United Soybean Board
Final Report Form – Technical Bulletin

Project # and Title	USB #2463-Use of soybean meal and soy protein concentrate as alternatives to fish meal in practical feeds for milkfish
Organization & Project Leader	SEAFDEC Aquaculture Department Relicardo M. Coloso, Ph. D.
Reporting Period	2009-2012

Introduction: Statement on the rationale and background for the studies

Milkfish, *Chanos chanos*, is an important food fish in Southeast Asia and its aquaculture potential is fast growing in the Philippines, Indonesia, Taiwan, and other Southeast Asian countries. The Philippines produced about 375,000 metric tons of milkfish in 2011 (BAS 2012) with 60% coming from brackish water ponds, 20% coming from freshwater cages and pens, and 20% coming from marine floating net cages. The intensification of milkfish aquaculture will require the use of a more cost-effective and low pollution formulated feed and a better production technology that would be sustainable for generations to come.

The primary goal of this project is to build demand for soy products in aquaculture markets in the Philippines and Southeast Asia by investigating the use of soy products, soybean meal (SBM) and soy protein concentrate (SPC), as alternatives to fish meal in practical feeds for milkfish. Before soy products can be applied as alternatives to fishmeal in milkfish feed, the protein to energy requirement of milkfish should be determined using practical diets. Once known, the maximum tolerance level of milkfish to soybean meal and soy protein concentrate using practical diets should be determined. Finally, a milkfish feed containing optimum levels of soybean meal and soy protein concentrate could be formulated and pilot-tested in marine floating net cages and shown to be shown to be both cost effective and environment-friendly. In addition, field-testing of this formulation could be done through the USB International Marketing Program.

The research was done in the facilities of the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC AQD) at Tigbauan, Iloilo which has a robust capability to conduct the research because of its decades-long involvement in milkfish nutrition and feed development as well as its active involvement in training and information dissemination on sustainable aquaculture in the Southeast Asian region.

A cost-effective and low pollution milkfish feed will benefit not only soybean producers and traders but also fish farmers, aquafeed manufacturers, fish nutritionists, academics, students, and other stakeholders of sustainable milkfish aquaculture.

Studies completed - brief summary of the number and type of studies conducted, including general study design and approach on how and where the studies were conducted, but without details of the materials and methods

1. The success or failure of the project was assessed by the production of a milkfish feed formulation with optimal levels of soybean meal (SBM) and soy protein concentrate (SPC) that shows good growth and survival (>400 g milkfish in 6 months of culture with a survival of 90%) and feed conversion ratio (FCR) < 2.0 in marine floating net cages.

2. Using this feed would also reduce feed wastage and nutrient loading into the surrounding water to a minimum and exacerbate problems of pollution of areas devoted to milkfish aquaculture.

3. Economic analysis would show improved profitability using the cost-effective feed for milkfish.

4. The project supported the strong R & D and extension activities of SEAFDEC AQD to promote sustainable milkfish aquaculture in the Philippines and in Southeast Asia. The extension manual and textbook on feed development and feeding management published by SEAFDEC AQD should be revised to include the use of soy products in milkfish feeds.

The following were the activities conducted in this project:

Activity 1: The objective of this activity was to determine the optimum dietary protein to energy requirement of milkfish in practical diets containing SBM

Milkfish juveniles (about 3 g average body weight, ABW) were fed practical diets containing fish meal, *Acetes* meal, and SBM and differing in protein to energy ratio. A 3 X 3 factorial experiment with three levels of protein, 30, 37.5, and 45%, and three levels of fat, 5, 10, and 15% were formulated. Carbohydrate level was kept at 20% mostly from bread flour. Fat source was cod liver oil and vitamin and mineral mixes at 3% each were included in the diets. Cellufil was used as filler. The amino acid composition of the diets was balanced based on the amino acid requirements of milkfish. Diets were analyzed for proximate composition and amino acid composition. The 12-wk feeding experiment had nine treatments and three replicates per treatment. Twenty fish were reared in each of 27 60-L oval fiberglass tanks containing 40 L of aerated, flow through sea water. They were fed at 10% of BW/d initially and then gradually decreasing to 4% of BW/d towards the end of the culture period. They were fed daily in three equal rations at 0800, 1200, and 1600H. The fish were weighed once a month. Mortalities were noted every day before cleaning the tanks and feeding. Water quality was monitored once a week for pH, dissolved oxygen, ammonia, nitrite, and phosphorus. Temperature and salinity were measured daily. At the end of the culture period, growth, survival, SGR, FCR, whole body composition, and PER were

determined. Data were analyzed using analysis of variance (ANOVA) for a 3 X 3 factorial experiment and differences were considered significant at $P < 0.05$.

