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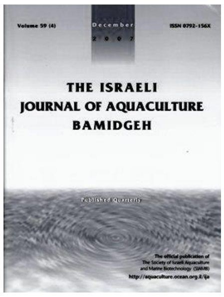
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2015.1237, 10 pages



Utilization of Mung Bean, *Vigna radiata* (Linnaeus) as a Novel Protein Source in Practical-Type Diets for Juvenile Milkfish, *Chanos chanos* (Forsskal): Effects on Growth, Feed Efficiency, Body Composition, and Histology of Gut and Liver

Mary Jane S. Apines-Amar[1]*, Relicardo M. Coloso[2], Ma. Novie G. Amar[1], Ma. Shirley M. Golez[1,3], Marj Gem B. Bunda[1], Cecilia J. Jaspe[1],

[1] *Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines*

[2] *Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines*

[3] *Present Address: Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines*

(Received 6.8.2015, Accepted 23.10.2015)

Key words: alternative protein source, fish, aqua feeds, feeding, legumes,

Abstract

A 15-week feeding trial was conducted to determine the optimum partial inclusion of mung bean protein in milkfish diet. Six isonitrogenous practical-type diets with mung bean included at 0%, 4%, 8%, 12%, 16%, and 20% of the diet equivalent to 0%, 3%, 7%, 10%, 13%, and 17% of the total dietary protein, respectively, were formulated. Milkfish with average body weight (ABW) of 8.5 ± 0.23g were distributed in eighteen tanks (6 treatments X 3 replications) with 10 fish each. The fish were fed the diets three times daily. Results showed that growth of milkfish was not adversely affected by the inclusion of mung bean protein at any dietary level. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly improved by the inclusion of mung bean at 20% of the diet. Nutrient compositions of the fish carcass were similar in all diets. Furthermore, no detrimental effects attributable to mung bean inclusion were seen in terms of protein retention, hepatosomatic index (HSI), and liver and midgut histology of the fish. Overall, mung bean is a promising protein source for milkfish and can be included up to 20% of the diet contributing as much as 17% of the total dietary protein without detrimental effects on growth, feed performance, PER, protein retention, HSI, and liver and intestinal histology.

* Corresponding author. Telefax: 6333-3158090 e-mail: janeamar09@gmail.com

Introduction

Milkfish, *Chanos chanos* (Forsskal) is farmed in brackish water ponds, freshwater pens, and in floating cages in marine and estuarine waters. In these culture systems, the success and sustainability of the industry is largely dependent on the availability of nutritionally adequate and inexpensive feeds. Feeds constitute about 60% of the operational cost and protein is the most expensive component. Fish meal has been the major protein source in aquaculture feeds because of its balanced amino acid and fatty acid profiles. However, due to the declining supply and rising cost of fish meal, alternative protein sources need to be identified to reduce the utilization and dependence on fish meal as the major protein source for aquaculture feed. Likewise, a stable supply of locally available protein sources could lead to the development of cheaper diets for sustainable aquaculture operations.

