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ORIGINAL RESEARCH ARTICLE

Biodegradation of Water hyacinth (*Eichhornia crassipes* Mart. Solms-Laubach) into value added Ruminant feed using White rot Fungi

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ABSTRACT

A 40 day experiment was carried out on the biodegradation of water hyacinth (WH) into value added ruminant feed using two white rot fungi (*Pleurotus florida* (PF) *and Pleurotus sajor caju* (PS)) in a Solid State Fermentation. Results revealed that crude protein (CP) increased significantly ($p<0.05$) from 11.65% in untreated WH to12.86% and 14.38% in WH treated with PF and PS respectively. This pattern was also observed for ether extract and ash. However, the Crude fibre (CF) decreased significantly ($p<0.05$) from 21.23% in untreated WH to 18.23 and 15.25% in WH treated with PF and PS respectively. Same trend was observed for neutral detergent fibre (NDF) and acid detergent fibre (ADF), which decreased from 66.80 and 36.90% in untreated WH to 63.17 and 32.96, and 64.32% and 33.21% in WH treated with PF and PS respectively. Fungi treatment significantly enhanced Organic matter digestibility (OMD) and metabolizable energy (ME) compared with the untreated WH ($p<0.05$). *In vitro* gas production did not differ significantly between the treatment means for 'a' and 'c' fractions, while the 'b' and 'a+b' fractions ranged significantly ($p<0.05$) from 11.80 and 15.60 ml in untreated WH to 12.12 - 13.00 and 15.85 – 16.73 ml in WH treated with PF and PS for 'b' and 'a+b' fractions respectively. Methane production decreased significantly from 4.00ml/200mg DM in untreated WH to 2.50 and 2.00 ml/200mg DM in WH treated with PF and PS respectively. This study revealed that fungi treatment of WH enhanced chemical composition and *in vitro* degradability. This improvement will enhance the value of WH, in endemic areas, for sustained ruminant production, especially during the off season..

Keywords: Biodegradation, nutritive value, Solid State Fermentation, white rot fungi, water hyacinth

INTRODUCTION

Water hyacinth is an aquatic plant that has been described as the most troublesome weed in the world (Mako, 2009, Wimalarathane and Perera, 2019, Ayanda *et al.*, 2020). It is called the ''World's worst invader'' ISSG (2005) because of its rate of multiplication. It contains nutrients that can meet the requirements of livestock especially ruminants (Akinwande, 2011, Ayanda *et al.*, 2020). The biological value of its protein is as high as that of conventional forages (Mako 2009, Ayanda *et al.*, 2020). It contains considerable quantities of cellulose and hemicellulose that can serve as potential source of energy for ruminant animals. The constraint of water hyacinth in animal nutrition is its poor feeding value, attributed to low dry matter digestibility of resulting from poor degradation of lignin-cellulose complex (LC) during rumen digestion. Solid State Fermentation of LC materials is receiving more attention because it resembles the natural conditions under which fungi grow on LC, hence the need to biodegrade water hyacinth. White rot fungi is reported to increase the nutrient content and digestibility of LC substrates (Mukherjee *et al.*, 2004 and Datsomor *et al*., 2022). Therefore the aim of this study is to use different white rot fungi (*Pleurotus florida* and *Pleurotus sajor-caju*) to improve the nutritive value of water hyacinth in order to enhance its digestibility.

MATERIALS AND METHODS

Sample collection

Water hyacinth was harvested from river Majidun in Odogbolu local government area of Ogun State. The roots were discarded, while the stem and leaves were thoroughly washed, chopped, and sundried to reduce the moisture content. The sundried residue was oven dried (65°C) to constant weight for dry matter determination.

The fungus

The sporophores of *Pleurotus florida* and *Pleurotus sajor-caju* growing in the wild were collected from the Botanical Garden, University of Ibadan. These were cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was then maintained on plate of potato dextrose agar (PDA).

Degradation of water hyacinth by *Pleurotus florida* and *Pleurotus sajor caju*

The jam bottles used for this study were thoroughly washed, and dried for 10 min at 100˚C. Exactly 25.00g of sun dried milled water hyacinth were weighed into each Jamb bottle and 70ml distilled water was added. The bottle was covered immediately with aluminium foil and sterilized in the autoclave at 121ºC for 15 min. Each treatment was replicated thrice.

