Chapter 4

4.0 Chemical Evaluation of Super Worm Meal for Juvenile Red Tilapia, *Oreochromis spp.*

4.1 Introduction

With the rapid increase in intensive aquaculture, demand for better quality aquafeed kept rising dramatically over the years. Furthermore, a total dependence on fish meal in the aquafeed industry contributes to increase in price. Hence, there is an urgent need for aquafeed manufacturing industry to reduce its dependency on fish meal as the sole protein source. Therefore, the dietary replacement of fish meal within existing aquafeed formulation with alternative protein sources should be seriously considered. Fish meal is still generally the recommended protein source because of its good nutritional quality and biological value to tilapia and other fish species to gain optimum growth (Watanabe *et al*., 1997). However, fish meal is often scarce and expensive, especially good quality brands. The relatively low production of FM but with increasing demand from feed manufacturing industry often lead to an increase in cost of fish production and a decrease in farmer’s net profit (El-Sayed, 2004). According to Tacon (1993), cost effective and practical aquafeeds can be produced without the use of fish meal with no apparent loss in fish growth in some species. Since animal protein sources lacked certain EAA profile content and plant proteins contain a variety of anti nutritional factors, insects seems to be suitable as dietary replacement within aqua feeds compounds as it serves similar nutritional value to get an optimum growth. Giant meal worm or better known as super worm, *Zophobas morio* has a high potential in substituting fish meal which is uncertain in terms of supply and increase in production cost. Ng *et al*. (2001) conducted a research on meal worm (*Tenebrio molitor*), a type of insect having similar physical characteristic to super worm and found that the diet with meal worm inclusion is significantly palatable to African catfish.

A feed ingredient which appears to be an excellent source in its chemical composition, is of little value unless it can be digested and absorbed well in target tissues to be converted into muscle tissue. Ideally, the analysis of ingredients should be done as comprehensive as possible and key variables should be considered important to all ingredients used in experiments. Besides, the method used for ingredient analysis should be consistent with those recommended by the Association of Official Analytical Chemist (AOAC, 1990).

Digestibility is determined by comparing the quantity of a nutrient consumed with that left in faeces at the end of the digestive process. In practical terms, the digestibility of the feed ingredients depends primarily on its chemical composition and the digestive capabilities of the species to which it is fed (Mc Googan and Reigh, 1996). In assessing diet digestibility, indirect method is the most recommended instead of direct method that is linked with some problems and errors. This is because the indirect assessment gives apparent digestibility (Glencross *et al.,* 2007). By adding an inert material (external marker) to the feed or measuring an inert natural component of the food (internal marker), apparent digestibility can be calculated by comparing the ratio of the marker in the food and faeces to the specific nutrient. Most digestibility studies conducted have used external markers and chromic oxide (Cr_2O_3) is the most commonly used marker and has been used extensively in studies with tilapia (Koprucu and Ozdemir, 2005; Guimaraes *et al*., 2007). Incorporation in diets at 0.5 - 1.0% levels, Cr_2O_3 has been demonstrated to be reliable indicator for digestibility studies in fish (Cho *et al*., 1974; De Silva and Anderson, 1995). Feedstuff substitution procedures described by Cho *et al*. (1982) as refined by Forster (1999) and Bureau *et al*. (1999) enable the apparent digestibility of a single ingredient in a multi-ingredient diet to be determined.

The present study was conducted to firstly evaluate the proximate analysis, amino acid and fatty acid profile of ingredients to be used, *Z. morio* in *Oreochromis sp.* diet before their subsequent use in growth study.

4.2 Materials and Methods

4.2.1 Experimental Set-up

Red tilapia juveniles of average weight 6.01 ± 0.04 g were stocked at 10 individuals per aquarium (30 L) in a closed water recirculation system. The fingerlings were obtained from Freshwater Hatchery Center, state of Pahang, Malaysia. There were three replicates for each treatment.

Fish was acclimatized for one week with the commercial diets until they were well adapted to the experimental condition. Fish were fed twice a day (0900 and 1700 hour) at 5% of their body weight. The experiment took 4 weeks to be completed until enough faeces were collected for analysis.

