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Evaluation of graded levels of cooked pigeon pea seed meal (*Cajanus cajan*) on the performance and carcass composition of Asian sea bass (*Lates calcarifer*)

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Abstract. A 12-week feeding trial was conducted to evaluate the effects of different graded levels of cooked pigeon pea (Cajanus cajan) seed meal on the growth performance and carcass composition of Asian sea bass (Lates calcarifer). Four isonitrogenous (40% crude protein) and isolipidic (8% lipid) diets were prepared incorporating different graded levels of cooked pigeon pea seed meal (PSM) at 0 (T1); 10 (T2); 20 (T3) and 30% (T4) with an equivalent amount of protein replacement in sea bass diets at 0, 5, 10 and 15% respectively. Fish fed T4 diet had the lowest value (13.81 g) for weight gain (WG) due to its poor palatability as sea bass obviously ignores the pellets offered during the feeding as compared to the rest of the treatments. The WG of fish (16.71 g) fed T1 diet (control) was comparable to fish fed T2 diet (16.38 g) but significantly (p < 0.05) higher than those fish fed T3 (14.28 g) and T4 (13.81 g) diets respectively. The highest specific growth rate (SGR) of sea bass was obtained in fish fed T1 diet, but the differences between groups T1, T2, T3 and T4 were statistically significant (p < 0.05). Fish fed T1 diet had the highest survival (93.33%) and feed intake (35.09 g/fish) among the different experimental diets, and was not significantly different from the T2 (10% PSM) diet. The incorporation of boiled PSM (10%) in T2 diet resulted in best feed conversion rate (FCR) and protein efficiency (PER) ratios comparable to the control fish (T1) but significantly higher (p < 0.05) than that of fish fed T3 and T4 diets. The crude protein in the carcass (17.44-17.86%) was not influenced by the different graded levels of boiled PSM in the diets (p > 0.05). No significant differences (p > 0.05) were found in the crude lipid among the different treatments. Results showed that PSM inclusion level at 10% in a 40% protein diet did not have adverse effects on the growth parameters, feed intake and carcass composition in sea bass. Key Words: sea bass, pigeon pea seed meal, carcass, proximate analysis.

Introduction. The use of plant protein sources in aquaculture diets has been commonly used for the past decades due to the increased cost and demand for fish meal (FM). Fish meal is one of the most expensive dietary protein source which is traditionally used in aquaculture diets (Woods 1999). Fish nutritionists tried to look for alternative plant protein sources such as grain legumes to replace FM in order to lower the cost of aquaculture diets (Martinez-Llorens et al 2009) and potentially reduce the nutrient levels in effluent water. Several reports have described the use of grain legumes in several feeding experiments (Kissil et al 2000; Booth et al 2001; Borlongan et al 2003; Tiril et al 2009). Several authors have described that utilization of soybean meal is considered the most widely used plant protein source (Akiyama 1991; Tacon & Akiyama 1997; Ganzon-Naret 2013c) amongst the various available plant protein feedstuffs because of its availability, high digestibility, reasonable price and for meeting the essential amino acid requirements of some fish species commercially grown. Other legumes specifically green peas (Pisum sativum) are known for their relatively high protein and energy content (Ganzon-Naret 2013a, 2013b). Soybean meal (SBM) and other grain legumes contain anti-nutritional factors (ANFs) notably; protease inhibitors, tannins, phytic acid, tannins and gossypols which reduce the nutrient utilization and their biological value (Liener 1980).

Other grain legumes which were tested and appropriate for aquafeed formulation include green peas (Cruz-Suarez et al 2001; Ganzon-Naret 2013), canola (Thiessen 2004), lupin (Robaina et al 1995), faba beans (Gous 2011) and sunflower seed meals (Sanz et al 1994). However, there is little or any known research on the use of pigeon pea (*Cajanus cajan*) as dietary protein source for sea bass (*Lates calcarifer*) and other marine fishes.

Pigeon pea is a perennial member of the family Fabaceae (Graham & Vance 2003). In the Philippines, pigeon peas are better known as "kadios" while in eastern Africa and Indian subcontinent they are known as gungo pea, gunga pea, Congo pea and "fio-fio". Today, pigeon peas are widely cultivated in all tropical and semitropical regions and they are important legume crops in the semiarid tropics (Vanaja et al 2010). The world's three main pigeon pea producing regions are the Indian subcontinent, eastern Africa and Central America. Being a legume, the pigeon pea enriches soil through symbiotic nitrogen fixation (Rogers et al 2009). Pigeon peas are very resistant to drought, so they can be grown in areas with less than 650 mm annual rainfall (Morton 1976).