Owing to the dwindling supply of fish meal, plant-based protein sources have been increasingly utilized to partially replace fish meal in the diets of many farmed aquatic species. Practical diets for cultured species such as milkfish have been developed to encourage the use of cheaper alternative protein sources to partially replace fish meal in diet formulations for these species. The replacement of fish meal with plant protein sources without adverse effects on fish growth and physiology is a strategy to lower the cost of feeds and minimize dependence of aquafeeds on fish meal (Kissil and Lupatsch, 2004). It was reported that up to 67% of fish meal in the diet could be replaced by commercial hexane-extracted soybean meal with methionine supplement without detrimental effects on growth and FCR of milkfish (Shiau et al., 1988). Apart from soybean meal, many other locally-available plant protein sources can potentially be used to replace fish meal with equally good results. Leguminous plants such as mung bean, *Vigna radiata* (Linneaus) (De Silva and Anderson, 1995) and African Yam beans (Olafia and Bello, 2011) have been identified to be suitable protein sources for fish diets. Mung bean is a drought-resistant plant and is cultivated in many tropical countries. It contains high levels of protein and energy and has a good amino acid profile comparable to soybean, kidney pea meal, and to an FAO/WHO reference protein (El-Adawy, 1996). Green mung bean was used as replacement for fish meal in Asian sea bass (Eusebio and Coloso, 2000) and milkfish diets (De la Pena et al., 1987) without deleterious effects on growth of the fish. Nonetheless, like many plant protein sources, mung bean contains anti-nutritional factors making it unsuitable for direct inclusion at high levels without proper processing. Mung bean in particular contains protease inhibitor, phytic acid, saponin, anti-thiamine factor, and causes flatulence (Kay, 1979). Fortunately, most of these substances can be inactivated by heat treatment (Mubarak, 2005). Therefore, pre-treatment of mung bean before feed formulation is necessary to improve its protein utilization. This study was conducted to evaluate the efficacy of mung bean (*Vigna radiata*) and determine its optimum inclusion level as a potential alternative protein source for milkfish. To carry out these objectives, a 15-week feeding trial using practical-type diets was conducted to measure growth, survival, FCR, PER, HSI, and liver and gut histology. Practical-type diets were chosen so that the results could be easily be adopted by the aquaculture feed industry.

Materials and Methods

Experimental diets. Dried mature mung bean seeds purchased locally in Iloilo City, Philippines were pre-treated by oven-roasting at 100ºC for 1 hour, and maintained at 60ºC overnight. The seeds were subsequently ground into a fine powder and sieved through a 60 μm mesh screen before being mixed with the other ingredients for processing into feed pellets. The proximate analysis of the mung bean showed 26.68% crude protein (Table 1). Six isonitrogenous (32% crude protein) practical-type diets were formulated with mung bean protein included at 0%, 4%, 8%, 12%, 16%, and 20% of the diet equivalent to 0%, 3%, 7%, 10%, 13%, and 17% of the total dietary protein, respectively (Table 2). The control diet (1) did not contain any mung bean protein. The rest of the ingredients were the same for all diets. Methionine and threonine were supplemented to balance the dietary essential amino acid content based on the amino acid requirements of milkfish. Rice bran was used as filler.

Table 1. Nutrient composition of mung bean, *Vigna radiata*

Parameters	*Present study*	*Other studies*			
		Oburuoga &	*Paul et al., 2011*	*Agugo & Onimawo, 2009*	*Mubarak, 2005*
Moisture (%)	6.26	10.85	12.07	ND	*9.75*
Crude protein(%)	26.68	27.67	21.57	22.90	*27.50*
Crude fat (%)	0.12	1.75	1.53	1.43	*1.85*
Crude fiber (%)	4.38	4.34	0.63	8.95	*4.63*
Carbohydrates (%)	64.56	53.38	60.35	61.47	*62.30*
Ash (%)	4.26	3.30	3.85	3.34	*3.76*

ND - No data

Table 2. Formulation (g/kg) and proximate composition (%) of the

Diet #	*1*	*2*	*3*	*4*	*5*	*6*
Mung bean level (%)	*0*	*4*	*8*	*12*	*16*	*20*
Fish meal	185	185	185	185	185	185
Soybean	300	300	300	300	300	300
Copra meal	15	15	15	15	15	15
Mung bean [a]	0	40	80	120	160	200
Corn starch	220	220	220	225	225	225
Cod liver oil	31	31	31	31	31	31
Soybean oil	31	31	31	31	31	31
Vitamin mix [b]	5	5	5	5	5	5
Mineral mix [c]	2.5	2.5	2.5	2.5	2.5	2.5
Asc P	0.1	0.1	0.1	0.1	0.1	0.1
L-methionine	2.4	2.1	2.1	1.9	1.8	1.7
L-threonine	3.2	2.9	2.7	2.6	2.4	2.2
Rice bran	224.8	165.4	125.6	80.9	41.2	1.4
Proximate composition (%)						
Moisture	0.7	1.2	1.4	0.4	1.0	0.8
Crude protein	32.5	31.9	32.4	33.3	32.9	33.3
Crude fat	9.1	8.6	7.7	7.4	6.9	6.6
Crude fiber	2.7	2.3	2.7	2.8	2.0	2.3
Ash	7.2	7.4	7.2	7.1	6.9	6.6
Nitrogen free extract	49.0	49.0	49.0	49.0	50.3	50.3

