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The use of green pea (*Pisum sativum*) as alternative protein source for fish meal in diets for Asian sea bass, *Lates calcarifer*

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Abstract. A 12-week feeding trial was conducted to investigate the effect of green pea *P. sativum* as alternative protein source for fish meal on the growth performance, feed utilization and phosphorus excretion for Asian sea bass, *Lates calcarifer*. Four isonitrogenous (40% crude protein) and isolipidic (10% lipid) diets were prepared with the increasing substitution levels of green pea (GP) for fish meal (FM) at 0% (P0), 10% (P10), 20% (P20) and 30% (P30) with corresponding dietary protein levels at 0, 2.6, 5.2 and 7.8% respectively in a 40% protein diet. The weight gain (WG) of fish (19.69 g) fed P0 diet (control without GP meal) was comparable to fish fed P10 diet (17.75 g) but significantly ($p < 0.05$) higher than those fish fed P20 (14.62 g) and P30 (13.17g) diets respectively. Fish fed P30 diet had the lowest value for WG which might be attributed to the poor palatability of the diet as sea bass obviously ignore the pellets offered during the feeding. Partial replacement (about 10%) of fish meal in P10 diet produced fish with feed conversión (FCR) and protein efficiency (PER) comparable to the control fish but significantly higher ($p < 0.05$) than that of fish fed P20 and P30 diets. Fish fed control diet had the highest survival (93.33%) and feed intake (41.40 g/fish). These results were comparable with sea bass fed P10 diets (93.33%; 38.34 g/fish). The decrease in the percentage protein in the carcass was associated with the increasing dietary levels of GP in the diets. No significant differences ($p > 0.05$) were found in the percentage of lipid among the different treatments. The peak time for phosphorus excretion was observed after the first feeding at the end of 8 hrs. Fish fed P0 diet had the highest phosphorus excretion which was attributed to its high level of FM component whereas fish fed P30 diet had the lowest. It appears that green pea can replace fish meal at the level of 10% in diets for sea bass without adverse effects on growth, feed utilization or body composition and this may also contribute to environmental protection as well as reduce feed cost to sustain aquaculture.

Key Words: sea bass, *Lates calcarifer*, green pea, phosphorus excretion, feeding.

Introduction. Sea bass *Lates calcarifer* (Bloch, 1790) is an economically important aquaculture species in Southeast Asia. It is a desirable fish with good flesh texture and taste, high market value and demand. They are protogynous hermaphrodites, developing first as females and then changing to males at 3-4 years of age. Juveniles with an average body weight of 20-50 g are usually stocked in grow-out ponds until they reached the marketable size of 300-600 g in about 4 to 7 months (SEAFDEC 2010). They had been raised successfully both in freshwater and brackishwater ponds as well as in marine cages. In some countries, all culture systems for sea bass used trashfish as feeds because of its low price and availability. As the supply of trashfish become limited and expensive, these affect the growth of sea bass industry. Studies by Boonyaratpalin et al (1998) found that the use of trashfish as sole feed to sea bass resulted in a decrease in feeding efficiency and slower growth rate. The feeding of trashfish itself is a significant source of pollution. Williams & Rimmer (2005) cited that feed losses associated with trashfish given to carnivorous fish have been reported to be as high as 38% as compared to around 10% for pelleted feeds. Moreover, studies conducted by Millamena & Toledo (2004) found that juvenile gold spot grouper (*Epinephelus coioides*) survived and grew well in formulated dry pellet diets than those fed with trashfish reared in laboratory tanks and net cages.

Fish meal is one of the most expensive ingredients of aquaculture diets and it is estimated that more than 50% of the variable costs for sea bass are feed costs (Woods 1999), so the profitability of production is significantly influenced by feed. However, due to the increased cost and demand for fish meal and its greater tendency to pollute the environment, it is desirable to replace FM with less expensive protein sources (Martinez-Llorens et al 2009) to improve the sustainability of aquaculture, as it would reduce dependence on fish source. Various animal and plant protein sources have been tested to completely or partially replace FM in practical fish and shrimp diets (Boonyaratpalin et al 1998; Eusebio & Coloso 1998; Kissil et al 2000; Borlongan et al 2003).

Among the legumes, soybeans (*Glycine max*) have been commonly used as an alternative protein source in fish feeds due to its high protein content and good amino acid profile (Lim & Dominy 1990). Previous studies with rainbow trout, *Oncorhynchus mykiss* and hybrid striped bass *Morone chrysops x M. saxatilis* (Webster et al 1999) indicated that inclusion of soybean meal completely in the basal diet resulted in poor growth and reduced protein utilization due to the presence of trypsin inhibitor.

