

Research Article

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Utilization of Raw, Dehulled, Autoclaved and Soaked Pea *Pisum Sativum* Seed Meals as Replacement for Fishmeal in Practical Diet Formulation for Juvenile Sea Bass in a Recirculating System

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Abstract

A 60-day feeding experiment on sea bass, *Lates calcariform* was conducted at the UPV Multi- Species Hatchery from September 23 - November 21, 2017. Healthy juveniles with an average initial weight of 0.09g were randomly stocked at twenty-five fish per tank at three replicates per treatment in fifteen 100-l conical tanks connected to a recirculating system under a photoperiod of 12h light and 12h dark. Five experimental diets were formulated to be isocaloric (40%) and isolipidic (10%) consisting of a Control Diet (without green pea); raw green pea, RGP (Diet 1); dehulled green pea, DGP (Diet 2); autoclaved green pea, AGP (Diet 3) and soaked green peas, SGP (Diet 4) to replace about 10-11% of the total protein in the diets. The percent weight gain (% WG) of sea bass fed control diet ($1544 \pm 2.60\%$) was significantly ($P < 0.05$) higher than diets with RGP, SGP and DGP, however fish fed AGP diets was not significantly different from those of the control group ($P > 0.05$). No significant difference ($P > 0.05$) was found in the specific growth rate (%SGR) among the groups of fish fed control, AGP and DGP diets. Similarly, the SGR were not also markedly different for fish fed DGP and SGP diets, nevertheless the values were the lowest for the RGP diets. The best feed conversion ratio (FCR) and protein efficiency ratio (PER) were observed in sea bass fed control diet at 1.74 and 1.88 respectively. The highest survival rate at the end of the 60-day feeding period was observed for DGP diet (85.33%) which was comparable to those obtained with the other diets (AGP, SGP and Control), however the value was lowest for RGP diet (73.33%). Based on these results FM based diets with AGP and DGP have similar effects on the growth performance and survival of juvenile sea bass, *L. calcariform*. It is quite clear that autoclaving and dehulling should be effectively used, not only for improving the nutritional quality of *P. sativum*, but also for reducing its anti-nutritional components as dietary protein source for sea bass.

Keywords: Green peas; Sea bass; Recirculating system; Dehulled peas; Growth

Introduction

Fish meal (FM) is the major component in fish diets due to its good amino acid profile, high digestibility and palatability. However due to its high demand and limited supply, FM resulted in a massive increase in prices, thus several investigators had to look for alternative plant protein sources for use by the fish growers in aquaculture industry [1-3]. Previous studies in fish had been carried out using plant protein components to partially or completely replace FM in for different fish species owing to its nutritional quality, availability and low cost [4-9].

Numerous studies have described peas as potential dietary protein ingredient in marine fishes and crustaceans such as European sea bass, *Dicentrarchus labrax* [10]; blue shrimp, *Litopenaeus stylirostris* [11]; juvenile pacific white shrimp, *Litopenaeus vannamei* [12]; milkfish, *Chanos-chanos* Forsskal [13]; juvenile tiger shrimp, *Penaeus monodon* [14] and in Atlantic salmon, *Salmo salar* [15]. Francis et al. [18] and Sharma et al. [17] reported the use of grain legumes as feed ingredients is limited due to the presence of trypsin inhibitors (TIA), phytic acid, tannins and

saponins which decrease the nutritive value of the legumes, thus reducing food intake and nutrient utilization in animals. In fact, several researchers had advocated the various processing methods such as heat treatment and other physical methods to eliminate the anti-nutritional components in legumes [18,19].

The objectives of this study were to process *P. Sativum* using the different physical and heat treatment methods in order to assess the same the use of these raw and processed green pea seed meals to partially replace fish meal as protein sources in formulated diets based on the growth performance and survival of sea bass in the recirculating system.

Materials and Methods

Preparation and processing of green pea seeds

Dry and mature seeds of green peas were obtained from the local market in Iloilo City, Philippines. The seeds were placed in trays made of bamboo splints and willowed using electric fan to remove the dusts and dirt's. They were kept in sealed plastic bags and stored in the refrigerator to prevent from molds and insect infestation. Three processing methods were employed for *P. sativum* seeds which include soaking, dehulling and autoclaving and was

carried out at the Wet Laboratory of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, UP Visayas, Miag-ao, Iloilo.

