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NUTRIENT COMPOSITION, IN VITRO GAS AND METHANE PRODUCTION OF Pleurotus SPECIES TREATED CROP RESIDUES FOR THEIR USE IN RUMINANT DIETS

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ABSTRACT

A promising approach for the provision of high-quality supplementary feed for dry season ruminant feeding is the use of fungal-treated crop residues. This current study, therefore, used 3 *Pleurotus* species (*P. ostreatus* -PO, *P. florida* – PF, and *P. sajor-caju*- PS) to treat 4 crop residues (cowpea chaff - CC, millet chaff - MC, groundnut haulm - GH, and maize stover -MST) over 20 days at room temperature. The treated residues were afterward dried and sub-samples were taken for chemical composition (Dry matter – DM, crude protein – CP, Neutral detergent fibre – NDF, and Acid detergent fibre – ADF) determination and *in vitro* studies (*in vitro* gas and methane gas production). The *Pleurotus* species improved the crop residues nutrient composition through increased CP values and decreased NDF and ADF values but the species varied in their residues preference. PO greatly improved GH and CC; PS greatly improved MC; and PF greatly improved MST. The *Pleurotus* species improved the *in vitro* gas production of millet chaff (57 – 107 ml/ g DM – PF; 123 ml/ g DM – PS; and 130 ml/ g DM – PO) only. The highest *in vitro* gas production in PO-treated MC was complemented with comparable methane gas production (3.28 – 5.19 ml/ g DM) and percentage methane in total gas production (7 – 7%) to intact residue at 72h of incubation. It can be concluded that PO-treated millet chaff can be used as improved supplementary dry season feed for better ruminant production.

Keywords: Dry season; chemical composition; crop residues; *Pleurotus* species; nutritive value.

INTRODUCTION

In Nigeria, ruminants majorly depend on natural pastures as their major source of feed. However, the availability of these pastures during the dry season is limited due to the harshness of weather and low rainfall (Brum et al., 2008), thus affecting animal growth, production, and performance. To augment the scarcity of this feed, farmers utilize crop residues due to their abundance as they are obtained from crops cultivated for human consumption (Illo et al., 2018). Among these crop residues, cowpea chaff, millet chaff, groundnut haulms, and maize stover have been extensively utilized in ruminant diets (Singh et al., 2011; Koura et al., 2016; Haile et al., 2017). However, these crop residues are still low in nutrient composition due to their low crude protein (CP) content as well as high fibre and lignin content. Thus, limiting the intake, digestibility, and utilization and consequently affecting better animal production, performance, and reproduction (Illo et al., 2018). Therefore, there is a need to improve or upgrade the nutritive value of these crop residues.

One of the possible ways of improving the quality of these crop residues is the adoption of the technique of lignin degradation. Lignin is a major component that binds with other polysaccharides in the feed, which make nutrients not efficiently degraded by rumen microbes. (Olafadehan *et al.*, 2009). Several methods such as physical, chemical, physico-chemical, and biological (bacteria, yeast, and fungi) methods have been used to achieve this purpose. The biological method with the use of fungi seems more promising as its usage requires lower cost does not cause any environmental hazards, has no residual effect, and produces enzymes that facilitate the process of degradation (Lee et al., 2009; Kumar and Sharma, 2017). Among the fungi, white rot fungi (WRF) produced better improvement as they degrade lignin with lesser effect on other polysaccharides needed as an energy source for the animals (Guillén et al., 2005). However, *Pleurotus* spp; a WRF usage has generated more attention in that it is safe when fed to animals; its feeding has improved intake and digestibility, it is edible, readily available and no pathogenicity has been recorded (Tuyen et al., 2013; Sharma and Arora, 2015; Niu et al., 2018). Despite its usage in several studies, there is still the need to investigate it with different substrates as they tend to exhibit different functions (i.e. enzymatic and degradability) even when presented with similar substrates. This study, therefore investigated the effects of three (3) Pleurotus spp. (P. ostreatus, P. florida, P. sajor-caju) on the nutrient composition, in vitro gas and methane gas production as well as the percentage methane in total gas production of four crop residues (Cowpea chaff, millet chaff, groundnut haulms, and maize stover).

