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Research Article
Feeding Values of Non-Biodegraded and Biodegraded Cassava Root Sievate for Goats Using *in vitro* Gas Production Technique

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Abstract

Background and Objective: The increased aesthetic nuisance caused by cassava root sievate has been a major concern to environmentalists and animal scientists in discovering means of adding value to this agro-waste. The study aimed to evaluate the nutritional, anti-nutritional, *in vitro* gas production and fermentation characteristics of biodegraded and non-biodegraded cassava root sievate for goats. **Materials and Methods:** *Pleurotus tuber-regium*, *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii*, were used to treat cassava root sievate for 45 days. The nutritional, *in vitro* gas production and fermentation characteristics of the biodegraded and non-biodegraded cassava root sievate, were evaluated. **Results:** The results showed that biodegraded cassava root sievate (BCRS) were significantly ($p>0.05$) higher in crude protein (CP), ash, nitrogen-free extract (NFE) and calcium while non-biodegraded cassava root sievate (NBCRS) were higher ($p>0.05$) in ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hydrogen cyanide (HCN) tannins, saponins. BSCR produced significantly higher ($p>0.05$) *in vitro* gas at 6, 12 and 24 hrs incubation time. Organic matter digestibility (OMD), *in vitro* dry matter digestibility (IVDMD), short-chain fatty acid (SCFA) and metabolisable energy (ME) were significantly ($p>0.05$) higher for the BSCR. **Conclusion:** It could be concluded that the use of white-rot fungi for biodegradation improved the quality and feeding value of cassava root sievate.

Key words: Cassava root sievate, white-rot fungi, solid-state fermentation, goats, *in vitro* digestibility, *in vitro* gas production and fermentation characteristics

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The huge amount of cassava root sievate (waste) generated in Nigeria as a result of processing cassava roots into a variety of cassava-based foods is contributing considerably to environmental affluence and aesthetic nuisance. This waste currently constitutes a disposal challenge and will be more problematic in the future as a result of population increase and more industrial cassava production. Cassava root sievate are usually discarded poorly as waste heaps near streams or homes where they are soaked, fermented and processed thereby producing a strong offensive smell and contaminations, hence the need to add value to this agro-waste. However, the low protein content, high cyanide content and high fibre contents are the limiting factor in the utilization of cassava root sievate by goats Jiwuba *et al*.[1]. Cassava root sievate utilization in goat feeding is hampered by its high lignin level. As a result, there is a need to improve the nutritional value of this agro-waste through biodegradation (use of white-rot fungi) to improve the overall performance of animals fed such diet.

The biodegradation of agricultural waste by an enzyme from microorganisms especially white-rot fungi (*Pleurotus* spp.) has been promising in degrading structural carbohydrates or structurally modifying proteins and their anti-nutritional properties to produce high-quality products. White-rot fungi, belonging to the wood-decaying *basidiomycetes*, as lignocellulolytic microorganisms can decompose and metabolize all plant cell constituents (cellulose, hemicellulose and lignin) by their enzymes Chukwuma *et al*.[2]. As a result, they can be genetically modified for growth on a specific substrate under specific cultural conditions to produce high protein value. Because of their capacity to grow in slurry or on solids, as well as their nutritional worth, they are as good as other high-protein traditional foods. *In vitro* digestibility techniques in ruminants provides a quick, low-cost and precise prediction of *in vivo* or conventionally determined digestibility. The *in vitro* approach does a better job of predicting digestibility than chemical composition because it accounts for all known and unknown factors affecting digestibility, which is not possible with existing chemical methods. *In vitro* digestion gas measurement focuses on the appearances of fermentation products. The gas approach allows the kinetics of fermentation to be examined on a single sample, requiring either a little amount of sample or a large number of samples to be analyzed at once by Pashaei *et al*.[3].

The possibility for turning agricultural waste into value-added feedstuffs for ruminants with some species of white-rot fungi (*Pleurotus ostreatus*, *Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus sajor caju* and *Lentinus subnudus*) have been investigated by various researchers Akinfemi *et al*.[4], Datsomor *et al*.[5]. Their experiments revealed that biodegradation of agro-wastes produced significantly higher crude protein content and a significant decrease in cellulose contents. Their research also revealed that white-rot fungi can improve short-chain fatty acid, metabolizable energy and digestibility. Therefore, using *Pleurotus tuber-regium*, *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii* to treat cassava root sievate will help to reduce environmental hazards and improve the nutritional value of this agro-waste. The objective of this study was therefore to evaluate the nutritional, anti-nutritional, *in vitro* gas production and fermentation characteristics of bio-degraded and non-bio-degraded cassava root sievate for goats.

