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Preliminary Guideline for Replacement of Fish Meal for Good Aquaculture Moving Towards Organic of Maejo Buk-Siam Hybrid Catfish

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The experiment was conducted to evaluate the fish culture of Maejo Buk-Siam Hybrid Catfish (*P. gigas x P. hypothamus* F2) with three different feed formulas (1=control, 2= 50% replacement for fishmeal with Napier grass and 3=50% replacement for fishmeal with Hydrilla) to find out the preliminary guideline for organic Maejo Buk-Siam Hybrid Catfish. The initial body weight of 2 months Maejo Buk-Siam Hybrid Catfish is 47.28±13.81 grams. Which were reared in 3x5 m^2 earthen pond (1.5 fish/m^2) at HuayTong and 6 months Maejo Buk-Siam Hybrid Catfish is 64.14±440 grams which were reared in 1x1 m^2 cages (4 fish/m^2) at PlaBuk knowledge base, Maejo university. The results showed that final body weight containing different feed formulas have no significant differences (p>0.05): 108.2±17.40, 122.17±35.10, 122.50±18.83, respectively in earthern pond at HuayTong and 642.92±54.86 ,685.70±41.19, 625.00±54.87 in cages at PlaBuk knowledge base, Maejo university. Brightness (L* Brightness), redness of meat (a*red), yellowness of meat (b*yellow), percentage of protein and lipid in meat and percentage of meat of fish with three different feed formulas were have no significant differences **(**p>0.05). While the smell of meat in fish was fed with dietary formula-3 has the value of satisfaction higher than control. These results verified that, it is possible to replacement for fishmeal with Napier grass or Hydrilla to real Maejo Buk-Siam Hybrid Catfish from 5-6 months. Consequently, those are beneficial to reduce production costs and develop the fish production for food security and preliminary guideline for Good Aquaculture Moving Towards Organic of Maejo Buk-Siam Hybrid Catfish as well.

Keywords: Maejo Buk-Siam Hybrid Catfish, Napier grass, Hydrilla , growth performance , organic aquaculture

Introduction

At present, white meat hybrid catfish has been popular among consumers as it is considered healthy food with high nutrition, a source of protein, vitamins, mineral as well as good fatty acids such as omega3 especially

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DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid), which are important for the development of brain and the prevention of coronary artery disease. Each year 3.5-4 thousands ton of catfish are needed in the national markets(www.nicaonline.com/webboard/index.php). Among the international markets in European nations, America, Malaysia and Singapore, the need of white meat fish and Pangasius catfish is between 500,000 to 1,000,000 tons per year(www.thefishsite.com/articles/963/recent-trends-in-Aquacultureproduction-in-Asia-and-Europe). The value of these fillet fish in the markets is over hundred thousand baht a year. However, nowadays the production of white meat hybrid catfish is not sufficient for the increasing needs of consumption.

Maejo Buk-Siam Hybrid Catfish is the generation 2-hybrid fish that is the offspring of a male Maekong Giant Catfish (*Pangasianodon gigas)* and a female Striped Catfish *(Pangasianodon hypophthalmus)*, the parent fish are hybrids that are the offspring of a male Maekong Giant Catfish and a female Striped Catfish. It is white meat catfish popular among consumers as it is considered healthy food high in nutrition, very good in growth and disease resistance. It can be reared in earthen ponds and cages (Mengamphan, 2016).

The researchers and the Department of Fisheries Technology and Aquatic Resources, Maejo University have collected the breeders of Maekong Giant Catfish since 1991 and could breed the first generation of Maekong Giant Catfish in the earthen ponds in year 2001 and 2002. In 2007 the second generation of Buk Maejo 75 could be bred, and in 2012 Maejo Buk-Siam Hybrid Catfish (the 2^{nd} generation offspring of a male Maekong Giant catfish and female Striped Catfish). From this first cultivation in earthen ponds in the world, the production of juveniles is sufficient for career development where these juveniles are reared in the communities to create more careers for people in the communities. However, the cultivation of Organic Maejo Buk-Siam Hybrid Catfish in order to develop careers requires a good aquaculture system i.e. certificate, production approval and reduction of cost such as the organic fish diet.

