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Growth response and feed intake of *Lates* calcarifer to four different dietary protein levels with green pea (*Pisum sativum*) under controlled laboratory condition

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Abstract. Asian sea bass, Lates calcarifer is strictly a carnivorous fish and has been considered a potential prospect for commercial culture due to its rapid growth rate, high market demand and ability to accept formulated feeds. Protein is the most expensive dietary component, and feeds represent about 50 % of operating costs in the intensification for the sea bass culture. A 75-day feeding trial was conducted with juvenile fish (initial mean weight = 0.62 g) at 10 % body weight to evaluate the effect of dietary protein levels on the growth response, feed intake and survival rate of sea bass. Four experimental diets were formulated to be isolipidic and isoenergetic with increasing levels of dietary protein from 35, 40, 45 and 50 %. The basal formulation contained animal and plant protein sources primarily from Danish fish meal, defatted soybean meal, shrimp meal, squid meal and green pea (Pisum sativum) meal, a non-conventional plant protein source which was incorporated in diets at 6.9 - 11.0 % of the total protein. The best growth and feed conversion ratio (FCR) were observed with sea bass fed diets containing 40 % crude protein. However the percentage weight gain was not significantly different from the group fed the 45 to 50 % protein diet. The whole body composition of sea bass was not significantly different among the dietary treatments. The highest hepatosomatic index (HSI) of 0.59 % was observed in fish fed diets containing 40 and 50 % crude protein and this was comparable to sea bass fed with 45 % crude protein diet, but significantly higher than those fed 35 % crude protein diet. Survival rate at the end of the feeding trial were not significantly affected (P < 0.05) by the different levels of protein. Analyses of the water quality parameters such as salinity, temperature, DO, pH, ammonia and nitrite were within the suitable range conducive for the growth of sea bass. Results obtained in the present study indicate that the optimum protein level for growth and feed utilization efficiency for sea bass juveniles was estimated to be around 40 % protein.

Keywords: Lates calcarifer, protein, growth, feed intake, green pea.

Introduction. Asian sea bass, Lates calcarifer (Bloch 1790) are important marine food fish species in Southeast Asia and Australia owing to their high commercial value. They are mostly raised in floating net cages (Tacon & Rausin 1989), and had been cultured in brackishwater ponds for more than 20 years. Sea bass are locally known as apahap, bulgan, burgan, solong-solong and salungsungan in the Philippines, and in Australia the common name is Barramundi which means large-scaled fish (Johnston 1998; Rimmer & Russell 1998). It is one of the most highly desired marine fish species for commercial culture because of its high market potential, fast growth, euryhaline nature and their fry can be easily grown in hatchery (Copland & Grey 1986). This species is distributed mainly in the Indo-west from Arabian Gulf through Asia and raised in aquaculture in Australia (NSW Fisheries 1997), Malaysia, India, Indonesia, Philippines, Vietnam, Israel, Thailand, United States and Poland. Internationally, annual barramundi production has been relatively static since 1998, at approximately 20,000 - 27,000 tonnes with Thailand as the major producer at about 8,000 tonnes per annum since 2001 followed by Indonesia, Malaysia and Taiwan who are also considered as major producers. The global average price of farmed barramundi has been around 3.70 USD/kg, over the past 10 years. The Australian barramundi is established relatively with an annual production of more than 4,000 tonnes. Considerable research has been made in Australia, Thailand, Philippines

and more recently in Israel, in defining the nutritional requirements of sea bass in order to improve its production.

Nutritional studies has advanced in recent years due to the development of new, balanced commercial diets that support good growth and health of fish. Consequently, protein is the most expensive and is the major component in fish feeds. Therefore it is important to determine accurately the protein requirements for each species and size of cultured fish (Wilson 1989; Steffens 1989). Excess of protein in fish diets maybe wasteful and cause unnecessary expenses (Ahmad et al 2004). According to Boonyaratpalin & Williams (2001), feeds and feeding are the critical factors that determine the economic viability for the commercial production of sea bass. Sea bass as a carnivore specie requires diet with high content of protein and energy (Ambasankar et al 2009). Feed formulations for sea bass utilize marine fish resources and fish oils along with plant protein sources to meet its protein requirement for optimum growth (Boonyaratpalin 1997).

