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# Use of raw and heat-treated mung bean seeds (*Phaseolus aureus)* as replacement for soybean meal protein in the diets for sea bass, *Lates calcarifer* fingerlings in tanks: effects on growth performance, nutrient utilization and survival rate

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**Abstract**. Four practical diets containing crude protein (CP) at 40% with or without raw and heat-treated mung bean (*Phaseolus aureus*) seed meals were formulated to replace 30% of soybean meal (SBM) protein in the diets for juvenile sea bass ( initial body weight = 1.04 g) during a 60-day feeding trial. The basal diet formulation contained Peruvian fish meal, shrimp meal, squid meal with defatted soybean meal as the major plant protein source. Diet 1, the control (CTRL) contained 32% SBM; Diet 2 raw mung beans (RwMB) contained 22.40% SBM with 17.63% RwMB; Diet 3 boiled mung beans (BoMB) contained 22.40%SBM with 18.32% BoMB; and Diet 4 autoclaved mung beans (AcMB) contained 22.40% SBM with 18.38% AcMB. Fish of similar sizes were randomly distributed into twelve 100 L conical fiberglass tanks with filtered aerated seawater, and each tank was stocked with 15 fish per treatment at three replicates. The best growth response and feed conversion ratio (FCR) were observed with sea bass fed Diet 4 (8.45 ± 0.4 g; 2.02) followed by Diet 3 (8.33 ± 0.2 g; 2.06); Diet 1 (8.26 ± 0.3 g; 2.08) and Diet 2 (6.59 ± 0.2 g; 2.44) groups. Similar results were also obtained for specific growth rate (SGR) and protein efficiency ratio (PER). Diet 4 (AcMB), Diet 3 (BoMB) and Diet 1 (CTRL) groups had comparable SGR (1.50 - 1.52% $day^{-1}$) and PER (2.02 - 2.08) values, but significantly ($P < 0.05$) higher than that of Diet 2, (RwMB) with SGR (1.34% $day^{-1}$) and PER (1.02) values respectively. The whole body composition of sea bass fed the various diets was not significantly ($P > 0.05$) different from each other. During the experimental period, seawater temperature ranged from 26 – 29 $^0$C, ph of 7.30 - 7.75, dissolved oxygen of 6.8 - 7.6mg $L^{-1}$, salinity of 29 – 32 ppt, ammonia-nitrogen ($NH_3$-N) of 0.02 - 0.035mg $L^{-1}$ while nitrite-nitrogen ($NO_2$-N) values ranged from 0.015 – 0.019mg $L^{-1}$. Survival rate (86.67%) of fish was not significantly ($P > 0.05$) affected by the different dietary treatments. No pathological and nutritional deficiency signs were observed other than growth depression in fish fed raw mung bean (Diet 2) at the end of the feeding trial. In conclusion, under the experimental condition, the results obtained showed that boiled and autoclaved mung bean seed–meal based diets were comparable to those fish fed on the control diet in improving the growth, feed consumption and survival rates, however significantly ($P < 0.05$) higher from those fish fed on Diet 2 (RwMB). The present findings suggest that boiled and autoclaved mung bean seed meals can be used as protein sources in practical diets to replace 30% of soybean meal protein without adversely affecting the growth of sea bass.
**Key Words**: plant protein source, growth rate, boiling, autoclaving, feed conversion ratio.