Aquaria were each fitted with oxygen inlets for aeration. 50% of water from all experimental aquaria was changed periodically each two days and a new dechlorinated water was added each time of water changing. Each aquarium contained bottom filter system fitted to an air pump for aeration to maintain a dissolved oxygen concentration of approximately 5.5 – 7.0 mg/L. Water quality parameters including dissolved oxygen, pH, nitrate and ammonia were monitored biweekly.

4.2.2 Diet Preparation

Two diets were formulated which were isonitrogenous (36% crude protein) using the WinFeed software which satisfy the nutrient requirement of Nile tilapia (De Silva *et al*., 1989; NRC, 1993). Two diets between fish meal based diet and super worm meal based diet were compared. The fish meal based diet was used as a reference or control diet upon the test diet, super worm meal based diet. These two diets for apparent digestibility were formulated to fulfill the 30% replacement portion each as described by Cho *et al*. (1985). Chromic oxide (Cr_2O_3) was used as an inert marker for the study at a concentration of 0.5% in the diets. The resulting mixture was pelleted using the mini pelleting plant machine (KCM-Y123M-4) with a 2 mm mesh sieve. The pellets were dried in an oven at 70 °C for 24 hours. They were packed in plastic bags, labelled and kept at room temperature in the laboratory until feeding time (Bureau *et al*., 1999).

4.2.3 Proximate Analysis

Proximate analysis of ingredients and diets samples were conducted using methods described in Chapter 3. Moisture, ash, protein and total fat content of super worm and fish meal were analyzed according to that recommended by the Association of the Official Analytical Chemist (AOAC, 1990). Amino acid and fatty acid profiles were conducted according to the previous procedure (Section 3.5).

4.2.4 Statistical Analysis

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

4.3 Results

4.3.1 Proximate Composition of Ingredient

Dry matter of super worm meal (SWM) demonstrated the highest among the ingredients with 92.49% and mushroom stalk meal (MSM) recorded the lowest value, 88.55%. Crude protein ranged from 0.60 to 55.64% with the fish meal the highest and corn starch the lowest among them. For crude lipid, corn starch stated the lowest value (0.14%) and SWM had the highest recorded value with 40.01%. Moisture content for the ingredients used ranged from 7.51 to 11.45%. The chemical analysis also showed that the ash level was highest in fish meal (FM) followed by mushroom stalk meal. SWM was the most expensive among the other ingredients with RM 20 / kg and MSM was considered as free, it was considered as waste agricultural by-product after harvest time of mushroom. FM price was RM 3.50 / kg was the most expensive compared to the other common raw ingredients used in feed formulation. With respect to crude protein and dry matter content of both diets tested, most values showed similar value with 37.45%, 93.71% and 36.53%, 94.33% respectively for each reference and test diet given. In contrast with the crude lipid mentioned, considerably a major and obvious difference in value with 3.96% for FM and 12.15% for SWM respectively. Reference diet that contained FM was analyzed for crude fiber and the value showed 3.55% of CF content, which was slightly lower than the test diet (5.21%). However, ash content of reference diet (12.72%) was higher compared to test diet (8%). With respect to crude protein and dry matter content of both diets tested, most values showed similar value with 37.45%, 93.71% and 36.53%, 94.33% respectively for each reference and test diet given. In contrast with the crude lipid mentioned, considerably a major and obvious difference in value with 3.96% for FM and 12.15% for SWM respectively. Reference diet that contained FM was analyzed for crude fiber and the value showed 3.55% of CF content, which was slightly lower than the test diet (5.21%). However, ash content of reference diet (12.72%) was higher compared to test diet (8%).

Table 4.1: Proximate analysis of reference and test diets (% as fed basis)

Components	**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

All known seventeen amino acids except tryptophan which considered destroyed during the acid hydrolysis process are present in this current study. All eight essential amino acids present have similar values except methionine which showed a large difference with 5.75 for SWM and 21.17 for FM respectively. SWM had higher value in arginine only and the rest of the essential amino acids values were lower than FM value. FM had higher histidine, threonine, valine, methionine, isoleucine, lysine, phenylalanine and leucine values. 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 4.2: A comparison of amino acid composition of super worm (*Zophobas morio*) meal and fish meal (mg/g crude protein)

