Chapter 7

7.0 Effect of Mushroom Supplementation as a Prebiotic Compound in Super Worm Based Diet on Growth Performance of Juvenile Red Tilapia

7.1 Introduction

One of most common way to treat most bacterial infections in aquaculture is to apply antibiotics treatment. However, the excessive use of antibiotics in industry has been criticized as it causes direct threat to the microbial gut system in fish and could possibly develop more resistant pathogenic bacteria. It also contributes to the accumulation of residual drugs in body tissue of cultured fish. Certain antibiotics have a negative effect on immune systems by causing the cultured organism to be more susceptible to viral infections. Of late, there is a trend that being prebiotic is proposed to be one of the health promoting ways in developing dietary supplementation strategies in diet preparation as an alternatives antibiotics. Prebiotic compound is referred to as a non digestible feed ingredient that beneficially affects the host by stimulating growth and improving its intestinal condition (Gibson and Roberfroid, 1995).

Gibson *et al*. (2004) stated that dietary carbohydrate such as fiber has a potential as prebiotic candidate with oligosaccharides as the most promising component. One commercial prebiotic, GroBiotic-A (consisting of a mixture of partially autolyzed brewer yeast, dairy ingredient component and dried fermentation products) has proven to be able to increase resistance of hybrid striped bass against a variety of viral pathogens (Li and Gatlin, 2004). Although prebiotic compounds are mostly plant derived additives and fibers, the prebiotic potential of oligosaccharides and other dietary fibers may have interesting applications in aquaculture to stimulate gut health as well as suppress deleterious bacteria. Effect of prebiotic on fish gut microbiota and morphology of the intestine have been investigated (Olsen *et al.,* 2001; Torrescillas *et al.,* 2007; Yilmaz *et al.,* 2007). In fish fed the MOS supplemented diets, growth was significantly

improved and increased head kidney macrophages activity (Torrescillas *et al.,* 2007). Yilmaz *et al*. (2007) showed that dietary MOS incorporation had no detrimental effects on the intestinal morphology in rainbow trout.

Several studies have demonstrated prebiotic effect on growth parameters in fish. Supplementation of Beluga (*Huso huso*) diet with 1 - 3% of inulin showed negative relationships between some performances indices including weight gain, specific growth rate, protein efficiency ratio, energy retention, feed efficiency and protein retention. Furthermore, the body composition may be affected by dietary prebiotic. However, the body protein of rainbow trout and hybrid tilapia concentration increases as the dietary level of MOS (mannanoligosaccharides) is increased from 1.5 to 4.5 g/kg (Genc *et al.,* 2007; Yilmaz *et al.,* 2007). In contrast, supplementing the diet with 10 g/kg of MOS resulted in a decrease in the body protein concentration of salmon (Genc *et al.,* 2007a). Sado *et al.* (2008) reported that 4 - 6 g inclusion level of MOS significantly improves weight of juvenile tilapia. It also shows approximately 15.4% increase and resistance against *Streptococcus agalactiae*. MOS is a beneficial feed supplement to Nile tilapia fry (Samrongpan *et al.,* 2008). Besides the feed price issue, tilapia farmers are also burdened with disease and growth problems. The most common disease of tilapia is caused by Gram positive bacteria namely, *Streptococcus agalactiae* (Darinya *et al.,* 2007). *Streptococcus* spp. is very common during the summer season in conjunction with limited and poor water quality supply. It is widely demonstrated that fish are more susceptible to disease agents. Chemotherapeutants, vaccines and immunostimulants are used to combat infectious outbreaks. More recently, the administration of prebiotic and probiotic has appeared to be a very promising control measurement for disease prevention in fish farming (Soroush *et al.*, 2011). Thus, introduction of prebiotic in the diet could help to solve this type of problems in the aquaculture industries.