Feed constitutes one of the highest operating expenditure towards intensification and the use of fishmeal (FM) is estimated to be more than 50 % of the variable cost in sea bass feed (Woods 1999). However, due to the increased cost and demand for fish meal it is desirable to replace FM with less expensive alternative plant protein sources (Woods 1999; Tacon & Jackson 1985; Alliot et al 1979). Plant ingredients such as green pea may be utilized in the feed formulation for sea bass, and typically these legumes are common agricultural products in the Philippines. Previous studies with milkfish (*Chanos chanos*) (Borlongan et al 2003) indicated that inclusion level of plant protein source of *P. sativum* can replace up to 20 % of the total protein in the milkfish diets. Studies conducted by Lovell (1989) and Tacon (1990) reported that *P. sativum* contains several anti-nutritional factors such as trypsin and proteinase inhibitors which decrease protein digestibility (Hertrampf & Piedad-Pascual 2000). Carnivorous fishes are sensitive to proteinase inhibitors (Takii et al 1997; Krogdahl et al 2010). Legume seeds such as green pea can replace FM at the level of 10 % in sea bass diets without adverse effects on fish growth, feed utilization and body composition (Ganzon-Naret 2013).

Currently, there is still less research information about the nutritional requirements and feeds for sea bass despite their economic importance. Understanding the protein requirement of sea bass may lead to the enhancement of their growth and production. Therefore, the present study was conducted to evaluate the effect of different dietary protein levels with green pea on the growth response, feed intake and survival rate of sea bass under controlled laboratory condition.

Material and Method

Experimental fish and rearing conditions. Sea bass which were obtained from the finfish hatchery of SEAFDEC, AQD, Tigbauan, Iloilo, Philippines were transported to the UPV Hatchery in Miag-ao, Iloilo. Prior to the start of the feeding trial fish were placed in 1,000 L circular fiberglass basin and fed a commercial diet to adjust to the experimental conditions and then after 2 weeks the fish were hand-graded to a uniform size. Sea bass juveniles (mean weight = 0.62 g) were randomly distributed at a stocking rate of 10 fish per tank in three replicate tanks per treatment in 12 - 100 L capacity conical fiberglass tanks containing 90 L filtered aerated seawater. The tanks were connected in a semiclosed recirculating system provided with sand and biological filters with continuous aeration. Fish were fed three times daily at 08:30, 12:30 and 16:30 at a feeding rate of 10 % body weight per day for 75 days. Fish were weighed individually at the start of the feeding trial and every 15 days until the end of the experiment to calculate fish growth and feed utilization. Dead fish were removed and recorded daily during the experiment. The pellet residues and feces in each tank were siphoned daily to maintain good water quality under controlled laboratory condition.

Dissolved oxygen levels and water temperature were measured *in situ* with portable oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, OH, USA) daily, which ranged from 6.8-8.3 mg L⁻¹ and 26-32 0 C, respectively. The water pH ranged between 6.8-7.4 during the experiment and salinity ranged 30-33 ppt. Total

ammonia-nitrogen and nitrite-nitrogen concentrations of the water samples were monitored every two weeks as described by Strickland & Parsons (1972) and the measured water quality parameters were within the acceptable ranges for the fish growth (Boyd 1984).