Theoretically the use of ingredient for organic aquaculture is that the producers have to use plant ingredients in aquaculture fish diet) Organic Agriculture Certification Thailand, 2012). The ingredients from plant have to be no less than 60 percent of natural ingredient or organic ingredients and they have to show very small effects on environment. This causes the limit in the production of organic fish diet. Therefore to study and select natural ingredients to produce aquaculture feed is very critical in terms of organic aquaculture. If local ingredients and water plants can replace fish meal in the diet, it can increase values and contribute this product to be the national brand. Moreover,

the fillet from the fish fed with these natural ingredients appears to have no muddy odor, contamination as well as have high quality and quantity.

Objectives: To study for replacement of fish meal in feed recipe with local ingredients, and studying the growth and meat quality of Maejo Buk-Siam Hybrid Catfish fed with local ingredient diet in order to find out for principle ways for organic aquaculture.

Materials and methods

Experimental Fish

1 .Earthen ponds were prepared with ridges to prevent the ponds from any chemical contamination. The ponds were required to have water system separated distinctly. The important aspect is that organic aquaculture system never has an effect on environment, society, community and water resources nearby. Expectedly, it requires defensive measure to prevent fish escape from the farm. Throughout the experiment period, the aquaculture standard according to Organic Standard 2012) Organic Agriculture Certification Thailand, 2012) and the Organic Aquaculture policy of the Department of Fisheries (Department of Fisheries, 2007) was acted accordingly.

2. Four earthen ponds, sized 3x5x1.5 meters, were prepared for Maejo Buk-Siam Hybrid Catfish aquaculture in Huay Tong Village community. A big earthen pond, sized 800 square meters and 1.3 meter depth, was prepared at Maekong Giant Catfish Knowledge Base, Faculty of Fisheries Technology and Aquatic Resources, Maejo University by draining the remaining water and let dried for a few days. The new water was filtered to trap pest fish before filling into the pond.

3. Maejo Buk-Siam Hybrid Catfish juveniles bred at Facultyof Fisheries Technology and Aquatic Resources, Maejo University were nursed for 2 months (sized 40 grams) before rearing in the experimental ponds in Huay Tong Village community. The hybrid catfish aged 6 months (sized 400 grams) were fed with the trial feed formulas with the local material as the replacement of fish meal in the recipe.

Experimental diets

Prepare 3 fish diet formulas for feeding Maejo Buk-Siam Hybrid Catfish as follows; the controlled pallet, 50% replacement of fishmeal with Napier grass and 50% replacement for fishmeal with Hydrilla) Diet formulas as presented in Table 1(. The nutrition analysis of the three feed formulas were then performed in order that the following analysis were performed, protein

analysis using micro-Kjeldahl, lipid analysis using dichloromethane extraction according to Soxhlet method, ash analysis by burning fish pellet in the muffle furnace at 550 degree Celsius for 12 hours, fiber analysis by bringing the weighed Crucible to burn in the furnace at 550 degree Celsius for 2 hours, and humidity analysis by bake drying in the oven at 105 degree Celsius for 24 hours according to AOAC (1995).

Feeding trial procedures and ample collection

1. Release Maejo Buk-Siam Hybrid Catfish with the average size of 47.28±13.81 grams into the earthen ponds in Huay Tong Village community, ratio 1.5 fish per a square meter. These fish were kept in the floating cages to adjustment and fed with the controlled pallet for 7 days before released into the earthen ponds and fed with the trial fish feed formulas.

2. Nine floating cages, sized 1x1x1 cubic meter were prepared and attached at the earthen ponds at the Maekong Giant Catfish Knowledge base. Each cage was placed 0.5 away from one another. Maejo Buk-Siam Hybrid Catfish aged 6 months with the average size of 64.14±440grams were released into the cage, 4 fish per a cage (ratio 4 fish per a square meter). These fish were kept in the floating cages to adjustment and fed with the controlled pellet for 7 days before released into the earthen ponds and fed with the trial diet.

3 .Study the growth rate of hybrid catfish fed with these three different feed formulas. CRD (Completely Randomized Design) was adopted in this experiment which was divided into three experimental units as follows.

Diet 1 the controlled pallet

Diet 2 50% replacement for fishmeal with Napier grass

Diet 3 50% replacement for fishmeal with Hydrilla

The feeding rate was 3-5 percent of the body weight per fish per day, 1-2 times in the morning and evening (8.00-9.00 hrs. and 17.00-18.00 hrs.)

