Chapter 6

6.0 Effects of Amino Acid Supplementation in Super Worm Based Diets on Growth Performance and Feed Utilization of Juvenile Red Tilapia

6.1 Introduction

Fish meal scarcity and its continual price increase have led to the interest among fish nutritionists to search for alternative protein sources. The development of practical diets from non FM protein sources has the potential to reduce feed. Generally, the common constraint of plant or animal derived protein sources are their deficiencies in one or more essential nutrients and most contain some anti nutritional factors (ANF) embedded within them. Imbalance of EAA profile may also be an obstacle among animal protein sources.

Supplementing crystalline amino acid in diets to optimize the AA requirement has been common in the terrestrial livestock industry has been achieved with good results (Lewis and Bayley, 1995). Inclusion of crystalline amino acid in feed has been used in aquaculture both in experimental diets and commercial feed production. Supplementation of the crystalline amino acid is an attempt to improve protein quality of fish feeds by the addition of essential amino acids. Teshima *et al.* (1986) determined that small amount of crystalline amino acid could be used in research diets fed to *M. japonicus* larvae. Supplementation of casein with crystalline L-arginine improved its nutritive value and yielded similar growth than fish fed to live feed control. Several investigators have attempted to improve protein quality of practical diets by supplementing amino acid deficient protein with crystalline amino acids (Teshima and Kanazawa, 1988; Webster *et al.,* 1995). Some EAAs, such as methionine and lysine are generally critical in the formulation of fish feed (Tacon, 1990). For most EAAs, deficiency is illustrated in the reduction of weight gain. In some species of fish, a deficiency of methionine or tryptophan leads to pathological symptoms because EAAs are not only incorporated into proteins but also used for the synthesis of other compounds (Lovell, 1989).

Tilapia has a requirement for sulphur-containing amino acids which can be met by either adding methionine alone or a proper mixture of methionine and cystine (Shiau, 2002). Dietary cystine can replace up to 50% of the total sulphur-containing amino acid requirements for *Oreochromis mossambicus* (Jauncey and Ross, 1982). There exists a relationship between methionine and cystine, two important sulphur-containing amino acids. Cystine can be considered as non essential amino acid because it can be synthesized by the fish through methionine, an EAA. Therefore, if methionine is fed without cystine, a portion of methionine is used for protein synthesis and another portion is converted to cystine. Once cystine is included into diet, it reduces the amount of methionine required. Thus, the fish has a total sulphur amino acid requirement rather than specific methionine requirement (Wilson, 1989). The total amino acid requirement containing methionine + cystine and also lysine in Nile tilapia nutrition has been reported to be 2.7% and 5.1% of the total protein respectively (Santiago and Lovell, 1988). Supplementation with DL-Methionine is an efficient way to compensate amino acid deficiency in fish (Shiau *et al.*, 1987) but the use of this substance in insect-based diets has yet to be investigated.

Dietary amino acid utilization requires that all amino acids are present in adequate concentrations at the site of protein synthesis. Hence, deficiency of an EAA limits the protein synthesis to the level of that particular EAA (Svevier *et al.*, 2001). For instance, deficiency of methionine causes trout to produce cataracts and suffer poor growth as well as survival (Keembiyehetty and Gatlin III, 1993). Supplementation of methionine naturally depends on feed ingredients used in formulation. Too much or too little level of methionine could cause severe effects on the fish itself. For instance, excess supplementation of methionine could cause low feed intake and consequently lead to growth retardation (Benevenga and Steel, 1984).

Several studies also demonstrated that fish utilize crystalline amino acid less efficiently than the protein bound form (Yamada *et al.*, 1981; Schuhmacher *et al.,* 1997; Sveier *et al.,* 2001). Crystalline amino acid has been reported to be successfully used to supplement EAA deficiency diets, improving fish growth and fish utilization efficiency (Murai *et al.,* 1986; Williams *et al.*, 2001). Jackson and Capper (1982) confirmed that free EAA are well utilized by *O. mossambicus*. El-Saidy and Gaber (2002) and Furuya *et al.* (2004) reported increased performance of Nile tilapia when diets are supplemented with crystalline amino acid. Webster *et al.* (1995) reported similar result in that the inclusion of essential amino acids in the diets of blue catfish (*I. furcatus*) resulted in high performance compared with the diets with high fish meal contents.

