

CHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY OF RICE STRAW TREATED WITH *PLEUROTUS OSTREATUS*, *PLEUROTUS PULMONARIUS* AND *PLEUROTUS TUBER-REGIUM*

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ABSTRACT

The nutritive value of rice straw treated with three different edible mushrooms: *Pleurotus ostreatus* (POR), *Pleurotus pulmonarius* (PPR) and *Pleurotus tuber-regium* (PTR) were studied through analysis of their proximate composition, mineral composition, crude fibre fractions and *in vitro* digestibility. Results of the proximate analysis showed an increase in the crude protein from 4.69 % in control to 7.69 % for PTR. Fungal treatment decreased crude fibre from 32.89 % in control to 19.96 % in PTR. Treatment effect on cellulose, neutral detergent fibre, acid detergent fibre and acid detergent lignin was significant. The mineral contents (g.kg⁻¹ DM) showed that PPR had the highest concentration of Ca (11.04) and Mg (5.00), and a significantly highest gas volume was obtained in PPR and the gas production rate constant (C) was not significant. The estimated metabolisable energy (ME) (MJ.kg⁻¹ Dm), organic matter digestibility (OMD %) and short chain fatty acid (SCFA) (µm) ranged from 6.47 (control) to 7.54 (POR), 51.17 (control) to 57.02 (POR) and 0.657 (control) to 0.848 (POR). Treatment effect on the insoluble but degradable fraction (b) was significant ranging from 22 mL in control to 28.33 mL in PTR. It is therefore concluded from this study that treatment of rice straw with different edible mushrooms improved the potential feeding value of the resultant substrate. Therefore, the product of fungal treatment has a good potential as feed resources for ruminants.

Key words: rice straw; nutritive value; edible mushroom; proximate composition; mineral composition; *in vitro* digestibility

INTRODUCTION

All over the world, different species of livestock are reared in an attempt to meet man's demand for animal protein. The high demand for protein occasioned by the increasing population can be tackled, simply by increasing ruminant livestock production. However, the ruminant livestock production in the developing countries, such as Nigeria is underdeveloped because livestock production is still very much at the subsistence level. For the few who are into commercial livestock production are faced with the problem of raising their animals predominantly on forages which are inherently poor in nutritive value. The availability of these forages is also seasonal. Ruminant livestock raised in this region, therefore tend

to reflect the cyclical variation in quantity and quality of these available forages (Bamikole and Babayemi, 2008). Although, ruminants are endowed with the ability to convert low quality feed into high quality protein and utilize feeds from land not suitable for cultivation of crops, however, the utilization of these low quality crop residues is hampered by its low protein content, fibre, digestibility, vitamin and minerals (Akinfemi, 2010a, b).

Most of the rice straws generated from rice farming in Nigeria are either fed to livestock, burnt or allowed to rot. Apart from the inherent problem of air pollution caused by burning, it also releases particle matter into the atmosphere. In recent time, there is global concern on human activities such as burning of wastes or refuse with the view of reducing impact of burning

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on ozone layer depletion. Such global concern, therefore, necessitated alternative option or method of recycling of waste or residues into beneficial products. The possibility of recycling rice straw into value added product therefore comes into view. The aim of this study therefore was to investigate the impact of edible mushrooms on the proximate composition, mineral content and *in vitro* digestibility of rice straw.

MATERIAL AND METHODS

Preparation of samples

Dried samples of rice straw were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The materials were milled and oven-treated at 65°C to constant weight for dry matter determination.

Fungi

The sporophores of *Pleurotus tuber-regium*, *Pleurotus pulmonarius* and *Pleurotus ostreatus* growing in the wild were collected from University of Ibadan botanical garden. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Degradation of rice straw by *P. tuber-regium*, *P. pulmonarius* and *P. ostreatus*

Preparation of substrate

The jam bottles used for this study were thoroughly washed, dried for 10 min. at 100°C. 25.00 g of the dried milled substrates were weighed separately into a jam bottle and 70 ml of distilled water were added. The bottle was immediately covered with aluminium foil and sterilized in the autoclave at 121°C for 15 min. Each treatment was done in triplicates.

