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Potential use of three legume seeds as protein sources and their effects on growth, nutrient utilization and body composition of Asian sea bass, *Lates calcarifer*

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Abstract. Locally produced legumes (pigeon pea - *Cajanus cajan*, yellow mungbean - *Phaseolus aureus*, and red kidney beans - *Phaseolus vulgaris* were assessed as potential protein sources to partially substitute fish meal in practical diets at three replicate samples for juvenile sea bass - *Lates calcarifer* (mean individual weight = 0.18 g) over a 75-day feeding trial. Four isonitrogenous (45% CP) and isolipidic diets (10% CL) were formulated on a dry matter basis consisting of a control diet (CTRL) with 38% fish meal without the test legumes, while other experimental diets containing different plant protein sources such as pigeon pea seed meal (PPSM), yellow mungbean seed meal (YMSM) and red bean seed meal (RBSM), replaced 18-20% of fish meal protein based on the CTRL diet which is equivalent to 11% of the total protein in the diet. Each of the legume test diet contained 33% fish meal and 8% defatted soybean meal. The experimental diets were analyzed for the concentrations of essential and non-essential amino acids (g $100g^{-1}$ protein). Results showed that the methionine level of the PPSM, YMSM and RBSM diets is slightly lower than the CTRL as in cystine. However the highest lysine concentration was found to be in CTRL (0.033 g $100g^{-1}$ protein) and the lowest was with the PPSM (0.026 g $100g^{-1}$ protein). By the end of the feeding trial, groups of sea bass fed CTRL diet had the highest weight gain (WG) and specific growth rate (SGR) of 6.46 g and 1.90% day^{-1} respectively, followed by YMSM fed group, whereas PPSM and RBSM diets induced significantly lower ($p < 0.05$) WG (3.34 g and 3.79 g) and SGR (1.65% day^{-1} and 1.76% day^{-1}) respectively. The best feed conversion ratio (FCR) was obtained with groups fed YMSM diet which was significantly lower ($p < 0.05$) from those of sea bass given PPSM and RBSM but comparable to that of fish fed CTRL diet ($p > 0.05$). Seabass fed YMSM diet had the highest protein efficiency ratio (PER) of 0.90 which did not differ significantly ($p > 0.05$) from the CTRL groups (0.87), however the lowest values for PER were obtained for the fish fed PPSM and RBSM ($p < 0.05$). Similarly, the highest condition factor (K) was recorded at the fish meal based control diet (CTRL) and the lowest value was recorded from juvenile sea bass fed pigeon pea seed meal (PPSM). The survival of sea bass for the different experimental groups ranged from between 66.67 and 83.33%. Proximate composition of the whole body showed a significant increase in the CP and CL contents of the fish fed diets containing YYSM which was comparable with fish fed CTRL diet. Poor growth rates were noted when fish meal protein was replaced by PPSM and RBSM. The overall results presented in this study showed that YMSM is the most promising protein source evaluated for for fish meal replacement at 20% with better growth performance and nutrient utilization and this may help reduce the operating exoenditures, waste output and better income for our fishfarmers.

Key Words: sea bass, yellow mungbean, red beans, amino acid, proximate analysis.

Introduction. Aquaculture is the fastest expanding food production system in the world. For the growth of feed industry, it is vital today to improve the high-yielding plant protein sources to replace fish meal (FM) protein due to its limited supply and higher market demand. Sea bass (*Lates calcarifer*) is strictly carnivore and most of its commercial production relied heavily on fish meal (>30%) which is very costly. Thus, it is important to develop cost-effective sea bass diets for a more profitable production. In order to ensure its sustainable development, the use of plant proteins in fish had been used to develop less expensive alternative protein source for fish meal. Feed technologists and nutritionists explore practical ways to develop cost effective feeds for the commercial bass production by reducing the amount of fish meal in diet formulations without adverse effects on growth (Tidwell et al 2005). Fish meal is the most expensive dietary protein source and is known to contain complete essential amino acids (EAA) that is needed to meet the protein requirement of most fish species (Woods 1999; Sogbesan & Ugwumba 2008; Abowei & Ekubo 2011). A good quality fish meal is a brown powder which contains 60-70% protein (Miles & Jacob 2012) and it represents the largest single cost item in the feed. Considerable research has focused on the use of plant protein as substitute for FM in the feed formulation in order to reduce the dependence on FM as a single protein source (El-Sayed 1999; Martinez-Llorens et al 2009; Tiril et al 2009).

