Effects of different feeding rates of dehulled green peas on growth, survival and feed utilization of Asian sea bass juveniles, *Lates calcarifer*

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Abstract. Feeding rate is an essential factor in the culture of both marine and freshwater species and it is one of the major concerns for research in aquaculture. This 60-day feeding trial was conducted at the UPV Multi-Species Hatchery in order to determine the optimal feeding rate for sea bass using dehulled green peas (DGP) at different levels at 4, 6, 8 and 10% body weight on the growth, survival and feed utilization of sea bass. Healthy juvenile with an average initial weight of 1.38±0.916 g were stocked in 12 conical fiberglass tanks (100 L capacity) at stocking density of 10 fish per tank in recirculating system. Test diet containing 20% DGP was formulated to be isonitrogenous and isolipidic (40% crude protein (CP) and 10% fat). Based on the results no significant differences (P>0.05) were observed on the growth performance, survival and feed utilization of sea bass on feeding rates up to 10%. The best feed conversion ratio (FCR) was observed in 6% (2.01±0.213) followed by 10% (2.09±0.832), and 8% (2.21±0.195); while fish fed at 4% feeding rate showed the poorer FCR. The highest survival rate (%) was recorded with fish fed on feeding rate at 10% but comparable to that of *L. calcarifer* fed at 4, 6 and 8%. Throughout the feeding trial, water temperature ranged between 25.6-29.9^{0}C, pH 6.0-8.2, dissolved oxygen 6.6-8.2 mg L^{-1}. Total ammonia and nitrite-nitrogen values ranged from 0.23-0.28 mg/L to 0.05 - 0.054 mg/L respectively. Cannibalism was observed in all the experimental groups 16.66–23.33% due to the presence of shooters in the population. Results suggest that the optimum feeding rate for *L. calcarifer* juveniles was 6% body weight/day with experimental diet containing 20% inclusion of DGP.
Key Words: anti-nutritional factors, cannibalism, parasites, fish meal, processing, feed conversion ratio, survival, water quality.

Introduction. Sea bass (*Lates calcarifer*) belonging to the family *Centropomidae* is one of the most important food fish in the Philippines. According to Sodikin (1986) *L. calcarifer* is a potential cultivable species because of its ability to tolerate environmental stress, has fast growth rate and good quality white meat. *L. calcarifer* is widely cultured in Australia and Southeast Asia, however the source of fingerling supply is not reliable, hence the availability of this fish species is primarily based on the hatchery production (FAO/SCSP 1982). Hatchery development and commercial production of *L. calcarifer* is an important indicator of a progressive aquaculture development in the Philippines (Lopez 2006). Generally *L. calcarifer* are highly cannibalistic due to the presence of shooters observed during the latter part of the hatchery phase (Parazo et al 1991; Singh et al 2005) and they are also euryhaline carnivorous fish (Barlow et al 1996; Singh 2000). They can be fed with artificial diet and rough fish and most of its seed supply is hatchery produced for the improved farming system. Since fish production can be increased through sustainable intensification, feed represents the largest single cost item with fishmeal (FM) as the major protein source in the current feed formulation (FAO 1983). However, due to the escalating cost of FM, several researchers had shifted using non-conventional protein sources to partially or completely replace FM in aquaculture feeds (Hardy 1996). Therefore, in order to reduce feed cost, several investigators have considered feeding rate as an important factor that affects the growth of fish and minimize feed wastes (Qin & Fast 1996; Dong-Fang et al 2003; Marimuthu et al 2011; El-

Saidy et al 2015). There are several factors that affect the feeding rate in the culture system such as the fish size, species, water quality and the types of feeds used (Cho et al 2003). Among legumes, pea seed (*Pisum sativum* L.) has become widely available as low cost protein source for aquatic feed (Hernandez Arias et al 2010). According to Alonso et al (1998) the utilization of this legume has been limited by its low protein digestibility, essential amino acid deficiency and the presence of certain anti-nutritional factors (ANFs). Among these ANFs are condensed tannins, phytic acids, protease inhibitors (trypsin and chymotrypsintrypsin), polyphenols and lectins which reduce the nutritive value of protein (Dvorak et al 2005). However, peas have shown to be of good protein source for rainbow trout (Thiessen et al 2003); Atlantic salmon (Aslaksen et al 2007); European sea bass (Gouveia & Davies 2000); juvenile tiger shrimps (Bautista-Teruel et al 2003) and for milkfish (Borlongan et al 2003). Dehulling is one of the most efficient conventional processing methods employed in the present study that reduces the ANFs in green peas through the mechanical removal of its seed coats or testa. Therefore, the objective of this study was to evaluate the effects of different feeding rates on the growth, survival and feed utilization of *L. calcarifer* fed with diet containing dehulled green peas (20%) in 60-day feeding trial.