Mushroom can be a potential prebiotic to be incorporated into fish diet as it contains carbohydrates and has also been associated with several health promoting effects (Oyetayo and Oyetayo, 2005). Mushrooms are food substances that are rich in non digestible dietary fiber which belongs to β-glucan, chitin and heteropolysaccharides (Mizuno, 1999). Apart from that, these substances also may prevent from viral infection by enhancing selectively the growth of probiotic bacteria in the large intestine of target species. Several attempts to include mushroom in livestock feed has been reported by Kaur *et al*. (2010) and Yousefian and Amri (2009) reported prebiotics that have the numerous beneficial effects in fish such as improved nutrient availability. The objective of this study was to evaluate mushroom supplementation as prebiotic compound into the super worm meal-based diets for red tilapia and to observe its effect on fish growth performances.

7.2 Materials and Methods

7.2.1 Experimental System and Fish

10 fingerlings of red tilapia with average body weight of 5.57 ± 0.15 g were stocked in triplicate in 30 L aquarium tanks each. Fish was acclimatized for one week with commercial diets until it adapted well with the experimental condition. Fish were then hand fed, twice a day (0900 and 1700h) at the rate of 10% of their body weight. The feeding trial period was eight weeks. The feed ratio was adjusted biweekly each time after the fry were weighed on an electronic top pan balance (AND EW-I Series).

Each aquarium contained a bottom filter system fitted with aeration by air pump to maintain a dissolved oxygen concentration in the water at the constant state of approximately 5.5 – 7.0 mg/L. Water from all experimental aquaria was changed biweekly.

7.2.2 Experimental Diets

Mushroom stalks were obtained from a commercial supplier (Gano Farm Homestay) at Tanjung Sepat district, state of Selangor, Malaysia where this agricultural by-product was given freely by the farm owner as it was considered as a waste product after the harvesting period. The mushroom was oven dried at 70 °C overnight to remove moisture. After drying, it was grounded to become meal and stored at room temperature before using in diet formulation. The mushroom stalk meal was subjected to proximate analysis.

Four experimental isonitrogenous diets (32% crude protein) were formulated using WinFeed version 2.8 Software (Least Cost Feed Formulation). The control diet was prepared with FM being replaced by SWM at 50% inclusion level. The other three diets containing the super worm meal at 50% replacement portion was supplemented with various level of mushroom stalk meal level, 0% (control), 10%, 15% and 20%.

7.2.3 Analysis of Experimental Data

The chemical analysis of diet, ingredients 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 3.8. Amino acid profiles were conducted according to the previous procedure (Chapter 3, 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 for each diet and expressed in unit of mg protein kJ^{-1}. Water quality parameters were measured using the method used by APHA (1992).

7.2.4 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.

7.3 Results

7.3.1 Proximate Composition of Diets

Table 7.1: Composition of super worm-based diets supplemented with mushroom stalk meal fed to *Oreochromis spp.* juveniles (g/kg)

Ingredients Mushroom stalk meal inclusion	**Diet 1 (0%)**	**Diet 2 (10%)**	**Diet 3 (15%)**	**Diet 4 (20%)**
Fish meal	15	15	15	15
Rice bran	27.87	18.06	13.16	8.26
Soy bean meal	25.13	24.94	24.86	24.74
Super worm meal	15	15	15	15
Corn starch	15	15	15	15
Di-calcium phosphate	1	1	1	1
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
Mushroom stalk meal	0	10	15	20
Nutrients (as fed basis)				
Dry matter	96.11	96.42	96.26	96.39`
Crude protein	33.58	33.44	33.84	31.91
Crude lipid	10.57	9.48	8.61	7.79
Crude ash	8.63	8.35	9.18	7.16
Crude fiber	2.44	3.32	4.97	5.12
NFE[1]	44.78	45.41	43.40	48.02
Gross energy[2]	473.21	464.70	450.50	450.79
P/E ratio (mg protein kJ^{-1})	17.48	18.12	18.96	17.81
Essential amino acid composition[3]				
Histidine	10.65 ± 0.08^{c}	8.26 ± 0.29^{b}	7.11 ± 0.01^{ab}	7.38 ± 0.24^{a}
Arginine	23.50 ± 0.15^{a}	22.74 ± 1.33^{a}	21.88 ± 1.27^{a}	22.08 ± 0.17^{a}
Threonine	16.03 ± 0.37^{a}	14.46 ± 0.81^{a}	14.26 ± 0.77^{a}	16.48 ± 0.38^{a}
Valine	18.82 ± 0.07^{c}	18.07 ± 0.11^{b}	17.99 ± 0.12^{b}	17.37 ± 0.17^{a}

