Bioconversion of sorghum stalk and rice straw into value added ruminant feed using *Pleurotus pulmonarius*

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Abstract: In these studies, attempts were made to investigate the bio-conversion of sorghum and rice straw into value-added ruminant feed using *Pleurotus pulmonarius* in solid state fermentation over a period of forty days. Samples were withdrawn every 10 days and analysed for chemical composition such as crude protein, cellulose, hemicelluloses, crude fats and neutral detergent fibre. The results obtained show a high positive correlation in the degradation of sorghum stalk and rice straw with an increase in the fermentation period. Sorghum stalk showed high digestibility compared to rice straw used for this study. The chemical composition results showed significant differences of ($P<0.05$), and high digestibility for the two substrates as the days of fermentation increases. Bioconversion of sorghum stalk and rice straw into value added ruminant feed using *Pleurotus pulmonarius*. Nature and Science 2012;10(4):10-16]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 2

Key words: *Pleurotus pulmonarius*, bioconversion, ruminant feed, rice straw and sorghum stalk

1. Introduction:

In Nigeria, the major feed for livestock is fibrous feedstuff. This fibrous feedstuffs or agricultural wastes despite being low in nutritive value are usually used for the feeding of live stocks. These agricultural wastes are limited in nutrients because of the presence of lignin, cellulose and hemicellulose and low levels of proteins, soluble carbohydrates and minerals (Jonathan *et al,* 2010).

Animal feeds are usually produced from agricultural products or by-products such as grains, cereals and their residues. However, it is necessary to add micro-ingredients to improve levels of essential amino acids, vitamins and minerals (Edelstein, 1982), hence, additives such as metabolic modifiers, anti-microbial agents, probiotics and special minerals are incorporated in order to supply essential these nutrients thereby enhancing growth and ultimately avoiding diseases (Wenk, 2000; Babayemi *et al*, 2004). In searching for cheap raw materials to be used in feed production, it is essential to search for substrates with excellent nutrients which will lead to high animal yield and maintain the economic viability of animal production. Therefore, it is desirable to use substrate of high quality materials as the basic ingredient of animal feeds, although, this may be limiting in these agricultural wastes. In the face of this challenge, a potential solution available is the possibility of utilizing microorganisms, mainly fungi (such as edible mushrooms), to biodegrade agro wastes to products with high nutrients, especially in regard to protein and vitamin contents, and with increased digestibility. These agricultural wastes are also lignocelluloses wastes which can be referred to as plant biomass. Plant wastes are made up of cellulose, hemicelluloses and lignin. Edible mushrooms are able to bio-convert a wide variety of lignocellulosic materials due to the secretion of extracellular enzymes (Buswell *et al.,* 1996; Rajarathman *et al.,* 1998). White Rot Fungi (WRF) is a physiological group comprising of fungi that are capable of biodegrading lignin. The name white rot is derived from the white appearance of the wood attacked by WRF, where lignin removal gives a bleached appearance (Pointing, 2001). *Pleurotus pulmonarius:* commonly known as the Indian Oyster, Phoenix Mushroom, or the Lung Oyster is a common example of WRF. It belongs to the Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Agaricales, Family:Tricholomataceae Genus: *Pleurotus*, Species: *pulmonarius*. In this study, attempts were made to investigate the bioconversion of two agro waste (rice straw and sorghum stalk) to animal feed additives using *Pleurotus pulmonarius*.

2. Materials and methods

2.1. Substrates sources and preparation

Sorghum stalk and Rice straw were obtained from Bodija market and International Institute of Tropical Agriculture (IITA) all in Ibadan, Nigeria. They were milled and oven dried at 65 ^{0}C until a constant weight was obtained for dry matter determination. Twenty five grams (25 g) of the substrates were weighed into each 500 ml Erlenmeyer's flask and 70 ml distilled water was added to moisten and then squeezed out into the bottles using cheese cloth. The bottles were immediately covered with aluminium foil and sterilized in the autoclave at 121 ^{0}C for 15 minutes. Each treatment was done in triplicate

2.2. Microorganism

Pleurotus pulmonarius used for this study was obtained from Forest Research Institute of Nigeria, Ibadan, Nigeria. The fruiting bodies of this fungus were tissue cultured to obtained mycelia starter culture which were used to produce spawn (Jonathan and Adeoyo, 2011)

2.3. Inoculation

Each bottle was inoculated with 10 g spawn of *Pleurotus pulmonarius* and covered with aluminium foil. They were incubated in the dark at 30 ^{0}C and relative humidity of 100% for 40 days and observed at an interval of 10 days. The biodegraded samples were oven dried at 80 ^{0}C to constant weight for chemical analysis and *in-vitro* digestibility.