Other legumes, such as green peas (*Pisum sativum*) has been reported to be a potential feed ingredient and is being used recently as an alternative protein source in fish diets. Borlongan et al (2003) confirmed that green pea (GP) meal could be used as dietary feed ingredient and can replace up to 20% of the total dietary protein in milkfish (*Chanos chanos*) diets. GP are common agricultural products in the Philippines and they are known for their crude protein content of 22-24%, lysine content at 1.6% and 14.3kJ/g digestible energy. In the study of Lovell (1989) it was reported that *P. sativum* contains several endogenous anti-nutritional factors such as protease inhibitors, phytohaemagglutinin, cyanogens, phytic acid, saponins and anti-vitamin E factor. The efficacy of green peas as feed ingredient had been already well established in some ruminant and non-ruminant species such as cattle, poultry and swine respectively.

Inclusion levels of plant protein *Leguminosae* in feed formulation (Tacon 1990) are often limited due to the presence of anti-nutritional factors and toxic substances. Anti-nutritional factors associated with soybean meal are due to the presence of trypsin inhibitors which decrease protein digestibility (Hertrampf & Piedad-Pascual 2000). Legumes contain adequate amounts of lysine.

Studies of using GP to feed carnivorous fish (sea bass) are very limited, therefore the scope of the present study was to evaluate the effect of green pea at different dietary levels of replacement for fish meal on the growth performance, feed efficiency and body composition for sea bass and to measure the phosphorus (P) excretion for fish fed different practical diets.

Material and Method

Experimental fish. Hatchery reared sea bass (*Lates calcarifer*) were obtained from SEAFDEC, AQD, Tigbauan, Iloilo, Philippines and these were transported to the UPV Hatchery in Miag-ao, Iloilo. One thousand fish were held in 1000 L circular tank for acclimation for 2 weeks under laboratory condition and fed the control (P0) diet twice daily prior to their transfer to the experimental tanks.

Feed ingredients and diet formulation. Four experimental diets containing different levels of green pea were formulated to be isonitrogenous and isolipidic as shown in Table 1. Analyses, however showed that experimental diets contained 40.44–41.05% protein, 10.11–10.98% lipid and the phosphorus content was 1.71–1.98% according to AOAC (1990). The basal diet formulation contained fish meal, defatted soybean meal, shrimp meal and corn meal as dietary protein sources. GP meal present in the diets were 0% (P0), 10% (P10), 20% (P20) and 30% (P30) with corresponding dietary protein levels at 0%, 2.6%, 5.2% and 7.8% respectively, in a 40% protein diet. The diet without GP meal served as the control diet (P0). Other dietary ingredients such as defatted soybean meal, shrimp meal, vitamin-mineral mix, cod liver oil and soybean oil were incorporated at the same levels in the experimental diets. As fish meal was replaced by GP meal, the amount of breadflour used as a binder, were adjusted accordingly to balance the dietary protein content.

In order to inactivate some anti-nutritional factors and increase the nutrient utilization of GP (Millamena et al 2002), the whole dry pea was oven-dried at 60^{o}C for 6 h, finely ground into a meal form and sieved through 60 mesh screen. Samples of feed ingredients were analyzed for proximate composition using the standard methods as described in AOAC (1990).

Table 1

Formulation (g/100 g diet) and proximate analyses of the experimental diets

Ingredients	*Diet No.*			
	P0	*P10*	*P20*	*P30*
Peruvian fish meal	40.00	36.00	32.00	28.00
Soybean meal, defatted	10.00	10.00	10.00	10.00
Shrimp meal	13.81	13.81	13.81	13.81
Corn meal	11.18	11.18	11.18	11.18
Vitamin-mineral mix	3.00	3.00	3.00	3.00
Cod liver oil	2.00	2.00	2.00	2.00
Soybean oil	2.00	2.00	2.00	2.00
Bread flour	18.01	11.18	4.35	0.40
Green peas	-	10.83	21.66	29.61
Proximate composition (% dry basis) of test diets				
Crude protein	40.56	41.05	40.66	40.44
Crude fat	10.88	10.11	10.67	10.98
Ash	13.20	12.00	11.59	11.39
Phosphorus	1.98	1.80	1.74	1.71

Table 2 shows the proximate composition of GP used in the feeding experiment. All dry diet ingredients were mixed thoroughly prior to the addition of vitamin-mineral premix. Soybean oil and cod liver oil at 1:1 ratio were then added to the dry ingredient mixture. Diets were then passed through a meat extruder with a 2mm die to form “spaghetti-like” strands and dried in the air convection oven at 60^{o}C to ≤ 10% moisture. Strands were cut into 2 mm pellets using a grinder and sieved using a # 40 sieve. Diets were stored in the refrigerator 4^{o}C until fed.

