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Replacement of Rice Bran with *Pleurotus florida* Stalks on Growth Performance of *Oreochromis niloticus* Fingerlings

(Penggantian Dedak Padi dengan Batang Cendawan *Pleurotus florida* dalam Prestasi Tumbesaran Anak Ikan Tilapia *Oreochromis niloticus*)

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ABSTRACT

*A 6-weeks feeding experiment was conducted to evaluate the effect of replacement of rice bran with mushroom stalk (*Pleurotus florida*) an agriculture waste, on growth performance in tilapia fingerlings (*Oreochromis niloticus*). Three isonitrogenous (32% crude protein) diets were formulated. Rice bran was replaced with 100% (Diet 1), 50% (Diet 2) and 0% (Diet 3, control) mushroom stalk. Each dietary treatment was tested in triplicate group of 12 fingerlings per tank arranged in completely randomized design. The result of this study showed that there was no significant difference (*$p>0.05$*) among all diets in terms of growth performance and feed utilization. Diet 1 gave the best results in BWG, SGR, FCR and PER. An economic evaluation indicated that Diet 1 gave the lowest production cost at RM2.03/kg followed by Diet 2 (RM2.18/kg) and Diet 3 (RM2.38/kg). Taking all the factors into consideration, the best diet was Diet 1 with 100% replacement of rice bran with mushroom stalks which is also the cheapest diet. This shows that* Pleurotus florida *stalks can replace 100% of rice bran in practical tilapia diets.*

Keywords: Carbohydrate; feed utilization; growth performances; Oreochromis niloticus*;* Pleurotus florida

ABSTRAK

*Suatu kajian tentang diet pemakanan selama 6 minggu telah dijalankan untuk menilai kesan potensi penggantian dedak padi dengan batang cendawan (*Pleurotus florida*) yang merupakan bahan buangan pertanian dalam prestasi tumbesaran anak ikan tilapia (*Oreochromis niloticus*). Tiga diet mengandungi kira-kira 32% protein diformulasikan. Dedak padi diganti dengan 100% (Diet 1), 50% (Diet 2) dan 0% (Diet 3, kawalan) batang cendawan. Setiap ujian dilakukan sebanyak 3 kali dengan setiap satu mengandungi 12 ekor ikan diatur secara rawak. Hasil kajian menunjukkan bahawa tiada perbezaan yang signifikan (*$p>0.05$*) bagi semua diet daripada segi aspek prestasi tumbesaran dan juga penggunaan nutrisi makanan. Diet 1 menunjukkan kadar tumbesaran yang baik bagi BWG, SGR, FCR dan PER. Anggaran kos menunjukkan Diet 1 menghasilkan kos yang paling rendah iaitu RM2.03/kg diikuti oleh Diet 2 (RM2.18/kg) dan Diet 3 (RM2.38/kg). Berdasarkan faktor ini, diet yang terbaik adalah Diet 1 yang menggantikan 100% dedak padi dengan batang cendawan dan ia juga merupakan diet yang paling murah. Ini menunjukkan bahawa batang cendawan* Pleurotus florida *boleh menggantikan 100% dedak padi dalam diet praktikal tilapia.*

Kata kunci: Karbohidrat; Oreochromis niloticus; *penggunaan nutrisi makanan;* Pleurotus florida*; prestasi tumbesaran*

INTRODUCTION

Tilapia is now popularly cultivated in almost 100 countries worldwide and has become one of the most important freshwater fish cultured for food (Shelton & Popma 2006). Harvest of tilapia increased from year to year, from 1980 (107459 mt), 1990 (379184 mt), 2000 (1,269964 mt) to 2006 (2,326413 mt) (FAO 2007). Tilapias have been domesticated at a fast rate and to a greater extend more than any other groups of fish (Fitzsimmons 2006). Tilapia has become the most important source of protein from fish and the yield has increased by 12% every year since 1984 (Fitzsimmons 2000; Shelton 2002).

