Sains Malaysiana 41(10)(2012): 1197–1203

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

(Kesan Suplementasi Cendawan sebagai Bahan Prebiotik dalam Makanan Berasaskan Ulat Roti terhadap Kadar Pertumbuhan Ikan Tilapia Merah)

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ABSTRACT

*A feeding trial was conducted to investigate the effect of mushroom supplementation as a prebiotic compound in an insect-based diet on the growth performance and feed utilization of red Tilapia (*Oreochromis *sp.). A total of 120 fingerlings were divided in triplicates for each treatment. Four experimental diets were offered to the fishes within a 56 days treatment period. Out of the four diets, three contained mushroom stalk meal (MSM) supplementation at various levels of inclusion of prebiotic compound (0.5% MSM; 1.0% MSM; 1.5% MSM) and the fourth a control diet without MSM inclusion. During the growth test, fish receiving diet containing prebiotic showed significantly higher (*p<0.05*) mean individual body weight in comparison with the control diet. The best effect was obtained in fish fed with Diet 2, where the specific growth rate (SGR) was 1.74%, feed conversion ratio (FCR) was 0.58, protein efficiency ratio (PER) was 5.17 and survival was 93.33%. No significant differences (*p>0.05*) were observed in whole body protein and ash contents among the fish feeding on these diets. All water quality parameters showed no significant difference (*p>0.05*) in all treatments. The result of this feeding trial indicated that the 10% supplementation level of MSM as a prebiotic for tilapia could be used in the insect-based diet,* Zophobas morio.

Keywords: Feed utilization; growth performance; mushroom stalk; prebiotic; red tilapia

ABSTRAK

Satu ujian pemakanan telah dibuat untuk mengkaji kesan cendawan sebagai bahan suplemen prebiotik dalam diet yang berasaskan serangga terhadap pertumbuhan dan penggunaan makanan pada tilapia merah, Oreochromis *sp. Sebanyak 120 benih ikan tilapia telah dibahagikan pada tiga ujian. Empat makanan ujian telah diberikan selama 56 hari tempoh uji kaji, dengan tiga makanan mengandungi tepung tangkai cendawan pada beberapa aras suplementasi yang berbeza (0.5% MSM; 1.0% MSM; 1.5% MSM) dan satu lagi sebagai makanan kawalan tanpa MSM. Sepanjang ujian, ikan yang menerima makanan yang mengandungi prebiotik menunjukkan berat badan individu yang tinggi yang signifikan (*p<0.05*) berbanding makanan kawalan. Kesan yang paling baik terdapat pada Diet 2 dengan kadar pertumbuhan spesifik (SGR) adalah sebanyak 1.74%, kadar pertukaran makanan (FCR) adalah 0.58, kadar kecekapan protein (PER) adalah 5.17 dan kemandirian adalah 93.33%. Tiada perbezaan yang signifikan (*p>0.05*) pada kandungan protein dan abu antara ikan yang diberi makanan tersebut. Kesemua parameter kualiti air menunjukkan tiada perbezaan yang signifikan (*p>0.05*) bagi kesemua ujian. Keputusan ujian pemakanan ini menunjukkan bahawa 10% MSM aras suplementasi bahan prebiotik untuk tilapia boleh digunakan dalam makanan berasaskan serangga,* Zophobas morio.

Kata kunci: Kadar pertumbuhan; pertukaran makanan; prebiotik; tangkai cendawan; tilapia merah

INTRODUCTION

Prepared diet not only provides essential nutrients to support growth and development of cultured fish but also had an influence in cultured organism by improving health, resistance to stress and disease causing agents (Gatlin 2002). The use of traditional means of combating disease such as the use of antibiotic for fish is costly and some farmers could not afford them. As a result, prebiotic is promoted to be one of the health promoting compounds in developing dietary supplementation strategies in diet preparation compared with antibiotics. Prebiotic compound is referred to as a non digestible feed ingredient that beneficially affects the host by stimulating the growth and improving its intestinal balance (Ringo et al. 2010).