Inoculation

Each bottle was inoculated at the centre of the substrate with mycelia disc and covered immediately. The inoculated substrates were kept in the dark cupboard in the laboratory at 30ºC and 100% RH. After 40 days of inoculation, the experimental bottles were harvested by autoclaving again to terminate the mycelia growth. Samples of the biodegraded substrates were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

In vitro gas production

Rumen fluid was obtained from three West African Dwarf female sheep through sunction tube before the morning feed. The animals were fed concentrate consisting of 40% corn bran, 35% wheat offal, 20% palm kernel cake, 4% oyster shell, 0.5% salt and 0.5% growers premix for three days prior to the collection of rumen liquor. Incubation was as reported (Menke and Steingass 1988) using 120 ml calibrated syringes in three batch incubation at 39 ^{0}C. 30 ml inoculums was introduced into 200 mg samples in the syringes containing cheese cloth strained rumen liquor and buffer ($NaHC0_3$ + Na_2HP0_4 + KCl + NaCl + $MgS0_4.7H_20$ + $CaCl_2.2H_20$)·(1:2, v/v) under continuous flushing with $C0_2$. Gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24, after 24h of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of gas produced at intervals was plotted against the incubation time, and from the graph, the gas production characteristics were estimated using the equation $Y= a+b(1-e^{-ct})$ described by Orskov and McDonald (1979)

where: Y= volume of gas produced at time 't', a = intercept (gas produced from insoluble fraction), 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 as established (Menke and Steingass 1988) and short chain fatty acids (SCFA, *u*mol) was calculated as reported (Getachew *et al*, 1999)

- ME = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF
- OMD = 14.88 + 0.889GV + 0.45CP + 0.651XA
- SCFA = 0.0239*GV – 0.0601

Where GV, CP, CF and XA are net gas productions (ml /200 mg DM), crude protein, crude fibre and ash of the incubated samples respectively

Chemical composition

DM was determined by oven drying the milled samples to a constant weight at 105ºC for 8hours. Crude protein was determined as kjeldahl nitrogen x 6.25.Ether extract and ash were determined according to AOAC (2012). Neutral detergent fibre and acid detergent fibre were determined as described by Van Soest *et al*. (1991).

Mineral Analysis

A total of ten minerals were analyzed. Treated and untreated WH were digested with HNO_3, $HClO_4$ mixture (20:5v/v), and the digest was made up to 100ml in standard volumetric flask with deionized water. Ca, K, Na, Mg, Zn, Mn, Fe, Cu and Pb in the digest were determined with the atomic absorption spectrophotometer model 420 (Gallenk CMP and CO LTD). Phosphorus in digest was estimated with vanadomolybrate solution. The colour developed was read with spectrophotometer at 420m/u.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and mean separated by Duncan multiple range tests using SAS (2012) package

RESULTS AND DISCUSSION

The result revealed significant ($p<0.05$) increase in crude protein contents of treated water hyacinth compared to untreated WH. It ranged from 11.65% in untreated WH to 12.86% and 14.38% in WH treated with PF and PS respectively (Table 1).

Table 1: Chemical composition (%) of biodegraded water hyacinth

Parameters	WHUT	WHPF	WHPS	SEM
Dry matter	77.90	76.82	77.73	2.20
Crude protein	11.65^c	12.86^b	14.38^a	1.20
Crude fibre	22.13^a	18.23^b	15.25^c	2.13
Ether extract	2.24^c	2.62^b	3.13^a	0.11
Ash	16.81^c	17.35^b	17.82^a	0.20
NDF	66.80^a	64.32^b	63.17^c	1 .00
ADF	36.90^a	33.21^b	32.96^c	0 .11

$^{abc=}$mean onthe same row with different super script differed significantly ($p<0.05$)

WHUT, water hyacinth untreated; WHPF, water hyacinth treated with *Pleurotus florida;* WHPS, water hyacinth treated with *Pleurotus-sajor-caju;* NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of mean.

Table 2: Macro and micro mineral content of biodegraded water hyacinth

Biodegraded water hyacinth	g/100 g DM					Ppm				
	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn	Pb
WHUT	0.65	0.21	0.41	0.17	0.15	527	52.6	12.2	680	17.2
WHPF	0.66	0.23	0.44	0.18	0.17	529	52.8	12.3	678	16.2
WHPS	0.67	0.25	0.42	0.18	0.18	530	52.9	12.4	679	16.3
SEM	0.29	0.03	0.02	0.02	0.03	10.0	1.12	0.30	9.10	0.25

WHUT, water hyacinth untreated; WHPF, water hyacinth treated with *Pleurotus florida;* WHPS, water hyacinth treated with *Pleurotus-sajor-caju.*

