested that more feed was necessary to provide the same amount of protein and then sustained growth. Average final mass results were then plotted according to daily protein supply (Figure 11). Whatever the dietary PE ratio, highest growth rate were obtained with the same protein supply. Moreover, expected maximal average mass at the end of experiment did not differ between to the diet.

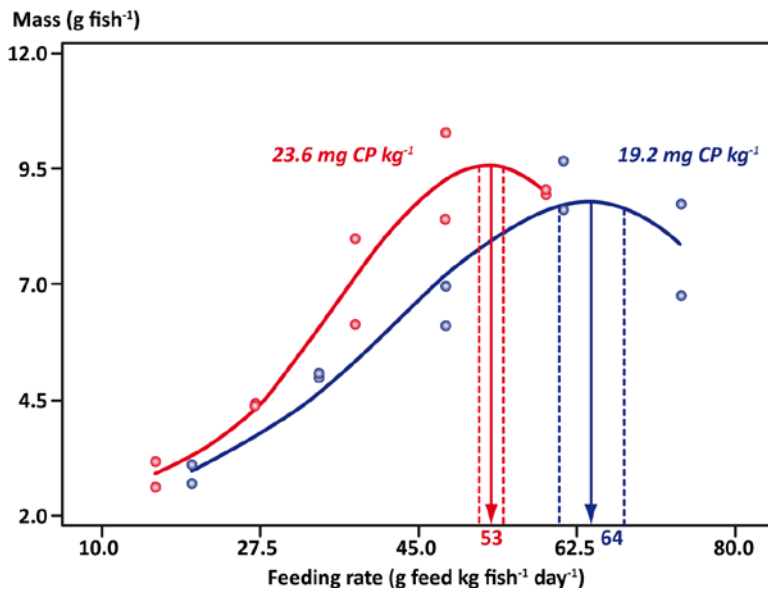


Figure 10 Average final mass of striped catfish juveniles (initial mass 1.8 ± 0.1 g) fed with increasing feeding rate and different dietary protein PE ratio, over 28 days. Arrows indicate optimal feeding level with 95% confidence interval.



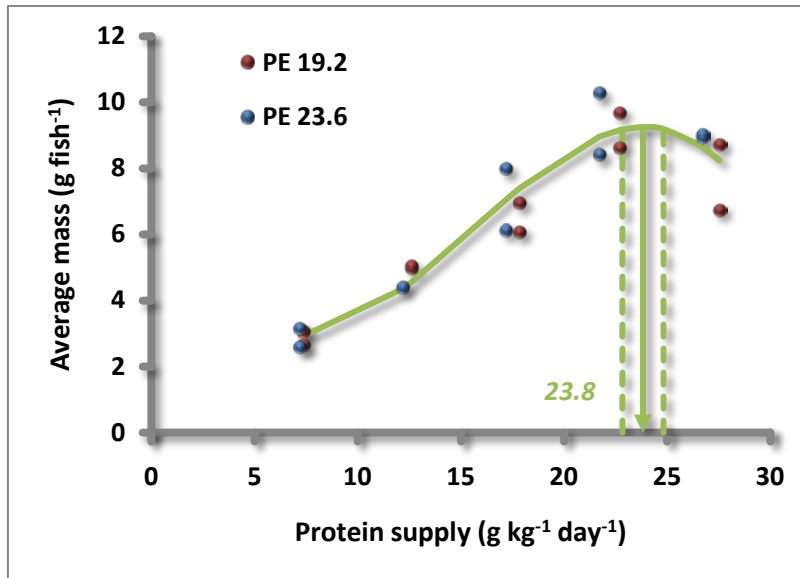


Figure 11 Average final mass according to daily protein supply of stripped catfish juveniles (initial mass 1.8 ± 0.1 g) fed different dietary protein PE ratio, over 28 days. Arrows indicate optimal protein supply with 95% confidence interval.

The results of this experiment allow confirming that the $15 \text{ g kg}^{-1} \text{ day}^{-1}$ protein supply used in the previous experiment was too low to support maximum growth for juveniles of stripped catfish. Protein requirement was then re-evaluated to $23.8 \pm 1 \text{ g kg}^{-1} \text{ day}^{-1}$ for stripped catfish with body mass ranging from 2 to 10 g. As no difference was observed between the two dietary PE ratios, new set of experiments were realized to test the new definition of protein requirement over a wider range of PE ratio.

