Enhancement of cowpea husk, an agrowaste, by white rot fungi towards the making of a potential ruminant feedstuff

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Abstract

A 30-day study was carried out to convert milled cowpea husk (CH) to a value added ruminant feed employing two white rot fungi, Pleurotus florida and Pleurotus sajor caju through a solid state fermentation procedure. Chemical composition of resulting substrate was determined. Also, *in vitro* gas production method was used to determine the digestibility of organic matter (DOM), short chain fatty acids (SCFA) and metabolisable energy (ME). Results showed that, crude protein (CP) increased from 14.90 % for the un-inoculated CH (Control) to 18.14 % for the *Pleurotus florida* biodegraded cowpea husk and to 16.70 % for the *Pleurotus sajor caju* biodegraded cowpea husk. Dry matter remained fairly unaltered ranging from 86.06 - 88.15 % i.e. not significant ($P > 0.05$). No specific trend was observed with ether extract and ash contents ranging respectively from 0.74 - 1.28 and 4.62 - 6.02 %. Conversely, for the fibre detergent fractions, Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin (ADL), hemicellulose and cellulose decreased significantly ($P < 0.05$) after the thirty-day period of fungal biodegradation. The short chain fatty acid was not significantly different ($P > 0.05$) but the digestible organic matter and metabolisable energy increased significantly ($P < 0.05$) compared to the Control. The gas volume showed similar trend, increasing from 5.0 to 8.5 mls/200 mg DM in the control and the Pleurotus florida degraded cowpea husk respectively. In conclusion, the study showed that white rot fungal treatment of cowpea husk improved chemical composition (especially the crude protein),organic matter digestibility and the metabolisable energy.The insoluble but degradable fraction was highest in the Pleurotus florida degraded sample and the gas production rate followed the same trend.

Keywords: Bio-degradation, composition, cowpea-husk, fungi, *in vitro* gas production

Introduction

Of all waste products that occur on earth surface, agro-industrial by-products and wastes (AIBPS) form a big chunk estimated to be about 50 billion tonnes per annum (Smith *et al*., 1983, cited by Akinfemi *et al*., 2009. In Nigeria, because of a long spell dry season when forages are scarce, farmers have little choice than to make use of the available AIBPS within the agro-climatic region. However, major problems with them include high fibre content, bulkiness or high moisture content, low levels of protein, minerals and vitamins. Among the common agro-wastes in Nigeria are rice bran, maize bran, cassava peels, brewer grain, palm kernel wastes, cocoa pods and shells and cowpea husk (or shell) of which is of interest in this study. Disposal of many of these wastes in the years past has been a thorny issue for environmentalists. However, research in the area of biotechnology or solid state fungal fermentation technique can offer a

respite. Fungi are capable of increasing the protein and soluble sugar while at the same time reducing the complex carbohydrates in these wastes (Jonathan *et al.*, 2012; Adenipekun and Dada, 2013; Aransiola and Fagade, 2015). Therefore this paper presents a study on the improvement of the nutritive value of cowpea husk by two white rot fungi i.e. *Pleureotus sajor caju* and *Pleurotus florida* following a 30-day fermentation. Substrates obtained were subjected to an *in-vitro* procedure to determine digestibility of organic matter, short chain fatty acids and metabolizable energy. The implications of these were discussed.

Materials and methods

Sample collection

Dry samples of cowpea husk were obtained from the Tai Solarin Teaching and Research Farm, Ijebu-Ode. Samples were milled and treated in the oven at 65^0 C to constant weight for dry matter determination.

Substrate preparation

Pleurotus florida and *Pleurotus sajor caju* were obtained from the Yaba College of Technology laboratory at Odo Iragunshin, Lagos State, Nigeria. Using the procedure of Jonathan and Fasidi, cited by Akinfemi *et al*., 2009; these were tissue cultured to obtain fungal mycelia, pure culture obtained and maintained on plates of potatoe dextrose agar medium at 25^0 until used. Jam bottles were used for biodegradation. They were washed, dried for 10 min at 100^0 C. Twenty five grammes (25 g) of the dried substrate was weighed into a bottle and 70 ml distilled water added. The bottle was immediately covered with aluminium foil and sterilized in the autoclave at 121^0 for 15 minutes (Akinfemi *et al*., 2009). Each treatment was prepared in triplicate.

