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Effects of incorporated swamp cabbage (*Ipomea aquatica*) and papaya (*Carica papaya*) leaf meals at different dietary levels in order to replace fish meal protein in practical diets for sea bass (*Lates calcarifer*)

Erlinda S. Ganzon-Naret

University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Miag-ao, Iloilo, Philippines. Corresponding author: E. S. Ganzon-Naret, erlindanaret@yahoo.com

Abstract. A 60-day feeding trial was conducted at the UPV Hatchery to evaluate the effects of diet containing different levels of swamp cabbage (*Ipomea aquatica*) and papaya (*Carica papaya*) leaf meal (LM) mixtures as dietary protein sources for replacing fish meal (FM) protein at 30-50% on the growth performance, feed utilization and survival rates for sea bass juveniles. This study was maintained under laboratory condition in 12-units conical fiber glass tanks (100 L) in a recirculating system provided with filtered and aerated sea water. Four isonitrogenous (40%) and isolipidic (9%) diets were formulated to contain 0 (Diet 1), 30 (Diet 2), 40 (Diet 3) and 50% (Diet 4) replacement of FM protein from a mixture of swamp cabbage (SwCLM) and papaya leaf meals (PLM). The diet containing 0% LM served as the control. Each dietary treatment was tested in triplicate groups of 10 juveniles per tank. The physico-chemical parameters monitored were temperature which ranged between 28-30^{0}C, dissolved oxygen 6.2-6.8 mg L^{-1}, pH 6.8-7.1 and 28-30ppt salinity. Ammonia (NH_3-N) and nitrite (NO_2-N) values were 0.11-0.76 ppm and 0.07-0.092 ppm respectively and within the optimum ranges for the growth of sea bass. Reactive PO_4^{-3} values ranged between 0.01-0.04 ppm. The results of the growth and feed utilization showed that there were no significant (P>0.05) differences among the fish fed Diet 1 (0%) and Diet 2 (30%) but were significantly higher (P<0.05) from those groups of fish fed on Diets 3 and 4. The lowest growth rate and feed utilization values were obtained in Diet 4. The control diet (Diet 1) recorded the highest body weight gain (WG) and specific growth rate (SGR). The feed conversion ratio (FCR) and protein efficiency ratio (PER) were not significantly different (P>0.05) among Diets 1 and 2, however their values differed significantly from those fish fed Diets 3 and 4. The present findings show that incorporating SwCLM and PLMs mixture in sea bass diets replacing 30% FM protein is a good potential protein source and probably low cost efficient diet without adverse effects on the growth for juvenile sea bass.
Key Words: Sea bass, growth, survival, swamp cabbage, papaya leaf meal.

Introduction. Sea bass (*Lates calcarifer*) also known as "barramundi," is a fast growing catadromous fish of high commercial value, strictly carnivorous and they are economically farmed in Southeast Asian countries, Taiwan, Australia and the Indo-Pacific region (Thirunavukkarasu et al 2004; Partridge et al 2008; Appelbaum & Arockiaraj 2010; Arockiaraj & Appelbauam 2010; Plaipetch & Yakupitiyage 2012). Sea bass has been cultured both in freshwater and brackishwater ponds and in marine cages with the ability to adapt in varied environmental conditions (Cheong 1989; Philipose et al 2010). Global aquaculture production of Mediterranean sea bass and sea bream increased by 7% in 2013 to 332,000 tones and a further 2% present increase in 2014 (www.thefishsite.com/fishnews/21482/goal-conference-2013). A major component of dietary protein for fish is normally provided by FM which is approximately 20-50% in practical diets. According to Munguti et al (2012) fish feeds account for the highest operational cost in aquaculture with protein being the most expensive diet. Fish requires high proportion of protein in the diet because they metabolize this macronutrient as one of their energy sources (Aladetohun & Sogbesan 2013). However, since the culture of

