

## Rice Bran Replacement in *Clarias gariepinus* Fingerlings Diets with *Pleurotus florida* Stalk

(Penggantian Dedak Padi dalam Diet Anak Ikan Keli  
dengan Menggunakan Batang Cendawan)

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### ABSTRACT

*The effect of replacement of ricebran by mushroom stalk (Pleurotus florida), an agriculture waste on growth performance of African catfish (Clarias gariepinus) fingerlings was investigated for 42 days. Three isonitrogenous (32% crude protein) experimental diets containing 100% (Diet 1), 50% (Diet 2) and 0% (Diet 3) mushroom meal were formulated. Each diet was randomly allocated to triplicate groups of fingerlings in experimental tanks, with each tank stocking 12 fishes. The fishes were fed twice daily to apparent satiation for 6 weeks. Good survival rates were obtained from fish fed with Diet 1 (94%) and Diet 3 (94%). There was no significant differences in body weight gain (BWG), specific growth rate (SGR) and feed conversion ratio (FCR) among all diets ( $p>0.05$ ). The fish fed with Diet 1 showed the best BWG, SGR, FCR and PER compared with Diet 2 and Diet 3. An economic evaluation indicated that Diet 1 gave the lowest production cost at RM2.03/kg followed by Diet 2 and Diet 3 at RM2.18/kg and RM2.38/kg, respectively. This study showed that the best diet was Diet 1 with 100% replacement of rice bran with mushroom stalks costing RM2.03/kg, the cheapest among the three diets.*

*Keywords: Carbohydrate; Clarias gariepinus; feed utilization; growth performances; Pleurotus florida*

### ABSTRAK

*Kajian ini dijalankan untuk mengkaji kesan penggantian dedak padi dengan tangkai cendawan (Pleurotus florida) yang merupakan bahan buangan agrikultur terhadap prestasi tumbesaran ikan keli (Clarias gariepinus) selama 42 hari. Tiga isonitrogen diet (32% protein kasar) iaitu Diet 1, Diet 2 dan Diet 3 dengan masing-masing mengandungi 100%, 50% dan 0% cendawan yang diformulasikan. Setiap diet dijalankan secara 3 replikasi dengan 12 ekor ikan telah diagihkan ke dalam setiap tangki eksperimen secara rawak. Ikan diberi makan dua kali sehari selama 6 minggu. Kadar kelangsungan hidup terbaik ditunjukkan oleh ikan yang diberi makan Diet 1 dan Diet 3 iaitu 94%. Tiada perbezaan signifikan diperhatikan dalam BWG, SGR dan FCR bagi kesemua diet ( $p>0.05$ ). Ikan keli yang diberi makan Diet 1 menunjukkan BWG, SGR, FCR dan PER terbaik berbanding Diet 2 dan Diet 3. Anggaran kos menunjukkan Diet 1 paling murah iaitu RM2.03/kg diikuti oleh Diet 2 dan Diet 3 dengan masing-masing RM2.18/kg dan RM2.38/kg. Hasil kajian menunjukkan Diet 1 yang mengandungi 100% penggantian dedak padi adalah yang terbaik dan termurah dengan harga RM2.03/kg antara ketiga-tiga diet.*

*Kata kunci: Clarias gariepinus; karbohidrat; penggunaan nutrisi makanan; Pleurotus florida; prestasi tumbesaran*

### INTRODUCTION

The ability to use dietary carbohydrates as an energy source differs among fish species. Most fresh and warmwater fish, including catfish, can use much higher levels of dietary carbohydrates than coldwater or marine fish. This may be attributed to the fact that warmwater fish has a much higher intestinal amylase activity than coldwater species (Wilson 1994). The polysaccharides dextrin and starch are well utilized by catfish (Wilson & Poe 1987). Studies indicated that catfish metabolize glucose in a manner similar to mammals but at a much slower rate. Although animals do not have a dietary carbohydrate requirement, catfish feeds should contain adequate amounts of grain or grain by-products that are rich in carbohydrates. Inclusion of carbohydrates is crucial to provide metabolic intermediates for the synthesis of other biologically important compounds (Wilson 1994). Thus, it is important

to provide appropriate level of carbohydrate in the African catfish, to spare protein energy.