Okah et al (2013) found that raw pigeon pea seeds are rich in protein about 18.5-31.1% and carbohydrate ranging from 36.0-66.0% depending on the variety which includes biological value from 61.6-69.8%. Pigeon peas contain amino acids such as methionine and lysine. In contrast to the mature seeds, the immature seeds are generally lower in all nutritional values, however they contain a significant amount of vitamin C and have a slightly higher fat content (Bressani et al 1986). Gupta et al (2006) pointed out that the protein content of the immature seeds is of a higher quality as compared to the mature seeds. The utilization of plant proteins, Leguminosae in the feed formulation for fish and livestocks are often limited due to the presence of anti-nutritional factors and toxic substances (Tacon 1990). Anti-nutritional factors associated with soybean meal are due to the presence of trypsin inhibitors which decrease protein digestibility (Hertrampf & Piedad-Pascual 2000). Thus, in order to improve the nutritional quality and effective utilization of legume grains, application of heat treatment must be employed such as cooking (boiling) and toasting to remove the anti-nutritional factors (Farran et al 2001; Ghadge et al 2008; Akande & Fabiyi 2010). According to Trugo et al (1990) cooking the legume seeds (*Phaseolus vulgaris*) for 60 minutes at 100°C was very efficient in inactivating over 90% of the trypsin inhibitors.

This study was therefore designed to evaluate the different graded levels of cooked pigeon seed meal (PSM) on the performance and carcass composition on sea bass.

Material and Method

Processing of test ingredient. The pigeon pea used in this study was obtained from the local market in Iloilo City, Iloilo, Philippines. The proximate analysis of boiled PSM (AOAC 1990) and amino acid composition were shown in Tables 1 and 2.

Table 1

Contents	PSM
Dry matter	93.40
Crude protein	21.32
Crude lipid	5.40
Crude ash	5.14
Crude fiber	7.29
NFE*	60.85

Proximate analysis (%) of boiled PSM

*Nitrogen free extract.

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Amino acid composition (g/100g dry sample) of boiled PSM

Amino acids	Boiled PSM
Lysine	7.86
Histidine	2.48
Arginine	6.93
Aspartic acid	13.70
Threonine	4.28
Serine	4.94
Glutamic acid	15.73
Proline	3.27
Glycine	3.94
Alanine	4.02
Cystine	0.84
Valine	4.17
Methionine	1.10
Isoleucine	2.93
Leucine	7.20
Tyrosine	2.93
Phenylalanine	5.90
Tryptophan	ND*

* ND = not determined

According to Millamena et al (2002) in order to inactivate some of the anti-nutritional factors and increase the nutrient utilization of leguminous seeds, the whole seeds of pigeon peas were boiled in the stainless steel pot for 2 h following the procedure as described by Carlini & Udedibie (1997). The boiled water was then discarded and the pigeon peas were removed, drained and dried in the oven at 60-70°C, finely ground and sieved through 60 mesh screen.

Fish and maintenance. Sea bass were obtained from the finfish hatchery of SEAFDEC 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 in 1000 L circular tank for 2 weeks and fed T1 diet (control) twice daily prior to the feeding trial.

Diet formulation. The experimental diets (Table 3) were formulated to be isonitrogenous (40% protein) and isolipidic (8% lipid). The cooked PSM (boiled) was incorporated at different graded levels (0, 10, 20 and 30%) to replace about 0, 5, 10 and 15% of the protein in sea bass diets.

The basal diet formulation contained fish meal, squid meal meal, *Acetes*, soybean meal (defatted) and corn meal as dietary protein sources. PSM (cooked) were incorporated at graded levels of 0% (T1), 10% (T2), 20% (T3) and 30% (T4) in a 40% protein diet. The diet without PSM served as the control diet (T1). Ingredients such as fish meal, squid meal, *Acetes*, vitamin and mineral mix were kept constant in all diets. Soybean and cod liver oils at a ratio of 1:1 were used as the lipid sources.

All dry diet ingredients were mixed thoroughly prior to the addition of vitaminmineral premix. Oils were then added with the dry mixture. Breadflour was cooked in 600 ml water, allowed it to cool before added to the ingredient mixture. The mixture was then passed through the meat pelletizer to obtain 2 mm-diameter pellet. The "spaghetti-like" strands were dried in the air convection oven at 60°C, stored in plastic bags and placed in the refrigerator at 4°C until used.