At every experimental unit, growth measurement; weight gain, weight gain per day, growing rate per day, and feed conversion ratio (FCR); was performed every 4-8 weeks for 4-12 months (from age 2 months reared in the earthen ponds in Huay Tong Village community to age 8 months and from age 6 month reared in the floating cages in the earthen ponds at Maekong Giant Catfish knowledge base to age 10 months).

Growth measurement was performed using Completely Randomized Design (CRD), One way ANOVA analysis and multiple comparison according to Turkey at the significant statistic level $p<0.05$ using instant program SAS.

Measurement of Fish meat quality

1. Colour was analysed using the colour measuring tool of Konica Minolta Chroma Meter, model CR 400. The fish meat on the left and right was cleaned and dried. The colour measuring tool was then used to measure the fish meat colour with the L* a* b* colour system (or called "CIELAB"). L* is the brightness at the area a*, and b* tells the colour trend.

+a* means it is in the Red colour trend, while –a* means it is in the Green colour trend

+b* means it is in the Yellow colour trend, while –b* means it is in the Blue colour trend

2. Nutrition analysis of fish meat includes protein analysis suing micro-Kjeldahl and fat analysis using dichloromethane extraction according to Soxlhet method and AOAC (1990)

3. Percentage of meat (%) = weight of fillet x 100/ body weight

4. The smell of meat in fish was measured by tasting and smelling. In this tasting and smelling test, the scale was used to score.

Table 1. Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients	**Diet 1**	**Diet 2**	**Diet 3**
Fishmeal	20	10	10
Hydrilla	0	10	0
Napier grass	0	0	10
Organic soybean	30	30	30
Rice bran	35	35	35
Organic broken-milled rice	15	15	15
Crude protein, % DM	36.10 ± 0.05^{c}	25.14 ± 0.01^{a}	26.27 ± 0.33^{b}
Crude lipid, % DM	6.93 ± 0.03^{c}	5.32 ± 0.03^{a}	5.52 ± 0.03^{b}
Ash, % DM	10.13 ± 0.04^{c}	7.75 ± 0.05^{a}	8.22 ± 0.05^{b}
Fiber, % DM	5.00 ± 0.11^{a}	6.72 ± 0.17^{b}	7.13 ± 0.02^{b}

Results

Growth performance

To study the growth of Maejo Buk-Siam Hybrid Catfish reared in earthen ponds at Huay Tong Village community, the average weight of Maejo Buk-Siam Hybrid Catfish aged 2 months was 47.28 ±13.81grams. Moreover

during November, the temperature which dropped to 20 Degree Celsius caused fish to eat less. However according to the statistic trial, the average body weights of Maejo Buk-Siam Hybrid Catfish aged 5 months, fed with the pallet in the three ponds have no statistically significant differences (p>0.05). Afterward the trial on the three different fish diet formulas was performed and found that the average body weight at age 8 months have no statistically significant difference (p>0.05). The average body weights of Maejo Buk-Siam Hybrid Catfish fed with different fish diet formulas throughout this experiment were presented in Table 2.

Table 2. Growth performance in weight (grams) of Maejo Buk-Siam Hybrid Catfish reared in earthen ponds at Huay Tong Village community during the experiment which feeding with three different fish diet formulas

Age	Diet 1	Diet 2	Diet 3
2 months	47.28±13.81[a] (N=32)	47.28±13.81[a] (N=32)	47.28±13.81[a] (N=32)
5 months	59.5±16.50[a] (N=2)	105.67±17.70[a] (N=3)	122.25±34.4[a] (N=4)
8 months	108.2±17.40[a] (N=5)	122.17±35.10[a] (N=6)	122.50±18.8[a] (N=4)

Data represented as LS Mean±S.E.M. of replicated cages. Value in the same row with different superscripts are significantly different (p>0.05). and the sample numbes shown in parentheses