Activity 2a: The objective of this activity was to determine the maximum tolerance of milkfish to SBM in practical diets

Milkfish juveniles (about 7 g ABW) were fed practical diets containing fish meal, and SBM at 0, 10, 20, 30, 40, or 50 % replacement for fish meal protein. The optimum dietary protein and energy ratio determined in Activity 1(USB #9463) was used in this activity (FY 2009 of USB # 9463; optimum protein and lipid levels determined to be about 34.7% and 9.8% (dry matter basis), respectively). The diets were isonitrogenous and isocaloric. Carbohydrate was kept at 38% mostly from bread flour, soybean meal, copra meal, and rice bran. Fat source was cod liver oil and soybean oil (1:1 ratio) and vitamin and mineral mixes (ASA-IM vitamin and mineral mixes) at 0.5 and 0.25 %, respectively, were included in the diets. Rice bran was used as filler. The amino acid composition of the diets was balanced based on the amino acid requirements of milkfish. Diets were analyzed for proximate composition and amino acid composition. The 12-wk feeding experiment was in a completely randomized design with six treatments and three replicates per treatment.

Twenty fish were reared in each of 18 250-L oval fiberglass tanks containing 200 L of aerated, flow through sea water. They were fed at 8% of BW/d initially and then gradually decreased to 3% of BW/d towards the end of the culture period. They were fed daily in three equal rations at 0800, 1200, and 1600H. The fish were weighed once every 3 weeks. Mortalities were noted every day before cleaning the tanks and feeding. Water quality was monitored once a week for pH, dissolved oxygen, ammonia, nitrite, and phosphorus. Temperature and salinity were measured daily. At the end of the culture period, growth, survival, SGR, FCR, whole body composition, and PER were determined. Data were analyzed using one-way analysis of variance (ANOVA), Duncan's multiple range test, and differences were considered significant at $P < 0.05$.

Activity 2b: The objective of this activity was to determine the maximum tolerance of milkfish to SPC in practical diets

Milkfish juveniles (about 7 g ABW) were fed practical diets containing fish meal, the optimum dietary protein and energy ratio determined in Activity 1 (FY 2009 of USB # 9463; optimum protein and lipid levels determined to be about 34.7% and 9.8% (dry matter basis), respectively), the optimal amount of SBM determined from Activity 2a, and SPC at 0, 10, 20, 30, 40, or 50 % replacement for fish meal protein present in the basal diet. Diets were made isonitrogenous and isocaloric. The bread flour content was varied to make the diets isocaloric. Fat source was cod liver oil and soy bean oil (at 1:1 ratio) and vitamin and mineral mixes (ASA-IM vitamin and mineral mixes) at 0.5 and 0.25 %, respectively, were also included in the diets. Rice bran was used as filler. Diets were analyzed for proximate composition and amino acid composition. The 12-wk feeding experiment was in a completely randomized design with six treatments and three replicates per treatment.

Twenty fish were reared in each of 18 250 L oval fiberglass tanks containing 200 L of aerated, flow through sea water. They were fed at 8% of BW/d initially and then gradually decreased to 3% of BW/d towards the end of the culture period. They were

fed daily in three equal rations at 0800, 1200, and 1600H. The fish were weighed once every 3 weeks. Mortalities were noted every day before cleaning the tanks and feeding. Water quality was monitored once a week for pH, dissolved oxygen, ammonia, nitrite, and phosphorus. Temperature and salinity was measured daily. At the end of the culture period, growth, survival, SGR, FCR, whole body composition, and PER were determined. Data were analyzed using one-way analysis of variance (ANOVA), Duncan's multiple range test, and differences were considered significant at $P < 0.05$.

Activity 3: The objective of this activity was to pilot test a suitable milkfish feed formulation with optimum SBM and SPC levels in marine floating net cages

Milkfish feed with optimum level of SBM and SPC determined from Activities 1 and 2a and 2b (FY 2009 and 2010) was formulated. Besides SBM and SPC, the feed contained fishmeal, cod liver oil, soybean oil, bread flour, vitamin and mineral mixture, and rice bran as filler. The feed was prepared at the SEAFDEC AQD Pilot Feed Mill using the extrusion process and was submitted for proximate analysis. The feeding experiment was conducted in marine floating net cages at SEAFDEC AQD Igang Marine Station at Igang, Guimaras, Philippines.

Milkfish juveniles with initial ABW of 40 g were used in this experiment. The experiment had two treatments (SEAFDEC-USB diet and commercial milkfish diet) with four replicates per treatment. The floating cage experiment was conducted in 8 units of 5x5x3 cu m bamboo floating net cages with a stocking density of 20/cu m. The fish were fed initially at 8% of their body weight per day and gradually decreased to 3% BW/d when they reached 300 g given in 3 equal rations at 0800, 1200, and 1600 H daily. One hundred fish per cage were sampled every 3 weeks for growth and adjustment of feed ration. Water quality was monitored each month for dissolved oxygen, temperature, salinity, pH, total suspended solids, ammonia, nitrite, hydrogen sulfide and phosphorus. At harvest after 180 days, ABW, survival, total production, FCR, whole body composition, and PER were determined. Means were compared using a t-test and differences were considered significant at $P < 0.05$. An economic analysis was done to determine profitability of feeding the SBM/SPC diet versus commercial feed.