Although prebiotic compounds are mostly plant-derived additives and contain fibers, the potential of oligosaccharides and other dietary fibers as a prebiotic may have interesting applications in aquaculture to stimulate gut health as well as to suppress deleterious bacteria. In addition to the prebiotic's effect on gut microbiota in fish, its effect on the morphology of intestine is also being studied (Torrescillas et al. 2007; Yilmaz et al. 2007).

Recent developments in prebiotic are aimed at mushroom as a potential prebiotic to be incorporated into fish diet as it contains carbohydrates and also has been associated with several health promoting effects (Oyetayo & Oyetayo 2005). Mushrooms are food substances that are rich in non digestible dietary fibre which belongs to β-glucan, chitin and heteropolysaccharides making this compound having as much as 10 – 50% in the dry matter (Mizuno 1999). Apart from that, this substance also may prevent any viral infection by enhancing selectively the growth of probiotic bacteria in the large intestine of target species.

In a previous study, 50% super worm meal (SWM) substitution showed a good performance in growth (Jabir et al. 2012). In this experiment, substitution level of SWM was taken as a basic with different level of mushroom supplementation. Not only to provide essential nutrients, the mushroom *Pleurotus sajor-caju* was chosen as a prebiotic compound to be formulated in SWM based diet in order to give more immunity to fish. Spent wood waste substrates degraded by *Pleurotus sajor-caju* has been reported to be used as carbohydrate source for animal feed (Bisaria et al. 1997). Mushrooms seem to be a good potential source of prebiotics as it contains several nutrients such as chitin, hemicelluloses, α and β-glucan, mannans, xylans and galactans (Aida et al. 2009). From this valuable findings, an attempt to include mushroom in livestock feed has been reported by Kaur et al. (2010). Yousefian and Amri (2009) in their review also reported prebiotics have the numerous beneficial effects in fish such as improved nutrient availability. The objective of this study therefore was to evaluate mushroom supplementation as a prebiotic compound into the SWM based diets for red tilapia and its effect on growth performances.

Materials and Methods

Experimental System and Fish

This study was conducted at the Freshwater Aquarium Laboratory of the Institute of Biological Sciences, University of Malaya. Red tilapia fingerlings with average weight of 5.57 ± 0.15 g were stocked in triplicate in 30 L aquarium tank. The fingerlings were obtained from Freshwater Hatchery Center, Bukit Tinggi, Malaysia. The feeding trial was conducted over a 56 day period. Fish was acclimatized for one week with commercial diets (Takara-Sakana II) until well adapted with the experimental condition. Each aquarium was supplied with a bottom filter system (Code no. 139, Guppy Plastic) fitted with aeration by air pump (EK – 8000, Eiko President) to maintain a dissolved oxygen concentration in the water at a constant state of approximately 5.5 – 7.0 mg/L. Water quality parameters namely dissolved oxygen, pH, nitrate and ammonia were monitored biweekly. Fish were hand-fed daily twice a day (0900 and 1700) at the rate of 10% of their body weight. 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 was measured for growth performance analysis and proximate analysis was done using fish carcass. Red tilapia fingerlings were randomly distributed at 10 fish per aquarium (three replicates/experiment).

Experimental Diets

Four isonitrogenous (32% crude protein) diets were formulated using WinFeed version 2.8 Software (Least Cost Feed Formulation). The control diet was prepared as FM replaced with SWM at 50% inclusion level. The other three diets containing the SWM at 50% replacement portion was supplemented with various level of mushroom stalk meal (MSM) level, 0% (control), 0.5%, 1.0% and 1.5%. Mushroom stalk meal from the species of *Pleurotus sajor-caju* was obtained from Gano Farm Homestay at Tanjung Sepat, Selangor, Malaysia. All ingredients were ground in a hammer mill (Disk Mill, FFC-454) thoroughly mixed to ensure the homogeneity of the ingredients. Water (10%) was added to the mixed ingredients. The resulting mixture was pelleted wet, using the mini pelleting plant machine (KCM, Y132M-4) with a 2 mm mesh sieve. The pellets were dried in an oven at 70°C for 24 h. They were packed in plastic bags, labelled and kept at room temperature in the laboratory until used for feeding. The composition of ingredients of experimental diets is shown in Table 1.

