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## Effects of *Pleurotus Tuber-Regium* Degraded Rice Husk on Growth, Nutrient Utilisation, Haematology and Biochemical Parameters In Nile Tilapia (*Oreochromis niloticus*) Fingerlings..

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### Abstract

A study was conducted to evaluate the effects of a biodegradation method on the nutritive value of undegraded rice husk (RH) and biodegraded rice husk (BRH) for Nile tilapia fingerlings. Undegraded rice husk (RH) and biodegraded rice husk (BRH) were incorporated into six isonitrogenous (35% CP) test diets 7.5%, 10% and 12.5% inclusion levels against maize. A diet without undegraded rice husk (RH) and biodegraded rice husk (BRH) served as control. The experimental diets were fed to triplicate groups of fish fingerlings (0.81g average body weight) for 70 days. The mean weight gain and specific growth rate of fish across the various inclusion levels were significantly better ( $P < 0.05$ ) than the control except at 12.5% level of inclusion in both RH and BRH. Feed conversion ratio (FCR) increased with the inclusion level of test ingredients (RH and BRH) but no significant difference ( $P > 0.056$ ) between RH diets and the control up to 10% inclusion but for the BRH beyond 7.5% FCR performance became significantly different. Investment cost analysis decreased significantly on addition of both RH and BRH to the different experimental diets. Blood parameters like PCV, Hb and lymphocyte in all experimental diets dropped below the control diet but as the level of the test ingredient increased so thus the above named parameters. Neutrophil on the other hand significantly increased along inclusion level on the RH diets. No regular pattern was observed in the biochemical parameters: alanine transaminase, alkaline phosphatase and aspartate transaminase at the various inclusion levels of test ingredients.

**Keywords:** Rice Husk, Biodegradation, Nile Tilapia, *Pleurotus tuber-regium*, Feed conversion ratio

## INTRODUCTION

High cost of conventional feedstuffs necessitates the need to look into the inclusion of non-conventional feedstuffs in aqua feeds as a way to reducing the cost of fish feeding.

The use of non-conventional feed ingredients has been advanced as a way of improving animal production in the tropics (Ogunji, 2004). The potential and utilization of non conventional feedstuffs in aquaculture feed have been reviewed by (Francis *et al.*, 2001; Ugwumba and Ugwumba, 2003,). Their levels of inclusion in aqua feed vary and largely depend on their availability, nutrient level, processing technique, species of fish and cultural farming pattern prevalent in the locality (Gabriel *et al.*, 2007). The recommended levels of non-conventional plant feeds (NCPF) vary and have so many factors that limit their higher level of inclusion (Nandeesha *et al.*, 1989). These are low protein content (Ibiyo and Olowosegun, 2004), amino acid imbalance (Eyo, 2001) and presence of anti-nutritional factors (Oresegun and Alegbeleye, 2001).

Rice husk (RH) is the dry outer covering of rice grain, which is always removed during the milling of rice (Aderolu and Oyedokun, 2009). It is of no direct nutritional value to man and in most mills; it is often discarded or allowed to rot away. Nigeria has the potential to produce about 200,000 metric tons annually (Aderolu and Oyedokun, 2009). Despite its abundance, nutritionists have neglected the use of rice husk in monogastric animal feed production because of the high fibre content, poor nutritive value and bulkiness (Dafwang and Shwaremen, 1996). This relatively high fiber content of RH may limit its use in tilapia feeds because this fish lack the ability to secrete cellulase, the main cellulose digesting enzyme (Buddington, 1980). Attempts at increasing the utilization of fibrous feed ingredients like RH include adequate fortification with micro nutrients (Onifade, 1993), supplementation with high quality protein and amino acids (Delorme and Wojak, 1982), physical and chemical pre-treatments and the use of microbial enzymes and antibiotics (Onilude, 1994; Wing-Keong *et al.*, 2002). The biological treatment of fibrous materials is not entirely new and the biotechnological techniques are gradually being introduced in the field of animal nutrition and production throughout the globe (Belewu and Popoola, 2007). Biodegradation of RH may reduce its fiber content and improve its nutritive value for fish. However, the effect of biodegradation processing upon RH nutritive value for tilapia remains uncertain. Hence, the present study was conducted to evaluate the effects of *P. tuber-regium* degraded rice husk, as an energy source on the feed intake, weight gain, feed conversion ratio, haematology and biochemical parameters in Nile tilapia (*Oreochromis niloticus*) fingerlings.