Activity 4: The objective of this activity was to pilot test the milkfish feed formulation with three types (starter, grower, and finisher) containing optimum levels of SBM and SPC in marine floating net cages

This activity followed the experimental design of Activity 3 except that milkfish were fed the formulated diet in phases: starter diet (with 34% and 9% crude protein and fat, respectively) (ABW 20-100 g), grower diet (with 32% and 9% crude protein and fat, respectively) (ABW 101-300 g), and finisher diet (with 30% and 9% crude protein and fat, respectively) (ABW 301->400 g). Two treatments (SBM/SPC diet and commercial diet) with four replicates per treatment were used. The floating cage experiment was conducted in 8 units of 5x5x3 cu m bamboo floating net cages with a stocking density of 20/cu m. The fish were fed initially at 8% of their body weight per day and gradually decreased to 3% BW/d when they reached 300 g given in 3 equal rations at 0800, 1200, and 1600 H daily. One hundred fish were sampled every 3 weeks for growth and adjustment of feed ration. Water quality was monitored each month for dissolved oxygen, temperature, salinity, pH, total suspended solids, ammonia, nitrite, phosphorus, and hydrogen sulfide. The sediment below the cages was also monitored

for appearance, organic matter, total nitrogen, hydrogen sulfide, and phosphorus level. At harvest after 105 days, ABW, survival, total production, FCR, whole body composition, and PER were determined. Means were compared using a t-test and differences were considered significant at $P \leq 0.05$. A sensory evaluation was done to compare milkfish fed the alternative soy based diet and the commercial diet. An economic analysis was done to determine profitability of feeding the SBM/SPC diet in phases versus commercial feed.

A suitable feed formulation will then be field tested in commercial scale through the USB International Marketing Program (Note: Budgetary requirements for this phase not included in this project). Feeds will be toll milled and delivered to the test areas that are preferably under commercial operation. Performance of the feed and economics in grow-out milkfish culture will be compared with those of corresponding commercial feed. Water quality will also be closely monitored similar to what would have been done for the pilot testing phase and impacts on aquaculture areas assessed.

Results - sequential summary of results, ending with recommendations on soy diet formulations, feeding protocols, economics and other related recommendations

