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Original Research Article

Bioconversion of Rice Straw as a Ruminant feed Using Three Strains of White Rot Fungi

ABSTRACT

Aims: To determine nutritive value of rice straw samples biodegraded by different fungal strains
Study Design: Completely Randomized Design
Place and Duration of Study: fungal inoculation of rice straw samples was carried out at the Federal Institute of Industrial Research Oshodi (FIIRO) Nigeria, for 21 days; proximate and fibre fractions analysis was done at the laboratory of the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria, while *in vitro* gas production was carried out at the laboratory of the Department of Animal Science, University of Benin, Nigeria.
Methodology: Samples of untreated rice straw (UTRS) were biodegraded for 21 days using three strains of edible mushroom *Pleurotus tuberregium* (PTTRS)*, Pleurotus pulmonarius* (PPTRS) *and Pleurotus ostreatus* (POTRS). The substrates were analyzed for changes in the proximate composition and crude fibre fractions. *In vitro* Gas Production (IVGP) was used to predict the Metabolizable Energy (ME), Organic Matter Digestibility (OMD), Short Chain Fatty Acids (SCFA), methane production (CH_4), soluble fractions (a), potential gas production (b), potentially degradable fraction (a+b), rate of fermentation (c) and incubation time (t).
Results: Proximate composition showed crude protein (CP), crude fibre (CF), ether extract (EE), ash and carbohydrate values to be 8.32, 15.32, 5.95 and 6.44%; 34.14, 24.72, 36.85 and 50.54%; 2.91, 3.93, 3.43 and 1.90%, 5.88, 12.38, 7.26 and 13.76% and 48.75, 43.65, 46.51 and 27.40% for UTRS, PTTRS, PPTRS and POTRS respectively. Crude fibre fractions for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose (HEM) and cellulose (CL) were 72.06, 77.43, 73.71 and 76.63%; 51.31, 6.14, 12.44 and 10.87%, 11.39, 1.43, 1.23 and 1.37%; 20.75, 71.29, 61.27 and 65.77% and 39.92, 4.71, 11.22 and 9.49% for UTRS, PTTRS, PPTRS and POTRS respectively. Estimates of ME, OMD, SCFA and CH4 were 4.23, 7.29, 7.32 and 9.10 MJ/kg DM; 31.34, 52.94, 45.39 and 57.83%; 0.18, 0.56, 0.56 and 0.78 umol/200 mg DM and 13.50, 9.33, 10.33 and 10.67 ml for UTRS, PTTRS, PPTRS and POTRS respectively. IVGP characteristics for a, a+b, b, c and t were 1.25, 2.38, 1.13 and 1.57; 9.25, 23.88, 24.13 and 24.07; 8.00, 21.50, 23.00 and 22.50; 0.11, 0.41, 0.35 and 0.35 and 21.00, 8.00, 9.00 and 9.00 hours for UTRS, PTTRS, PPTRS and POTRS respectively
Conclusion: Based on the result, *Pleurotus tuberregium* degraded rice straw (PTTRS) was adjudged superior to the others due to the higher crude protein, ether extract, potentially degradable fractions, high gas production per unit time and lower crude fibre and methane production.

Key words: In vitro, Nutrients, Rice straw, Degraded, Mushroom

1. INTRODUCTION

Huge amounts of agricultural waste are produced from crop farms all over the world annually [1], one of which is rice straw. Rice straw, a residue arising from rice farming, is produced in copious quantities worldwide annually. It is a residue of the rice plant left after the grains have been harvested [2]. Despite the great quantity produced, it is reported to be low in nitrogen, rich in polysaccharides and has a high lignin and silica content, limiting voluntary intake and reducing degradability by ruminal micro organisms all