Growth Parameters

Growth performance and nutrient utilization were expressed in terms of weight gain (WG), specific growth rate (SGR), survival rate, feed conversion ratio (FCR) and protein efficiency ratio (PER) as follows:

WG = W2 – W1, where W2 = mean final weight,
W1 = mean initial weight.

FCR = Feed Offered / Live Weight Gain

SGR = (ln W2 – ln W1 /T) × 100;
where W2 = final weight of fish,
W1 = initial weight of fish,
T = experimental period (day)

PER = Live weight gain (g) / Protein fed (g)

Survival (%) = F2 / F1 × 100;
F1 = number of fish at the beginning of experiment,
F2 = number of fish at the end of the experiment.

All calculations were based on the triplicate tank treatment.

Chemical Analysis

All proximate analysis composition and chemical composition of fish fillet and diets were analysed based on AOAC procedure (2002). The analysis consisted of dry matter by drying in an oven (Carbolite) at 105°C for 24

TABLE 1. Composition of super worm based diets supplemented with mushroom stalk meal fed to red tilapia juveniles

Ingredients mushroom stalk meal inclusion	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (15%)	Diet 4 (20%)
FM	15	15	15	15
Rice bran	27.87	18.06	13.16	8.26
SBM	25.13	24.94	24.86	24.74
SWM	15	15	15	15
Corn starch	15	15	15	15
Di-calcium phosphate	1	1	1	1
Vitamin premix[1]	0.2	0.2	0.2	0.2
Mineral premix[2]	0.3	0.3	0.3	0.3
Chromic oxide	0.5	0.5	0.5	0.5
MSM	0	10	15	20
Nutrients				
Dry matter	96.11	96.42	96.26	96.39`
Crude protein	33.58	33.44	33.84	31.91
Crude fat	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[3]	44.78	45.41	43.40	48.02
Gross energy[4]	473.21	464.70	450.50	450.79
P/E ratio (mg protein kJ^{-1})	17.48	18.12	18.96	17.81
Amino acids[5]				
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[ab]	14.67±0.08[a]
Leucine	26.95±0.12[b]	27.00±0.23[b]	26.52±0.01[ab]	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]

FM = Fish meal; SBM = Soy bean meal; SWM = Super worm meal; MSM = Mushroom stalk meal
[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.
[3] NFE = 100 – (% protein + % fat + % ash + % fiber),
[4] Gross energy (GE) was calculated as 5.65, 9.45, 4.1 kcal/g for protein, fat and NFE respectively (NRC 1993)
[5] All values are means ± SE for triplicate feeding groups and values in the same row with different superscripts are significantly different ($p < 0.05$). [1] 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.

h, crude protein was determined (as g N × 6.25) by the Kjeldahl method (FOSS Tecator Digestor Auto), fat by the Soxhlet method (FOSS Soxtec 2055), ash by combustion at 550°C in a muffle furnace (Naberthem) for 24 h, crude fiber after an alkali and acid digestion and nitrogen-free extract (NFE) by the difference [NFE = 100 – (% protein + % fat + % ash + % fiber)] according to AOAC (1995). 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). Amino acid profiles were conducted using the HPLC (Jasco, CO-2065 Plus, Intelligent Column Oven) using column (Purospher STAR RP-18 encapped, 5 µm). The amino acids were determined by comparing peak retention times to a known standard. Protein to energy ratio was calculated over each diet and expressed in unit of mg protein Kj^{-1}.

STATISTICAL ANALYSIS

Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS version 12.0. The data were subjected to an analysis of variance and Duncan multiple-range test and was used to evaluate specific differences between treatments test at 5% probability level. Differences were considered significant at $p<0.05$.

RESULTS

The study demonstrated that there were no significant differences ($p>0.05$) in the initial weight of fish. The mean final weight, weight gain and SGR of Diet 2 and 3 were not significantly different ($p>0.05$) but were the highest value recorded among the others with 10.79 g and 10.33 g in final weight gain, respectively. SGR (specific growth rate) 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 and the lowest was recorded in Diet 1 and Diet 2. It is obvious that survival rate fed for the four experimental diets varied from 93.33 – 73.33% (Table 2). The highest value was showed in Diet 2 (93.33%) followed by Diet 1 (83.33%) and the lowest value was showed 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.

