Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# Chemical Composition and Nutrient Digestibility of Super Worm Meal in Red Tilapia Juvenile

M. D. Abd Rahman Jabir*, S. A. Razak and S. Vikineswary

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Corresponding author: abdrahmanjabir@gmail.com

ARTICLE HISTORY

Received: October 20, 2011
Revised: December 07, 2011
Accepted: January 15, 2012

**Key words:**
Digestibility coefficient
Fish meal
Red tilapia
Super worm meal

**ABSTRACT**

The chemical composition and nutrient digestibility of super worm (*Zophobas morio*) meal were determined for fish feed formulations. Experiments were conducted to compare super worm meal (SWM) with fish meal (FM) as main protein sources for fish diets. Super worm had lower protein content (42.83%) compared to fish meal (52.64%). SWM contained high percentage (40.01%) of lipids along with quality protein and this made it a suitable replacement for FM. SWM contained seventeen amino acids including the essential amino acids. All eight essential amino acids present were similar in values except for methionine which showed a large difference with 5.75 (mg/g crude protein) and 21.17 (mg/g crude protein) for SWM and FM respectively. SWM contained higher percentage of arginine and glutamic acid while the rest of the essential amino acids were lower than those present in FM. The fatty acid profile of SWM also showed a good polyunsaturated to saturated fatty acid ratio (0.87). Apparent digestibility coefficients (ADC) of protein in SWM diet was lowest (50.53±6.08%) and significantly different ($P<0.05$) from that of FM diet (77.48±0.53%). Lipid digestibility of SWM based diet was significantly lower (69.76±3.72%) than that of FM value (91.51±0.21%). However, SWM-based diets fulfilled the requirements of fish recommended by FAO.



**To Cite This Article:** Jabir MDAR, SA Razak and S Vikineswary, 2012. Chemical composition and nutrient digestibility of super worm meal in red tilapia juvenile. Pak Vet J, 32(4): 489-493.

## INTRODUCTION

Total dependence of the fish feed industry on fish meal (FM) has escalated the cost of nutritionally balanced aqua feeds. This necessitates the replacement of FM with an alternative nutrient such as the super worm meal (SWM) to reduce the cost of fish feed. Therefore, the dietary replacement of FM in fish feeds with the alternative protein sources is considered. FM is often scarce and expensive due to its use in poultry feeds and uncertain supply during the whole year (Gumus *et al.*, 2009). The relatively low production of FM and with increasing demand from feed manufacturing industry often lead to an increase in the production cost (El-Sayed, 2004).

Since various animal protein sources lack the full essential amino acid (EAA) profile and plant proteins contain a variety of anti nutritional factors. Therefore, insect meal may substitute the FM in fish diets. The super worm (*Zophobas morio)* meal has a high potential in substituting FM which is uncertain in terms of supply and increase in production cost. Now-a-days, researchers are making attempts to use unconventional locally available sources of proteins rather than depending too much on fish meal (Lenka *et al.*, 2010). Ng *et al.* (2001) conducted a research on meal worm (*Tenebrio molitor)*, a type of insect similar to super worm and found that the diet with meal worm inclusion was palatable to African catfish. This insect is worldwide in its distribution and can also be found locally in Malaysia as it has adapted well with the local tropical climate (Ghaly and Alkoaik, 2009). Recently, some other insects have also been identified as alternative protein source to FM (Adesulu and Mustapha, 2000). Therefore, *Z. morio* should be evaluated nutritionally to replace FM in fish diets for economical fish production. Few studies have evaluated insect meals to replace FM in fish diets (Fasakin *et al.*, 2003; Ajani *et al.*, 2004). Finke (2002) reported the nutrient composition of commercially raised insects for animal feedings.

Determination of digestibility of feedstuff is necessary to evaluate their potential for fish growth (Koprucu and Ozdemir, 2005). However, their digestibility is dependent upon its chemical composition and the digestive capability of the species to which it is fed. The present study was conducted to evaluate the apparent digestibility coefficients of dry matter, crude protein and crude lipid of SWM compared with FM.

## MATERIALS AND METHODS

The experiment was conducted at the Freshwater Aquarium, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia. Red tilapia, *Oreochromis spp.* juveniles weighing 6.01±0.04g were obtained from the Freshwater Hatchery Center, Bukit Tinggi, Malaysia. The feeding trial was conducted over a period of 56 days. One week prior to the experiment, 150 fish were acclimatized to the laboratory conditions and fed with commercial diet. At start of the experiment, 10 fish initially proceed to proximate carcass analysis. For each treatment, three replicates were used and in each replicate 10 juveniles were stocked. After each biweekly weighing, ratio sizes were adjusted according to their body weights for the next period of feeding. At the end of the experiment, the fish were measured for growth performance and proximate analysis of their carcass.

