Chapter 5

5.0 Evaluation of Different Mixtures of Super Worm Meal as Dietary Protein Source in Practical Diet of Juvenile Red Tilapia

5.1 Introduction

Feed represents the largest expenditure in semi-intensive and intensive fish culture system. The dependency on fish meal in semi-intensive and intensive aquaculture represents a severe constraint towards development and intensification of this industry in many regions. On the global view, aquaculture has grown dramatically about three folds compared to capture fisheries and terrestrial farmed meat production systems respectively since 1970 (FAO, 2007b). However, world fish meal production is not expected to increase due to source depletion. It has remained static without showing production increase (Tacon *et al.*, 2006). Availability of fish meal in the global market is limited and consequently the price has dramatically increased (Hardy, 2006). Therefore, in order to develop an economically viable aquaculture industry, alternative sources of high protein content will have to be identified to replace the costly and fluctuating quality of fish meal. For both economic and practical reasons, fish feeds should use locally available protein sources, especially from those unsuitable for direct human consumption (Glencross *et al.,* 2007).

A number of published reports are available regarding the suitability of insects as alternative protein sources in aquafeeds (Ogunji *et al.*, 2006; Oyegoke *et al.,* 2006; Sogbesan *et al.,* 2006; Sogbesan and Madu, 2008; Sogbesan and Ugwumba, 2008; Hassan *et al.,* 2009; Ijaiya and Eko, 2009). Super worm (*Zophobas morio*) meal is widely used as a feed supplement for birds and fish. It is also locally available in abundance. The nutritive potential and utilization of this insect larvae as fish feed ingredient especially in tilapia have not been adequately recorded yet. Thus, Finke (2002) reported an overview of complete nutritional composition of commercially raised insect used as food for insectivores. The response of tilapia and other fish to diets containing insect meal has been investigated by various authors. Insect meal has been used as substitute for fish meal in diets as demonstrated by Ng *et al.* (2001). Attempts to reach substitution levels of more than 50% of the fish meal protein by mixing two or more protein sources has been scarce although some of the results looks promising (Borgeson *et al*., 2006; Fontainhas-Fernandes *et al.,* 1999; Jackson *et al.,* 1982). However, El-Saidy and Gaber (2003) stated that a plant protein mixture in equal proportions (25% each with lysine and methionine supplementation) can completely replace fish meal in diets of Nile tilapia.

This study was conducted to evaluate the possibility of replacing a significant proportion of FM protein partially in the diets of red tilapia using locally available insect, super worm (*Zophobas morio*) meal and to access its effects on growth, feed utilization and body composition.

5.2 Materials and methods

5.2.1 Experimental Systems and Animal

Red tilapia juveniles with average weight of 5.57 ± 0.15 g were stocked in triplicate treatment in 30 L of aquaria. All fifteen aquaria were used in this experiment. Water quality parameters were monitored at acceptable range every week during the experiment.

Fish was acclimatized for one week with a commercial diet (Takara Sakana-II, Fish Food, Kian Weng Trading Co.) until they adapted well with the experimental condition. Fish were then fed twice a day (0900 and 1700h) at the rate of 10% of their body weights. The duration of the experiment was 8 weeks. The feed ration was adjusted biweekly each time after the fry were weighed on an electronic top pan balance

(AND EW-I Series). Feeding was carried out twice daily throughout the experimental period.

At the start of the experiment, 10 fries of similar average body weight were sacrificed and frozen. At the end of the experimental period, fish were randomly removed from the aquaria, sacrificed and frozen for carcass composition analysis.

5.2.2 Diet Preparation

Fresh super worm (*Zophobas morio*) was obtained from a local aquarium shop. They were then sacrificed humanely in a freezer for one hour, packed in plastic bags, sealed and oven-dried at 70 °C overnight to remove moisture. The dried super worm were milled and stored at 8 °C until used in the diet preparation. The powder form of super worm was subjected to proximate analysis to evaluate its nutritional content.