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of which serve as a hindrance to its effective utility as a livestock feed [3] [2]. Biodegradation of crop residues using edible mushroom has been reported to give rise to products with enhanced nutritive value that promote farm animal performance [4]. Thus rice straw could also be converted to a useful animal feed resource through biological treatment. This is particularly useful to ensure provision of animal protein sources for the teeming world population. Animal nutritionists are continuously evaluating forages and other feed resources so as to provide solutions to problems arising from practical feeding of livestock in specific situations and on a year round basis. The *in vitro* gas production technique of assessing nutritive value of feeds and feedstuffs, based on use of syringes [5] has become popular due to its advantages of convenience, less requirement of time, less cost and ability to analyze many samples almost simultaneously. [6] used the *in vitro* gas production method to assess the replacement value of *Panicum maximum* (PM) with *Ficus polita* (FP) at four levels in two seasons (dry and wet). Chemical composition and qualitative analysis of saponin and tannins were determined. In vitro gas production of PM and FP were carried out over a 24 hour incubation. Metabolizable energy, organic matter digestibility and short chain fatty acids were predicted. Results indicated that FP contained high levels of protein in the dry and wet seasons. Values for crude fibre, ether extract, ash and neutral detergent fibre in the dry and wet seasons were 22.0 and 19.0 g/100 g DM, 20.5 and 17.8 g/100 g DM, 7.8 and 10.0 g/100 g DM, 67.0 and 88.0 g/100 g DM respectively. Qualitative contents of secondary metabolites showed that FP contained low amounts of saponin in the dry season while tannin was absent in both seasons. In vitro gas production, Metabolizable energy, organic matter digestibility short chain fatty acids and methanogenesis were not significantly affected by levels of inclusion of FP in forage diets. The aim of this study was to evaluate rice straw samples ensuing from biodegradation by three edible mushrooms *Pleurotus tuberregium, Pleurotus pulmonarius and Pleurotus ostreatus* by assessing the proximate composition, crude fibre fractions, in vitro gas production and nutrient estimations.

2. MATERIALS AND METHODS

2.1 Sample collection

Samples of dried rice straw were collected from crop farms around the environs of the University of Agriculture, Makurdi, Benue State. The samples were milled and oven dried at 65º C until a constant weight was obtained for any dry matter determination.

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2.2 The 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 [7]. The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

2.3 Degradation of rice straw by different fungi

2.3.1 Preparation of Substrates

The jam bottles used for this study were thoroughly washed, dried for 10 min. at 100 ^{0}C. 25.00 g of the dried milled substrate was weighed individually into each jam bottle and 70 ml distilled water was added. The bottle was immediately covered with tin foil and sterilized at 121 ^{0}C for 15 min. Each treatment was done in triplicate.

2.3.2 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 ^{0}C and 100 % relative humidity (RH). At day 21 of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradations were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

2.3.3 *In vitro* gas production

This was carried out at the Animal Science laboratory of University of Benin. Rumen fluid was obtained from three West African Dwarf male 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 [5] in 120 ml calibrated syringes in three batches at 39 ^{0}C. To 200 mg sample in the syringe was added 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (9.8 g $NaHCO_3$ + 2.77 g Na_2HPO_4 + 0.57 g KCL + 0.47 g NaCl + 0.12 g $MgSO_4.7H_2O$ + 0.16 g $CaCl_2$. $2H_2O$ in a ratio (1:4 v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs. After 24hr of incubation, 4 ml of NaOH (10M) was introduced to estimate the amount of methane

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produced [8]. 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}) [9], where Y = volume ofgas 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 DM) and organic matter digestibility (OMD %) were estimated [5] and short chain fatty acids (SCFA) was calculated [10].ME MJ/kg DM = 2.20 + 0.136 *Gv + 0.057* + 0.0029*CFOMD = 14.88 + 0.88Gv + 0.45CP + 0.651XA;SCFA = 0.0239*Gv – 0.0601;Where Gv, CP, CF and XA are net gas production (ml /200 mg DM), crude protein, crude fiber and ash of the incubated sample respectively.

