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Utilization of *Moringa oleifera* leaf meals as plant protein sources at different inclusion levels in fish meal based diets fed to *Lates calcarifer*

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Abstract. A feeding experiment was carried out to utilize *Moringa oleifera,* Lam leaf meals at varying inclusion levels in the fish meal based diets for Asian sea bass, *Lates calcarifer* to determine its effects on growth, feed conversion ratio (FCR), protein efficiency ratio (PER), proximate body composition and survival rates. *L. calcarifer* is a carnivorous fish that grows fast and is totally dependent on the use fishmeal (FM) as the main protein feed. Currently, the price of this feed is escalating, due to increasing competition of this conventional protein for livestock and human consumption as well as for aquaculture diets and industrial use thus making their costs too high thereby reducing the profitability of fish farming and its yield in terms of quantity and quality. Four isonitrogenous test diets were formulated to contain 40 % crude protein (CP). *M. oleifera* leaf meals (MOLM) were incorporated in sea bass diets at 0 % (T1); 10 % (T2); 20 % (T3) and 30 % (T4). The control diet (MOLM-0) contained 35 % fish meal without *M. oleifera* leaf meal. Groups of fifteen sea bass fingerlings with an initial weight of 2 g were randomly distributed at three replicates into each of twelve 100 L cylindro-conical fiberglass tanks and fed thrice a day for 75 days. At the end of the trial, feed utilization efficiency and survival rates of sea bass fed diets with up to 30 % dietary inclusion level of MOLM were not significantly different ($P>0.05$) from the diet with 20 % dietary inclusion level of *M. oleifera* (MOLM-20). The highest percentage weight gain (%), protein efficiency ratio had been observed in sea bass fed with control diet (0 % MOLF) than fish fed 10, 20 and 30 % MOLM diets. Weight gain and specific growth rate generally declined as dietary inclusion level of MOLM was increased from 20 – 30 % in fish meal based control diet for sea bass. The feed conversion ratio (FCR) and protein efficiency ratio (PER) for MOLM-0 and MOLM-10 diets were significantly different ($P<0.05$) among the diets fed MOLM-20 and MOLM-30. Proximate body composition of Asian sea bass showed that crude protein, crude ash decreased as the dietary inclusion level of MOLM was increased from 10 to 30 %. However, body crude lipid showed an opposite trend as the level of fish meal replacement with MOLM was increased. It was concluded that MOLM at a maximum level of 10 % could be acceptable as plant protein replacement in fish meal based diets without adverse effects on the growth performance and health welfare to *L. calcarifer*.

Key Words: Sea bass, fishmeal, proximate body composition, crude protein, survival rate.

Introduction. Aquaculture is the fastest growing sector and is mostly destined for human consumption and currently provides 50 % of the total global food fish consumption (De Silva & Turchini 2008; FAO 2010). Most of the aquafeeds are largely based on fish meal which is a significant protein source for carnivorous fish, especially sea bass (Williams & Rimmer 2005; Glencross 2006; Tacon et al 2011; Plaipetch & Yakupitiyage 2012). According to Tacon et al (2011) Asian sea bass feed contains approximately 20 – 50 % fishmeal (FM). With the continued growth for intensive sea bass production, the need for suitable diets using alternative plant protein sources has become a necessity in a particular country in order to develop less expensive formulation that will maintain efficient growth at least cost per unit gain (Martinez-Llorens et al 2009; Tiril et al 2009). Several alternative protein sources are being investigated to replace fish meal in sea bass diets which include meals of soybean, canola, lupin, pigeon pea, green peas, yellow mungbean and other leaf meals (Higgs et al 1995; Adamidou et al 2009; Ganzon-Naret 2013). Numerous options had been advocated by the researchers to direct their attention to utilize non-conventional feedstuff with emphasis on leaf meals as