Effect PE ratio on feed efficiency with constant protein supply

Effect of dietary PE ratio was tested on juvenile stripped catfish receiving the same amount of dietary protein, $23.8 \text{ g kg}^{-1} \text{ day}^{-1}$. Dietary protein was provided by a mixture of fishmeal and soybean meal using corn and palm oil to adjust dietary PE ratio. Daily protein supply was adjusted for each diet by adaption of feeding levels according to their protein content. The highest (28 mg kJ^{-1}) and the lowest (17 mg kJ^{-1}) limit for PE ratio were defined to avoid excessive feeding levels (less than $60 \text{ g kg}^{-1} \text{ day}^{-1}$).



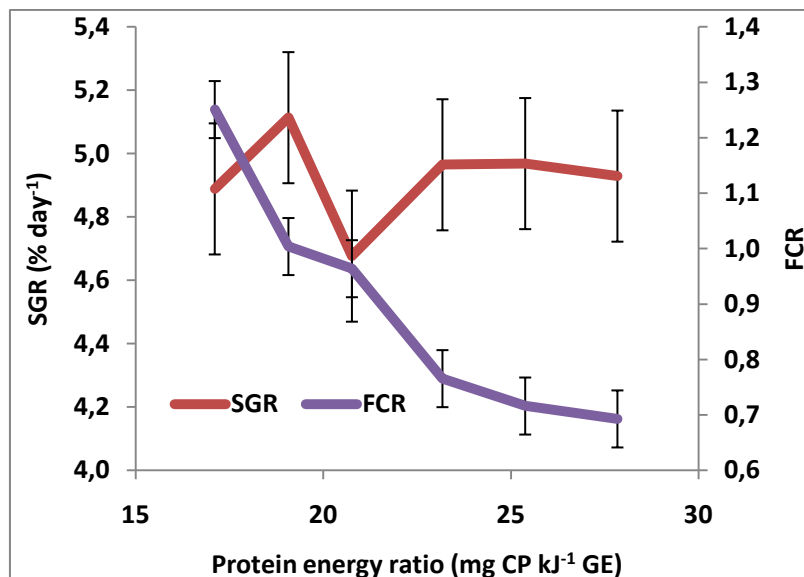


Figure 12 Specific growth rate (SGR) and food conversion ratio (FCR) of juvenile striped catfish (initial mass 1.13 ± 0.04 g) receiving the same protein supply from feed with different dietary PE ratio, over 35 days.

Differences in growth rate among all dietary PE ratios were not significant, while FCR was inversely correlated to dietary protein³ (Figure 12). In addition, the comparison of growth curve parameters (not shown here) did not show any effect associated to the type of diet. These results indicate that striped catfish may be fed on wide range of feed quality⁴ as far as the right protein supply is maintained to sustain growth. Moreover, optimal protein supply may be determined with a large variety of diet as the growth of striped catfish did not depend of dietary protein content when feeding levels were not limiting.

Based on these observations, optimal protein supplies were determined for striped catfish until market size from experimental determination of optimal feeding rate with commercial diet, at BBAT Jambi. Then, a feeding chart was prepared to help striped catfish farmer for the calculation of optimal feeding rate according to the mass of the fish and the protein content of available feed (Ediwarman *et al.*, 2009).

Influence of protein quality

This re-evaluation of protein requirement for striped catfish allowed continuing experiments previously engaged on utilization of plant as alternative protein source. In the light of the results obtained with the studies on optimal protein supply, experiments were focused on interaction between amino-acid profile of proposed dietary protein and effective protein supply required for optimal growth. Growth and feed utilization were compared on fish fed on feed with low-lysine and high-lysine content at different feeding level to modify protein supply at Depok.

The results of the successive experiments performed for this purpose were strongly impaired by a high variation among the different batches of fish to maintain comparable high growth rate. However the

³ This is consequence of the necessary decrease of feeding level to maintain the protein supply of high protein content feed corresponding to higher PE ratio.

⁴ Lower dietary PE ratios are usually associated to cheaper feed as their protein content is lower.



comparison of growth rates on fish from the same origin and in the same rearing conditions shown that protein supply required to obtain higher expectable growth rate had a tendency to increase with the low-lysine content diet. So, even this must be confirmed by others experiments with a higher reproducibility of growth, indication are given that utilization of dietary protein with lower indispensable amino-acid profile may be at least partially compensated by an increase in protein supply and then feeding level.