Material and Method. This study was a sequel to the previous study conducted at the Wet Laboratory of the Institute of Aquaculture regarding the utilization of raw, dehulled, autoclaved and soaked peas in *L. calcarifer* diets. The raw mature green peas were processed by dehulling using corn mill to separate the seed coats or its testa from their cotyledons. The ingredient was ground into a powder form, sieved through a 60 mesh sieve and dried in the air convection oven at 60^{o}C to about 10% moisture content. The proximate chemical composition of dehulled green peas was analyzed at the Centralized Analytical Lab of SEAFDEC Aquaculture Department following the AOAC (2000).

Experimental diet. The basal diet (g/100 g diet) contained fish meal (32.63 g), shrimp meal (3.0 g), squid meal (2.0 g) as animal protein sources with soybean meal and *Gracilaria* sp. at 14.0 and 3.0 g respectively. The amount of breadflour was adjusted at 12.27 g when dehulled green pea (DGP) was added at a level of 20.0 g. Lipid sources such as cod liver oil together with corn oil was mixed at a ratio of 1:1 while vitamin and mineral mixtures were added at 2.0 g each with butylated hydroxytoluene (BHT) which acts as anti-oxidant at 0.5 g/100 g diet. The experimental diet was formulated to contain 40% crude protein (CP) and 10% lipid. The proximate composition of the experimental diet was determined according to AOAC methods (2000).

Fish and feeding trial. The feeding trial was conducted from February 10 – April 9, 2018 at the Multi-Species Hatchery, Institute of Aquaculture, College of Fisheries & Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo. *L. calcarifer* juveniles were first procured at Trapiche, Oton to be used for the feeding trial. However, after one week of acclimatization, mass mortalities occurred and fish were observed to have an abnormal swimming behavior and becoming darker in color. Live specimens were brought to the Fish Health Section, SEAFDEC, AQD, Tigbauan, Iloilo for diagnosis of Viral Nervous Necrosis (VNN) using brain tissue samples processed and analyzed with the use of nested polymerase chain reaction (PCR) IQ 2000^{TM} VNN Detection and Prevention System (GeneReach Biotechnology Corp. Taiwan). The fish were found positive (+) for VNN and the stocks were discarded immediately. Disinfection of equipment, tanks, nets, siphon and hoses were done using 50 mg L^{-1} of sodium hypochlorite for 30 min to 1 h at room temperature to prevent from contamination. Another batch of *L. calcarifer* juveniles were procured and transported from SEAFDEC to Miag-ao, Iloilo. Fish were subjected to parasitological examination as shown in Figure 1 and microbiological diagnosis. Healthy juveniles were then acclimated to laboratory condition for two weeks using commercial feed and slowly weaned to DGP diet. Periodical size grading of fish was done in order to get rid of cannibalism and obtain maximum survival rate and growth. Twelve 100-L culture tanks in a recirculating system were stocked each with 10 fish with an average initial weight of 1.38±0.916 g. The

experimental diet was fed to triplicate groups thrice daily (09:00, 13:00 and 17:00) at different feeding rates of 4, 6, 8 and 10% body weight during the 60-day feeding trial. Feces and uneaten feeds were siphoned and about 50% of the water volume was replaced daily with filtered aerated seawater. Throughout the feeding trial, water temperature ranged between 25.6-29.9^{0}C determined by a thermometer; pH 6.0-8.2 was determined with pH meter; dissolved oxygen level at 6.6-8.2 mg L^{-1} with the use of YSI DO meter. Total ammonia and nitrite-nitrogen values ranged from 0.23-0.28 mg/L to 0.05-0.054 mg/L respectively following the procedures as described in Strickland & Parsons (1972). Fish were weighed every 15 days to adjust the feeding ration.

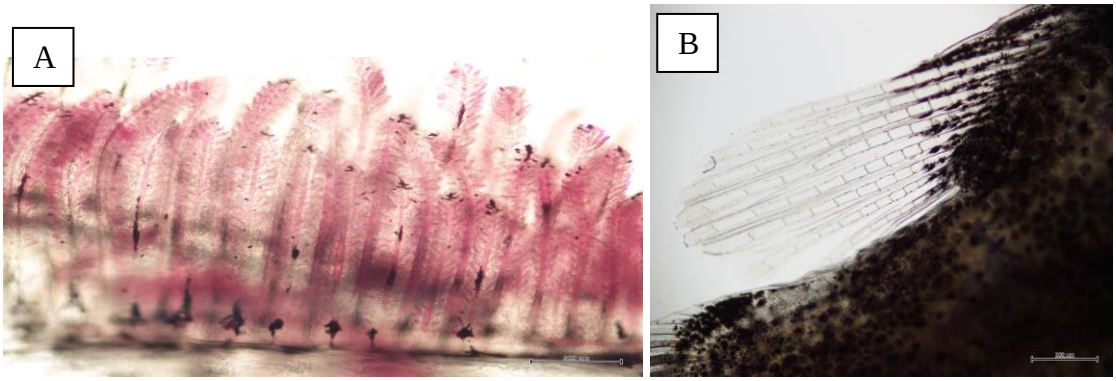


Figure 1. Gills (A) and body (B) of sea bass juveniles are negative for parasites (original).