2.4. Determination of the microbiological quality

The microbiological quality of the substrates were carried out on the untreated substrates and the final treated substrates (40 days) to determine the total count of the aerobic organisms, *Escherichia coli* and coliforms present in the substrates using the plate count method.

2.5. Chemical composition

Dry matters (DM) were determined according to Pearson (1975). Crude protein (CP), ether extracts (EE) (Crude fat) and ash content (AC), were determined according to AOAC method (1995). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method described by Van Soest *et al.* (1991). Hemicellulose was calculated as the difference between NDF and ADF while cellulose is the difference between ADF and ADL (Zadrazil, 1982).

2.6. In-vitro gas production

Rumen fluid was obtained from male and female goats using the method of Babayemi and Bamikole (2006) using suction tube from goats previously fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% Pannicum maximum at 5% body weight. The rumen liquor was collected into the thermo flask that had been pre warmed to a temperature of 39 °C from the goats before they were offered the morning feed. Incubation procedure was as reported by Menke and Steingass (1988) using 120 ml calibrated transparent plastic syringes with fitted infusion clip. The sample weighing 200 mg (n = 3) were weighed into a pre weighed insacco bag and sealed using a nylon sealer, thereafter inserted into the syringes. 30 ml inoculum containing cheese cloth strained rumen liquor and buffer (g/l) of 9.8 $NaHCO_3$, 2.77 Na_2HPO_4 , 0.57 KCl , 0.47 NaCl, 2.16 $MgSO_4$•7H2O, 16 $Cacl_2$•2H2O) (1:2 v/v) was dispensed using another 50 ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The infusion clip in the syringes was then locked so as to prevent escape of gas. Incubation was carried out at 39 ± 1 °C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h. At post incubation period, 4 ml of NaOH (10 M) was introduced to estimate the methane production as reported by Fievez *et al.* (2005). The insacco bags were washed thoroughly and oven dried to a constant weight to determine their percentage digestibility using the formula:

$$\frac{\text{initial weight} - \text{Final weight}}{\text{initial weight}} \times \frac{100}{1}$$

The post incubation parameters such as metabolizable energy, organic matter digestibility and short chain fatty acids were estimated at 24 h post gas collection according to Menke and Steingass (1988). The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample against the incubation time and from the graph, the gas production characteristics were estimated using the equation:

Y = a+b (1-e-ct) as described by Orskov and McDonald (1979).

Where Y = volume of gas produced at time t, c, = intercept (gas produced from the insoluble fraction (b), t = incubation time.

Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136Gv + 0.057CP + 0.0029 CF (Menke and Steingass, 1988), while organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA (Menke and Steingass, 1988).

Short chain fatty acids (SCFA) were obtained as 0.0239 V – 0.0601 (Getachew *et al*., 1999)
Where Gv, CP CF and XA are total gas volume, Crude protein, crude fiber and ash, respectively.

2.7. Statistical analysis

Data obtained were subjected to analysis of variance and test of ANOVA significance was carried out by Duncan's multiple range tests.

3. Results and Discussion

Table 1 represents the microbiological quality of the untreated and 40 days treated sorghum stalk and rice straw, showing no appreciable increase in the total aerobic count, total coliform and *Escherichia coli* count in the treated substrates.

Table 1. Microbial Population of untreated substrates and 40 days treated substrates.

	Total aerobic count	Total coliforms	*Escherichia coli*
URS	150	39	22
RS	82	2	0
USS	125	34	14
SS	16	2	1

USS=untreated sorgum stalk, SS= Sorghum stalk,
URS= Untreated rice straw, RS= Rice straw.

As presented in Table 2 there was significant ($P<0.05$) increase in the crude protein (CP) contents of the substrates treated with *P. pulmonarius* with an increase in fermentation days, with a 4.50% recorded in the control, 4.60% at day 10, 4.78% at day 20 and 9.36% after forty days fermentation for rice straw. Similar trend was also observed in the sorghum stalk substrate. Results similar to this have been reported by Khan *et al*. (1981) and Jonathan (2002). The increase in crude protein might be due to an increase in fungal mycelia as the organism (*P. pulmonarius*) grows on the substrates during the degradation of the various substrates since mushroom mycelia have been observed to be rich in proteins (Jonathan and Adeoyo, 2011). This increase could also be due to the release of polysaccharide bound proteins (Belewu and Balewu, 2005; Jonathan *et al.,* 2006).