protein substitute for fish meal in fish feeds such as *Moringa oleifera* (Richter et al 2003; Olaniyi et al 2013), *Pennisetum clandestinum* (Hlophe & Moyo 2014), *Leucaena leucocephala* (Osman et al 1996; Bairagi et al 2004), *Ipomoea batatas* leaves (Adewolu 2008; Lochmann et al 2013), *Medicago sativa* (Yousif et al 1994) and *Manihot esculenta* leaf meal (Eusebio & Coloso 2000; Chhay et al 2010; Nnaji et al 2010). However, the use of leaf meals is limited due to their high fiber content and presence of toxic factors. In order to reduce the need for fish meal ingredient in sea bass diet, *M. oleifera* is a great potential source of protein, an excellent source of vitamins, minerals and amino acids. It possess some medicinal properties that could combat against malnutrition, treatment for cardiovascular diseases, anti-ulcer, anti-inflammatory, food for human consumption, reproductive health, and for other industrial purposes (Khalafalla et al 2010; Moyo et al 2011; Gadzirayi et al 2012). *M. oleifera* is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family *Moringaceae* which contains high crude protein in the leaves (251 g kg^{-1} DM) with negligible content of tannins and other anti-nutritive compounds (Nouala et al 2006). The english common names include: moringa, drumstick tree (Ranachandran et al 1980) and horseradish tree, or ben oil tree having an impressive range of medicinal uses with high nutritional value throughout the world (Sanchez-Machado et al 2010). In Africa, Moringa is called the "Miracle tree of life", savior, and in their local language "Nebedaye," which means "never die," (http://www.mamamoringa.com/moringa_oleifera.html). It is a fast-growing, drought-tolerant tree, and widely cultivated in tropical and sub-tropical areas where its young seed pods and leaves are used as a vegetable (Martin 2007). The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese and other essential nutrients (Anwar et al 2007).

In the Philippines, *M. oleifera* is known as "malunggay," and is a popular plant known for its nutritional value as well as herbal medicine. It can grow as high as 9 m with erect white trunks and its leaves contain "pterygospermin" a compound having antimicrobial, antibacterial and anti-fungal properties (http://ww.medicalhealthguide.com /articles/malunggay.htm). Large quantities of "malunggay" are available all year round in the Philippines at a cheaper cost and their leaves are used mostly for human consumption as protein source. Moringa can be used in the treatment of prostate cancer and skin cancer and nutritional content of its leaves varies with location (Fuglie 2000; Anjorin et al 2010). Research has indicated that *M. oleifera* leaf meals had been used widely in animal feed especially for pigs and poultry, mainly to supplement vitamins and trace minerals (Zaichun 1990). It is also a viable alternative plant protein source that could reduce the use of dietary fish meal for carnivorous fish due to its nutritional value of high pepsin and total soluble protein. *M. oleifera* leaf meal can replace groundnut cake up to 12.5 % in the nutrition for African catfish, *Clarias gariepinus* (Olaniyi et al 2013) while at low inclusion level of Moringa up to 10 % in tilapia diet (Richter et al 2003) significantly increased the growth performance.

Currently, the use of *M. oleifera* has been under investigation for non-ruminants and monogastric animals as protein feed source, however there is no available information on the use of as leaf meal to replace fish meal in the diet for Asian sea bass. The aim of this study was to determine the effect of utilizing *M. oleifera* leaf meal Lam at different inclusion levels in fish meal based diets for Asian sea bass on the growth, feed conversion ratio, protein efficiency ratio, body proximate composition and survival rate.

Material and Method

Preparation of M. oleifera leaf meal. A number of batches of fresh leaf-stem *M. oleifera* were purchased from the wet market in Iloilo, Iloilo City, Philippines. The leaves were rinsed in a clean water to remove dirt and were dried by spreading out thinly on a concrete floor under a shady area to prevent the loss of vitamins. The leaves were then covered with mosquito netting to protect them from pests and other contaminants with constant mixing. After 3 days of drying, the leaves became brittle and they were ground into powder using mortar and pestle and further sifted to remove the remaining stems.

The clean dried powder was stored in air tight container and kept at room temperature. The nutrients and amino acid composition of *M. oleifera* leaf meal (AOAC 1990) were presented in tables 1 and 2.

Table 1

Nutrient composition (% dry matter) of MOLM

Nutrients	*% Composition*
Dry matter	93.36
Crude protein	29.10
Crude lipid	8.50
Crude fiber	8.10
Ash	11.80
NFE*	42.50
Metabolizable energy (Kcal/100g)**	339.20
Calcium	0.49
Phosphorus	0.36
Potassium	1.38

*Nitrogen-free extract: **Metabolizable energy was calculated based on the standard physiological value of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8 kcal/g fat (Brett & Groves 1979).