## MATERIALS AND METHODS

The experiment was carried out at the Fish Nutrition Unit of the Department of Marine Science, University of Lagos, Akoka, Lagos, Nigeria.

### Collection of Rice husk and Inoculation

The rice husk was obtained at a local rice mill industry at Owode-Egba in Ogun State, Nigeria. The rice husk was divided into small portions; water was added into each portion to make it moist. All the portions were mixed together and covered with polythene for fermentation to take place. It was allowed to ferment for seven days to aid the release of nutrients (Fasidi *et al.*, 2008). The dried sclerotium of *P. tuber-regium* was cut into chumps and soaked in water for few hours (2-3 hours). The soaked sclerotium was used to inoculate the rice husk. The inoculated rice husk was covered with polythene to give the desired heat required for proper spread of the mycelium. The mycelium was allowed to grow for fifteen days (Fasidi *et al.*, 2008) to allow for the even spread and biodegradation of rice husk. The undegraded rice husk and the biodegraded rice husk were analysed for their proximate composition (Table 1).

**Table 1: Proximate composition of raw and biodegraded rice husk.**

	Undegraded Rice Husk (RH)	Biodegraded Rice Husk(BRH)
Moisture	7.4	7.6
Crude Protein	1.18	2.06
Crude Lipid	1.12	1.14
Ash	13.16	11.13
Crude Fibre	28.27	23.88
Nitrogen Free Extract	48.87	47.19

### Diet Formulation

Based on the nutrient composition of the feedstuffs, seven iso-calorific and iso-nitrogenous experimental diets were formulated. Undegraded rice husk (RH) and biodegraded rice husk (BRH) were incorporated into the six test diets (RH1, RH2, RH3, BRH1, BRH2 and BRH3) as an energy source at a rate of 7.5, 10.0 and 12.5% respectively. A diet without undegraded rice husk and biodegraded rice husk served as control as shown in Table 2.

**Table 2: Gross composition of experimental diets containing undegraded rice husk (RH) and biodegraded rice husk (BRH) at various replacement level with maize .**

Ingredients	Experimental Diets						
	Control	RH,7.5%	RH,10%	RH,12.5%	BRH,7.5%	BRH,10%	BRH,12.5%
Fish meal (72%)	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Groundnut cake	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	25.00	28.00	28.00	28.00	28.00	28.00	28.00
Maize	36.00	28.50	26.00	23.50	28.50	26.00	23.50
Wheat offal	3.00	-	-	-	-	-	-
Rice husk	-	7.50	10.00	12.50	-	-	-
Degraded rice husk	-	-	-	-	7.50	10.00	12.50
Dicalcium sulphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit. Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<b>Calculated Nutrients Value of the Feed</b>							
Crude Protein (%)	35.31	35.48	35.44	35.39	35.54	35.44	35.39
Lipids (%)	5.90	5.64	5.65	5.60	5.64	5.62	5.60
Dietary Fibre (%)	3.35	5.08	5.20	5.33	5.05	5.16	5.29
Energy-Protein Ratio (Kcal/g)	10.14	9.90	9.90	9.90	9.9	9.9	9.9

### Fish Feeding

Nile tilapia (*Oreochromis niloticus*) (0.81g average body weight) were obtained from a private fish farm, Novos Farm at Agric area, Iyana-Oba, Lagos. The diets were fed to triplicate groups of 10 fish at 5% body weight/day, twice a day (0900 and 1600 h) for 70 days. Fish were weighed collectively at 14-day intervals, their average weights recorded and the daily rations were adjusted accordingly.

### Diet Performance Evaluation

Growth performance and nutrient utilization of fish fed the experimental diets were determined in terms of Final Individual Weight, Survival (%), Specific Growth Rate (SGR %/ day), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Net Protein Utilisation (NPU) responses and was calculated as:

$$\text{Mean Weight Gain (g)} = \text{Mean Final body Weight} - \text{Mean Initial body Weight}$$

$$\text{Weight Gain (\%)} = \frac{\text{Final weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100$$

$$\text{SGR (\%/day)} = \frac{\ln \text{ final body weight} - \ln \text{ initial body weight}}{\text{Time (days)}}$$

$$\text{FCR} = \frac{\text{dry weight of feed fed}}{\text{Fish weight Gain}}$$

$$\text{NPU} = \frac{\text{Net protein in Carcass}}{\text{Protein Fed}}$$

$$\text{FI} = \frac{\text{Feed Intake During Experimental Period}}{\text{No of Days}}$$

### **Carcass Analysis**

Three (3), Nile tilapia per treatment were sacrificed at the beginning and end of the feeding trial respectively and analysed for their carcass composition (AOAC, 2004).