Fish were hand-fed, twice a day (0900 and 1700 hour) at 5% of their body weight. Water quality parameters including dissolved oxygen, pH, nitrate and ammonia were monitored biweekly to ensure their optimum levels for appropriate growth of Tilapia.

Diets were formulated about 36% crude protein using the WinFeed version 2.8 software in which to satisfy the nutrient requirement of Nile tilapia (NRC, 2011). Each test diet contained 70% of reference diet and 30% of test ingredient (Cho *et al.*, 1982). Two test diets were formulated using FM as a reference on the test diet, SWM based diet. These two diets used for ADC study were formulated to fulfill the 30% replacement portion each. Chromic oxide ($Cr_2O_3$) was used as an inert marker for this study at a concentration of 0.5% in each prepared diet. The resulting mixture was pelleted using the mini pelleting plant machine (KCM-Y123M-4) before drying in the oven at 70°C for 24 hours. The composition of ingredients of reference and test diets is shown in Table 1.

**Table 1:** Proximate composition of reference and test diets (g/kg) for the digestibility study

| Ingredients | Reference diet | Test diet |
|---|---|---|
| Dry matter (%) | 93.71 | 94.33 |
| Crude protein (%) | 37.45 | 36.53 |
| Crude lipid (%) | 3.96 | 12.15 |
| Crude fiber (%) | 3.55 | 5.21 |
| Ash (%) | 12.72 | 8.00 |

[1]The vitamin premix supplied the following per kg diet:Vitamin A, 500IU; Vitamin $D_3$, 100IU; Vitamin E, 75000 mg; Vitamin $K_3$, 20000 mg; Vitamin $B_1$,10000 mg; Vitamin $B_3$, 30000 mg; Vitamin $B_6$, 20000 mg; Vitamin $B_{12}$, 100 mg; Vitamin D, 60000 mg; Niacin, 200000 mg; Folic Acid, 500 mg; Biotin, 0.235 mg.[2]The mineral premix supplied the following per kg diet: Selenium, 0.2 g; Iron, 80 g; Manganese 100 g; Zinc, 80 g; Copper, 15 g; Potassium Chloride, 4 g; Magnesiun Oxide, 0.6 g; Sodium Bicarbonate,1.5 g; Iodine, 1.0 g; Cobalt, 0.25 g.

Diets were analyzed in triplicate for proximate composition (AOAC, 2002) and chromic oxide was determined using the method mentioned by Furukawa and Tsukahara (1966). The ADC of dry matter, protein and lipid of diets were calculated based on Koprucu and Ozdemir (2005). Fatty acid methyl esters (FAME) were analyzed using a HP 6890 Series (Hewlet Packard) gas chromatography equipped with flame ionization detector and fused silica capillary column (30 m x 0.32 mm) with 0.25 µm (Model BPX70). The carrier gas was helium (1.6 ml/min). Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standard from SUPELCO. Amino acid profiles were determined by using HPLC work station (Jasco, CO-2065 Plus, Intelligent Column Oven) equipped with Purospher STAR RP-18 encapped column (5mm). The amino acids were determined by comparison of peak retention times to known standards.

Data thus obtained were subjected to one-way ANOVA using SPSS version 12.0. Differences between the means were compared using Duncan's post hoc test at 5% probability level.

## RESULTS

The HPLC analysis demonstrated a better amino acid profile in FM as compared to super worm meal (SWM). All seventeen amino acids were obtained except tyrptophan that considered destroyed during the acid hydrolysis process (Fig. 1 and 2). All eight essential amino acids present had similar values except methionine which showed a large difference with 5.75 and 21.17 (mg/g) for SWM and FM, respectively. SWM contained higher percentage of arginine and glutamic acid while the rest of the essential amino acids were lower than those present in FM (Table 2). All the values were significantly different (P<0.05) from each other but not with histidine. The total amino acids were 578.53 and 526.99 mg/g crude protein in FM and SWM respectively, which was less than the 864.2 mg/g crude protein in chicken egg that is considered as a main protein source in the human diet. The total amount of the EAAs found in SWM and FM was 199.20 and 288.40 mg/g crude protein respectively, which were higher values recommended by FAO/WHO (1991), (113 g protein for adults).