Table 2

Amino acid composition (g/100 g protein) of *Moringa oleifera* leaf meal

Amino acids	*MOLF*
Alanine	3.00
Arginine*	1.81
Aspartic acid	1.34
Cysteine	0.01
Glutamic acid	2.45
Glycine	1.526
Histidine*	0.697
Isoleucine*	1.181
Leucine*	1.948
Lysine*	1.629
Methionine*	0.302
Phenylalanine*	1.637
Proline	1.198
Serine	1.085
Threonine*	1.36
Tyrytophan*	0.491
Tyrosine	2.639
Valine*	1.409

*Essential amino acids.

Feed ingredients and diet formulation. Four isonitrogenous and isolipidic diets were formulated at 40 % protein and 8 % lipid respectively. A control diet (MOLM-0) contained 35 % fish meal as the primary source of animal protein without the MOLM, and this was compared with test diets MOLM-10, MOLM-20 and MOLM-30, where *M. oleifera* leaf meal was added approximately at 10 – 30 % in the test diets. Each diet contained shrimp meal at 12 % as an attractant and the amount of defatted soybean meal was the same in all the experimental diets. The levels of corn meal and wheat flour were adjusted to maintain the same dietary protein when MOLM was incorporated at 10, 20 and 30 % replacing protein at 12.21 to 36.59 % in fish meal based control diet. The vitamin and mineral mix were also kept constant in all diets while corn oil and cod liver oil as lipid sources were mixed at a ratio of 1:1.

The test diets as shown in table 3 were prepared by thoroughly mixing the powdered dry ingredients with the vitamin-mineral mix except the wheat flour and they were blended using a homogenizer. Wheat flour was gelatinized and cooked in 600 mL water, allowed to cool and added to the mixture. The resulting mixture was then passed through the meat pelletizer to obtain 2 mm pellet. The "spaghetti-like" strands were dried overnight in the air convection oven at 60 ^{o}C, placed in plastic bags and stored in the refrigerator at 4 ^{o}C until used.

Table 3

Composition and proximate analyses of the test diets used during the experiment over a period of 75 days (g/100 g dry weight)

Ingredients	*Treatments*			
	MOLM-0	*MOLM-10*	*MOLM-20*	*MOLM-30*
Peruvian fish meal	35.00	30.71	26.45	22.17
MOLM	-	10.00	20.00	30.00
Shrimp meal	12.00	12.00	12.00	12.00
Soybean meal, defatted	12.00	12.00	12.00	12.00
Corn meal	10.00	10.00	7.55	1.83
Wheat flour	23.00	17.29	14.00	14.00
Corn oil	2.00	2.00	2.00	2.00
Cod liver oil	2.00	2.00	2.00	2.00
Vitamin mix	2.00	2.00	2.00	2.00
Mineral mix	2.00	2.00	2.00	2.00
Proximate composition (%)				
Crude protein	41.02	40.71	40.02	39.65
Crude lipid	7.71	8.16	8.57	8.89
Crude fiber	1.78	2.52	3.21	3.84
Crude ash	11.41	12.60	12.90	13.20
NFE	39.94	38.71	37.97	37.06
Metabolizable energy (Kcal/100 g)	378.07	376.22	373.95	371.84

Chemical analyses. The chemical composition of MOLM, other dietary feed ingredients and diets were analyzed by standard methods (AOAC 1990). Moisture was analyzed by drying the sample in the air convection oven at 105 ^{o}C overnight. Crude protein was analyzed by means of Kjeltec 2200 after acid digestion (% crude protein = % nitrogen x 6.25); crude lipid after extraction with petroleum ether by the Soxhlet method. The ash content in diets was analyzed by combustion of samples in a muffle furnace at 550 ^{o}C for 12 h. Likewise, calcium, phosphorus and potassium were determined in the MOLM ingredient by AOAC method. Crude fiber was determined using the instrument Fibertec^{TM}. Based on the chemical analyses, the crude protein content of the diets ranged from 39.65 to 41.02 %; crude lipid 7.71 - 8.89 %; crude fiber 1.78 - 3.84 %; crude ash from 11.41 to 13.20 % while the diets remained isocaloric with values ranging from 371.84 to 378.07 (Kcal/100 g) as shown in table 3. The amino acid analysis of MOLM was determined by HPLC after hydrolysis of 5.0 mg protein sample with 1 mL of 6N HCl in vacuum-sealed hydrolysis vials at 110 ^{o}C for 22 hours. Norleucine was added to HCl as an internal standard. As shown in table 2, there were 18 amino acids found in the MOLM, and 10 of which are essential amino acids (EAA). The highest level of EAA content in moringa were leucine, arginine, phenylalanine, lysine and valine while the limiting amino acids were tryptophan and methionine at 0.491 and 0.302 (g/100 g protein) respectively.