MATERIALS AND METHODS

Study area: The inoculation was conducted at the Tissue Culture Laboratory of National Root Crops Research Institute, Umudike, Abia State, from February-March, 2018. The *in vitro* studies and nutritional and anti-nutritional studies were conducted at the Department of Animal Science Laboratory, University of Benin, Edo State from June-July, 2018. Umudike is located at latitude 05°28[1] North and longitude 07°31[1] East and lies at an altitude of 122 meters above sea level. It lies within the tropical rainforest zone characterized by average annual rainfall of 2,177 mm in 148-155 rain days. The average ambient temperature was 25.5°C with minimum and maximum temperatures of 22 and 29°C, respectively. Relative humidity ranged from 76-87% by Jiwuba *et al*.[6].

Sourcing of cassava root sievate: The cassava root sievate (variety, TME419) were sourced from Akawa, Nneato, Umunneochi L. G. A. Abia State. The cassava root sievate is a by-product of cassava root. They were gotten after the cassava roots meant for *fufu* production were peeled or not, washed clean and soaked in clean water for 3-5 days to ferment as to reduce the hydrogen cyanide level and also to soften the roots before sieving. Thereafter, the retted cassava roots were sieved, the sievate (waste) collected and sundried for about 7 days to reduce the moisture contents to about 10-15% and possible anti-nutrients that were not removed during the retting process. The sundried cassava root sievate were coarsely milled using a blur mill to reduce the particle size and create a greater surface area for microbial activity.

Inoculation of cassava root sievate: The inoculation room was thoroughly swept, washed and disinfected using Izal in water at the rate of 1 L Izal to 4 L of water. The

floor was mopped free of water and the doors, allowed to dry and locked up for two weeks to kill any surviving contaminant. Thereafter, the milled cassava root sievate (CRS) were wetted with water at the rate of 1.0 kg sievate to 1.0 litre of water and thoroughly mixed to enable complete wetting of the CRS. White rot fungi species (*Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii*) were obtained from Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos while Tubers of *Pleurotus tuber-regium* (PTR), were purchased from Orie Ugba market, Umuahia, Umuahia North L.G.A. Abia State.

For the *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii*, thirty kilograms of CRS were soaked in the plastic basin with clean water for 5 hrs to ensure that the residues absorbed enough water. The soaking was to ensure the washing off of soil particles. After the water was drained from the substrates they were allowed to stand for 3 hrs. Thereafter, 500 g portions each of CRS were weighed and transferred into three different cleaned, labelled aluminium trays measuring 15×11×4 cm. The open end of the aluminium trays was covered with polyethylene films and aluminium foil. The trays and their contents were steam-pasteurized for four hours in a 200 L metal barrel. After, the trays were allowed to cool to room temperature before inoculation was done. Inoculation was done by carefully lifting the foil covering each tray and quickly broadcasting one gram (1 g) portion of *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii* spawn grain onto substrates using a disinfected inoculation spoon. A sterilized inoculation pin was used to evenly distribute the spawn onto the substrates and they were quickly covered and was held firmly with rubber band and cello tape. The trays were arranged in shelves in the incubation room so that fermentation could commence.

For the *Pleurotus tuber-regium* (PTR), the tubers were weighed, washed, dissected to smaller bits and soaked in water for two hours after which they were removed and put in white transparent buckets and covered for three days to enable spore formation of the tubers. Spores of PTR were inoculated into the 4th wetted CSR at the rate of 1.0 kg spores to 3.0 kg CRS. The ends of the poly-ethene sheets were brought together and sealed using masking tape to create an airtight environment. Water was poured on the room floor and some were left in buckets after which doors of the inoculation room were closed. After 45 days, the mass of composted CRS now colonized by mycelium of the fungi showing whitish growths was taken out of the inoculation trays from the inoculation room and sun-dried by spreading them thinly on a drying surface to terminate the growth of the fungi and to dry the material. The materials were put in sacks and stored until required for use.