On the other hand, the Crude fiber (CF), Neutral detergent fiber (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were decreasing as the days of fermentation progresses. The NDF decreased from 64.6% and 68.9% in the controls to 54.2% and 55.8% for sorghum stalk and rice straw respectively after a period of 40 days treatment.

Table 2. Chemical composition (g/100Dm) of untreated and 10 – 40 days *Pleurotus pulmonarius* treated Sorghum stalk and Rice straw.

Parameter	Dry matter	Crude protein	Ether extract	Ash content	Crude fiber	NDF	ADL	ADF	Cellulose	hemicellulose
Controls										
RS	74.2^{a}	4.50^{d}	1.36^{c}	12.2^{a}	41.2^{a}	68.9^{a}	26.4^{a}	51.1^{a}	24.7^{a}	17.8^{b}
SS	70.7^{a}	5.31^{c}	1.17^{d}	7.27^{a}	31.2^{a}	64.6^{a}	28.2^{a}	45.5^{b}	17.3^{b}	19.1^{a}
10days										
RS	68.2^{b}	4.60^{d}	1.52^{b}	9.50^{b}	37.4^{a}	66.9^{a}	24.0^{b}	46.4^{b}	22.4^{a}	20.5^{a}
SS	64.5^{b}	5.44^{c}	1.22^{c}	6.52^{c}	27.0^{b}	63.7^{a}	26.4^{a}	44.2^{b}	17.8^{b}	19.5^{a}
20 days										
RS	64.2^{b}	4.78^{d}	1.55^{b}	8.50^{b}	32.0^{a}	64.2^{a}	21.4b	42.8^{b}	21.4^{a}	21.4^{a}
SS	62.3^{b}	6.03^{b}	1.36^{c}	4.75^{d}	24.6^{b}	60.9^{b}	23.8^{b}	42.6^{b}	18.8^{b}	18.3^{b}
30 days										
RS	62.4^{b}	5.32^{c}	1.54^{b}	5.60^{c}	26.3^{b}	60.7^{b}	18.5^{b}	38.4^{c}	19.9^{b}	22.3^{a}
SS	58.4^{c}	7.49^{b}	1.45^{b}	3.85^{d}	19.8^{c}	57.9^{b}	19.2^{b}	39.9^{c}	20.7^{a}	18.0^{b}
40 days										
RS	58.7^{c}	9.36^{a}	1.68^{a}	2.88^{d}	23.2^{c}	55.8^{b}	13.7^{c}	33.9^{c}	20.2^{a}	21.9^{a}
SS	52.6^{c}	8.62^{a}	1.71^{a}	2.92^{d}	22.2^{c}	54.2^{b}	14.7^{c}	36.8^{c}	22.1^{a}	17.4^{b}

$^{a, b, c}$ means values followed on the same column for each substrates are significantly different by Duncan's Multiple range test ($P<0.05$).
RS = Rice straw, SS = Sorghum stalk.

ADF decreased from 45.5% and 51.1% in the controls to 36.8% and 33.9% for sorghum stalk and rice straw respectively after forty days, while ADL reduced from 28.2% and 26.4% in the controls to 14.7% and 13.7% for sorghum stalk and rice straw respectively. The decrease observed in this study has been reported earlier by Tamara *et al*. (1996) and Jonathan *et al*. (2010). This decrease could be due to

the production of various lignocelluloses enzymes such as laccase, manganese peroxidase and lignin peroxidise, during the vegetative and reproductive phases Tamara *et al.* (1996). Jonathan *et al* (2008) also suggested that the extent of lignin degradation by these fungi may be related to their ability to produce lignin degrading enzymes such as lignin peroxidase and manganese peroxidase. Zadrazil (2000) observed that increase in lignin content in plants materials used as feed correlates with the decrease of digestibility for rumen microorganisms.