**Introduction**. Protein is the most expensive component and important nutrient in fish feeds since it plays a very important role in the synthesis of enzymes, hormones and antibodies. It is also essential for growth and development of fish and provides energy for maintenance (Keembiyehetty & Gatlin 1992; Lim 1997; Erglin et al 2010). According to Lim (2003) seabass (*Dicentrarchus labrax*) requires dietary protein requirement from 42-52% depending on fish size, protein quality, protein to energy ratio and feeding management. Several investigations in fish nutrition had been already reviewed and compiled previously by the National Research Council (NRC 1993). Feed is the most expensive operational cost for highly intensive fish culture which could be accounted for two-thirds of the variable cost (Jauncey 1982; Chebbaki et al 2010). Intensive production of sea bass depends heavily on the use of fish meal (FM) as protein source due to its high digestibility and palatability, high biological value, excellent essential amino acid and serving as an excellent source of fatty acids and energy (Thompson et al 2004; Glencross et al 2007; Ganzon-Naret 2013a; Mohanta et al 2013). High quality FM normally contains 60 to 72% crude protein by weight. Typical diets for fish may contain 25% to 42% total protein (www.thefish site.com/articles /200/the-benefits-of-fish-meal-in-aquaculture diet). However, due to the steady rise in cost and growing demand for FM, recent efforts have been directed to utilize inexpensive practical diets to minimize overhead production costs thereby increasing its profitability (Woods 1999). Several studies in marine fish and shrimps had been already investigated using soybean meal as alternative plant protein source to partially or completely replace FM in practical diets because of its high crude protein content, good amino acid profile, abundance and availability (Tacon & Akiyama 1997; Hertrampf & Piedad-Pascual 2000; Gatlin et al 2007; Morken et al 2012). The main draw back however in utilizing plant proteins is the presence of various endogenous anti-nutritional factors such as trypsin, chymotrypsin, amylase inhibitors, flatulence factors, saponins, gossypols and lectins which reduce the nutrient utilization and biological value in fish diets (Liener 1980; Savelkoul et al 1992; Storebakken et al 2000; Francis et al 2001). A naturally occurring anti-nutritional factor in uncooked soybeans is the heat-labile Kunitz-trypsin inhibitor that prevents the enzyme trypsin from breaking down dietary protein in the intestine of the animals (Liener 1994). In particular fat deposition in sea bass were shown to be affected when substantial levels of dietary FM were replaced by single plant protein source such as maize gluten or soy derivatives (Tulli et al 1999; Dias et al 2005; Messina et al 2007). It is well documented that other plant protein sources which were tested and formulated in aquatic feeds include cowpea, *Vigna catiang*, black gram, *Phaseolus* mungo seeds, cereal by products, aquatic plants, sunflower seed meal and sesame seed cake for tilapia, *Oreochromis niloticus* (De Silva & Gunasekera 1989; Keembiyehetty & de Silva 1993; El-Sayed 1999; Abdel-Hakim et al 2008), grain legumes in Australian silver perch, *Bidyanus bidyanus* (Booth et al 2001); rapeseed protein concentrate for gilthead sea bream, *Sparus aurata* (Kissil et al 2000); and various types of soybean products, sun flower seed meal, canola meal, green pea, *Pisum sativum* which had been already reported previously to replace FM in the diet of Asian sea bass (Boonyaratpalin et al 1998; Chebbaki et al 2010; Plaipetch & Yakupitiyage 2012; Ganzon-Naret 2013b). Mung beans (*Phaseolus aureus* synonym: *Vigna radiata*) belong to the family *"Leguminosae,"* also called *"Fabaceae,"* are mainly grown for edible seeds and also known as grain legumes (Hussain & Burhanddin 2011). Mung beans play an important role in human nutrition because they are rich source of nutrients with 23.7% crude protein (CP), crude fat (CF) of 1.9%, certain amount of minerals and vitamins and essential amino acids which are comparable with that of soybean and kidney beans (Deshpande 1992; El-Adawy 1996; Habibullah et al 2007). They are tropical legumes widely grown in Asia particularly in the Philippines, Thailand, India and Pakistan. Sprouted *Phaseolus aureus* contain vitamin C and they are rich in lysine. Mild processing techniques such as soaking, cooking and sprouting could improve the quality of mung beans by reducing their anti-nutritional factors such as raffinose, phytic acid and tannins while increasing the quantities of glucose, galactose, sucrose, folic acid and inorganic phosphorus (www.naturechoice.co. za/bio-friendly/beans-peas-legumes/mung-beans/). It is highly recommended that grain legumes should be subjected for heat treatment for the improvement of its nutritional quality, detoxify the seeds, enhanced the digestibility of dry matter, protein and lysine for its effective utilization (Van der Poel 1990; Wiryawan & Dingle 1998; Owusu et al 2002). Some earlier studies observed that application of heat treatment such as cooking (boiling and roasting, autoclaving) on grain legumes of *Phaseolus vulgaris, Vicia sativa seeds, Cajanus cajan* could reduce the anti-nutritional factors and inactivate the amount of trypsin inhibitors above 90% (Trugo et al 1990; Farran et al 2001; Ghadge et al 2008; Akande & Fabiyi 2010). Although there were studies carried out on the replacement of soybean meal protein by other plant protein sources in other marine finfishes, only limited knowledge is available on the nutritional value and the physiological effect of raw and heat-treated mung bean seed meals, *P. aureus* on the growth performance of Asian sea bass, *Lates calcarifer*.

Therefore, the aim of this study is to determine the effect of replacing soybean meal protein with raw and heat-treated mung bean seed meals on the growth performance, nutrient utilization and survival rate of juvenile sea bass reared in tanks.

## Material and Method

***Preparation and formulation of experimental* diets**. The proximate analysis and the amino acid composition (g/100 g sample) of raw and heat-treated mung bean seed meals are presented in Tables 1 & 2. The test material used in this study was mung bean (*Phaseolus aureus*) seeds grown in Iloilo Province, Iloilo, Philippines and obtained in the local market. A number of kilos of whole raw mung beans were washed to remove dirt and other impurities. Mung bean seeds were soaked in tap water at a ratio of 1:10 (v/w) for 10 h at room temperature ($24^0$C).

Table 1

Proximate analysis of raw and heat-treated mung bean seed meals (% dry weight)

| *Components* | *RwMB* | *BoMB* | *AcMB* |
|---|---|---|---|
| Dry matter | 93.43[a] | 93.40[a] | 93.42[a] |
| Crude protein | 23.96[a] | 23.06[b] | 22.98[b] |
| Crude lipid | 1.20[a] | 1.18[a] | 1.18[a] |
| Crude ash | 3.88[a] | 3.64[b] | 3.62[b] |
| Crude fiber | 3.28[a] | 3.16[a] | 3.26[a] |
| NFE* | 67.68[a] | 68.96[a] | 68.96[a] |

*Nitrogen free extract.