Diet preparation. Four isolipidic and isoenergetic experimental diets were formulated to contain increasing levels of 35 %, 40 %, 45 % and 50 % crude protein. The basal formulation contained danish fish meal, soybean meal (defatted), squid meal and shrimp meal as dietary protein sources. Green pea (GP) was incorporated in diets at the level of 12 - 16 % which is equivalent at 6.9 - 11 % of the total protein. The whole dry pea was dried in the oven for 6 h at 60 °C and was processed to a fine powder. The pre-weighed dry ingredients were carefully mixed using the homogenizer with oils at a ratio 1:1 (cod liver oil : soybean oil) were then blended to the mixed dry ingredients. The wheat flour was cooked and subsequently added to the mixture after cooling to attain the desired consistency for pelleting. Each diet was then extruded in a meat grinder to form 2 mm pellets and dried in an air convection oven at 40 °C for moisture content less than 10 %. The resulting diets were stored in a refrigerator until use. The ingredients and proximate composition of the four experimental diets are presented in Table 1.

Table 1 Ingredients and proximate composition (g/100g dry matter) of experimental diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4			
Danish fish meal	23.00	27.00	32.00	36.00			
Soybean meal (defatted)	14.00	18.00	22.00	26.00			
Shrimp meal	5.00	5.00	5.00	5.00			
Squid meal	5.00	5.00	5.00	5.00			
Green pea	16.00	16.00	16.00	12.00			
Wheat flour	20.00	14.00	8.00	4.00			
Rice bran	6.00	4.00	1.00	1.00			
Soybean oil	2.50	2.50	2.50	2.50			
Cod liver oil	2.50	2.50	2.50	2.50			
Vitamin premix	3.00	3.00	3.00	3.00			
Mineral premix	3.00	3.00	3.00	3.00			
Proximate composition							
Protein	35.75	40.92	45.54	50.45			
Crude fat	9.22	9.26	9.31	9.37			
Nitrogen free extract	46.29	39.87	36.28	29.63			
Crude Fiber	2.52	3.21	1.62	2.73			
Ash	6.22	6.74	7.25	7.82			
Metabolizable energy (kcal/100 g) ¹	387.39	389.79	399.13	399.74			

¹Metabolizable energy was calculated based on the standard physiological values of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8 kcal/g fat (Brett & Groves 1979).

Chemical analysis. Experimental diets and feedstuffs were analyzed for proximate composition according to AOAC (1990). Dry matter was determined by oven-drying the samples to a constant weight at 105 °C. The nitrogen content was analyzed using a micro-Kjeldahl apparatus (Labconco Corporation, USA) and crude protein was determined by multiplying the nitrogen content by 6.25. Lipid extractions were undertaken by petroleum ether extraction in a Soxhlet extraction system. Ash was determined by combusting the dry samples in a muffle furnace at 550 °C for 12 h. Crude fiber was determined using a Fibretec System 1020 Hot Extractor. Ten fish were pooled from the initial population for the analysis of the whole body composition and after the feeding experiment, random sample of six fish from each tank were collected and frozen at -60 °C for subsequent proximate analyses.

Growth parameters and feed utilization. The growth performance of sea bass feed diets with different protein levels was evaluated by calculating the percentage weight gain (% WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), hepatosomatic index (HSI) and survival rate (%).

% WG = [(final weight (g) – initial weight (g)/initial weight (g)] \times 100

SGR (% day $^{-1}$) = [In final weight (g) – In initial weight (g)/no. of culture days] x 100

FCR = feed intake (g)/weight gain

PER = wet weight gain (g)/dietary protein intake (g)

HSI(%) = weight of liver (g)/ body weight (g) x 100

Survival rate (%) = No. of fish recovered/No. of fish stocked x 100

Statistical Analysis. The data were subjected to analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used as a *post hoc* test to compare between means at $P \le 0.05$. The SPSS version 16 software was performed for statistical analysis.

Results and Discussion. The growth responses and feed utilization efficiency of *L. calcarifer* with different protein level diets with green pea are shown in Table 2.