Table 2

Proximate composition of *Pisum sativum*

Proximate composition	*% dry weight*
Moisture	10.3
Crude protein	24.1
Crude lipid	1.2
Crude fiber	3.8
NFE**	57.2
Ash	3.4
Calcium	0.13
Phosphorus	0.52

** Nitrogen-free extract.

Fish and feeding management. The 12 weeks feeding experiment was conducted in September 16 to December 15, 2011 at the Multi-species Hatchery of the University of Philippines Visayas UPV), College of Fisheries and Ocean Sciences (CFOS), Institute of Aquaculture (IA), Miag-ao, Iloilo, Philippines. Two (2) weeks after the acclimation period, hatchery-bred sea bass (mean body weight of 0.51–0.63 g; total length (TL) of 3.28–4.00 cm) were randomly distributed at a density of 15 fish per tank for a total of three replicates per treatment. The feeding trial was conducted in one hundred-liter capacity, twelve (12) conical fiberglass tanks containing 90 L filtered aerated seawater. The tanks were connected in a semi-closed recirculating system provided with sand and biological filters with continuous aeration. Fish in each treatment were then fed three times daily at 09:00, 12:00, 16:00 h at a feeding rate of 15%, 10% and 8% of the total body weight for the first month, second month and third month respectively. Feed consumption and mortality were recorded daily. All the fish in each tank were counted and weighed every 15 days to adjust the feeding ration throughout the 12-week feeding trial and the total lenght (cm) was measured. At the start of the experiment, ten fish were weighed from the same population for the initial whole body composition and during termination about six fish from each tank were weighed, killed and frozen at -60^{o}C for the final body composition.

Water quality parameters measured were dissolved oxygen, 6.5–8 mg L^{-1}; temperature, 25–30^{o}C; pH, 6.5–7.5 and salinity ranged 30-32 ppt. The values were within the ranges suitable for the growth of sea bass. Ammonia-nitrogen and nitrite nitrogen were monitored every 15 days using the Nitrogen analyzer (Skalar).

Phosphorus excretion. During the last feeding trial, the results on phosphorus (P) excretion of sea bass fed different diets were determined in the rearing water at the peak hours after feeding. In order to determine the peak hour, a preliminary measurement for phosphorus excretion was carried out for two consecutive days for an 8-h period according to Tantikitti et al (2005) and Jindal et al (2009). Water samples about 1 L were collected from each tank immediately after the first meal at 09:00 h at every 2, 4, 6 and 8 h. The water samples were then stored at 20^{o}C until further analysis for the phosphate concentration (Strickland & Parsons 1972).

Calculation and analyses. Growth response and feed efficiency of sea bass in relation to the different treatments were determined by weight gain (final weight - initial weight), specific growth rate (SGR % day^{-1}) [(In final weight – In initial weight)/time (days) x 100], feed conversion ratio (FCR) [total dry weight of feed (g)/total wet t weight gain (g)] and protein efficiency ratio (PER) [wet weight gain/protein intake]. Survival was determined and data were calculated using the arcsin square root.

All data were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT), to determine the significant differences among treatments ($p < 0.05$). The statistical analyses were carried out using the SPSS Software Program for Windows, Version 10.0.

Results and Discussion. Growth performance, FCR, PER and survival of sea bass offered diets containing different levels of GP after 12 weeks of feeding are summarized in Table 3.