2.4 Chemical composition

Dry matter was obtained by oven drying the milled samples to a constant weight at 105º C for eight hours. Crude protein was determined as Kjadhal nitrogen x 6.25. ether extracts, crude fibre and ash were determined according to [11] method. Neutral detergent (NDF), Acid detergent fibre (ADF) and Acid Detergent Lignin (ADL) was determined using the method described by [12]. Hemicellulose was calculated as the difference between NDF and ADF while cellulose was calculated as the difference between ADF and ADL

2.5 Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Minitab Statistical software which also separated the means where significant differenced occurred (at 95% probability).

3. RESULTS

3.1 Proximate Composition

The proximate composition of rice straw treated with different fungal species is shown in Table 1. There was a significant (P = 0.01) increase in the crude protein (CP) of the fungal treated RS compared to the untreated RS. The highest CP was by the PTTRS, followed by POTRS while CP of PPTRS was lowest. Crude fibre was significantly (P = 0.04) reduced in PTTRS and increased in PPTRS and POTRS. Ether extract was also significantly (P = 0.01) affected, being highest in PTTRS, followed by PPTRS and least in POTRS. Ash percent was significantly (P = 0.00) highest in POTRS,

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followed by PTTRS and lowest in PPTRS, while the carbohydrate fraction was significantly (P = 0.03) lowest in POTRS, followed by PTTRS and then PPTRS.

3.2 Crude Fibre Fractions

The crude fibre fractions are shown in Table 2. Neutral detergent fibre (NDF) was significantly (P = 0.03) highest in PTTRS, followed by POTRS and then PPTRS. Acid detergent fibre (ADF) values were significantly (P = 0.00) reduced in the order PPTRS, POTRS and then, PTTRS. Acid detergent lignin (ADL) percent was significantly (P = 0.00) least in PPTRS, followed by POTRS and lastly PTTRS. Hemicellulose percent was significantly (P = 0.00) highest in PTTRS, followed by POTRS and PPTRS, while cellulose percent was significantly (P = 0.02) least in PTTRS, followed by POTRS and PPTRS.

3.3 Nutrient Estimations

The Metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA) and Methane production are shown in Table 3. The ME was significantly (P = 0.00) improved, being highest in POTRS while values of PTTRS and PPTRS were similar to each other. The OMD was significantly (P = 0.04) increased, being highest in POTRS, followed by PTTRS and then PPTRS. The SCFA values were also significantly (P = 0.04) improved, following pattern exhibited by ME. Methane

Table 1. Proximate Composition of Rice Straw Degraded by Different Fungal Species

Parameter	UTRS	PTTRS	PPTRS	POTRS	SEM
Crude protein (%)	8.32^{b}	15.32^{a}	5.95^{d}	6.44^{c}	0.11
Crude fibre (%)	34.14^{c}	24.72^{d}	36.85^{b}	50.54^{a}	0.45
Ether extract (%)	2.91^{c}	3.93^{a}	3.43^{b}	1.90^{d}	0.09
Ash (%)	5.88^{d}	12.38^{b}	7.26^{c}	13.76^{a}	0.24
Carbohydrate (%)	48.75^{a}	43.65^{c}	46.51^{b}	27.40^{d}	0.63

^{a,b,c,d} = Means in same row with different superscripts vary significantly (P<0.05)

UTRS = Untreated rice straw; PTTRS = *Pleurotus tuberregium* treated rice straw

PPTRS = *Pleurotus pulmonarius* treated rice straw; POTRS = *Pleurotus ostreatus* treated rice straw

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Table 2. Crude Fiber Fractions of Rice Straw Degraded by Different Fungal Species (%)