[a] Purchased from a local dealer.
[b] ASA F2 Vitamin mix (unit/kg diet): Vitamin A-6,000 IU, Vitamin D3-1,000 IU, Vitamin E-100 IU, Vitamin B1-40 mg, Vitamin B2-40 mg, Vitamin B6-25 mg, Vitamin B12-10 mg, Niacin-200 mg, Ca-Pantothenate-100 mg, Biotin- 0.2 mg, Folic Acid-9 mg, Ethoxyquin-2.5mg.
[c] ASA F1 Mineral mix (unit/kg diet): Iron-200 mg, Manganese-50 mg, Zinc-200 mg, Copper-20 mg, Iodine-9 mg, Cobalt-0.1 mg, Selenium-1 mg.

Experimental fish, feeding, and rearing conditions. Milkfish juveniles purchased from a nearby fish farm in Leganes, Iloilo, Philippines (average weight 8.5 ± 0.23g) were used in the experiment. The fish were acclimatized under laboratory conditions for 2 weeks before the start of the feeding trial. One hundred eighty fish were randomly stocked at 10 fish each in 250-L capacity concrete circular tanks filled with sand-filtered seawater. Each dietary treatment was replicated 3 times. The feeding trial was conducted in a flow-through culture system for 15 weeks. The daily feed ration was divided into 3 equal parts given at 8:00AM, 12:00Noon, and 4:00PM initially at 6%, and later reduced to 3%, of the fish body weight. Although feeding rate was predetermined, feeding was stopped when the fish were satiated. Unconsumed feed was weighed and recorded to calculate actual food consumption. Fish were sampled for growth every 3 weeks by individually weighing all the fish from each tank. The feed ration was adjusted immediately after each sampling for growth. Water temperature, salinity, dissolved oxygen (DO), pH, ammonia, nitrite, and phosphorous were monitored regularly throughout the experiment and averaged 28ºC, 25g/L, 5.9 mg/L, 8.5, 0.4 mg/L, 0.1 mg/L, and 0.1 mg/L, respectively.

Growth and survival. At the end of the 15-week feeding trial, fish from each tank were counted and weighed individually to measure the differences in growth and survival between treatments using the following formulae:

Weight gain (WG) (%) = ((final weight –initial weight)/initial weight) x 100

Specific growth rate (SGR) = ((ln final weight – ln initial weight)/no. of days) x 100
Survival rate (%) = (final no. of fish/initial no. of fish) x 100

Feed consumption was calculated from the recorded accumulated daily feed consumed by each fish. Feed performance was measured based on feed conversion ratio (FCR) computed as follows:
Feed conversion ratio (FCR) = feed consumed, g (dry wt.)/weight gain, g (wet wt.)

Collection and analysis of samples. At the beginning of the experiment, 20 fish samples were taken at random from a pool of 250 fish for analysis of body composition, and five fish per tank (15 fish per diet group) were randomly taken as final samples for proximate analysis (moisture, ash, crude protein, crude fat, and crude fiber) at the end of the feeding trial. Feed and fish carcass proximate compositions were analyzed following the Official Methods of Analysis (AOAC, 1995). An additional 3 fish per tank (9 fish per diet) were collected for the measurement of hepatosomatic index (HSI), and for liver and intestine histology. The liver samples were excised and weighed for collection of HSI data. The whole liver together with the intestine (cut from the middle section between the stomach and the posterior intestine) were placed in 10% buffered formalin solution and sent to the histology laboratory for processing. Subsequently, the samples were dehydrated and embedded in paraffin following standard histological procedures. Sections were stained with hematoxylin and eosin and mounted on glass slides, and were examined with a light microscope (Motic Red 223) equipped with a digital camera (Moticam 10) and Motic Images Plus image analysis software. Images were taken and examined for the presence of histological abnormality in the liver and midgut tissues.