Five experimental diets isonitrogenous (32% crude protein) were formulated using WinFeed version 2.8 Software (Least Cost Feed Formulation). In the diets, SWM was used to replace FM gradually as alternative protein sources at various inclusion levels, 0% (control), 25%, 50%, 75% and 100%, as shown in Table 5.1. Prior to preparing the experimental diets, all ingredients were grounded in a hammer mill (Disk Mill, FFC-454) and all the dry ingredients of each diet were thoroughly mixed to ensure the homogeneity of the ingredients. Water was then added to the mixed ingredients. The resulting mixture was pelletized wet using the mini pelletizing plant machine (KCM, Y132M-4) with a 2 mm mesh sieve. The pellets were dried in an oven at 70 °C for 24 hours. They were then packed in plastic bags, labelled and kept at room temperature in the laboratory until used for feeding.

5.2.3 Analysis of Experimental Data

Growth performances and feed utilization were measured as described in Section 3.7.

5.2.4 Monitoring of Water Quality

Parameters of water quality were measured using the method of APHA (1992). Ammonia and nitrate was determined bi weekly using Spectroquant Pharo 300 (Merck, USA). Water temperature and pH were recorded daily using pH meter. Dissolved oxygen was recorded daily using DO meter (YSI Model 58, Yellow Springs, OH).

5.2.5 Proximate Analysis

Moisture, ash, protein and total fat content of diets were analysed according to the Association of the Official Analytical Chemist (AOAC, 1990). Amino acid profile analysis was conducted according to the previous procedure (Section 3.5). Gross energy was calculated using the following factors: crude protein = 5.65 kcal/g, crude lipid = 9.45 kcal/g and NFE = 4.1 kcal/g (NRC, 1993). Protein to energy ratio were calculated over each diet and expressed in unit of mg protein kJ^{-1}.

5.2.6 Statistical Analysis

Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS version 12.0. Differences among the means were compared using Duncan's post hoc test at 5% probability level.

5.3 Results

5.3.1 Proximate Composition of Diets

Table 5.1: Composition of experimental diets fed to *Oreochromis spp.* juveniles (g/kg)

Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
SWM inclusion level	**(0%)**	**(25%)**	**(50%)**	**(75%)**	**(100%)**
Fish meal	30	22.5	15	7.5	0
Soybean meal	22.06	24.35	26.65	28.94	31.23
Rice Bran	31.44	29.15	26.85	24.56	22.27
SWM	0	7.5	15	22.5	30
Corn starch	15	15	15	15	15
Vitamin Premix	0.2	0.2	0.2	0.2	0.2
Mineral Premix	0.3	0.3	0.3	0.3	0.3
Di-Calcium Phosphate	1	1	1	1	1
Cost of feed (RM/kg)	2.59	3.83	5.15	6.43	7.71
Nutrients (% as fed basis)					
Dry matter	92.27	92.29	93.12	92.62	92.16
Crude protein	34.18	31.31	32.62	31.88	30.85
Crude lipid	7.05	7.41	11.21	13.05	15.93
Crude ash	12.81	11.27	9.51	8.06	6.45
Crude fiber	2.95	2.82	4.24	4.21	4.18
NFE[1]	43.01	47.19	42.42	42.80	42.59
Gross energy[2]	436.08	440.41	464.16	478.92	473.11
P/E ratio (mg protein Kj^{-1})	19.83	17.90	17.68	16.69	15.44
Essential amino acids composition[3]					
Histidine	5.23 ± 0.45[a]	5.52 ± 0.13[a]	6.80 ± 0.19[b]	7.16 ± 0.01[b]	8.22 ± 0.68[c]
Arginine	22.96 ± 0.01[c]	21.48 ± 0.41[b]	21.29 ± 0.15[b]	19.66 ± 0.12[a]	19.08 ± 0.11[a]
Threonine	23.15 ± 0.11[e]	13.14 ± 0.08[a]	14.76 ± 0.11[d]	13.60 ± 0.05[b]	14.12 ± 0.15[c]
Valine	13.19 ± 0.14[a]	15.86 ± 0.05[b]	17.17 ± 0.05[d]	16.16 ± 0.05[c]	16.23 ± 0.06[c]
Methionine	26.29 ± 2.79[b]	3.75 ± 0.01[a]	3.63 ± 0.04[a]	3.08 ± 0.07[a]	3.52 ± 0.06[a]
Isoleucine	14.32 ± 0.06[e]	13.12 ± 0.04[b]	13.90 ± 0.02[d]	13.38 ± 0.04 [c]	12.83 ± 0.07[a]
Leucine	24.43 ± 0.25[d]	22.49 ± 0.01[b]	23.66 ± 0.13[c]	22.62 ± 0.03[b]	19.37 ± 0.01[a]
Phenylalanine	16.49 ± 0.35[a]	15.79 ± 0.25[a]	15.92 ± 0.02[a]	15.74 ± 0.25[a]	15.94 ± 0.02[a]
Lysine	13.74 ± 0.12[b]	13.25 ± 0.03[a]	15.83 ± 0.02[c]	15.65 ± 0.06[c]	18.82 ± 0.02[d]