### **Economic Analysis**

Economic Analysis was estimated as:

**Incidence Cost Analysis (ICA)**= Cost of Feed/ Mean Weight Gain

**Profit Index(PI)** = Cost of Fish/ Cost of Feed

### **Haematological Analysis**

The haematological parameters of the fish blood were done using the method of Joshi *et al.* (2002).

### **Enzyme Assays**

Three enzymes were determined (aspartic acid transaminase, alanine transaminase and alkaline phosphatase) using the procedure from QCA diagnostic test-kits.

### **Water Quality Monitoring**

Water temperature and dissolved oxygen were measured using a combined digital dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio); pH was monitored weekly using pH meter (Mettler Toledo – 320, Jenway UK).

### **Statistical Analysis**

All data were analysed with one-way ANOVAs using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Where ANOVA revealed significant difference ( $P < 0.05$ ), Duncan multiple – range test (Zar, 1996) was applied to characterize and quantify the differences between treatments

### **Results**

Table 3 showed the Growth and Economic Performance of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH). The MWG and SGR showed significant difference ( $P < 0.05$ ) across the test diets, while the highest MWG and SGR were recorded by RH1 respectively and the least by BRH3. The two parameters decreased with increasing inclusion of RH and BRH in the diets. No regular

pattern was recorded for the feed intake though, there was significant difference in the quantity of feed consumed. The highest consumption was recorded by BRH2 and the least by the control group. The FCR recorded a slight significant difference, while the CTR recorded the least FCR, the highest value of FCR was recorded by BRH3.

**Table 3: Growth and Economic Performance of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH).**

Parameters	Experimental diets						
	CTR	RH1	RH2	RH3	BRH1	BRH2	BRH3
AVGINWT	0.81 <sup>a</sup>	0.81 <sup>a</sup>	0.81 <sup>a</sup>	0.81 <sup>a</sup>	0.81 <sup>a</sup>	0.83 <sup>c</sup>	0.82 <sup>b</sup>
AVGFNWT	4.09 <sup>b</sup>	4.65 <sup>e</sup>	4.30 <sup>c</sup>	3.94 <sup>b</sup>	4.42 <sup>d</sup>	4.34 <sup>cd</sup>	3.80 <sup>a</sup>
MWG	3.28 <sup>b</sup>	3.84 <sup>e</sup>	3.49 <sup>c</sup>	3.18 <sup>b</sup>	3.61 <sup>d</sup>	3.51 <sup>cd</sup>	2.98 <sup>a</sup>
SGR	2.31 <sup>c</sup>	2.49 <sup>f</sup>	2.38 <sup>d</sup>	2.28 <sup>b</sup>	2.42 <sup>e</sup>	2.36 <sup>d</sup>	2.19 <sup>a</sup>
FI	4.83 <sup>a</sup>	5.79 <sup>c</sup>	5.53 <sup>b</sup>	6.19 <sup>d</sup>	5.48 <sup>b</sup>	6.59 <sup>e</sup>	5.92 <sup>c</sup>
FCR	1.47 <sup>a</sup>	1.51 <sup>a</sup>	1.58 <sup>a</sup>	1.95 <sup>b</sup>	1.52 <sup>a</sup>	1.87 <sup>b</sup>	1.99 <sup>b</sup>
FCE	0.68 <sup>c</sup>	0.66 <sup>bc</sup>	0.63 <sup>b</sup>	0.52 <sup>a</sup>	0.66 <sup>bc</sup>	0.53 <sup>a</sup>	0.50 <sup>a</sup>
IC	0.23 <sup>c</sup>	0.15 <sup>a</sup>	0.20 <sup>b</sup>	0.25 <sup>d</sup>	0.19 <sup>b</sup>	0.246 <sup>cd</sup>	0.25 <sup>d</sup>
PI	19.47 <sup>bc</sup>	25.76 <sup>c</sup>	21.43 <sup>d</sup>	18.79 <sup>b</sup>	21.61 <sup>d</sup>	17.35 <sup>cd</sup>	19.76 <sup>d</sup>
AVGINWT:	Average initial weight						
AVGFNWT:	Average final weight						
MWG:	Mean weight gain						
SGR:	Specific growth rate						
FCR:	Feed conversion ratio						
FCE:	Feed conversion efficiency						
FI	Feed intake						
IC:	Incidence cost						
PI:	Profit index						

The economic parameters studied (incidence cost and profit index) showed significant difference ( $P < 0.05$ ) across the test diets. RH1 showed the highest profit index followed by BRH1, while the least was recorded by RH3.