MATERIALS AND METHODS

Pleurotus spawn source

The *Pleurotus* species (*Pleurotus ostreatus, Pleurotus florida and Pleurotus sajor-caju*) pure spawns were purchased from the Plant pathology Department of Forestry Research Institute of Nigeria (FRIN), Jericho Road, Ibadan Oyo state, Nigeria

Preparation of forages and inoculation with *Pleurotus* species

Dried cowpea chaff, millet chaff, and groundnut haulm were purchased from Eleweran market, Abeokuta, Nigeria while maize stover was harvested from the University Farm, near FADAMA area, Federal University of Agriculture, Abeokuta, Nigeria. The maize stover was chopped to approximately 5cm and dried in an open space until a constant weight was achieved. About 10g of each of the residues were weighed into 9 cleaned and dried Erlenmeyer flasks (250 ml) representing 3 Pleurotus species with 3 replicates, soaked with clean water for 24hrs to absorb moisture, and thereafter drained to remove excess water. The flask's inner tip was immediately covered with cotton wool and aluminum foil paper and then sterilized in an autoclave set at 121°C for 15 minutes. After autoclaving, the flasks were allowed to cool down, inoculated with 2 % of each Pleurotus specie spawn (w/w) under aseptic conditions, and covered. The flasks were then kept on benches in a clean dark room in the laboratory at room temperature for 20 days. Control flasks which include the residue without no autoclaving or Pluerotus spp (intact) and residue with autoclaving but no fungi (control) were made available. After 20 days of inoculation, the flasks excluding the intact flasks were autoclaved again to terminate the mycelia growth. The biodegraded samples in the flasks were dried at 55- 60°C until a constant weight was achieved, milled using a 2mm sieve, and then kept in small bags for chemical analyses and in vitro study.

Chemical analyses

Part of the samples of the intact, autoclaved, and *Pleurotus* species treated crop residues were ovendried at 100°C for 24 hours for DM determination (AOAC, 1990). Samples were ashed at 550°C for 5hrs in the muffle furnace for Ash determination (AOAC, 1990) methods. Crude protein was determined following the procedure of Bradford (1976). Neutral detergent fibre and acid detergent fibre were determined using the method described by Van Soest *et al.* (1991) and Goering and Van Soest (1970) without using sodium sulphite and Dekalin.

In vitro gas and methane production

In vitro incubation study was conducted in accordance with the procedure of Menke and Steingass (1988). Rumen content was collected from a freshly slaughtered cow in Odo-eran abbatoir, Abeokuta, Ogun State into thermoflask. On arrival in the laboratory, the content was strained using four layers of cheesecloth to obtain rumen fluid (RF). Also, buffer solution containing 9.8 NaHCO3 + 9.33 Na2HPO4.12H2O + 0.5 $NaCl + 0.6 KCl + 0.01 (CaCl_2 anhydrous) + 0.1$ (MgCl₂ anhydrous) was prepared and kept in a waterbath set at 39°C. RF and buffer solution were then mixed at 1:2 ratio respectively to obtain the buffered inoculum (BI). The BI pH was adjusted using 1M HCl if found to be above 7.0 ± 0.2 . The BI in the bottles were slightly closed and kept in a water bath set at 39 ⁰C until when ready for use. The crop residues

weighing 0.2 g (n =3) for three (3) selected post incubation time (24h, 48h, and 72h) were weighed into 50 ml calibrated transparent syringes and 20 ml of the BI was added. The end of the syringe tip was immediately locked using a 4 way male slip stop cock and the syringes were placed in an incubator set at 39±1°C for 72h to determine the in vitro gas production of the various treatments. Blank syringes that contain only BI were provided for correction of gas volume. The gas production was measured at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69 and 72hrs. At the end of 24h, 48h and 72h respectively, some syringes (n = 3) were selected and 4ml of NaOH (10M) was introduced into the gas phase level to estimate the methane gas production as reported by Fievez et al. (2005).