Many studies in fish and shrimp diets have shown that soybean meal possesses an acceptable amino acid profile for growth in many fish due to its high protein content, high digestibility and reasonable price (Tacon & Akiyama 1997; Ganzon-Naret 2013a) among the various plant protein sources. Boonyaratpalin et al (1998) found that diets with soybean (*Glycine max*) meal substituting 37.5% fish meal protein gave an acceptable growth to sea bass, though with a slight difference in comparison to that of fish fed a fish meal based diets. According to Sogbesan et al (2006) the utilization of non conventional feedstuffs of plant origin and other grain legumes (Liener 1980; Tacon 1990; Mikic et al 2009) had been limited as a result of the presence of anti-nutritional factors such as tannins, phytic acid, protease inhibitors, haematoglutinin and saponegin. These anti-nutritional factors negate growth and other physiological activities when incorporated at higher levels in the diets (Oresegun & Alegbeleye 2002). Recently some legume grains which were tested in livestock and poultry; and monogastrics such as fish were faba beans (*Vicia faba*) (Gous 2011); green peas (*Pisum sativum*) (Ganzon-Naret 2013b); sunflower seed meals (*Helianthus annuus*) (Olivera-Novoa et al 2002; Akintayo et al 2008); and kidney beans - *Phaseolus vulgaris* (Absalom et al 1999). Obasa et al (2003) and Ganzon-Naret (2014) evaluated the use of pigeon pea - *Cajanus cajan* in the diets of Nile tilapia (*Oreochromis niloticus*) fingerlings and juvenile sea bass respectively. Results showed that pigeon pea seed meal (PPSM) incorporated at a level of 10% in a 40% protein diet did not have adverse effect on the growth performance, feed intake and carcass composition in sea bass.

Pigeon peas are very resistant to drought (Morton 1976; Saxena et al 2011; Dutta et al 2011), and they can be grown in areas with less than 650 mm annual rainfall. It is a perennial member of the family Fabaceae (Graham & Vance 2003). Pigeon pea is a very important legume food crop which are widely grown in the semi-tropical and tropical regions (Vanaja et al 2010). They contain antitryptic factors and tannins, both of which are thought to depress growth performance, reduces digestibility and protein content (Visitpanich et al 1985). According to Okah et al (2013) pigeon peas are the main protein sources for more than a billion of people and contain approximately 18-31% crude protein and are rich in lysine, however they are limited in sulfur amino acids such as methionine and cysteine and contain carbohydrates from 36.0-66.0%. Pigeon peas are better known as "kadios" in the Philippines while in eastern Africa and Indian subcontinent they are known as gungo pea, gunga pea, Congo pea and "fio-fio." According to Rogers et al (2009) pigeon peas proved to be a better performing nitrogen fixers. The world's three main pigeon pea producing regions are the Indian subcontinent, eastern Africa and Central America. Pigeon peas are cultivated worldwide on 4.92 million hectares with an annual production of 3.65 Mt and productivity of 898 kg ha^{-1} (http://www.agriculturalproductsindia.com/cereals-pulses/cereals-pigeon-pea.html). In Asia, India, Nepal, China and Myanmar are the major producers of pigeon peas.

Yellow mung bean (*Phaseolus aureus*) (*Phaseolus aureus*) is mainly cultivated in Asia. It is popularly known in the Philippines as "munggo" and it is a good source of Vitamins A, B, C and E, calcium, iron, magnesium, potassium and amino acids. Mung beans contain 20% protein, foliate and dietary fiber. It also has anticancer qualities and considered as the most popular bean in the whole world for its bean sprouts, and is known by several names like green bean, sabut moong, nga choy, golden gram, green gram, bundo (http://www.agriculturalproductsindia.com/cerealspulses/cerealsmungbean.html). Today China and India are the main producers of mung beans.

Kidney bean (*Phaseolus vulgaris*) is one of the many varieties of common beans, red in color and has a shape quite similar to that of a human kidney. The nutrients found in kidney beans are vitamins, minerals, folate and amino acids such as tryptophan, threonine, isoleucine, arginine, histidine and glycine (http://www.agriculturalproductsindia.com/cerealspulses/cerealskidneybean.html).

Generally protein sources from legume grains had significantly lower essential and non-essential amino acid content than those of soybean and fish meal (Ozkan et al 2011). However food legumes contain relatively low levels of starch and high levels of fiber compared to cereals. Some authors (Ghadge et al 2008; Akande & Fabiyi 2010) have reported that application of heat must be employed such as sun-drying, boiling and toasting of legume grains in order to inactivate the anti-nutritional factors present, thus improving the nutritional quality, palatability and optimum utilization for the legumes. In the Philippines, very few studies have been conducted on the use of locally available legume seed meals such as pigeon pea, mung bean and kidney bean as potential protein sources in diets for Asian sea bass.

The present study aimed to assess the use of locally available legume seed meals such as pigeon pea, *Cajanus cajan*; yellow mung bean, *Phaseolus aureus* and red kidney bean, *Phaseouls vulgaris* as potential protein sources and its effects on growth, nutrient utilization and body composition of Asian sea bass under controlled laboratory condition.

Material and Method. This feeding trial on sea bass fry was conducted for 75 days from June 2 to August 15, 2013 at the UPV Multi-Species Hatchery of the Institute of Aquaculture, College of Fisheries and Ocean Sciences in Miag-ao, Iloilo, Philippines.