Methionine	9.07±0.21[b]	5.64±0.02[a]	5.23±0.07[a]	5.86±1.19[a]
Isoleucine	17.18±0.14[c]	15.24±0.18[b]	14.86±0.12[a]	14.67±0.08[a]
Leucine	26.95±0.12[b]	27.00±0.23[b]	26.52±0.01[a]	26.00±0.13[a]
Phenylalanine	22.58±0.03[a]	18.48±1.97[a]	20.29±0.92[a]	17.57±1.22[a]
Lysine	20.44±0.16[b]	19.04±0.24[a]	19.03±0.10[a]	18.54±0.46[a]

* All values are means of two 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.

7.3.2 Growth Performance and Feed Utilization

No significant differences ($P>0.05$) in the mean final weight were observed among treatments although the highest value recorded in fish fed Diet 2. However, weight gain showed a significant difference between Diet 2 and Diet 3. FCR value in Diet 2 also significantly higher compared to other diets. SGR varied among the treatments ranging from 1.63 – 1.74% day^{-1}. Diet 4 showed slight decrease in weight gain and SGR value as level of mushroom stalk meal incorporation was increased. No significant difference ($P>0.05$) in PER and FCR among the diets was observed. The highest FCR was observed in Diet 4 followed by Diet 3, Diet 1 and Diet 2. It is obvious that survival rate of fish fed with the four experimental diets varied from 93.33 – 73.33%. The highest value was shown in Diet 2 (93.33%) followed by Diet 1 (83.33%) and the lowest value was shown in Diet 4 (76.67%) followed by Diet 3 (73.33%). There was no significant difference in survival rate ($P>0.05$) among the treatments.

Table 7.2: Growth performances and feed utilization of red tilapia juveniles fed with experimental diets

Components	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (15%)	Diet 4 (20%)
Initial weight, g	3.83±0.06[a]	4.07±0.17[a]	4.08±0.02[a]	4.00±0.10[a]
Final weight, g	9.95±0.60[a]	10.79±0.12[a]	10.33±0.33[a]	9.95±0.11[a]
Weight gain, %	160.05±0.54[b]	165.11±0.29[b]	152.94±0.33[a]	148.00±0.20[a]
SGR[1]	1.69±0.08[a]	1.74±0.09[b]	1.65±0.06[a]	1.63±0.06[a]
FCR[2]	1.58±0.05[a]	1.58±0.02[a]	1.62±0.03[a]	1.64±0.02[a]
PER[3]	5.15±0.46[a]	5.17±0.22[a]	4.24±0.22[a]	4.91±0.17[a]
Survival, %	83.33±3.33[b]	93.33±3.33[c]	73.33±3.33[a]	76.67±6.67[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).

7.3.3 Whole Body Composition

At the end of the growth trial, there were no significant differences ($P>0.05$) in whole body protein and ash contents among fish of different treatments. These values were higher than initial whole body composition with the exception of ash content which fluctuated slightly among treatments. Crude lipid in fish fed with Diet 3 was significantly higher ($P<0.05$) compared to fish fed with Diet 4. Fish fed with Diet 3 had the highest dry matter followed by fish fed Diet 1, Diet 4 and Diet 2.