Statistical analysis and calculation

One-way ANOVA was used to analyze the chemical composition data and in vitro study data for each crop residue All data sets for the *in vitro* study parameters for each crop residue was statistically analyzed separately using the Completely Randomized Design procedure on Minitab 16 software. A p < 0.05 was considered to indicate statistical significance. Means were separated using the Tukey *post-hoc* test. The percentage of methane in total gas production was calculated by dividing the methane gas (ml/g) by the gas produced (ml/g) multiplied by 100.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of intact, autoclaved and Pleurotus spp-treated crop residues. The crop residues both treated and untreated presented significant (p > 0.05) different variable ash, CP, NDF, and ADF values that ranged from 61.10 - 146.40 g/ kgDM, 21.67 – 130.04 g/kg DM, 549.85 – 688.09 g/kg DM, and 407.41 – 525.98 g/kg DM respectively. The fibre values were slightly lower than the ranges of 617.6 - 714.4 kg DM (NDF) and 421.1 - 523.5 g/kg DM (ADF) for cowpea shells treated with Pleurotus specie (Kinfemi et al., 2009); ranges of 645.9 - 706.3 g/kg DM (NDF) and 416.7 - 515.8 g/kg DM (ADF) for maize cobs treated with Pleurotus specie (Akinfemi, 2010); and ranges of 597.9 – 929.4 g/ kg DM (NDF) and 416.8 - 661.9 g/kg DM (ADF) for maize stover, rice straw, oil palm frond and sugarcane bagasse treated with different fungus (Tuyen et al., 2012). The variations can be linked to the substrate used, time and kind of treatment, fungal strains and species, and growth conditions etc. (van kuijk et al., 2015; Sharma et al., 2017). The autoclaving slightly (p < 0.05) reduced the CP, ash and fibre fraction contents of the crop residues when compared to the intact residues. The obtained results were in line with the findings of Udensi et al. (2010) when the effect of soaking and boiling, and autoclaving on the nutritional quality of Mucuna flagellipes was examined. The reduction in fibre fractions might be that autoclaving disrupts the residues structural component while the loss in CP might be that the disruption was accompanied by

leaching out or solubilisation of solubles such as nitrogenous compounds (Udensi *et al.*, 2010; Siah *et al.*, 2014) that was eventually lost in the process through evaporation or denaturing etc.

The *Pleurotus* species greatly (p < 0.05) decreased the fibre fractions (NDF and ADF) content and increased the CP, and ash contents of the residues more than the intact and autoclaved residues. The fibre reduction can be attributed to the fungal physical possession of rhizoids that penetrate complex intra-cellular tissues of substrates for disruption of internal plant structures which are further broken down by enzymes released by

the fungi (Mahesh and Mohini, 2013; Puniya *et al.*, 2015). The CP increase might be due to the addition of fungal biomass which is proteinous in nature on the treated residues (Maza *et al.*, 2015). Among the *Pleurotus* species, P. *ostreatus* recorded greater fibre reduction in groundnut haulms, and cowpea chaff than other species while it produced a similar fibre reduction with *P. sajor-caju* in millet chaff and maize stover than *P. florida*. The variations show that the degree to which fungi degrade lignocellulosic materials varies even when provided with the same growth conditions (Eriksson *et al.*, 2012).

 Table 1. Chemical composition (g / kg DM) of intact, autoclaved (i.e. control) and 20 days Pleurotus species treated residues