The extreme variations in the culture practices and the availability of natural food have made it impossible to formulate feeds to efficiently supplement the contribution of natural food (Lim 1989). Nowadays, most researchers focused on consumption of less expensive and readily available resources in fish feed, without sacrificing nutritional and quality of feed (El-Sayed 1999). The ability of fish to utilize carbohydrate is low compared with the ability of other domestic animals (Christiansen & Klungsoyr 1987; NRC 1981, 1983). Nevertheless, the Nile tilapia is commonly regarded as opportunistic omnivores with a strong tendency towards herbivory.

Due to changes in environmental conditions, unstable source and increase in cost of raw materials, it is likely that the source and composition of aquaculture feeds will change considerably in the near future. Plant protein and carbohydrate have been considered as the important alternative ingredients for formulated fish feeds.

Regardless of the protein source, the use of carbohydrate-rich diets has been considered economical as fish would utilize the inexpensive carbohydrate as a source of energy, thus sparing the absorbed protein for growth. Several studies have shown that fish grew satisfactorily and with less pathological signs when fed carbohydrate-free diet. However, carbohydrates are always included in fish feed because they are the most abundant and least expensive source of energy. Besides functioning as pellet binders, they are also precursors for the formation of various metabolic intermediates which are essential for growth and have a sparing effect on the utilization of dietary protein (NRC 1993).

Rice bran is one of the ingredients commonly incorporated in aquaculture diets and act as a carbohydrate source in providing energy in diet. Even though carbohydrates are the least expensive form of dietary energy for man and domestic animals, their utilization by fish varies and remains somewhat obscure. Rice bran is the bran layer and germ of rice grain with hulls or broken rice at levels that are unavoidable in milling rice grain. It has higher protein content (13.5%) than the grain (7%) itself.

Rice bran has been used extensively in fish diets as a carbohydrate source. However, recent global trends indicated that the price of rice bran is also increasing annually although not as rapid as that of fishmeal. Hence, an alternative source for rice bran has to be found before the price becomes uneconomical. Fortunately, Malaysia, being a tropical country is blessed with many alternatives due to its diversity in flora and fauna composition. One of the alternatives is the mushroom *Pleurotus florida*. As a by-product of the rapidly increasing mushroom cultivation industry, mushroom stalks are usually fed to farm animals in combination with other feeds. Buwjoom and Yamauchi (2005) have investigated the effects of shiitake mushroom stalk meal on growth performance in broilers. However, sometimes due to overproduction, the mushroom stalks are simply dumped into landfills as keeping it in the farm could contribute to pollution when it starts to rot.

Mushrooms are rich in proteins, vitamins and minerals and popularly known as the 'vegetarian's meat'. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman 1976) as it contains all the nine essential amino acids required for the human body (Hayes & Haddad 1976). This benefit contributes to a well-balanced diet and good dietary content (Manzi et al. 1999). As protein contributes the single most expensive item in fish diets, it is imperative to incorporate only the amount necessary for normal maintenance and growth in order to reduce the cost of production and maximize the return on investment. Other source of energy can come from the carbohydrates incorporated in the diets.

This experimental feeding trial was conducted to investigate the effect of replacing rice bran with mushroom stalk on the growth performance of Nile tilapia (*Oreochromis niloticus).*

MATERIALS AND METHODS

EXPERIMENTAL DETAILS

Fresh mushroom stalks were obtained from a local commercial supplier. After harvesting, it was immediately dried in an oven at 100°C overnight and grinded to a powder form. The mushroom stalk, together with all the main raw materials were analyzed for its proximate composition for dry matter, moisture, crude protein, lipid and ash content (Table 1). Tilapia fingerlings (average weight, 1 g) used for this study were obtained from Bukit Tinggi Aquaculture Extension Centre. Prior to the experiment, the fish were acclimatized in the experimental tanks for seven days. Throughout the six weeks experiment, 12 fish with an average weight of 1 g were randomly distributed in nine experimental tanks with 27 L capacity filled with water treated with anti-chlorine and equipped with filtration units and supplemented with aerators. The tanks were allocated to each experimental diets in triplicates. All the three experimental diets were formulated using the Winfeed (version 2.8) computer program based on isonitrogenous crude protein (32%) content (Table 2). The dried pellet for each test diet was also subjected to proximate analysis using the standard method according to AOAC (1990) (Table 2).