* All values are means of three replicates ± SEM for triplicate feeding groups and values in the same row with different superscripts are significantly different ($P < 0.05$). [1] NFE = 100 – (% protein + % fat + % ash + % fiber), [2] Gross energy (GE) was calculated as 5.65, 9.45, 4.1 kcal/g for protein, fat and NFE respectively (NRC, 1993)[3] 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.

5.3.2 Growth Performances and Feed Utilization

The study showed that there was no significant difference ($P>0.05$) in the initial weight of the fish fed with all the diets treated. The mean final weight, WG and SGR of fish fed with the diets containing 25% and 50% SWM were significantly higher ($P<0.05$) than those fish fed with 0%, 75% and 100% SWM diet. The growth performance was reduced with a diet containing higher 75% SWM. Highest weight gain and SGR were clearly recorded in tilapia fed with diet 2, followed by diet 3 which did not significantly differ ($P>0.05$). Diet 2 and 3 gave the highest weight gain and was better utilized by the fish as it gave the following biological parameter: SGR, 1.02 and 1.01% day^{-1}; FCR, 1.25 and 1.36; PER, 1.97 and 1.92 respectively. Feed utilization in red tilapia was also affected by the SWM inclusion level. Final weight, weight gain and SGR values of fish fed with Diet 2 and 3 were higher compared to those fed with other diets. The best FCR was observed at 25% inclusion of SWM in the tilapia diet.

PER of fish fed with diet 2 and 3 were significantly higher ($P<0.05$) than other diets. The highest PER was recorded by diet 2 (25% SWM) with 1.97 while the lowest value of 1.10 was recorded in 100% SWM based diet (Table 3). All diets gave 100% survival.

Table 5.2: Growth performance and feed utilization of *Oreochromis spp.* juveniles fed with experimental diets*

Components	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Initial weight, g	$5.57±0.15^{a}$	$5.71±0.24^{a}$	$5.81±0.04^{a}$	$5.53±0.27^{a}$	$5.59±0.11^{a}$
Final weight, g	$9.18±0.42^{ab}$	$10.11±0.26^{c}$	$10.24±0.15^{c}$	$9.17±0.43^{ab}$	$8.49±0.18^{a}$
Weight gain, %	$64.81±0.27^{a}$	$76.88±0.11^{b}$	$76.24±0.15^{b}$	$65.82±0.41^{b}$	$51.34±0.26^{a}$
SGR[1]	$0.88±0.04^{ab}$	$1.02±0.41^{b}$	$1.01±0.29^{b}$	$0.90±0.08^{ab}$	$0.75±0.06^{a}$
FCR[2]	$1.47±0.05^{bc}$	$1.25±0.02^{a}$	$1.36±0.03^{b}$	$1.42±0.02^{bc}$	$1.50±0.03^{c}$
PER[3]	$1.34±0.12^{a}$	$1.97±0.15^{b}$	$1.92±0.12^{b}$	$1.39±0.15^{a}$	$1.10±0.04^{a}$
Survival, %	100.00	100.00	100.00	100.00	100.00

* All values are means of three replicates ± SEM for triplicate feeding groups and values in the same row with different superscripts are significantly different ($P < 0.05$). [1] SGR = (ln W2 – ln W1 /T) x 100; [2] FCR = Food Fed / Live Weight Gain; [3] PER = Live weight gain (g) / Protein fed (g).