The catfish, a freshwater fish, can be found in many sub-tropical and tropical countries. It is one of the most popular species cultivated in Malaysia due to its affordable price and ease of culture. A report published by FAO (1992) indicated that catfish is increasingly becoming an important commercial species in Africa, Europe and Asia. Unfortunately, the increasing price of conventional ingredients of feed and global increase of petroleum oil price has made it difficult for catfish culturist to make profits using commercial pelleted feeds. Low cost but good quality feeds are needed when farmers wish to increase their farm productivity.

In Southeast Asia, rice bran is one of the most common by-products used to feed fish (Jhingran & Pullin 1985). However, recently the price of rice bran had also increased

but not as much as fish meal. Another very cheap by-product are mushroom stalks from the mushroom industry. After harvesting the main upper parts, the lower parts are usually discarded or sometimes mixed with other feeds and fed to animals such as cattle and swine. If produced in very large quantities some might end up thrown in landfills and can cause pollution.

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 by the human body (Hayes & Haddad 1976). *Pleurotus* species are commonly called Oyster mushrooms. There are about 40 species of this mushroom. Oyster mushrooms now ranked second among the important cultivated mushrooms in the world (Chang 1991). Mass production of mushroom cultivation resulted in mushroom stalks being considered as an agro-industrial waste. It is considered wasteful to just discard mushroom stalks as it still contains some good nutritional value. Studies done by Abd-Rahman et al. (2012) found that 10% supplementation level of mushroom can be used as a prebiotic compound in tilapia diet.

The objective of the present study was to carry out a nutritional research on African catfish, *Clarias gariepinus*. The aim was to improve the cost-effectiveness of feeds for this species and to evaluate its growth performance using an agro-industrial waste, the mushroom stalk of the *Pleurotus florida* as a replacement for ricebran.

## MATERIALS AND METHODS

### EXPERIMENTAL DIET

Fresh mushroom stalks (*Pleurotus florida*) were obtained from a commercial mushroom farm in Banting, Selangor. Grinded mushroom and all the raw materials were analyzed for proximate analysis to obtain the content of crude protein, crude lipid, crude fiber, moisture, dry matter, carbohydrate and ash using the standard method according to the AOAC (1990) (Table 1). All the experimental diets were formulated using the Winfeed (version 2.8) computer program based on isonitrogenous crude protein (32%) content. Diet 1 substitutes 100% of rice bran with

mushroom meal, Diet 2 substitute 50% of rice bran with 50% mushroom meal, Diet 3 constitute 100% rice bran (Table 2). Proximate analysis was performed on all the diets and showed in Table 2.

Fingerlings of *C. gariepinus* were obtained from a commercial supplier in Sungai Buloh, Selangor, Malaysia. Upon arrival at the laboratory, the catfish fingerlings were acclimatized in rearing tanks for one week and they were fed twice daily with commercial floating catfish fingerling feed (Takara Sakana- II) containing 32% protein. Some fish were sacrificed and kept in a freezer for whole body material proximate analysis. At the end of the acclimation period, the fishes were randomly stocked into 9 aquaria at a density of 12 fish per aquarium (27L capacity). Throughout the 42 days, the aquaria were filled with de-chlorinated tap water and half of the aquarium water was changed twice daily. Each aquarium was continuously aerated. During the experimental period, the fishes were hand-fed twice daily between 10.00-11.00 am and 17.00-18.00 pm at 10% body weight and the mortality rate was recorded. Any dead fish were removed immediately to prevent water contamination. Measurements were made once a week for dissolved oxygen, pH, temperature, total ammonia and total nitrate. Fish carcasses within each group were pooled and were analyzed for whole body proximate analysis using standard procedures (AOAC 1990). All samples were analyzed in duplicates.