Table 3

Growth, feed utilization and survival of seabass after 12 weeks of feeding*

Parameters	*Diet No.*			
	P0	*P10*	*P20*	*P30*
Initial mean weight (g)	0.51	0.65	0.58	0.63
Final mean weight (g)	20.2	18.4	15.2	13.8
Weight gain (g)	19.69^{a}	17.75^{a}	14.62^{b}	13.17^{b}
SGR (%/day)	1.91^{a}	1.59^{c}	1.66^{b}	1.58^{b}
FCR	2.10^{a}	2.16^{a}	2.40^{b}	2.49^{bc}
PER	1.18^{a}	1.15^{a}	1.03^{b}	1.00^{bc}
Feed intake (g/fish)	41.40^{a}	38.34^{a}	35.16^{b}	32.79^{b}
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 results showed that there were significant differences in growth, FCR and PER of sea bass after 12 weeks of feeding. The highest weight gain (WG), specific growth rate (SGR), PER and survival were obtained in sea bass fed P0 and P10 diets. The best FCR were also obtained in the same diets. Growth and feed intake of fish that were fed diets P20 and P30 were significantly ($p < 0.05$) lower than those fed P0 and P10 diets while FCR increased. The lowest SGR (1.58%) and PER (1.00) were observed in fish fed diets with up to 30% inclusion level of GP (P30) but were not significantly different ($p > 0.05$) from those fish fed P20 diet. Fish fed P30 diets appeared to ignore the pellets offered during feeding which led to a decline in feed intake as well as to poor FCR and therefore resulted in slower growth. However diets containing low levels of GP at 10% were readily accepted by sea bass. Moreover, after 12 weeks of feeding, the survival rate of fish fed P0 (93.33%) and P10 (93.33%) diets have similar values and this was significantly higher ($p < 0.05$) than those fed P20 (80%) and P30 (86.67%) diets. The carcass proximate composition (%) of sea bass fed control and various test diets is shown in Table 4.

The protein content of fish fed P20 and P30 diets were 18.79% and 18.26% respectively, and was significantly lower ($p < 0.05$) than those fed P0 (21.15%) and P10 (19.26%) diets. The final group of sea bass fed each diet showed higher protein (18.26-21.15%) and lipid (4.16-4.85%) content for the whole body composition after feeding. The lipid content (4.85%) was highest in the control diet group as compared to those fed on the green pea meal diets (4.16-4.56%). P30 group had the highest phosphorus content while P20 group had the lowest as compared with control group.

Table 4

Whole body proximate composition (%) of sea bass fed the various test diets[a]

	Dry matter	*Crude protein*	*Crude lipid*	*Ash*	*Phosphorus*
Initial	23.03	13.56	3.96	4.15	0.79
P0	22.70	21.15 ^{a}	4.85^{a}	5.70^{a}	0.96^{b}
P10	22.30	19.26^{a}	4.16^{a}	5.68^{a}	0.94^{b}
P20	21.65	18.79^{b}	4.56^{a}	5.61^{a}	0.92^{b}
P30	20.29	18.26^{b}	4.18^{a}	5.80^{a}	1.02^{a}

[a]Means in the same column with different superscripts are significantly different at $p < 0.05$.

The peak time of phosphorus (PO_4) excretion for sea bass was monitored at every 2 h over the 8 h sampling period after feeding them with various experimental diets (Figure 1). Fish fed P0 diet had the phosphate excretion followed by fish fed P10 and P20 while P30 fish fed group was the lowest. The highest peak of phosphorus excretion for all the treatments was noticeable at 8-h after feeding.

Proximate analyses of GP (24.1% CP) as shown in Table 2 indicates that it can be used as an alternative protein source of protein to replace fishmeal (Borlongan et al 2003; Eusebio 1991) in practical diets for sea bass. In addition, the NFE (carbohydrate content) value for GP which was 57.2% could be an excellent source of energy and possibly reduce the feeding cost. The efficiency of GP as an ingredient had been already evaluated for non-ruminant animals particularly swine and poultry. Earlier work has confirmed that GP meal proved to be an acceptable ingredient for grower stage production of milkfish (Borlongan et al 2003) and juvenile *Penaeus indicus* (Eusebio & Coloso 1998) provided that their antinutritional components have been removed.

In summary, replacement of FM with GP meal beyond 10% in sea bass diet significantly reduced growth performance and survival while FCR was significantly increased (Table 3). Fish fed control diet (P0) had the highest WG (19.69 g) and SGR (1.91%/day) but this did not differ significantly ($p > 0.05$) with the WG of sea bass fed P10 diet (17.75 g). There were no significant differences between the WG of fish fed P20 and P30 diets, however, growth was significantly lower than that of the control fish. These results agree with those of Tacon & Akiyama (1997) who have reported that high levels of soybean meals in fish and marine shrimp diets resulted in poor growth and lower feed conversion efficiency. The lower WG and SGR for sea bass fed diets at 20%