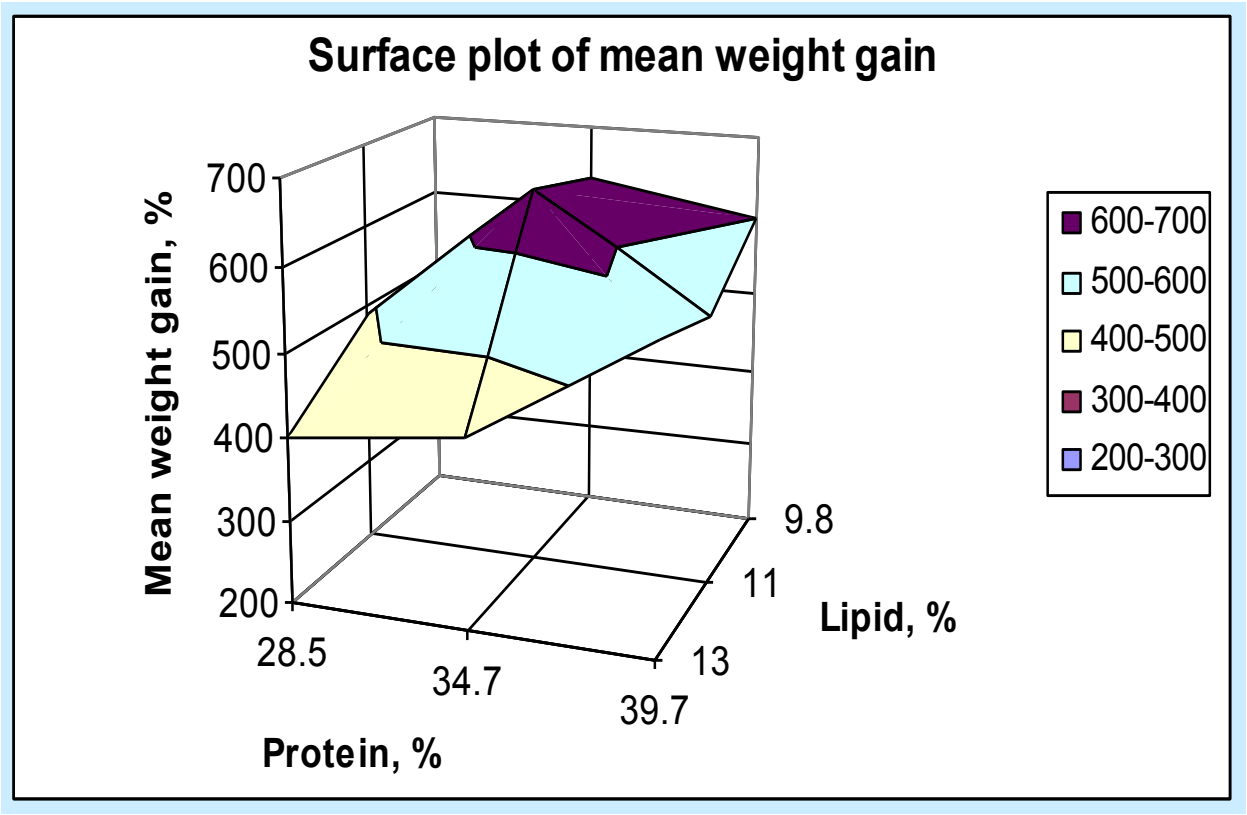


Figure 1. Surface plot of mean weight gain (%) as a function of dietary lipid (%) and protein (%) in practical diets fed to milkfish *Chanos chanos* juveniles for 12 weeks.

Data from surface response curve and univariate analysis of variance for a 3 X 3 factorial design suggest that optimum levels of dietary protein and fat for milkfish juveniles fed diets containing fishmeal and soybean meal are at 34.7% (36.16 analyzed value) and 9.8% (9.47, analyzed value), respectively.

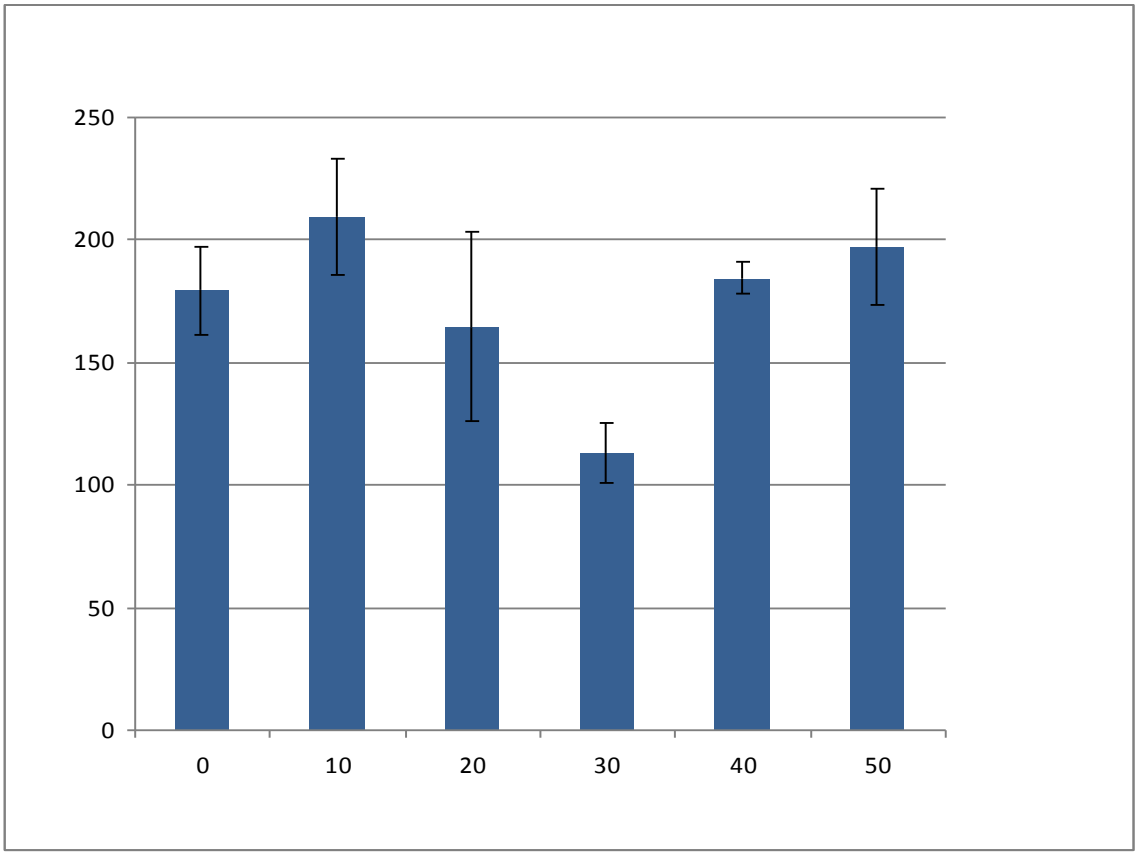
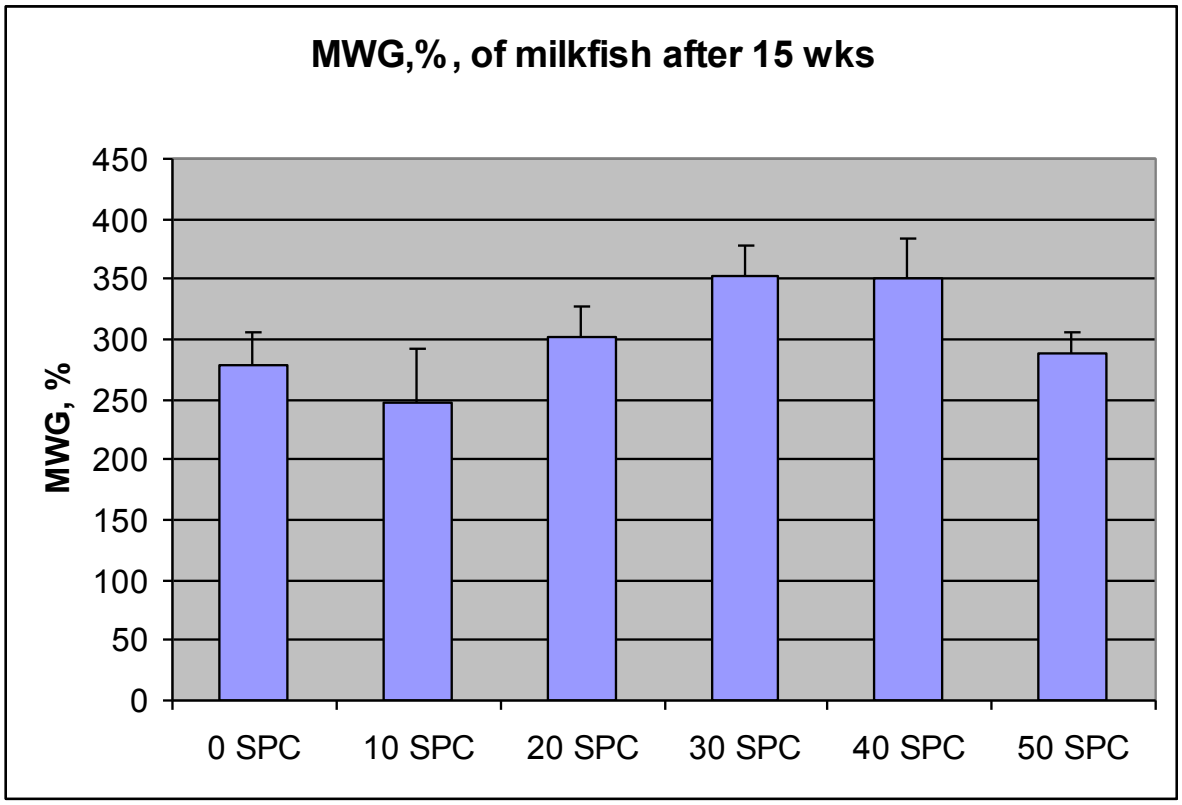