Chemical composition: Triplicate samples of dried biodegraded and non-biodegraded cassava root sievate were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), ash, ether extract, organic matter (OM) and metabolizable energy (ME) according to the methods of AOAC[7]. The fibre fractions such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al.*[8]. Nitrogen free extract was determined as:

$$\text{NFE (\%)} = 100-(\text{crude protein (\%)}+\text{crude fibre (\%)}+\text{ether extract (\%)}+\text{ash (\%)})$$

Organic matter was calculated as:

$$\text{OM (\%)}=\text{crude fibre (\%)}+\text{crude protein (\%)}+\text{ether extract (\%)}$$

Gross energy determination: The gross energy was calculated using the formula, according to Nehring and Haenlein[9]:

$$T = 5.72Z_1+9.50Z_2+4.79Z_3+4.03Z_4\pm0.9\%$$

Where:
T = Gross energy
Z_1 = Crude protein
Z_2 = Crude fat
Z_3 = Crude fibre
Z_4 = Nitrogen free extract

Anti-nutrient determinations: The silver titration method as described by Oboh *et al.*[10] was used to determine the cyanide content

- Tannins were determined according to the methods of Petchidurai *et al.*[11]
- Phytate determination was according to the methods of Agostinho *et al.*[12]
- Oxalate determination was according to the methods of Karamad *et al.*[13]

Saponins determination: 2.5 g of sample (W_1) was weighed into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated inside a hot water bath for 4 hrs with continuous stirring at 55°C. The mixture was filtered and the residue was re-extracted with another 200 mL of aqueous ethanol. The extract was evaporated to 40 mL inside the water bath at 90°C. The extract was transferred into a 250 mL separating funnel and 20 mL of diethyl ether will be added and shaken vigorously. The aqueous layer was

recovered, while the layer was discarded, the purification process was repeated. About 60 mL of n-butanol was added, the combined extracts were washed twice with 10 Ml of 5% aqueous sodium chloride. The remaining solution was heated in the water bath. After evaporation, the sample was transferred into a known weight of petri dish (W_2) and then dried in the oven to a constant weight (W_3). The percent saponins were calculated as follows:

$$\text{Saponins} = \frac{W_3 - W_1}{W_2}$$

Minerals composition: About 1 g of each sample was ashed in a muffle furnace at 600°C. The ashed samples and silica dishes were removed and transferred into the desiccator to cool after which, the sample was dissolved with 1 mL of concentrated HNO_3. Little distilled water was added and filtered into a clean small plastic bottle using the number 43 Whatman filter (Merck KGaA, Darmstadt, Germany). Distilled water was later used to dilute the solution up to 50 mL. Atomic absorption spectrophotometer was used to determine the concentration of calcium, phosphorus magnesium, sodium and potassium.

Collection and preparation of rumen fluid: *In vitro* gas production of biodegraded and non-biodegraded cassava root sievate meals were done by collecting rumen fluid from WAD goats through a suction tube after the goats were fed for 14 days with each of the five samples (NBCRS, PPBCRS, POBCRS, PEBCRS and PTRBCRS). The rumen liquor was collected between 7:00-8:00 am before feeding the WAD goats into the thermo flask that had been pre-warmed to a temperature of 39°C.

Preparation of the buffer solution: The buffer solution prepared was McDougall's solution (g L^{-1}) which consist of 9.8 $NaHCO_3$+2.77g $NaHPO_4$+0.57g KCl+0.47g NaCl+2.16 $MgSO_3$. $7H_2O$+16 $CaCl_2$. $2H_2O$) (1:4 v/v) under continuous flushing with CO_2 (to minimize changes in microbial population and to avoid O_2 contamination) was added using another 50 mL plastic calibrated syringe.

Preparation of the rumen liquor-buffer solution: The rumen liquor-buffer solution was mixed at the ratio of 1:4 (v/v) for the incubation.

Preparation of the syringes for incubation: Incubation was carried out according to Menke and Steingass[14] using 120 mL calibrated transparent plastic syringes with a fitted silicon tube. The sample weighing 200 mg was carefully dropped into syringes and thereafter, 30 mL each of the inoculum containing cheesecloth strained rumen liquor and buffer solution was added. The syringe was trapped and pushed upward by the piston to eliminate air in the inoculum. The silicon tube fitted to the syringe was tightened by a metal clip to prevent the escape of gas. Incubation was carried out at 39±1°C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 hrs.