Asian sea bass is totally dependent on the use of FM, perhaps this resulted to high the price of fish due to the escalating cost and uncertain availability of FM (Lim et al 1997; Naylor et al 2000). Feeds account 50-60% of the total variable costs (Keembiyehetty & de Silva 1993; Anderson et al 1997; Richter et al 2003; Gabriel et al 2007; Bichi & Ahmad 2010; Olaniyi et al 2013) which affect the profitability and sustainability of the species being cultured. Therefore, research interest had directed and prompted our researchers to identify for cheap and locally available non-conventional plant protein sources in order to attain more economically sustainable and viable production, environmentally friendly and without adverse effect on the flesh quality of fish.

Leaf meals are among the unconventional and cheapest sources of plant proteins that may tend to reduce the high cost of feed (De Silva & Andersons 1995). Hence, several studies had been already conducted utilizing various sources of leaf meal proteins (Ng & Wee 1989; Eusebio & Coloso 2000 on cassava leaf meal; Olaniyi et al 2013; Ganzon-Naret 2014 on *Moringa oleifera* leaf meal; Adewolu 2008 on sweet potato, *Ipomoea batatas* leaf meals; Bairagi et al 2004 on ipil-ipil leaves, *Leucaena leucocephala*; Reyes & Fermin 2003 on papaya, *Carica papaya*) as partial or complete replacement for FM. The particular leaf meals of interest as potential dietary protein sources for sea bass were *Ipomoea aquatica* and *Carica papaya*.

Ipomoea aquatica is a semi-aquatic plant found throughout the tropical and subtropical regions of the world and can be cultivated either in dry or flooded soils. It is known in English as water spinach, swamp cabbage (SwC) water morning glory, Chinese watercress, water convolvulus or kangkong (Filipino, Malay), Ong choy (Cantonese), Pak Bung (Thai), Raun Muong (Vietnam), Tongsin Tsai (Mandarin) and Paani-palak (India). Water spinach has been cultivated for human food, fed to pigs and other animals in Southeast Asia. According to Ly (2002) water spinach is a potential source of feed protein which contains 29% crude protein and has been used successfully in pigs and rabbits. Further, *I. aquatica* contains very low amount of anti-nutritional factors such as trypsin inhibitor, tannin, phytate and calcium oxalate (Mandal et al 2008). Fresh leaves of *I. aquatica* have a crude protein content between 20 and 30% (Men et al 2010) and ash of around 12% in DM (Gohl 1981). Water spinach is closely related to sweet potato, the leaves are dark green and are usually heart-shaped depending on its variety and has the potential to supply the much needed minerals, high in Vitamins A and C and considered food with medicinal effects: reduce cholesterol, treatment for jaundice and liver problems, anti-diabetic and beneficial for the eyes (Kala & Prakash 2004; Chitsa et al 2014; http://www.clovergarden.com/ingred).

In the Philippines, papaya (*Carica papaya* Linn.), is considered as one of the most important fruit crops in the Philippines because of its great economic potential. The fruit is rich in Vitamins A and C, iron, calcium, protein, carbohydrates and phosphorous (Ikram et al 2014). Its enzyme known as papain, is commonly used by pharmaceutical and cosmetic industries (Da-Silva et al 2010). In Japan, several studies have been conducted that papaya leaf tea can prevent and kill cancer cells in cervix, prostate, liver, breast and lungs (Otsuki et al 2010; Nguyen et al 2013). The papaya leaves are great cancer healing agent and also the best cure for dengue fever (www.community.omtimes.com /profiles/blogs/15-uses-for-papaya-leaves.) Papaya plant thrives best in areas with dry climate (25-30°C) and grows best in soils with pH of 5.0-6.5 (www.bpi.da.gov.ph/ bpioldsitel/guide_papaya.php). According to Taiwo et al (2005) papaya leaves (PL) given to cross bred weaned rabbits had a better feeding value compared with Guinea grass (*Megathyrsus maximus*) and tridax daisy (*Tridax procumbens)*. Omole et al (2004) concluded that papaya peels and leaves fed to giant West African snails gave the best results in feed intake, weight gain and shell increment without adverse effects when compared to other tropical by-products (mango, plantain and cocoyam). Studies was conducted with the use of pawpaw (*Carica papaya* leaf meal on the partial replacement of soybean meal on *Cortunix japonica*, Japanese quails, results showed no detrimental effects on birds' growth (Kanyinji & Zulu 2014).