Protein sources and diet formulation. In the current study, dried legume seeds of pigeon pea, yellow mungbeans and red kidney beans were obtained from the local market in Iloilo City, Philippines. Every batch of the whole dry mature legume seeds about 100-200 g were arranged in a single layer on a baking pan in the oven and they were roasted for 40 to 50 minutes at 400^{o}F in order to reduce a significant portion of phytate by about 40% and other anti-nutrients present (Igbedioh et al 1994; Khattab & Arntfield 2009). The legumes were then stirred occassionally every 15 to 20 minutes, allowed them to cool at room temperature and were placed in the airtight container and stored at 4^{o}C. The seeds were milled in a fine powder and sieved using a 60 mesh screen. The test ingredients (Table 1) such as roasted pigeon pea seed meal (PPSM), yellow mung beans (YMSM), red beans seed meal (RBSM), Danish fish meal (FMd) were analyzed for proximate analyses using the standard method as prescribed by AOAC (2000). Crude protein was analyzed using the

Table 1

Proximate composition (%) of fish meal and legume meals as dietary protein sources used in the experimental diets for sea bass

Protein sources	*Proximate composition (%)*			
	Crude protein	*Crude lipid*	*Crude fiber*	*Ash*
Fish meal, Danish	72.00	10.00	1.20	11.00
Pigeon pea	23.80	1.30	4.00	4.10
Yellow mungbean	24.30	1.10	4.00	3.70
Red beans	26.30	0.90	4.10	4.30

Kjeldahl distilling apparatus (Kjeltec system 1002, Tecator, Sweden); crude lipid using the Ether Extraction Method for 16 h; ash was determined by combusting dry samples in a muffle furnace at 550^{o}C for 6 h; crude fiber using the instrument Fibertec and moisture content was estimated by drying the samples to a constant weight using the air convection oven.

Four isonitrogenous (45% crude protein) and isolipidic (10%) diets were formulated as presented in Table 2 with legume seed meals used as dietary protein sources replacing 18-20% of the fish meal (FM) protein equivalent to 11% of the total protein in the diet. The control diet (CTRL) contained 38% fish meal and 10% defatted soybean meal. Diets PPSM, YMSM and RBSM each contained 33% fish meal and 8%

soybean meal with the remainder of the protein from *Acetes* and squid meal at 10 and 5% respectively. Diets were prepared by first mixing the dry ingredients one at a time prior to the addition of vitamin and mineral premixes while lipid source containing a mixture of soybean oil and cod liver oil at a ratio of 1:1 was then blended to the dry mixture. The amount of breadflour was adjusted accordingly PPSM, YMSM and RBSM were incorporated in the diets. The breadflour used as a binder was gelatinized to obtain a dough like consistency, allowed to cool, before added to the mixture. The moist mixture was then extruded using a meat grinder with a 2-mm die. The pellets were dried in the oven at 40-60^{o}C and stored at 4^{o}C before feeding.

Table 2

Composition and proximate analyses of the experimental diets used during the 75-day feeding trial (g 100g^{-1} dry weight)

Ingredients	*Diets*			
	Ctrl	*PPSM*	*YMSM*	*RBSM*
Fish meal, Danish	38.00	33.00	33.00	33.00
Soybean meal, defatted	10.00	8.00	8.00	8.00
Acetes sp.	7.00	10.00	10.00	10.00
Squid meal	5.00	5.00	5.00	5.00
Pigeon pea seed meal		21.00		
Yellow mungbean seed meal			22.00	
Red bean seed meal				21.29
Rice bran (filler)	14.00	6.00	5.00	5.71
Bread flour	16.00	7.00	7.00	7.00
Soybean oil	3.00	3.00	3.00	3.00
Cod liver oil	3.00	3.00	3.00	3.00
Vitamin premix*	2.00	2.00	2.00	2.00
Mineral premix**	2.00	2.00	2.00	2.00
Proximate composition (%)				
Crude protein	45.21	45.02	45.19	45.63
Crude lipid	11.00	10.59	10.55	10.51
Ash	7.23	7.15	7.45	7.18
Crude fiber	2.53	2.63	2.59	2.63

*Each kg of vitamin premix contains vitamin A = 1,200,000 IU; vitamin D3 = 200,000 IU; vitamin E = 20,000 IU; vitamin B1 = 8000 mg; vitamin B2 = 8000 mg; vitamin B6 = 5000 mg; vitamin B12 = 1% 2000 mcg; niacin = 40,000 mg; calcium panthothenate = 20000 mg.
**Each kg of mineral premix contains iron = 40,000 mg; manganese = 10,000 mg; zinc = 40,000 mg; copper = 4000 mg; iodine = 1800 mg; cobalt = 20 mg; selenium = 200 mg.