Table 2

Amino acid composition (g/100 g dry sample) of raw and heat-treated mung bean seed meals used during the 60-day feeding trial

| *Amino acids* | *RwMB* | *BoMB* | *AcMB* |
|---|---|---|---|
| Lysine | 4.16 | 4.08 | 4.01 |
| Histidine | 2.46 | 2.52 | 2.50 |
| Arginine | 6.35 | 6.28 | 6.38 |
| Aspartic acid | 13.45 | 13.69 | 13.68 |
| Threonine | 3.12 | 3.18 | 3.16 |
| Serine | 4.94 | 4.90 | 4.88 |
| Glutamic acid | 21.50 | 21.38 | 21.39 |
| Proline | 4.15 | 4.22 | 4.17 |
| Glycine | 4.22 | 4.31 | 4.27 |
| Alanine | 4.32 | 4.52 | 4.50 |
| Cystine | 0.72 | 0.74 | 0.68 |
| Valine | 5.30 | 5.28 | 5.33 |
| Methionine | 1.95 | 1.87 | 1.91 |
| Isoleucine | 4.74 | 4.54 | 4.40 |
| Leucine | 8.32 | 8.48 | 8.52 |
| Tyrosine | 3.22 | 3.19 | 3.15 |
| Phenylalanine | 5.64 | 5.68 | 5.70 |
| Tryptophan | 0.95 | 0.90 | 0.86 |

The seeds were then drained and rinsed six times with distilled water. After soaking, one-third (1/3) portion of the rinsed mung beans were mashed, dried in the oven and then ground into meal while two-thirds (2/3) portion of soaked mung beans were subjected further for heat-treatments such as boiling and autoclaving. The seeds were cooked in a beaker with tap water at $100^oC$ at a ratio of 1:10 (w/v). The water was allowed to boil on the hot plate before the seeds were added and the cooking time was 1 h until they became soft as felt between fingers. The cooked mung beans were then mashed, dried, ground to fine powder passing through a 60 mesh sieve and stored in a plastic container until use at $4^oC$. Another portion of the seeds were autoclaved for 30 minutes at 15 atmospheric pressure ($121^0C$) at a depth of 2 cm. The seeds were then processed, ground, and sieved and the powdered meal was stored ready for use.

Four isonitrogenous diets were formulated to contain 40% crude protein as presented in Table 3.

Table 3

Formulation and chemical proximate composition of experimental diets (g/100 g dry weight)

| *Ingredients* | *Diets* | | | |
|---|---|---|---|---|
| | *CTRL* | *RwMB* | *BoMB* | *AcMB* |
| Peruvian fish meal | 14.00 | 14.00 | 14.00 | 14.00 |
| Squid meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Shrimp meal | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean meal, defatted | 32.00 | 22.40 | 22.40 | 22.40 |
| Raw mung bean | - | 17.63 | - | - |
| Boiled mung bean | - | - | 18.32 | - |
| Autoclaved mung bean | - | - | - | 18.38 |
| Corn meal | 9.00 | 3.97 | 3.28 | 3.22 |
| Bread flour | 17.00 | 14.00 | 14.00 | 14.00 |
| Soybean oil | 2.00 | 2.00 | 2.00 | 2.00 |
| Cod liver oil | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin mix[1] | 2.00 | 2.00 | 2.00 | 2.00 |
| Mineral mix[2] | 2.00 | 2.00 | 2.00 | 2.00 |
| *Chemical analysis (%)* | | | | |
| Crude protein | $41.21^a$ | $40.44^b$ | $40.92^b$ | $40.38^b$ |
| Crude lipid | $7.86^a$ | $7.04^b$ | $7.23^b$ | $7.47^b$ |
| Crude ash | $6.30^a$ | $6.11^b$ | $6.08^b$ | $6.02^b$ |

[1]Each kg contain: Vitamin A – 1,200,000 IU; Vitamin $D_3$ – 200,000; Vitamin E – 20,000; Vitamin $B_1$ – 8,000 mg; Vitamin $B_2$ – 8,000 mg; Vitamin $B_6$ – 5,000 mg; Vitamin $B_{12}$ - 2,000 mg; Niacin – 40,000 mg; Calcium Panthothenate – 20,000 mg; Biotin 40 mg; Folic acid – 1,800 mg; Ethoxyquin – 500 mg.

[2]Each kg contain: iron – 40,000 mg; manganese – 10,000 mg; zinc – 40,000 mg; copper – 4,000 mg; iodine – 1,800 mg; cobalt – 20 mg; selenium – 200 mg.