Parameters				Treatme				
Forages	Nutrients	Intact	Control	P. ostreatus	P. florida	P. sajor-caju	SEM	P <
GH	DM(g/kg)	859.89 ^b	838.95°	934.18 ^a	935.32ª	927.13ª	11.10	0.000
				g / kg DM				
	Ash	120.62 ^b	115.72 ^b	135.16 ^a	135.01 ^a	146.40 ^a	3.15	0.000
	СР	112.78 ^b	107.32 ^b	130.04 ^a	116.81 ^{ab}	121.24 ^{ab}	2.42	0.007
	NDF	652.92 ^a	635.79 ^b	549.85 ^d	593.16 ^c	554.76 ^d	11.13	0.000
	ADF	462.29 ^a	450.92 ^b	407.71 ^e	439.15 ^c	427.40^{d}	5.13	0.000
CC	DM(g/kg)	909.86ª	920.92ª	893.71 ^b	912.04ª	873.53°	4.56	0.000
	Ash	76.69 ^c	71.55 ^d	118.25 ^a	82.40 ^b	114.34 ^a	5.27	0.000
	СР	54.12 ^b	50.24 ^b	86.35 ^a	61.42 ^b	84.26 ^a	4.19	0.000
	NDF	639.14 ^a	623.65 ^b	590.67 ^d	611.39°	602.64 ^c	4.55	0.000
	ADF	505.69ª	498.83 ^{ab}	464.82°	487.86 ^b	469.29 ^c	4.52	0.000
MC	DM(g/kg)	917.03ª	912.42ª	900.28 ^b	898.52 ^b	897.87 ^b	2.92	0.000
	Ash	90.45 ^{bc}	88.56°	96.54 ^b	90.49 ^{bc}	108.57^{a}	2.02	0.000
	СР	24.71 ^{ab}	21.67 ^b	28.49 ^{ab}	28.07 ^{ab}	32.78 ^a	1.31	0.05
	NDF	673.53ª	654.23 ^b	627.44°	645.48 ^b	622.67 ^c	5.00	0.000
	ADF	525.98ª	514.32 ^b	442.56 ^c	503.72 ^b	446.64 ^c	9.41	0.000
MST	DM(g/kg)	949.84ª	945.94ª	928.36°	935.62 ^b	932.51 ^{bc}	2.23	0.000
	Ash	66.47°	61.10 ^d	77.91 ^b	83.16 ^a	74.82 ^b	2.16	0.000
	СР	27.49 ^b	24.35 ^b	37.20 ^a	41.63 ^a	37.29 ^a	1.88	0.000
	NDF	688.09ª	666.66 ^b	627.89°	622.38°	624.47 ^c	7.14	0.000
	ADF	502.74 ^a	493.47 ^a	420.93 ^b	413.07 ^b	417.03 ^b	10.74	0.000

DM (dry matter), CP (Crude protein), NDF (neutral detergent fibre), ADF (Acid detergent fibre), ADL (Acid detergent lignin), HEM (hemicellulose), CEL (cellulose), TA (total antioxidant), SEM (Standard error of means) *a.b.c* Values within a *row with different superscripts are significantly different*

Figure 1 shows the *in vitro* gas production of the intact (untreated), autoclaved, and *Pleurotus* species treated crop residues after 0h, 12h, 24h, 36h, 48h, 60h and 72h of incubation. The crop residues had variable (p < 0.05) total gas production (tGP) as influenced by autoclaving, and *Pleurotus* species treatments. The residues tGP ranked from lowest to highest values as follows: ~80 ml/ g DM (MST), ~120 ml /g (GH), ~130 ml/g DM (MC) and ~180 ml/ g DM (CC). The gas produced is a reflection of carbohydrate fermentation more than protein fermentation (Chumpawadee *et al.*, 2007). Also,

gas produced have been identified to be highly correlated with *in vitro* dry matter and organic matter degradability (Nitipot and Sommart, 2003). This indicates that the residues varied in their availability of soluble carbohydrates to rumen microbes for possible degradation. The obtained tGP values were lower than the ranges of 175 - 250 ml /g DM at 72h of incubation (Kinfemi *et al.*, 2009); ranges of 245 – 315 ml/g DM at 11h of incubation (Akinfemi, 2010); and ranges of ~160 – 270 ml/g OM for corn stover treated with different fungal strain (Zuo *et al.*, 2018). The variation might be

due to time of incubation, substrates available degradable carbohydrates, fungal strain and species, and length of substrate degradation by fungus (Arora and Sharma, 2009).

Autoclaving of GC significantly (p > 0.05) improved the gas production than intact and *Pleurotus* species treated GC. Autoclaving and *Pleurotus* species treatment of CC and MST did not improve the gas production compared to the intact CC and MST respectively. *Pleurotus* species treatment of MC increased the gas production than intact and autoclaved MC. The reduced *in vitro* gas production by most of the *Pleurotus* spe treated crop residues contradicts the report of Chumpawadee *et al.* (2007), Kinfemi *et al.* (2009) and Akinfemi (2010). The obtained result was similar to the report of Zuo *et al.* (2018) where the tGP of corn stover was improved by

Irpex lacteus and P. ostreatus but not improved by P. cystidiosus. The variation in gas produced by the treated crop residues might be attributable to availability of chitin and or fungal secretion of metabolites/compounds that inhibits degradation by rumen microbes (Zuo et al., 2018). Among the residues, only MC in vitro gas was improved with fungal treatment and the Pleurotus species improved MC following this trend from highest to lowest, P. ostreatus, followed by P. sajor- caju and lastly by P. florida. The differences might be linked to the species degrading potential (Patel et al., 2009) as well as the chemical composition of the treated MC (Niu et al., 2018). The ability of the Pleurotus species to improve the nutritive value of millet chaff as dry season supplementary feed for ruminant may be a good way forward.