In the previous study, most of the super worm-based diets were deficient in one or more essential amino acid particularly methionine resulting in a lower performance compared to fish meal-based diet. In this study, this diet was formulated on the basis of 50% SWM inclusion and then supplemented with crystalline amino acid, DL-Methionine. The objective of this study therefore was to investigate whether supplementing an amino acid to the super worm meal based diets for red tilapia could improve growth and feed utilization.

6.2 Materials and Methods

6.2.1 Experimental System and Animal

The rearing system consisted of eight modified glass aquaria of 30 L capacity based on Guelph system designed to collect faecal material of the fish. Each diet treatment was done in triplicate.

Red tilapia, *Oreochromis spp.* juveniles weighing 6.00 ± 0.07 g were stocked at 10 fish per aquarium. Fish were obtained from Freshwater Hatchery Center, Bukit Tinggi district, state of Pahang, Malaysia. The feeding trial was conducted over 56 days. One week prior to the experiment, 80 fish were acclimatized to their rearing environment and fed with a commercial diet (Takara Sakana-II, Fish Food, Kian Weng Trading Co.). Within this period, all dead and apparently stressed fish were removed. At the start of the experiment, a sample of 10 fish was removed from the total population for proximate carcass analysis.

Water in all experimental aquaria was changed periodically. Each aquarium contained bottom filter system fitted with aeration by air pump to maintain a dissolved oxygen concentration in the water at a constant range of 5.5 – 7.0 mg/L. Water quality parameters including dissolved oxygen, pH, nitrate and ammonia were monitored biweekly.

6.2.2 Diet Preparation and Feeding Regime

Four test diets were prepared. The chemical composition and formulation of diets are shown in Table 6.1. The diets containing the super worm meal was supplemented with various levels of DL-Methionine level, 0% (control), 0.5%, 1.0% and 1.5%. In this experiment, only the limiting EAA (methionine) was supplemented (Shiau, 2002). The procedure of pelleting the diets was similar to what was described in chapter 3. Fish were fed daily manually twice a day (0900 and 1700 h) at the rate of 10% of their body weight. After each biweekly weighing, ration was adjusted according to body weight for the next period.

6.2.3 Analytical Procedures and Analysis of Experimental Data

The proximate analysis of diet, ingredients, faeces and carcass was performed according to the procedures described in Chapter 3, section 3.4. Growth performance and feed utilization were calculated as described in Chapter 3, section 3.7. Amino acid profiles were conducted according to the previous procedure (Chapter 3, Section 3.5). Protein to energy ratio were calculated over each diet and expressed in unit of mg protein kJ^{-1}.

6.2.4 Statistical Analysis

Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS version 12.0 as described in section 3.9. Data was subjected to an analysis of variance and Duncan multiple-range test was used to evaluate specific differences between treatments test at 5% probability level.

6.3 Results

6.3.1 Proximate Composition of Diets

The diets were supplemented with DL-Methionine and made to be isonitrogenous (35% crude protein content). Crude protein of all the diets remained in the range of 34.87% to 35.52%. This trend of similar value among the diets was followed by crude lipid content value and ranged between 10.56% and 11.15%. Crude ash content was higher in Diet 2 (10.29%) and Diet 4 was noted to be the lowest value among others with 9.93%. Crude fiber, nitrogen free extract and gross energy values in all diets tested were close to each other, with slight variation but in the range between 3.34 – 3.70%, 40.06 – 41.01% and 464.14 – 470.31 kcal/g respectively. The essential amino acid (EAAs) showed that the methionine level of each diet was of closely similar value after supplementation.