### ANALYSIS OF GROWTH PARAMETER, NUTRIENT UTILIZATION AND STATISTICAL ANALYSIS

Growth parameters and nutrient utilization were calculated using the following formulas:

- Weight gain (WTG) = final mean weight g (Y) – initial mean weight g (X)
- Body weight gain (BWG) = [100(Y-X)] / X
- Specific growth rate (SGR) = [ ln (Y) – ln (X) X 100 ] / No of days (t)
- Food conversion ratio (FCR) = weight of dry feed fed (g) / Live weight gained (g)
- Protein efficiency ratio (PER) = Gain in weight of test fish (g) / Protein consumed (g)

All data were subjected to analysis of variance (ANOVA) using SPSS software version 17.0. All differences

TABLE 1. Proximate analysis of raw materials used in the experimental diets

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

\*All values are means ± SE for triplicate groups

TABLE 2. Ingredients used and proximate composition of experimental diets

Ingredient g kg <sup>-1</sup> (as fed basis)	Diet 1	Diet 2	Diet 3
Mushroom meal	315.1	168.2	0
Rice bran	0	170	340
Fish meal	150	150	150
DCP	10	10	10
Palm oil	31.1	17.8	21.9
Soy bean	388.8	379	373.1
Corn starch	100	100	100
Vitamin premix <sup>a</sup>	2	2	2
Mineral premix <sup>b</sup>	3	3	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

<sup>a</sup>vitamin A 50.000MTV; Vitamin D3 10.000MTV; Vitamin E 75.000gm; Vitamin K3 20.000gm; vitamin B1 10.000gm; vitamin B2 30.000gm; vitamin B6 20.000GM; Vitamin 12 0.100gm; calcium D- Pantathenate 60.000gm; nicotinic acid 200.000gm; folic acid 5.000gm; biotin 235.000gm. <sup>b</sup>selenium 0.200gm; iron 80.000gm; manganese 100.000gm; zinc 80.000gm; copper 15.000gm; potassium chloride 4.000gm; magnesium oxide 0.6000gm; sodium bicarbonate 1.500gm; iodine 1.000gm; cobalt 0.250 gm.

\*All values are means  $\pm$  SE for triplicate groups

were regarded as significantly different at  $p < 0.05$  among treatment groups. The results are presented as means  $\pm$  SE (standard error).

## RESULTS AND DISCUSSION

### SURVIVAL RATE, GROWTH AND EVALUATION OF GROWTH PARAMETER

All the experimental diets were accepted well by the *C. gariepinus*. Good survival rate was also achieved in all experimental diets. Highest percentage of survival rate, about 94.44% was noted in both Diet 1 and Diet 3, followed by Diet 2 (88.89%) (Table 3). However, an excellent survival rate (100%) was reported in the *C. gariepinus* fed varying dietary levels of processed cassava leaves (Bichi & Ahmad 2010). They attributed it to their good handling

practice and water quality management throughout the experimental period. The suitability of *Manihot esculenta* leaves as inclusion in *Clarias gariepinus* also contributed to the excellent survival rate. In our study, fish mortality could be due to handling stress. The initial means value of weight of fish for Diet 1 was 1.43 g, Diet 2 was 1.47 g and Diet 3 was 1.52 g. At the end of the experiment, the weight performance of fish fed with Diet 1 was highest (9.22g), followed by Diet 3 and Diet 2 which are 8.77 g and 8.06 g, respectively. Weight performance between all three tested diets showed no significant difference ( $p > 0.05$ ).