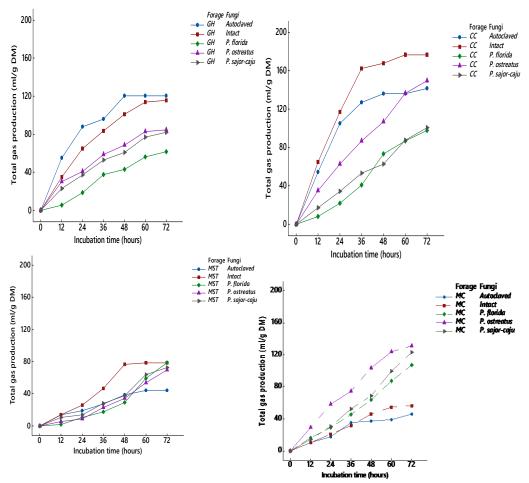


Figure 1: Total gas production (ml / g OM) of intact, autoc*laved (i.e. control) and Pleurotus* treated crop residues after 72 h of incubation in rumen fluid. GH: groundnut haulms; CC: cowpea chaff; MST: maize stover; MC: millet

Table 2 shows the methane gas production of intact, autoclaved and *Pleurotus* species-treated crop residues at 24h, 48h, and 72h of incubation. The crop residues had variable (p < 0.05) methane gas production (tGP) as

influenced by autoclaving, and *Pleurotus* specie treatments. Autoclaving of CC, MST, and MC significantly (p < 0.05) reduced the methane gas production more than intact residues but not in GH. This

might reflect the availability of lesser structural carbohydrates in the autoclaved crop residues than in the intact crop residues. Higher methane gas is produced in the rumen when highly structural feed is fed (Kim et al., 2013). In most cases, autoclaved crop residues recorded lesser methane gas production than Pleurotus-treated crop residues except in GH and P. ostreatus-treated CC. The reduced methane gas production can be linked to reduced degradation as autoclaved crop residues recorded lesser tGP. The few exceptions recorded have autoclaved residues been well degraded. Pleurotus species treatment of GH, CC and MST significantly (p < p(0.05) reduced the methane gas production more than the intact residues but slightly increase the methane gas produced by MC. This can also be linked with substrates degradation by rumen microbes as reflected in the tGP. However the reduced methane gas production was not in line with Kinfemi et al. (2009) and Akinfemi (2010) where reduced methane gas was not caused by reduction

in total gas production. The obtained trend of methane gas in this study was similar to the observation of Tuyen et al. (2013) where fungal treatment of some selected crop residues led to either increased or decreased methane gas production. The differences in methane gas production as observed in this study could be linked to either shift in the volatile fatty acid (acetic, butyric or propionic) pathway by the fungal treatment or by the total gas produced (Kamalak et al., 2002). Acetic and butyric production are positively correlated with methane production while propionic is negatively correlated (Kim et al., 2013). On the other hand, the methane gas production of *Pleurotus* species treated MC was not really different from the intact MC despite their high tGP. This indicates that the inclusion of treated MC in ruminant diet is capable of increasing degradability more than the intact MC with similar methane gas production.