About 5kg of RGP seeds were soaked in distilled water at a ratio of 1:10 for 12 h at room temperature, dried and milled to pass through the 60-mesh sieve. Another portion of the seeds were dehulled using mortar and pestle then dried before milling. The third batch of seeds were spread in the aluminum trays at a depth of 1-2cm and heat treated in the autoclave at 15psi for 60 min. at 121°C. The samples were dried, cooled and milled to pass through a 60 mm sieve while the remaining portion of the whole unprocessed RGP seeds, about 5kg were milled into powder form.

Samples of raw and processed green pea meals were analyzed for proximate composition following the procedure as described by the Association of Official Analytical Chemists (AOAC 2000) as shown in Table 1. In the present study, the amino acid composition of the test ingredients (Table 2) was determined by hydrolyzing the 5.0 mg of protein sample using nor leucine as an internal standard added to the HCl and was carried out using the high-performance liquid chromatography (HPLC) and analyzed by the Feeds & Foods Nutrition Research Center at the Pukyong National University in Busan, South Korea.

Table 1: Proximate composition of raw and processed green pea seeds (g/100g DM)*.

Treatment	Crude Protein	Crude Fat	Crude Fiber	Ash	Moisture	NFE**
RGP	21.56±0.13 ^c	1.06±0.01 ^a	5.92±0.17 ^c	3.17±0.002 ^a	10.21±0.08 ^c	70.46±0.35 ^b
DGP	22.31±0.35 ^c	1.08±0.12 ^a	1.23±0.06 ^a	3.16±0.07 ^a	10.69±0.06 ^d	72.22±0.34 ^c
AGP	19.39±0.17 ^a	1.31±0.13 ^a	6.77±0.06 ^d	2.79±0.49 ^a	4.74±0.12 ^a	67.58±0.18 ^a
SGP	20.66±0.11 ^b	1.18±0.09 ^a	5.17±0.04 ^b	3.11±0.01 ^a	5.67±0.04 ^b	69.88±0.05 ^b

Mean of two replicate samples ± standard error of the mean (SEM). Treatment means within a column followed by different superscripts are significantly different (P<0.05).

** NFE – Nitrogen-free extract.

Table 2: Amino acid content (g/100 g dry weight) of raw and processed green pea meals as dietary protein sources.

Amino Acids	RGP	DGP	AGP	SGP
Arginine ^a	0.091	0.039	0.005	0.046
Lysine ^a	0.021	0.031	0.034	0.019
		4		
Histidine	0.001	0.004	0.01	0.003
Isoleucine ^a	0.002	0.001	0.007	0.005
Leucine ^a	0.001	0.001	0.015	0.006
Methionine ^a	0.001	0.001	0.003	0.002
Phenylalanine ^a	0.001	0.002	0.03	0.007
Threonine	0	0	0	0
Tryptophan	0	0	0.015	0
Valine	0.012	0.009	0.008	0
Aspartic acid	0	0	0	0
Glutamic acid	0	0	0	0
Serine	0	0	0	0
Proline	0.004	0.006	0.005	0.011
Glycine	0.002	0.003	0.003	0.006
Tyrosine	0.002	0.002	0.023	0.007

Cystine	0	0	0	0
Total	0.146	0.107	0.201	0.162

^aEssential amino acids for sea bass (Tibaldi et al. 1994; Baki et al. 2017) ingredients consisting of raw and processed seed meals were incorporated in the diets at 19.92-20.20% which is equivalent to 10.08 – 11.15% replacement of the total protein.

Table 3: Composition (g/100g diet) and proximate analyses (%) of experimental diets.