Figure 2. Growth of milkfish juveniles fed practical diet with increasing levels of soy bean meal

Data from Activity 2a suggest that milkfish juveniles may tolerate up to 50 % of total protein from soybean meal as long as its requirements for limiting amino acids are met.



- **Figure 3. Results of Activity 2b suggest that milkfish can tolerate up to 30 % soy protein concentrate in practical diets containing optimal SBM level.**

- **The protein from fishmeal in milkfish diet can be reduced to 30% of total protein and about 60% can come from soy products, soybean meal and soy protein concentrate, without any adverse effect on growth and survival.**

Diet R, 0% SPC – 277; S, 10% SPC- 248; T, 20% -302; U, 30%, - 353%; V,40%-351; W, 50% - 288

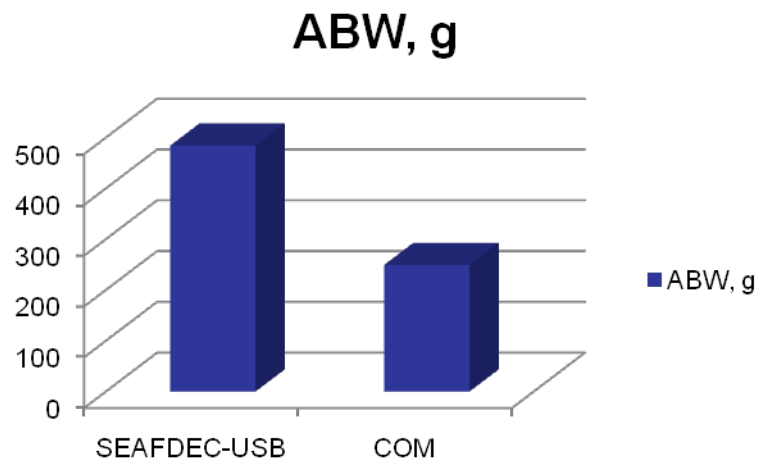


Figure 4 a. Average body weight (ABW), g, of milkfish fed the SEAFDEC-USB diet or commercial diet for 19.3 weeks