Based on the chemical analyses, the crude protein of the diets ranged from 45.02-45.63%; 10.51-11.00% for crude lipid; crude fiberfrom 2.53–2.63 while the ash value ranged from 7.15 to 7.45%. Samples of diets were submitted to a commercial laboratory (Feeds and Foods Nutrition Research Center (FFNRC), Pukyong National University, Busan, South Korea) for amino acid analyses.

Experimental fish. Eight hundred pieces, forty-five day old sea bass fry were obtained from SEAFDEC hatchery in Tigbauan, Iloilo, Philippines and were transported to the Multi-species hatchery of the Institute of Aquaculture, University of the Philippines Visayas in Miag-ao, Iloilo. The fish were acclimatized for 2 weeks in 1000 L circular tank with seawater and fed to satiation with CTRL diet two times (2x) a day before they were transferred to experimental tanks.

Feeding trial. Ten sea bass fingerlings with an average weight of 0.18 g and total length (TL) of 2.32 cm were randomly assigned at three replicates in twelve conical fiberglass tanks containing 90 L filtered seawater equipped with a recirculating system. Sea bass were fed the formulated diets at a daily feeding rate of 20-25% of the total body weight and adjusted every 15-day interval during sampling for 75 days. Feeding ration was divided into three equal feedings at 08:30, 13:00 and 16:30.

Water temperature, salinity, dissolved oxygen and pH were measured daily in all tanks. Values were within the ranges acceptable for growth: DO 6.0-7.2 ppm, pH 7.1-7.8, temperature and salinity ranged from 26-29°C and 30-32 ppt., respectively. Total values for ammonia-nitrogen and nitrite-nitrogen were measured once a week and values obtained were negligible. Mortality was recorded daily and dead fish were removed. At the end of the feeding trial, all fish samples from each tank were pooled for the proximate analyses of the whole body composition following the standard method of AOAC (2000).

Parameters of growth and feed utilization. Growth performance was determined and feed utilization of sea bass in response to the control and test diets was calculated as follows:

$$\%\ \text{Weight gain} = \frac{(Wf - Wi)}{Wi} \times 100$$

where: Wf = final weight (g); Wi = initial weight (g)

Specific growth rate (% day^{-1}) = [(In final weight – In initial weight)/time (days) x 100]

Food Conversion Ratio shows the amount of feed required to achieve a unit weight increase in fish. It was calculated by the equation:

FCR = total dry weight of feed (g)/total wet weight gain (g)]

Protein Efficiency ratio = [wet weight gain/protein intake]
where: protein intake = food supplied x crude protein (%)

Survival rate (%) = No. of fish survived/total no. of fish at the beginning x 100

$$\text{Condition factor (K)} = 100 \times \frac{\text{fish weight (g)}}{\text{fish length (in cubic centimeters)}}$$ (Nash et al 2006)

Statistical analysis. All the data obtained were subjected to one-way analysis of variance (ANOVA) Differences among treatments were compared by the Duncan's Multiple Range Test (DMRT) and considered significant at $p < 0.05$. The statistical analyses were carried out using the SPSS Software Program for Windows, Version 16.0. Survival was calculated using the arcsin square root.

Results and Discussion. The proximate composition of locally available legumes and Danish fish meal is shown in Table 1. The protein contents of the leguminous seed meals were very low (23.80-26.80%) as compared to fish meal (FM). Red kidney bean had the highest protein content among the tested legume seeds while pigeon pea had the lowest. The crude lipid content of the legumes ranged from 0.90-1.30% while fish meal contained the highest crude lipid (10.0%). The crude fiber values of legume seed meals were higher than that of the FM while the highest ash value among the feed ingredients tested as protein sources was FM.

All diets were isonitrogenous with protein content of 45% and isolipidic containing 10% lipid. The amino acid analysis of the experimental diets (Table 3) showed that the methionine level (0.004 g 100g^{-1} protein) of the CTRL diet is slightly higher than the PPSM (0.002 g 100g^{-1} protein); YMSM (0.003 g 100g^{-1} protein) and RBSM (0.002 g100g^{-1} protein) diets. Also the lysine concentration in the PPSM (0.026) and RBSM (0.027) diets was relatively low than those of the other diets in the YMSM (0.031) and CTRL (0.033) expressed in g 100g^{-1} protein. The red kidney bean diet had the highest arginine content (0.055 g 100g^{-1} protein) followed by yellow mungbean diet (0.054 g 100g^{-1} protein) even higher than that of the control (0.048 g $100g^{-1}$ protein) while pigeon pea diet had the lowest (0.045 g $100g^{-1}$ protein).