There were no significant differences ($p>0.05$) in whole body protein and ash contents among the diets. These values were higher than initial whole body composition with the exception of ash content (Table 3). The percentage of crude lipid in fish fed Diet 2 (2.34%) was significantly ($p<0.05$) higher compared with fish fed Diet 4 (1.34%) which has the lowest value among others but was not significantly different ($p>0.05$) with the fish fed Diet 1 (2.29%) and Diet 3 (2.17%). For dry matter content, fish fed Diet 3 was significantly higher ($p<0.05$) (22.64%) compared with fish fed Diet 1 (21.26%) but Diet 2 and Diet 4 were not significantly different ($p>0.05$) at 20.35% and 20.09% respectively.

Water quality parameter for all treatments showed no significant difference ($p>0.05$). All the values were in the acceptable range as suggested by El-Shafai et al. (2004) and all fish did not show any pathological signs of depression during the experiment. From Table 6, 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 were recorded, respectively.

DISCUSSION

It was found that dietary supplementation of prebiotic compound showed a positive growth performance with the level of 10% mushroom inclusion exhibiting the greatest weight gain (Figure 1). Among the growth performance

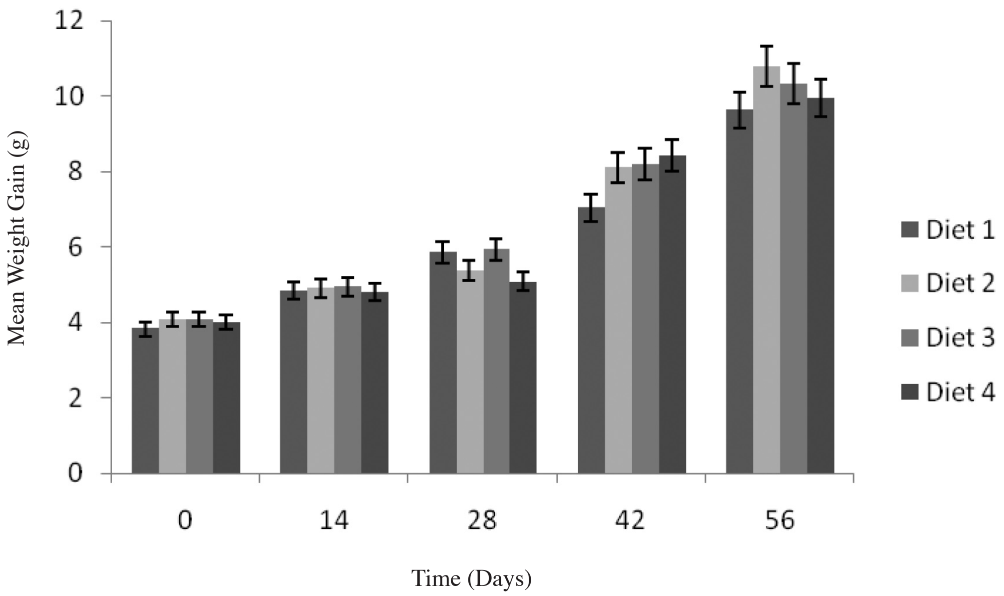

FIGURE 1. Growth performance of red tilapia fingerlings *Oreochromis* sp. fed with the experimental diets over a 56-day trial: super worm meal based diet without mushroom stalk meal supplementation (Diet 1); super worm meal based diet supplemented with 10% mushroom stalk supplementation (Diet 2); super worm meal based diet supplemented with 15% mushroom stalk supplementation (Diet 3), super worm meal based diet supplemented with 20% mushroom stalk supplementation (Diet 4)

analysis (Table 2), Diet 2 and Diet 3 showed better growth response but was not significantly different (p>0.05). The improved growth observed in the present study was in agreement with studies done with hybrid striped bass (Li & Gatlin 2004) and rainbow trout (Staykov et al. 2007). However, the effect of dietary prebiotic supplementation on aquatic organism growth exhibits negative results in certain reports (Grisdale-Hetland et al. 2008; Mahious et al. 2006). Fish fed with Diet 4 (20% mushroom stalk meal supplementation) showed slight decrease in weight gain compared with Diet 2 and 3. Reduced growth rate was recorded in terms of weight gain and increased FCR value that caused fish to have lower feed intake and consequently deterioration of water quality. According to 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. The survival rate and FCR values of all diets were not significantly different (p>0.05). This was in agreement with a report from Samrongpan et al. (2008) who mentioned that mannan–oligosaccharides has not affected the Nile tilapia fry in terms of survival rate and feed conversion ratio.