The basal diet formulation contained Peruvian fish meal, shrimp meal, squid meal and with defatted soybean meal (SBM) as the major plant protein source. The CTRL diet (Diet 1) contained SBM without either the raw, boiled or autoclaved mung bean seed meals. Diet 2 contained raw mung bean seed meal (RwMB) replacing 30% of the SBM protein. Diet 3 contained boiled mung bean seed meal (BoMB) replacing 30% of the SBM protein while Diet 4 contained autoclaved mung bean seed meal (AcMB) replacing 30% of SBM protein. Prior to the addition of vitamin and mineral premix, dietary ingredients were ground into fine powder passing through the 60 mm mesh sieve, weighed and were homogeneously mixed for 15 min. Lipid sources such as soybean oil and cod liver oil were added to the mixture at a ratio of 1:1 while bread flour as a carbohydrate source was gelatinized in 600 mL water using a hotplate, allowed it to cool at room temperature before this was added to the mixture to produce a stiff dough. The dough was made to pass through the meat pelletizer to obtain a 2 mm pellet and then dried in the air convection oven at 60$^0$C, stored in plastic bags (Ziploc) and kept in the refrigerator at 4$^0$C. Crude protein, crude lipid, moisture and ash content of the mung bean seed meals and experimental diets were determined by standardized methods as described by the Association of Official Analytical Chemists (AOAC 1995). Moisture was determined by oven-drying the sample at 105$^o$C until the weight was constant. Crude protein content was analyzed by the Kjeldahl method using semi-automatic Kjeldahl System after acid digestion while crude lipid by ether-extraction method using Soxhlet. Ash content was determined by incinerating the samples at 550$^0$C in muffle furnace for 24 h. Amino acid analysis was determined by hydrolyzing the 5.0 mg of protein samples with 1.0 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110$^0$C for 22 h with Norleucine as an internal standard added to the HCl and analysis was carried out using the high performance liquid chromatography (HPLC). Based on the chemical proximate analyses (Table 3) the crude protein content of the diets ranged between 40.38 - 41.21%; crude lipid 7.04 - 7.86% and crude ash 6.02 - 6.30%.

***Experimental fish and condition***. The experiment was conducted in a recirculating sytem at the University of the Philippines Visayas Multi-Species Hatchery, Miag-ao, Iloilo, Philippines. Juvenile sea bass were obtained from SEAFDEC, Tigbauan, Iloilo, and were transported to the hatchery. Prior to the experiment, the fish were acclimatized in 1000 L circular tank for 2 weeks and fed CTRL diet twice daily. Hatchery-bred fish of similar sizes (initial weight of 1.04±0.01 g) were randomly distributed into 12 conical tanks (100 L) with filtered aerated seawater, and each tank was stocked with 15 fish. Each of the experimental diet was assigned to each tank at three replicates. Water was changed daily at 50% and uneaten feed and feces were siphoned to ensure good water quality. Fish in each treatment were fed to satiation thrice a day and the amount of feed was recorded. Seawater was monitored for its temperature of 26 - 29$^0$C; dissolved oxygen of 6.8 - 7.6 mg $L^{-1}$; values for pH and salinity ranged from 7.30 to 7.75 and 29 – 32 ppt respectively. Ammonia-nitrogen ($NH_3$-N) and nitrite nitrogen ($NO_2$-N) were analyzed following the method described by Strickland & Parsons (1972) every two weeks and the values were within the range of recommended level for farmed sea bass. Each tank was covered with a black plastic sheet to protect the fish from external stress and the photoperiod was maintained at 12 h light and 12h dark. All the fish in each tank were counted and weighed every 15 days to adjust the ration and the feeding trial lasted for 60 days.

***Chemical analyses for whole body composition***. At the end of the experiment, 10 fish were killed and pooled from each dietary group, homogenized and analyzed for the whole body composition. Crude protein, crude lipid, ash content and moisture in the whole body were determined by AOAC (1995).

***Calculations and statistical analysis***. The data obtained for growth performance, feed utilization and survival rate of fish were evaluated by calculating percentage weight gain (% WG), specific growth rate (SGR), feed conversion ratio FCR), protein efficiency ratio (PER) and survival rate (%) using the following formulae:

WG (%) = final weight (g) - initial weight (g)
SGR (% day$^{-1}$) = [(final weight (g) – initial weight (g)/time (days) x 100]
FCR = [total dry weight of feed (g)/total wet weight gain (g)]
PER = [wet weight gain (g)/protein intake (g)]
Survival rate (%) = No. of fish survived/No. of fish stocked x 100

All the data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used as a post hoc test to compare between means 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**. Growth, FCR, PER and survival rate of juvenile sea bass fed various experimental diets with and without raw and heat-treated mung bean seed meals after 60 days of feeding are summarized in Table 4.