Table 6.1: Composition of super worm-based diets supplemented with DL-Methionine fed to *Oreochromis spp.* juveniles (g/kg)

Ingredients	**Diet 1 (0%)**	**Diet 2 (0.5%)**	**Diet 3 (1.0%)**	**Diet 4 (1.5%)**
Fish meal	15	15	15	15
Rice bran	24.74	24.10	23.47	22.83
Soy bean meal	23.26	23.40	23.53	23.67
SWM	15	15	15	15
Corn starch	15	15	15	15
Di-calcium phosphate	1	1	1	1
Squid meal	5	5	5	5
Vitamin premix	0.2	0.2	0.2	0.2
Mineral premix	0.3	0.3	0.3	0.3
Chromic oxide	0.5	0.5	0.5	0.5
DL-Methionine	0	0.5	1.0	1.5
Nutrients (% as fed basis)				
Dry matter	91.68	91.43	91.83	92.17
Crude protein	34.87	35.35	35.34	35.52
Crude lipid	10.82	10.60	10.56	11.15
Crude ash	9.94	10.29	10.02	9.93
Crude fiber	3.36	3.70	3.39	3.34
NFE[1]	41.01	40.06	40.69	40.06
Gross energy[2]	467.41	464.14	466.29	470.31
P/E ratio (mg protein kJ^{-1})	18.82	19.24	19.14	18.92
Essential amino acids composition[3]				
Histidine	7.58 ± 0.89^a	7.84 ± 0.04^a	6.47 ± 0.04^a	7.21 ± 0.15^a
Arginine	24.29 ± 3.25^a	23.89 ± 1.87^a	21.47 ± 0.12^a	21.72 ± 0.48^a
Threonine	1.76 ± 0.28^a	1.79 ± 0.17^a	1.46 ± 0.12^a	1.46 ± 0.01^a
Valine	19.32 ± 1.06^a	18.79 ± 0.83^a	17.23 ± 0.08^a	17.01 ± 0.16^a
Methionine	5.68 ± 1.17^a	8.69 ± 0.28^b	13.31 ± 0.09^c	17.44 ± 0.05^d
Isoleucine	14.98 ± 0.48^b	14.64 ± 0.56^b	13.65 ± 0.04^b	13.46 ± 0.04^a
Leucine	26.04 ± 0.68^b	25.48 ± 0.94^b	24.23 ± 0.09^b	23.35 ± 0.04^a
Phenylalanine	15.69 ± 0.69^a	15.68 ± 0.43^a	14.41 ± 0.08^a	14.26 ± 0.20^a
Lysine	16.30 ± 0.54^a	17.99 ± 0.37^b	15.76 ± 0.12^a	15.27 ± 0.05^a

* All values are means ± SEM for triplicate feeding groups and values in the same row with different superscripts are significantly different ($P < 0.05$). [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] Values are mean of duplicate determination ± SE ; 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.

6.3.2 Growth Performance and Feed Utilization

All the diets were well accepted by all fish. No significant differences (P>0.05) in the final weight of fish and SGR with Diet 3 and Diet 4 showing higher values in terms of final weight. In contrast, weight gain of fish Diet 2, Diet 3 and Diet 4 was higher (P<0.05) than fish fed with Diet 1. SGR of fish fed with Diet 1 (1.02) was lower compared to other diets and Diet 3 was recorded as the highest (1.53). The growth performance of fish increased as level of methionine supplementation increased dramatically but slightly decreased in Diet 4. Diet 2 and 3 were best utilized by the fish as it gave the following biological parameter: SGR, 1.35 and 1.53 day^{-1}; FCR, 1.59 and 1.86; PER, 2.22 and 1.87 respectively.

The data also showed that FCR of fish fed with the diets offered was not significantly different (P>0.05). The highest protein efficiency ratio (PER) was recorded by Diet 1 (0% methionine supplementation) with 3.35 while the lowest value of 1.87 was recorded in Diet 3 (1.0% methionine supplementation). Survival rate was not significantly affected (P>0.05).