These values are higher than value range of 2.2 to 10.3% and 1.9 to 8.9% reported for WH treated with PS and *Pleurotus ostreatus* respectively (Mukherjee *et al*., 2004). These values are also higher than the findings of Dotsomor *et al.* (2022), who reported an increase in CP from 5.1% in untreated rice straw to 6.5 and 6.6% in rice straw treated with *P. ostreatus* and *P. chrysoporium* respectively. This result also agrees with findings of Ramirez-Bribiesca *et al.* (2010) who reported increased CP content of corn straw treated with *P. ostreatus*. Increase in CP content could be attributed to secretion of extracellular enzymes and synthesis of mycelia protein as degradation progressed (Mukherjee *et al*., 2004). Although several species of higher fungi possess ligninolytic activity, *Pleurotus* sp. is the most studied fungi since they improve digestibility (Kundu *et al*., 2005). A similar trend was also observed for ash, ranging from 16.81% in untreated WH to 17.35and 17.82% in WH treated with PF and PS respectively. Since ash determination is a measure of mineral level, it can be inferred that Solid State Fermentation (SSF) contributed to the elevation of mineral levels in the fermented WH. Similar improvement of ash and ether extract content during SSF has been reported by Shi *et al*. (2021). However, there was significant decrease in the crude fibre content ranging from 22.13% in untreated WH to 18.23 and 15.25% and in WH treated with PF and PS respectively. This also corroborates the findings of Mahesh and Mohini (2013). *Pleurotus sajor-caju* showed maximal increase in CP, ether extract and ash enrichment. This agrees with the findings of Mukherjee *et al*., (2004). Results for macro and micro mineral content of biodegraded water hyacyinth presented in Table 2, highlight no significant difference ($p>0.05$) between the treatment means. The Ca, P, k & Mg content obtained here is within the recommended level that meets the requirements of sheep and goats (NRC 2002).

Table 3 shows the estimated gas production parameters of treated and untreated WH. No significant difference was observed for SCFA, this agrees with report by Akinfemi *et al*. (2009). SCFAs are end products of carbohydrate fermentation and this contributes to the energy supply for the host animal (Hoffman *et al*., 2005). OMD varied significantly ($p<0.05$) ranging from 48.50% in untreated WH to 52.12 and 53.89% in WH treated with PF and PS respectively. Same trend was observed for ME, the lowest value (5.68 MJ/Kg DM) in untreated WH is similar to value reported for WH elsewhere (Mako, 2009). The increased value of 7.56 and 8.39 MJ/Kg DM was obtained in WH treated with PF and PS respectively. The extensive degradation of crude fibre and hence, the fibre fractions by the fungi most likely contributed to the increased ME. Methane decreased significantly from 4.00ml in untreated WH to 2.50 and 2.00ml in WH treated with PF and PS, although methane production indicates energy loss to the animal, it was observed that fungi treatment suppressed methane production. This agrees with the findings of Akinfemi (2010). Presented on Table 4 is the *in vitro* gas fermentation characteristic of treated and untreated WH. Fraction 'a' indicates the amount of gas produced from soluble degradable fraction of the samples. This is the fraction that the rumen microbes ferment first to obtain energy. There was no significant ($p>0.05$) differences for this fraction among the treatment means, with the lowest value (3.70 ml/200mg DM) being recorded for untreated WH, while numerically, the WH treated with PF and PS had increased values of 3.72 and 3.73 ml/200mg DM respectively

Table 3: *In vitro* gas parameters of biodegraded water hyacinth

Parameters	WHUT	WHPF	WHPS	SEM
ME	5.65^{c}	7.56^{b}	8.39^{a}	0.50
OMD	48.52^{c}	52.12^{b}	53.89^{a}	2.25
SCFA	0.52	0.54	0.55	0.10
CH_4	4.00^{a}	2.50^{b}	2.00^{c}	0.20

$^{abc=}$mean on the same row with different super script differed significantly ($p<0.05$)

ME, metabolizable energy (MJ/Kg DM); OMD, organic matter digestibility (%); SCFA, short chain fatty acid (µmol); CH_4, methane (ml/200g DM); SEM, standard error of mean.