Fish husbandry and feeding management. The experiment was conducted at the indoor facility of the Multi-Species Hatchery of the Institute of Aquaculture, University of the Philippines Visayas in Miag-ao, Iloilo. A group of 300 sea bass fingerlings were obtained from the private hatchery and acclimatized in 1000 L circular tank for 2 weeks

prior to the experiment. During the acclimation, the fish were fed MOLM-0 control diet twice per day to satiation. Hatchery-bred sea bass with an average body weight of 2 g, weighed to the nearest 0.1 g were randomly distributed at a stocking rate of 15 fish into each of twelve 100 L conical fiber glass tanks with three replicates in a semi-closed recirculating system provided with filtered aerated seawater. The average initial weight of sea bass was all uniform in size, so no significant difference ($P>0.05$) found was found in body weight among treatments. Fish in each treatment were fed three times daily at a feeding rate of 10 % total body weight for 75-day feeding experiment. The fish tanks were thoroughly cleaned and 50 % of the water was changed to maintain good water quality. The fish were counted and weighed together every 15 days to adjust the feeding ration. At the end of the trial, five fish from each tank were sacrificed, pooled under the same treatment, homogenized and analyzed for the carcass composition.

Water quality parameters were monitored daily and average dissolved oxygen, temperature, pH and salinity were 6.8 - 7.4 ppm, 27 – 30 ^{o}C, 7.2 - 7.5 and 29 – 32 ppt, respectively. NH_3-N (0.05 - 0.06 ppm) and NO_2-N (0.028 - 0.035 ppm) values were within the normal ranges as reported conducive for the growth of sea bass.

Calculations and data analysis. Growth, feed and nutrient utilization of seabass were calculated by using the following equations:

BWG (body weight gain) = [(final weight - initial weight)/initial weight] x100
SGR (Specific growth rate % day^{-1}) = [(ln final weight – ln initial weight)/time (days)] x 100
FCR (Food Conversion Ratio) = [(total feed (g)/net weight gain (g)]
PER (Protein Efficiency Ratio) = wet weight gain/protein intake
Survival rate (%) = No. of fish survived/total no. of fish at the beginning x 100

ANOVA was used to determine the effect of different test diets on the growth and feed utilization for sea bass and DMRT was applied to evaluate the differences among treatment means at $P<0.05$. All the statistical analyses were carried out using the SPSS Version 16.0. Survival was calculated using the arcsin square root.

Results and Discussion. The growth performance, survival and feed utilization of sea bass fed experimental diets for 75 days are presented in table 4.

Table 4

Growth, survival and feed utilization of sea bass juveniles fed different diets for 75 days

Parameters	*Treatments*			
	MOLM-0	*MOLM-10*	*MOLM-20*	*MOLM-30*
Initial mean weight (g)	2.00	2.00	2.00	2.00
Final mean weight (g)	9.18^{a}	9.02^{a} `	7.25^{b}	6.44^{c}
Weight gain (g)	7.18^{a}	7.02^{a}	5.25^{b}	4.44^{c}
BWG (%)	359.00^{a}	351.00^{a}	262.50^{b}	222.00^{c}
SGR (%/day)	0.88^{a}	0.87^{a}	0.75^{b}	0.68^{c}
FCR	1.98^{a}	2.17^{a}	2.38^{b}	2.97^{c}
PER	1.32^{a}	1.29^{a}	1.08^{b}	1.02^{b}
Survival (%)	86.66^{a}	73.33^{b}	73.33^{b}	73.33^{b}

*Means of three replicate samples. Values in the same row with different superscripts are significantly different ($p<0.05$).