Methane determination: At the end of 24 hrs of incubation, 4 mL of NaOH (10 M) was introduced to estimate the amount of methane produced according to the methods described by Fievez *et al.*[15], metabolizable energy (ME), organic matter digestibility (OMD), dry matter digestibility (DMD) and Short-Chain Fatty Acids (SCFA) were estimated according to the methods of Menke and Steingass[14]. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Calculations:

ME (MJ kg^{-1} DM) = 2.20+0.136GV+0.057CP+0.0029 CF
OMD (%) = 14.88+889GV+0.45 CP+0.651XA according to Babayemi and Bamikole[16]
SCFA = 0.0239 V-0.0601 according to Getachew *et al.*[17]:

$$\text{IVDMD}(\%) = \frac{1-\left[\left(\text{Residue} + \text{filter paper}\right) - \text{filter paper}\right] - \text{blank}}{\text{Sample weight}} \times \text{DM}$$

Where:
Blank = (Blank+filter paper)-filter paper
GV, CP, CF and XA = Total gas volume, crude protein, crude fibre and ash, respectively

The experimental design was a completely randomized design (CRD). Data obtained were analyzed using Analysis of Variance (ANOVA) as described by SAS[18]. Significant means were separated using the Duncan Multiple New Range Test Duncan[19] at $p<0.05$.

RESULTS AND DISCUSSION

The chemical composition of non-biodegraded cassava root sievate, (NBCRS), *Pleurotus tuber-regium* cassava root sievate (PTRCRS), *Pleurotus pulmonarius*, cassava root sievate (PPCRS), *Pleurotus eryngii* cassava root sievate (PECRS) and *Pleurotus ostreatus* cassava root sievate (POCRS) are presented in Table 1. The fungi degraded cassava root sievate were significantly ($p<0.05$) higher in crude protein, ash and nitrogen-free extract (NFE) but significantly ($p<0.05$) lower in

ether extract, crude fibre, NDF, ADF and ADL than the NBCRS. The CP values were highest ($p<0.05$) in PTRCRS (7.37%) and lowest in NBCRS (2.87%). CF values was highest in NBCRS (19.66%), PECRS (15.30%), PPCRS (12.81%), POCRS (11.97%) and lowest in PTRCRS (9.90%). Ether the ether extract was significantly ($p<0.05$) higher in NBCRS when compared with the other treatments. PTRCRS had the highest ($p<0.05$) ash content which differed significantly ($p<0.05$) from PPCRS, PECRS and NBCRS but similar to ($p>0.05$) to POCRS. PTRCRS and PPCRS are significantly ($p<0.05$) higher than NBCRS for NFE but similar ($p>0.05$) to PECRS and POCRS. Organic matter was higher ($p<0.05$) in NBCRS, PPCRS, PECRS than PTRCRS and POCRS. NDF were significantly ($p<0.05$) higher in NBCRS than the fungi degraded CRS. Acid detergent lignin (ADL) was significantly ($p<0.05$) higher in NBCRS and POCRS than those of PTRCRS, PPCRS and PECRS. The significant ($p<0.05$) improvement reported in this study for CP was corroborated by the findings of Barde *et al.*[20] and Ochepo[21] for white-rot fungi degraded cassava peels. The higher CP value reported in this study for PTRCRS may be attributed partly to the faster colonization of the substrate by the PTR, which ensured faster and early release of polysaccharide bound protein and due to higher deposition of sclerotia (tuber). Bamigboye *et al.*[22] in an earlier study noted that the tuber of PTR is very rich in proteins and Andrea *et al.*[23] noted that the extracellular enzymes secreted by this mushroom contain amorphous homo and heteropolysaccharides, which are associated with proteins. Generally, the significant improvement in CP values observed in this study by the white-rot fungi degradation CRS have been reported Akinfemi *et al.*[24], Barde *et al.*[20], Ochepo[21] earlier using other agro by-products as substrates. The improved CP values among the biodegraded CSR may be attributed to the ability of the fungi to increase the bioavailability of the protein, capture of excess nitrogen by aerobic fermentation, increased fungal biomass, secretion of enzymes by the fungi and their consequent metabolism and addition of fungal mycelia into the substrates, a view corroborated by Bimrew[25], Akinfemi *et al.*[24], Ochepo[21], Amoateng *et al.*[26]. In this present study, it was observed that the CF, NDF, ADF and ADL were significantly ($p<0.05$) higher in NBCRS than the biodegraded groups. Similar observations were made by Akinfemi *et al.*[24], Akinfemi *et al.*[4], Datsomor *et al.*[5], Barde *et al.*[20]. The lower values reported for the CF and the fibre fractions may be due to the hydrolytic nature of the fungi species, which could have delignified the lignocelluloses complex and utilised them by microbes during fermentation thus converting them to more useful components like protein or NFE. This conforms with the report of Carvalho *et al.*[27] that enzymes from most fungi can improve not only the non-starch polysaccharides but also the crude proteins, minerals and fatty acids. Akinfemi *et al.*[4], Barde *et al.*[20] and Ochepo[21] have earlier reported improved ash values on agro by-products degraded using *Pleurotus* spp. The improvement may be attributed to the high mineral composition of *Pleurotus* spp. Akindahunsi and Oyetayo[28] in an earlier study reported high mineral composition in *Pleurotus* spp. NFE is used to evaluate non-fibrous carbohydrates, like sugars and starches and the significant improvement among the *Pleurotus spp.* have earlier been reported by Akinfemi *et al.*[4]. The increase in NFE may be the result of cellulase enzymes like laccase, lignin peroxidase and manganese peroxidases secreted by cellulolytic fungi, which degraded the non-digestible fibre thus converting them to more digestible carbohydrates.