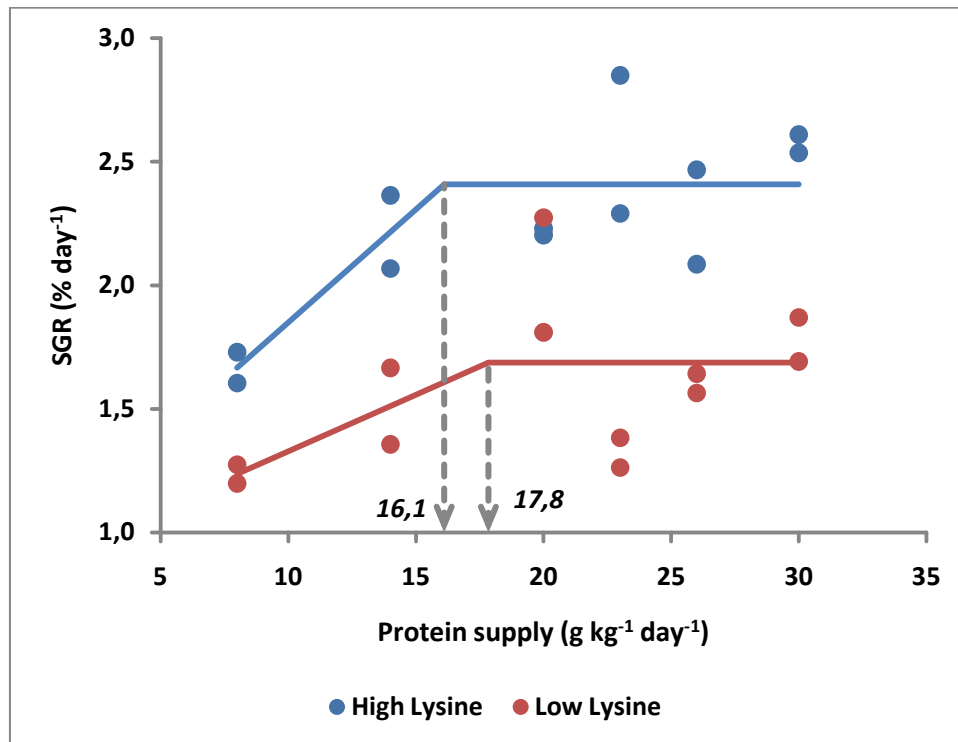


Figure 13 Specific growth rate of juveniles striped catfish (initial mass 6.5 ± 0.1 g) fed on low-lysine or high-lysine content diet with different protein supplies over 33 days. Arrows indicate minimum protein supply to reach highest expectable growth for each diet.

Bioconversion

The bioconversion program conducted by IRD team since 2004 leads to the production of new feedstuff candidate for dietary protein source in fish feed: larvae of black soldier fly (BSF), *Hermetia illuscens* (Hem et al., 2008). The potential of this new feedstuff, initially produced on chicken or swine manure, was largely evaluated for fish, chicken, pork and others reared species (Brodi and Cole, 2007, Newton *et al.*, 2005, Sheppard, 2002, Sheppard and Newton, 2000, Bondari and Shepard, 1987, Bondari and Sheppard, 1981). However as the present production of BSF larvae was based on bioconversion of palm kernel meal (PKM), a by-product of palm oil industry, new evaluation of the product was necessary with the fish species reared in Indonesia. At first, the potential of BSF larva as protein source for fish was evaluated by analysis of the nutrient compounds compare to other major feedstuff available in Indonesia. Afterwards, the efficiency of protein from PKM, the initial by-product, and BSF larva was tested on fish. Finally, the

limits of utilization of BSF larva, as alternative protein in compound feed or as substitute to pellet, were determined on different species of fish.

Chemical composition of BSF larvae

Proximate analysis, amino-acid and fatty composition were performed on a meal prepared from crushed and freeze dried larvae of BSF. The results were compared to other common feedstuffs used in fish feed and to palm kernel meal in table 2. Meal from BSF larvae are characterized by rather high crude protein content (only 10 points less than soybean meal) associated to a high fat level. Fatty acids are mainly saturated ones (78.8%), with 51.0% of total fat corresponding to lauric acid. Analyses of fiber show large discrepancy between the two methods, crude fiber or Weende method and TDF. This may be associated to presence of hardened proteinaceous matrix embedded with chitin in the exoskeleton of BSF larvae which was released during strong enzyme digestion process of TDF analysis.

Table 2 Proximate composition, amino-acid, fatty-acid and energy content of meal from black soldier fly larva (BSF) compare to other feedstuffs.