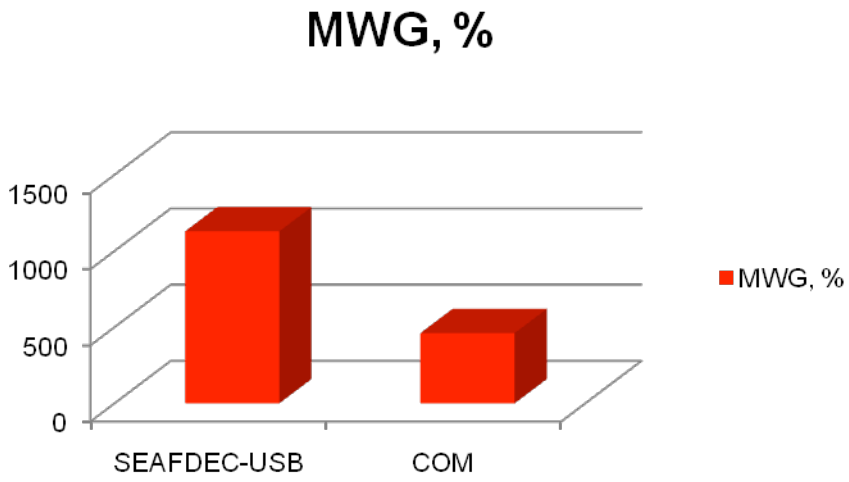


Figure 4 b. Mean weight gain of milkfish fed the SEAFDEC-USB diet or commercial diet for 19.3 weeks

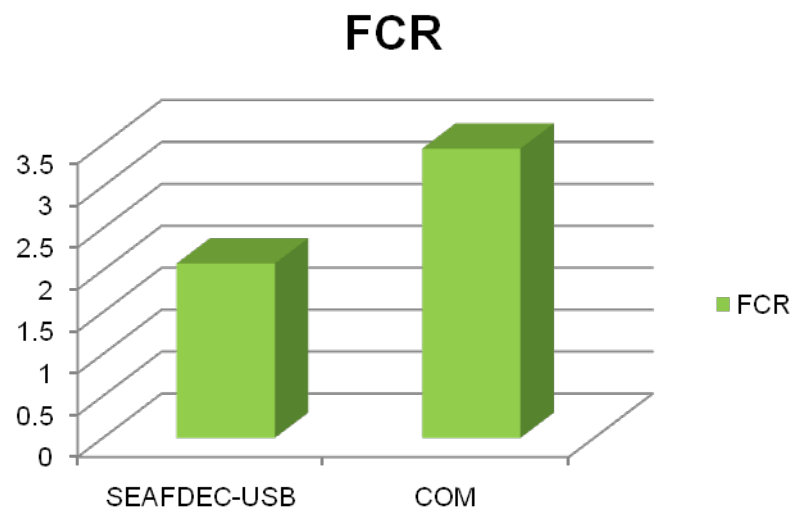


Figure 4c. Feed conversion ratio of milkfish fed the SEAFDEC-USB diet or commercial diet for 19.3 weeks.

The previous results showed better growth and feed conversion efficiency in fish fed the SEAFDEC-USB diet compared with those fed commercial diet.

After 135 days of feeding, the following data were obtained (mean of 4 replicates per treatment):

Diet 1 (SEAFDEC-USB soy based diet) – Average body weight (ABW, g), 484; Weight gain (WG, %), 1129; Feed conversion ratio (FCR), 2.08; mortality (%), less than 1%. Survival in both treatments was 98% after subtracting mortalities during sampling.

Diet 2 (Commercial feed) – ABW, 249; WG, 461; FCR, 3.45; mortality, less than 1%

The differences in ABW, WG, and FCR were statistically significant at $P < 0.05$.

The fish fed the commercial diet were fed until they reach about 400g.

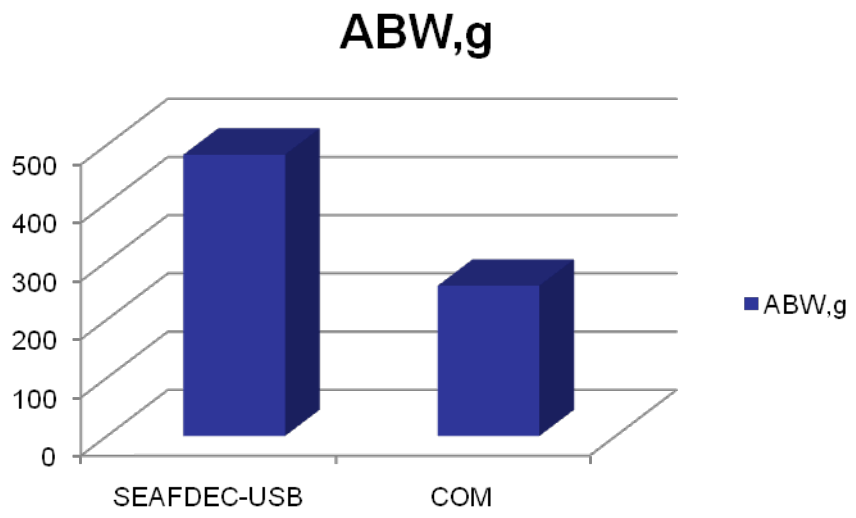


Figure 5 a. ABW, g, of milkfish fed the SEAFDEC-USB diet or commercial diet in phases (starter, grower, finisher) for 15 weeks.