Fraction	UTRS	PTTRS	PPTRS	POTRS	SEM
NDF	72.06[d]	77.43[a]	73.71[c]	76.63[b]	0.02
ADF	51.31[a]	6.14[d]	12.44[b]	10.87[c]	1.20
ADL	11.39[a]	1.43[b]	1.23[d]	1.37[c]	1.35
HEM	20.75[d]	71.29[a]	61.27[c]	65.77[b]	0.03
CL	39.92[a]	4.71[d]	11.22[b]	9.49[c]	2.31

[a,b,c,d] = Means in same row with different superscripts vary significantly (P<0.05)

UTRS = Untreated rice straw; PTTRS = *Pleurotus tuberregium* treated rice straw

PPTRS = *Pleurotus pulmonarius* treated rice straw; POTRS = *Pleurotus ostreatus* treated rice straw

NDF = Neutral detergent fiber ADF = Acid detergent fiber ADL = Acid detergent lignin HEM = Hemicellulose; CL = Cellulose

Table**3. Metabolizable Energy, Organic Matter Digestibility, Short Chain Fatty Acids and Methane Production of Rice Straw Degraded by Different Fungal Species**

Treatment	ME (MJ/kg DM)	OMD (%)	SCFA (umol/200 mg DM)	Methane (ML)
UTRS	4.23[c]	31.34[d]	0.18[c]	13.50[a]
PTTRS	7.29[b]	52.94[b]	0.56[b]	9.33[d]
PPTRS	7.32[b]	45.39[c]	0.56[b]	10.33[c]
POTRS	9.10[a]	57.83[a]	0.78[a]	10.67[b]
SEM	0.13	0.51	0.05	0.20

[a,b,c,d] = Means in same column with different superscripts vary significantly (P<0.05)

UTRS = Untreated rice straw; PTTRS = *Pleurotus tuberregium* treated rice straw

PPTRS = *Pleurotus pulmonarius* treated rice straw; POTRS = *Pleurotus ostreatus* treated rice straw

ME = Metabolizable energy OMD = Organic matter digestibility SCFA = Short chain fatty acids

production was significantly (P = 0.01) reduced due to fungal treatment, being lowest in PTTRS, followed by PPTRS and then POTRS.

3.4 *In vitro* gas Production

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The *in vitro* gas production characteristics are shown in Table 4. Gas production from soluble fractions (a) was not significantly (P = 0.02) affected by fungal treatment. Gas production from insoluble fraction (b) was significantly (P = 0.02) affected by fungal treatment, being highest in PPTRS and POTRS, which were similar to each other, but higher than PTTRS which was also significantly higher than the UTRS. Total gas production (a+b) was significantly (P = 0.04) affected by fungal treatment such that values of PTTRS, PPTRS and POTRS were similar to each other, but were significantly different from UTRS. Gas production rate (c) was significantly (P = 0.01) highest in PTTRS, followed by PPTRS and POTRS which were similar to each other, but significantly different from UTRS. Time lag values (t) were significantly (P = 0.00) depressed by fungal treatment, being lowest in PTTRS, followed by PPTRS and POTRS which were similar to each other, but significantly lower than UTRS.

In vitro gas production in 24 hours is shown in Table 5. In general, gas production rate from three to 24 hours was significantly affected by fungal treatment. At three hours significantly (P = 0.04) highest gas production was by PTTRS, followed by PPTRS and then POTRS which was also higher than UTRS. At six hours, PTTRS, PPTRS and POTRS produced values similar to each other but significantly (P = 0.00) higher than UTRS. At nine hours, PTTRS value was significantly (P = 0.03) higher than values of PPTRS and POTRS, which were similar to each other, but higher than UTRS. At 12hrs, values of PTTRS and POTRS were the highest and similar to each other, but significantly (P = 0.04) higher than UTRS. At 15hrs, POTRS produced significantly (P = 0.04) highest value, followed by others in the order PTTRS, PPTRS and lastly UTRS. Gas production values at 18 and 21 hours

Table 4. *In vitro* Gas Production Characteristics of Rice Straw Degraded by Different Fungal Species (ml/200 mgDM)