Characteristics (g kg ⁻¹ dry matter)	BSF larvae	Fish meal ¹	Soybean meal ¹	Palm kernel meal ²	Rice bran ¹
NFE	-	-	344	582	456
Crude fiber/Chitin	227	-	84	127	129
TDF ³	119	-	-	-	-
Crude protein	388	720	483	193	142
Lysine	18.3	54.7	30.7	7.1	6.2
Methionine+cystine	24.0	27.4	14.4	6.0	4.7
Tryptophan	5.2	7.6	6.5	2.0	1.4
Threonine	14.3	31.3	19.0	6.6	4.9
Leucine	24.4	55.4	37.0	13.3	10.0
Isoleucine	14.7	33.8	24.3	7.4	5.7
Valine	20.5	39.9	24.8	11.2	8.6
Histidine	8.6	16.5	11.9	3.5	3.8
Arginine	17.3	41.0	36.1	26.1	10.8
Phenylalanine+tyrosine	27.0	52.5	41.5	13.3	13.2
Fat	416	104	20	49	153
Saturated fatty acid	328	20.2	2.6	39.9	9.0
Monosaturated fatty acid	77	52.4	3.5	7.4	66.7
LCFPA n-6	-	0.5	-	-	-
LPFA n-6	10.8	1.8	9.7	1.1	61.1
LCFPA n-3	-	13.8	-	-	-
LPFA n-3	0.8	14.9	1.4	-	1.2
Minerals	89	170	68	50	119
Gross Energy (MJ kg⁻¹)	25.6⁴	20.3	19.7	16.5⁴	9.1

¹ from Guillaume et al. (2001); ² Amino-acid profile recalculated from Tang (2001) and fatty acid from palm kernel oil profile (Wikipedia, http://en.wikipedia.org/wiki/Palm_oil#cite_note-21); ³ Total dietary fiber, AOAC 991.43 method (AOAC International., 1998); ⁴ Calculated from crude protein, fat and NFE contents, 23.6, 39,5 and 17.2 kJ g⁻¹, respectively (N.R.C, 1993)



Protein quality is usually evaluated on the basis of IAA profiles expressed in g per 16 g nitrogen which correspond to the percentage of crude protein as determined by Kjeldahl method. The IAA profile of BSF larvae protein was compared to other feedstuff with respect to the requirements for channel catfish (Wilson and Moreau, 1996) usually retained as reference for all catfish due to a relative homogeneity between fish species. Whatever the source of protein, the proportion of each IAA fulfills catfish requirements except for lysine (Figure 14). Lysine proportion in BSF larvae protein is just below the limit (93%) but still higher than for PKM and rice bran. Thus, as far as suitable amount of dietary proteins may be supplied, the BSF larvae should be a valuable source of protein for catfish.

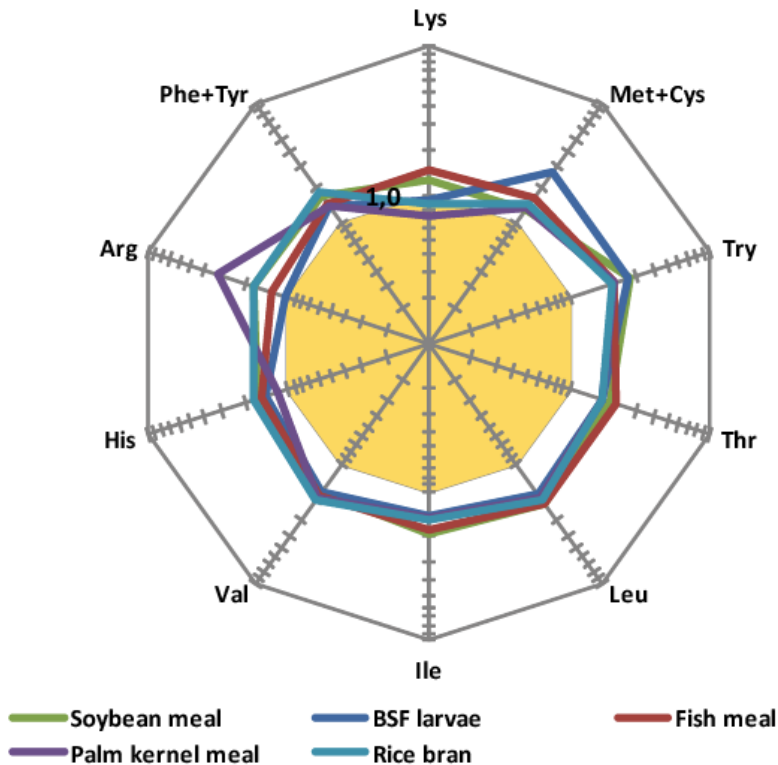


Figure 14 Indispensable amino-acid profiles of BSF larva protein compare to other protein sources as percentage of catfish requirements, shaded area (Wilson and Moreau, 1996). Logarithm scales are used to avoid large discrepancies between each amino-acid.

Compare to initial by-product, analytical results indicated that bioconversion process led to a potential feedstuff with twice higher protein content and slight improvement of protein quality but still high fat level with fatty acid composition strongly correlated to PKM. However, these contestations had to be confirmed on the basis of experimental test on fish.

Protein efficiency from PKM and BSF larvae

Experimental protocols based on dietary protein substitution by alternative protein source to test protein efficiency from each feedstuff. Even this type of protocol led to preparation and distribution of diets with increasing proportion of alternative feedstuff, the presentation of results will be focused on the fate of protein and then the amount of protein which can be supplied an alternative feedstuff.