The highest final weight (FW) and weight gain (WG) were 9.18 g and 7.18 g respectively for the control group (MOLM-0), but the differences between treatments MOLM-0, MOLM-20 and MOLM-30 were significantly different ($P<0.05$). No significant differences were found in FW and WG of fish fed MOLM-0 and MOLM-10 ($P>0.05$). SGR followed the same trend. At the end of the experimental period, the best FCR was obtained in fish fed MOLM-0 and MOLM-10 diets and these were significantly different ($P<0.05$) from the groups of sea bass fed MOLM-20 and MOLM-30 diets. The poorest FCR of 2.97 was

observed in fish juveniles fed MOLM-30 diet with the level of moringa increased up to 30 % in the diet. In the present study, the PER values of the different diets ranged from 1.02 to 1.32 at the end of the experiment. PER of feed was significantly higher in diets MOLM-0 and MOLM-10 than those of diets MOLM-20 and MOLM-30 ($P<0.05$). The survival rate was high in all treatment diets and ranged from 73.33 to 86.66 %. The carcass protein, lipid, ash and moisture contents at the end of the study are presented in table 5.

Table 5

Proximate carcass composition (%) of sea bass fed various experimental diets

Experimental diets	*Proximate composition (%)*[a]			
	MOLM-0	*MOLM-10*	*MOLM-20*	*MOLM-30*
Crude protein	19.72[a]	19.38[a]	18.46[b]	18.18[b]
Crude lipid	5.54[b]	5.59[b]	5.90[a]	5.93[a]
Crude ash	4.23[a]	4.20[a]	4.19[a]	4.16[a]
Moisture	76.36[a]	76.31[a]	76.28[a]	76.25[a]

It was observed that after 75 days of feeding the body crude protein of fish showed a significant decreasing trend (19.72 - 18.18 %) as the level of inclusion of MOLM was increased in the fish meal based control diet ($P<0.05$). Further, a significantly higher carcass crude lipid was clearly noticeable for fish fed MOLM-20 (5.90 %) and MOLM-30 (5.93 %) as compared to those fed with MOLM-0 (control group) and MOLM-10 diets at 5.54 and 5.59 % respectively ($P<0.05$). Differences in body ash were not significantly different ($P>0.05$) among fish fed different test diets, but showed a decreasing trend at 20 and 30 % level of MOLM inclusion in juvenile sea bass diets. The moisture content was highest in the MOLM-0 diet (76.36 %) as compared to those fed on the moringa leaf meal diets which ranged from 76.25 - 76.31 %, however, no significant differences were observed among the dietary treatments ($P>0.05$).

In the current study, the chemical composition of *M. oleifera* leaf meal showed that the crude protein content was 29.10 %. This value obtained for CP was slightly higher compared to the values of 25.0 %, 26.44 % as reported by Richter et al (2003) and Olaniyi et al (2013) respectively. Abo-State et al (2014) found that the CP of dried *M. oleifera* leaf meal was 30.57 %. In contrast, Ogbe & Affiku (2011) reported that CP value of MOLM was only 17.01 %. The difference in values might be associated with the difference in the processing methods of moringa leaves, soil types, harvesting time, maturation stage and the frequency of defoliation. The leaves of *M. oleifera* are rich in minerals, ascorbic acid and carotenoids and they can be used as substitute to some leguminous seeds such as soybean meal since they provide 2600g kg^{-1} protein (Makkar & Becker 1996; Ferreira et al 2008). According to Ayotunde et al (2004) *M. oleifera* could be considered as potential protein source for inclusion in fish diets at low levels. The results obtained in the present study show that there were 10 essential amino acids found in the MOLM, however the methionine and tryptophan EAA content were of limited value in moringa, 0.302-0.491 g/100 g protein respectively. The efficiency of MOLM as feed ingredient had been already evaluated in some ruminants like goats (Aregheore 2002), for non-ruminants as in broiler chickens (Olugbemi et al 2010; Gadzirayi et al 2012), tilapia, *Oreochromis niloticus* (Afuang et al 2003; Richter et al 2003); catfish, *Clarias gariepinus* (Olaniyi et al 2013) and in fancy carp, *Cyprinus carpio* (Yuangsoi & Masumoto 2012).