Hepatosomatic index (HSI), protein efficiency ratio (PER) and productive protein values (PPV) were computed as follows:
HSI = Weight of Liver (g)/Weight of Fish (g) x 100
PER = Weight gain (g)/Protein intake (g)
PPV = [final body protein (%) x final wt. (g) – initial body protein (%) x initial wt. (g)]
wt. gain (g) x total protein intake (g)

Statistical analysis. Results were analyzed statistically using SYSTAT, SPSS software. Data on weight gain, FCR, PER, PPV, and survival were analyzed by one-way ANOVA and the differences between dietary treatments were compared using Tukey's test. Values on survival were square root transformed and checked for normality before performing the statistical analysis. The growth trial results were analyzed with a General Linear Model. Results were considered statistically significant at P<0.05.

Results

Experimental feeds, fish growth and survival. The formulation and proximate composition of the experimental diets are presented in Table 2. Dietary protein contents were similar for all treatments and closely matched the formulated values. Dietary fat content decreased with increasing mung bean protein in the diet but all satisfied the fish requirements. Calculated values for essential amino acids (EAA) were similar for all diets and were within the required levels for milkfish except for isoleucine in Diets 1, 2, and 3, which were slightly lower than the requirement of milkfish (Table 3). As the amount of mung bean in the diet increased, dietary isoleucine in Diets 4, 5, and 6, became sufficient for the fish.

Table 3. Amino acid contents of the experimental diets (% protein) [a]

Mung bean level (%)	*0%*	*4%*	*8%*	*12%*	*16%*	*20%*
Essential						
Arginine	5.4	5.6	5.7	5.8	6	6.1
Histidine	2.4	2.4	2.5	2.6	2.6	2.7
Isoleucine	3.7	3.8	3.9	4.0	4.1	4.2
Leucine	6.4	6.6	6.8	7.0	7.2	7.4
Lysine	5.8	6.0	6.2	6.4	6.5	6.8
Methionine	2.8	2.7	2.7	2.7	2.7	2.7
Phenylalanine	4.2	4.3	4.5	4.6	4.8	4.9
Threonine	4.6	4.6	4.6	4.6	4.6	4.5
Tryptophan	0.6	0.6	0.7	0.7	0.8	0.8
Valine	4.1	4.3	4.4	4.6	4.7	4.8

[a] Calculated values based from the published EAA of protein sources: mung bean (Mubarak 2005); fish meal (SEAFDEC AQD, Iloilo, Philippines); soybean meal (Peñaflorida1989); copra meal (PCARRD 1984); rice bran (Yamazaki et al. 1988).

The growth of the fish and feed conversion ratio (FCR) were significantly improved in Diet 6 with mung bean supplied at 20% of the diet (Fig. 1; Table 4).