Amino acid composition	Super worm meal	Fish meal	Chicken egg	Tilapia requirement[2]
Aspartic acid	70.08 ± 5.89	111.69 ± 5.32	89.2	-
Glutamic acid	125.53 ± 3.70	181.76 ± 4.75	121.3	-
Serine	5.41± 0.27	31.13 ± 1.12	67.2	-
Glycine	24.55 ± 0.53	43.42 ± 1.62	30.2	-
Histidine*	13.86 ± 0.25	14.74 ± 0.44	20.9	17.2
Arginine*	21.91 ± 0.02	11.09 ± 0.49	57.0	42.0
Threonine*	21.23 ± 0.26	26.99 ± 0.58	44.7	37.5
Alanine	37.88 ± 0.32	45.91 ± 1.51	50.3	-
Proline	25.71 ± 0.21	28.24 ± 0.69	N.I	-
Tyrosine	37.05 ± 0.13	23.47 ± 0.22	38.1	-
Valine*	29.37 ± 0.17	34.42± 0.06	54.2	51.2
Methionine*	5.75 ± 0.02	21.17 ± 0.54	28.1	26.8[3]
Cystine	0.86 ± 0.11	2.50 ± 0.31	19.0	-
Isoleucine*	21.41 ± 0.04	29.19 ± 0.54	48.8	31.1
Leucine*	30.21 ± 0.04	47.71 ± 1.24	81.1	33.9
Phenylalanine*	21.93 ± 0.95	33.93 ± 0.46	48.2	37.5[4]
Lysine*	34.25 ± 0.15	69.74 ± 1.44	65.9	51.2
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 ($P<0.05$) [2] Experimentally determined data for *Oreochromis niloticus* from Santiago (1986) [3] Value includes of cystine of ingredients [4] Value includes of tyrosine of ingredients

SWM contained a high concentration of lipids and analysis was made to determine the fatty acid profile of super worm's lipid. Results in Table 4.3 also revealed that the extracted total lipid from super worm sample was dominated by saturated fatty acids (49.65% of total fatty acids) mainly represented by palmitic (C16:0, 17.29% of total fatty acids) and stearic acid (C18:0, 5.69% of total fatty acids). Monounsaturated fatty acids amounting altogether to 26.93% of total fatty acids, were mostly represented by oleic acid (C18:1, 34.10% of total fatty acids) and polyunsaturated fatty acids were present at 23.42% of total fatty acids respectively. The same table also revealed that SWM contained polyunsaturated fatty acids, mostly linoleic (C18:2n-6, 23.42%) which was higher from polyunsaturated fatty acid of FM content.

Table 4.3: Fatty acid composition of super worm (*Zophobas morio*) meal and fish meal (% of total fatty acids)

Fatty acids	**Super worm meal**	**Fish meal**
Myristic acid (C14:0)	0.09	14.32
Myristolic acid (C14:1)	0.07	6.71
Palmitic acid (C16:0)	17.29	24.64
Palmitolic acid (C16:1)	0.45	13.19
Heptadecanoic acid (C17:0)	0.58	2.04
Stearic acid (C18:0)	5.69	7.72
Oleic acid (C18:1)	34.10	10.17
Linoleic acid (C18:2n-6)	23.42	1.19
Arachidoic acid (C20:0)	1.12	1.36
Eicosanoid acid (C20:1)	0.96	3.76
Erucic acid (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

4.4 Discussion

SWM had slightly higher crude protein of 42.83% than 19.01% as reported by Finke and Winn (2004). This disparity can be attributed to the factors such as source, stage of harvesting, methods of processing and drying as suggested by Ojewola *et al.* (2005). Crude lipid of SWM, super worm meal obviously was higher (40.01%) compared to other ingredients. This might be as a result of feed that was fed to this insect larvae during their growth. Also, SWM had favorable ash content (3.54%) which was evident of the presence of exoskeleton made of chitin. Super worm is one of the insects that contain chitin but predominantly chitin consists of protein compound in cuticle (Kramer *et al.*, 1995). Finke (2007) found that the average chitin in meal worm is around 49.8 mg/kg on dry matter basis. Sakai *et al.* (1992) found that chitin could help to stimulate macrophage activities in rainbow trout. In contrast, Shiau and Yu (1999) found that chitin does not promote growth and nutrient digestibility in tilapia. Chitin can also bind dietary lipid resulting in reducing plasma cholesterol and triglycerides (Koide, 1998). Consequently, the intestinal absorption of lipid is reduced.

Powell and Rowley (2006) found that supplementation of pure chitin also does not affect the survival and immune reactivity of adult shore crab (*Carcinus maenus*).