Table 4

Effect of different experimental diets on growth performance, feed utilization and survival rate of juvenile sea bass

| *Parameters* | *Diets* | | | |
|---|---|---|---|---|
| | *CTRL* | *RwMB* | *BoMB* | *AcMB* |
| Initial body weight (g) | 1.04±0.01 | 1.04±0.01 | 1.04±0.01 | 1.04±0.01 |
| Final body weight (g) | 8.26±0.3[a] | 6.59±0.2[b] | 8.33±0.2[a] | 8.45±0.4[a] |
| WG | 7.22±0.72[a] | 5.55±1.02[b] | 7.29±0.11[a] | 7.41±0.08[a] |
| %WG | 826.00±22.7[a] | 659.00±7.8[b] | 833.00±11.5[a] | 845.00±17.0[a] |
| SGR (%/day) | 1.50±0.2[a] | 1.34±0.1[b] | 1.51±0.1[a] | 1.52±0.2[a] |
| FCR | 2.08[a] | 2.44[b] | 2.06[a] | 2.02[a] |
| PER | 1.20[b] | 1.02[c] | 1.21[b] | 1.24[a] |
| Feed intake (g $fish^{-1}$) | 15.02±0.38[a] | 13.54±0.2[b] | 15.02±0.34[a] | 14.97±0.38[a] |
| Survival rate (%) | 86.67±2.4[a] | 86.67±1.2[a] | 86.67±2.0[a] | 86.67±2.6[a] |

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

Heat processing such as autoclaving and boiling significantly (P < 0.05) reduced the protein and ash content in mung bean seeds as compared to raw mung beans (Table 1), however heat treatment did not have greater effect on the crude lipid. No significant (P > 0.05) differences were observed in crude protein or Nitrogen Free Extract (carbohydrate) among heat-treated mung beans. These observations are in agreement with those reported by Bau et al (1997) on soybeans. Several studies have confirmed that ordinary cooking and autoclaving caused a slight increase in protein content in two varieties of soybeans and further lowering of its phytic acid content (Igbedioh et al 1994; Ramadan 2012). Based on the results presented in Table 2, mung bean protein had relatively low sulfur containing amino acid (methionine) of 1.87, 1.91 and 1.95 (g/100 g sample) for BoMB, AcMB and RwMB respectively. The results confirmed those reported by Mubarak (2005) who found that cooking reduced the methionine content as compared with those in the raw mung bean. Also the effects of treatments such as boiling and autoclaving of mung beans tended to lower the concentrations of lysine (4.08 - 4.01 g) and tryptophan (0.90 - 0.86 g) as compared to the RwMB containing lysine and tryptophan of 4.16 and 0.95 g/100 g respectively. Coloso et al (1999) reported that juvenile sea bass requires arginine 3.6 g, lysine 4.4 g, methionine/cysteine 2.9 g and tryptophan of 0.5 g for their optimum growth. In the present study, the chemical proximate analyses (Table 3) of the experimental diets had crude protein of 40.38 - 41.21%, crude lipid (CL) of 7.04 - 7.86% and crude ash of 6.02 - 6.30%. Results of the proximate composition of the experimental diets showed that the protein levels of diets RwMB, BoMB and AcMB were not significantly different from each other, while that of the CTRL diet (Diet 1) was significantly (P < 0.05) higher than the other groups. Crude lipid and ash contents were significantly highest in the CTRL diet. All the experimental diets were consumed avidly by juvenile sea bass. The water quality parameters monitored during the feeding trial were all within the acceptable tolerant range recommended for sea bass in aquaculture (Blancheton 2000; Colt 2006; Roque d' Orbcastel et al 2010).

Results of the percentage weight gain (Table 4) was highest in AcMB seed-meal based diet (845±17%), but this value was not statistically (P > 0.05) significant than those of fish fed BoMB (833±11.5%) and CTRL (659±7.8%) diets, however significantly (P < 0.05) higher in the group of sea bass fed RwMB diet (659±7.8%). This suggests that heat-treated mung beans (BomB & AcMB) proved to be an acceptable ingredient replacing 30% of the SBM protein in the practical diets for juvenile sea bass. Raw MB may contain appreciable amount of tannin which might be associated with lower nutritive value and biological availability of proteins, carbohydrates, amino acids, vitamins and minerals (Desphande & Cheryan 1985; Makkar et al 1987). Noor et al (1980) also found that uncooked mung bean contained trypsin inhibitory activity of 24.1 - 27.7 TIU/mg protein which inhibits trypsin enzyme action during proteolytic digestion. The same trend was also observed for SGR in both heat-treated mung bean seeds and control diets however, this trend was more obvious in diet containing raw mung beans (1.34±0.1%) that could be attributed due to the presence of anti-nutritional factors in diets which could hamper the growth performance in fish and other monogastric animals (Dhurandhar & Chang 1990; Atienzo-Lazos et al 2011). Teguia et al (2003); Nergiz & Gokgoz (2007) noted that aqueous cooking increased the nutritive value of common beans and enhanced growth performance and nutrient utilization satisfactorily in non-ruminant animals. The best feed conversion ratio (FCR) and protein efficiency ratio (PER) were obtained in the AcMB (2.02; 1.24) fed group followed by BoMB (2.06; 1.21), CTRL (2.08; 1.20) and RwMB (2.44; 1.02) groups. The data revealed that the final survival rate (SR) of juvenile sea bass for 60 days was 86.67%, indicating that there were no significant (P > 0.05) differences in SR among all treatments

The data on the whole body proximate composition of seabass juveniles fed with different diets are shown in Table 5.