Eusebio & Coloso (1998) found that prawns *Penaeus indicus* could utilize soybean meal-based diet when papaya leaf meal replaced 9% of the soybean protein. Prawns fed the test diets had a non-significantly lower weight gain, growth and survival rate than those fed the control diet. In contrast to the study on the feeding trial of Asian sea bass, the inclusion level of PLM at 13 to 18% in the diet gave a lower fish growth (Eusebio & Coloso 2000). Although, some leaf meals had been already evaluated solely as potential dietary protein source in fish diets to reduce cost, few studies was carried out using different mixtures of SwC and PLMs as dietary non-conventional plant protein sources for sea bass juveniles.

In the Philippines, research priority in aquaculture is the development of cost effective feed using locally available, cheap and unconventional protein sources due to the scarcity and high cost of FM. Thus, the aim of this experiment was to assess the different mixture levels of SwCLM and PLMs as dietary protein sources in replacing FM protein in sea bass diets, to determine the nutritive value of these two leaf meals. Furthermore, the effects of experimental diets on the growth performance and nutrient utilization of juvenile sea bass were evaluated under laboratory condition.

Material and method

Preparation of swamp cabbage (Ipomea aquatica) and papaya (Carica papaya) leaf meals. Table 1 & 2 shows the proximate analyses (AOAC 1995) and amino acid composition of SwCLM and PLMS. Fresh leaves of swamp cabbage and papaya were removed from their stems, washed with tap water to remove dirt and other debris. The leaves were soaked then for 12 h, dried in an air convection oven at 80^0C, powdered by means of feed mill and sieved through a fine meshed screen.

Table 1

Proximate composition (% dry weight) of SwCLM and PLM

Analyses	*SwCLM*	*PLM*
Dry matter	92.26	93.02
Crude protein	28.18	21.36
Crude fat	6.05	11.78
Crude fiber	11.20	11.45
Ash	11.92	13.87
NFE	42.65	41.54

Table 2

Amino acid composition (g/100 g protein) of SwCLM and PLM

Amino acids	*SwCLM*	*PLM*
Alanine	6.37	6.22
Arginine	7.43	7.20
Aspartic acid	12.62	12.86
Cysteine	0.00	0.00
Glutamic acid	16.73	15.85
Glycine	5.46	5.96
Histidine	4.09	4.59
Isoleucine	4.82	4.87
Leucine	8.77	8.32
Lysine	7.10	6.20
Methionine	0.09	0.05
Phenylalanine	4.86	5.69
Proline	5.39	4.96
Serine	4.52	4.22
Threonine	4.32	5.68
Tryptophan	0.00	0.00
Tyrosine	3.41	3.45
Valine	4.02	3.88
Hydroxyproline	0.00	0.00

Diet preparation and formulation. All the experimental diets contained 40% protein (isonitrogenous) and 9% lipid (isolipidic) as presented in Table 3. The basal diet without SwCLM and PLM served as Diet 1 (control) which contained 35% FM as the primary source of animal protein. The other three test diets (Diet 2, Diet 3 and Diet 4) contained the different mixture of SwC and PLMs replacing FM protein at 30%, 40% and 50% respectively. Feed ingredients were weighed according to the formulation composition as shown in Table 3. Each diet contained squid meal (7%) and shrimp meal (5%) as an attractant and the amount of defatted soybean meal (12%) was added in the same amount in all the experimental diets. The amount of corn meal and wheat flour were adjusted to maintain the same levels of dietary protein when SwC and PLMs were incorporated in sea bass diets. The dry ingredients were homogeneously mixed prior to the addition of vitamin-mineral premix and thereafter lipid sources such as cod liver oil and corn oil (1:1) were poured to the dry mixture. The dough was extruded using a 2 mm die, dried in the oven at 60^{0}C and kept in the refrigerator at 4^{0}C until use. An anti-oxidant, butylated hydroxytoluene (BHT) was included in the feed formula at 0.5 g/100 g to reduce feed spoilage.