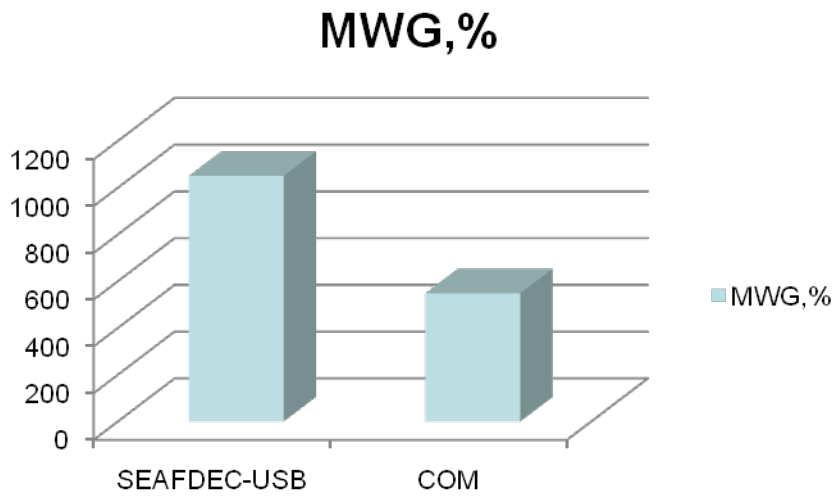


Figure 5 b. Mean weight gain, %, of milkfish fed the SEAFDEC-USB or commercial diet in phases (starter, grower, finisher) for 15 weeks.

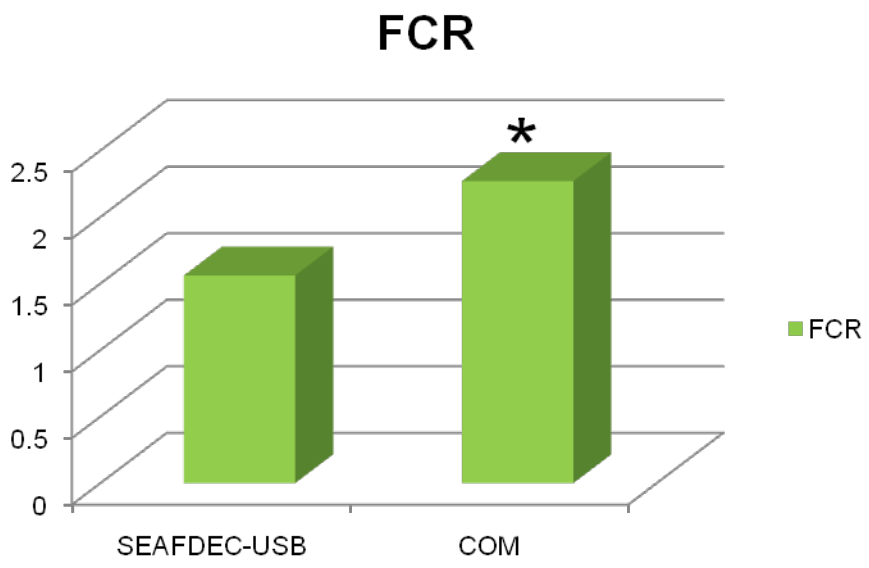


Figure 5 c. Feed conversion ratio of milkfish fed the SEAFDEC-USB diet or commercial diet in phases (starter, grower, finisher) for 15 weeks.