To study the growth of Maejo Buk-Siam Hybrid Catfish reared in the floating cages hung in the earthen pond at Maekong Catfish Knowledge Base, Faculty of Fisheries Technology and Aquatic Resources**,** Maejo University, the average weight of Maejo Buk-Siam Hybrid Catfish aged 6 months was 64.14±440grams, which have significant differences (p<0.05). At the beginning the fish were fed with the pallet for medium-sized fish for 2 months, and found that the average body weight of the hybrid fish aged 8 months have statistically significant differences (p<0.05). Afterward the trial on the three different fish diet formulas was performed and found that the average body weight of the hybrid fish aged 9 months , which were reared in each floating cage for 1 month have no statistically significant difference (p>0.05). Accordingly the average body weight of the hybrid fish aged 10 months have no statistically significant difference (p>0.05). The average body weights of the Hybrid Catfish aged 10 months shows no statistically significant difference (p>0.05), and the average body weight of Maejo Buk-Siam Hybrid Catfish fed with different fish diet formulas throughout this experiment were presented in Table 3.

The results reveals that Weight gain, average daily growth (gram per fish per day), diet changed into meat and survival rate of Maejo Buk-Siam Hybrid Catfish fed with different trial fish diets in different floating cages (Table 4) show statistically significant indifferences among the three feed formulas (p>0.05).

Table 3. Growth performance in weight (grams) of Maejo Buk-Siam Hybrid Catfish reared in in the floating cages hung in the earthen pond at Maekong Catfish Knowledge Base, Faculty of Fisheries Technology and Aquatic Resources**,** Maejo University during the experiment which feeding with three different fish diet formulas.

Age	Diet 1	Diet 2	Diet 3
6 months	466.67±25.62 [a] (N=12)	450.00±15.08[a] (N=12)	403.33±3.33 [b] (N=12)
8 months	555.82±28.17[a] (N=11)	504.25±22.24[ab] (N=12)	474.75±18.01[b] (N=12)
9 months	627.5.82±42.61[a] (N=11)	641.67±24.31[a] (N=12)	549.78±35.10[a] (N=9)
10 months	642.92±54.86[a] (N=11)	685.70±41.19[a] (N=10)	625.00±54.87[a] (N=7)

Data represented as LS Mean±S.E.M. of replicated cages. Value in the same row with different superscripts are significantly different (p>0.05) and the sample numbes shown in parentheses.

Table 4. Weight gain, Specific growth rate; % perday, Average daily growth; gram/fish/day, Feed conversion rate; FCR and survival rate; % of Maejo Buk-Siam Hybrid Catfish reared in in the floating cages hung in the earthen pond at Maekong Catfish Knowledge Base, Faculty of Fisheries Technology and Aquatic Resources**,** Maejo University during the experiment (age 8-10 months) which feeding with three different fish diet formulas.

	iet 1	et 2	et 3
eight gain (grams)	176.25±58.20[a]	265.17±99.50[a]	211.94±30.72[a]
ecific growth rate	1.25±0.41[a]	1.76±0.61[a]	1.61±0.18[a]
verage daily growth	1.47±0.49[a]	2.21±0.83[a]	1.77±0.26[a]
ed conversion rate; FCR	6.88±3.38[a]	4.45±1.30[a]	3.97±0.41[a]
rvival rate (%)	91.67±8.33[a]	83.33±16.67[a]	58.33±8.33[a]

Data represented as LS Mean±S.E.M. of replicated cages. Value in the same row with different superscripts are significantly different (p>0.05).

The quality of fish meat

Fish meat measurement of Maejo Buk-Siam Hybrid Catfish aged 12 months shows that the fish meat colours,; brightness (L*brightness), redness of meat (a*red) and yellowness of meat (b*yellow); of Maejo Buk-Siam Hybrid Catfish fed with these three fish diet formulas have no significant differences (p>0.05). The analysis of protein and fat shows that Maejo Buk-Siam Hybrid Catfish fed with these three formulas have no significant differences in protein and fat quantity(p>0.05). Accordingly, the percentage of fish meat of Maejo Buk-Siam Hybrid Catfish fed with these three fish diet formulas have no significant differences (p>0.05). The data were shown in Table 5.

The smell of fish meat performed by smelling and tasting test and scaled score reveals that 102 testers gave the satisfactory scores on the taste and smell of of Maejo Buk-Siam Hybrid Catfish fed with these three fish diet foemulas as presented in Table 6. The most satisfactory score was present in the fish meat of Maejo Buk-Siam Hybrid Catfish fed with fish diet formula 3 being 643 points. Secondary 377 points were given to the fish meat of of Maejo Buk-Siam Hybrid Catfish fed with fish diet formula 2, and finally they gave 276 points to the fish meat of of Maejo Buk-Siam Hybrid Catfish fed with the fish diet formula 1.