Table 3

Composition (g/100 g diet) and proximate analyses of experimental diets for sea bass juveniles

Ingredients	*Treatments*			
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*
Peruvian FM	35.00	24.50	21.00	17.50
Squid meal	7.00	7.00	7.00	7.00
Shrimp meal	5.00	5.00	5.00	5.00
Defatted soybean meal	12.00	12.00	12.00	12.00
SwCLM	-	13.66	16.20	20.27
Papaya LM	-	17.73	21.02	26.31
Wheat flour	18.50	7.00	6.28	1.00
Corn meal	12.00	2.61	1.00	0.42
Vitamin-mineral mix	6.00	6.00	6.00	6.00
Cod liver oil	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00
BHT	0.50	0.50	0.50	0.50
Proximate composition (%)				
Crude protein	41.21	39.79	38.67	38.36
Crude fat	8.80	9.48	9.49	9.79
Crude fiber	6.11	1.66	4.64	5.18
Ash	11.20	8.27	10.43	10.59
NFE*	32.68	40.80	36.77	36.08
Dry matter	91.81	91.78	91.35	91.68
Metabolizable energy** (Kcal/100g)	443.68	389.54	371.27	370.00

*Nitrogen free-extract;
** based on 4.5 kcal g^{-1}, 3.3 kcal g^{-1} and 8 kcal g^{-1} protein, carbohydrate and fat, respectively (Brett & Groves 1979).

Chemical analyses. Proximate analyses for crude protein, crude fat, crude fiber and ash were determined using the standard methods (AOAC 1995) for duplicate samples. Moisture was done by drying the samples overnight in the oven at 105^{0}C. Crude protein was analyzed by means of Kjeltec 2200 after acid digestion, crude fat after extraction with petroleum ether using the Soxhlet apparatus, ash using the muffle furnace at 550^{0}C while crude fiber was determined using the hot extractor. The NFE is derived by subtracting the sum of other proximate components, crude protein, crude fat, ash, crude fiber on dry weight basis from 100. The amino acid composition of leaf meals were analyzed using the HPLC. About 2 mg of dry sample was hydrolyzed for 22 h at 110^{0}C using 4 N-methanesulfonic acid according to Simpson et al (1976). The hydrolysate was then adjusted to a pH of 2.2 and injected into the HPLC unit with anion-exchange resin column (Teshima et al 1986). Norleucine was used as the internal standard for the quantitative analyses.

