THE KING TUBER MUSHROOM (*Pleurotus tuber-regium*) ASSISTED FERMENTATION OF RICE HULL COULD BE USED TO PRODUCE RUMINANT FEEDSTUFF IN THE PASTORALIST CONFLICT PRONE SOUTHERN NIGERIA

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ABSTRACT

The objective of the study was to improve the nutrient values of locally available rice hull biomass wastes (BWs) by subjecting them to solid-state fermentation using the king tuber mushroom, Pleurotus tuber-regium (PTR). The RHL was composted for seven days and thereafter inoculated with the mushroom spores and fermented anaerobically to achieve mycelia colonization during 20, 30, and 40 days in triplicates in a completely randomized design (CRD). At the end of each period, the RHL was sun-dried, and subjected to physicochemical analysis to determine the effects of the SSF on the RHL nutrient values. The P. tuber-regium was able to colonise the RHL by the 20th day with formation of pin heads which dried by the 40th day. There was significant decrease ($p<0.05$) crude protein, crude fiber, ether extract and gross energy values of the RHL from control values across fermentation periods. The 40th day nitrogen free extract and ash values however increased by 90.39 and 6.69% respectively over the control values. There were also significant decreases in the 20th day fiber fraction values ($p<0.05$) over the control value by a range of 7.34% (neutral detergent fiber) to 20.74% (hemicellulose). There was also a significant nutrient improvement ($p<0.05$) in the total digestible nutrient (TDN) and the TDN:CP ratio values over the control value (39.11 to 42.70% and 3.45 to 4.27%) at the 20th day of fermentation. Based on the TDN: CP results, the 20th day fermented RHL could be used as protein and energy feedstuff for meat type ruminants with limited energy supplementation. PTR assisted SSF could therefore serve as an innovative method of converting RHL into functional feedstuff for sedentary ruminant production.

Keywords: *Pleurotus tuber-regium*, Biomass wastes, Climate change, Rice hull/husk

INTRODUCTION

Climate change events in the Sahel and semi-arid zones of West Africa have led to the migration of pastoralists and their and their animals to the Guinea savannah and rainforest zones of the region in search of feed and water (United Nation Environmental Program, 2011). This has resulted in frequent conflicts with indigenous crop farmers due to scarcity of pastoral lands and therefore unsustainability of pastoral animal production in these zones, although sedentarilization of the animal has been advocated as the practical solution to the problem, it has the throw back of being band-based and requiring extensive forage cultivation. Several biomass wastes in the form of crop and forest residues as well as agro-industrial by-products have been identified as candidate non-conventional feed resources (NCFRs) in Nigeria through decades of research (Uchegbu *et al.*, 2017; Esonu, 2009; Okoli, 2020). Agricultural biomass wastes have traditionally been utilized for various purposes, including fertilizers, biofuels, building materials, and artisanal crafts such as baskets and mats. Limited use as raw animal feed by smallholder farmers is also reported. Locally, these wastes have been transformed into organic fertilizers through composting and employed as substrates for mushrooms, industrial chemicals, and fibers (*Okoli, 2020*). Innovative methods like producing feed-grade ash, biochar, activated charcoal *(Nwogu, 2022; Ohanaka, 2022),* biogas, biofuels, and briquettes (*Edo, 2023),* as well as cost-effective animal feedstuffs (*Obasi, 2017),* have been explored to enhance their value.

Despite favorable physicochemical properties for use as feed, limitations such as poor nutritional quality, variability in nutrients, and high transportation costs hinder their application (*Esonu, 2009).* These materials are generally high in fiber, low in protein, and contain anti-nutrients that negatively impact digestibility and animal performance (*Amata, 2014; Uchegbu et al., 2017)*. Research on improving their feeding value has achieved limited success, with inclusion levels in monogastric diets reaching only 20–30% *(Uchegbu et al., 2017*).