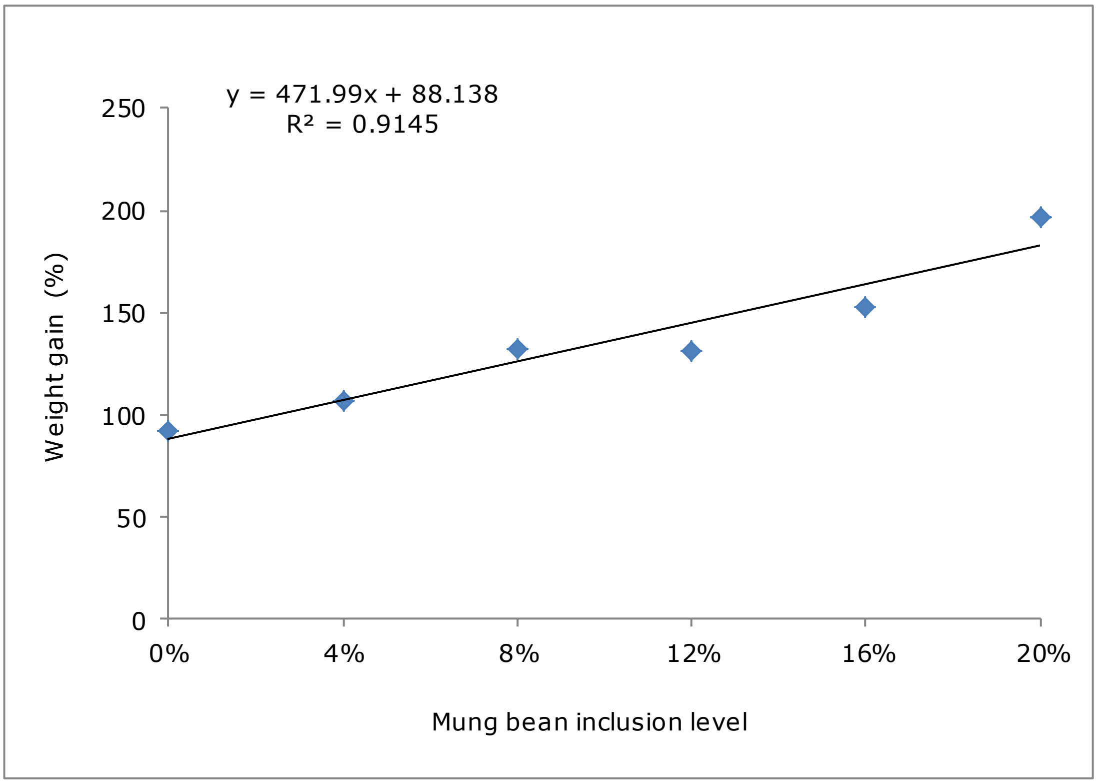


Fig. 1. Growth of milkfish fed the experimental diets for 15 weeks. Means (n=3) are significantly different (P<0.05).

Table 4. Growth, FCR and survival of milkfish fed the experimental diets for 15 weeks[a]

Mung bean level (%)	*Initial weight (g)*	*Final Weight (g)*	*Weight Gain (g)*	*SGR[b] (%/day)*	*FCR[c]*	*Survival (%)*
0	8.7±0.3 [a]	17.9±1.9 [b]	9.3±1.7 [b]	0.7±0.04 [b]	3.2±0.3[a]	83±2.7 [b]
4	8.7±0.4 [a]	19.9±2.7 [ab]	11.3±2.6 [ab]	0.8±0.06 [ab]	3.2±0.3[a]	87±3.3 [b]
8	8.6±0.4 [a]	19.9±2.07 [ab]	11.3±2.0 [ab]	0.8±0.05 [ab]	3.1±0.2[ab]	100±0.0 [a]
12	8.4±0.3 [a]	19.3±2.8 [ab]	11.0±2.2 [ab]	0.8±0.06 [ab]	3.2±0.4[a]	97±3.3 [a]
16	8.6±0.3 [a]	21.8±3.4 [ab]	13.2±3.4 [ab]	0.9±0.07 [ab]	2.8±0.3[ab]	83±3.05 [b]
20	8.4±0.2 [a]	26.2±2.0 [a]	17.9±1.7 [a]	1.1±0.03 [a]	2.7±0.1[b]	90±3.6[ab]

[a] Means±SE (n=3) within a column with different superscript letters are significantly different (P<0.05).
[b] Specific growth rate.
[c] Feed conversion ratio.

Neither survival of the fish (Table 4), nor nutrient composition of the fish carcass (Table 5) was affected by the treatments.

Table 5. Body composition (%) of milkfish after feeding the experimental diets for 15 weeks*

Diet # *Mung bean level*	*Diet 1* *0%*	*Diet 2* *4%*	*Diet 3* *8%*	*Diet 4* *12%*	*Diet 5* *16%*	*Diet 6* *20%*
Moisture	72.4±1.6 [ab]	74.0±1.4 [a]	73.1±1.1 [a]	69.8±1.2 [b]	71.6±1.7 [ab]	70.4±2.8 [ab]
Crude Protein	62.6±1.1 [a]	61.8±1.1 [a]	62.8±0.4 [a]	62.0±0.6 [a]	60.9±1.8 [a]	61.1±1.1 [a]
Crude Fat	19.9±1.0[a]	21.3±3.2[a]	20.5±1.5 [a]	21.7±1.4 [a]	23.1±3.6 [a]	22.0±2.0 [a]
Crude Fiber	0.7±0.17 [c]	0.7±0.3 [c]	0 .7±0.1[bc]	0.7±0.2 [b]	0.8±0.1 [b]	1.0±0.3 [a]
Ash	13.2±1.0 [a]	11.6±0.6 [ab]	11.8±0.1 [ab]	12.0±0.3 [ab]	10.2±1.8 [b]	10.4±0.7 [b]

*Means±SE (n=3) within a row with different superscript letters are significantly different (*P*<0.05).