Fermentation Characteristics	UTRS	PTTRS	PPTRS	POTRS	SEM
a	1.25	2.38	1.13	1.57	0.44
a+b	9.25^{b}	23.88^{a}	24.13^{a}	24.07^{a}	0.78
b	8.00^{c}	21.50^{b}	23.00^{a}	22.50^{a}	0.46
c	0.11^{c}	0.41^{a}	0.35^{b}	0.35^{b}	0.78
t	21.00^{a}	8.00^{c}	9.00^{b}	9.00^{b}	0.50

^{a,b,c,d} = Means in same row with different superscripts vary significantly (P<0.05)

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UTRS = Untreated rice straw; PTTRS = *Pleurotus tuberregium* treated rice straw

PPTRS = *Pleurotus pulmonarius* treated rice straw; POTRS = *Pleurotus ostreatus* treated rice straw

a = Soluble fraction

b = Potential gas production
a+b = Potentially degradable fraction

c = Rate of fermentation

t = incubation time

Table 5. *In vitro* Gas Production of Rice Straw Degraded by Different Fungal Species (ml/200 mgDM)

Incubation Period (hr)	UTRS	PTTRS	PPTRS	POTRS	SEM
3	2.00^{d}	4.50^{a}	3.00^{b}	2.50^{c}	0.65
6	2.67^{b}	22.00^{a}	21.00^{a}	21.00^{a}	0.78
9	5.00^{c}	24.00^{a}	23.00^{b}	23.00^{b}	0.50
12	5.00^{c}	25.00^{a}	24.00^{b}	25.00^{a}	0.50
15	6.00^{d}	25.00^{b}	24.00^{c}	28.00^{a}	0.29
18	7.00^{d}	25.00^{c}	26.00^{b}	28.00^{a}	0.41
21	8.00^{d}	25.00^{c}	26.00^{b}	30.00^{a}	0.29
24	10.00^{c}	26.00^{b}	26.00^{b}	35.00^{a}	0.65

^{a,b,c,d} = Means in same row with different superscripts vary significantly (P<0.05)

UTRS = Untreated rice straw; PTTRS = *Pleurotus tuberregium* treated rice straw

PPTRS = *Pleurotus pulmonarius* treated rice straw; POTRS = *Pleurotus ostreatus* treated rice straw

showed similar pattern of POTRS being the significantly (P = 0.04) highest, followed by PTTRS, PTTRS and lastly UTRS. At 24 hours, POTRS value was (P = 0.04) highest, followed by values of PPTRS and PTTRS, which were similar to each other, but higher than UTRS.

4. DISCUSSION

4.1 Proximate Composition and Fibre Fractions

The highest CP level of PTTRS implies that the fungus effectively degraded the rice straw and colonized it causing the CP level to rise due to CP of the fungal biomass and enzymes. According to [13], increase in CP levels of fungal treated materials is caused by secretion of certain extra cellular

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enzymes which are proteinous in nature during the materials breakdown and its subsequent metabolism. That the CP of PPTRS and POTRS was lower than that of UTRS implies that the fungi were less effective in degrading rice straw. Crude fibre of PPTRS decreased showing that the fungus was effective in breaking down the fibre portion of the rice straw. [3], using white rot to increase the degradability of straw is at the expense of easy assessable carbohydrates, such as cellulose and hemicellulose, and some white rot fungi preferably attack lignin, without degrading cellulose and hemicellulose. In this study, the fungi attacked lignin and cellulose, not hemicellulose, leading to more hemicellulose in the degraded straw. Increase in CF of PPTRS and POTRS means the fungi degraded other nutrients in the rice straw, making the CF to be higher than that of rice straw. The highest EE percent of PTTRS and PPTRS means the fungi contributed more EE while in POTRS; it used EE as a nutrient in its development. According [14], some micro organisms consume some fatty acids as suitable energy for growth. The general increment in ash values of the treated straw means more minerals and vitamins were available after the degradation. This observation is consistent with the report of [7] who reported improved values for ash when maize husk was degraded using different species of white rot fungi. The Lower carbohydrate fraction in all the treated rice straw means that the fungi used carbohydrate as source of energy.