Table 4 presented the haematological indices of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH). All the haematological parameters tested showed significant difference ( $P < 0.05$ ) across the test diets. There was a drop in the volume of PCV and Hb against the control and gradual increase was subsequently observed with increased inclusion of RH and BRH. There was gradual increase in neutrophil with increased RH and gradual decrease with increased BRH; opposite of this was observed with lymphocyte. No value was recorded for eosinophil except in BRH2.

**Table 4: Haematological indices of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH).**

Parameters	Experimental Diets						
	CTR	RH1	RH2	RH3	BRH1	BRH2	BRH3
PCV%	41.00 <sup>c</sup> ±0.57	32.67 <sup>a</sup>	34.00 <sup>a</sup> ±1.15	38.00 <sup>b</sup> ±1.15	36.67 <sup>b</sup> ±0.88	37.67 <sup>b</sup> ±0.33	38.67 <sup>bc</sup> ±0.66
Hb	14.37 <sup>c</sup> ±0.43	10.46 <sup>a</sup>	11.37 <sup>b</sup> ±0.06	12.07 <sup>c</sup> ±0.08	12.77 <sup>d</sup> ±0.03	12.87 <sup>d</sup> ±0.06	12.83 <sup>d</sup> ±0.03
Neutrophil(x10 <sup>3</sup> .µL <sup>-1</sup> )	51.00 <sup>a</sup> ±0.57	56.00 <sup>c</sup>	58.67 <sup>d</sup> ±0.88	61.00 <sup>de</sup> ±0.57	63.00 <sup>e</sup> ±1.15	52.67 <sup>ab</sup> ±0.66	54.33 <sup>bc</sup> ±0.94
Lymphocyte(x10 <sup>3</sup> .µL <sup>-1</sup> )	48.00 <sup>d</sup> ±0.57	45.67 <sup>c</sup>	42.00 <sup>b</sup> ±0.57	41.33 <sup>b</sup> ±0.88	37.33 <sup>a</sup> ±0.88	42.00 <sup>b</sup> ±0.57	45.00 <sup>c</sup> ±0.57
Eosinophil(x10 <sup>3</sup> .µL <sup>-1</sup> )	0.00 <sup>a</sup> ±0.00	0.00 <sup>a</sup> ±	0.00 <sup>a</sup> ±0.00	0.00 <sup>a</sup> ±0.00	0.00 <sup>a</sup> ±0.00	5.00 <sup>b</sup> ±0.57	0.00 <sup>a</sup> ±0.00

PCV:Packed Cell Volume, Hb: Haemoglobin Content

The results of biochemical indices of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH) is presented in table 5. There were significant differences (P<0.05) in AST, ALT and ALP. The activities of the enzymes AST and ALT was highest in BRH1. The least value was recorded in RH1. The highest level of ALP activity were recorded in RH1 and RH3 recorded the least value of ALP.

**Table 5: Biochemical indices of *Oreochromis niloticus* fed undegraded rice husk (RH) and biodegraded rice husk (BRH).**

Parameters	Experimental Diets						
	CTR	RH1	RH2	RH3	BRH1	BRH2	BRH3
AST (IU/L)	92.00 <sup>b</sup>	85.33 <sup>a</sup>	97.83 <sup>bc</sup>	100.17 <sup>c</sup>	126.33 <sup>d</sup>	122.17 <sup>d</sup>	124.67 <sup>d</sup>
ALT (IU/L)	35.22 <sup>d</sup>	8.67 <sup>a</sup>	18.00 <sup>b</sup>	30.33 <sup>c</sup>	38.33 <sup>e</sup>	36.67 <sup>de</sup>	38.33 <sup>e</sup>
ALP (IU/L)	56.62 <sup>b</sup>	79.90 <sup>d</sup>	54.97 <sup>b</sup>	42.14 <sup>a</sup>	75.42 <sup>c</sup>	77.50 <sup>c</sup>	75.33 <sup>c</sup>