Recent studies have however shown that the abundant biomass wastes such as rice hulls /husk (RHL) could be processed into ruminant feedstuff with the aid of white rot fungus. This study reports the nutrient values of RHL subjected to solid-state fermentation (SSF) with the aid of *pleurotus tuber-regium* (PTR) mushroom in order to improve its nutrient value for ruminant feeding.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the Federal University of Technology (FUTO) in Imo State, in Southeastern Nigeria. The state is divided into 27 Local Government Areas (LGA), which are further grouped into three agricultural zones namely, Owerri, Orlu and Okigwe. Imo state is situated in the rainforest vegetational belt of Nigeria, and longitude $6^0$151 and $7^0$341 E, at an altitude of about 90m above sea level, except elevations of about 200m at the Okigwe highlands. The statistical analysis was carried out at Precision food and feed analysis laboratory, Iyana Agbala, Ejikeyi, Ibadan.

Sources and Preparations of Experimental Materials

Rice hulls (RHL) were sourced from a rice mill in Orji, Owerri North Local Government Area (LGA), Imo State. The material was screened using a magnet to eliminate metal fragments, followed by sorting to remove debris and other impurities followed by seven days of composting. Locally obtained *Pleurotus tuber-regium* tubers were thoroughly washed to remove dirt, immersed in water for one hour, and then incubated in covered transparent plastic buckets for three days to facilitate spore (spawn) formation. Subsequently, the tubers were sliced into smaller, spore-containing segments as described by Wuanor and Carew (2018).

Inoculation of *P. tuber-regium*

The composted RHL was loaded onto wooden trays constructed with 5.08 × 5.08 cm wooden sides and wire mesh bottoms, which were lined with white polyethylene sheets disinfected with methylated spirit. The spores of *P. tuber-regium* were inoculated into the composted RHL at a ratio of 100 g of spores to 500 g of RHL, and the polyethylene sheets were sealed to create an airtight environment for fermentation.

Experimental Design

The solid-state fermentation of the rice hull was for the periods of 40 days divided into three stages of 20, 30 and 40days. The study was divided into four (4) treatments (T_1, T_2, T_3, and T_4); with T1 the unfermented biomass wastes serving as control, while T_2 - T_4 represented 20 days fermentation – 40 days fermentation respectively. Each of the biomass wastes treatments was replicated three (3) times in a completely randomized design (CRD). At the end of each fermentation period, the samples were sun-dried to halt further mycelial development and stored at room temperature for later laboratory analysis. The physical and proximate composition of the RHL were determined using standardized procedures outlined by AOAC International (2016). Metabolizable energy values were calculated using results from the proximate analysis following the modified Atwater equation (AAFCO, 1997).

The formula is ME (Kcal/100g) = 10 × (3.5 x %CP) + 8.5 x %EE) + (3.5 x %NFE)

Data Analysis

The data obtained from the study were analyzed using Analysis of Variance (ANOVA), and the differences among means were evaluated using Duncan's Multiple Range Test, as described in the Statistical Package for Social Sciences (SPSS, 2002) User's Guide.

RESULTS AND DISCUSSION

Proximate Composition

Table 1 illustrates the proximate composition of rice hulls (RHL), highlighting significant alterations ($p<0.05$) across all parameters by the 20th day of fermentation. By the 40th day, notable decreases ($p<0.05$) were observed in crude protein (CP), ether extract (EE), and crude fiber (CF) values compared to the control. In contrast, total ash (TA) and nitrogen-free extract (NFE) values significantly increased ($p<0.05$). The fermentation process with *P. tuber-regium* appears to enhance CF, TA, and NFE content while diminishing CP and EE content. Specifically, the 40th-day fermentation data indicated reductions in CP, EE, and CF by 15.60%, 24.66%, and 24.72%, respectively, relative to control values, while TA and NFE increased by 6.69% and 90.39%, respectively.