Table 2
Growth responses and feed utilization efficiency of *Lates calcarifer* fed diets containing various levels of dietary protein with green pea after 75 days of feeding*

Parameters	Diet				
Farameters -	1	2	3	4	
Initial weight (g)	0.62±0.02a	0.62±0.02a	0.62±0.02a	0.62±0.02a	
Final weight (g)	$14.01 \pm 0.012b$	17.89±0.019a	17.83±0.015a	17.85±0.017a	
Weight gain (%)	2159.67±1.2b	2785.48±3.8a	2775.81±2.7a	2779±2.85a	
SGR (%/day)	$1.81 \pm 0.17b$	1.94±0.21a	1.94±0.21a	1.94±0.21a	
Survival (%)	80	80	80	80	
HSI	$0.46 \pm 0.13b$	$0.59 \pm 0.43a$	$0.58 \pm 0.34a$	$0.59 \pm 0.25a$	
Feed intake (g/fish)	$31.46 \pm 11.03b$	$34.36 \pm 2.37a$	34.76±2.76a	$34.97 \pm 3.3a$	
FCR	$2.35 \pm 0.09a$	$1.99 \pm 0.12b$	$2.02 \pm 0.04b$	$2.03\pm0.039b$	
PER	$1.22 \pm 0.062b$	1.26±0.015a	$1.10 \pm 0.014c$	$0.99 \pm 0.018c$	

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

During feeding experiment sea bass readily accepted and consumed the experimental diets. The total feed intake values for fish receiving diets containing protein levels of 35, 40, 45 and 50 % were 31.46 \pm 11.03, 34.36 \pm 2.37, 34.76 \pm 2.76 and 34.97 \pm 3.3 g of feed respectively. The highest growth responses and feed utilization were found in fish offered diets containing 40 % protein, however growth rate expressed both as percentage of weight gain and SGR and HSI was not significantly different for fish fed diets containing 45 or 50 % protein. The poorest growth and feed conversion rate were recorded for the lowest dietary protein (35 %) level in the diet as compared to those fed diets containing 40 to 50 % protein. PER (1.26 \pm 0.015) was highest in 40 % protein diet and this was significantly different (P < 0.05) from the groups of fish fed diets containing 35, 45 and 50 % protein. Overall, sea bass fed with 16 % inclusion level of green pea in 35 % protein diet had significantly lower growth rates and higher FCR compared to other dietary treatments (P < 0.05). Moreover after 75 days of feeding, the survival rate (80 %) of fish fed different dietary treatments did not differ significantly (P > 0.05) from each other, hence not affected by the levels of protein.

The proximate analysis for the whole body composition of sea bass fed with different experimental diets is presented in Table 3. No significant differences were found in the proximate whole body composition of sea bass fed graded levels of dietary protein. The proximate analysis (%) indicated that the crude lipid content of the fish decreased with increasing dietary protein levels at the end of the experiment. Further, it was observed in the different diets containing green pea at 12 - 16 % did not affect the palatability, digestibility of the diets as well as the body composition of fish.

Table 3 Proximate analysis (%) of the body composition of sea bass fed experimental diets with different levels of protein^a

Diet ^b	Dry matter	Crude protein	Crude lipid	Ash
1	22.70 ^b	17.47 ^a	4.94 ^a	2.50 ^a
2	22.30 ^b	17.50 ^a	4.92^{a}	2.52 ^a
3	22.65 ^b	17.48 ^a	4.89^{a}	2.54 ^a
4	22.34 ^b	17.46 ^a	4.88^{a}	2.53 ^a
Initial	24.80 ^a	14.78 ^b	4.85 ^a	2.58 ^a

^a Means in the same column with different superscripts are significantly different at P<0.05.