Table 6.2: Growth performances and feed utilization of *Oreochromis spp.* juveniles fed with experimental diets*

Components	**Diet 1 (0%)**	**Diet 2 (0.5%)**	**Diet 3 (1.0%)**	**Diet 4 (1.5%)**
Initial weight, g	6.00 ± 0.07^{b}	5.12 ± 0.26^{ab}	4.78 ± 0.04^{a}	5.33 ± 0.55^{ab}
Final weight, g	10.75 ± 2.38^{a}	10.95 ± 0.74^{a}	11.28 ± 0.43^{a}	11.37 ± 0.58^{a}
Weight gain, %	79.16 ± 2.31^{a}	113.86 ± 0.47^{b}	135.98 ± 0.38^{b}	112.01 ± 1.13^{b}
SGR[1]	1.02 ± 0.37^{a}	1.35 ± 0.02^{a}	1.53 ± 0.05^{a}	1.33 ± 0.27^{a}
FCR[2]	1.15 ± 0.37^{a}	1.59 ± 0.03^{a}	1.86 ± 0.02^{a}	1.67 ± 0.17^{a}
PER[3]	3.35 ± 1.63^{a}	2.22 ± 0.79^{a}	1.87 ± 1.10^{a}	2.28 ± 0.44^{a}
Survival, %	80.00 ± 28.84^{a}	95.00 ± 7.07^{a}	95.00 ± 7.07^{a}	90.00 ± 0.00^{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). [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).

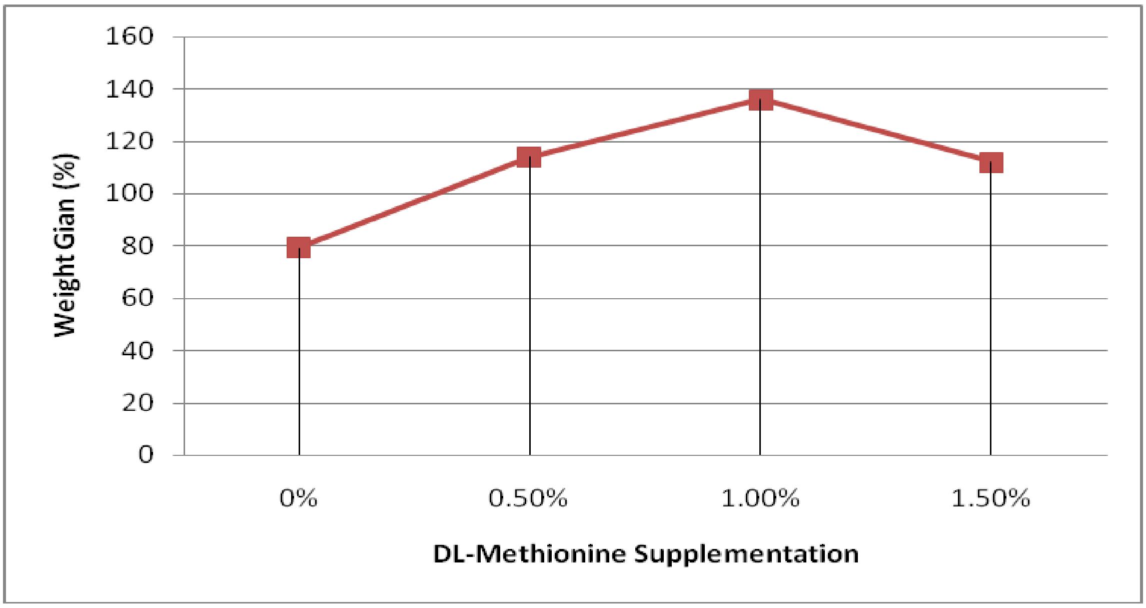

Figure 6.1: Growth performance of juvenile tilapia *Oreochromis spp.* fed with the experimental diets over a 56-day trial