The mineral composition of NBCRS and fungi degraded CRS is presented in Table 2. There was a significant difference ($p<0.05$) for calcium while phosphorus, potassium, magnesium and sodium were not ($p>0.05$) influenced by the treatments. Minerals are important in animal nutrition, hence the need to analyze the mineral content of the samples upon biodegradation. Calcium was higher in the fungi degraded CRS groups in comparison with the NBCRS. In addition, there were improvements ($p>0.05$) in the values of phosphorus, potassium and magnesium in the fungi degraded CRS. The higher mineral values of fungi degraded samples are in agreement with the findings of Belewu and Babalola[29] but disagree with the reports of Akinfemi *et al.*[4]. The increase in the mineral content may conform with the increase in the ash values of the biodegraded CRS. The improved values may be attributed in part that fungi accumulate minerals from the environment. Oei[30] in earlier study reported higher mineral content in different mushrooms. The differences in the mineral content observed in this study may be attributed to the different species of fungi used.

The anti-nutritional factors of non-biodegraded and fungi degraded cassava root sievate is shown in Table 3. HCN, tannins and saponins values were significantly ($p<0.05$) reduced by the fungi degradation. Phytates and oxalates though not significantly ($p>0.05$) influenced but showed lower values for the fungi degraded CRS. Cassava and its by-products are generally high in anti-nutritional factors (ANFs), predominantly HCN. The results showed the ability of *Pleurotus* spp., to secrete enzymes capable of degrading HCN, tannins, saponins, phytates and oxalates. The HCN content of NBCRS were significantly higher ($p<0.05$) when compared with the biodegraded CRS, with PTRCRS showing the highest reduction in the cyanide content. The reduction in the cyanide content of the biodegraded CRS might be due to the synergistic effect of loss of cyanogenic glycosides on

Table 1: Chemical compositions of non-biodegraded and fungi degraded cassava root sievate

Parameters (%)	T_1 (NBCRS)	T_2 (PTRCRS)	T_3 (PPCRS)	T_4 (PECRS)	T_5 (POCRS)	SEM
Dry matter	91.51	91.03	91.87	92.02	91.01	0.28
Crude protein	2.87^{c}	7.37^{a}	5.22^{b}	4.91^{b}	5.50^{b}	0.68
Crude fibre	19.66^{a}	9.90^{d}	12.81^{c}	15.30^{b}	11.97^{cd}	1.44
Ether extract	4.14^{a}	2.42^{b}	2.37^{b}	2.22^{b}	2.45^{b}	0.32
Ash	2.83^{c}	5.55^{a}	4.63^{b}	4.54^{b}	4.78^{ab}	0.39
Nitrogen free extract	61.62^{b}	65.88^{a}	66.85^{a}	65.05^{a}	65.40^{a}	0.88
Organic matter	88.29^{a}	85.56^{b}	87.25^{a}	87.48^{a}	85.56^{b}	0.48
Gross energy (Kcal g^{-1})	3.98	3.55	3.83	3.84	3.83	7.60
NDF	48.92^{a}	21.72^{b}	24.63^{b}	21.75^{b}	25.51^{b}	4.61
ADF	31.14^{a}	11.69^{c}	14.53^{bc}	14.13^{bc}	15.90^{b}	2.98
ADL	9.34^{a}	5.26^{b}	5.35^{b}	5.40^{b}	6.56^{ab}	0.75