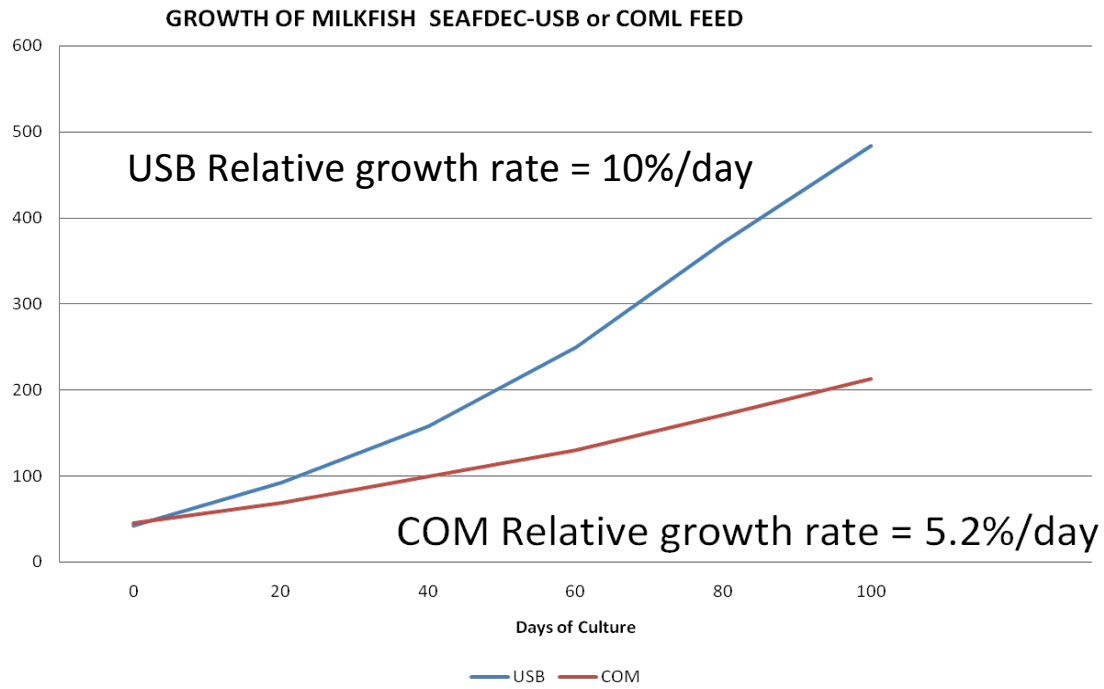


Figure 5 d. Relative growth rate of milkfish fed the SEAFDEC-USB diet or commercial diet in phases (starter, grower, finisher) for 15 weeks.

After 15 weeks (105 days) of culture (27 July 2012) the following data were obtained:

SEAFDEC-USB Diet:
ABW- 483 g; % Mean wt gain- 1050; Survival-95% (final harvest data); FCR-1.56

COMMERCIAL Diet:
ABW- 258 g; % Mean wt gain- 548; Survival- 97% (estimate); FCR- 2.27

Differences between the two treatments in ABW, % Mean weight gain, and FCR were highly significant ($P < 0.01$, t-test). Survival rates were high in both treatments and were not significantly different from each other. At this point, the fish fed the SEAFDEC-USB diet were harvested since they already reached the desired ABW of >400 g and the fish were sold to the local market and the staff of the Center.

The fish fed the commercial diet were fed until they reached the desired harvest weight of >400 g. At the last sampling (05 Oct 2012, 160 days of culture), the following data were obtained on fish fed the commercial diet:

COMMERCIAL DIET:
ABW- 441 g; % Mean wt gain – 1002; Survival 90% (final harvest data); FCR – 3.25

Thus, FCR SEAFDEC USB – 1.56 at 105 DOC and FCR COM diet – 3.25 at 160 DOC

Simple economic comparison showed - NET Income SEAFDEC-USB $448.73 per 5X5X3 m cage
and NET Income COM diet (-$144.75) per 5X5X3 m cage

The breakeven price for the commercial diet was PhP 109.01 ($2.72)/kg; and PhP 69.67 ($1.74)/kg for the SEAFDEC-USB diet.

Water quality as well as sediment quality monitoring in areas where the floating net cages were situated showed low to moderate increases in ammonia-nitrogen, phosphate and hydrogen sulfide concentrations over the culture periods in 2011 as well as in 2012. Thus, the experimental set up had minimal impact on the surrounding marine environment.

Conclusions - summarize overall value of research results and application opportunities by industry

In 2009, the optimum levels of crude protein and fat in milkfish practical diet were determined to be 34% and 9%, respectively. Using these levels of protein and fat in the test diets in the following experiment, the amount of defatted SBM that can replace fishmeal was found to be about 50%. In the third experiment using basal diets that contained optimal levels of protein, fat, and SBM, the optimal level of SPC replacement for the remaining fishmeal in the diet was determined to be about 30%. A milkfish practical feed containing optimum levels of SBM and SPC was pilot-tested in marine floating net cages in 2011. Results showed better growth and feed conversion efficiency in fish fed the SEAFDEC-USB diet compared with those fed commercial diet. In 2012, the use of the milkfish diet containing SBM and SPC was optimized by phased feeding using starter, grower and finisher diets which resulted in much lower FCR of 1.56 for the SEAFDEC-USB diet. Better feeding and management strategies that will ensure cost efficiency, lower pollution in the surrounding environment, and sustainability will be further tested in 2013. The field testing of this formulation through the ASA International Marketing Program, though not officially included in this project, is being conducted. A cost-effective and low pollution milkfish feed will benefit not only soybean producers and traders but also fish farmers, feed manufacturers, fish nutritionists, academics, students, and other stakeholders of sustainable milkfish aquaculture.

The information obtained is important in developing cost-effective milkfish practical diets with optimum levels of soy products, soybean meal and soy protein concentrate, for use in marine floating net cages and other systems available for milkfish culture.