Results from the present study showed that the dietary protein level at 40-50 % significantly influenced the growth performance and feed utilization of sea bass. The best growth response of sea bass was obtained at 40 % dietary protein level. The poorest growth in terms of weight gain (%) and specific growth rate for *L. calcarifer* were recorded for the lowest dietary protein (35 %) level in the diet after 75 days of feeding. The same trend was observed at protein efficiency ratio (PER) which was highest at 40 % dietary protein level. PER in the present study agrees with the previous studies conducted on tilapia species (Shiau & Huang 1989; Ahmad et al 2004) where PER values were significantly affected by protein level at 40 % (Table 2). Furthermore, it was observed that at protein level of 40 % (Table 2) there was a better protein utilization and was significantly higher (P < 0.05) from the rest of the experimental groups. The FCR decreased with increasing dietary protein levels and ranged from 1.99 to 2.03 (40 - 50 % protein diets) but 35 % crude protein (CP) diet showed significant increase of FCR = 2.35. The best FCR was recorded in the 40 % CP diet. No significant differences were observed in the PER and FCR values (40 – 50 %) of three diets.

There were no significant differences in the proximate whole body composition after the feeding experiment. Fish fed 40 % protein diet had higher crude protein content (17.50 %) in their carcass than did fish fed protein levels at 35 % (17.47 % CP), 45 % (17.48 % CP), and at 50 % (17.46 % CP) respectively, however no significant differences in the whole body samples were observed (Table 3). These results agree with those communicated by Lazo et al (1998), who have reported that with increasing dietary protein levels (30, 35, 40 or 45 % crude protein) in juvenile Florida pompano (*Trachinotus carolinus*) there was no significant differences in the biochemical analyses of the whole body samples. The crude lipid content of the whole body decreased as the protein content of diet increased.

The increase in dietary protein level had been associated with higher growth rates in carnivorous fishes such as discus (*Symphysodon spp.*) (Chong et al 2000); swordtail (*Xiphophorus helleri*) (Kruger et al 2001); rainbow trout (Tiews et al 1976); Arctic char (Tabacheck 1986); Atlantic salmon (*Salmo salar*) (Carter et al 2000) and red drum (*Sciaenops ocellatus*) (Jirsa et al 1997). The growth and feed utilization increased with dietary protein level and were highest for fish fed diet containing 40 % protein. Similarly, the results are in agreement with those studied in different carnivorous species that the best growth was observed at 40 % dietary protein level such as in rainbow trout, Atlantic salmon and red drum (Reigh & Ellis 1992; Olli et al 1995). For maximum growth and feed conversion, sea bass juveniles required a minimum dietary protein level of 40 %. Increases in dietary protein level have often been associated with higher growth rates in

^b Values represent means of three replicates.

many species, however the best growth, FCR and PER for sea bass were obtained using the diet containing 40 % protein. In the present study, the reduction of growth at higher protein levels between 45 and 50 % in sea bass in terms of its final weight and percentage weight gain may be caused by catabolism of excessive amino acid by the fish (Lloyd et al 1978; Jauncey 1982; Ramezani 2009). Similarly, other authors reported that decrease in weight gain of fish fed diets containing protein levels above the dietary requirement have been also observed in several other fish species (Chong et al 2000; Kim et al 2004; Ergiin et al 2010).

Overall results show that inclusion level of green pea at 12 - 16 % could be used as dietary protein source in sea bass diet without adverse effect on growth under controlled laboratory condition.

Conclusions. The results of the present study showed that improved growth response and feed utilization of juvenile sea bass was obtained when the fish fed on the 40 % CP diet with green pea under controlled laboratory condition. FCR increased as dietary protein content is increased at 40 - 50 % CP diet. However the FCR values (1.99 - 2.03) were significantly lower as compared to the values observed for fish fed 30 % CP diet (2.35). PER of fish decreased significantly with increasing dietary protein level at 45 - 50 % crude protein diet which had been attributed to changes in metabolism. Likewise, diets with low protein level at 40 % had a higher PER than those fed on the 35 %, 45 % or 50 % CP diets which could be linked to a better protein utilization.

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