AST.....Aspartic Acid transaminase

ALT.....Alanine transaminase

ALP.....Alkaline phosphatase

## Discussion

According to Jauncey (2000), the most important characteristic of feedstuffs is the bioavailability of nutrients, particularly digestible protein and digestible energy. The growth performance of a fish is dependent on feedstuff nutrient composition and availability, feed composition and fish ability to utilize the available nutrient. When rice husk was biodegraded with sclerotium of *Pleurotus tuber-regium* for fifteen days, the process resulted in improvement in the proximate composition of the biodegraded rice husk. This result agreed with the work of Nwanna *et al.* (2005) who fermented bambara nuts and El Sayed (2003) who fermented water hyacinth. It is expected that growth of tilapia fed diets containing similar levels of digestible protein and digestible energy should be identical (Fagbenro and Davies, 2001). Growth retardation and poor feed conversion ratio was, however, observed in the diets containing RH and BRH. El-Sayed (2003) fed fermented water hyacinth to Nile tilapia and reported poor

performance when compared to that of control. This could be due to high fibre level which accumulates into increased cell wall materials and non-soluble polysaccharides which invariably limit the rate of digestion and nutrient absorption (Aderolu and Oyedokun, 2009). According to Sayer *et al.*, (1991), high fibre diets result in increase in weight of excreta and poor nutrient absorption. Keembiyehetty and De Silva (1993) also reported decrease in weight gain at high fibre load inclusion. More so, that Nile tilapia has been identified as lacking the ability to secrete cellulase, the main cellulose digesting enzyme (Buddington, 1980). Increased feed intake was observed in the test diets and this could be justified by the work of Ponigrahi and Powel (1991), that for efficient growth rate, feed intake must correspondingly increase to meet up with anticipated growth rate of the animal in question. Fish fed diets containing RH had better FCR, MWG, and SGR than BRH. Similar results were obtained by Wing-Keong *et al.*, (2002) in tilapia fed PKC, where the fermented PKC failed to have a better weight gain and FCR over raw and enzyme-treated counterparts. Though, the explanation given by the authors was probably due to the presence of mycotoxin and the amino acid deficiency of fungi protein in the fermented PKC as confirmed by Lim *et al.* (2001), on fermented PKM with *Aspergillus flavus*.

Economic evaluation revealed that the cost of producing fish (IC) was significantly different between the treated RH, BRH and the control but the least value was recorded by RH1 and highest by BRH3. The reasons for the least cost in RH1 was as a result of the low cost of purchasing rice husk which at some times can be free and also due to the presence of low fibre in the diet which is not likely to hinder nutrient absorption in the fish; and the high cost in BRH3 is due to the time taken to degrade the rice husk by the mushroom, time taken to dry it and also the relatively high fibre content in the diet which may hinder nutrient absorption according to (Aderolu and Oyedokun 2009).

Haematology has been developed and well utilized in assessing the health of man and livestock. Svobodova *et al.*, (1991) opined that ichthyo-haematology would be useful in the assessment of suitability of feeds and feed mixture, evaluation of fish conditions, determination of toxic effect of substances as well as diagnosis of disease. Haematological characteristics of most fish have been studied with the aim of establishing normal value range and any deviation from it may indicate a disturbance in the physiological process (Rainzapaiva *et al.*, 2000). In the present study, values of PCV and Hb were observed to have dropped in test diets compared to the control. This may be as a result of stress induced by the high fibre in the diet as reported by Rainzapaiva *et al.*(2000). This study corroborates with the work of other researchers who have also reported a decrease in haemocrit and haemoglobin content with increase level of test ingredients. Blom *et al.*, (2001) and Richard *et al.*, (2003), reported that blood infection might reduce haematocrit value and erythrocyte count. Martins *et al.*, (2002; 2008), reported increase in number of neutrophil due to stress factor. In this



study, there was significant increase ( $P < 0.05$ ) in neutrophil of fish fed diets containing RH and BRH. The results also agreed with the work of Fagbenro *et al.* (2010) who fed raw sunflower and sesame seedmeal to *Clarias gariepinus* and reported increases in the neutrophil value as a result of stress induced by anti-nutrients present in them. The inclusion of RH led to a significant reduction in the lymphocytes at increasing level of inclusion. Since the fish did not show any sign of external discomfort, the reduction may probably be as a result of stress induced by anti-nutritional factors or probable shortfall in some essential amino acids.

The serum enzyme in RH and BRH groups did not follow a particular pattern; this had the same pattern with the work of Mona *et al.*, (2007) who worked on effect of vanadium toxicity on *Clarias lazera*. The values of AST and ALT fall within the range reported for healthy Nile tilapia by Manuel *et al.* (2007).

In conclusion, the results obtained from this experiment showed that both 7.5% of RH and BRH can be incorporated into the diets of *O. niloticus* without any adverse effect on growth, nutrient utilization, haematology and biochemical parameters in Nile tilapia (*Oreochromis niloticus*) fingerlings.

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