Table 1: Effects of *P. tuber-regium* Fermentation on the Proximate Composition of RHL

Parameters	Fermentation periods (Days)				
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	SEM
CP (%)	11.35^{a}	10.02^{b}	10.15^{b}	9.58^{b}	0.25
EE (%)	3.65^{a}	0.28^{d}	1.32^{c}	2.75^{b}	0.39
CF (%)	47.46^{a}	42.65^{b}	35.73^{c}	37.13^{c}	1.43
TA (%)	18.54^{b}	16.58^{c}	19.02^{b}	19.78^{a}	0.37
NFE (%)	14.88^{c}	24.09^{b}	28.31^{a}	28.33^{a}	0.71

^{abc} Means with different superscript in a row are significantly different (p<0.05), CP = Crude protein, EE = Ether extract, CF = Crude fiber, TA = Total ash, NFE = Nitrogen free extract

In comparison, *Wuanor and Carew (2017)* reported increases in CP (8.32–11.33%), EE (2.91–2.87%), CF (34.14–28.78%), ash (5.88–10.90%), and NFE (48.75–46.12%) in rice straw fermented with *P. tuber-regium* for 30 days. Unlike their findings, this study demonstrated significant CP reductions alongside an increase in NFE. These compositional changes are likely to impact the nutritional quality and suitability of *P. tuber-regium*-fermented RHL as a feed component (Vadiveloo *et al.*, 2009).

The protein values (9.58 – 11.35) recorded in this study is higher than the values (4.69 - 7.69%) reported by *Akinfemi and Ogunwole (2012) who worked on the chemical composition and in vitro digestibility of rice straw treated with P. ostreatus, P. pulmonarius and P. tuber-regium. The present result also varies with the report of Aderolu et al. (2007) who reported progressive increase across all their proximate parameters except NFE that decreased with fermentation period. They also reported similar decrease in crude fiber values (32.89 - 19.96%) across their treatment as recorded in this study.* Kuan *et al.* (2012) reported lower range of values in protein (5.14 to 5.84%), fat (0.85 – 0.99%) and ash (15.35 – 15.55%) but higher range of carbohydrate (71.30 – 71. 94%) than the values from the present study. Their fat value is also higher than the 20th day value reported in this study. Partama *et al.* (2019) in their review of the nutrient composition of rice hulls reported a crude protein value of 3.1%, nitrogen free extract (29.2%), crude fiber (35%), fat (2.7%) and ash (17.5%) within the range of values recorded in this study except for their crude fiber value which is lower than the present value.

Fiber Fraction Concentration

The results presented in table 2 show a general reduction in all the parameter values of the RHL by the 20th day of fermentation, with the exception of ADL that increased. All the parameter values subsequently increased with the increasing days of fermentation such that the 40th day values of NDF, ADF and ADL were significantly higher (p<0.05) than the other values. The 40th day value of hemicellulose although similar to the control value was however significantly higher than the others. Specifically, the NDF and ADF decreased significantly below their control values. These findings indicate the poor degradation of the rice hull by the *P. tuber-regium*.

Table 2: Effects of *P. tuber-regium* Fermentation on the Fiber Fractions in the Rice Hull/Husk

Parameters	Fermentation periods (Days)				
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	SEM
NDF (%)	69.61^{b}	64.50^{c}	69.25^{b}	80.67^{a}	1.80
ADF (%)	50.96^{bc}	49.70^{c}	53.46^{b}	60.07^{a}	1.27
ADL (%)	20.28^{d}	23.31^{c}	34.89^{b}	37.00^{a}	2.19
Cellulose (%)	30.68^{a}	26.39^{ab}	18.57^{c}	23.07^{b}	1.46
Hemicellulose (%)	18.66^{ab}	14.79^{b}	15.79^{b}	20.60^{a}	0.85

^{abc} Means with different superscript in a row are significantly different (p<0.05), NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin

Akinfemi and Ogunwole (2012) reported higher percentage fiber fraction reduction figure for rice straw treated with *P. tuber-regium* for 60 days. They reported percentage reductions in NDF, ADF, ADL and cellulose to the 12.26, 16.28, 22.81 and 14.40% respectively, while the hemicellulose value remained similar to the control value. Belewu and Banjo (1999) also reported 31.86, 76.81 and 31.33% lignin, hemicellulose and cellulose losses in rice husk treated with *sojor caju* for 42 days. Dairo *et al.* (2017) equally reported that ADF value decreased from 17.45

to 12.57% (27.97% decrease) while NDF values were similar after 21-day biodegradation with *P. ostreatus.* This is however contrary to the results of the present study which recorded 14.94 percent increase in ADF value at the 20th day of fermentation. *P. ostreatus* may therefore be a better degrader of rice husk than *P. tuber-regium*. Glushankova *et al.* (2018) have also reported the fiber fractions in RHL. The cellulose values recorded in the present study is however lower than the value range of 28 – 38% reported by these authors except the control value (30.68%) which fell within their range, indicating the effect of fermentation on RHL in the present study. They also reported higher value of hemicellulose (28%) than the value recorded in this study. Wu *et al.* (2015) however reported higher value range of 33.97 – 36.49% cellulose, 22.41 – 26.37% hemicellulose but lower lignin (11.50 – 14.34%) in composted rice husk from china. These variations in values may be attributed to different rice varieties, experimental locations, as well as the grain processing methods (Huang and Lo, 2018).

TDN and TDN: CP Ratio

The results of total digestible nutrient (TDN) in the biomass waste, which is the sum of the digestible fiber, protein, lipid and carbohydrate components of a feedstuff or diet (Lemus, 2020) are presented in table 3. TDN is usually a measure of the energy content of the feedstuff. There was a general significant increase ($p<0.05$) in the TDN values of the biomass wastes by the 20th day of fermentation and a subsequent progressive significant decrease thereafter ($p<0.05$). Thus, the 40th day values were significantly lower ($p<0.05$) than the other values. The TDN values at the 20th day of fermentation recorded an increment of 9.18% compared to the control. Rashy and Martin (2024) reported that TDN value of 55.00% and above are indicative of high-quality feedstuff ideal for meeting the energy requirements of beef cattle. The TDN results of both the raw and fermented biomass wastes would therefore not meet the energy requirements of beef cattle, and would require appropriate supplementation before they could be used in feeding such animals.

The TDN:CP ratio shows significant increase ($p<0.05$) in the values on the 20th day fermentation, which subsequently decreased with increasing fermentation days. All the values were below the NRC (1984) recommended lower limit of 5.6. The result show that the feedstuff is low in energy content, and will therefore require both protein and energy supplementation to meet the dietary requirements of beef cattle and small ruminants (Moore *et al.*, 1970). Moore and Kunkle (1998) suggested that the TDN:CP ratio should be kept close to 7.0 to obtain some degree of balance in the intake of protein and energy in beef cattle. Restitrisnani *et al.* (2013) reported that a TDN:CP ratio of 5.94 which was derived from 9.20% CP and 54.67% TDN supported normal growth in goats. Increasing the CP and TDN content of a diet will enhance rumen fermentation, increase feed digestibility and utilization, and therefore feed efficiency (Chanthakhoun *et al.*, 2012).

Table 3: Effects of *P. tuber-regium* Fermentation on the Total Digestible Nutrient and Total Digestible-to-Crude Protein Ratio of the Biomass Wastes

Biomass wastes	Fermentation periods (Days)				
	T1 (0)	T2 (20)	T3 (30)	T4 (40)	SEM
TDN	39.11^{b}	42.70^{a}	39.13^{b}	31.38^{c}	1.26
TDN:CP	3.45^{bc}	4.27^{a}	3.88^{ab}	3.28^{c}	0.13

^{abc} Means with different superscript in a row are significantly different ($p<0.05$), RHL = Rice hull/husk

CONCLUSION

It was concluded from this study that *P. tuber-regium* could colonize the milled seven days composted rice hull/husk (RHL). Twenty days of *P. tuber-regium* fermentation of the composted RHL was adjudged optimal based on its improvements of their proximate, fiber fraction and total digestible nutrient (TDN) values. Based on the TDN: Crude protein (CP) ratios results of the fermented biomass waste, the 20 days fermented RHL will require significant energy and limited protein supplementation. Furthermore, the bio-conversion of RHL to potential ruminant feedstuffs could enhance continuous supply of feedstuff throughout the year thereby limiting seasonal migration and unnecessary herders-crop farmers' conflict.

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