The results of cellulose content of sorghum stalk and rice straw showed that the cellulose content of sorghum stalk increased from 17.3 % in the control to 22.1 % after a fermentation period of 40 days while that of rice straw decreased from 24.7% in the control to 22.2% after the 40 days fermentation time. The hemicelluloses content of sorghum stalk was also found to be decreasing with an increase in fermentation time (19.5% at10 days, 18.3% at 20 days, 18.0% at 30 days and 17.4% at 40 days) while that of rice straw increased from 17.8% in the control to 21.9% after forty days fermentation period. Similar results have been reported by Chen *et al*. (1995). This preferential degradation of cellulose and hemicellulose could be as a result of the type of substrate, duration of degradation and physiological behaviours of the fungi used as suggested by Chen *et al*. (1995) and Jonathan and Akinfemi (2011).

3.1 Gas volume

The quantity of gas was measured at 3, 6, 9, 12, 15, 18, 21 and 24h for the untreated and 10 – 40 days treated sorghum stalk and rice straw and the result is presented Figure 1a and 1b. The results revealed that for sorghum stalk, there is a general increase in gas production for all the days of treatments in which samples were withdrawn and analysed with the highest being 24 h of forty days (24 m/200mg Dry Matter), followed by 24 h of 30 days (22 ml/200mg Dry Matter) while the least volume of gas (10 ml/200mg Dry Matter) was recorded in the untreated sorghum stalk (Fig 1a). Similar trend was also observed for the rice straw substrate (Figure 1b). Similar results were reported by DeBoever *et al.* (2005). The authors observed that gas production was negatively related with NDF content and positively with starch. Furthermore, Sallam *et al.* (2007) reported that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. The variation in gas production observed between the treated and untreated substrate could be traceable to the depletion of lignin content of the treated substrate as shown in (Table 2). Furthermore, variation in gas production in the two substrate observed in this study could be due to the difference in the nature and level of fiber presented in the substrate as suggested by Babayemi *et al.* (2004a) and potency of the rumen liquor used for incubation (Babayemi, 2007). Gas volume has also been shown to have close relationship with feed intake (Blummel and Becker 1997) and growth rate (Blummel and Orskov 1993).

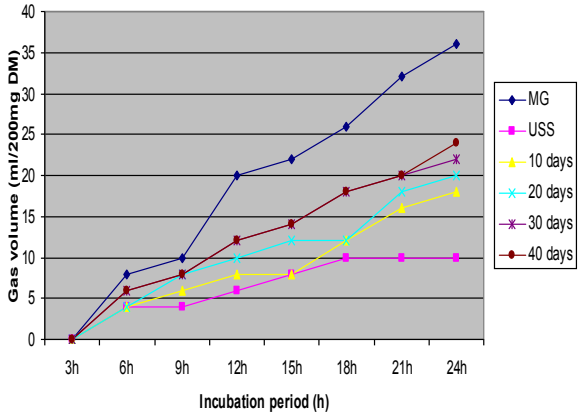

Figure 1a: Gas volume for untreated and 10 – 40 days treated sorghum stalk using maize grain (MG) as a standard.
MG = maize grain, URS = untreated sorghum stalk.

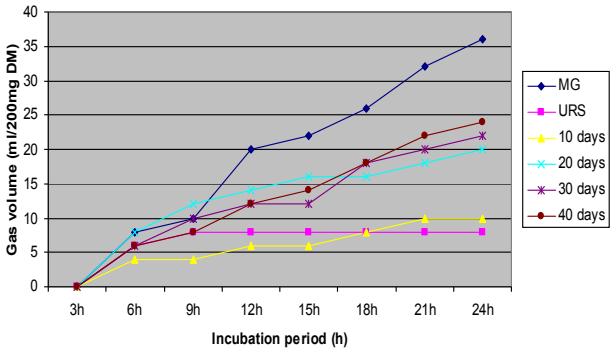

Figure 1b: Gas volume for untreated and 10 – 40 days treated rice straw using maize grain (MG) as a standard.
MG = maize grain, URS = untreated rice straw.

Beuvink and Spoelstral, (1992) reported that gas is produced mainly when feedstuff carbohydrate are fermented to acetate and butyrate with fermentation to propionate yielding gas only from buffering of the acid, therefore silage which produce high amount of propionate should produce low gas volumes.

The results of Methane production as presented in Fig 2 also shows increasing methane production as the days of fermentation increases with the highest production methane volume of 11 ml/200mg Dry Matter and 12 ml/200mg Dry Matter observed in rice straw and sorghum stalk respectively after 40 days of fermentation treatement.