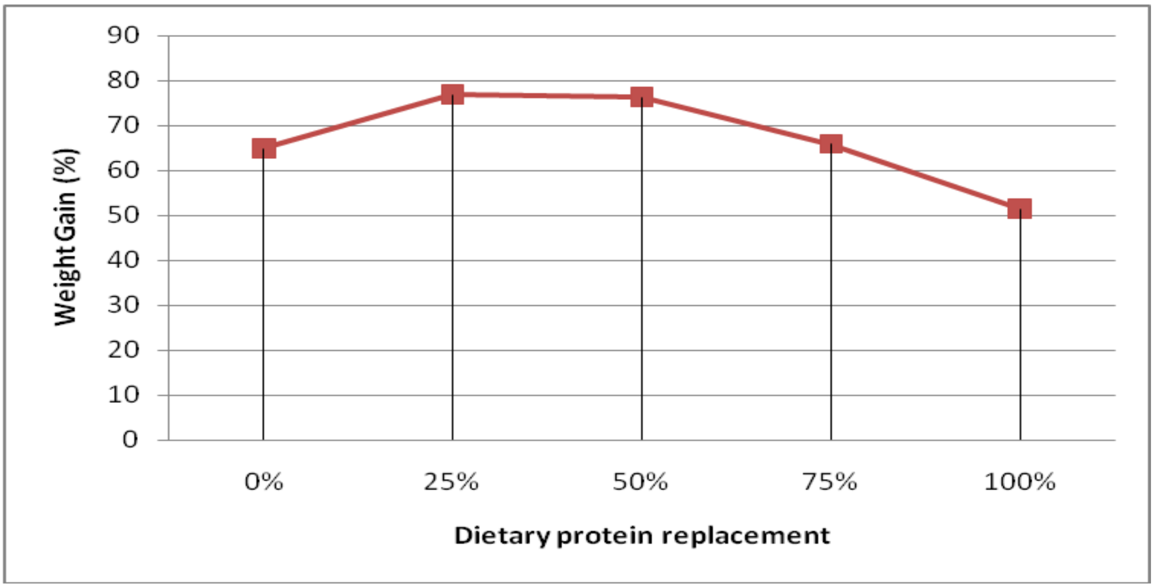

Figure 5.1: Growth of juvenile tilapia *Oreochromis spp.* fed with the experimental diets over a 56-day trial

5.3.3 Water Quality Parameter

Water quality parameters indicated that water temperature ranged from 25.50 – 25.87 ◦C, dissolved oxygen from 5.33 – 5.86 mg/l, pH from 7.25 – 7.36, total ammonia from 0.37 – 0.79 mg/l and total nitrate concentration from 0.71 – 1.84 mg/l. There was no significant difference in all treatments (P>0.05) during the whole experimental period indicating that the experimental diets did not affect water quality of experimental fish.

Table 5.3: Water quality parameter during experimental period

Components	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
DO (mg/l)	5.86±0.19[a]	5.59±0.24[a]	5.33±0.19[a]	5.45±0.19[a]	5.43±0.36[a]
pH	7.26±0.31[a]	7.25±0.24[a]	7.39±0.26[a]	7.36±0.26[a]	7.35±0.27[a]
Tempt (◦C)	25.87±0.58[a]	25.85±0.56[a]	25.63±0.39[a]	25.50±0.28[a]	25.57±0.22[a]
NH_4 (mg/l)	0.52±0.40[a]	0.37±0.27[a]	0.48±0.37[a]	0.52±0.32[a]	0.79±0.44[a]
Nitrate (mg/l)	0.71±0.12[a]	0.73±0.15[a]	1.24±0.51[a]	1.84±0.51[a]	1.13±0.31[a]

* All values are means of three replicates ± SEM for triplicate feeding groups and values in the same row with different superscripts are significantly different (P < 0.05)

5.3.4 Whole Body Composition

Final body composition of tilapia fed with various experimental diets resulted in higher protein, lipid and moisture compared to its initial values. Dietary protein level affected the whole body composition (P<0.05). The whole body protein content of fish fed with diets containing 25%, 50%, 75% SWM was higher (P<0.05) than that of fish fed with Diet 1 (0% SWM). However, crude ash content in the final body composition of experimental fish increased with the increase in dietary SWM. The fish fed with both diets 2 and 3 showed higher protein but lower lipid content in comparison with those fed with other diets. Partial replacement of SWM in diets did not reduce final body composition of dry matter, protein and fat contents compared to the control treatment. Ash content irregularly fluctuated in fish among treatments at the end of the experiment.