Experimental set-up and maintenance. A group of 2000 sea bass juveniles, *Lates calcarifer* was obtained from the private hatchery and these were transported immediately at UP Visayas Multi-Species Hatchery in Miag-ao, Iloilo, Philippines. The fish were maintained in 2 x 1000 L circular tanks and fed with control diet for 2 weeks. Fish weighing 1 to 1.2 g were size graded, sorted into groups of ten fish (1.09 g mean weight) and were randomly distributed into each of twelve 100 L conical tanks at three replicates. The feeding experiment was maintained in a recirculating system with 90 L filtered aerated seawater under the natural light and dark cycle during the study period. Each culture tank was cleaned to remove uneaten feeds and feces and approximately 50% of the total water volume was changed daily with filtered sea water. During the first week after stocking, any mortality observed was replaced immediately with fish having similar weight. The fish were fed three times daily (at 09:00, 13:00 and 16:00) for 60 days. Sampling of sea bass in each tank was done every 15 days by getting its total weight rather than individual weights to minimize handling and stress and also to adjust the amount of feeds to be given. Dead fish were removed and recorded daily. Water quality parameters were checked three times weekly for dissolved oxygen (6.2-6.8 mg L^{-1}) using a YSI model 58 oxygen meter, water temperature (28-30^{0}C) was measured using a thermometer, salinity (28-30 ppt) with Atago refractometer, pH (6.8-7.1) was determined with electronic pH meter. Total ammonia (NH_3-N) and nitrite (NO_2-N) were determined every two weeks using the spectrophotometer according to the procedure described by Strickland & Parson (1972) and the values obtained ranged between 0.12-0.96 ppm and 0.10-0.11 ppm respectively and within the acceptable limits for the indoor production of fish (Boyd 1984). During the experimental period, reactive phosphate (PO_4^{-3}) ranged between 0.01-0.04 ppm.

Parameters and calculations. Growth, nutrient utilization and survival of juvenile sea bass for 60 days feeding trial were calculated using the following formulae:

$$WG = \text{final weight (g)} - \text{initial weight (g)}$$

$$SGR\ (\%\ day^{-1}) = [\ln \text{final weight (g)} - \ln \text{initial weight (g)} / \text{no. of culture days}] \times 100$$

$$FCR = \text{feed intake (g)} / \text{weight gain (g)}$$

$$PER = \text{wet weight gain (g)} / \text{dietary protein intake (g)}$$

$$\text{Survival rate (\%)} = \text{No. of fish recovered} / \text{No. of fish stocked} \times 100$$

Statistical analysis. Data were presented as mean±SEM and subjected to one way analysis of variance (ANOVA) using SPSS version 16 Software Program. Duncan's Multiple Range Test (DMRT) was used as a post-hoc test to compare between treatment means at $P<0.05$. Survival was calculated using the arcsin square root.

Results and Discussion. Based on proximate analyses (Table 1), crude protein, crude fat and nitrogen free extract of swamp cabbage and papaya leaf meals were 28.18%, 21.36%, 6.05%, 11.78%, 42.65% and 41.54% respectively. Results of the analyzed amino acid content as presented in Table 2 showed that both leaf meals were deficient in tryptophan and sulfur containing amino acids such as methionine and cysteine. Leaf meals of SwC and PLMs were found to be high in protein, easily digested and with no toxins (SEAFDEC 1994). The proximate analyses which include protein (38.36–41.21%), fat (8.80-9.79%), fiber (1.66-6.11%), ash (8.27-11.20%) and NFE (32.68-40.80%) of the four experimental diets are presented in Table 3. Metabolizable energy (ME) ranged between 370.00–443.68 kcal g^{-1} was calculated from physiological fuel values of 4.5, 3.3 and 8 kcal g^{-1} for protein, carbohydrate and fat respectively (Brett & Groves 1979). Initial weight (Table 4) did not differ significantly among treatments (P>0.05). Similarly, the highest final body weight was recorded for the fish fed Diet 1 and the lowest value so far was obtained for groups of sea bass fed Diet 4. After 60 days, weight gain (WG) and specific growth rate (SGR) were comparable with those fish in Diet 2, however this was significantly (P<0.05) different from those groups of fish fed Diets 3 and 4. The survival rate of Asian sea bass juveniles during the growth trial was 80% and showed no significant variation among all the dietary treatments. Data on growth performance and feed efficiency of sea bass juveniles in terms of initial weight, final weight, weight gain, SGR, FCR PER and survival are presented in Table 4. Sea bass fed the other two test diets, Diet 3 and Diet 4 showed a lower value for the final body weight of 6.23 g and 5.65 g respectively. The performance of fish fed Diet 4 in terms of growth and feed efficiency was the lowest. The best feed conversion ratio (FCR) and protein efficiency ratio (PER) were obtained in sea bass groups fed Diet 1 (2.01, 1.60) followed by Diet 2 (2.07, 1.58), Diet 3 (2.52, 1.34), and Diet 4 (2.76, 1.42) groups respectively.