Table 7.3: 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	18.85	21.26 ± 0.21[b]	20.35 ± 0.11[a]	22.64 ± 0.01[c]	20.09 ± 0.87[a]
Protein	68.91	86.79 ± 0.82[a]	88.39 ± 0.47[a]	90.27 ± 0.55[a]	87.97 ± 1.45[a]
Lipid	2.43	2.90 ± 0.09[b]	2.93 ± 0.07[b]	2.80 ± 0.17[b]	1.67 ± 0.28[a]
Ash	27.65	26.08 ± 2.99[a]	27.29 ± 0.21[a]	27.64 ± 0.22[a]	26.35 ± 3.46[a]

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

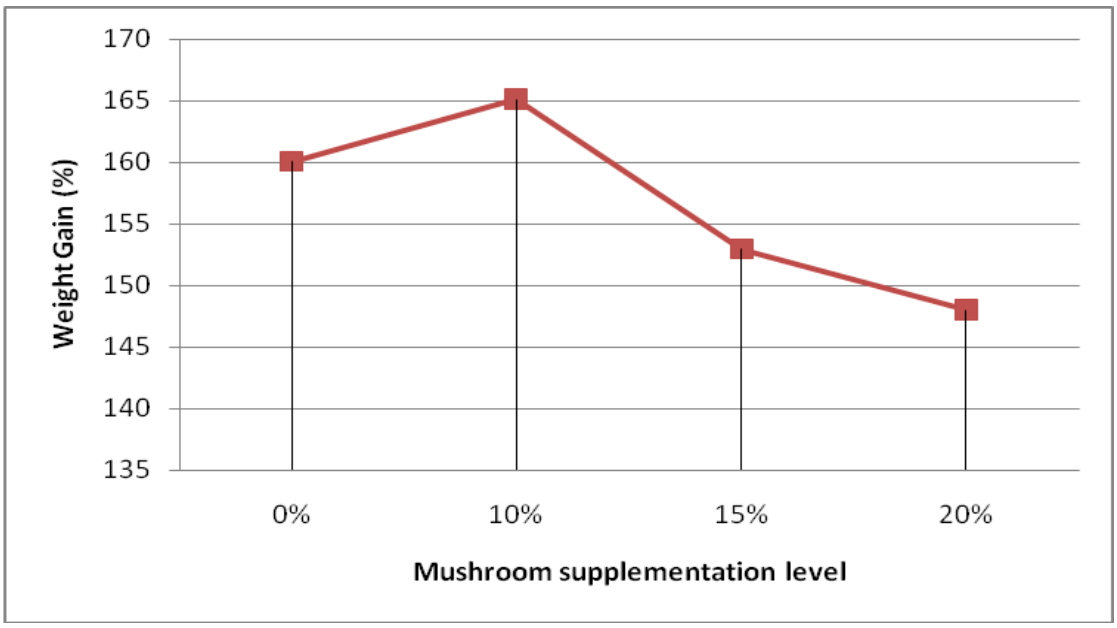

Figure 7.1: Growth performance of juvenile tilapia *Oreochromis spp.* fed with the experimental diets over a 56-day trial.

7.3.4 Water Quality Parameter

No significant differences (P>0.05) in water quality parameter were seen among treatments. All the values were within the acceptable range and fish did not show any pathological signs of depression during the trial period. Water temperature, DO (dissolved oxygen), pH, ammonia and nitrate ranged from 26.81 – 26.42 ◦C, 6.94 – 6.64 mg/l, 7.06 – 6.84, 0.97 – 0.78 mg/l and 4.31 – 3.41 mg/l respectively.

Table 7.4: Water quality parameters during experimental trial period

Components	Diet 1	Diet 2	Diet 3	Diet 4
DO (mg/l)	6.94 ± 0.11^{a}	6.81 ± 0.16^{a}	6.68 ± 0.19^{a}	6.81 ± 0.21^{a}
pH	6.97 ± 0.02^{a}	7.06 ± 0.09^{a}	6.95 ± 0.31^{a}	6.84 ± 0.13^{a}
Tempt (◦C)	26.78 ± 0.71^{a}	26.81 ± 0.74^{a}	26.42 ± 0.72^{a}	26.55 ± 0.78^{a}
NH_4 (mg/l)	0.96 ± 0.26^{a}	0.97 ± 0.27^{a}	0.78 ± 0.18^{a}	0.83 ± 0.32^{a}
Nitrate (mg/l)	3.48 ± 0.51^{a}	3.56 ± 0.12^{a}	3.41 ± 0.66^{a}	4.31 ± 0.47^{a}