Table 5.4: Whole body composition of red tilapia fed with experimental diets (% as dry matter basis)

Component	Initial	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Dry matter	21.10	22.81 ± 0.41[a]	22.91 ± 1.50[a]	21.30 ± 1.46[a]	23.11 ± 0.56[a]	22.14 ± 0.64[a]
Protein	54.08	86.18 ± 0.00[a]	95.21 ± 1.16[b]	92.89 ± 0.05[b]	90.66 ± 0.97[b]	88.85 ± 0.59[a]
Lipid	3.85	18.16 ± 0.09[d]	11.42 ± 0.22[a]	12.63 ± 0.17[b]	15.98 ± 0.12[c]	17.80 ± 0.12[d]
Ash	17.46	19.35 ± 3.19[a]	11.15 ± 2.15[a]	16.59 ± 0.95[b]	18.70 ± 0.24[a]	17.92 ± 1.91[a]

* All values are means ± SEM for triplicate feeding groups and values in the same row with different superscripts are significantly different (P < 0.05).

5.4 Discussion

Numerous studies on use of insects as alternative to fish meal have been described. Adesulu and Mustapha (2000) reported the use of housefly maggot meal as a substitute for fish meal in tilapia and African catfish diets. Bondari and Sheppard (1987) observed that channel catfish and blue tilapia fed on soldier fly larvae for 10 weeks are acceptable as food by consumers. Growth and organoleptic quality are not affected when common carp are fed to non defatted silkworm pupae, a major by-product of the sericulture industry in India (Nandeshaa *et al.*, 2000). Ng *et al.* (2002) demonstrated that meal worm, *T. molitor* larvae meal is highly palatable to the African catfish (*Clarias gariepinus*) and could replace up to 40% of the fish meal component without reducing growth performance of fish.

The results of the present study clearly indicated that the growth performance and feed utilization of red tilapia were affected by the different experimental diets used in this study. Fish growth was best with 25% and 50% replacement of fish meal protein with SWM. Highest growth performance was obtained from the group fed with Diet 2 which indicated that 25% inclusion of SWM can be considered as the most optimum level for SWM inclusion in the diet of *Oreochromis spp.* juvenile. The reduced weight gain as observed when SWM content in the diet was higher than 50% suggested that high level of SWM inclusion led to growth reduction. One of the reasons for this inferior growth of fish fed with diet up to 50% could be attributed to feed consumption by fish. St. Hilaire *et al.* (2007) using rainbow trout *Oncorhynchus mykiss* also observed similar decreased in feed intake in response to fish meal replaced by fly prepupae. However, Sealey *et al*. (2011) commented the differences of feed intake may be attributable to decreased nutrient availability of fly pupae. The fish also seemed to show varying growth performance individually in the replicated tanks. The growth of fish also is highly variable especially in terms of size, being greatly dependent upon a variety of environmental factors such as water temperature and other factors such as degree of competition, the amount and the quality of feed ingested, the age and the maturity of the fish (Moyle and Cech, 2000). Wang *et al.* (1998) suggested that feeding frequency approach can solve the size variation among fish treated. They also suggested that apparent satiation feeding may not lead all fish to be satiated but frequent feeding between certain interval times may increase feeding opportunities of subordinate fish because the individual dominant fish is less aggressive after satiation feeding. Problem of poor growth and size variability could be due to riboflavin deficiency (Murai and Andrews, 1978) or limiting amino acid content (Nengas *et al.*, 1999; NRC, 1993). This assumption denies the high level fat content that resulted in reduced growth performance of fingerlings.