To calculate the cost of fish diet production, it was found that the cost of the feed formula 3 is the lowest, being 28.30 baht per kilogram. Secondary, it was feed formula 2, being 29.60 baht per kilogram, whereas the cost of feed formula 1 was 33.70 baht per kilogram.

Table 5. The fish meat colours; brightness (L*brightness), redness of meat (a*red),yellowness of meat (b*yellow), % protein % lipid and % fillet of Maejo Buk-Siam Hybrid Catfish reared in in the floating cages hung in the earthen pond at Maekong Catfish Knowledge Base, Faculty of Fisheries Technology and Aquatic Resources**,** Maejo University fed with these three fish diet formulas.

	Diet 1	**Diet 2**	**Diet 3**
L*brightness	40.32±0.76[a]	42.48±1.84[a]	42.28±1.78[a]
a*red	8.38±1.81[a]	6.83±0.42[a]	8.43±2.08[a]
b*yellow	9.45±0.33[a]	7.00±0.60[a]	7.77±0.76[a]
% protein	18.03±0.37[a]	18.41±0.46[a]	18.52±0.33[a]
% lipid	2.32±0.88[a]	2.03±1.08[a]	1.59±0.69[a]
% fillet	41.2±1.59[a]	40.32±0.99[a]	36.21±2.80[a]

Data represented as LS Mean±S.E.M. of replicated cages. Value in the same row with different superscripts are significantly different (p>0.05).

Discussion

The result of Maejo Buk-Siam Hybrid Catfish growth when fed with the three different fish diet formulas shows that the local ingredients such as Napier grass (sun dried) or Hydrilla (sundried) can replace 50% of fish meal. Scince, at the beginning the young juveniles were fed with pallet for 3 months since in young juveniles the need of protein is 30-40 per cent, whereas the mature fish requires only 22-35 per cent of protein (Mengamphan, 2016). The growth rate among the Maejo Buk-Siam Hybrid Catfish fed with Napier grass or Hydrilla shows no significant differences in the average body weight from the Maejo Buk-Siam Hybrid Catfish fed with the controlled pallet since at the period the juveniles need less protein, which is only 25-35 percent (Mengamphan, 2016). The percentage of protein in the fish diet formula 1 is significantly different ($p<0.05$) from the fish diet formulas 2 and 3, which are 36.10 ± 0.05, 25.14 ± 0.01 and 26.27 ± 0.33 respectively (Table 1). Therefore this should be considered another way to reduce cost of fish diet by replacing fish meal with local ingredients such as Napier grass or Hydrilla, which accords with the experiment to replace fishmeal with Napier grass (fresh) to rear Maejo Buk-Siam Hybrid Catfish (Kitcharoen *et al.*, 2014; Meekaew *et al*., 2016). Maejo Buk-Siam Hybrid Catfish were fed with three different fish diet formulas (Formula1 The controlled pallet, Formula 2 50% replacement of fishmeal with Napier grass, Formula 3 100% replacement of fishmeal with Napier grass) in order to seek for ways to aquaculture Maejo Buk-Siam Hybrid Catfish. The feeding ratio throughout this experiment was 3-5 percent of the body weight per day. At the beginning of the experiment Maejo Buk-Siam Hybrid Catfish aged 9 months, weighted averagely 606.89 ± 126.38 grams, were released into the floating cages sized one square meter at the ratio 3 fish per one square meter. After the floating cages were hung in the earthen ponds for 120 days, it was found that the final body weights of Maejo Buk-Siam Hybrid Catfish fed with the three different fish diet formulas showed no significant differences ($p>0.05$), which were 710.00 ± 24.68, 636.67 ± 48.42 and 614.44 ± 30.78 grams respectively. However it is obvious that in Maejo Buk-Siam Hybrid Catfish aged 10 months and 11 months, the final body weight of the hybrid catfish fed with the three different fish diet formulas showed statistically significant differences ($p<0.05$). The hybrid fish fed with the controlled pallet showed the highest average final body weight, and the hybrid fish fed with the diet formulas 2 and 3 respectively. When multiple comparing, it was found that the average weight of Maejo Buk-Siam Hybrid Catfish fed with the controlled pallet was not significantly different from the average weight of Maejo Buk-Siam Hybrid Catfish fed with the fish diet formula 2 , but

significantly different from the average weight of Maejo Buk-Siam Hybrid Catfish fed with the fish diet formula 3.