Table 3

Amino acid composition of the diets (g 100 g^{-1}) used in the growth experiment

Analysis	*Treatments*			
	CTRL	*PPSM*	*YMSM*	*RBSM*
Phosphoserine	0.003	0.003	0.003	0.003
Taurine	0.153	0.123	0.130	0.118
Phosphoethanolamine	0.000	0.000	0.000	0.000
Urea	0.000	0.000	0.000	0.000
L-Aspartic acid	0.002	0.002	0.004	0.002
L-Threonine	0.034	0.032	0.032	0.028
L-Serine	0.013	0.011	0.012	0.011
Asparagine	0.000	0.000	0.000	0.000
L-Glutamic acid	0.036	0.036	0.032	0.033
L-a-Aminodipic acid	0.000	0.000	0.000	0.004
L-Proline	0.079	0.083	0.087	0.084
L-Glycine	0.074	0.067	0.074	0.066
L-Alanine	0.158	0.141	0.15	0.139
L-Citruline	0.010	0.006	0.008	0.006
L-α–Aminobutyric acid	0.004	0.003	0.004	0.002
L-Valine	0.050	0.052	0.047	0.045
L-Cystine	0.003	0.001	0.002	0.000
L-Methionine	0.004	0.002	0.003	0.002
L-Isoleucine	0.033	0.028	0.033	0.029
L-Leucine	0.069	0.065	0.070	0.061
L-Tyrosine	0.020	0.021	0.024	0.02
L-Phenylalaine	0.029	0.036	0.028	0.024
β-alanine	0.004	0.005	0.004	0.004
DL-β-Aminoisobutyric acid	0.005	0.008	0.006	0.004
γ-Aminobutyric acid	0.007	0.006	0.006	0.005
L-Histidine	0.003	0.005	0.005	0.006
3-Methyl-L-Histidine	0.000	0.000	0.000	0.000
1-Methyl-L-Histidine	0.000	0.000	0.000	0.000
L-Carnosine	0.002	0.008	0.005	0.005
L-Orinithine	0.005	0.003	0.003	0.003
L-Lysine	0.033	0.026	0.031	0.027
L-Arginine	0.048	0.045	0.054	0.055
Total	0.878	0.821	0.855	0.793

Results of the feeding trial are summarized in Table 4. Sea bass fed CTRL diet showed the highest weight gain and specific growth (6.46 g and 1.90% day^{-1}, respectively). Growth among the legume seed meal diets was significantly ($p < 0.05$) higher for the fish fed YMSM than for those fed diets containing RBSM and PPSM, however this was significantly lower than those of the control. Results indicated that the best ($p < 0.05$) FCR recorded were obtained by the YMSM (2.48) followed by CTRL (2.55) diets, these values were found to be significantly different than those of the PPSM (2.90) and RBSM (3.00).

The same trend was also reported with protein efficiency ratio (PER) which was found to be 0.90, 0.87, 0.76 and 0.74, respectively. In the current study, sea bass fed CTRL diet (2.40) showed the best condition factor (K) while the poorest (1.60) was observed in the PPSM diet. Survival rates of 83.33% and 80.00% for CTRL and YMSM diets respectively were significantly higher ($p < 0.05$) than those fish fed diets RBSM and PPSM. At the end of the feeding experiment fish fed CTRL diet showed the highest crude protein (7.5%) and crude fat (2.0%) than the rest of the experimental groups. Replacing

20% of fish meal protein with yellow mungbean seed meal led to increased crude protein (7.3%) and crude fat (1.9%) on the body composition of sea bass as presented in Table 4, but this was not the case with diets RBSM or PPSM.

Table 4
Growth performance and proximate body composition of sea bass juveniles fed control and test diets over 75 days*

Growth performance	*Diets*			
	CTRL	*PPSM*	*YMSM*	*RBSM*
Initial weight (g)	0.17	0.17	0.19	0.17
Final weight (g)	6.63[a]	3.51[c]	5.10[b]	3.96[c]
Weight gain (g)	6.46[a]	3.34[c]	4.91[b]	3.79[c]
% Weight gain	3604[a]	1898[c]	2630[b]	2599[b]
Specific growth rate (%)	1.90[a]	1.65[c]	1.89[a]	1.76[b]
FCR	2.55[c]	2.90[b]	2.48[c]	3.00[a]
PER	0.87[a]	0.76[b]	0.90[a]	0.74[b]
Condition factor (K)	2.40[a]	1.60[c]	2.06[b]	1.84[c]
Survival (%)	83.33[a]	66.67[c]	80.00[a]	76.66[b]
Body composition (%)				
Moisture	78.2[a]	79.0[a]	77.6[a]	78.6[a]
Crude protein	7.5[a]	6.5[b]	7.3[a]	6.8[b]
Crude fat	2.0[a]	1.5[b]	1.9[a]	1.6[b]
Ash	5.9[a]	5.8[b]	5.6[c]	5.7[c]

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

Legume seeds are good sources of energy, fiber, minerals, vitamins and essential fatty acids, however most of their proteins are low in sulfur amino acids, cystine and methionine (Bartsch & Valentine 1986; Batterham et al 1986). Some factors that limit the incorporation of these ingredients at high levels in fish diets are its low protein content, amino acid imbalance and presence of anti-nutritional factors (Wee 1991). The best known of the anti-nutritional factors found in legumes are trypsin inhibitor and lectins (Gupta 1987; Gilani et al 2005) which inhibit digestive enzymes and may also precipitate proteins. According to De Silva & Anderson (1995) it is essential to have the digestibilty knowledge of the main ingredients, as well as of the whole diet.

