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Comparative Utilization of Biodegraded Rice Husk in the Diets of *Clarias gariepinus*

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Abstract: Possible improvement in the nutritional composition of Rice Husk (RH) was attempted through Solid State Fermentation with the use of fungus, *Trichoderma viridii*. A randomised complete block design experiment was set up to compare the utilization of the fermented RH (FRH), raw RH and a control diet, using parameters like performance and nutrient utilization alongside some economic indicators to arrive at conclusion. Seven isonitrogenous diets were produced using graded levels of RH (5.0, 7.5 and 10%) of both raw and the fermented which were compared to a control diet. The process of fermentation resulted in improvement in the crude protein value of RH by 97% and reduction in the crude fibre composition by 45%. Probably as a result of the fermentation process, fishes fed FRH had better FCR (0.98-1.51), PER (1.84-2.78) and SGR (1.84-1.94) than their counterparts fed raw RH but these values are significantly lower than the control diet. The Feed Intake (FI), Feed Conversion Ratio (FCR) and Protein Intake (PI) increased as level of inclusion of the RH increases. The same observation is equally true for the Profit Index, Benefit Cost Ratio and the Incidence Cost.

Key words: Biodegraded, rice husk, *Clarias gariepinus*, fungus

INTRODUCTION

Agriculture development is given serious impetus by the present government in Nigeria but the realization of this dream is seriously impaired by inadequate supply of feedstuffs particularly the protein sources. Serious attention has been given to the use of plant proteins especially legume seeds but problems like, presence of anti-nutritional factors, competition with utilization by man and other livestock and amino acid imbalance have to be tackled with.

Cereals bran and offals like rice bran, maize and wheat brans unlike legume seeds are not presently being consumed by man and they are produced in abundance. Rice Husk (RH) is the dry outer covering of rice grain, which is always removed during the milling of rice. It is of no direct nutritional value to man and in most mills, it is often discarded or allowed to rot away. In some areas however, it may be collected and used as litter material or used in fire making. Since rice offal makes up to 40% of parboiled rice, Nigeria has the potential to produce about 200,000 metric tones annually (Fagade, 2000).

In spite of RH abundance, nutritionists have neglected the use of RH in monogastric animals feed production because of the fibrousness, poor nutritive value and bulkiness (Dafwang and Shwaremen, 1996; Ajiboso *et al*., 2007). RH contains 2.9-3.6% crude protein, 0.8-1.2 ether extract, 39-42% crude fibre and 15-22% ash (Oyenuga, 1968).

Attempts at increasing the utilization of fibrous feed ingredients like RH include adequate fortification with micro nutrients (Aletor, 1999), supplementation with high quality protein and amino acids (Henuk and Dingle, 2003; Amaefule *et al*., 2006), physical and chemical pre-treatments and the use of microbial enzymes and antibiotics (Zyla *et al*., 1999; Wing-Keong *et al*., 2002).

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Research on the utilization of RH in fish diet is not common as far we know, but its utilization in chicken has been well researched into Moshad *et al*. (2003). The use of rice bran on the other hand in fish diet has been well documented. Unlike RH, rice bran is less fibrous and higher in protein and oil content (Fagbenro and Adebayo, 2002).

The present study sought to enhance the nutritive value of RH by pre-heating with microbial enzyme to digest the Non-Soluble Polysaccharide (NSP) so as to improve the bioavailability of nutrients and increase the protein content by solid-state fermentation with the fungus *Trichoderma viridii.* Diter *et al*. (2000) and Mehrdad *et al*. (2006), had earlier reported significant increase nutrient bioavailability with different species of *Trichoderma* fungus on some fibrous feedstuffs but the final product has only been tested on meat and egg type chicken but yet on fish (Onu *et al*., 2006).