The idea of prebiotic in aquaculture feed originated from the observation that inulin and oligosaccharides stimulated 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 until earlier studies being conducted in 1995 (Hanley et al. 1995). Some reviews on potential of dietary fiber used as prebiotic in aquaculture were done by Ringo et al. (2010). Earlier studies in prebiotic application was done by using the commercial prebiotic, Grobiotic that significantly increased feeding effectiveness, improved the survival rate, immunological response and the resistance against pathogen in striped bass (Peng & Gatlin 2003). Lactic acid bacteria are regarded as beneficial organism living in the fish intestinal system. It is because they produce bacteriocins which suppress the development of fish potential pathogen and thereby positively affect microflora of fish. Some reports such as Szilagyi (2002) revealed that prebiotic compounds may promote the existence of lactic acid-producing bacteria and can enhance the resistance to development of potential pathogen.

There were several studies that showed feed efficiency and protein efficiency ratio were improved in fish fed with the diets supplemented with prebiotics. Results from Zhou et al. (2010) indicated that prebiotic-supplemented diets improved the height of microvilli in red drum. The microvillus height was correlated with improvement of growth and feed utilization of target fish by enhancing apparent digestibility coefficient (ADC) of nutrient uptake.

As shown in Table 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 that exhibited significant difference between Diet 4 and others. However, dry matter of carcass composition showed significant increase in comparison with the Diet 1 (control) in all dietary treatments. Similarly, Genc et al. (2007) found that dry matter and protein content of fish fillet increased with increasing level of dietary MOS (mannan–oligosaccharides).

In conclusion, the improvement of growth performance and survival with 10% prebiotic supplemented diet (Diet 2) may result in promising productivity in various aquaculture enterprises leading to a beneficial return of investment. Growth performance obtained by Diet 2 treatment was the best and feeding with this dietary treatment with supplementation of prebiotic compound makes fish culture worthy because it comes with a combination of medication treatment in the diet against infections. The use of natural immunostimulants such as mushroom in fish culture for prevention of fish diseases showed a promising new development and could solve the problem of massive use of antibiotics. The results obtained in the present study indicated a distinct improvement of growth with the addition of prebiotic.

TABLE 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, g	6.13±0.54[a]	6.72±0.29[a]	6.24±0.33[a]	5.95±0.20[a]
SGR[1]	1.69±0.08[a]	1.74±0.09[a]	1.65±0.06[a]	1.63±0.06[a]
FCR[2]	0.58±0.05[a]	0.58±0.02[a]	0.62±0.03[a]	0.64±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[ab]	93.33±3.33[b]	73.33±3.33[a]	76.67±6.67[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)

TABLE 3. Whole fish body composition of red tilapia fed to experimental diets

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	68.34 ± 0.82[a]	70.41 ± 0.47[a]	69.84 ± 0.55[a]	70.30 ± 1.45[a]
Fat	2.43	2.29 ± 0.09[b]	2.34 ± 0.07[b]	2.17 ± 0.17[b]	1.34 ± 0.28[a]
Ash	27.65	20.54 ± 2.99[a]	21.74 ± 0.21[a]	21.39 ± 0.22[a]	21.06 ± 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)

ACKNOWLEDGEMENTS

The authors would like to thank University of Malaya for providing financial support through a grant (PS270/2010A) and the University of Malaya Research Grant (UMRG130/10AFR). Thanks are also due to Freshwater Fisheries Research Centre, Jelebu and staffs of the Faculty of Food Science and Biotechnology, UPM (Universiti Putra Malaysia) for their technical assistance and access to laboratory equipments. Special thanks to Gano Farm Homestay, Tanjung Sepat, Selangor, Malaysia for supplying the mushroom stalk used in this experiment.

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Received: 2 December 2011
Accepted: 21 May 2012