Inoculation

Each bottle was inoculated at the centre and surface of the substrate with two 15 mm mycelia disc and covered immediately. Bottles were kept in a dark cupboard in the laboratory at 30^0 C and 100 % relative humidity. On the 30^{th} day of inoculation, the experimental bottles were autoclaved again to terminate mycelial growth. Next, biodegraded samples were oven dried to constant weight and withdrawn for chemical analysis and in-vitro gas production technique to determine the DOM, SCFA and ME.

In-vitro gas production

Samples were incubated *in-vitro* with rumen fluid in calibrated glass syringes according to the method of Menke and Steingass (1988). Rumen liquor was obtained from threee mature sheep through suction tube before feeding in the morning. They were fed with 20 % concentrate feed and 80 % *P. maximum*, twice daily with access to water. About 200 mg of milled samples were weighed into 100 ml calibrated glass syringes in duplicate. The syringes were pre-warmed (39° C) for 1 h, before addition of 30 ml of buffered rumen fluid into each. All syringes were incubated in a water bath maintained at 39° C. Incubation was stopped after 24 h and gas volume (Gv) was noted. Organic matter digestibility (OMD %) and metabolizable energy (ME, MJ/ Kg DM) were calculated using the equations of Menke and Steingass (1988) viz.: OMD = 14.88 + 0.889* Gv + 0.45* CP + 0.65CA and ME = 2.20 + 0.136* Gv + 0.057* CP + 0.0029CF. Where Gv is 24 h net gas production (ml/200 mg DM), CP, CA and CF are respectively, crude protein, crude ash and crude fibre (% DM). Volume of 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, a = intercept (gas produced from soluble fraction), b = gas produced from insoluble fraction, (a + b) = final gas produced, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

Chemical composition

Of the samples, dry matter (DM), crude protein(CP), crude fibre (CF), ether extract (EE) and ash were determined by the standard procedure of AOAC (2012). Also, another set of samples were analyzed for fibre, [neutral detergent fibre (NDF) and acid detergent fibre (ADF) by the procedure of Van Soest *et al.* (1991). Every analysis was done in triplicate.

Statistical analysis

Data obtained were analyzed by ANOVA procedure of SAS (2012) and significant different means separated by Duncan's multiple range tests of the same software.

Results and discussion

Table 1 presents the chemical composition of the *Pleurotus florida* and *Pleurotus sajor caju* biodegraded cowpea husk (CH). The chemical composition of the CH varied. All the proximate fractions were significantly different ($P < 0.05$). Organic matter was in the range 93.98 % to 95.38 %. Crude protein increased from14.9 % in the un-inoculated cowpea husk (UCH) to 18.4 % in the *P. florida* degraded cowpea husk (CHF). Conversely, the NDF decreased from 79.26 % (in UCH) to 72.55 % (in CHF). Ether extract was in the range of 0.74 % to 1.28 % while ash ranged from 4.62 % to 6.02 %. Results in this study indicates a marked increase in CP as a result of biodegradation by fungi (*P. florida* and *P. sajor caju*). This is consistent with other findings like Akinfemi et al. (2009), Adenipekun and Fasidi (2005), Adenipekun and Dada (2013), Aransiola and Fagade (2015). The increase in CP, in the opinion of Kadiri (1999), cited by Adenipekun and Dada (2013), might be a result of secretion of proteinous extra cellular enzymes into the waste (CH) during their breakdown and subsequent metabolism. The two fungi decreased the NDF and ADF or the fibre fraction of the agro-waste. This could be due to cellulose enzymes being secreted by cellulotic fungi or some fungi utilizing cellulose in their process of metabolism. It has been observed that some white rot fungi produce extracellular lignin-modyfying enzymes such as laccase, lignin peroxidase and manganese peroxidases (Isikhumhen and Nerud (1999), cited by Adenipekun and Dada (2013). The low EE fraction in CH suggests that this agrowaste coul probably be used in feed formulation as an energy source without the fear of acidosis developing. DePeters et al. (1997), cited by Areghore and Abdulrazak (1995) had reported that inclusion of by-products high in EE could precipitate problem.

Presented in Table 2 are estimates for short chain fatty acids (SCFA), digestible organic matter (DOM) and metabolizable energy (ME) of the agrowaste CH. The SCFA was not significantly different ($P > 0.05$) but the DOM and ME increased significantly ($P < 0.05$) compared to the Control. The same observation was made in a similar study by Akinfemi et al. (2009). Since SCFA is an end product of carbohydrate digestion in the ruminant animal, it is therefore an indication of energy content. In essence, biodegradation improved the nutritional status of CH.

Table 1: Chemical composition (g / 100g DM) of *Pleurotus florida* and *Pleurotus sajor caju* degraded cowpea husk.