Growth performance in terms of weight gain (%) and SGR showed that performance of the fish fed control diet and the test diet having 10 % inclusion level of MOLM (MOLM-10) was highly significant to those fish fed with MOLM-20 and MOLM-30 dietary treatments ($P<0.05$). A number of studies showed that *M. oleifera* leaf meal could be used to substitute FM up to 10 % level in *Clarias gariepinus* (Ozovehe 2013) and this are also in agreement with the findings obtained by Tagwireyi et al (2008) for *Oreochromis niloticus* fry without any negative effects on the growth and feed efficiency.

The proximate carcass composition of sea bass in terms of crude protein and crude ash for MOLM-0 and MOLM-10 diets differed significantly ($P<0.05$) among fish fed

diets MOLM-20 and MOLM-30. However, the carcass crude lipid showed an increasing trend as inclusion level of moringa was increased from 20 to 30 % to replace fish meal protein. This agreed with the previous study conducted by Glencross (2003) on the use of canola meal in red sea bream.

Result from this study showed that methionine, an essential amino acid (EAA) was detected at a level of 0.302 g/100 g protein in moringa leaves which might be considered a limiting amino acid. Therefore the methionine content of the experimental diets with dietary inclusion levels of moringa from 20 to 30 % used in the present study revealed that diets MOLM-20 and MOLM-30 were deficient in methionine thus they suppressed growth and feed utilization in sea bass. Low methionine levels may reflect a poor quality protein diet (Gaber 2006). According to Lim (2003) the EEA requirements of sea bass expressed as percentage of dietary protein are arginine (4.1), lysine (4.8), methionine + cystine (2.6), threonine (4.4) and tryptophan (0.2).

Conclusions. *M. oleifera* leaves could be used in juvenile sea bass diets up to 10 % without adverse effect on growth performance, feed utilization and carcass analyses. Improved in growth and maximum utilization of feed could had been achieved if sulfur containing amino acid, methionine was supplemented to the diets containing moringa up to 30 % dietary inclusion level to improve protein digestibility.

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References

Abo-State H., Hammouda Y., El-Nadi A., Abozaid H., 2014 Evaluation of feeding raw *Moringa* (*Moringa oleifera* Lam.) leaves meal in Nile tilapia fingerlings (*Oreochromis niloticus*) diets. Global Veterinaria 13(1):105-111.

Adamidou S., Nengas I., Henry M., Grigorakis K., Rigos R., 2009 Growth, feed utilization, health and organoleptic characteristics of European sea bass (*Dicentrarchus labrax*) fed extruded diets including low and high levels of three different legumes. Aquaculture 293:263-271.

Adewolu M. A., 2008 Potential of sweet potato (*Ipomoea batatas*) leaf meal as dietary ingredient for Tilapia zilli fingerlings. Pak J Nutr 7:444-449.

Afuang W., Siddhuraju P., Becker K., 2003 Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of *moringa* (*Moringa oleifera* Lam.) leaves on *Oreochromis niloticus*. Aquac Res 34:1147-1159.

Anjorin T. S., Ikokoh P., Okolo S., 2010 Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int J Agric Biol 12:431-434.

Anwar F., Latif S., Ashraf M., Gilani A. H., 2007 Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res 21(1):17-25.

Aregheore E. M., 2002 Intake and digestibility of *Moringa oleiífera- batiki* grass mixture by growing goats. Small Rum Res 46:23-28.

AOAC, 1990 Official Methods of Analysis. Association of Official Analytical Chemists, 15th edition, Washington, D. C., 1298 pp.

Ayotunde E. O., Fagbenro O. A., Adebayo O. T., 2004 Toxicity of aqueous extracts of drumstick *Moringa oleifera*, seeds to Nile tilapia, *Oreochromis niloticus*, fingerlings and adults. Technical Session: Health Management and Disease, ISTA, Manila, Phillippines, pp. 200-208.

Bairagi A., Sarkar Ghash K., Sen S. K., 2004 Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquac Res 35:436-446.

Brett J. R., Groves T. D. R., 1979 Physiological energetics. In: Bioenergetics and growth. Hoar W. S., Randal D. J., Brett J. R. (eds), 8:279-352. New York Academic Press.