**Table 2:** Amino acid composition of SWM and FM (mg/g crude protein) [1]

| | SWM | FM | Chicken Egg |
|---|---|---|---|
| Aspartic acid | 70.08±5.89[a] | 111.69±5.32[b] | 89.2 |
| Glutamic acid | 125.53± 3.70[a] | 181.76±4.75[b] | 121.3 |
| Serine | 5.41±0.27[a] | 31.13±1.12[b] | 67.2 |
| Glycine | 24.55±0.53[a] | 43.42±1.62[b] | 30.2 |
| Histidine* | 13.86±0.25[a] | 14.74±0.44[a] | 20.9 |
| Arginine* | 21.91±0.02[a] | 11.09±0.49[b] | 57.0 |
| Threonine* | 21.23±0.26[a] | 26.99±0.58[b] | 44.7 |
| Alanine | 37.88±0.32[a] | 45.91±1.51[b] | 50.3 |
| Proline | 25.71±0.21[a] | 28.24±0.69[b] | N.I |
| Tyrosine | 37.05±0.13[a] | 23.47±0.22[b] | 38.1 |
| Valine* | 29.37±0.17[a] | 34.42±0.06[b] | 54.2 |
| Methionine* | 5.75±0.02[a] | 21.17±0.54[b] | 28.1 |
| Cystine | 0.86±0.11[a] | 2.50±0.31[b] | 19.0 |
| Isoleucine* | 21.41±0.04[a] | 29.19±0.54[b] | 48.8 |
| Leucine* | 30.21±0.04[a] | 47.71±1.24[b] | 81.1 |
| Phenylalanine* | 21.93±0.95[a] | 33.93±0.46[b] | 48.2 |
| Lysine* | 34.25±0.15[a] | 69.74±1.44[b] | 65.9 |
| Total AA | 526.99 | 578.53 | 864.2 |
| Total EAA | 199.20 | 288.44 | 448.9 |

* Essential amino acids; [1] Values are mean of two replicates ± SEM, Means on the same row with the different superscripts are significantly different (P<0.05); essential amino acid requirements of Nile tilapia (%) according to NRC (1993): tryptophan 1.00, lysine 5.12, histidine 1.72, arginine 4.20, threonine 3.75, valine 2.80, methionine 2.68, isoleucine 3.11, leucine 3.39, phenylalanine + tyrosine 3.75.

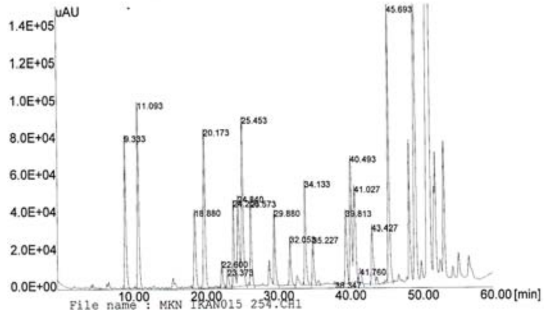


**Fig. 1:** Chromatogram of FM amino acid profile

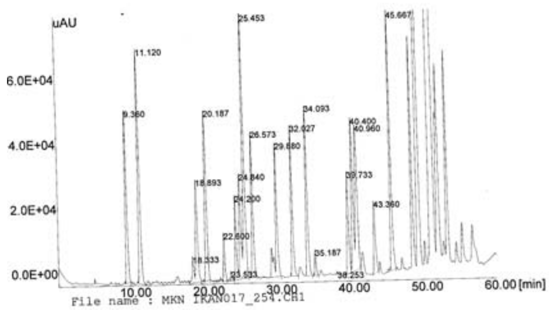


**Fig. 2:** Chromatogram of SWM amino acid profile

**Table 3:** Fatty acid composition of SWM and FM (% of total fatty acids)

| Fatty acids | SWM | FM |
|---|---|---|
| C14:0 | 0.09 | 14.32 |
| C14:1 | 0.07 | 6.71 |
| C16:0 | 17.29 | 24.64 |
| C16:1 | 0.45 | 13.19 |
| C17:0 | 0.58 | 2.04 |
| C18:0 | 5.69 | 7.72 |
| C18:1 | 34.10 | 10.17 |
| C18:2n-6 | 23.42 | 1.19 |
| C20:0 | 1.12 | 1.36 |
| C20:1 | 0.96 | 3.76 |
| C22:1 | 0.59 | 6.96 |
| % SFA | 49.65 | 40.79 |
| % MUFA | 26.93 | 53.39 |
| % PUFA | 23.42 | 5.82 |
| PUFA / SFA | 0.87 | 0.11 |

SFA - Saturated fatty acid; MUFA - Monounsaturated fatty acid; PUFA - Polyunsaturated fatty acid.