Protein efficiency ratio (PER) increased as the level of dietary mung bean increased and was highest at 20% mung bean inclusion (Table 6).

Table 6. Hepatosomatic index, protein efficiency ratio, and protein productive value in milkfish fed the experimental diets for 15 weeks [a]

Mung bean level (%)	*HSI* [b]	*PER* [c]	*PPV* [d]
0	1.1 ± 0.08 [a]	0.6 ± 0.03[b]	2.8 ± 0.13 [a]
4	1.1 ± 0.11 [a]	0.7 ± 0.06 [b]	2.8 ± 0.20 [a]
8	1.1 ± 0.01 [a]	0.7 ± 0.05 [b]	2.9 ± 0.20 [a]
12	1.2 ± 0.17 [a]	0.7 ± 0.06 [b]	2.9 ± 0.07 [a]
16	1.1 ± 0.07 [a]	0.7 ± 0.07 [ab]	3.0 ± 0.23 [a]
20	1.1 ± 0.04 [a]	0.9 ± 0.02 [a]	3.0 ± 0.21 [a]

[a] Means±SE (n=3) within a column with different superscript letters are significantly different ($P<0.05$).
[b] Hepatosomatic index.
[c] Protein efficiency ratio.
[d] Protein productive value.

PER of diet 6 was significantly higher than the control and the other treatments except diet 5 (16% mung bean inclusion level). Protein retention as measured by the protein productive value (PPV) followed the same increasing pattern with dietary mung bean level but no significant differences were observed among and between diet groups (Table 6). Finally, the hepatosomatic index was unaffected showing similar values among treatments (Table 6).

Liver and intestinal morphology. Hematoxylin and Eosin (H&E) stained sections sampled at the end of the 15-week feeding trial showed no significant morphological changes in the liver and intestine of milkfish. The liver showed normal architecture with absence of discernible pathological changes in the hepatocytes, lobules, bile ducts, blood vessels, and sinusoids. No fatty infiltration, swelling, or necrosis of the liver was evident (Figs. 2A, B). Similarly, histological lesions such as hyperplasia, infiltration of inflammatory cells, stunting/fusion of villi, vacuolation, enteritis, or necrosis were not observed in the midgut epithelial tissues of any fish examined, including the mucosa, submucosa, the lamina propia, and the underlying soft muscle tissues (Figs. 2C, D).