Chhay T., Borin K., Sopharith N., Preston T. R., Aye T. M., 2010 Effect of sun dried and fresh cassava leaves on growth of tilapia (*Oreochromis niloticus*) fish fed based diets of rice bran mixed with cassava root meal. Livest Res Rural Dev vol. 22, Article #43. Retrieved August 12, 2014 from http://www.lrrd.org/lrrd22/3/chha 22043.htm

De Silva S. S., Turchini G. M., 2008 Towards understanding the impacts of the pet food industry on world fish and sea food supplies. J Agric Environ Ethics 21:459-467.

Eusebio P. S., Coloso R. M., 2000 Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. J Appl Ichthyol 16(2):56-60.

FAO (Food and Agriculture Organization of the United Nations), 2010 The State of the World Fisheries and Aquaculture. Fisheries and Aquaculture Department, FAO, Rome 197 pp.

Ferreira P. M. P., Farias D. F., Oliveira J. T., Carvalho A. F. U., 2008 *Moringa oleifera*: bioactive compounds and nutritional potential. Rev Nutr 21(4):431-437.

Fuglie L. J., 2000 New uses of *Moringa* studied in Nicaragua. Echo Development Notes # 68. http://www.echotech.org/network/modules.php?

Gaber M. M., 2006 Partial and complete replacement of fish meal by broad bean meal in feeds for Nile tilapia, *Oreochromis niloticus*, L., fry. Aquac Res 37:986-993.

Gadzirayi C. T., Masamha B., Mupangwa J. F., Washaya S., 2012 Performance of broiler chickens fed on mature *Moringa oleifera* leaf meal as a protein supplement to soybean meal. Int J Poult Sci 11(1):5-10.

Ganzon-Naret E. S., 2013 The use of green pea (*Pisum sativum*) as alternative protein source for fish meal in diets for Asian sea bass, *Lates calcarifer*. AACL Bioflux 6(4): 399-406.

Glencross B. D., 2003 Pilot assessment of the potential for canola meal and oil use in aquaculture feeds, final report for the grains research and development corporation no. 5. Department of Fisheries, Western Australia.

Glencross E., 2006 The nutritional management of barramundi, *Lates calcarifer*- a review. Aquac Nutr 12:291-309.

Higgs D. A., Dosanjh B. S., Prendegast A. F., Beams R. M., Hardy R. W., 1995 Use of rapeseed/canola protein products in finfish diets. In: Nutrition and utilization technology in aquaculture. Lim C. E., Sessa D. J. (eds), AOCS Press, USA.

Hlophe S. N., Moyo N. A. G., 2014 A comparative study on the use of *Pennisetum clandestinum* and *Moringa oleifera* as protein sources in the diet of the herbivorous *Tilapia rendalli*. Aquacult Int 22:1245-1262.

Khalafalla M. M., Abdellatef E., Dafalla H. M., Nassrallah A. A., Aboul-Enein K. M., Lightfoot D. A., El-Deeb F. E., Elo-Shemy H. A., 2010 Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. Afr J Biotechnol 9(49):8467-8471.

Lim C. E., 2003 Nutrient requirements of European sea bass (*Dicentrarchus labrax*) and gilt head sea bream (*Sparus aurata*). U.S.A. Department of Agriculture. Technical Bulletin. http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=157340.

Lochmann R. T., Islam S., Philipps H., Adam Z., Everette J., 2013 Effects of dietary sweet potato leaf meal on the growth, non-specific immune response, total phenols and anti-oxidant capacity in cannel catfish (*Ictalurus punctatus*). J Sci Food Agric 93(6):1365-1369.

Makkar H. P. S., Becker K., 1996 Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim Feed Sci Technol 63:211-228.

Martin L. P., 2007 The moringa tree. Echo. North Fort Myers, FL 33917, USA. Available at: http://www.echonet.org/ (Accessed 24 December 2013).

Martinez-Llorens S., Vidal A. T., Garcia I. J., Torres M. P., Cerda M. J., 2009 Optimum dietary soybean meal for maximizing growth and nutrient utilization of on-growing gilthead sea bream (*Sparus aurata*). Aquac Nutr 15:320-328.

Moyo B., Masika P. J., Hugo A., Muchenje V., 2011 Nutritional characterization of *Moringa* (*Moringa oleifera* Lam.) leaves. Afr J Biotechnol 10(60):12925-12933.