Protein efficiency of PKM was tested on *P. djambal* juveniles. Feeds were formulated with PKM as protein substitute compare to fishmeal and soybean, with particular attention to reduce fishmeal contents. According to the limit for feeding rate and dietary protein content target (300 g CP kg⁻¹), substitution of dietary protein by PKM could not exceed 18%. Growth rate and feed conversion ratio were not affected by protein substitution with PKM as high as 15.8% and 13.3%, respectively. Significant loss of performances were then observed for the higher substitution levels.

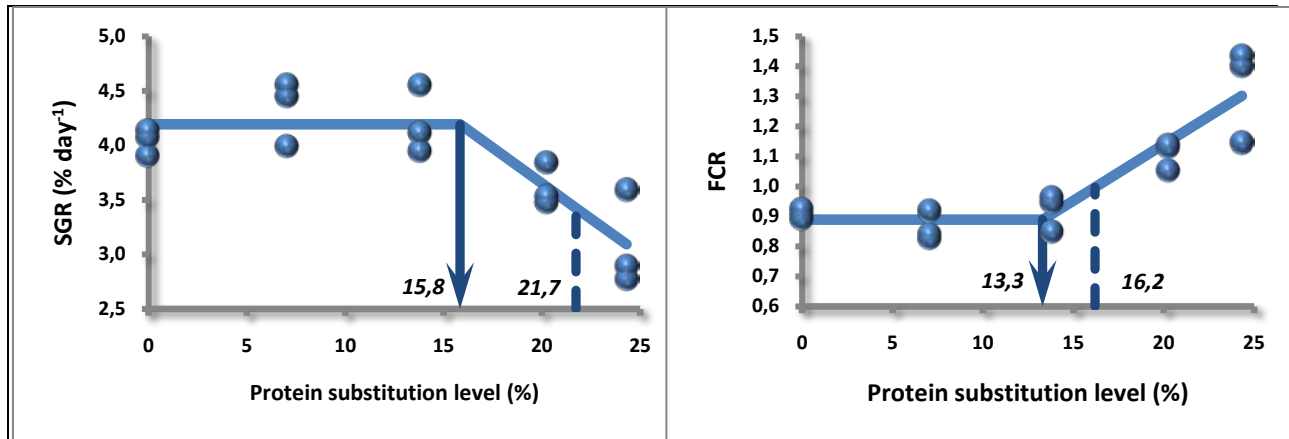


Figure 15 Growth and food utilization of *Pangasius djambal* juvenile fed on diets with increasing level of protein substitution by palm kernel meal, over 28 days. Plain arrow indicate break point estimate and dashed line their 95% confidence upper limit.

Similar experiment was realized to test protein efficiency of BSF larva meal on the same species. Considering fat and then energy content of BSF larva meal, the highest protein substitution level was 22%. Difference were not significant among all treatments even a slight diminution of growth rate was observed with all diets containing BSF (Figure 16). Thus, BSF larva meal may substitute at least 22% of protein in diet for *P. djambal*.

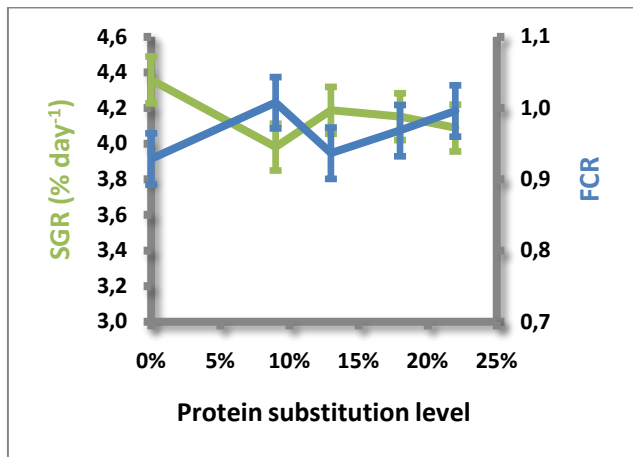


Figure 16 Growth and food utilization of *Pangasius djambal* juvenile fed on diets with increasing level of protein substitution by BSF larva meal, over 28 days.

Protein substitution level was limited for both feedstuffs either according to protein efficiency for PKM and energy content for BSF. A third experiment was then realized to test the effect of protein

substitution using both products simultaneously. A new test diet was prepared where 17% of dietary protein was substitute by PKM and 24% by BSF larva. Higher protein substitution levels were tested to anticipate any synergic action due to probable complementarities of these feedstuffs. Compare to results with a control diets with neither BSF nor PKM, growth rate and food conversion ratio were not altered with diet containing both feedstuffs (Figure 17). Moreover results were better than for dietary protein substitution with PKM or BSF only.