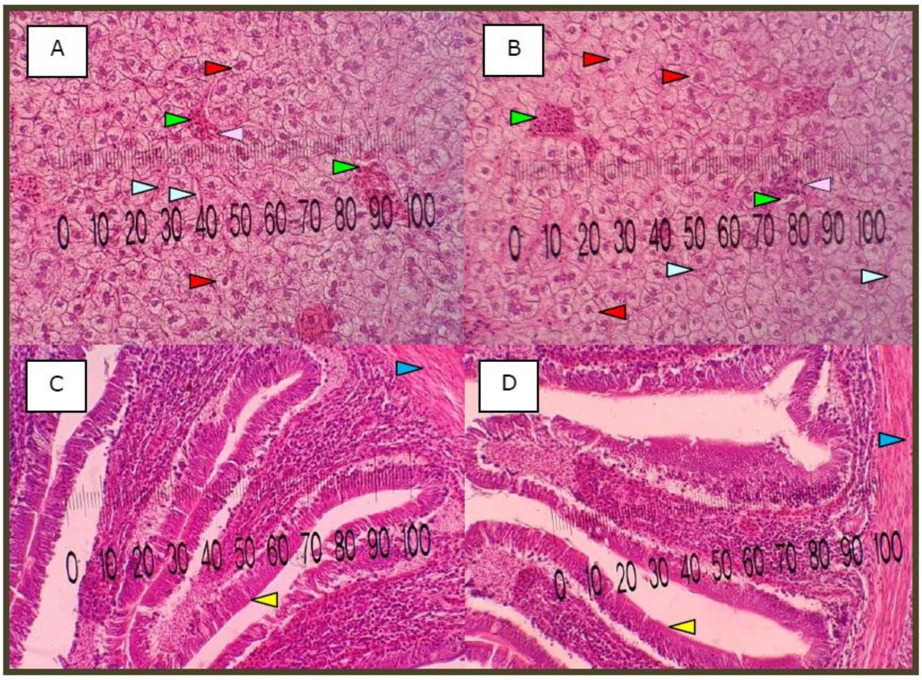


FIGURE 2. Histology of milkfish liver and intestine A) Liver (Control, 40x); B) Liver (20% Mung bean, 40x); ►hepatocytes, ►bile duct, ►portal vein, ►sinusoids C) Intestine (Control, 40x); D) Intestine (20% Mung bean, 40x); ►villi, ►submucosa after feeding the experimental diets for 15 weeks.

Discussion

This study clearly demonstrates that mung bean with a protein content of 26.68% is a promising dietary protein source for milkfish. Feedstuffs that contain at least 20% protein are considered potential protein sources (Allen and Arnold, 2000). Although mung bean fulfills this criterion, it contains antinutritional substances such as antitrypsin (Desphande et al., 1982) that hinder the digestibility of certain nutrients. The trypsin inhibitor in mung beans is similar to one of the two trypsin inhibitors found in black-eyed peas but different from that of soybeans (Chrispeels and Baumgartner, 1978). Colored seed coats such as those in mung bean contain higher levels of tannins than beans with white coats (Elias et al., 1979). These antinutritional factors are known to have adverse effects on animal growth but this was not observed in the present study as pre-treatment by roasting of mung beans at high temperatures prior to feed preparation might have helped reduce, if not eliminate, the antinutritional substances present in the beans. A 20-30% and 33-67% reduction in phytic acid and tannins, respectively was observed in the heat-treated mung beans compared to the raw mung beans (Mubarak, 2005). The superior growth and FCR with the provision of 20% of the diet from mung beans in the present study suggests that mung beans at this level can be efficiently utilized by milkfish and converted to tissue growth. In fact, growth was found to be directly correlated with dietary mung bean levels with significant differences detected between the control and the highest mung bean inclusion at 20%. Since all diets were isonitrogenous, their varying performance could be attributed to the quality of the protein as determined by the composition of the essential amino acids (EAA). The EAA profile of the diets in this study was improved by increasing mung bean levels thereby satisfying the requirements of milkfish for adequate growth. Increasing mung bean inclusion levels also decreased levels of rice bran used as filler that might have enhanced nutrient digestibility thereby contributing to the observed improved growth of fish in the group fed the highest level of mung beans. When 25% fish meal protein was replaced with mung bean in formulated diet for milkfish no adverse effects were observed on the growth, survival, and feed efficiency, in the fish (De la Peña et al., 1987). The feed formulation in the present study used a combination of different protein sources which differed from the study quoted above which used fish meal as the single dietary protein source. In Nile tilapia, best results were obtained when mung bean replaced 25% of the dietary fish meal (De Silva and Gunasekera, 1989). The addition of mung bean at 18% of dietary protein did not affect the growth of sea bass (Eusebio and Coloso, 2000). Conversely, it was reported that 18-20% replacement of fish meal by mung bean protein negatively affected the growth, FCR, and PER, of sea bass (Ganzon-Naret, 2013). The different processing methods employed prior to feed preparation might have contributed to the different findings in the above study. Regardless of dietary treatment, the feed intake of fish in the present study did not change suggesting that mung bean had no deleterious effects on the palatability of the formulated diet. Low feed intake due to poor palatability of plant ingredients was reported in other fish species (Hajen et al., 1993).