The fish were hand-fed at 5% of their body weight twice daily at 0830 and 1730 h. Thirty min after the end of feeding time, the feed residue was removed to maintain water quality. Water was changed at 50% of the tanks daily. Water quality from each tank was monitored weekly before water change, feeding and

TABLE 1. Proximate analysis of raw materials used in the feed formulations

Sample	Dry matter (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Fishmeal	89.25	10.755	55.64	5.32	12.75		
Mushroom stalk	88.94	11.06	9.44	1.42	6.48	13.77	68.89
Rice bran	89.61	10.359	11.19	9.15	9.17	31.04	39.45
Corn starch	90.4	9.605	0.6	0.14	0.05		
Soya bean meal	88.7	11.3	53.04	1.34	7.6		

*All values are means. $n = 3$

TABLE 2. Ingredients used and proximate composition of experimental diets

Ingredient g/kg diet (as fed basis)	Diet 1	Diet 2	Diet 3
Mushroom meal	31.51	16.82	0
Rice bran	0	17	34
Fish meal	15	15	15
DCP	1	1	1
Palm oil	3.11	1.78	2.19
Soy bean	38.88	37.9	37.31
Corn starch	10	10	10
Vitamin premix[a]	0.2	0.2	0.2
Mineral premix[b]	0.3	0.3	0.3
Proximate composition (% dry basis)			
Protein	30.47	31.66	32.79
Lipid	4.38	6.27	9.34
Fiber	7.37	5.28	3.01
Dry matter	97.23	96.81	97.00
Ash	8.04	9.17	11.36
Moisture	2.77	3.19	3.00
Carbohydrate	49.74	46.72	43.50

[a]vitamin A 50.000 MTV; Vitamin D3 10.000 MTV; Vitamin E 75.000 gm; Vitamin K3 20.000 gm; vitamin B1 10.000 gm; vitamin B2 30.000 gm; vitamin B6 20.000 gm; Vitamin 12 0.100 gm; calcium D- Pantathenate 60.000 gm; nicotinic acid 200.000 gm; folic acid 5.000 gm; biotin 235.000 gm.
[b]selenium 0.200 gm; iron 80.000 gm; manganese 100.000 gm; zinc 80.000 gm; copper 15.000 gm; potassium chloride 4.000 gm; magnesium oxide 0.6000 gm; sodium bicarbonate 1.500 gm; iodine 1.000 gm; cobait 0.250 gm.
*All values are means. $n = 3$

sampling. Water temperature (°C) and dissolved oxygen (mg/L) of each tank was measured by using a Martini D.O meter (MI 605). Water pH was measured by using the YSI Environmental pH meter. Total alkalinity of the culture water was measured by using a spectrophotometer. Mortality was observed every day for the determination of survival rate in each aquarium for the different diets tested. At the end of the feeding trial, several fish from each aquarium, including the initial, Diets 1, 2 and 3 were randomly collected according to its group and tested for whole body proximate analysis. All the raw materials, test diets and fish samples were tested according to the standard method of AOAC (1990).

CALCULATION AND STATISTICAL ANALYSIS

Fish growth performance and protein utilization were analyzed in terms of body weight gain (BWG), feed conversation ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). These parameters were calculated as follows:

Weight gain (WTG) = final mean weight, g (Y) – initial mean weight, g (X); percentage weight gain (PWG) = (100 (Y-X)) / X; specific growth rate (SGR) = (ln (Y) – ln (×) X 100) / No of days (t); food conversion ratio (FCR) = weight of dry feed fed (g) / Live weight gained (g) and protein efficiency ratio (PER) = Gain in weight of test fish (g) / Protein consumed (g).

All the data were subjected to one-way analysis of variance (ANOVA) followed by a comparison of means using the Least Significant Difference (LSD) Test. All differences were regarded as significantly different at $p<0.05$ among treatment groups.