Table 2. Methane gas production (ml / g DM) of intact, autoclaved (i.e. control) and *Pleurotus* species treated grasses after 24, 48 and 72 hours of incubation

Parameters	5			Treatments				
Crop residues	Incubation time	Intact	Autoclaved	P. ostreatus	P. florida	P. sajor-caju	SEM	P value
	(hours)							
GH	24	6.97 ^a	8.28 ^a	1.69 ^b	0.25 ^b	2.37 ^b	0.88	0.000
	48	12.49 ^a	14.03 ^a	4.39 ^b	1.72 ^b	4.56 ^b	1.34	0.000
	72	20.49 ^a	17.25 ^a	9.21 ^b	4.30 ^b	8.98 ^b	1.68	0.000
CC	24	25.07ª	19.15 ^b	8.58°	1.09 ^d	2.64 ^d	2.53	0.000
	48	55.94ª	34.91 ^b	20.51°	10.03 ^d	7.40 ^d	4.86	0.000
	72	66.58 ^a	42.04 ^b	45.74 ^b	19.31°	20.01°	4.94	0.000
MC	24	0.90 ^b	0.61 ^b	2.70 ^a	1.30 ^b	1.04 ^b	0.34	0.000
	48	2.26 ^{bc}	1.26 ^c	5.19 ^a	3.79 ^{bc}	4.65 ^b	1.40	0.003
	72	3.28 ^{ab}	1.72 ^b	5.19 ^a	3.61 ^{ab}	3.88 ^a	2.28	0.000
MST	24	0.71	0.47	0.09	0.11	0.21	0.09	0.069
	48	6.53ª	2.22 ^b	2.07 ^b	2.03 ^b	3.50 ^{ab}	0.52	0.003
	72	8.45 ^a	2.95 ^b	6.53 ^{ab}	8.46 ^a	7.40 ^{ab}	0.67	0.021

GH (Groundnut haulms), CC (Cowpea chaff), MC (Millet chaff), MST (Maize stover), SEM (Standard error of means) ^{*a.b.c*} Values within a row with different superscripts are significantly different

The proportion of CH₄ in total gas production after 72h of incubation of the intact, autoclaved, and *Pleurotus* species-treated crop residues is presented in Figure 2. The autoclaving and *Pleurotus* treatment of GH, CC, and MST recorded a lower percentage methane in total gas production than the intact ones but produced similar responses in MC. The lower percentage (%) of methane in the autoclaved and most of the *Pleurotus* species treated crop residue might be due to low methane gas production. This agrees with the reports of Kinfemi *et al.* (2009) and Akinfemi (2010) where reduced percentage of methane in tGP was due to lower methane gas production for *Pleurotus* species treated cowpea chaff and maize cobs treated respectively. The similar % methane gas in tGP recorded in MC despite

the high tGP of *Pleurotus* species treated MC, especially *P. ostreatus* indicates that *P. ostreatus* improvement of MC nutritive value will lead to decreased methane gas generation. Autoclaved MST and MC showed the least % methane in tGP, *P. florida* treated GH showed the least % methane in tGP; and *P. florida* or *P. sajor caju* treated CC showed similar least % methane in tGP. The variations in % methane in tGP as recorded in this study is similar to the findings of Tuyen *et al.* (2013) where the treated agricultural by-products gave variable responses in % methane in tGP. This could be attributed to differences in the chemical contents, tGP and methane gas production of the crop residues.

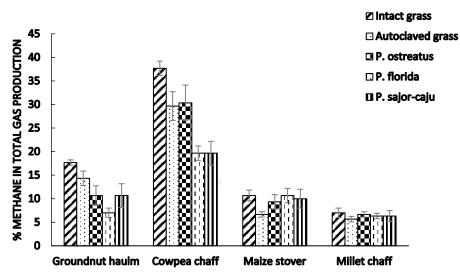


Figure 2: The percentage of methane in total gas produced by intact, autoclaved, and *Pleurotus* species-treated grasses incubated for 72h

CONCLUSION AND RECOMENDATIONS

All of the Pleurotus species improved the crop residues nutrient composition but their residues preference varied. P. ostreatus produced better improving effect on groundnut haulms and cowpea chaff; P. sajor-caju produced better improving effect on millet chaff; and P. florida produced better improving effect on maize stover. In comparison to the intact residues, *Pleurotus* species were only able to improve the *in vitro* gas production of millet chaff and higher improvement was recorded in P. ostreatus treated MC. The improvement was coupled with negligible less methane gas production and comparable percentage methane in in vitro gas production. Therefore, based on this study's findings, the use of P. ostreatus treated millet chaff can be recommended as supplementary improved feed for dry season ruminants feeding.

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