Inoculation

Each bottle was inoculated at the centre of the substrate with 2, 10.00 mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30°C and 100 % relative humidity (RH). At day 21 of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradation were oven dried to turn to constant weight for chemical analysis and *in vitro* digestibility.

In vitro gas production

Rumen fluid with pH of 6.5 was obtained from three West African Dwarf female goats through suction tube via the oesophagus before morning feed. The animals were fed with 40 % concentrate (40 % corn,

10 % wheat offal, 10 % palm kernel cake, 20 % groundnut cake, 5 % soybean meal, 10 % brewers grain, 1 % common salt, 3.75 % oyster shell and 0.25 % fishmeal) and 60 % Guinea grass. Incubation was carried out (Menke and Steingass, 1998) in 120 ml calibrated syringes in three batches at 39°C. The inoculums (30 ml) containing cheese cloth strained rumen liquor and buffer (9.8g NaHCO₃ + 2.77g Na₂HPO₄ + 0.57g KCl + 0.47g NaCl + 0.12g MgSO₄·7H₂O + 0.16g CaCl₂·2H₂O) was added to 200 mg sample in the syringe in a ratio (1:4 v/v) under continuous flushing with CO₂. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs. After 24 hrs of incubation, 4 ml of NaOH (10M) was added to estimate the amount of methane produced (Fievez *et al.*, 2005). The average volume of gas produced from the blanks was deducted from the total volume of gas produced. Fermentation characteristics were estimated using the equation $Y = a + b(1 - e^{-ct})$ (Orskov and McDonald, 1979), where Y = volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), b = gas production rate constant for the insoluble fraction, (a + b) = final gas produced, C = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy (ME, MJ.kg⁻¹ DM) and organic matter digestibility (OMD %) were estimated (Menke and Steingass, 1998) and short chain fatty acids (SCFA) were calculated (Getachew *et al.*, 1999) as follows:

$$ME \text{ MJ/kg DM} = 2.20 + 0.136 *G_v + 0.057* + 0.0029*CF$$

$$OMD = 14.88 + 0.88G_v + 0.45CP + 0.651XA;$$

$$SCFA = 0.0239*G_v - 0.0601;$$

where G_v = net gas production (ml/200mg DM), CP = crude protein, CF = crude fibre and XA = ash.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) and significant difference occurred means were separated (Duncan (1955) using Statistical Analysis System (SAS) package.

RESULTS AND DISCUSSION

Table 1 shows the result of the proximate composition and cell wall constituents of fungal treated rice straw. Crude fibre content was lowest in PTR and highest in control with significant differences (P<0.05) between control, POR and PTR. The CP content in the untreated group (control) was lower than the fungal treated rice straw. The EE content of 1.66 g.kg⁻¹ DM was significantly (P<0.05) lowest in control and was highest in PTR with a value of 2.33 g.kg⁻¹ DM. Variation in EE content of the fungal treated straw was not significant (P>0.05). Treatment effect on NDF, ADF, ADL and

cellulose was significant ($P>0.05$). Treatment effect on hemicelluloses was not significant.

The mineral composition of fungal treated rice straw, as shown in Table 2, indicates that the impact of fungal treatment on Na and Cu was not significant ($P>0.05$). The value recorded for Mg in control was significantly ($P<0.05$) lower compared with the values recorded for the fungal treated straw. However, the variations in POR, PPR and PTR were not significant ($P>0.05$). Treatment effect on K, Mn, Fe and P was significant ($P<0.05$).