**Table 4:** ADC (%) of protein, lipid and dry matter in the experimental diets[1]

| Components | FM | SWM |
|---|---|---|
| Dry matter (%) | 63.96±0.85[a] | 43.85±6.89[b] |
| Crude protein (%) | 77.48±0.53[a] | 50.53±6.08[b] |
| Crude lipid (%) | 91.51±0.21[a] | 69.76±3.72[b] |

[1] Values are mean of three replicates ± SEM, Means on the same row with the different superscripts are significantly different (P<0.05).

SWM sample had 26.93% of saturated fatty acids, 49.65% of monounsaturated fatty acids and 43.42% of polyunsaturated fatty acids (PUFA) (Table 3). Moreover, SWM contained PUFA, mostly linoleic acid (C18:2n-6, 23.42%) which was higher than PUFA of FM.

The ADC of crude protein differed significantly (P<0.05) between FM and SWM-based diets (Table 4). Generally, FM-based diet was highly digested by fish as compared to SWM-based diet with ADC of dry matter and crude lipid each showing similar trends with FM-based diet having the higher value and followed by SWM-based diet (Table 4).

## DISCUSSION

The result showed that SWM had slightly higher crude protein of 42.83%. This value was higher compared to Finke and Winn (2004) who obtained a result of 19.01%. But, Jabir *et al.* (2012) also made a nutrient analysis on super worms and reported that it had 47.43% crude protein and 40.01% crude lipid. This disparity is attributed to factors such as source, stage of harvesting, methods of processing and drying (Ojewola *et al.,* 2005). Crude lipid of SWM obviously was higher (40.01%) compared to other ingredients. This is because of the feed consumed by the super worm during its growth. SWM had an outer exoskeleton made up of chitin and chitin was proven scientifically as a toxin binder (Khajarern *et al.*, 2003). In this study, whether the ash content of SWM (3.54%) is related to the presence of chitin still need further study. Finke (2007) found that the average chitin in super worm was estimated to be 49.8 mg/kg on dry matter basis. In fact, Shiau and Yu (1999) carried out the experiment to study the effects of chitin on growth and nutrient digestibility in tilapia and found that lower body weight of fish were recorded after being fed with this chitin-based diet. Powell and Rowley (2006) has found that supplementation of pure chitin did not affect the survival and immune reactivity of adult shore crab (*Carcinus maenus*). The findings of chitin's effect on aquatic organism varied.

The nutritive value of protein of any ingredients depended substantially on the protein capacity to fulfill the needs of organisms with respect to essential amino acids. Li *et al.* (2008) reported on the importance of amino acids in fish nutrition that are necessary for the development of a balanced aqua feed. Lysine, methionine and cystine are the essential amino acids that made *Z. morio* a superior alternative protein source particularly for tilapia (Santiago and Lovell, 1988). Generally, the levels of amino acids and fatty acids of SWM demonstrated here were slightly lower than FM. This was probably due to the loss of nutrients during the drying process. For sulphur-containing amino acids, dietary methionine in *Z. morio* was lower (5.75 mg/g) compared with fish meal (21.17 mg/g). Non essential amino acid could be synthesized from the essential amino acid precursor. Methionine could be synthesized from cystine conversion (NRC, 2011). Thus, for fish diets of *Z. morio* likely could contribute or spare the synthesis of low level methionine. Arginine content (21.91 mg/g) in SWM was higher and this EAA is highly required due to its function in stimulating growth and its health-promoting effect to the fish. Buentello and Gatlin (2001) reported the dependent of catfish to the dietary arginine in its resistance towards *Edwardsiella ictaluri*.