There was no significant difference in body weight gain (BWG), specific growth rate (SGR) and food conversion ratio (FCR) for fishes fed with all experimental diets. This indicates that the growth and conversion efficiency in *Clarias gariepinus* are not affected by the different levels of mushroom meal as carbohydrate source

TABLE 3. Growth performance and feed efficiency of *Clarias gariepinus* fed with the different diets

	Diet 1	Diet 2	Diet 3
Initial weight (g/fish)	1.433 $\pm$ 0.906 <sup>a</sup>	1.473 $\pm$ 0.145 <sup>a</sup>	1.52 $\pm$ 0.115 <sup>a</sup>
Final weight (g/fish)	9.217 $\pm$ 0.413 <sup>a</sup>	8.06 $\pm$ 0.651 <sup>a</sup>	8.767 $\pm$ 0.423 <sup>a</sup>
Body weight gain, BWG (%)	544.779 $\pm$ 19.187 <sup>a</sup>	453.47 $\pm$ 53.893 <sup>a</sup>	484.021 $\pm$ 56.227 <sup>a</sup>
Specific growth rate, SGR (%)	4.435 $\pm$ 0.710 <sup>a</sup>	4.052 $\pm$ 0.227 <sup>a</sup>	4.18 $\pm$ 0.223 <sup>a</sup>
Feed conversion ratio, FCR (%)	1.673 $\pm$ 0.038 <sup>a</sup>	1.932 $\pm$ 0.014 <sup>a</sup>	1.918 $\pm$ 0.015 <sup>a</sup>
Protein efficiency ratio, PER (%)	13.727 $\pm$ 0.233 <sup>b</sup>	11.479 $\pm$ 0.599 <sup>a</sup>	11.196 $\pm$ 0.653 <sup>a</sup>
Survival rate (%)	94.44 $\pm$ 0.333 <sup>a</sup>	88.89 $\pm$ 0.667 <sup>a</sup>	94.44 $\pm$ 0.333 <sup>a</sup>

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

incorporated in the experimental diets. This is similar to a previous finding, of Garling and Wilson (1977) who reported that diets containing different carbohydrate-to-lipid ratios in *Ictalurus punctatus* showed no significant difference in weight gain, feed conversion or protein and energy deposition. In tilapia, *Tilapia zilli*, there was no significant effect on growth and performance of fish fed with different levels of carbohydrate-to-lipid ratios (El-Sayed & Garling 1988).

In terms of protein efficiency ratio (PER), it was significantly affected by different experimental diets. Increased PER values were obtained with the increasing levels of mushroom inclusion as carbohydrate source in diets with a range of 11.20-13.72. Similar findings were also found in *Labeo rohita* fingerling fed with dextrin or sucrose used as carbohydrate source, as PER generally increased with increasing level of carbohydrates (Erfanullah & Jafri 1995). Other previous studies also reported high PER in fish fed diets containing low protein and high carbohydrates for *Cyprinus carpio* (Khan & Jafri 1991; Ogino & Saito 1970; Santiago & Reyes 1991), tilapia (Mazid et al. 1979) and *Anguilla anguilla* (Degani 1987; Hidalgo et al. 1993). This could be due to high carbohydrate composition in the mushroom stalk (68.89%) which provides sufficient energy for the fish metabolism and demonstrating the effect of protein-sparing effect. Therefore, the expensive fish meal as the main protein source in the experimental diets should be optimally utilized for protein synthesis by the fish (Arockiaraj et al. 1999). Knowledge of the optimal level of protein and protein-sparing effects of non-protein nutrients such as carbohydrate can be used effectively in reducing feed cost (Shiau 1997).

Diet 1 which had 100% mushroom meal gave the best result in BWG (5.45%), SGR (4.43%), FCR (1.67) and PER (13.72) compared with other diets. The result clearly indicated that fish showed better utilization of diet with complete replacement of rice bran with mushroom meal as an alternative carbohydrate source. This is in contrast with Erfanullah and Jafri (1998), who reported that high carbohydrate-low lipid diet for *Clarias batrachus* resulted in reduced growth and poor feed efficiencies. In a recent study, replacement of raw cocoyam for maize meal at 25% level in the *Clarias gariepinus* diets as an alternative carbohydrate source was optimal in growth performance and feed efficiency (Zaid & Sogbesan 2010). Lin et al. (1997) also reported that better carbohydrate utilization by

*Acipenser transmontanus* and *Oreochromis niloticus* x *O. aureus* through feeding strategy and carbohydrate source.