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

7.4 Discussion

The idea to include prebiotic in aquafeed originated from the observation that inulin and oligosaccharides stimulate the growth of bifidobacteria selectively in human nutrition. However, application of prebiotic in aquatic organism was limited in its use due to lack of information (Hanley *et al.,* 1995). A review on the potential of dietary fiber used as prebiotic in aquaculture was done by Ringo *et al.* (2010). An earlier report showed that commercial prebiotic, Grobiotic^{TM} significantly increases feeding effectiveness, improves the survival rate, immunological response and the resistance against pathogen in striped bass (Peng and Gatlin, 2003). Lactic acid bacteria are regarded as beneficial organism living in the fish intestinal system because they produce bacteriocins which suppress the development of potential fish pathogen and thereby positively affect microflora of fish. Prebiotic compound has been shown to promote the existence of lactic acid producing bacteria and enhance the resistance to development of potential pathogen (Szilagyi, 2002). Zhou *et al.* (2010) indicated that prebiotic-supplemented diets improve the height of microvilli in red drum which correlated with improvement of growth and feed utilization of target fish by enhancing ADC of nutrient uptake.

It is clear from the present study that dietary supplementation of prebiotic compound showed a positive growth performance with the level of 10% mushroom exhibiting the greatest weight gain. Among the growth performance analysis, fish fed with Diet 2 and Diet 3 showed better growth response in weight gain($P<0.05$). The improved growth observed in the present study was in agreement with studies done with hybrid striped bass (Li and Gatlin, 2004), rainbow trout (Staykov *et al.,* 2007) and juvenile white shrimp (Zhou *et al.,* 2007). Conversely, the effect of dietary prebiotic supplementation on growth of aquatic organism exhibited negative improvement in

certain reports (Mahious *et al.*, 2006; Grisdale-Hetland *et al.*, 2008). Fish fed with Diet 4 (20% mushroom stalk meal supplementation) showed slight decrease in weight gain compared with Diet 2 and Diet 3. Depressed growth rate was detected in terms of weight gain and increased FCR value that caused fish to have lower feed intake and consequently deterioration of water quality. Based on a report by Vetter (2007), some bioactive compounds embedded in mushroom can potentially affect the digestibility of nutrients. High fiber content of mushroom may explain the result of the lower nutrient intake in the present study. In this present study, survival rate of fish were not significantly different ($P>0.05$). This was in agreement with a report from Samrongpan *et al.* (2008) who mentioned that mannan-oligosaccharides did not affect the Nile tilapia fry in terms of survival rate and feed conversion ratio.

As shown in Table 7.3, carcass composition was not affected by dietary treatments at the end of the feeding trial. Crude protein, crude lipid and ash of fish carcass were not significantly different between dietary treatments with the exception of crude lipid of fish fed with Diet 4 although the values showed an increased trend up to 10% mushroom stalk meal supplementation. However, dry matter of carcass composition showed significant increase in comparison with the Diet 1 (control) in all dietary treatments. Similarly, Genc *et al.* (2007a) found that dry matter and protein content of hybrid tilapia fillet increased with the increasing level of dietary MOS (mannan-oligosaccharides). Samrongpan *et al.* (2008) suggested that MOS is a beneficial feed supplement for Nile tilapia fingerlings against *Streptococcus agalactiae.* In contrast, the reduced value of the whole body protein and lipid contents in Diet 4 may be due to lower amino acid utilization and diet digestibility as described by Grisdale-Hilland *et al.* (2008) when Atlantic salmon fed diets supplemented with MOS. This study also needs to look into the effect of prebiotic action on haematological

parameters as previously showed by Sado *et al.* (2008) using MOS on juvenile Nile tilapia.

In conclusion, the inclusion of 10% mushroom stalk meal supplementation gives the best result in improving the weight gain and growth performance. Further studies should focus on bacteria challenge after feeding with prebiotic diet and an evaluation be done on hematological and biochemical aspects. Therefore, more detailed studies of the microbial community in gastrointestinal tract of the cultured fish are needed to assess the effectiveness of prebiotic supplementation.