Nnaji J. C., Okuye F. C., Omeje V. O., 2010 Screening of leaf meals as feed supplements in the culture of *Oreochromis niloticus.* African Journal of Food, Agriculture, Nutrition, and Development 10(2):2112-2123.
Nouala F. S., Akinbamijo O. O., Adewumi A., Hoffman S., Muetzel S., Becker K., 2006 The influence of *Moringa olifera* leaves as substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay. Livestock Research for Rural Development 18(121). Article available at: http://www.lrrd.org/lrrd18/9/noual 8121.htm.
Ogbe A. O., Affiku J. P., 2011 Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. J Microbiol Biotechnol Food Sci (3):296-308.
Olaniyi C. O., Ajani N. O., Adetomi M. N., 2013 Growth performance and nutrient utilization of *Clarias gariepinus* fed *Moringa oleifera* leaf meal. Journal of Natural Sciences Research 3(8):99-104.
Olugbemi T. S., Mutayoba S. K., Lekule F. P. 2010 Effect of *Moringa oleifera* inclusion in cassava based diets fed to broiler chickens. Int J Poult Sci 9:363-367.
Osman M. F., Eglal-Omar A., Nour A. M., 1996 The use of *leucaena* leaf meal in feeding Nile tilapia. Aquac Int 4(1):9-18.
Ozovehe B. N., 2013 Growth performance, hematological indices and some biochemical enzymes of juveniles *Clarias gariepinus* (Burchell 1822) fed varying levels of *Moringa oleifera* leaf meal diet. J Aquac Res Development 4:166 doi:10. 4172/2155-9546.1000166.
Plaipetch P., Yakupitiyage A., 2012 Use of yeast-fermented canola meal to replace fishmeal in the diet of Asian sea bass *Lates calcarifer* (Bloch 1790). J Aquac Res Development 3:2.
Ranachandran C., GopalaKrishnan P. K., Peter K. V., 1980 Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. Econ Bot 34(3):276.
Richter N., Siddhuraju P., Becker K., 2003 Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 217:599-611.
Sanchez-Machado D. I., Nunez-Gastelum J. A., Reyes-Moreno C., Ramirez-Wong B., Lopez-Cervantes J., 2010 Nutritional quality of edible parts of *Moringa oleifera*. Food and Methods 3:175-180.
Tacon A. G. J., Hasan M. R., Metian M., 2011 Demand and supply of feed ingredients for farmed fish and crustaceans. Trends and prospects. FAO Fisheries and Aquaculture. Technical Paper 56.
Tagwireyi T., Mupangwa J. F., Jepsen J., Mwera P., 2008 Effect of feeding *Moringa oleifera* leaf meal on the growth performance of *Oreochromis niloticus* fry. 3rd International Research and Practice in Appropriate Technology-Energy Solution in the Era of Climate Change, November 12-15, Kigali, Rwanda.
Tiril S. U., Karayucel I., Alagil F., Dernekbasi S., Yagci F. B., 2009 Evaluation of extruded chickpea, common bean and red lentil meals as protein source for juvenile rainbow trout (*Oncorhynchus mykiss*). J Anim Vet Adv 8(10):2079-2086.
Williams K. C., Rimmer M. A., 2005 The future of feeds and feeding of marine finfish in the Asia-Pacific region: the need to develop alternative aquaculture feeds. Regional workshop on low value “trashfish” in the Asia-Pacific region, June 7-9, 2005, Hanoi, Vietnam.
Yousif O. M., Alhadhrami G. A., Passaraki M., 1994 Evaluation of dehydrated alfalfa and saltbush (*Atriplex*) leaves in diets for Tilapia (*Oreochromis aureus* L.). Aquaculture 126:341-347.
Yuangsoi B., Masumoto T., 2012 Replacing *Moringa* leaf (*Moringa oleifera*) partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*). Songklanakarin Journal of Science and Technology 34(5):477.
Zaichun X., 1990 Availability and use of shrubs and tree fodders in China. In: Shrubs and tree fodders for farm animals. Devendra C. (ed), pp. 295-302, Proceedings of a workshop in Denpasar, Indonesia, 24-29 July 1989, IDRC-276e, IDRC, Canada.
http://www.medicalhealthguide.com/articles/malunggay.htm
http://www.mamamoringa.com/moringa_oleifera.html

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