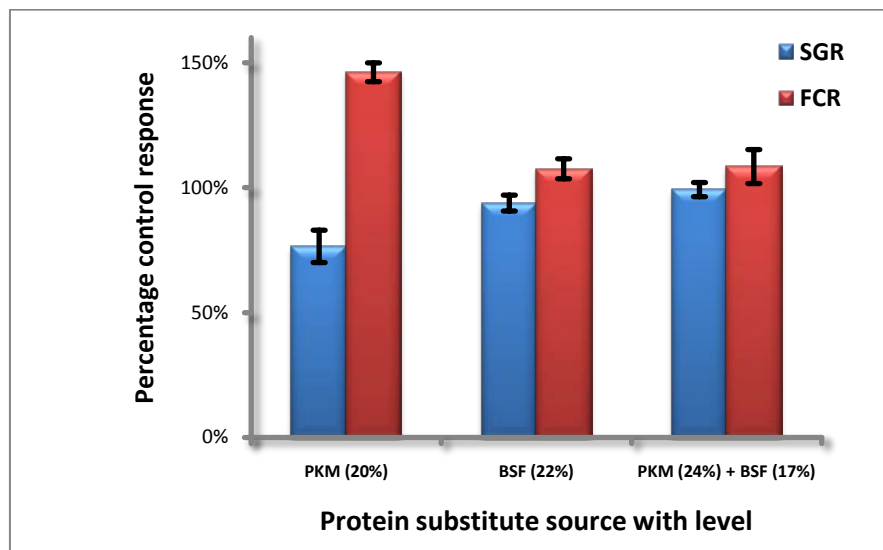


Figure 17 Growth and food utilization of *Pangasius djambal* juvenile fed on diets with fix level of protein substitution by PKM or BSF larva meal only or together, over 28 days. Results are presented as percentage of answers obtains during the same experiment with a control BSF-free and PKM-free diet.

PKM and BSF larva can be used efficiently as dietary protein source for *P. djambal*. However, limit for utilization of PKM appears lower than with BSF larvae. Substantial benefit may be expected if the feedstuffs were used simultaneously. Considering 3:1 ratio as rough estimate for efficiency of bioconversion of PKM, the preparation of one ton of PKM+BSF containing feed will allow the valorization of three tons of PKM.

Limits for utilization of BSF larvae

Limits for utilization of BSF larvae were determined when used either as meal for dietary protein substitution in formulation of compound feed, or as a whole fresh or alive for substitution of common feed (pellet or trash fish). Experiments were completed on various species of fish reared in Indonesia: two catfishes, *P. hypophthalmus*, *P. djambal*, a tilapia, *Oreochromis sp.* and the giant snake head, *Channa micropeltes*. For this purpose the growth of fish were follow over longer period until nearly 100 grams.

Responses of fish to increasing dietary protein substitution by BSF larva meal differed according to species. Typical broken-line response was observed for the striped catfish as for *P. djambal* in previous experiments. On contrary, growth response of tilapia was continuously altered with increasing level of BSF larva meal (Figure 18).



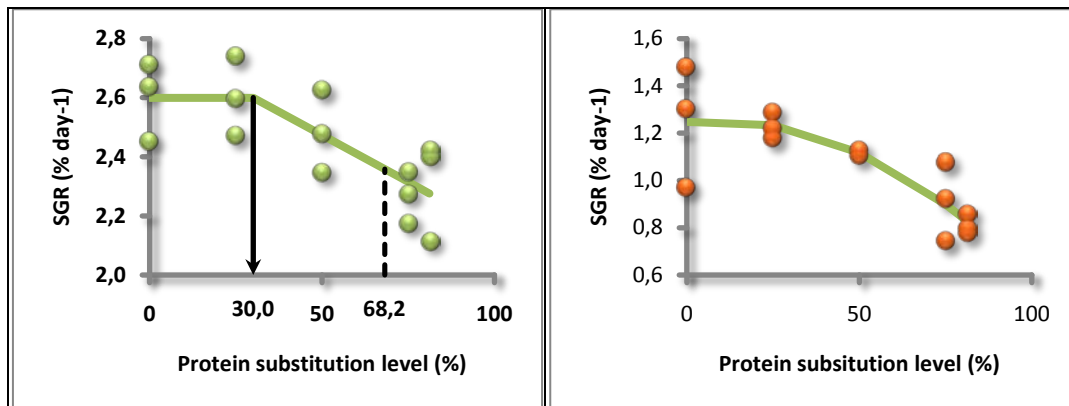


Figure 18 SGR of striped catfish (left) and tilapia (right) according to dietary protein substitution level by BSF larva meal, over 84 for catfish and 90 days for tilapia.