Based on the chemical analyses, the crude protein content of the diets ranged from 41.94-43.69%; 8.34-9.37% for crude lipid while the ash value ranged 11.41-13.20% (Table 3).

Table 3

Ingredients		Treatr	ments	
	Τ1	T2	Т3	Τ4
Peruvian fish meal	30.0	30.0	30.0	30.0
Squid meal	5.0	5.0	5.0	5.0
Acetes sp.	8.0	8.0	8.0	8.0
Soybean meal, defatted	26.0	19.0	14.0	11.0
Pigeon pea (cooked)	0.0	10.0	20.0	30.0
Corn meal	8.0	5.0	3.0	1.0
Wheat flour	13.0	13.0	10.0	5.0
Soybean oil	2.5	2.5	2.5	2.5
Cod liver oil	2.5	2.5	2.5	2.5
Vitamin mix	2.0	2.0	2.0	2.0
Mineral mix	3.0	3.0	3.0	3.0
Proximate composition (%)				
Crude protein	43.69	42.62	42.02	41.94
Crude lipid	8.34	8.64	8.96	9.37
Crude ash	11.41	12.60	12.90	13.20

Composition and proximate analyses of the experimental diets used in the 12-week feeding trial (g/100g dry weight)

Feeding management. After the acclimation period of two weeks, hatchery-bred sea bass (mean body weight of 0.49–0.52 g) were stocked randomly in twelve conical fiber glass tanks (100 L capacity) at a density of 15 fish per tank for a total of three replicates per treatment. The tanks were connected in a semi-closed recirculating system with filtered aerated seawater. Fish in each treatment were then fed three times daily at a feeding rate of 15%, 10% and 8% of the total body weight for the first month, second month and third month respectively. Mortality and feed consumption were recorded daily. The fish in each tank were counted and weighed as a group every 15 days for 3 months (July 15 to October 14, 2009) to adjust the feeding ration. At the end of the experiment, nine (9) fish were pooled, from each treatment, homogenized and analyzed for the carcass composition.

The seawater was analyzed for dissolved oxygen (6.2–7.5 mg L^{-1}); temperature of 26–30°C; pH of 7.1-7.6 and salinity of 30-32 ppt. Ammonia-nitrogen and nitrite nitrogen were monitored every 15 days using the Nitrogen analyzer (Skalar), the values were within the ranges conducive for the growth of the fish.

Evaluation of growth parameters and calculation. Growth parameters and feed efficiency of fish in relation to the different graded levels of cooked PSM were determined such as:

Weight gain - WG (g) = (final weight - initial weight)

Specific Growth Rate - SGR (% day⁻¹) = [(In final weight – In initial weight)/time (days) x 100]

Food Conversion Ratio - FCR = [total dry weight of feed (g)/total wet weight gain (g)]

Protein Efficiency ratio - PER = [wet weight gain/protein intake]

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

Statistical analysis. All the data were subjected to analysis of variance (ANOVA). Differences among treatments were compared by the Duncan's Multiple Range Test (DMRT) and considred 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 survival, growth, FCR and PER of sea bass fed various experimental diets containing different graded levels of cooked PSM after 12 weeks of feeding are summarized in Table 4.

Table 4

Growth performance of sea bass	juveniles to the var	rious experimental die	ets after 12		
weeks of feeding					

Baramotors	Treatments			
Falameters	T1	T2	Т3	Τ4
Initial mean weight (g)	0.49	0.52	0.52	0.52
Final mean weight (g)	17.20	16.90	14.80	13.70
Weight gain (g)	16.71 ^a	16.38 ^a	14.28 ^b	13.18 ^c
SGR (%/day)	1.72 ^a	1.68 ^a	1.61 ^b	1.11 ^c
FCR	2.10 ^a	2.16 ^a	2.40 ^b	2.49 ^{bc}
PER	1.19 ^a	1.16 ^a	1.04 ^b	1.00 ^{bc}
Feed intake (g/fish)	35.09 ^a	35.38 ^a	34.27 ^b	32.81 ^c
Survival (%)	93.33 ^a	93.33 ^a	80.00 ^c	86.67 ^b