NDF and hemicellulose fractions generally increased due to fungal treatment means the fungi secreted enzymes less effective in breaking down the components. [15] reported that hemicellulose percent was increased when rice straw was treated with *Trichoderma reesi*. The generally reduced levels of ADF, ADL and cellulose demonstrate the fact that the fungi were more effective in degrading those fractions in the straw.

4.2 Nutrient Estimation

The estimated Metabolizable energy increased signifying that fungal treatment produced more nutritive products. This result is not unexpected because ME estimation makes use of gas volume, protein, fat and crude fibre fraction of the feeds.

Organic matter digestibility also improved due to fungal treatment. This is reasoned to be due to reduction in lignin which inhibits digestibility and the high ash value and gas volume used in estimating the organic matter digestibility.

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Short chain fatty acid production increase in the treated materials is reasoned to be due to higher absolute gas production, which was most evident in the 24 hour of incubation. [10] reported close association between SCFA and gas production *in vitro*. During incubation of feedstuff with buffered rumen fluid *in vitro*, carbohydrates are fermented to SCFA and gases, mainly carbon dioxide and methane and microbial cells. This report implies the release of more energy courtesy of fungal treatment.

Methane production decreased due to fungal treatment. Generally, methane represents source of energy waste or loss, as the animals have to eructate or belch it to prevent bloat [16]. This reduction in methane production makes the treated materials more environmentally friendly, as they would contribute less to global warming capable of frustrating food production in agricultural areas due to drought or flood [17].

4.3 In vitro Gas Production

Gas produced from soluble fraction (a) did not significantly differ, but was higher in PTTRS showing its tendency to release more energy from the soluble fractions than the other materials. The final gas produced (a+b) increased in the treated materials. This result is consistent with those of other works [7] [18]. The higher gas production from insoluble, but degradable fraction (b) of the treated materials translates to higher carbohydrate fraction made available consequent upon fungal treatment. The improved rate of gas production (c) in fungal treated materials signified more carbohydrate availability per unit time and also how fast the treated materials would release nutrients for animal use. [19] had reported that rate of gas production determines digestion time and consequently how long a potentially digestible material would occupy space. Thus, the higher gas production rate of the treated materials would make them to be easily digested and other materials fed consequently leading to better performance by animals fed the materials. In general, gas production can be regarded as an indicator of carbohydrate degradation and the low gas production in the UTRS could be explained by lignin binding to the carbohydrate [20]. On the other hand higher gas production of the treated straw is further explained by higher CP and lower CF (especially PTTRS) and the generally reduced lignin. According to [21], lignin protects carbohydrates from attack by rumen microbes and negatively influences *in vitro* gas production. Gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation and carbohydrate because gas production from protein

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fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible [20].

The reduced time lag of the treated materials implies that it takes less time for nutrients to be released. This would be beneficial as the animals will be able to tap nutrients in the materials faster, not having to wait for long before benefiting from feed intake.

5 Conclusion

It was concluded that treatment of rice straw with *Pleurotus tuberregium* significantly improved its nutritive value as there was increase in crude protein and gross energy, while crude fibre and fibre fractions decreased. Also, the fungal treatment of rice straw significantly improved *in vitro* gas production and Metabolizable energy, organic matter digestibility and short chain fatty acids. Additionally, methane production was significantly decreased by fungal treatment of the rice straw, thereby making the resultant product more environmentally friendly and of less potential in contributing to global warming.

6 RECOMMENDATION

It was recommended that fungal treatment of rice straw before feeding to animals should be encouraged due to the additional benefit of reduced methane production.

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