3.2 Invitro organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA)

Organic matter digestibility (OMD), Metabolizable energy (ME) and Short chain fatty acid (SCFA) estimated for the untreated and treated substrates is represented in table 3 and the percentage digestibility in Figure 3. The results showed high values estimated for OMD in all treated substrates with highest values occurring in 30 – 40 days treated substrates. OMD has been shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003).

The highest value obtained for OMD was seen in 40 days PFS and PFR. The high volume obtained for OMD in this study suggests that the microbes in the rumen and animal have high nutrient uptake (Chumpawudee *et al.,* 2006). The reduced CF contents (Table 2) of the fungal treated substrates probably influenced improvement in OMD, since high NDF and ADL contents in feedstuffs result in lower fiber degradation (Van Soest, 1988).

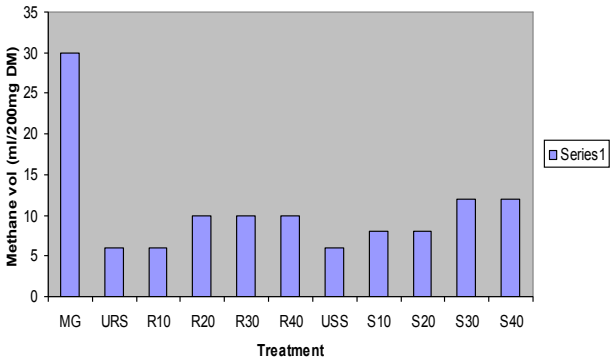

Figure 2: Methane production for untreated and 10 – 40 days treated rice straw and sorghum stalk using maize grain (MG) as a standard.
MG = maize grain, USS = untreated sorghum stalk, S = treated sorghum.
URS = untreated rice straw, R = treated rice.

Table 3. Estimated Organic matter digestibility (OMD), (%), Short chain fatty acid (SCFA), ME = Metabolizable energy.

	OMD	ME	SCFA
USS	30.803^{a}	3.953^{b}	0.1789^{a}
S10	37.413^{c}	5.036^{ab}	0.3701^{cd}
S20	38.286^{d}	5.334^{c}	0.4179^{e}
S30	40.117^{ab}	5.676^{e}	0.4657^{d}
S40	41.780^{a}	6.020^{d}	0.5135^{a}
URS	31.892^{cd}	3.664^{c}	0.1311^{c}
R10	31.935^{e}	3.931^{a}	0.1789^{ab}
R20	40.165^{d}	5.285^{b}	0.4179^{cd}
R30	40.280c	5.571^{c}	0.4657^{e}
R40	42.087^{b}	6.061^{e}	0.5135^{c}

USS = Untreated sorghum stalk, URS = Untreated rice straw. $^{a, b, c}$ means values followed on the same column for each substrates are significantly different by Duncan's Multiple range test ($P<0.05$).

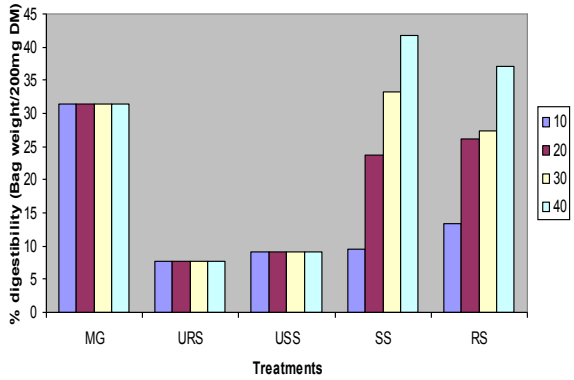

Figure 3: Percentage digestibility (200mg/ DM) of untreated and 10 – 40 Days treated rice straw and sorghum stalks with maize grain (MG) as a standard. USS = untreated Sorghum stalk, URS = Untreated rice straw, RS = Rice straw, SS = *P. florida* Sorghum stalk.

The levels of SCFA indicate the energy available to the animal and it contributes nearly 80% of animal daily energy requirement (Fellner, 2004). SCFA was directly proportional to metabolizable energy (Menke *et al.*, 1979). The result agrees with this because the SCFA increases as fermentation increase giving highest values in 40 days treated substrates. The ME was higher in all the treated substrates with highest values coming from the 40 days treated substrates and this indicates that there is an improvement in energy station of the substrates and thus, the potential of been incorporated in conventional feed mixtures.

4. Conclusion

From the present study, *Pleurotus pulmonarius* shows potential positive increase degradation in the lignin contents of the fungal treated wastes. This is of great importance when considering the use of these agro wastes as possible use as animal feeds

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