The inclusion of pigeon pea meal or red kidney meal in diets at 11% of the total protein in this study did not significantly affect the growth and feed utilization of the sea bass juveniles. The PPSM and RBSM used in this study were roasted for 40-50 minutes at 400^oF before these ingredients were incorporated in sea bass diets respectively at 21 and 21.29 g $100g^{-1}$ diet. However fish offered these diets showed poor growth and survival which were significantly lower than those of CTRL and YMSM. According to Huisman et al (1990), common beans or red kidney beans included at 20% dietary level in pigs resulted in lower protein digestibility and hampers animal performance. This can be attributed possibly to the presence of growth inhibiting substances in roasted beans of PPSM and RBSM. Legume seeds were not dehulled while they were roasted, therefore the tannin content present in the seed coat could possibly explain the lower nutritive values and biological availability of proteins, amino acids and carbohydrates (Deshpande & Cheryan 1985). Different processing methods had been already employed for legumes such as toasting boiling and soaking in order to reduce the concentration of anti-nutritional factors. This confirmed the report of Ogonji et al (2005) that soaking the legumes for 16 hours, sundried thereafter enhanced the best weight gain and haematological values of African catfish (*Clarias gariepinus*) larvae with pigeon pea. Concerning the results of feed utilization in terms of FCR (2.90) and PER (0.76) in the present study, this is in contrast with Tangtaweewipat & Elliot (1989) that the FCR of meat chicken fed diets containing 10-30% pigeon pea were not significantly different from those fed a control diet. Likewise Elias et al (1976) reported that since PSM contains 10.1 total unit of trypsin inhibitors it requires further processing for dehulling and extrusion cooking to improve its nutritional value.

In this study, although the essential amino acids of control diet was higher than those of legume based diets, the growth of fish fed CTRL diet was comparable with that of fish fed YMSM. The poorest growth was in fish fed PPSM. These results agree with those of Eusebio & Coloso (2000) reported that mungbean can be used as protein source up to 18% without affecting growth of sea bass. Furthermore, De Silva et al (1989) have shown that mungbean could be used as partial replacement for fish meal in Nile tilapia and the best results were obtained at 20-25% fish meal replacement. In *Penaeus indicus*, Eusebio & Coloso (1998) observed that 9% replacement of soybean meal protein with mungbean resulted in a significantly lower weight gain, growth rate and survival rate than those fed the control diet.

The effects of the experimental diets on the whole body protein and fat contents containing fish meal (CTRL) and yellow mungbean meal (YMSM) diets showed significant difference ($p < 0.05$) compared to other experimental diets (PPSM and RBSM).

Conclusions. From the aforementioned results, it could be concluded that yellow mungbean seed meal could be a potential protein source to replace fish meal protein at 20% in the juvenile sea bass (0.18 g) without adverse effect on growth, survival and whole body composition. It is further suggested that other processing methods should be employed in addition to roasting for other legume seeds of pigeon pea and red kidney beans to improve its nutrient availability and to remove its anti-nutritional factors that hinder its protein digestibility. Feed efficiency and growth could had been enhanced in this study if these were supplemented with essential amino acids such as lysine, methionine and cystine in the diets.

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References

Abowei J. F. N., Ekubo A. T., 2011 A review of conventional and unconventional feeds in fish nutrition. British Journal of Pharmacology and Toxicology 2(4):179-191.

Absalom K. U., Omoregie E., Igbe A. M., 1999 Effect of kidney bean (*Phaseolus vulgaris*) on the growth performance, feed utilization and protein digestibility of Nile tilapia. Journal of Aquatic Science 14:55-59.

Akande K. E., Fabiyi E. F., 2010 Effect of processing methods on some anti-nutritional factors in legume seeds for poultry feeding. International Journal of Poultry Science 9(10):996-1001.

Akintayo I. A., Obasa S. O., Alegbeleye W. O., Bangbose A. M., 2008 Evaluation of toasted sunflower (*Helianthus annus*) seed meal in the diets of African catfish (*Clarias gariepinus*) fingerlings. Livestock Research for Rural Development 20(10). Retrieved March 14, 2014, from http://www.lrrd.org/lrrd20/10/akin20157.html

AOAC, 2000 Official Methods of Analysis. Association of Official Analytical Chemists, International, 17th edition. Edited by William Horwitz; 2 volumes, 1141 pp.

Bartsch B. D., Valentine S. C., 1986 Grain legumes in dairy cow nutrition. Proceedings of the Australian Society of Animal Production 16:32-34.

Batterham E. S., Andersen L. M., Burnham B. V., Taylor G. A., 1986 Effect of heat on the nutritional value of lupin (*Lupinos angustifolius*) seed meal for growing pigs. British Journal of Nutrition 55:169-177.