Even negative impact was expected, savings associated to dietary protein substitution by BSF larva meal may reduce significantly the cost of feeding. Besides a necessary economical evaluation for production of BSF larva meal, the future determination of worthy limits will need a quantification of the impact on growth and FCR according to the level of dietary protein substitution by BSF. Growth models were then adjusted to data and the expectable delays for a fish to reach similar size than with control diet were calculated according to dietary substitution level. Corresponding adjusted models were plotted for striped catfish and tilapia in figure 19. Even at the highest substitution level, incorporation of BSF in the diet of striped catfish will increase only a third of the rearing time of fish to reach nearly 100 g and only 7% more food will be required. On the contrary impacts on tilapia are more important, as rearing time will be 1.5-fold longer and required feed 1.6 higher.

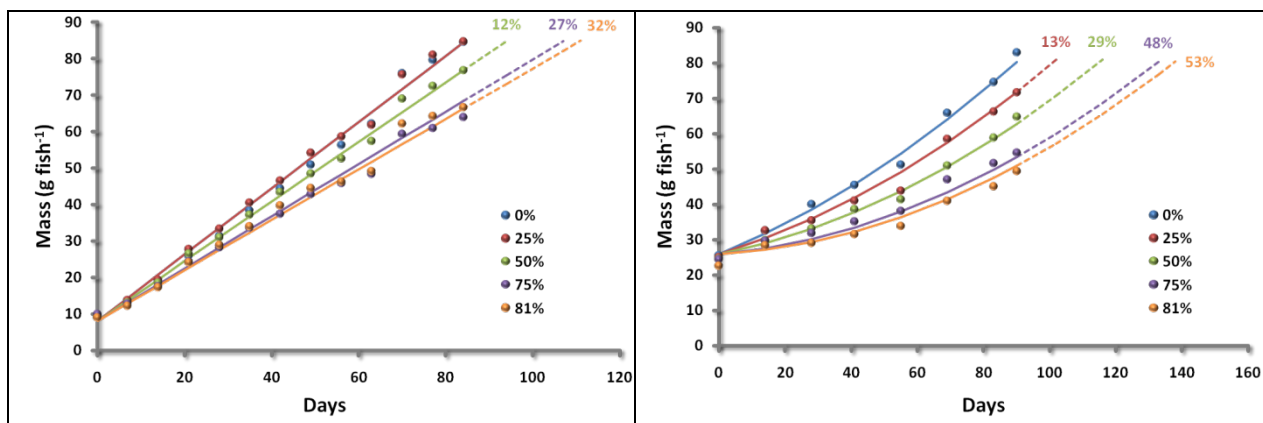


Figure 19 Growth model for striped catfish (left) and tilapia (right) according to dietary protein substitution by BSF larva meal. Percentage at the top of curves indicate the increase of time required for fish to reach the same mass than with 0% dietary protein substitution level.

Substitution⁵ of pellet by live BSF larva was studied on *P. djambal* in hapas and in aquariums. Compare to inclusion in feed, the substitution of pellet by live BSF larva was not limited by formula and then allowed 100% substitution level. The apparent impacts on growth were more important than by inclusion directly in the diet. Growth curve analyses indicate higher impact in hapa than in aquarium (Figure 20). Expected time required for the same increase in body mass was multiplied by two in hapa and only 60% in

⁵ To maintain equivalent feeding supply, substitution rate were calculated on dry matter basis.



aquarium, while corresponding amount of feed should be 62% and 46% higher, respectively. The better results obtained in aquarium might be associated to the fact that access to uneaten BSF larva was maintained even after the time of feeding. Utilization of live BSF larva with tilapia confirmed the results obtained by inclusion of BSF meal in the diet. Growth was strongly impaired and increase of time for the same body mass increase was even not estimable (data not shown here).