Table 4

Growth performance and feed efficiency of juvenile sea bass fed four experimental diets (0%, 30%, 40% and 50%) with or without mixture of swamp cabbage and papaya leaf meals for 60 days

Specification	*Treatments*			
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*
Initial weight (g)	1.09±0.01	1.09± 0.02	1.09±0.01	1.09±0.02
Final weight (g)	7.68±0.2^{a}	7.45±0.23^{a}	6.23±0.37^{b}	5.65±0.34^{c}
Weight gain (%)	6.59±1.2^{a}	6.36±3.5^{a}	5.14±2.8^{b}	4.56±1.8^{c}
SGR	1.41±0.16^{a}	1.39±0.09^{b}	1.25±0.13^{b}	1.18±0.21^{c}
FCR	2.01±0.12^{a}	2.07±0.03^{a}	2.52±0.02^{b}	2.76±0.14^{c}
PER	1.63±0.03^{a}	1.58±0.13^{a}	1.34±0.04^{b}	1.42±0.15^{c}
Survival (%)	80.00	80.00	80.00	80.00

Data represent the mean of three replicates. Values in the same row with different superscript are significantly different (P<0.05).

The results of the present study showed that sea bass juveniles fed well and accepted Diet 2 containing swamp cabbage mixed with papaya leaf meals at 30% replacement for FM protein. A significant decrease (P<0.05) in growth was observed in sea bass groups fed Diets 3 and 4 and this was attributed to the low palatability of the diets due to the increased levels of dietary plant ingredient mixture at 40-50% replacement of FM protein. According to El-Sayed (1994) and Alceste (2000), the inclusion of plant ingredients as dietary feed component usually varies according to fish species and its particular life stage. In contrast, Lim & Dominy (1989) reported that the growth of tilapia (*S. mossambicus*) was improved when 50% of the FM protein was replaced by the cottonseed meal. On the other hand Pantastico & Baldia (1980) found that 100% replacement of FM with *Leucaena* leaf meal in tilapia resulted in a significant growth. Since the diets in the present study were formulated to be isonitrogenous (40% crude protein) and isolipidic (9%), the growth was presumably affected by the different crude protein level in the diets after the chemical analyses. The crude protein level of the experimental diets based on proximate analyses (Table 3) decreased with the increased level of replacement of SwC and PLMs mixture for FM protein. However, the level of crude fat in the diets increased as the dietary level of replacement of SwC and PLMs mixture was also increased. The same trend was also observed for the crude fiber in the diets. Phiny et al (2008) reported that growth and feed performance were better in monogastric animal such as pigs when fed mulberry leaves was mixed with water spinach as compared with water spinach as the only forage. Earlier findings by different authors (Mamun et al 2003; Manvar & Desai 2013) found that methanol extract of *Ipomea* *aquatica* showed good diuretic activity in Swiss albino mice. According to De Silva et al (1990), he concluded that it is better to use ingredients particularly leaf meals at 15-20% rather than higher level of inclusion.

Conclusions. The present study showed that a mixture of *I. aquatica* and *C. papaya* leaf meals at 40-50% replacement of FM protein in sea bass diets resulted with adverse effects on fish growth and reduced its feed efficiency. It is further recommended that another study shall be carried out in juvenile sea bass by replacing FM protein as low as 30% mixture of plant ingredients. This study indicates that up to 30% level of SwC and PLMs mixture could be included in the practical diets for *L. calcarifer* without affecting its growth and feed utilization.

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Authors:
Erlinda Souribio Ganzon-Naret, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Iloilo, 5023 Miag-ao, e-mail: erlindanaret@yahoo.com

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