The effects of raw and fermented RH on performance, nutrient utilization and economy of production of *Clarias gariepinus* was investigated in this present study. It was hoped that enhancing the nutritive value of RH through fungi fermentation could result in RH being incorporated at higher levels in *Clarias gariepinus* feeds.

MATERIALS AND METHODS

Rice husk was collected from a local rice mill in (Abeokuta, Ogun State) Nigeria around January to February 2007. Adequate quantity was collected to avoid problems associated with different batches (Aderolu, 2000).

The fungus *Trichoderma viridii* was collected from the culture collection, Department of Botany and Microbiology, University of Ibadan. It had earlier been worked on and confirmed by Aderolu (2000) as having a very high celluloytic enzyme secretion on RH compared to other species of *Trichoderma*.

Fermentation Procedure

The process of biodegradation which lasted for 10 days following the procedure below. Ten kilogram of RH was autoclaved at 70°C for 30 min, allowed to cool and was later inoculated with vibrant culture of the fungus.

A broth solution was prepared using 1.5 g of KH_2PO_4 with 3 g of malt agar and 2 g of sucrose all dissolved in 1,500 mL of distilled water plus the fungus. Autoclaved RH was then inoculated with spore suspension of *T. viridii.* It was later packed in an aluminum container, well covered and left at room temperature (28-33°C).

At the end of the tenth day, the fermented RH was oven-dried at 80°C for 24 h in a forced-air oven in order to terminate the growth of the fungus. Proximate and detergent fibre analyses were later carried out on both the fermented and the raw RH (Table 1) according to the method of AOAC (2000).

Diet Formulation and Experimental Procedures

Experimental Diets

Seven experimental diets of approximately isonitrogenous and isocalorific nutrient levels were formulated. The control diet had neither the raw nor the fermented RH, diets 1-3 had graded levels of the raw RH (5, 7.5 and 10% respectively), while diets 4-6 had the fermented RH at the same graded levels (Table 2). All the ingredients were thoroughly mixed in a Hobart A-200T mixing and pelleting machine to obtain a homogeneous pellet which was later crushed to the right sizes, the juvenile fishes could pick easily. The pelleted diets were then air-dried at 31-35°C for 3 days and stored in air-tight polythene bags prior to use.

Table 1: Chemical composition of raw and fermented RH (% on dry basis)

Nutrients	Raw RH	Fermented RH
Crude protein	3.06	6.04
Crude fibre	36.50	20.25
Ether extract	5.70	1.75
Ash	20.40	15.70
Nitrogen-free extract	40.30	5.70
Nutrient detergent fibre	69.43	20.40
Acid detergent fibre	49.13	39.89
Hemicellulose	20.26	13.32
Acid detergent lignin	13.28	11.34
Cellulose	35.89	28.52

Table 2: Feed composition of experiment diets

		Diet					
		1	2	3	4	5	6
Ingredients	Control	5% Raw RH	7.5% Raw RH	10% Raw RH	5% FRH	7.5% FRH	10% FRH
Fish meal (72%)	25.000	25.000	25.000	25.000	25.000	25.000	25.000
Soybean meal	36.000	36.000	36.000	36.000	36.000	36.000	36.000
Maize	32.000	30.000	27.500	25.000	30.000	27.500	25.000
Wheat offal	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Rice husk (Raw)	-	5.000	7.500	10.000	-	-	-
Rice husk (fermented)	-	-	-	-	5.000	7.500	10.000
Dicalcium phosphate	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Soy oil	1.500	1.400	1.300	1.200	1.400	1.300	1.200
Premix	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Salt	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Calculated (%) CP	36.860	36.810	36.640	36.470	36.960	36.860	36.760
Cost of production ($ kg^{-1})	0.886	0.883	0.877	0.872	0.884	0.876	0.873