Ingredient	Control	Diet1	Diet2	Diet2	Diet4
Peruvian fishmeal	40	32.49	32.63	32.4	32.44
Soybean meal	14	14	14	14	14
Shrimp meal	3	3	3	3	3
Squid meal	2	2	2	2	2
Gracilariasp.	3	3	3	3	3
Rice bran	4.6	4.6	4.6	4.6	4.6
Green pea, raw	-	19.92	-	-	-
Green pea, dehulled	-	-	20	-	-
Green pea, autoclaved	-	-	-	20.8	-
Green pea, soaked	-	-	-	-	20.69
Bread flour	24.9	12.49	12.27	11.7	11.77
Corn oil	2	2	2	2	2
Cod liver oil	2	2	2	2	2
Vit.mix*	2	2	2	2	2
Mineral mix†	2	2	2	2	2
BHT	0.05	0.05	0.05	0.05	0.05
Proximate analyses	-	-	-	-	-
Moisture	4.8	4.2	3.76	3.89	4.6
Crude protein	40.18	41.12	39.99	40.52	39.88
Crude lipid	10.79	10.52	10.2	10.83	10.56
Ash	11.08	10.82	12.06	10.74	10.61

*ASA F2 Vitamin Feed Premix Powder

†ASA F1 Mineral Feed Powder Premix

Five experimental diets (Table 3) were formulated to be iso-nitrogenous and isolipidic (40% protein and 10% fat). The basal diet contained fish meal with equal amounts of shrimp meal, squid meal, soybean meal and Gracilaria sp. added into each diet. The test ingredients consisting of raw and processed seed meals were incorporated in the diets at 19.92-20.20% which is equivalent to 10.08 - 11.15% replacement of the total protein. Bread flour was used as a binder and the amount was adjusted when RGP, DGP, AGP and SGP were added to maintain similar dietary protein contents in the diets. The dry ingredients were mixed carefully prior to the addition of vitamin, mineral and BHT (Butyl hydroxytoluene). Corn oil and cod liver oil (1:1) were added to the mixture. Diets were prepared following the FDS Manual (1994) and the dry extruded pellets were placed in plastic containers and stored at 40C before feeding. Proximate analyses of experimental diets were determined by the standard methods as described by AOAC (2000).

Fish and experimental condition

The experiment was conducted in a recirculating system at the University of the Philippines Visayas Multi-Species Hatchery, Miagao, Iloilo. Juvenile sea bass were obtained from SEAFDEC,

Tigbauan, Iloilo. Fish were acclimated to the recirculation system at least 2 weeks before the trial. Healthy juveniles (Table 4) of similar sizes (initial body weight, IBW = 0.09±0.01 g) were randomly distributed at a stocking rate of 25 fish per tank into 15 -100L conical tanks. Aeration was supplied to each tank 24h daily.

Table 4: Microbiological and parasitological results of juvenile sea bass* used for the experiment.

A	Microbiological:		A. Bacterial count (CFU/ml)
Sample	Total Plate Count	Luminous Bacterial Count	Presumptive <i>Vibrio</i> Count
Sea bass	0 at 10 ¹	0 at 10 ¹	0 at 10 ¹
B	A. Parasitological: The whole body and gill of the fish samples were negative for parasites.		

*Live sea bass were transported to SEAFDEC Fish Health Section. for analyses.

Three tanks were randomly assigned to each diet group for a 60-day feeding experiment. Feed was given thrice daily (09:00; 13:00 and 16:00h) at a feeding rate of 25% and was adjusted every

15days. Seawater was monitored for its temperature of 25-28°C using a thermometer; dissolved oxygen was measured by YSI DO meter and it ranged from 6.5 to 7.4 ppm; pH of 7.3-7.7 and salinity which range from 28-32ppt were determined using the pH meter and refractometer (Atago) respectively. The values for ammonia – nitrogen (NH₃-N) and nitrite-nitrogen (NO₂-N) were measured from the water samples taken inside the tanks every 15 days and the values were within the ranges appropriate for the growth of sea bass. Uneaten feeds and feces were siphoned, and mortality was recorded daily. All the surviving fish in each tank were counted and group weighed using a precision balance to the nearest 0.001 g (Shimadzu, TXB 522L) every 15 days to adjust the feeding ration.