Boonyaratpalin M., Suraneiranat P., Tunpibal T., 1998 Replacement of fish meal with various type of soybean products in diets for Asian sea bass, *Lates calcarifer*. Aquaculture 161:67-78.
De Silva S. S., Anderson T. A., 1995 Fish nutrition in aquaculture. Chapman and Hall Aquaculture Series, London, 319 pp.
De Silva S. S., Gunasekera R. M., Atapattu D., 1989 The dietary protein requirement of young tilapia and an evaluation of the least cost dietary protein levels. Aquaculture 80:271-284.
Deshpande S. S., Cheryan M., 1985 Evaluation of vanillin assay for tannin analysis of dry beans. J Food Sci 50:905-910.
Dutta S., Kumawat G., Singh B. P., Gupta D. K., Singh S., Dogra V., Gaikwad K., Sharma T. R., Raje R. S., Bandhopadhya T. K., Datta S., Singh M. N., Bashasab F., Kulwal P., Wanjari K. B., Varshney K., Cook D. R., Singh N. K., 2011 Development of genic-SSR markers by deep transcriptome sequencing in pigeon pea [(*Cajanus cajan* (L.) Millspaugh]. BMC Plant Biol 11:17.
El-Sayed A. F. M., 1999 Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture 179(1-4):149-168.
Eusebio P. S., Coloso R. M., 1998 Evaluation of leguminous seed meals and leaf meals as plant protein sources in diets for juvenile *Penaeus indicus*. Israeli Journal of Aquaculture - Bamidgeh 50:47-54.
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.
Elias L. G., Cvistales F. R., Bressani R., Miranda H., 1976 Chemical composition and nutritive value of some grain legumes. Nutrition Abstracts and Reviews Series B 47:603-864.
Ganzon-Naret E. S. 2013a 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.
Ganzon-Naret E. S., 2013b Growth response and feed intake of *Lates calcarifer* to four different dietary protein levels with green pea (*Pisum sativum*) under controlled laboratory condition. ABAH Bioflux 5(2):137-144.
Ganzon-Naret E. S., 2014 Evaluation of graded level of cooked pigeon pea seed meal (*Cajanus cajan*) on the performance and carcass composition of Asian sea bass (*Lates calcarifer*). ABAH Bioflux 6(1):1-9.
Ghadge P. N., Shewalkar S. V., Wankhede D. B., 2008 Effect of processing methods on qualitites of instant whole legume: pigeon pea (*Cajanus cajan* L). Agricultural Engineering International: the CIGR E-journal Manuscript FP 004, Vol. X.
Gilani G. S., Cockell K. A., Sepehr E., 2005 Effects of anti-nutritional factor on protein digestibility and amino acid availability in foods. J AOAC Int 88(3):967-987.
Gous R. M., 2011 Evaluation of faba bean (*Vicia faba* cv. Fiord) as a protein source for broilers. South African Journal of Animal Science 41(2):71-78.
Graham P. H., Vance C. P., 2003 Legumes: importance and constraints to greater use. Plant Physiology 131:872-877.
Gupta Y. P., 1987 Anti-nutritional and toxic factors in food legumes: a review. Plant Foods for Human Nutrition 37:201-228.
Huisman J., van der Poel A. F. B., Mouwen J. M. V. M., van Weerden E. J., 1990 Effect of variable protein contents in diet containing *Phaseolus vulgaris* beans on performance, organ weight and blood variable in piglets, rats and chickens. British Journal of Nutrition 64(3):755-764.
Igbedioh S., Olugbemi K., Akpapunam M., 1994 Effects of processing methods of phytic acid level and some constituents in bambara groundnut (*Vigna subterranea*) and pigeon pea (*Cajanus cajan*). Food Chemistry 50(2):147-151.
Khattab R. Y., Arntfield S. D., 2009 Nutritional quality of legume seeds as affected by some physical treatment part 2. Antinutritional factors. LWT-Food Science and Technology 42:1113-1118.
Liener I. E., 1980 Toxic constituents of plant foodstuffs. Academic Press, New York and London, 502 pp.

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*). Aquaculture Nutrition 15:320-328.

Mikic A., Peric V., Dordevic V., Srebric M., Mihailovic V., 2009 Anti-nutritional factors in some grain legumes. Biotechnology in Animal Husbandry 25:1181-1188.

Miles R. D., Jacob J. P., 2013 Fish meal in poultry diets: understanding the production of this valuable feed ingredient. IFAS Extension pp. 1-3, PS 12/PS 007. Available at: edis.ifas.ufl.edu/ps007.

Morton J. F., 1976 The pigeon pea (*Cajanus cajan* Millsp.), a high protein tropical bush legume. Horticultural Science 11(1):11-19.

Nash R. D. M., Valencia A. H., Geffen A. J., 2006 The origin of Fulton's condition factor – setting the record straight. Fisheries 31:236-238.