Fatty acids play an important role in finfish nutrition. Their main functions were as a source of energy and also for fish bioenergetics and physiology (Trushenski *et al*., 2006). Data from the recent result of SWM showed that PUFA / SFA ratio in the lipids of SWM was higher (0.87). Recent report made by Nandeesha *et al*. (2000) revealed pupa oil to be rich in short chain unsaturated fatty acids and is an excellent energy source in diet of common carp. Fatty acid methyl ester (FAME) profile in Table 3 was similarly parallel with that reported by Pereira *et al.* (2003). Palmitic acid and myristic acid present in SWM and FM were scientifically proven to elevate low density lipoprotein (LDL) cholesterol (Connor and Connor, 2007). Ratio of polyunsaturated to saturated fatty acid (PUFA / SFA) has been widely used to determine the cholesterol lowering potential of food. Also, the ratio of n-3 to n-6 fatty acids has also been proposed as an indicator of fish health status (Sargent *et al.*, 1999). The present result of PUFA / SFA ratio (0.87) was associated with the desirable level of cholesterol suggested by Akinnawo and Ketiku (2000). SWM contained high lipid that can be used to replace the fish oil as well. In this study, linoleic acid (C18:2n-6) of SWM was higher in value (23.42%) than FM. This finding is meant to be essential to fish, which lack the ability to synthesize this biological compound to meet biological demands of fish (Tocher, 2003). In fact, other long chain PUFA such as arachidonic acid (C20:0), constituted 1.12 % in SWM and emerged as a required dietary for eicasonoids precursor (Sargent *et al.*, 2002).

SWM digestibility of dry matter, protein and lipid were significantly lower than FM digestibility for juvenile red tilapia. Results in Table 4 also indicated that FM was highly digestible with an apparent protein digestibility of 77.48%. The apparent digestibility of dry matter was also lower than the value of 84–89% reported by Eusebio *et al.* (2004). The ADC of dry matter may be affected by the type of raw material used. The ADC crude protein was less than reported by Koprucu and Ozdemir (2005) who observed the digestibility for crude protein of fish meal for tilapia was 90.5%. The significantly low ADC crude protein of SWM may be mainly attributed to its low protein content and poor amino acid profile as shown in Table 2. However, Pike *et al.* (1990) suggested that digestibility of FM may be improved by applying low temperature in the drying process. Cheng and Hardy (2002) suggested to the fish feed formulators to be wise in utilizing specific source rather than simply buying ingredients such as FM and SWM because ADC crude protein varied widely from the source obtained. In addition, the high ADC crude protein registered confirmed the Nile tilapia's ability of digesting animal protein well.

Koprucu and Ozdemir (2005) reported the ADC lipid range for tilapia was 72-90% for corn gluten, soy bean meal, rapeseed meal, sunflower seed meal, sorghum, barley and wheat bran. The ADC of lipid (69.76%) for SWM in this study was lower than reported by Koprucu and Ozdemir (2005).

**Conclusion:** The results of this study showed that the nutrient content and digestibility of SWM need to be slightly improved in order to be a good alternative feed for red tilapia. This alternative protein source can become a viable choice to guarantee it to be of the same quality as in FM-based fish diet. This result can be used to aid in the formulation of cost effective diets for red tilapia using SWM for partial or complete replacement of FM.

**Acknowledgement:** The author would like to thank the University of Malaya for providing financial support through grants (PS270/2010A) and (UMRG: RG130/ 10AFR). Also, thanks the Freshwater Fisheries Research Center staffs for their technical assistances.

## REFERENCES


Adesulu EA and AK Mustapha, 2000. Use of housefly maggots as a fishmeal replacer in tilapia culture: a recent vogue in Nigeria. 5th International Symposium on Tilapia Aquaculture, Rio de Janeiro, Brazil, pp: 138.

Ajani EK, LC Nwanna and BO Musa, 2004. Replacement of fishmeal with maggot meal in the diets of Nile tilapia, *Orocheromis niloticus*. World Aquac, 35: 52-54.

Akinnawo O and AO Ketiku, 2000. Chemical composition and fatty acid profile of edible larvae of *Circina forda* (Westwood). Afr J Biomed Res, 3: 93-96.

AOAC, 2002. Official Methods of Analysis. 13th Ed, Association of Official Analytical Chemists, Washington, DC, USA.

Buentello JA and DM Gatlin, 2001. Effects of elevated dietary arginine on resistance of channel catfish to exposure to *Edwardsiella ictaluri*. J Aquat Anim Health, 13: 194-201.

Cheng ZJ and RW Hardy, 2002. Apparent digestibility coefficients and nutritional value of cotton seed meal for rainbow trout (*Onchorhynchus mykiss*). Aquaculture, 212: 361-372.

Cho CY, SJ Slinger and HS Barley, 1982. Bioenergetic of salmonids fishes: energy intake, expenditure and productivity. Comp Biochem Physiol (B), 73: 25-41.