Calculation and Statistical analysis

Five experimental diets were used in the feeding experiment. The diet was tested in each tank at three replicates. All the data were analyzed by a one-way ANOVA for a completely randomized design (CRD) using a Statistical Analysis Software Program of SPSS Version 18. The Duncan's Multiple Range Test (DMRT) was used to determine the differences between the treatment means. Survival was computed using the arcs in square root. And results were considered significant at a level of $P < 0.05$. Growth and feed utilization parameters were calculated as follows:

$$\text{SGR } (\% \text{ day}^{-1}) = (\ln W_f - \ln W_i) \times 100 / T \text{ and}$$

$$\text{WG } (\%) = (W_f - W_i) / W_i \times 100$$

where W_f is the final weight (g), W_i is the initial weight and T is the culture period (days).

$$\text{FCR} = \text{dry feed intake} / \text{wet weight gain}$$

$$\text{PER} = \text{weight gain} / \text{protein intake.}$$

$$\text{Survival } (\%) = \text{Final number of fish} / \text{Initial number of fish} \times 100.$$

Results

The proximate composition of raw and processed green pea seeds are presented in Table 1. The moisture contents between

the raw and processed peas were significantly different ($P < 0.05$) among treatments. Autoclaving and soaking significantly ($P < 0.05$) decreased the moisture content at 4.74 and 5.67% respectively. It was noted that the ash content of AGP and SGP were considerably low which ranged from 2.79 - 3.11% as compared with DGP and RGP which ranged from 3.16 - 3.17%, however no significant ($P > 0.05$) differences were observed among the treatments. The crude protein level for DGP was significantly higher ($P < 0.05$) as compared with two other treatments at 19.39% and 20.66 % for AGP and SGP respectively and was not significantly ($P > 0.05$) different from the value obtained for RGP (21.56%). The highest crude fat (1.31%) was obtained in AGP and was not significantly ($P > 0.05$) different among the RGP, DGP and SGP values. Based on the crude fiber content of raw and processed pea samples, DGP (1.23) showed the lowest significant ($P < 0.05$) value followed by RGP and SGP with the highest value obtained for AGP (6.77). Among the processing methods such as autoclaving and soaking, dehulling significantly ($P < 0.05$) improved the NFE of GPs in the present study.

Table 2 shows the amino acid composition of raw and processed GPs. The total free amino acids of the given four GPs samples ranged from 0.107 - 0.201 g/100 g dry weight. Also, the essential amino acid content of all test GP samples was generally low, except for the arginine content in RGP which was 0.091 mg/100g, however the value was not significantly ($P > 0.05$) different from DGP, AGP and SGP at 0.039, 0.005 and 0.046 mg/100g respectively. The proximate analyses of control and experimental diets (Diets 1-4) is shown in Table 3. All diets were formulated to be isonitrogenous and isolipidic (40% protein and 10% fat). Results showed that crude protein ranged from 39.88 to 41.12% while the values for crude fat were 10.20 to 10.83%.

At the start of the feeding trial juvenile sea bass were analyzed for microbiological and parasitological examination at the Fish Health Section, SEAFDEC, AQD, Tigbauan and the samples were found to be free from bacterial diseases and parasites (Table 4). Table 5 shows the growth response and survival of sea bass. after 60 days of culture period.

Table 5: Growth and survival of juvenile *L. calcarifer* fed diets containing protein from raw and processed GPs for 60days in a recirculating system (initial weight of 0.09 ± 0.01 g)*.

Diet No.	Final Mean Weight (g)	Weight gain (%)	SGR (%)	FCR	PER	Survival (%)
Control	1.48 ± 0.23^c	1544.44 ± 2.6^c	4.65 ± 0.3^c	1.74 ± 0.2^a	1.88 ± 0.04^b	82.7 ± 6.1^b
Diet 1	0.85 ± 0.11^a	848.15 ± 1.2^a	3.74 ± 0.22^a	3.41 ± 0.2^c	1.37 ± 0.05^a	73.3 ± 4.6^a
Diet 2	1.18 ± 0.14^b	1211.11 ± 1.5^b	4.21 ± 0.11^b	1.86 ± 1.3^a	1.85 ± 0.04^b	85.3 ± 8.3^b
Diet 3	1.41 ± 0.28^c	1470.37 ± 3.1^c	4.57 ± 0.36^c	1.81 ± 0.1^a	1.87 ± 0.05^b	84.0 ± 4.0^b
Diet 4	1.11 ± 0.19^b	1103.70 ± 1.8^b	4.17 ± 0.29^b	1.93 ± 0.2^b	1.82 ± 0.06^b	84.0 ± 8.0^b

*Means of three replicate samples. Values in the same row with different superscripts are not significantly different ($P > 0.05$).