Obasa S. O., Dada A. A., Alegbeleye W. O., 2003 Evaluation of pigeon pea (*Cajanus cajan*) as a substitute for soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*) fingerlings. Nigerian Journal of Animal Production 30(2):265-270.

Ogonji J. O., Uwadiegwu N., Osuigwe D. I., Wirth M., 2005 Effects of different processing methods of pigeon pea (*Cajanus cajan*) on the haematology of African catfish (*Clarias gariepinus*) larvae. Conference on International Agricultural Research for Development, Stuttgart-Hohenheim, October 11-13, pp 1-7.

Okah U., Ibeawuch I. A., Herbert U., 2013 Nutrient intake and digestibility by west African dwarf sheep fed graded levels of pigeon pea seed meal. Iranian Journal of Applied Animal Science 3(2):263-268.

Olivera-Novoa M. A., Olivera-Castillo L., Martinez-Palacios C. A., 2002 Sunflower seed meal as protein source in diets for *Tilapia rendalii* (Boulanger, 1896) fingerlings. Aquaculture Research 33(3):223-230.

Oresegun A., Alegbeleye W. O., 2002 Serum and tissue thiocynate concentration in tilapia (*Oreochromis niloticus*) fed with cassava peel based diets supplemented with DL-methionine. Journal of Aquaculture in the Tropics 17(2):93-100.

Ozkan C. O., Atalay A. I., Guven I., Kaya E., Sagocak A., 2011 Crude protein and amino acid compositions of some protein sources used livestock production in South of Turkey. Asian Journal of Animal and Veterinary Advances 6:750-753.

Rogers A., Ainsworth E. A., Leakey A. D. B., 2009 Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? Plant Physiology 151:1009-1016.

Saxena R. K., Cui X., Thakur V., Walter B., Close T. J., Varshney R. K., 2011 Single feature polymorphisms (SFPs) for drought tolerance in pigeonpea (*Cajanus* spp.). Funct Integr Genomics 11(4):651-657.

Sogbesan O. A., Ugwumba A. A. A., 2008 Nutritional value of some non-conventional animal protein feedstuffs used as fish meal supplement in aquaculture practices in Nigeria. Turkish Journal of Fisheries and Aquatic Sciences 8:159-164.

Sogbesan O. A., Adebisi A. A., Falaye B. A., Okaeme B. N., Madu C. T., 2006 Some aspects of dietary protein deficiency in semi-intensive cultured fishes. A review. J of Arid Zone Fish 2(1):80-89.

Tacon A. G. J., 1990 Standard methods for the nutrition and feeding of farmed fish and shrimp. The essential nutrients. Vol. 1. Argent Laboratories Press, Redmond, Washington, 454 pp.

Tacon A. G. J., Akiyama D. M., 1997 Feed ingredients. In: Crustacean Nutrition. Advances in World Aquaculture, 6: World Aquaculture Society, Baton Rouge, USA. D'Abramo L. R., Concklin D. E., Akiyama D. M. (eds), pp. 411-472.

Tangtaweewipat S., Elliott R., 1989 Nutritional value of pigeon pea (*Cajanus cajan*) meal in poultry diets. Animal Feed Science and Technology 25:123-135.

Tidwell J. H., Coyle S. D., Bright L. A., Yasharian D., 2005 Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass *Micropterus salmoides*. Journal of the World Aquaculture Society 36(4):454-463.

Tiril S. U., Karayucel I., Alagil F., Dernekbasi S., Yagci F. B., 2009 Evaluation of extruded chickpea, common vean and red lentil meals as protein source for juvenile rainbow trout (*Oncorhynchus mykiss*). Journal of Animal and Veterinary Advances 8(10):2079-2086.

Vanaja M., Ram Reddy P. R., Lakshmi N. J., Abdul Razak S. K., Vagheera P., Archana G., Yadav S. K., Maheswari M., Venkateswarlu B., 2010 Response of seed yield and its components of red gran (*Cajanus cajan* L. Millsp.) to elevated CO_2. Plant, Soil and Environment 56:458-462.

Visitpanich T., Batterham E. S., Norton B. W., 1985 Nutritional value of chickpea (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*) meals for growing pigs and rats. II. Effect of autoclaving and alkali treatment. Australian Journal of Agricultural Research 36:337-345.

Wee K. L., 1991 Use of non-conventional feedstuffs of plant origin as fish feeds-is it practical and economically feasible? In: Fish nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition. De Silva S. S. (ed), Asian Fisheries Society, Manila, Philippines, pp. 13-32.

Woods T. A., 1999 Largemouth bass – production budget. Commonwealth of Kentucky Aquaculture Plan, Frankfort, Kentucky, USA, 33 pp.

*** http://www.agriculturalproductsindia.com/cerealspulses/cerealsmungbean.html.

*** http://www.agriculturalproductsindia.com/cerealspulses/cerealskidneybean.html.

*** http://www.agriculturalproductsindia.com/cereals-pulses/cereals-pigeon-pea.html.

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