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

The highest growth performance and feed utilization were observed in fish fed T1 and T2 diets (WG = 16.71; 16.38; SGR = 1.72; 1.68) respectively which were influenced by the different graded levels of cooked PSM. Fish fed the highest graded level of PSM from 20% (T3) to 30% (T4) were significantly lower (p < 0.05) in terms of growth performance as compared to fish fed T1 and T2 diets. The growth of the fish fed diet containing 20% graded level of PSM was significantly higher than that of fish fed 30% (T4). The total feed intake during the 12 weeks experiment was achieved by group of fish fed T2 (35.38 g/fish), followed by T1 (35.09 g/fish) and were found to be significantly higher (p < 0.05) than did fish fed the T3 and T4 diets. Sea bass fed the control diet (T1) showed the best FCR and PER (2.10 and 1.19, respectively). Survival rate of 93.33% was significantly higher (p < 0.05) for both the fish fed control without PSM (T1) and with 10% inclusion of PSM (T2) while the poorest (80.00%) was observed in the 30% PSM diet (T3). The data on the carcass proximate composition (%) of seabass fed with the four different diets are shown in Table 5.

Table 5

Carcass proximate composition (%) of sea bass juveniles fed the various experimental diets (values are not significantly different at $p > 0.05^{a}$)

Experimental diets	Proximate composition (%) ^a			
	Τ1	T2	Т3	Τ4
Crude Protein	17.84	17.60	17.46	17.44
Crude lipid	5.62	5.70	5.72	5.68
Crude ash	3.10	3.12	3.16	3.18
Moisture	75.23	75.16	76.18	76.36

The crude protein, lipid, ash and moisture contents in the carcass composition of sea bass fed with various experimental diets and control diet were not influenced by the graded level of PSM at the end of the experiment. Fish fed the control diet (T1) showed the highest crude protein (17.84%) and lower lipid level (5.62%), but no significant differences (p > 0.05) were observed among the treatments (0-30% PSM diets).

In the current study, FCR and PER of sea bass were improved in the control diet (T1) and as the dietary level of PSM was incorporated at 10% in a 40% protein diet a better performance in the feed intake was achieved. This suggests that cooked PSM proved to be an acceptable ingredient in practical diets for juvenile sea bass. Based on the proximate analysis (Table 1) cooked pigeon pea contains 21.32% crude protein and 5.40% crude lipid. Lysine is particularly high in PSM about 7.86% (Table 2). According to Deosthale & Rao (1981) pigeon pea has protein content which ranged from 19.8 to 23.6%, methionine from 1.2% to 1.9% and trytophan from 0.43 to 0.62%. In addition, pigeon pea contained a higher amount of nitrogen free extract (60.85%) which can be

used as a source of energy (FNRI 1980), thus reducing the cost of feeds. The efficiency of PSM as feed ingredient has been investigated in some monogastric animals such as in growing pigs (Castro et al 1987; Mckbungwan et al 2003), broilers (Tangtaweewipat & Elliot 1989) and in Japanese quails (Yisa et al 2013). The PSM used in this study were boiled in the steel pot for 2 h and dried overnight in the oven at 60-70°C. According to Amaefule et al (2005) boiled PSM could replace 20 and 43.9% maize and soybean meal, respectively in the diet of weaner rabbits, constituting up to 20% of the whole ration. In this study the highest growth and feed intake were obtained in sea bass fed control diet (T1) and with 10% incorporation of PSM (T2) in contrast to the growth experiments in rabbits (Amaefule et al 2005) wherein the boiled pigeon pea seed (BPSM) meal did not significantly influence their performance as well as their carcass characteristics. This is in agreement 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. Elias et al (1976) reported that PSM contains trypsin inhibitors which are estimated to be 10.1 total unit of trypsin inhibitors in contrast to a much higher content of 25.5 total unit of trypsin inhibitors in soybeans.

The carcass composition of sea bass among the different experimental diets did not differ significantly among the treatments. This also indicates the higher the level of pigeon pea inclusion from 20 to 30% in sea bass diet, they must be supplemented with sulfur amino acid such as methionine and cystine from other protein sources (Aremu et al 2006).

Conclusions. The results of the present study show that boiled PSM could be used as dietary protein source for sea bass. The level of dietary inclusion of boiled PSM at 10%, replacing 5% of the protein in the diet is considered as the optimum level for the growth and feed utilization for sea bass. Growth and feed efficiency could have been improved in fish if graded levels of boiled PSM at 20-30% were supplemented with essential amino acids such as lysine, methionine and cystine with squid meal added in the diets as an attractant.

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