NBCRS: Non biodegraded cassava root sievate, PTRCRS: *Pleurotus tuber-regium* cassava root sievate, PPCRS: *Pleurotus pulmonarius* cassava root sievate, PECRS: *Pleurotus eryngii* cassava root sievate, POCRS: *Pleurotus ostreatus* a cassava root sievate, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, SEM: Standard error of means and $^{a-d}$means within the same row with different superscripts are significantly different ($p<0.05$)

Table 2: Mineral compositions of non-biodegraded and fungi degraded cassava root sievate

Parameters (%)	T_1 (NBCRS)	T_2 (PTRCRS)	T_3 (PPCRS)	T_4 (PECRS)	T_5 (POCRS)	SEM
Calcium	1.51^{b}	2.53^{a}	2.46^{a}	2.42^{a}	2.55^{a}	0.17
Phosphorus	0.77	1.11	1.17	1.23	1.14	0.09
Potassium	0.70	0.87	0.99	1.19	1.16	0.16
Magnesium	0.07	0.08	0.08	0.09	0.09	0.00
Sodium	0.04	0.04	0.05	0.06	0.04	0.00

NBCRS: Non biodegraded cassava root sievate, PTRCRS: *Pleurotus tuber-regium* cassava root sievate, PPCRS: *Pleurotus pulmonarius* cassava root sievate, PECRS: *Pleurotus eryngii* cassava root sievate, POCRS: *Pleurotus ostreatus* a cassava root sievate, SEM: Standard error of means and $^{a-b}$means within the same row with different superscripts are significantly different ($p<0.05$)

Table 3: Anti-nutritional factors of non-biodegraded and fungi degraded cassava root sievate

Parameters (%)	T_1 (NBCRS)	T_2 (PTRCRS)	T_3 (PPCRS)	T_4 (PECRS)	T_5 (POCRS)	SEM
Hydrogen cyanide (mg kg^{-1})	19.05^{a}	7.15^{d}	9.30^{bc}	13.14^{b}	9.96^{c}	1.77
Tannins	1.51^{a}	0.18^{b}	0.17^{b}	0.23^{b}	0.24^{b}	0.22
Phytates	0.54	0.21	0.17	0.24	0.34	0.08
Oxalate	2.27	0.68	0.68	0.58	0.61	0.36
Saponins	3.40^{a}	2.23^{b}	2.46^{b}	2.48^{b}	2.22^{b}	0.20

NBCRS: Non biodegraded cassava root sievate, PTRCRS: *Pleurotus tuber-regium* cassava root sievate, PPCRS: *Pleurotus pulmonarius,* cassava root sievate, PECRS: *Pleurotus eryngii* cassava root sievate, POCRS: *Pleurotus ostreatus* a cassava root sievate, SEM: Standard error of means and $^{a-c}$means within the same row with different superscripts are significantly different ($p<0.05$)

hydrolysis by linamarase Aweke *et al.*[31], metabolic activities of innate microorganisms Aro[32], the ability of the microorganisms to secrete extracellular enzymes (amylase, xylanase and linamarase), increase in cell mass and formation of a hydrolytic complex bind force to the cyanide compound. The high tannin degradation by the *Pleurotus* spp. maybe attributed to tannase activity (tannin acyl hydrolase, EC 3.1.1.20) which abound in *Pleurotus* spp. Batra and Saxena[33] have reported the activity of this enzyme in the polyphenols degradation. Alteration of the substrates by the fungi mainly contributed to the observed decrease in tannin content for the treatments. The reduction in tannins observed in this study is in agreement with reports of Oei[30], Alemawor *et al.*[34] who reported a significant reduction in tannins content among biodegraded cassava peels and cocoa pods husk, respectively. The significant reductions of saponin in this study conform with the findings of Dei *et al.*[35]. Difo *et al.*[36] noted that a decrease in various anti-nutrient levels could be due to the production of various enzymes during the vegetative and reproductive phases of the fungi.