The data on gas volume and in vitro gas production characteristics are shown in Table 3; the data on estimated OMD, SCFA and ME are presented in Table 4. Gas volume increased significantly ($P<0.05$) in all the fungal treated compared with untreated (control). Gas volume at 24 hrs of incubation increased from 30 ml in control to 38 ml in POR. Similar trend of increase in gas volume was recorded at 72 hrs of incubation while gas volume at 48 hrs of incubation increased from 38 ml in control to 54 ml in POR. At all incubation times, POR had the highest gas volume followed by PPR. The estimated

Table 1: Proximate composition and cell wall contents (g.kg⁻¹ DM) of fungal treated rice straw

Component	Control	POR	PPR	PTR	SEM
Dry matter	93.00 ^a	86.75 ^b	84.21 ^c	86.00 ^b	0.03
Crude protein	4.69 ^b	7.39 ^a	7.18 ^a	7.69 ^a	0.12
Crude fibre	32.89 ^a	20.96 ^b	21.59 ^b	19.96 ^c	0.17
Ether extract	1.66 ^b	2.09 ^a	2.13 ^a	2.33 ^c	0.07
Ash	11.95 ^a	8.26 ^d	8.31 ^c	9.26 ^b	0.003
Nitrogen free extract	48.81 ^b	61.30 ^a	60.79 ^a	61.38 ^a	0.13
Neutral detergent fibre	69.96 ^a	61.67 ^c	62.79 ^b	61.38 ^d	0.003
Acid detergent fibre	56.28 ^a	48.12 ^c	49.78 ^b	47.12 ^d	0.003
Acid detergent lignin	12.54 ^a	10.06 ^c	10.15 ^b	9.68 ^d	0.003
Cellulose	43.74 ^a	38.06 ^c	39.63 ^b	37.44 ^d	0.005
Hemicellulose	13.68 ^b	13.55 ^c	13.01 ^d	14.26 ^a	0.005

Row means with different superscripts differ significantly at ($P<0.05$), $n=3$

POR = *Pleurotus ostreatus* treated rice straw, PPR = *Pleurotus pulmonarius* treated rice straw,

PTR = *Pleurotus tuber-reguim* treated rice straw, SEM = Standard error of mean

Table 2: Mineral compositions (mg.kg⁻¹) of major minerals and trace minerals (ppm) of fungal treated rice straw

Component	Control	POR	PPR	PTR	SEM
Major minerals					
Na	0.0609 ^a	0.0501 ^c	0.0350 ^b	0.0400 ^d	0.010
K	0.957 ^a	0.789 ^c	0.817 ^b	0.760 ^d	0.002
Ca	2.24 ^d	9.20 ^c	11.04 ^a	9.60 ^b	0.020
P	0.39 ^c	1.57 ^a	0.61 ^b	0.400 ^c	0.017
Mg	2.35 ^b	4.23 ^a	5.00 ^a	4.30 ^a	0.170
Trace minerals					
Cu	0.005	0.012	0.014	0.017	0.001
Fe	0.45 ^d	0.64 ^a	0.60 ^b	0.47 ^c	0.002
Zn	0.021 ^b	0.053 ^a	0.050 ^a	0.030 ^b	0.002
Mg	0.29 ^d	0.43 ^a	0.41 ^b	0.32 ^c	0.002

Row means with different superscripts differ significantly at ($P<0.05$), $n=3$

POR = *Pleurotus ostreatus* treated rice straw, PPR = *Pleurotus pulmonarius* treated rice straw,

PTR = *Pleurotus tuber-reguim* treated rice straw, SEM = Standard error of mean

OMD was significantly ($P < 0.05$) highest in the fungal treated compared with the control. The value was ranged from 51.17 % in control to 57.02 % in POR. Moreover, estimated SCFA and ME was highest in POR followed by PPR and PTR, and lowest in control. Variations in the gas production rate constant (C) between the control and the fungal treated were not significant values ($P > 0.05$) recorded in the insoluble but fermentable fraction (b) as affected by fungal treatment was significant ($P < 0.05$). However, the values recorded for POR and PTR were not significant ($P < 0.05$).