Discussion

The highest moisture content was observed for the DGP (10.69%), which was consistent with the findings obtained for the dehulled raw mungbean flour at 10.14% [20]. It was also apparent

that the moisture content in the AGP was significantly decreased as a result of processing the seeds at high temperature of 210°C for 60 min. Thermal processing may improve the nutritional value and reduce the level of anti-nutritional components. in GP meals.

Results of the proximate analyses showed that protein content of DGP was significantly higher from those values obtained in SGP and AGP. These results are comparable with the findings of [21,22] in horsegram seeds [23] found that dehulling significantly improved the protein digestibility of green gram, cowpea, lentil and chickpeas at 2.2, 5.1, 13.2 and 16.7% respectively, which could be explained due to the removal of hull portion and concentration of endosperm after dehulling. It was noted that the NFE content in the dehulled sample was significantly higher among the various samples, this can be attributed possibly due to the removal of the hulls.

The result indicates that the different processing methods did not affect the fat content of green peas which is due to the insoluble nature of fat. These observations are in agreement with those reported by [24] in lentils (*Lens culinaris*) and [25] for lima beans (*Phaseolus lunatus*).

Data presented in Table 2 shows the amino acid composition of raw and processed green peas. Green pea's protein contains relatively low concentration of essential and non-essential amino acids as compared to the different varieties of *P. sativum* tested by [27] in Asia and with the FAO/WHO Reference Pattern [26]. There were no significant changes in the free amino acids among all the GP samples. Like other legumes, *P. sativum* seeds are deficient in sulphur containing amino acids (methionine and cysteine) and to a lesser extent tryptophan and threonine which was similar to the results obtained by [28] in *Phaseolus* seeds. Dehulling and autoclaving caused a slight increase in lysine, whereas there was no effect ($P>0.05$) on the availability of arginine.

In the present study, the growth performance in terms of final weight, weight gain (%) and SGR of fish fed Diet 3 was comparable to those fish fed the Control Diet without green peas. This could be the result that green peas which were incorporated at 20.80% as shown in Table 3 were heat-treated in an autoclave at 121°C for 15 psi for 60 min which eliminated the condensed tannin content prior to its addition in sea bass diets. These results conform with the reports of [29] on the mirror carp fed diets containing 10-30% heated *Vicia peregrina* seeds. The presence of tannin lowers the nutritive value and reduces the biological availability of proteins, carbohydrates, amino acids, vitamins and minerals in legumes [30,31]. The growth of seabass offered the raw green pea-based diet (Diet 1) had significantly lower growth performance and this can be attributed possibly to the presence of growth inhibiting substances present in raw peas. This observation is also consistent with the findings of [13] for milkfish. In this study, processing such as dehulling and soaking had no significant effect on the growth and survival of *L. calcariform*, this may be due on the variety of peas used, although the nutritional value of green peas was slightly improved. Diet 1 containing raw green peas resulted in significantly poorer feed utilization for fish as evidenced with the values obtained for the FCR and PER as compared with the other treatments, this may be attributed to the presence of anti-nutrients in the unprocessed meal sample added to the diet. Akanje et al [32] found that the FCE and PER were markedly reduced in broiler

chickens fed with raw cowpea. Although green peas are considered to contain anti-nutritive factors, the survival rate is high using the different processed peas (Diets 2-4) in the formulation for sea bass diets [33-36]. Fish accepted the diets readily without adverse effects on the growth performance.

This study demonstrated that processed GP meals can partially replace fish meal as a protein source up to 20% in diets with no adverse effects on the growth performance and survival of *L. calcariform*. It is also recommended that *P. sativum* should always be heat-treated (autoclave) to retain its nutritive value and enhance the feed utilization in fish. The anti-nutritional factors present in the raw green peas were responsible for the poorest growth and feed intake during the feeding experiment. Considering that legumes contain relatively low amino acids, therefore raw and processed green pea meals incorporated in sea bass diets should be supplemented with essential amino acids if the total replacement of protein is more than 10%.

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Conflict of Interest

No conflict of interest.

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