Table 5

Proximate composition (%) of sea bass juveniles fed different experimental diets

| *Experimental diets* | *Proximate composition (%)*[a] | | | |
|---|---|---|---|---|
| | *CTRL* | *RwMB* | *BoMB* | *AcMB* |
| Crude protein | 21.84[a] | 18.60[b] | 22.12[a] | 22.36[a] |
| Crude lipid | 7.58[b] | 7.08[c] | 7.72[b] | 8.12[a] |
| Crude ash | 6.15[a] | 6.06[b] | 6.24[a] | 6.38[a] |
| Moisture | 72.13 | 72.12 | 72.18 | 72.15 |

[a]Values within each row with the same superscripts are not significantly different (P > 0.05).

The crude protein, lipid and ash content in the whole body composition of juvenile sea bass were greatly influenced by inclusion of heat-treated and raw mung beans in the practical diets for sea bass. In this study, dietary levels of 18.38% AcMB and 18.32% BoMB replacing 30% of SBM protein in the diets had highest level of crude protein with values of 22.36% and 22.12% respectively. It showed that they were almost the same and higher than that of the CTRL diet with the value of 21.84%, this is an indication that heat-treated MB seeds were able to improve the whole body composition relatively to control. Lower protein content was observed in fish fed RwMB diet. This may have been due to the low digestibility and the decrease in feed consumption noted for this group. Mung beans boiled for 1 h at $100^0$C and autoclaving at 15 atmospheric pressure at 30 min at $121^0$C could be recommended to replace 30% of SBM protein in practical diets for sea bass.

**Conclusions**. The results of the present study showed that replacement of SBM protein at 30% by meals of raw mung beans induced a deteriorating effect on the growth performance and nutrient utilization of sea bass. The anti-nutritional factors (ANFs) present in the raw mung beans which were not properly deactivated are responsible for the poorest growth (% weight gain, SGR) and feed conversion ratio for sea bass during this feeding trial.

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## References


Abdel-Hakim N. F., Lashin M. E., Al-Azab A. A., Nazmi H. M., 2008 Effect of replacing soybean meal protein by other plant protein sources on growth performance and economical efficiency of mono sex Nile tilapia (*Oreochromis niloticus*) cultured in tanks. Proceedings 8th International Symposium on Tilapia in Aquaculture, held in Cairo International Convention Center, Cairo, Egypt, October 12-14, 2008, pp. 739-753.

Akande K. E., Fabiyi E. F., 2010 Effect of processing methods on some anti-nutritional factors in legume seeds for poultry feeding. Int J Poult Sci 9(10):996-1001.

Association of Official Analytical Chemists (AOAC) 1995 Official methods of analysis of Official Analytical Chemists International. 16th edn., Association of Official Analytical Chemists, Arlington, 1298 pp.

Atienzo-Lazos M., Delgado E., Ochoa-Martinez A., Aguilar-Palazuelos E., Martinez B. F., Ramirez-Wong B., Gallegos-Infante A., Medrano-Roldan H., Solis-Sotto A., 2011 Effect of moisture and temperature on the functional properties of composite flour extrudates from beans (*Phaseolus vulgaris*) and nixtamalized corn (*Zea mays*). J Anim Prod Adv 1(1):9-20.

Bau H., Villaume C., Nicolas J., Mejean L., 1997 Effect of germination on chemical composition, biochemical constituents and anti-nutritional factors of soybeans (*Glycine max*) seeds. J Sci Food Agric 73:1-9.

Blancheton J. P., 2000 Development in recirculating system for Mediterranean fish species. Aquac Eng 22:17-31.

Boonyaratpalin M., Suraneiranat P., Tunpibal T., 1998 Replacement of fishmeal with various types of soybean products in the diets for the Asian sea bass, *Lates calcarifer*. Aquaculture 161:67-78.

Booth M. A., Allan G. L., Frances J., Parkinson S., 2001 Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*. IV. Effects of dehulling and protein concentration on digestibility of grain legumes. Aquaculture 196:67-85.

Chebbaki K., Akharbach H., Talbaoul E., Abrehouch A., Aitali A., Sedki S., Ben Bani A., Idaomar M., 2010 Effect of fish meal replacement by protein sources on the extruded and pressed diet of European sea bass juvenile (*Dicentrarchus labrax*). Agriculture and Biology Journal of North America, pp. 704-710.

Coloso R. M., Murillo-Gurrea D. N., Borlongan I. G., Catacutan M. R., 1999 Sulphur amino acid requirement of juvenile Asian sea bass *Lates calcarifer*. J Appl Icthyol 15(2):54-58.

Colt J., 2006 Water quality requirement for reuse systems. Aquac Eng 34:143-156.

Deshpande S. S., 1992 Food legumes in human nutrition: a personal perspective. Crit Rev Food Sci Nutr 32:333-363.

Desphande S. S., Cheryan M., 1985 Evaluation of vanillin assay for tannin analysis of dry beans. J Food Sci 50:905-908.

De Silva S. S., Gunasekera R. S., 1989 Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diet on consumption, growth performance and carcass composition in *Oreochromis niloticus* (L.) fry. Aquaculture 80(1-2):121-133.

Dhurandhar N. V., Chang K. C. 1990 Effect of cooking in firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans *Phaseolus vulgaris*. J Food Sci 55(2):470-474.