Higher PER in milkfish with the use of mung bean could be attributed to its high protein digestibility and lower phytic acid content (Nair et al., 2013). This supports our present results which showed that better growth was achieved with the highest mung bean protein in the diet. Further, high (though not statistically significant) protein retention as measured by the protein productive value (PPV) in this study suggests that mung bean was able to provide high quality protein required by milkfish, and did not in any way hinder its ability to utilize the diets.

The recorded mortalities in the present study, although significant among treatments, were not influenced by the mung bean inclusion levels. Highest survival was recorded in the groups given 8-12% mung bean with no significant difference detected between the higher mungbean levels and the control. In shrimp, low survival could be attributed to an imbalance in the amino acid (AA) component of the protein sources (Peñaflorida, 1989), whereas deficiency of AA is generally shown by reduction in weight (Cowey, 1994). As the AA profile of the diets in the present study was balanced by AA supplementation, the reason for the different survival rates could not be clearly determined.

Liver is the main organ for metabolism in animals including fish, and the hepatosomatic index (HSI) is a useful biomarker to detect the hazardous effects of diet and environmental stressors, and energy reserves in fish (Lunger et al., 2006; Luo et al., 2006). In the present study, HSI values were similar among all groups indicating that mung bean in the diet did not cause toxicity in the fish that may result in inflammation or swelling of the liver. This also implies that the energy reserves of the fish were not adversely influenced by the use of dietary mung bean. High accumulation of fat resulted in high viscerosomatic index in Nile tilapia fed soybean as substitute for fish meal (Koumi et al., 2008). In the present study, such accumulation of fat in the liver was not observed in milkfish fed mung bean even at the highest inclusion level.

Absence of toxicity was further confirmed by histological observations of the liver and intestine of the fish fed mung bean diets. Toxicity is normally detected by pathology in the intestinal tissues such as changes in villus crypt and height, loss of epithelial cells, villi fusion, hyperplasia, and enteritis or changes in liver tissues such as fatty infiltration, loss of glycogen granules, hepatocytomegaly, occlusion of the bile ducts, degenerative vacuolation, toxic hepatitis, and general changes in the overall organization of the liver (Takashima and Hibiya, 1995). The above histological changes have been used as criteria for assessing the beneficial or adverse effects of feed ingredients or of specific antinutritional or toxic factors present in feedstuffs (Heikkinen et al., 2006). At the levels tested in this study, milkfish were not affected by the inclusion of mung bean in the diet formulation. Similar replacement of dietary fish meal protein by mung bean did not cause changes in the liver morphology of sea bass (Ganzon-Naret, 2013). Heating (boiling) effectively removed antinutritional factors such as trypsin inhibitors of kidney beans resulting in 50% replacement of soybean protein without adversely affecting the internal organs of broiler chickens (Emiola et al., 2007). Moreover, in *P. monodon*, pre-treated dietary mung bean did not cause abnormalities in the hepatopancreas and midgut of shrimp (Kumaraguru Vasagam et al., 2007). Other physical treatments such as microwave cooking and autoclaving, can also effectively remove antinutritional factors (Khattab and Arntfield, 2009). Therefore, optimum processing conditions to effectively reduce or eliminate toxic components should be determined for certain types of feedstuffs used as protein sources. In the present study, roasting of mung beans for 1 h at 100ºC followed by storing at 60ºC overnight seemed effective in eliminating toxic factors.

Overall, this study demonstrates that mung bean is a promising alternative protein source for milkfish. Partial inclusion of mung bean at 20% of the diet (17% of the total dietary protein) had no detrimental effects on the growth, feed performance, protein efficiency ratio, protein retention, hepatosomatic index, and liver and intestine histology in milkfish. Based on the results of this study, inclusion level of mung bean at 20% of the diet to replace 17% of the total protein is recommended.

Acknowledgements

This study was funded by the Department of Science and Technology (DOST) through the Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD). We would also like to express our appreciation to the University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture (UPV-CFOS-IA) and the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC AQD) for the use of their research facilities. The authors would also like to thank James David Lopez and Audie Golez for their assistance with this study.

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