Analysis. In the feeding trial, the DGP diet was fed to four groups of fish at different feeding rates by a completely randomized design (CRD). Differences among dietary treatments were tested by one-way ANOVA. The data were analyzed on the SPSS Version 18 and survival rate was performed on the arcsin square root transformation. Differences between mean values were compared by Duncan's Multiple Range Test (DMRT) to determine significant differences among treatments ($P<0.05$). The growth and feed utilization of *L. calcarifer* in relation to the different dietary treatments were determined by percentage weigh gain (final weight – initial weight) x 100/initial weight; specific growth rate (SGR % day) [(In final weight- In initial weight) time^{-1} (days) x 100)]; feed conversion ratio (FCR) = (total dry weight of feed (g)/total wet weight gain (g). Survival rate (%) was determined using the formula: 100 x (final count of fish/initial count of fish). Cannibalism was calculated by monitoring the difference in fish numbers between each count, reduced by number of mortalities as described by Qin & Fast (1996).

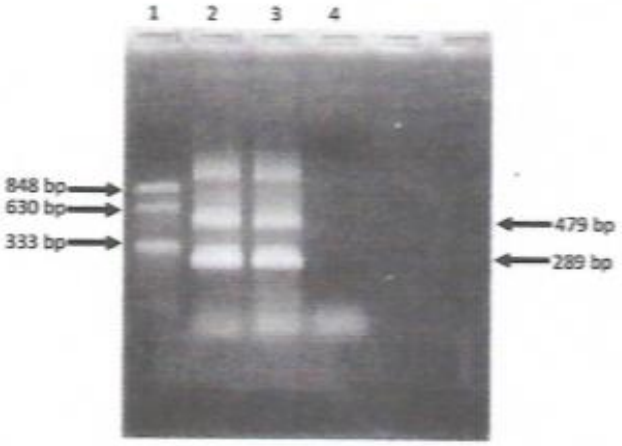
Results and Discussion. Parasitological and microbiological examination of *L. calcarifer* showed that their gills and body (Figure 1) were free from parasites as well as the lumninous bacterial count was recorded to be 0 at 10^{-1} CFU/mL. Samples of brain tissues were removed from the live specimens and analyzed for the Viral Nervous Necrosis (VNN) using the nested PCR as given in Figure 2. The proximate chemical analysis of dehulled green peas showed moisture content of 10.69%, crude protein of 22.31%, crude fat, 1.08%, crude fiber 1.23%, ash 3.16% and nitrogen free extract (NFE) of 72.27%. The crude fiber content of the dehulled pea sample had a lower value; this is because of the removal of the soluble and insoluble fiber present in the seed coats during the process of dehulling (Ramadan 2012; Ganzon-Naret 2018). Likewise, it was observed that the effect of dehulling improved the NFE content to 72.27%, this is attributed to the reduced level of fiber content in dehulled seeds. These observations are in agreement with the previous works conducted by Kerr et al (2000) on copeas and Raghuvanshi et al (2011) on mungbeans. The crude protein content of the dehulled sample was 22.31%.

Case Code: 17 F1-19 (17 PCRF-07)

Source: Trapiche, Oton

Species: *L. calcarifer*

Test for VNN using IQ2000 VNN Detection and Prevention System, GeneReach Biotechnology Corp., Taiwan



Lane #	Sample	Result of nested-step PCR
1	Marker	
2	Positive	+
3	SB	+
4	Negative control	-

Diagnostic Interpretation:

a. Band formed at 289 bp and/or 479 bp: **Positive (+)**

b. Band formed only at 665 bp: **Negative (-)**

c. Molecular weight marker: 848 bp, 630 bp and 333 bp

Figure 2. Analysis of the brain sample for the VNN using the nested PCR IQ2000^{TM}.