Dias J., Alvarez M. J., Arzel J., Corraze G., Diez A., Bautista J. M., Kaushik S. J., 2005 Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp Biochem Physiol A Mol Integr Physiol 142(1):19-31.

El-Adawy T. A., 1996 Chemical, nutritional and functional properties of mungbean protein isolate and concentrate. Minufiya J Agric Res 21(3):657-672.
El-Sayed A. F., 1999 Alternative dietary protein sources for farmed tilapia, *Oreochromis spp.* Aquaculture 179(1-4):149-168.
Erglin S., Gilroy D., Tekesoglu H., Gilroy B., Celik I., Tekinay A. A., Bulut M., 2010 Optimum dietary protein level for Blue streak hap, *Labidochromis caeruleus*. Turkish J Fish Aquat Sci 10:27-31.
Farran M. T., Dakessian P. B., Darwish A. H., Uwayjan M. G., Dbouk H. K., Seliman F. T., Ashkarian V. M., 2001 Performance of broilers production and egg quality parameters of laying hens fed 60% raw and treated common vetch (*Vicia sativa*) seeds. Poult Sci 80:203-208.
Francis G., Makkar H. P. S., Becker K., 2001 Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197-227.
Gatlin D. M., Barrows F. T., Brown P., 2007 Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac Res 38:551-579.
Ganzon-Naret E. S., 2013a The potential use of legume-based diets supplemented with microbial phytase on the growth performance and feed efficiency of sea bass*, Lates calcarifer*. AACL Bioflux 6(5):453-463.
Ganzon-Naret E. S., 2013b The use of green pea (*Pisum sativum*) as alternative protein source for fish meal in diets for Asian sea bass, *Lates calcarifer*. AACL Bioflux 6(4): 399-406.
Ghadge P. N., Shewalkar S. V., Wankhede D. B., 2008 Effect of processing methods on qualities of instant whole legume: pigeon pea (*Cajanus cajan* L). Agricultural Engineering International: the CIGR E-journal Manuscript FP 004, vol. X, pp. 1-8.
Glencross B. D., Booth M., Allan G. L. 2007 A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquac Nutr 13:17-34.
Habibullah, Abbas M., Shah H. U., 2007 Proximate and Mineral composition of mung beans. Sarhad J Agric 23(2):463-466.
Hertrampf J. W., Piedad-Pascual F., 2000 Handbook on ingredients for aquaculture feeds. Kluwer Academic Publishers, The Netherlands, 573 pp.
Hussain I., Burhanddin M., 2011 Optimization of germination conditions for germinated mungbean flour by response surface methodology. Afr J Food Sci Technol 2(10):232-239.
Igbedioh S. O. Olugbemi K. T., Akpapunan M. A., 1994 Effect of processing methods on phytic acid level and some constituents in bambara groundnut (*Vigna subterranea*) and pigeon pea (*Cajanus cajan*). Food Chem 50:147-151.
Jauncey K., 1982 The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). Aquaculture 27:43-54.
Keembiyehetty C. N., Gatlin D. M. III, 1992 Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops x M. saxatilis*). Aquaculture 104:271-277.
Keembiyehetty C. N., De Silva S. S., 1993 Performance of juvenile *Oreochromis niloticus* (L.) reared on diets containing cowpea, *Vigna catiang*, and black gram, *Phaseolus* mungo, seeds. Aquaculture 112(2-3):207-215.
Kissil G. W., Lupatsch I., Higgs D. A., Hardy R. W., 2000 Dietary substitution of soy and rapeseed protein concentrate for fishmeal, and their effects on growth and nutrient utilization in gilthead seabream, *Sparus aurata* L. Aquac Res 31:595-601.
Liener I. E., 1980 Toxic constituents of plant foodstuffs. Academic Press, New York and London, 502 pp.
Liener I. E., 1994 Implications of anti-nutritional components in soybean foods. Crit Rev Food Sci Nutr 34:31-67.
Lim C., 1997 Replacement of marine animal protein with peanut meal in diets for juvenile White shrimp, *Penaeus vannamei*. Journal of Applied Aquaculture 7(3):67-78.