Connor WE and SL Connor, 2007. The importance of fish and docosahexanoic acid in Alzheimer disease. Am J Clin Nutr, 85: 929-930.

El-Sayed AFM, 2004. Protein Nutrition of Farmed Tilapia: Searching for Unconventional Sources. New Dimensions on Farmed Tilapia. Proc 6th Int Symp Tilapia in Aquac, 12-16 September 2004, Manila, Philippines, pp: 364-378.

Eusebio PS, RM Coloso and EP Mamauag, 2004. Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coides* (Hamilton). Aquac Res, 35: 1261-1269.

Fasakin EA, AM Balogun and OO Ajayi, 2003. Evaluation of full fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac Res, 34: 733-738.

Finke M, 2007. Estimate of chitin in raw whole insects. Zoo Biol, 26: 105-115.

Finke M and D Winn, 2004. Insects and related anthropods: A nutritional primer for rehabilitators. J Wildlife Rehabil, 27: 14-27.

Finke M, 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol, 21: 286-293.

Furukawa A and H Tsukahara, 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull Jap Soc Sci Fish, 32: 502-504.

Ghaly AE and FN Alkoaik, 2009. The yellow mealworm as a protein novel source. Am J Agric Biol Sci, 4: 319-331.

Gumus E, Y Kaya, BA Balci and BB Acar, 2009. Partial replacement of fish meal with tuna liver meal in diets for common carp fry, *Cyprinus carpio* L. 1758. Pak Vet J, 29: 154-160.

Jabir MDAR, SA Razak and S Vikineswary, 2012. Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. Afr J Biotechnol, 11: 6592-6598.

Khajarern JM, S Khajarern, TH Moon and JH Lee, 2003. Effect of dietary supplementation of fermented chitin-chitosan on toxicity of mycotoxin in ducks. Asian-Aust J Anim Sci, 16: 706-713.

Koprucu K and Y Ozdemir, 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). Aquaculture, 205: 308-316.

Lenka S, SS Giri and BN Paul, 2010. Nutrient digestibility and gastro-intestinal enzyme activity of *Cyprinus carpio* fingerlings fed water washed neem seed cake incorporated diets. Indian J Anim Sci, 80: 486-489.

Li P, K Mai, J Trushenski and G Wu, 2008. New developments in fish amino acid nutrition: towards functional and environmentally oriented aqua feeds. Amino Acids, 37: 43-53.

Nandeesha MC, B Gangadhara, TJ Varghese and P Keshavanath, 2000. Growth response and flesh quality of common carp, *Cyprinus carpio* fed with high levels of nondefatted silkworm pupae. Asian Fish Sci, 13: 235-242.

Ng WK, FL Liew, LP Ang and KW Wong, 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquac Res, 32: 273-280.

NRC, 2011. Nutrient Requirement of Fish. National Research Council, National Academy Press, Washington DC, USA.

Ojewola GS, FC Okoye and OA Ukoha, 2005. Comparative utilization of three animal protein sources by broiler chickens. Int J Poult Sci, 4: 462-467.

Pereira NR, OS Filho, M Matsushita and NE Souza, 2003. Proximate composition and fatty acid profile of *Bombyx mori* L. chrysalis toast. J Food Comp Anal, 16: 451 - 457.

Pike IH, G Andorsdottir and H Mundhein, 1990. The role of fish meal in diets for salmonids. International Association of Fish Meal Manufacturers. Technical Bulletin, No. 24. pp: 1-35.

Powell A and AF Rowley, 2007. The effects of dietary chitin supplementation on survival and immune reactivity of adult shore crab, *Carcinus maenus*. Comp Biochem Physiol A (Mol Integr Physiol), 147: 122-128.

Santiago CB and RT Lovell, 1988. Amino acid requirements of growth of Nile tilapia. J Nutr, 118: 1540-1546.

Sargent JR, G Bell, L McEnvoy, D Tocher and A Estevez, 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture, 177: 191-199.

Sargent JR, DR Tocher and JG Bell, 2002. The lipids. In: Fish Nutrition, 3rd Ed., (Halver JE and RW Hardy, eds): Academic Press, San Diego, California, pp: 181-257.

Shiau SY and YP Yu, 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus x O. aureus*. Aquaculture, 179: 439-446.

Trushenski JT, CS Kasper and CC Kohler, 2006. Challenges and opportunities in finfish nutrition. North Am J Aquac, 68: 122-140.

Tocher DR, 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci, 11: 107-184.