Similarly weight gain, growth rate per day (gram per fish per day) and the feed conversion ratio shows that 50% of Napier grass can replace fish meal, and the growing rate will not be significantly different in each age. The use of soybean meal to replace fish meal also shows similar trend in aquaculture. In other words, the fish diet replaced fish meal with soybean meal at 0 and 25 percent, the growing rate including the final body weight, weight gain per day and percentage of weight gain show no significant difference, whereas it shows statistically significant differences in Black ear catfish (*Pangasius larnaudi)*, Giant perch, Giant gourami, hybrid catfish and Nile tilapia when replacing fish meal with 50, 75 and 100 per cent of soybean meal (Shiau *et al.*, 1990). This could be the result of the essential amino components; methionine and lysine; in the diet which is inappropriate and may cause the imbalance in essential amino acids. This also causes slow growth. Similarly the replacement of Napier grass, a protein resource in plants, may cause the imbalance in the essential amino acids. Shrestha, M.K. and C.N.R. Yadav (1998) found that the aquaculture of grass carp reared with other silver carp, bighead carp and common carp in Monoculture and Poly culture system, the replacement of Napier grass can be performed. However feed conversion ratio shows in high level 17.3 -21.90, which is considered high , whereas this experiment on the replacement of fishmeal with 50 per cent of Napier grass was only 7.35 ± 1.52 and 15.75 ± 2.25 when replacing the fish meal with 100 per cent of Napier grass. And it shows the highest FCR when there was no replacement of fish meal with Napier grass (3.63 ± 0.02), and it has statistically significant differences ($p<0.05$) according to what Tongsiri *et al.* , (2010) had found in the study of Russian Catfish aquaculture with the replacement in fish diet with banana, guava and coconut husk. When comparing with the controlled trial, no statistically significant differences were found ($p>0.05$).

In the aspect of growth and cost reduction, the result shows that fish meal replaced fish diet formulas have both lower and higher in costs when compared with the pallet (data not plublished). The Provincial Fisheries Operation Center had tested the replacement of Napier grass Pak Chong1 in fish diet and fed to Nile Tilapia, *Barbonymus schwanefeldi* (Bleeker), butterfish, Pangasius, Cyprinidae and *Trichogaster pectoralis*. The first 3 months, rice bran was given and caused more water moss and made the water green. Afterward, chopped Napier grass Pak Chong1 was given as fish diet to the hybrid catfish throughout 1 year. The experiment on the replacement of fish meal with Napier grass Pak Chong1 or Hydrilla, which are considered local ingredients, in the fish diet for

Maejo Buk-Siam Hybrid Catfish reveals that this is effective in terms of cost reduction.

To examine fish meat quality of Maejo Buk-Siam Hybrid Catfish aged 10 months, the quality of the hybrid catfish fed with these three different fish diet formulas is as follows. The colour qualities of the fish meat including brightness (L* brightness), Redness (a* Red) and yellowness *b*), protein, fat and meat percentage have no statistically significant difference (p>0.05). To examine the smell of fish meat by tasting, smelling and scaling, it was discovered that the highest satisfactory score was showed in the smell of Maejo Buk-Siam Hybrid Catfish fed with fish diet formula3, being 653 points; secondary Maejo Buk-Siam Hybrid Catfish fed with fish diet formula2, being 377 points; and Maejo Buk-Siam Hybrid Catfish fed with fish diet formula1, being 276 points. This possibly indicates that the replacement of Napier grass or Hydrilla for fish meal in the fish diet to feed Maejo Buk-Siam Hybrid Catfish does not effect on the quality of the fish meat. This study is, therefore, considered a fundamental way for Good Aquaculture Moving toward Organic Maejo Buk-Siam Hybrid Catfish, beneficial in reducing cost and increasing potential in the competitive markets. It is to develop and improve the fish to become safety food as well as to determine the organic aquaculture principle for farmers in the future.

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