The nutritive value of protein of any ingredient mainly depended on its protein capacity to fulfill the needs of organisms with respect to essential amino acids. Strom and Eggum (1981) stated that lysine, methionine and cystine are the most important amino acid for fish from a nutritional viewpoint. The availability of essential amino acids especially histidine in *Z. morio* matches with the requirements listed by Santiago (1986). Santiago (1986) had compared EAA profiles of egg with the tilapia requirement for EAA. Mambrini and Kaushik (1994) suggested that total non essential amino acids (NEAA) should be less than 60% of total protein in *O. niloticus* feeds when alanine, glutamic acid or glycine were fed separately and less than 50% when these three amino acids were used in combination. Considering the 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 can also be synthesized from the essential amino acid precursor such as cystine synthesis can be done by methionine conversion (NRC, 1993). It has been estimated that cystine can spare 40 – 60% of methionine in the diets for various fish (Wilson, 2002). For *Z. morio* likely could not contribute or spare the synthesis of methionine from cystine due to low content of cystine. Thus, supplementation of crystalline amino acid to balance methionine content is the best approach (Sveier *et al.,* 2001). 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*. Choo *et al.* (1991) reported that excessive leucine could cause decreased feed intake and protein deposition of rainbow trout. Leucine content (30.21 mg/g) in SWM is not excess the tilapia requirement range and no sign of depressed growth is recorded. The present results indicated that this insect contained seventeen known amino acids, including all the essential amino acids and non essential amino acids. The effectiveness of amino acids in fish was reported by several earlier workers for Japanese flounder (Kim *et al.*, 2003), rainbow trout (Riley *et al.*, 1996) and cobia (Salze *et al.*, 2008).

Fatty acids play an important role in finfish nutrition. Their main functions are particularly as a source of energy for fish bioenergetics and physiology (Trushenski *et al.*, 2006). Tilapia is considered to require greater amount of n-6 fatty acids rather than n-3 fatty acids in their diets (Takeuchi *et al.*, 2002). Santiago and Reyes (1993) reported beneficial aspect of n-6 fatty acids that can enhance spawning success and fry production in *O. niloticus*. High level of n-3 fatty acid is reported to decrease growth performance of tilapia (Kanazawa *et al.*, 1980). However, it was observed that amount of n-6 fatty acid is high in linoleic acid content (23.42%). Olsen *et al.* (1990) reported that *O. niloticus* has the ability to convert both linoleic and linolenic acids to the longer chain and physiologically important highly unsaturated fatty acid (HUFA) via a series fatty acid conversion and elongation. Recent report made by Nandeesha *et al.* (1999) revealed that silkworm pupa oil is rich in short chain unsaturated fatty acids and has been found to be an excellent energy source of diet in common carp. Clearly, replacement of animal derived lipid is advantageous as replacement of animal derived protein sources. Sealey *et al.* (2011) replaced portion of fish oil with oil derived from black soldier larvae in the diet of rainbow trout and found no deleterious effect on growth and food conversion efficiency. Thus, replacement of fish oil improved nutritional quality of fish muscle and oxidative fillet stability. Data from the result showed that PUFA / SFA ratio in the lipids of super worm meal was significantly higher (0.87) than the recommended 0.45 for a healthy diet. Ratio of polyunsaturated to saturated fatty acid (PUFA / SFA) has been widely used to determine the cholesterol lowering potential of feed. As far as nutrition is concerned, fat intake could be interpreted based on the optimal ratio between saturated and unsaturated fatty acids. In this study, linoleic acid (C18:2n-6) of SWM was higher in value (23.42%) than FM (1.19%). However, studies found that the absence of inadequate dietary supply of 18-C fatty acids of n-3 and n-6, desaturation of 18:1(n-9) will occurr and more unsaturated series of n-9 will accumulate into tissue lipids (Jobling, 1994). This finding is meant to be essential to fish, which lack the ability to synthesize this biological compound to meet biological demands (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).

In conclusion, the result of this study showed that the nutrient content of super worm meal need to be slightly improved in order to be a good alternative for red tilapia. FM was obviously better digested than SWM and the disparity can be improved. Further studies need to be conducted on SWM utilization as a substitute of FM to determine the distribution of fatty acid concentrations in fish muscle and the effect on fillet quality.