The result was in conformity with the studies conducted by Ghavidel & Prakash (2007) that both protein and digestibility increased following dehulling of cowpea, lentil and chickpeas which might be attributed to the removal of the hull portion of the seeds due to dehulling. The water quality parameters like temperature, pH dissolved oxygen (DO), ammonia (NH_3) nitrogen and nitrite were optimum for the growth of *L. calcarifer* during the feeding trial. The values obtained were comparable with the works of Rimmer & Russell (1998) on the culture of *L. calcarifer*. After the feeding trial (Table 1) the highest percentage weight gain (121.10%) was observed in *L. calcarifer* fed with 6% DGP diet followed by (104.80%) at 10%; (102.25%) at 8%, whereas the lowest value (84.32%) was recorded in 4% feeding rate. However, the different feed application rates did not significantly affect growth ($P<0.05$). In contrast to the previous works of several authors, it was reported that feeding rates vary according to species and their developmental stages. Fish species like *Oreochromis niloticus* in polyculture with *Cyprinus carpio* and *Hypophthalmichthys molitrix* and the larval rearing of hybrid red tilapia (*Oreochromis mossambicus x Oreochromis niloticus*) showed an increase in growth performance, feed utilization at a feeding rate of 5% (Abdelghany & Ahmad 2002; El-Saidy et al 2015);

Colossoma macropomum showed production performance at 10% feeding rate (Silva et al 2007) while daily feed application at 2% enhanced the growth performance of *Sander lucioperca* (Wang et al 2009).

Table 1

Effect of different feeding rate on the growth performance, feed utilization and survival of *Lates calcarifer* juveniles using artificial diet with dehulled green peas in a recirculating system

Feeding rate (%)	*Final weight (g)*	*Weight gain (%)*	*SGR (% day^{-1})*	*FCR*	*Survival (%)*	*Cannibalism (%)*
4%	2.54±0.1^{a}	84.32±7.2^{a}	1.02±0.1^{a}	2.46±0.2^{b}	76.67^{a}	23.33^{a}
6%	3.08±0.9^{a}	121.10±4.3^{a}	1.30±0.3^{a}	2.01±0.2^{a}	80.00^{a}	20.00^{a}
8%	2.80±0.6^{a}	102.25±3.9^{a}	1.16±0.2^{a}	2.21±0.2^{ab}	80.00^{a}	20.00^{a}
10%	2.81±.08^{a}	104.80±1.5^{a}	1.19±0.1^{a}	2.09±0.8^{a}	83.33^{a}	16.66^{a}

Mean values given in the same column having the same superscripts are not significantly different at ($p<0.05$). Each value is the mean of three replicates (SEM±T).

Harpaz et al (2005) reported that *L. calcarifer* reared in freshwater showed no improvement in growth performance when fed at 6% feeding rate while the optimum growth was obtained at 4% feeding rate. In the present study, the highest SGR (% day^{-1}) was observed in 6% feeding rate (1.30%) however no significant differences ($P<0.05$) were noticed among the different treatments. At lower feeding level of 2% the SGR was 1.02%. Several authors have reported that higher SGR was obtained for fish species fed with 7% body weight day^{-1} rather than with 3% body weight day^{-1} like channel catfish (*Ictalurus punctatus*), European seabass (*Dicentrarchus labrax*) and for cobia (*Rachycentron canadum*) according to Ng et al (2000), Eroldogan et al (2004) and Sun et al (2006) respectively.

FCR ranged from 2.01 to 2.46. In general, the best FCR among the different feeding rates was at 6% body weight day^{-1} (2.01) which differ significantly ($P<0.05$) from 4% feeding rate. Similarly, feed utilization parameters of groups of fish fed the feeding rates of 8% and 10% body weights exhibited the same trend. At lower feeding level at 4%, FCR was 2.46 and the growth rate of fish juveniles was 1.02. These results are in accordance with El-Saidy & Gaber (2005). Salama & Al-Harbi (2007) found that the FCR of fingerlings of *L. calcarifer* reared on hypersaline condition increased with increase in feeding rate while better FCR and PER were achieved with increase in feeding frequencies. According to Marian et al (1982) and Anderson & Fast (1991), feed ration greater than the optimum feeding rate as required by certain fish species would result in the food wastage/uneaten feeds with higher FCR and diminishes water quality. *L. calcarifer* juveniles showed increased survival rates (76.67-83.33%) with increasing feeding rates, these findings are in line with the results obtained by El-Sayed (2002) on *O. niloticus*, however they were not significantly different among the treatments. Cannibalism ranged from 16.66 to 23.33%. In the present study, it was observed that the level of feeding rates influenced the rate of cannibalism with respect to the size variation of fish. In several fish species, the heterogeneity in size often leads to social dominance resulting in cannibalism due to the aggressive behavior of fish (Fox 1975; Hecht & Appelbaum 1988).

Conclusions. The results obtained from the feeding trial suggest that from the nutritional point of view, the inclusion of dehulled green peas at 20% as replacement for fish meal in the artificial diet did not have adverse effect on the growth, feed utilization and survival of *L. calcarifer*. Dehulled green peas may be used successfully as protein source for *L. calcarifer* juveniles as long as supplementation of crystalline amino acids such as methionine and cysteine must be incorporated in the artificial diets when formulating feeds containing dehulled green peas. It can be concluded that artificial diet

containing 40% CP and 10% fat at 6% body weight day^{-1} is recommended for maximizing growth and feed utilization for *L. calcarifer*.

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