RRH: Raw Rice Husk FRH: Fermented Rice Husk, Vitamin A, 10,000,000 IUD, D3, 2,000,000 IUD, E, 23,000 mg, K3, 2,000 mg, B1, 3000 mg, B2, 6000 mg, niacin, 50,000 mg, calcium pathonate, 10,000 mg, B6, 5000 mg, B12, 25.0 mg, folic acid, 1,000 mg, biotin, 50.0 mg, choline chloride, 400,000 mg, manganese, 120,000 mg; iron, 100,000 mg, copper, 8,500 mg, iodine, 1500 mg, cobalt, 300 mg, selenium, 120 mg, antioxidant, 120,000 mg

Experimental Procedure

Seventy *Clarias* juveniles obtained from a local fish dealer were acclimatized to laboratory condition for a period of 14 days and fed 2 mm coppens floating feed (45% CP) during the period. The feeding trial was conducted in flow-through aquaria dimension 55×35×31 cm containing 40 L of dechlorinated water. Water quality parameters in each tank were monitored each week throughout the experimental period. It ranges between 27-29°C, 7.5-7.8 and 4.2-4.4 mg L^{-1} for temperature, pH and dissolved oxygen, respectively.

The fishes were fed 4% their body weight per day and twice daily (9:00 and 16:00 h). Weighing of the fish was done on weekly basis and the next feeding rate is adjusted to the new weight.

The mean weight data were used to assess the growth performance as follows:

Mean Weight Gain (MWG) = Final body weight−Initial body weight (Morais *et al*., 2001)

$$\text{Specific Growth Rate (SGR)} = \frac{(\text{Ln}W_2 - \text{Ln}W_1)}{T_2 - T_1} \times 100$$

where, W_1, W_2 are initial and final weights and T_1, T_2 are initial and final periods, respectively.

$$\text{Food Conversion Ratio (FCR)} = \frac{\text{Total dry feed fed}}{\text{Total wet weight gain}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gained}}{\text{Protein fed}}$$

$$\text{Protein Intake (PI)} = \text{Weight of feed fed} \times \text{Protein content of feed}$$

Economy of Feed Production and Raising of Fishes

The cost was based on current prices of feed ingredients in the experimental locality (Nigeria) as of the time of the experiment. The economic evaluations of the diets were calculated from the method of New (1989) as:

$$\text{Estimated investment cost analysis} = \text{Cost of feeding (\$)} + \text{Cost of juveniles stocked (\$)}$$

$$\text{Profit index} = \frac{\text{Value of fish (\$)}}{\text{Cost of feeding (\$)}}$$

$$\text{Net profit} = \text{Sales} - \text{Expenditure}$$

$$\text{Benifit Cost Ratio (BCR)} = \frac{\text{Total sales}}{\text{Total expenditure}}$$ (Mazid *et al*., 1997)

$$\text{Incidence of Cost (IC)} = \frac{\text{Cost of feed}}{\text{kg of fish produced}}$$

Statistical Analysis

The experimental design was a complete randomised design and all the data were subjected to Analysis of Variance (ANOVA), Ducan Multiple Range Test (Ducan, 1955) was used to evaluate the mean differences among individuals diets at 0.05 significant level.

RESULTS

The treatment of the RH with fungus *Trichoderma viridii* resulted in almost double the value of crude protein (3.06-6.04%). The solid state fermentation resulted in the hydrolysis of the Non-Soluble Polysaccharide (NSP) which is attested to by the drop in values of the acid and neutral detergent fibre, Hemicellulose and the Cellulose content of the RH (Table 1). The biodegradation process also resulted in reduction in the value of ether extract and ash from 5.70-1.75% and 20.40-15.70, respectively. The feed composition of the experimental diets is shown in Table 2. The diets were isocalorific and isonitrogenous.

Data regarding growth, performance and nutrient utilization by *Clarias gariepinus* juvenile fed experimental diets are shown in Table 3. The average final weight of fishes fed the fungus-treated RH were higher than their counterpart fed the raw RH even at the same inclusion levels (33.50-29.00 at 5% inclusion level) and it was also noticed that the weight gain decreases as the level of RH rises across the diets(29.00-20.46 between 5-7.5% inclusion level of RH).