This result is lower and at variance to the value (2.50 ml/200mg DM) for *Persea americana* leaf, as reported by (Mako *et al*., 2018). The 'b' fraction indicates the fraction that is insoluble but degradable. This fraction is the major source of gas generated during fermentation, it is the fraction that rumen microbes ferment after the

rapidly fermented fraction 'a' had been depleted. This fraction varied significantly ($p<0.05$) among the treatment means, with the lowest value (11.80 ml/200mg DM) being recorded for untreated WH, while WH treated with PF and PS recorded 12.12 and 13.00 ml/200mg DM respectively. These values compared well with the value obtained for WH collected from river (Mako, *et al*., 2009). The 'a+b' fraction indicates the potential degradability of the treated and untreated WH. It followed the same trend with the 'b' fraction with the lowest value (15 60 ml/200mg DM) being recorded for untreated WH, while WH treated with PF and PS recorded higher values of 15.85 and 16.73 ml/200mg DM respectively. These values are comparable and in agreement with values reported by Mako *et al*., (2011). The rate of degradation 'c' amongst the treated and untreated WH was not significantly different.

Table 4: *In vitro* gas characteristics of biodegraded water hyacinth

Parameters	WHUT	WHPF	WHPS	SEM
A	3.70	3.72	3.73	0.50
B	11.80^{b}	12.12^{b}	13.00^{a}	0.20
a+b	15.60^{b}	15.85	16.73	0.23
C	0.050	0.048	0.049	0.10

a,b,c, means on the same row with different superscript differed significantly ($p<0.05$)

a, soluble degradable fraction; b, insoluble degradable fraction; a+b, potential degradability; c, rate of degradation; SEM, standard error of mean.

Presented in Fig 1, is the cumulative gas production obtained from treated and untreated WH. Gases produced during fermentation are waste products and of no nutritive value to ruminants, but gas production tests are routinely used in feed research as gas volumes are related to both the extent and rate of substrate degradation (Blummel *et al*., 1997). The highest (20.37ml) gas produced was obtained in WH treated PS followed closely by WH treated with PF (19.77ml) compared to the value of 18.31ml obtained in untreated WH. This is expected since gas production has positive correlation with crude protein (Sallam *et al*., 2007). Hence fungi treatment enhanced value addition of water hyacinth.

CONCLUSION

In conclusion, the investigated white rot fungi significantly degraded the lignocellulose and increased the crude protein content of water hyacinth. Based on the nutritional value of water hyacinth, Pleurotus cajor caju had the greatest ability to degrade lignin and improve nutritional quality. Hence, biological treatments can be employed for improving the feeding value of low quality feed resources, as revealed in this study. The use of *Pleurotus spp* added value to water hyacinth.

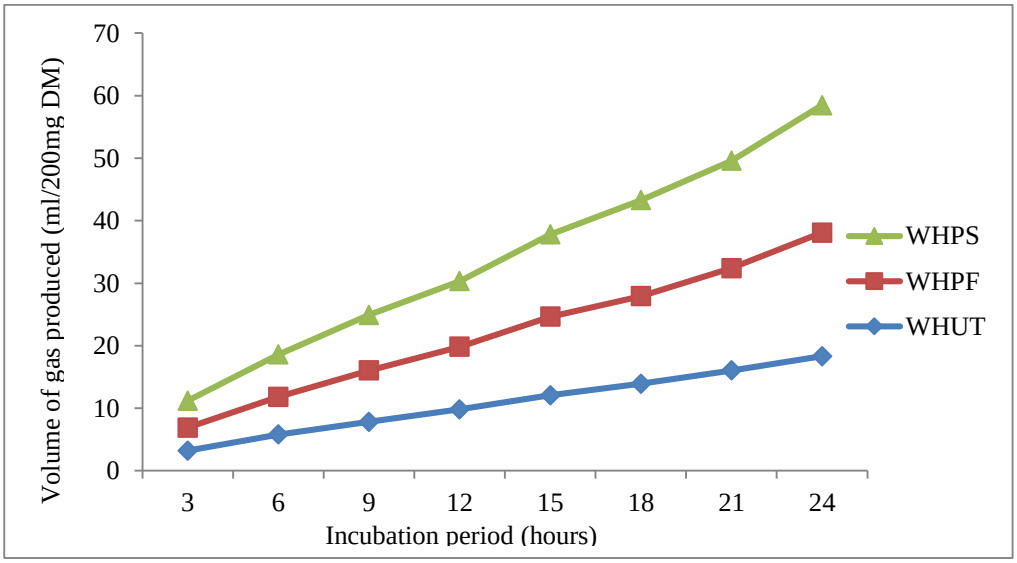

Figure 1 : Gas production of biodegraded water hyacinth

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