6.3.3 Whole Body Composition

There were no significant differences (P>0.05) in whole body protein, lipid and ash contents among the fish fed with those diets. These values were higher than initial whole body composition with the exception of ash content which resulted in a slight fluctuation value among treatments. The percentage of crude protein in fish fed with Diet 3 (76.65%) was significantly higher (P<0.05) compared to fish fed with Diet 1 (72.71%) which was the lowest value among others but not significantly different (P>0.05) with the fish fed with Diet 2 (73.97%) and Diet 4 (74.32%). For dry matter content, fish fed with Diet 1 was significantly higher (P<0.05) (27.79%) compared to fish fed with Diet 3 (23.35%) but not significantly different (P>0.05) with fish fed with Diet 2 (26.03%) and Diet 4 (24.54%). The whole body of lipid content of fish varied 5.23% - 6.38%from but the differences were not significant.

Table 6.3: Whole fish body composition of red tilapia fed with experimental diets (% as dry matter basis)

Components	Initial	Diet 1	Diet 2	Diet 3	Diet 4
Dry matter	21.10	27.79 ± 0.97[b]	26.03 ± 1.22[b]	23.35 ± 0.54[a]	24.54 ± 1.15[b]
Protein	54.08	100.69 ± 0.97[a]	100.00 ± 1.22[a]	100.00 ± 0.54[a]	98.48 ± 1.15[a]
Lipid	3.85	5.78 ± 0.06[a]	6.38 ± 0.38[a]	5.23 ± 0.03[a]	5.32 ± 1.35[a]
Ash	17.46	16.16 ±14.98[a]	18.57 ± 6.28[a]	21.43 ±1.64[a]	20.32 ± 0.31[a]

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

6.3.4 Water Quality Parameter

All the water quality parameters Table 6.4 showed no significant difference ($P>0.05$) in all treatments. All the values were acceptable and the fish did not show any pathological signs of depression during trial.

Table 6.4: Water quality parameters during experimental period

Components	Diet 1	Diet 2	Diet 3	Diet 4
DO (mg/l)	5.34 ±0.48[a]	4.96 ± 0.21[a]	4.83 ± 0.29[a]	4.95 ± 0.49[a]
pH	7.60 ± 0.41[a]	7.44 ± 0.52[a]	7.43 ± 0.42[a]	7.39 ± 0.46[a]
Tempt (◦C)	26.22 ± 1.63[a]	26.42 ± 1.31[a]	26.40 ± 1.04[a]	25.42 ± 1.68[a]
NH_4 (mg/l)	0.31 ± 0.38[a]	0.18 ± 0.22[a]	0.28 ± 0.28[a]	0.26 ± 0.16[a]
Nitrate (mg/l)	1.25 ± 0.22[a]	1.71 ± 0.25[a]	0.85 ± 0.18[a]	1.75 ± 0.95[a]

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

6.4 Discussion

Supplementing crystalline amino acid in fish diets gives different results. According to Shiau *et al.* (1989), male tilapia (*O. niloticus* x *O. aureus*) fed diets in which 100% of the fish meal was replaced with soybean meal (SBM) either with or without methionine supplement has a significantly lower weight gain, FCR and protein digestibility compared with groups fed with fish meal as a sole protein source. Bai and Gatlin (1994) recorded that supplementation of L-lysine to a diet with 25% crude protein from soybean does not improve the growth of channel catfish. However, DL-Methionine supplementation has been demonstrated beneficial in other fish (Murai *et al.,* 1982; Pongmaneerat *et al.,* 1993). The variation in crystalline amino acid requirement within and among fish species has been observed and this variation due to the use of different basal diets, different feeding levels and environmental conditions as well as using different stage of fish.