RESULTS AND DISCUSSION

There was no significant difference ($p>0.05$) in terms of survival rate among all diets. Fish fed with Diet 3 (83.33%) had the highest survival rate followed by Diet 1 (77.78%) and Diet 2 (69.44%) (Table 3). Although fish fed with Diet 3 showed the poorest growth performance, in terms of survival rate it was the highest with more than 80% fish survival. Shiau and Peng (1993) reported that survival rate of fish was not affected by dietary treatment in their study of protein-sparing effect by carbohydrates in diets for *Oreochromis niloticus* × *Oreochromis aureus*. In contrary, excellent survival rate (100%) was reported in the African catfish fed varying dietary levels of processed cassava leaves (Bichi & Ahmad 2010). They accorded this result to their good handling practice and water quality management throughout the experimental period. In our study, fish handling and water quality management could contribute to the cause of fish mortality. According to our observation, at the beginning of the experiment, survival of fish decreased because some of them were trapped inside the air filter machine due to their smaller size. Besides, the changes in environmental conditions from natural to experimental conditions also contributed to the mortality of fish. About 30% of water in the experimental tanks was changed daily in order to maintain the quality of water as its deterioration could increase stress to the fish.

The initial means value of weight of fingerling fish for Diet 1 was 1.08 g, Diet 2 was 1.14 g and Diet 3 was 1.28 g. At the end of the experiment, the weight performance of fish fed with Diet 3 was highest (6.02 g), followed by Diet 2 (5.46 g) and the least was those fed with Diet 1 (5.35 g). There was no significant difference ($p>0.05$) in terms of weight performance among the diets.

The result of the present study indicated that growth performance and feed efficiency in *Oreochromis niloticus* were not affected by the replacement of rice bran by mushroom stalk as an alternative carbohydrate source. The replacement of mushroom stalk at three different level (100, 50 and 0%) in the diets showed no significant differences for body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) (Table 3). Similar findings were reported by Nematipour et al. (1992) and Brauge et al. (1993), in which no significant effect were observed for SGR, feed gain ratio and protein retention in hybrid striped bass and rainbow trout fed with different carbohydrate to lipid ratios. However, our results were in contrast with the findings of Ali and Al-Asgah (2001) in that the growth performance and feed efficiency of *Oreochromis niloticus* fed with different levels of carbohydrate to lipid was affected significantly.

Carbohydrate utilization in fish is affected by the source and complexity of carbohydrate and the presence of carbohydrate-metabolizing enzymes (NRC 1983; Singh et al. 2006; Wilson 1994). Lin et al. (1997) reported that better carbohydrate utilization by sturgeon than tilapia may be related to differences in their natural diets. Our present study showed that *Oreochromis niloticus* was able to utilize high carbohydrate diets as our diets contained carbohydrate ranging from 43.50-49.74%. Our result was also in agreement with Teshima et al. (1985) who concluded that *Oreochromis niloticus* fingerlings grew optimally with diets containing 30-40% protein, 12-15% lipid and 30-40% dietary carbohydrates. Anderson et al. (1984) and Qadri and Jameel (1989) also reported in their study that high-carbohydrate diet (40%) did not retard growth of *Oreochromis niloticus*. In our study, fish fed with Diet 1 had improved performance in BWG, SGR, FCR and PER compared with Diets 2 and 3. Highest weight gain, feed efficiency ratio and PER was achieved in fish fed with diet where starch was used as carbohydrate source (Shiau & Peng 1993). The ability of *Oreochromis niloticus* to digest high-digestible carbohydrate diet may be due to its nature as freshwater omnivorous species. Omnivorous and herbivorous species such as catfish and carp appear more able to use dietary carbohydrate as an energy source (Brett & Groves 1979). Popma (1982) reported that *Oreochromis niloticus* was able to digest over 70% of the energy of raw corn starch. According to Wilson (1994), freshwater and warmwater fish had better ability than marine fish in carbohydrate digestion. The difference may be related to the relative amount of amylase activity present in the digestive system of the various species.