The proximate composition of fungal treated rice straw presented in this study showed that changes in the CP contents compared favourably with those reported for some fungal treated residues favourably with those reported for some fungal treated residues (Akinfemi *et al.*, 2010c). Fungal treatment increased the CP and ash contents of the straw compared with the control. Such apparent increase could be due to the proliferation of fungi during degradation (Farkas, 1979; Belewu and Belewu, 2005). This agrees with the report published by Farkas (1979) and Jacqueline and Viser (1996), who noted

that the extracellular enzymes secreting fungus contain amorphous home and heteropolysaccharides, which are associated with fungal protein. Some authors (Zadrazil, 1993; Belewu and Okhawere, 1998, and Akinfemi *et al.*, 2010b) reported that colonization of substrates by fungal mycelia results in increase in their nutritional values. The variations in the CP content as affected by the fungi used may be attributed to strain differences, length of fermentation and the physiological behaviour of the fungi. All the fungi used were effective in degradation of CF because the hyphae of these fungi were capable of penetrating deep into the cells of the straw. This means that fungi not only grow on the surface of the substrate but also penetrated deep into the substrates. This observation is consistent with such findings (Shoukry *et al.*, 1985), in which CF decreased while CP increased. This trend is consistent with decrease in NDF, ADF and ADL (Albores *et al.*, 2006).

Earlier reports (Karunananda *et al.*, 1995) concluded that lignifications of structural polysaccharides not only inhibited ruminal microbial digestion of polysaccharides by forming 3-D matrix, but

Table 3: Gas volume and *in vitro* gas production characteristics

Component	Control	POR	PPR	PTR	SEM
C h ⁻¹	0.032	0.021	0.105	0.030	0.02
b (ml)	22.00	28.00	25.00 ^b	28.33 ^a	0.38
Gv 24hrs	30.00 ^d	38.00 ^a	35.00 ^b	33.00 ^c	0.37
Gv 48hrs	38.00 ^c	54.00 ^a	46.00 ^b	47.00 ^b	0.33
Gv 72hrs	44.00 ^d	58.00 ^a	56.00 ^b	52.00 ^c	0.33
CH ₄ (ml)	12.00 ^a	10.00 ^b	9.00 ^c	10.00 ^b	

Means along the same row with different superscript are significant ($P < 0.05$), n=3

POR = *Pleurotus ostreatus* treated rice straw, PPR = *Pleurotus pulmonarius* treated rice straw,

PTR = *Pleurotus tuber-reguim* treated rice straw, SEM = Standard error of mean

Table 4: Estimated organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolisable energy (ME) of fungal treated rice straw

Component	Rice Straw				SEM
	Control	POR	PPR	PTR	
ME (MJ.kg-1 DM)	6.49 ^d	7.54 ^a	7.14 ^b	6.86 ^c	0.05
SCFA (μM)	0.657 ^d	0.0848 ^a	0.776 ^a	0.729 ^c	0.01
OMD (%)	51.17 ^c	57.02 ^a	54.32 ^b	52.62 ^c	0.30

Row means with different superscripts differ significantly at ($P < 0.05$), n=3

POR = *Pleurotus ostreatus* treated rice straw, PPR = *Pleurotus pulmonarius* treated rice straw,

PTR = *Pleurotus tuber-reguim* treated rice straw, SEM = Standard error of mean

also depicted liquefied tissues which formed a physical barrier. This prevented accessibility of highly digestible tissues to the action of hydrolytic enzymes of the rumen microorganisms. Furthermore, the decrease in cellulose, as affected by fungi, in this study suggested that the substrate is acceptable to the degrading fungi. It provides the fungi with the energy source for growth, as reported elsewhere (Rolz *et al.*, 1986).

The mineral composition of the treated substrate indicated that Ca and Mg are the most abundant. This was probably contributed by the fungi used. Reports of Onwuka and Akinsoyinu (1988) suggested that the presence of mineral elements in animal is vital for the animals' metabolic process. The results obtained for Ca in this study after fungal treatment were higher than those reported later (Ayodeji, 2005; Ngamsaeng *et al.*, 2006 and Oni *et al.*, 2010).