Lim C. E., 2003 Nutrient requirements of European sea bass (*Dicentrarchus labrax*) and Gilt-head sea bream (*Sparus aurata*). In United States Department of Agriculture (USDA) Technical Bulletin (www.ars.usda.gov/research/publications.htm?seq_no_115=157340)
Makkar H. P. S., Singh B., Dawra K. K., 1987 Tannin-nutrient interactions – a review. Int J Anim Sci 2:127-139.
Messina M., Tulli F., Messina C., Tibaldi E., 2007 Varying plant protein sources in the diet of sea bass *Dicentrarchus labrax* differently affects lipid metabolism and deposition. Ital J Anim Sci 6(Suppl. 1):806-808.
Mohanta K. D., Subramanian S., Korikanthimath V. S., 2013 Evaluation of different animal protein source in formulating the diets for blue gourami, *Trichogaster trichopterus* fingerlings. Aquaculture Research and Development 4(2):1-7.
Morken T., Kraugerud O. F., Sorensen M., Storebakken T., Hillestad M., Christiansen R., Overland M., 2012 Effects of feed processing conditions and acid salts on nutrient digestibility and physical quality of soy-based diets for Atlantic salmon (*Salmo salar*). Aquac Nutr 18:21-34.
Mubarak A. E., 2005 Nutritional composition and anti-nutritional factors of mungbean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chemistry 89:489-495.
Nergiz C., Gokgoz E., 2007 Effects of traditional cooking methods on some anti-nutrients and in vitro protein digestibility of dry bean varieties (*Phaseolus vulgaris* L.) grown in Turkey. Int J Food Sci Technol 42:868-873.
Noor M. I., Bressani R., Elias L. G., 1980 Changes in chemical and selected biochemical components, protein quality and digestibility of mungbean (*Vigna radiata*) during germination and cooking. Plant Foods Hum Nutr 30:135-144.
NRC (National Research Council) 1993 Nutrient requirements of fish. National Academy of Sciences, Washington D.C., 128 pp.
Owusu A. A., Baidoo S. K., Nyachati C. M. 2002 Effect of heat processing on nutrient digestibility in pea and supplementing amylase and xylanase to raw, extruded or micronized pea-based diets on performance of early-weaned pigs. Can J Anim Sci 82:367-374.
Plaipetch P., Yakupitiyage A., 2012 Use of yeast-fermented canola meal to replace fishmeal in the diet of Asian sea bass *Lates calcarifer* (Bloch, 1790). J Aquac Res Development 3:2 (http://dx.doi.org/10.4172/2155-9546.1000125).
Ramadan E. A., 2012 Effect of processing and cooking methods on the chemical composition, sugar and phytic acid of soybeans. Food and Public Health 2(1):11-15.
Roque d' Orbcastel E. R., Lemarie G., Breuil G., Petochi T., Marino G., Triplet S., Dutto G., Fivelstad S., Coeurdacier J. L., Blancheton J. P., 2010 Effects of rearing density on sea bass (*Dicentrarchus labrax*) biological performance, blood parameter and disease resistance in a flow through system. Aquat Living Resour 23:109-117.
Savelkoul F. H. M. G., Van Der Poel A. F. B., Tamminga S., 1992 The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibotors in legume seeds during germination. A review. Plant Foods for Human Nutrition 42(1):71-85.
Storebakken T., Refstie S., Ruyter B., 2000 Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Soy in animal nutrition. Drackley J. K. (ed), pp. 127-170, Federation of Animal Science Societies, Savoi, IL.
Strickland J. D. H., Parsons T. R., 1972 A Practical handbook of seawater analysis. Bulletin 167, Second Edition.
Tacon A. G. J., Akiyama D. M., 1997 Feed ingredients. In: Crustacean nutrition. Advances in World Aquaculture, 6: World Aquaculture Society, Baton Rouge, USA. D'Abramo L. R., Concklin D. E., Akiyama D. M. (eds), pp. 411-472.
Teguia A., Japou I. B., Kamsu E. C., 2003 Response of broiler chickens to *Vigna unguiculata* (L), Walp (cowpea) and *Phaseolus vulgaris* (black beans) and *Voandzeia cubterranean* (Bambara groundnut) as feed ingredients in replacement of meat meal. Anim Feed Sci 11:127-133.

Thompson K. R., Muzinic I. A., Engler I. S., Morton S., Webster C. D., 2004 Effects of feeding practical diets containing various protein levels on growth, survival, body composition, and processing traits of Australian red claw crayfish *Cherax quadricarinatus*, and on pond water quality. Aquac Res 35:659-668.
Trugo L. C., Ramos L. A., Trugo N. M. F., Souza M. C. P., 1990 Oligosaccharide composition and trypsin inhibitor activity of *Phaseolus vulgaris* and the effect of germination on the alpha–galactoside composition and fermentation in the human colon. Food Chemistry 36:53-61.
Tulli F., Tibaldi E., Commin A., 1999 Dietary protein sources differently affect plasma lipid levels and body fat deposition in juvenile sea bass. In: Piva G., Bertoni G., Satoh S., Bani P., Calamari L. (eds), Recent Progress in Animal Production Science: I. Proc. A.S.P.A. XIII Congress, Piacenza, Italy, pp. 782-784.
Van der Poel A. F. B., 1990 Effect of processing on anti-nutritional factors and protein nutritional value of dry beans (*Phaseolus vulgaris* L.*):* A Review. Anim Feed Sci Technol 29:179-208.
Wiryawan K. G., Dingle J. G., 1998 Recent research on improving the quality of grain legumes for chicken growth. Anim Feed Sci Technol 76:185-193.
Woods T. A., 1999 Largemouth bass – production budget. Commonwealth of Kentucky Aquaculture Plan, Frankfort, Kentucky, USA.
*** http://www.the fishsite.com/articles/200/the-benefits-of-fish-meal-in-aquaculturediet
*** http://www.naturechoice.co.za/bio-friendly/beans-peas-legumes/mungbeans/

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