In this study, there was a significant difference in the body composition in protein and lipid contents (Table 4). The results clearly showed that lipid contents in the fish fed with all the experimental diets had two fold increments at the end of the experimental period. Similar result was found in another study by Anderson et al. (1984) whereby carcass fat was higher in fish fed on diets containing assailable carbohydrate. Deposition of high lipid contents in the fish was due to the availability of sufficient energy provided by the inclusion of high carbohydrate in those diets (Habib et al. 1994). Similar findings were also reported by Wee and Ng (1986) who used cassava as an energy source in pelleted feed for tilapia. However, reduced lipid deposition was noted in walking catfish fed with high carbohydrate-low lipid diets (Erfanullah & Jafri 1998). The capability to utilize different carbohydrate source varies among fish species according to their mode and feeding habits (Arockiaraj et al. 1999; Wilson 1994).

According to Robinson and Li 1996, optimal requirement of carbohydrate in *Clarias gariepinus* was in the range of 25%-35%. In this study, the fish were able to digest higher carbohydrate diets from 43.50%-49.74% range. This might be attributed to their feeding nature as freshwater omnivorous. Popma (1982) and Wilson and Poe (1985) reported that *Ictalurus punctatus* were able to digest over 70% of the energy of raw corn starch. Ali and Jauncey (2004) also reported that *Clarias gariepinus* can perform well in diet containing carbohydrate ranging from 27% to 38%. It appears that freshwater fish are able to utilize high digestible carbohydrate at more than 20%, which is the optimal value for marine fish. This could be attributed to the relative amount of amylase activity present in the digestive system of the various freshwater species (Wilson 1994). Previous study by Shimeno et al. (1977) showed that amylase activity in the digestive system of *Cyprinus carpio* (a freshwater fish) was about 80 times greater than yellowtail (a marine fish).

An economic evaluation of all the three diets resulted in Diet 1 giving the lowest production cost at RM2.03/kg followed by Diet 2 and Diet 3 at RM2.18/kg and RM2.38/kg, respectively (Table 5).

This study has demonstrated the potential of mushroom stalk up to 100% complete replacement levels in

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

	Initial fish		Final	
	Control	Diet 1	Diet 2	Diet 3
Protein (%)	77.24± 0.15 <sup>b</sup>	76.78± 0.14 <sup>b</sup>	68.35± 0 <sup>a</sup>	73.91± 0.05 <sup>a</sup>
Lipid (%)	7.51± 0.06 <sup>a</sup>	15.31± 0.21 <sup>b</sup>	18.87± 0 <sup>b</sup>	16.54± 0.07 <sup>b</sup>

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

TABLE 5. Cost of each diet per 100 gram

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

the *Clarias gariepinus* diets without compromising growth performance. The use of such high levels of non-protein energy in the practical diets for *Clarias gariepinus* could be an effective means of reducing feed costs.

#### CONCLUSION

The best growth performance (BWG and SGR), feed utilization efficiency (FCR and PER) and high survival rate was achieved in Diet 1. Taking all the factors into consideration, the best diet was Diet 1 with 100% replacement of rice bran with mushroom stalks under cheaper cost of approximately RM2.03/kg. Hence, mushroom stalk has the potential to completely replace rice bran as a carbohydrate source in reducing the cost of fish feed production.

#### ACKNOWLEDGEMENT

The authors would like to thank Mr Hanan and colleagues from the Freshwater Fish Research Centre, Glami Lemi for providing technical assistance and also to Gano Farm, Banting, Selangor for providing the mushroom stalk. The authors are grateful for financial support through the award of a University of Malaya Research Grant (UMRG, RG130/10AFR) to SAR.

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Received: 11 May 2012

Accepted: 25 January 2013