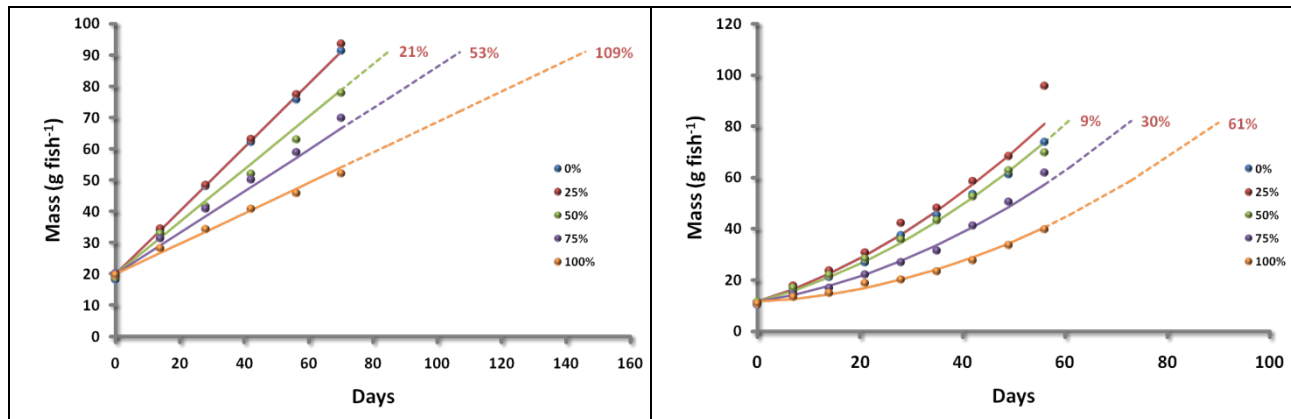


Figure 20 Growth model for *Pangasius djambal* in hapa (left) and in aquarium (right) according to pellet substitution by BSF larvae. Percentage at the top of curves indicate the increase of time required for fish to reach the same mass than with 0% pellet substitution level.

Similar analysis was performed with substitution of trash fish diet by live BSF larva on giant snakehead between 5 to 27 g, over 56 days. As for tilapia, growth was strongly impaired and delay estimated to 200% of the highest observed growth rate.

As no mortality was observed on fish fed with BSF larva-containing pellet or with live BSF larva, this feedstuff may be regarded as suitable for fish. Impact on growth differs within the studied species in both cases, with better acceptance by the two catfishes, *P. hypophthalmus* and *P. djambal*. If better results were obtained by inclusion of BSF larva meal in pellet, the limit of dietary protein substitution induced with this way of utilization did not allow advocating this method. In fact considering the protein content, limit was obtain with similar dietary protein substitution level using the two ways of presentation.

Experiments on BSF larva were realized at LORIBIHAT Depok for short term experiments, and BBAT Jambi for long term ones.

Miscellaneous activities

Limited activities were also realized in relation with the program develop by partners, such as analysis of feeding behavior of clown loach and the effect of the nature and number of feedstuffs used for the preparation of home-made feed.

Stomach contents of clown loach from Musi were analyzed according to size, sex, season and place. The results indicated a large predomination of insect larvae in the prey selected by the fish. This suggest that clown was a bottom feeder, grazing the surface of rocks or gravels in the river. Occurrence of bivalves

was only observed in a big female fish, indicating the possibility for the fish to switch from picking up to digging up behavior if need.

A comparison of feed prepared with two, three or four ingredients was realized on the striped catfish using the different feedstuff available in Jambi. Performance was not improved by the multiplication of feedstuffs and better results were obtained with a mixture of fish meal and rice bran.

Experimental and laboratories facilities

During this 6-year period, special attention was paid to improvement of laboratory facilities in Depok as well as optimization of method in BBBAT Sukabumi or BBAT Jambi.

In Depok, besides the transfer of biochemical equipment from Pasar Minggu, a fully functional laboratory for proximate analyses was installed in late 2008 (Figure 21). With more than 300 analyses in June 2009, this equipment was used by different programs carried out in Depok to complete analysis of feed and feedstuff. Comparison of results with others laboratories were also realized to control these new facilities.



Figure 21 Equipment newly available in Depok laboratory for determination of proximate analysis: Oven and furnace (A), Kjeldahl mineralization and Soxhlet extraction (B), Kjeldahl distillation and titration (C), and crude fiber analysis (D)

Capacity building improvement

Special attention was paid to supervising of Indonesian student as well as BRKP or BBAT staffs. Ten S1 were supervised or co-supervised, Universitas Indonesia (Ika Ayuningtyas, Yestha Jwalita), IPB (Rini Afifah, Siti Rachmi), UNSRI (Maya Angraini, Susanti Monika, Soikar Hariadi, Ida Hadayani), and UNPAD (Danang Prasetyo, Hasbiallah); two S2, IPB (Ediwarman, Dain Retnosari); and one S3 was planned but unfortunately aborted. The two research partners, Nina Meilisza (LORIBIHAT, Depok) and Ediwarman



(BBAT Jambi) were also invited to participate to an international symposium for presentation of communication and poster.

List of publications

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- Baras, E., Hafsaridewi, R., Slembrouck, J., Priyadi, A., Moreau, Y., Pouyaud, L. & Legendre, M. (2009) Why is cannibalism so rare among cultured larvae and juveniles of *Pangasius djambal*? Morphological, behavioural and energetic answers. *Aquaculture*. Submitted
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