There was no significant difference between fishes on control diets and those fed 5% treated RH ($p>0.05$) in term of the average Feed Intake (FI), Feed Conversion Ratio (FCR), PER and SGR. A

Table 3: Performance and nutrient utilization of *Clarias gariepinus* juvenile fed raw and fermented RH

		Diet					
Parameters	Control	1	2	3	4	5	6
Mean final weight	44.00	39.50	30.16	31.50	45.25	35.34	41.00
Mean initial weight	10.30	10.50	9.70	8.70	11.70	10.20	10.50
Mean weight gain	33.70^{a}	29.00^{b}	20.46^{d}	22.80^{d}	33.50^{a}	25.14^{c}	30.50^{b}
Average feed intake	25.40^{d}	28.29^{b}	30.12^{b}	34.47^{a}	26.86^{c}	27.76^{bc}	29.80^{b}
Feed conversion ratio	0.75^{c}	0.98^{b}	1.04^{b}	1.51^{a}	0.80^{c}	1.10^{b}	0.98^{b}
Protein intake	9.36^{d}	10.41^{c}	11.04^{b}	12.57^{a}	9.93^{cd}	10.23^{c}	10.96^{bc}
Protein efficiency ratio	3.60^{a}	2.79^{b}	1.85^{c}	1.81^{c}	3.38^{a}	2.46^{b}	2.78^{b}
Specific growth rate	2.07^{a}	1.90^{a}	1.63^{d}	1.84^{b}	1.93^{a}	1.74^{c}	1.94^{a}

All treatments on the same row with dissimilar superscripts are significant difference at $p<0.05$

Table 4: Economic analysis of *Clarias gariepinus* juvenile graded levels of raw and fermented RH

		Diet					
Parameters	Control	1	2	3	4	5	6
Mean cost of feeding	0.023^{c}	0.025^{bc}	0.026^{b}	0.030^{a}	0.024^{c}	0.024^{c}	0.026^{b}
Mean cost of juvenile	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Estimated investment cost	0.173^{c}	0.175^{bc}	0.176^{a}	0.180^{a}	0.174^{c}	0.174^{c}	0.176^{b}
Mean yield	0.254^{a}	0.238^{b}	0.212^{d}	0.219^{cd}	0.252^{a}	0.226^{c}	0.242^{b}
Net profit	0.081^{a}	0.063^{b}	0.039^{d}	0.039^{d}	0.078^{a}	0.052^{c}	0.062^{b}
Profit index	11.040^{a}	9.520^{b}	7.300^{d}	7.300^{d}	10.500^{a}	9.420^{b}	9.310^{b}
Benefit cost ratio	1.470^{a}	1.360^{b}	1.220^{d}	1.220^{d}	1.450^{a}	1.300^{c}	1.380^{b}
Incidence cost	0.680^{d}	0.860^{c}	1.320^{a}	1.320^{a}	0.720^{d}	0.960^{b}	0.850^{c}

All treatments on the same row with dissimilar superscripts are significant difference at $p<0.05$

comparison of the treated and raw test ingredients at same inclusion level also showed some level of significant difference with the fishes on treated ingredient showing better performances in the parameters mentioned earlier

On the other hand, the SGR and Protein Efficiency Ratio (PER) decrease as the inclusion level of the RH increase (1.90-1.84 and 2.79-1.81, respectively). Significant difference ($p<0.05$) was observed in the SGR and PER diets. The Protein Efficiency Ratio values were higher for the treated RH compared to the raw RH diets (Table 3).

Furthermore the mean cost of feeding and the estimated investment cost increased significantly across the graded levels of the test ingredients. Despite the decrease in cost of feed production as inclusion rate of the RH increases (Table 4).