Extensive studies have been carried out to investigate the protein-sparing effect of carbohydrate on the growth in many species such as channel catfish (Garling & Wilson 1976), rainbow trout (Pieper & Pteffer 1979), juvenile tilapia (Shiau & Peng 1993) and *Cirrhinus mrigala* (Singh et al. 2006). Shiau and Peng (1993) suggested that starch or dextrin could spare some protein when the dietary protein level was low. Our PER in Diets 1 and Diets 2 was superior to Diet 3 which had the lowest carbohydrate content. The high carbohydrate level in Diets 1 and 2 was probably due to the inclusion of mushroom stalk meal. Mushroom stalk meal has higher carbohydrate content than rice bran, which is 68.89 and 39.45%, respectively. The highest PER was achieved in Diet 1 which contained the highest carbohydrate (49.74%). The presence of mushroom stalk meal as non-protein energy clearly improved the efficiency of protein synthesis. This will enable the more expensive protein fraction to be optimally used for growth rather than for energy by fish (Shiau 1997).

Poor FCR values were observed in all the diets indicating the poor utilization of the experimental diets by the fish. The poor utilization of diets by fish may be attributed to the fiber composition in the diets. Ali and Al-Asgah (2001) suggested that elevated crude fiber content in fish diets may exert a negative effect on nutrients digestibility. Fiber levels above 10% are undesirable in tilapia diets (Anderson et al. 1984). It appears that *Oreochromis niloticus* had poor utilization of fiber-containing diets ranging from 3.01-7.37%. On the

TABLE 3. Growth performance and feed efficiency of *Oreochromis niloticus* fed with different diets

	Diet 1	Diet 2	Diet 3
Initial weight (g/fish)	1.087 ± 0.098^{a}	1.137 ± 0.107^{a}	1.283 ± 0.188^{a}
Final weight (g/fish)	5.35 ± 0.574^{a}	5.46 ± 0.3^{a}	6.02 ± 0.683^{a}
Body weight gain (BWG)(%)	392.465 ± 26.619^{a}	391.205 ± 61.919^{a}	373.328 ± 17.772^{a}
Specific growth rate (SGR)	3.789 ± 0.13^{a}	3.749 ± 0.314^{a}	3.698 ± 0.09^{a}
Feed conversion ratio (FCR)	3.36 ± 0.014^{a}	3.402 ± 0.027^{a}	3.423 ± 0.015^{a}
Protein efficiency ratio (PER)	6.843 ± 0.201^{a}	6.543 ± 0.349^{a}	6.244 ± 0.194^{a}
Survival rate (%)	77.778 ± 5.556^{a}	69.445 ± 2.778^{a}	83.333 ± 4.811^{a}

*All values are means ± SEM for triplicate groups. Means on the same row with different superscripts are significantly different ($p<0.05$)

contrary, inclusion of 9% fiber in the diets of *Oreochromis niloticus* improved the growth, FCR, PER and NPR values as observed by Al-Ogaily (1996). El-Sayed (1991) also reported a sharp reduction in fish performance of *Oreochromis niloticus* fed diets containing high fiber content of around 20%.

Proximate analysis on the whole body composition at the initial and final stage of experiment for fish fed with 3 different diets is shown in Table 4. There was significant difference (p<0.05) in terms of protein and carbohydrate content. The results on the body composition of fish were in correlation with growth performance. Protein and carbohydrate content was affected significantly by the inclusion of mushroom meal, as an increase in carbohydrate content was observed in all experimental diets. However, only fish fed with Diet 1 had an increase in protein content. As more energy was provided by carbohydrate, the proportion of protein energy in the diets decreased and this led to an increase in protein retention in the body (Anderson et al. 1984). This indicates that rice bran replacement by mushroom meal did not affect protein synthesis.