The best FCR will be suitable for growth gain as the fish could convert each gram of feed consumed to be deposited in 1kg of body protein of the carcass. In this present study, the FCR ranged from 1.25 to 1.50 and was within the acceptable range recommended by De Silva and Anderson (1995). The poor conversion ratio reported in Diet 4 and Diet 5 may be attributed to the feeding management, culture system, experimental condition, improper balance of amino acids, high carbohydrates and reduction in pellet quality (Ovie and Ovie, 2007). An adverse effect on growth and poor survival of aquatic organism could be explained by excessive dietary lipid with recommendation of 8% of dietary lipid to ensure best growth and survival (Briggs *et al.*, 1994). FCR and PER in terms of feed utilization efficiency were influenced by dietary treatments. The higher SGR and better FCR were obtained by Diet 2. This result was in agreement with the study managed by Gumus (2009). SGR, FCR and PER of red tilapia were improved slightly when they were fed the diet containing 25% and 50% SWM inclusion in the experimental diets. As the SWM replacement level increased up to

75%, SGR, FCR and PER decreased. PER and FCR also were generally related to digestibility of nutrients. PER is also related to how well the protein sources in a diet could provide the essential amino acid requirement. In addition, the relatively low PER of fish fed with diet containing 75% and 100% SWM compared to fish fed with diet containing 25% and 50% indicates that excess protein was used for metabolic purposes other than growth (Lee *et al.,* 2001). The higher survival rate recorded indicated that feeding *Oreochromis spp.* juvenile with SWM based diets could enhance the survival of fish. A similar trend of 100% survival rate was also reported by Ogunji *et al.* (2008) when *Oreochromis niloticus* fingerlings was fed with maggot meal. In fact, Holm and Torrisen (1987) reported that living organisms incorporated into animal feed such as zooplankton do enhance the survival and healthy state of fish at early stage. They also classified insect also as living organism to be used in the animal feed formulation study.

The whole body protein and lipid contents were significantly higher in fish fed with 25% SWM as compared to fish fed with lower (Diet 1) and higher (Diet 5) SWM inclusion. Fish fed with all experimental diets had higher percentages of protein, lipid and dry matter contents whereas ash was lower in the initial fish sample. These suggested that red tilapia efficiently ingested, digested and assimilated SWM protein as an alternative feedstuff. Body composition of red tilapia fry fed with diets containing various levels of SWM did not significantly differ in this present study. These findings are in agreement with the values reported by Hassan *et al*. (1993) and Nengas *et al*. (1999). The findings of Weatherup and McCracken (1999) and Hasan and Amin (1997) also supported the present result that the final lipid levels were higher and ash was lower than the initial composition. In the present study, the lipid content was slightly higher and ash was lower in fish fed with Diet 4 and 5. The findings were also in agreement with Ogunji and Wirth (2002) who reported that decreased growth and body protein retention were observed in *Oreochromis spp.* fingerlings fed with diets containing extremely low crude protein content of 0.18% dry matter and with a P/E ratio of 0.42. Lovell (1989) also suggested that fish fed with excessive energy content could reduce the feed consumption resulting in reduced growth. In the present study, the uneaten feed was found at dietary lipid treatment up to 11%. Furthermore, the dietary P/E ratio recommended as an optimal growth for tilapia has been established between 16.26 mg kJ^{-1} and 19.43 mg kJ^{-1} (Mazid *et al*., 1979; De Silva *et al*., 1989). The present study showed that the P/E ratio is between this range. P/E ratio seem to be practical index in fish feed formulation since this ratio acted as a mark to avoid fatty fish due to excess energy and excess discharge of ammonia excretion in water (Du *et al.*, 2009).

The present study indicated that 25% and 50% SMW can be included within 32% crude protein diet in *Oreochromis spp.* fingerlings. However, the reduced growth in the present study indicates that additional research is needed to indentify nutritional limitations of this ingredient. Furthermore, the duration of this trial was limited to 8 weeks only and in other studies, the FCR of fish fed with insect known as black soldier fly showed the best value over longer experimental period (Bondari and Sheppard, 1987). A larger trial over a longer period time should be conducted in order to confirm the results of this initial study.