PARAMETERS	UCH	CHF	CHS	SEM
Dry Matter	88.15	86.74	86.06	1.01
Crude Protein	14.90^{c}	18.14^{a}	16.70^{b}	1.05
Ether Extract	0.74^{b}	1.28^{a}	0.79^{b}	0.01
Ash	4.62^{c}	5.87^{b}	6.02^{a}	0.50
Crude Fibre	37.29^{a}	31.16^{b}	30.88^{b}	0.20
Nitrogen Free Extract	30.60^{b}	30.29^{b}	31.67^{a}	0.20
Neutral Detergent Fibre	79.26^{a}	72.55^{b}	73.02^{b}	0.35
Acid Detergent Fibre	54.38^{a}	47.57^{b}	45.05^{c}	1.25
Acid Detergent Lignin	22.55^{a}	20.10^{b}	19.00^{c}	0.50
Cellulose	31.83	27.47	26.05	0.42
Hemi cellulose	24.88	24.98	27.98	0.01

a ,b ,c = Means on the same row differently superscripted are significantly different ($P < 0.05$) , UCH = Uninoculated Cowpea Husk (Control) , CHF = *Pleurotus florida* degraded cowpea husk , CHS = *Pleurotus sajor caju* degraded cowpea husk , SEM = Standard Error of Mean.

Table 2: Organic matter digestibility (%), short chain fatty acids (ml) and metabolizable energy (MJ / Kg DM) of *Pleurotus florida* and *Pleurotus sajor caju* bio-degraded cowpea husk.

PARAMETERS	UCH	CHF	CHS	SEM
Short chain fatty acids	0.11	0.26	0.19	0.04
Organic matter digestibility	26.85^{b}	33.43^{a}	32.00^{a}	1.31
Metabolizable energy	4.03^{b}	5.27^{a}	4.52^{b}	0.20

a, b, c= Means on the same row differently superscripted are significantly different ($P < 0.05$) , UCH = Uninoculated Cowpea Husk (Control) , CHF = *Pleurotus florida* degraded cowpea husk , CHS = *Pleurotus sajor caju* degraded cowpea husk , SEM = Standard Error of Mean

The net gas produced over a 24 h period by the three samples can be seen in Table 3. Net gas produced ranged from 5.0 to 8.5 ml/y24 h and significant difference ($P < 0.05$) observed among the samples; the uninoculated sample (Control) producing the least. Gas production reflects content of fermentable carbohydrate and available nitrogen. Net gas produced is a parameter for predicting digestibility and microbial protein synthesis by rumen microbes. Also, gas production is the result of fermentation of carbohydrates into acetate, propionate and butyrate (Getachew et al., 1999).

Table 3: In –vitro gas production (ml / 200mg DM) of *Pleurotus florida* and *Pleurotus sajor caju* bio-degraded cowpea husk for a period of 24 hours.

	INCUBATION PERIOD (Hrs)							
PARAMETERS	3	6	9	12	15	18	21	24
UCH	3.5^{c}	4.5^{c}	4.5^{b}	5.0^{b}	5.0^{c}	5.0^{c}	5.0^{c}	5.0^{c}
CHF	6.5^{a}	6.5^{a}	6.5^{a}	6.5^{a}	6.5^{a}	7.0^{a}	8.5^{a}	8.5^{a}
CHS	5.0^{b}	5.0^{b}	5.0^{b}	6.0^{b}	6.0^{b}	6.5^{b}	6.5^{b}	6.5^{b}
SEM	0.25	0.21	0.31	0.27	0.40	0.35	0.32	0.27

a, b, c= Means on the same row differently superscripted are significantly different ($P < 0.05$) , UCH = Uninoculated Cowpea Husk (Control) , CHF = *Pleurotus florida* degraded cowpea husk , CHS = *Pleurotus sajor caju* degraded cowpea husk , SEM = Standard Error of Mean

Conclusion

In conclusion, the agro-industrial by-products and wastes (AIBPS) investigated in this study possessed reasonable CP (14.9 %) which increased to 16.7 % and 18.1 % and concomitantly resulted in decreased NDF, ADF, ADL and cellulose as a result of biotechnological skill of biodegradation using fungi *P.sajor caju* and *P. florida.* Due to manipulation,whereas the SCFA was not altered, the DOM and ME increased leading to enhancement of the AIBPS. Net gas production trend showed evidence of improvement on uninoculated samples.Finally, this study demostrated that, fungal treatment of cowpea husk resulted in value added and subsequent possible increased utilization as a forage.

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