Generally, the major minerals were within the range of value previously reported (McDowell, 1985). The values are adequate to meet the requirement for growth, reproduction and milk in West Africa dwarf sheep and goats (Babayemi, 2006). The calcium and phosphorus ratio were not within the approved 1:1 to 2:1 range recommended (McDowell 1985). All the trace mineral contents in the present study were extremely deficient in the treated straw. This therefore implies that the feed may be fortified with minerals in form of either salt lick or diet inclusion (Babayemi, 2006).

The fermentation of the insoluble but degradable fraction (b) increased with fungal treatment, a reflection of the beneficial effect of the fungi used. Furthermore, the high fermentation of the insoluble but degradable fraction (b) observed in the treated straw may possibly be influenced by the carbohydrate fractions readily available to the microbial population (Chumpawadee *et al.*, 2007), a reflection of its improved nutritive value.

The cumulative gas volume at 24, 48 and 72 h after incubation was higher in the treated substrates. The gas volumes ranked from the highest to the lowest were as follows: POR, PPR, PTR and control. Menke *et al.*, (1979) suggested that gas volume at 24 h after incubation is in indirect relationship with metabolisable energy in feedstuffs. Others (Sommart *et al.*, 2000) suggested that gas volume is a good parameter to predict digestibility, fermentation of end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Report elsewhere (Sommart *et al.*, 2000; Nitipot and Sommart, 2003) indicated that *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume. Gas volume has also shown to have a close relationship with feed intake (Blummel and Becker, 1997) and growth rate (Blummel and Orskov, 1993).

The higher gas volume recorded in the treated straw was likely to have been caused by its reduced

contents of cell wall, especially ADF and ADL. Lignin has been implicated in rations with depressed digestibility (Van Soest, 1994) due to its effect on lowering the rate of microbial colonization of such high fibre feed (Silva and Orskov, 1988). This implies good digestibility potential for the fungal treated rice straw when harnessed as feed resources for ruminant livestock.

Although gas production is a nutritionally wasteful product (Mauricio *et al.*, 1999), but provides a useful basis from which metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) may be predicted.

High OMD was observed in POR and PPR suggesting that the microbes in the rumen and animal have high nutrient uptake (Chumpawadee *et al.*, 2007). The higher fibre content in control probably resulted in lower OMD since high NDF and ADL content in feedstuffs result in lower fibre degradation (Van Soest, 1988). In general, tropical crop residues have a large proportion of lignified cell walls with low digestibility.

Higher production of gas and the eventual preponderance of SCFA in the fungal treated rice straw probably showed an increased proportion of acetate and butyrate but may mean a decrease in proximate production (Babayemi *et al.*, 2004b). However, since the treated straw yielded better SCFA than the control suggests a potential to make energy available to the ruminants (Babayemi *et al.*, 2006).

The estimated ME was found to be comparable to that reported for fungal treated millet stover (Akinfemi *et al.*, 2010a), melon husk (Akinfemi *et al.*, 2010b), sorghum stover (Akinfemi *et al.*, 2010c) and maize cob (Akinfemi *et al.*, 2010d). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew *et al.* 1998; Getachew *et al.*, 2002; Aiple *et al.*, 1996). Others (Krishnamoorthy *et al.*, 1995) suggested that *in vitro* gas production technique should be considered for estimating ME in tropical feedstuffs. Evaluating ME using *in vitro* technique reduces cost, time and is comparable to those evaluated by *in vivo* method.

CONCLUSION

This study validated earlier report that *in vitro* gas production technique can be used to evaluate the potential value of feedstuffs. Besides, fungal treatment of rice straw not only improved the CP contents but also enhanced digestibility: fungal treated rice straw have a good potential as feed resources for ruminant animals and could be used in combination with other feedstuffs. However, more work may be required before application to *in vivo* studies.

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