inclusion level of GP or higher might have been attributed to the nutritional imbalance of amino acid content in the diets. Studies with sea bass have also reported some success to partial replacing of FM by plant protein sources (Tibaldi et al 2006). The low survival (%) of fish fed diets with increasing levels of GP at P20 (80%) and P30 (86.67%) respectively, might have been due to stress encountered during fish sampling. Replacement of FM with high levels of legume seeds, *Vigna catiang* (cowpea) and *Phaseolus mungo* (black gram) in diets of juvenile *Oreochromis niloticus*, caused significant decrease in growth performance (Keembiyehetty & De Silva 1993). According to Liener (1979) legume seeds contain anti-nutritional factors such as trypsin inhibitor which inhibit enzyme trypsin during proteolytic digestion. PER decreased while FCR increased with increasing dietary levels of GP beyond 10% replacement for FM. Furthermore, it was noted that the reduction in feed intake of approximately 8.3% and 14.0% in fish fed P20 and P30 diets respectively as compared to those fish fed P10 diet was attributed to reduced palatability. It has been shown that in fish fed P20 and P30 diets some fish refused to accept the diets and eventually became susceptible to cannibalism by those individuals that would accept the diet and became physically larger. Previous studies of Choi et al (2004) have found that olive flounder could utilize diet at 30% replacement of FM protein with soybean supplemented with amino acids and attractants. Hajen et al (1993) also found a reduction in feed intake when chinook salmon (*Oncorhynchus tshawytscha*) fry were fed diets containing soybean meal at 15% and 30% of the diet.

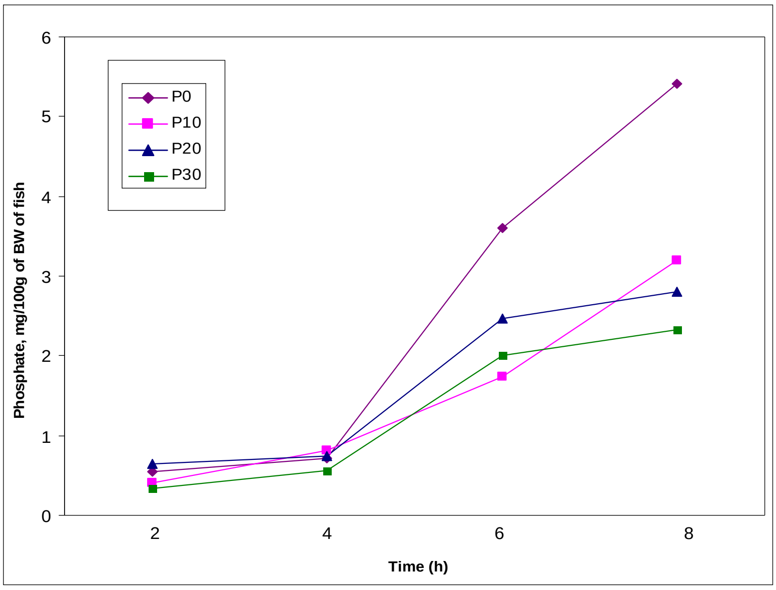

Figure 1. Phosphorus excretion of sea bass at every 2-h over an 8-h sampling with different experimental diets.

The whole body composition as shown in Table 4 indicated that fish fed control diet (PO) had the highest crude protein (21.15%) which was comparable with P10 diet (19.26%) but significantly higher than those fed P20 (18.79%) and P30 (18.26%) diets. Crude ash and lipid did not differ significantly ($p > 0.05$) among the various treatments. The phosphorus content of fish fed P30 diet (1.02) was significantly higher than those of other diets.

Results on phosphorus excretion of fish over the 8-h sampling period showed that fish fed P0 control diet exhibited the highest phosphate concentration (mg PO_4-P/100 g of fish), followed by P10, P20 and P30 diets at 54.12, 32.01, 28.19 and 23.36 respectively (Figure 1). Lall (1991) reported that FM has the richest source of phosphorus among the commercial feedstuffs used in feed formulation. The differences in the phosphorus levels of the various experimental diets were greatly contributed by fish meal.

Conclusions. The results of the present study indicate that among the different dietary levels of GP evaluated, the 10% level of dietary inclusión shows the best potential for replacing fish meal in diets for sea bass. When substituting the GP for 20 to 30% of the fish meal, diet palatability was reduced and growth of the fish were significantly decreased. However, the growth and feed efficiency for sea bass could have been possibly improved if practical diets with high levels of GP (20 to 30%) was supplemented with essential amino acids such as lysine, methionine, cystine and also addition of attractants. Sea bass appears to be a promising species for aquaculture due to its good growth rates, ease of handling and it can readily accept formulated feeds.

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