The total cost of each of the three different diets was analyzed to determine its economic implications. All the cost was based on the prevalent prices for raw materials in Ringgit Malaysia (RM). An economic evaluation indicated that Diet 1 gave the lowest production cost at RM2.03/kg followed by Diet 2 (RM2.18/kg) and Diet 3 (RM2.38/kg) (Table 5).

From Table 6, obviously there is no significant difference in terms of water quality in all experimental diets. It can be concluded that partial replacement of fish meal in all diets did not affect the water quality of the experimental tanks.

This study has demonstrated the potential of mushroom stalk to completely replace rice bran in the diets of *Oreochromis niloticus* without compromising growth performance. The use of such high levels of non-protein energy in the production diets for *Oreochromis niloticus* could be an effective means of reducing feed costs.

TABLE 4. Whole body proximate composition (% fresh weight basis) at initial and final periods of experimentation with different diets

	Initial	Final		
		Diet 1	Diet 2	Diet 3
Protein (%)	82.83 ± 0.78^{a,b}	84.11 ± 0.44^{b}	80.28 ± 0.96^{a}	79.90 ± 1.01^{a}
Carbohydrate (%)	1.08 ± 0.05^{a}	2.06 ± 0.09^{b}	2.02 ± 0.15^{b}	2.26 ± 0.17^{b}

*All values are means ± SEM for duplicate groups. Means on the same row with different superscripts are significantly different (p<0.05)

TABLE 5. Cost involved of each per 100 g of diets

Ingredients	Cost (RM/Kg)	Diet 1		Diet 2		Diet 3	
		Total in 100 g	Cost (RM/g)	Total in 100 g	Cost (RM/g)	Total in 100 g	Cost (RM/g)
Rice bran	1.2	0	-	17	0.0204	34	0.0408
Mushroom stalk	-	31.51	-	16.82	-	0	-
Soya bean meal	2.2	38.88	0.08554	37.9	0.0834	37.31	0.0821
Fish meal	3.2	15	0.048	15	0.048	15	0.048
Corn starch	3	10	0.03	10	0.03	10	0.03
Palm oil	2.7	3.11	0.0084	1.78	0.0048	2.19	0.0059
Premix vitamin	80	0.2	0.016	0.2	0.016	0.2	0.016
Premix mineral	25	0.3	0.0075	0.3	0.0075	0.3	0.0075
DCP	8	1	0.008	1	0.008	1	0.008
Total	100.3	100	0.203	100	0.2181	100	0.2383

TABLE 6. Water quality parameter at the end of the experimental period

Components	Diet 1	Diet 2	Diet 3
pH	6.990 ± 0.006^{a}	6.970 ± 0^{a}	6.990 ± 0.017^{a}
Temperatute (°C)	30.000 ± 0.047^{b}	29.530 ± 0.058^{a}	29.650 ± 0.035^{a}
Dissolved oxygen (mg/L)	5.350 ± 0.169^{a}	5.280 ± 0.186^{a}	5.700 ± 0.259^{a}
Ammonia (mg/L)	0.020 ± 0.001^{a}	0.030 ± 0.011^{a}	0.020 ± 0.002^{a}
Nitrate (mg/L)	0.117 ± 0.005^{a}	0.212 ± 0.006^{b}	0.267 ± 0.013^{c}

*All values are means ± SE for triplicate groups. Means on the same row with different superscripts are significantly different (p<0.05)

Conclusion

The total replacement of rice bran with mushroom stalks (*Pleurotus florida*) did not affect the growth performance of the tilapia fingerlings (*Oreochromis niloticus*). In terms of cost involved, an economic evaluation indicated that Diet 1 with 100% replacement of rice bran with mushroom stalk gave the lowest production cost compared with the rest of the diets. It can be concluded that mushroom stalk has the potential to completely replace rice bran in the practical diets of *Oreochromis niloticus* and further reduced the cost of fish feed production. Thus, an agricultural waste, the unwanted post-harvest mushroom stalk which could potentially contribute to leachates production and contribute to pollution due to its decomposition if thrown into landfills can be effectively used in the aquaculture industry to reduce the production cost. This is in line with the green circular economy.

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