With regard to all economic production parameters studied, there was no significant difference ($p<0.05$) between the control diet and the 5% treated RH diet. It was also observed that the result from the diet containing 10% RH treated, competed favourably with the 5% raw RH diet.

Economic indicators like Incidence, Cost, Benefit Cost Ratio and Profit Index decreased at higher level of inclusion of the test ingredient but the values for the raw RH are higher than for the fermented RH (Table 4).

DISCUSSION

The protein content of RH could be substantially increased by solid state fermentation with the fungus *T. viridii*. This increase is due to microbial protein synthesis. Nevertheless, loss of lipid during the fermentation process was also observed and this nutrient was probably converted into the resultant fungi biomass (Iyayi and Aderolu, 2004).

According to Ramachandran *et al.* (2005), the fermentation of *P. mungo* seed meal by *Bacillus* specie resulted in significant reduction in crude fibre value and also some anti-nutritional factors like Tannin and Phytic acid contained within the seed.

Similar results on RH fermentation were obtained by Belewu and Okhawere (1998) and Iyayi and Aderolu (2004). *T. viridii* species are well recognized as celluloytic fungi with a high level of cellulose activities (Halliwel *et al*., 1985; Iyayi and Aderolu, 2004).

The ability of an organism to utilize nutrients especially protein will positively influence its growth performance (Sogbesan and Ugwumba, 2006). This is justified by the best PER and low FCR, the decrease in the rate of weight gain and as inclusion level of RH increases could then be explained as thus; despite similar protein levels in all the diets, the quality of microbial protein could not be compared absolutely with fish meal. This could probably be due to the bitter taste of microbial protein, at high level inclusion, high nucleic acid and in balanced amino acid composition (Davies and Wareham, 1988). It could also be probably due to high fibre level which cumulates into increased cell wall materials and non-soluble polysaccharides which invariably limit the rate of digestion and nutrient absorption. According to Sauer *et al.* (1991), high fibre diet result in increase in weight of excreta and poor nutrient absorption. The decrease in weight gain at high fibre load inclusion has also been reported by Keembiychetty and De Silva (1993). This could probably be due to the fermentation process which has drastically reduced the fibre load and the NSP content. The addition of microbial protein through the growth of the fungi could also be additional point. Increased feed intake at high level of inclusion of test ingredient could be justified by the findings of Ponigrahi and Powel (1991), for efficient growth rate, feed intake must correspondingly increase to meet up with anticipated growth rate of the animal in question.

Fishes fed diets containing fermented RH had better SGR, FCR and PER. Similar results were obtained by Mukhopadhyay and Ray (1999) when he fed fermented sesame seed meal to fish and Ramachandran and Ray (2007), when they fed fermented black seed to Rohu fingerlings. Other results on improved utilization of fermented feed ingredients include Ramachandran *et al.* (2005), when they fed up to 40% fermented grass pea seed to Rohu fingerlings.

Tilapia fed fermented PKM failed to have better weight gain and FCR when compared to raw- and enzyme-treated counterpart (Wing-Keong *et al*., 2002), the explanation given by the authors was probably the presence of mycotoxin and the amino acid defficiency of fungi protein in the fermented PKM as confirmed by Lim *et al.* (2001) from his fermented PKM with *Aspergillus flavus*.

Despite the reduction in cost of feed as a result of the use of RH, economic evaluation revealed that the cost of producing fishes (IC) was significantly different between the treated RH and the control but marginal similarity was noticed between the control and 5% fermented RH.

In conclusion, results obtained from this investigation showed that 5% level of fermented RH can be incorporated into the diets of *C. gariepinus* without any adverse depression on growth or any deleterious effects on health of the fish. Also that fermented RH when included in *Clarias gariepinus* juvenile gave better production and economic performances than the raw RH. This is an indication that it may be feasible to reduce the amount of expensive conventional feedstuffs such as fish meal and maize, invariably resulting in reduction in the cost of aquaculture production.

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