There were no significant differences in growth performance and feed utilization among fish fed with SWM based diets supplemented with methionine (Table 6.2) except for weight gain of Diet 2 to 4 showed a significant different. Polat (1999) reported that an excess dietary supplementation of Methionine up to 0.5% in a tilapia diet depresses its growth rate. Li and Robinson (1998) and Yamamoto *et al.* (2005) stated that supplementation of EAA in an insufficient diet of channel catfish and rainbow trout respectively improves feed efficiency but does not enhance fish growth. However, fish fed 1.5% methionine supplementation showed slight decrease in weight gain compared those fed lower methionine level. This might be attributed to disproportionate free amino acid absorption rates and the leaching of dietary crystalline AA during feeding of fish. Yamada *et al.* (1981) reported that free amino acids are absorbed faster than protein bound amino acids. In order to minimize leaching occurring, Lovell (1998) and Tantikitti and March (1995) recommended that feeding with smaller quantities of feed be done at the right moment has yet to be proven to increase protein retention efficiency. Liu *et al.* (2002) reported that pre coated L-lysine were tested and achieved significantly higher values of growth performances compared to uncoated diets. In that experiment showed that coating of the amino acid supplemented diets can avoid AA leaching. The lower growth recorded in Diet 4 than those methionine-supplemented diets, this can be considered as an improvement because these values (WG, FCR, PER, SGR) were higher and better from the control diet (0 % methionine supplementation). In other cases, methionine supplementation had caused an increase in free methionine sulphoxide (MSO) in serum of *Oncorhynchus mykiss* (Nose, 1974) and *Dicentrarchus labrax* (Thebault, 1985). It seems there is a pathway of methionine degradation of MSO in some fish species that lead to fish toxicity. Lovell (1984) revealed that the depressive effect of excess methionine supplementation was due to inhibitory effect of MSO in glutamine synthesis, which has a role in detoxification of NH_3.

The lower weight gain of fish fed a diet without and excess methionine supplementation could be explained by lower feed voluntary intake as described by Mambrini *et al.* (1999). Excess methionine also could lead to high serine in plasma causing to fish toxicity (Harper *et al.*, 1970). In addition, survival of fish (80 – 100%) was considered high with no sign of specific diseases such as cataract related to methionine deficiency or its excess (Cowey *et al*., 1992). In the present study, FCR and PER values for fish fed with Diet 2 and Diet 3 showed that the fish well utilized and absorbed amino acid supplementation in each diet. FCR increased with increasing amino acid supplementation level with 0.5% supplementation showed the best value. Similar trend has been reported by Furuya *et al*. (2004) using diet supplemented by essential amino acid in feeding Nile tilapia.

Dietary amino acid supplementation resulted in an increase in whole body protein and whole body lipid in the fish although there were no significantly differences. This is similar to Webster *et al.* (1995) who found no significant differences in the percentage of moisture, protein and lipid in blue catfish fed L-Methionine supplemented diets. Conversely, Robinson (1991) reported that amino acid supplementation decreased the whole body lipid in channel catfish diets with increasing level of lysine. Supplementation of methionine in all levels resulted in an increase in protein and fat values as compared to initial body composition with no supplementation of methionine (control). This result was similar with the reports by El-Saidy and Gaber (2002) in Nile tilapia and Mukhopadhyay and Ray (2001) in rohu. The lowest protein content was found in the Diet 4. It was due to excessive amino acids supplementation beyond optimal level would be no longer used for protein synthesis but was used for catabolism for lipid synthesis and deposited as tissue fat. In addition, similar results achieved by Yang *et al.* (2010) as they found whole body protein content was low at beyond optimum level of methionine supplementation in grass carp.

In conclusion, the present study revealed that supplementation of 0.5% and 1.0% DL-Methionine effectively improved growth performance and feed utilization of Nile tilapia juvenile compared to control diet, SWM-based diet. It is further concluded that red tilapia fingerlings are able to effectively utilize a diet having 35% protein supplemented with 0.5% DL-Methionine for maximizing growth response. However, more detailed experiments on DL-Methionine in different dietary protein level are required to understand its efficiency on growth response of *Oreochromis* spp. and on synthesis of biochemical constituents.