The volume of *in vitro* gas produced at different incubation times by non-biodegraded and biodegraded cassava root sievate is presented in Table 4. The treatments were significantly ($p<0.05$) influenced at 6, 12, 18, 21 and 24 hrs. The significant ($p<0.05$) difference observed in this study agrees with the findings of Okpanachi *et al.*[37]. At 3, 9 and 15 hrs, the volume of *in vitro* gas produced by non-biodegraded and fungi degraded CRS were not influenced ($p>0.05$). It was observed that the *in vitro* gas produced tend to increase with incubation time (hr) and the possibility of more gas beyond 24 hrs. The potency of the rumen liquor used for incubation, nature and level of fibre may be responsible for the amount of gas produced during fermentation. Amanzougarene *et al.*[38] noted that the amount of gas produced is dependent on the nature of the carbohydrates. In this study, it was observed that all the *Pleurotus* spp., used, improved gas production, hence, indicating a better digestibility of the biodegraded substrates. Sommart *et al.*[39] submitted that gas volume is a good index to predict digestibility, fermentation end product and microbial

Table 4: Volume of *in vitro* gas produced at different incubation time by non-biodegraded and fungi degraded cassava root sievate

Parameters (hrs)	T_1 (NBCRSM)	T_2 (PTRCRS)	T_3 (PPCRS)	T_4 (PECRS)	T_5 (POCRS)	SEM
3	9.33	12.67	12.00	10.00	10.67	0.70
6	20.00^{c}	26.67^{a}	21.33^{c}	23.33^{b}	23.33^{b}	1.15
9	28.00	34.67	28.00	31.33	32.00	1.18
12	35.33^{c}	40.00^{a}	34.67^{c}	34.00^{c}	38.00^{b}	1.36
15	40.00	47.33	40.00	39.33	44.67	1.68
18	43.33^{b}	50.67^{a}	42.67^{b}	41.33^{b}	50.67^{a}	1.80
21	44.00^{c}	63.33^{a}	46.67^{bc}	49.33^{b}	56.00^{ab}	2.74
24	48.67^{d}	64.00^{a}	53.33^{c}	54.00^{c}	57.33^{b}	2.5

NBCRS: Non biodegraded cassava root sievate, PTRCRS: *Pleurotus tuber-regium* cassava root sievate, PPCRS: *Pleurotus pulmonarius* cassava root sievate, PECRS: *Pleurotus eryngii* cassava root sievate, POCRS: *Pleurotus ostreatus* a cassava root sievate, SEM: Standard error of means and $^{a-c}$means within the same row with different superscripts are significantly different ($p<0.05$)

Table 5: *In vitro* digestibility characteristics of non-biodegraded and biodegraded cassava root sievate

Parameters	T_1 (NBCRSM)	T_2 (PTRCRS)	T_3 (PPCRS)	T_4 (PECRS)	T_5 (POCRS)	SEM
Methane (mL/200 mg DM)	23.33	24.00	24.67	20.67	23.33	0.99
OMD (%)	68.95^{d}	82.44^{a}	73.05^{c}	72.47^{c}	76.57^{b}	2.22
IVDMD	11.83^{d}	32.23^{a}	18.00^{c}	15.63^{c}	24.83^{b}	4.66
SCFA (UM)	1.10^{d}	1.47^{a}	1.21^{c}	1.23^{c}	1.31^{b}	0.06
ME (MJ kg^{-1} DM)	10.03^{c}	11.21^{ab}	10.67^{b}	10.76^{b}	12.12^{a}	1.22

NBCRS: Non biodegraded cassava root sievate, PTRCRS: *Pleurotus tuber-regium* cassava root sievate, PPCRS: *Pleurotus pulmonarius* cassava root sievate, PECRS: *Pleurotus eryngii* cassava root sievate, POCRS: *Pleurotus ostreatus* a cassava root sievate, OMD: Organic matter digestibility, IVDMD: *In vitro* dry matter digestibility, SFCA: Short chain fatty acids, ME: Metabolizable energy, SEM: Standard error of means and $^{a-c}$means within the same row with different superscripts are significantly different ($p<0.05$)

protein synthesis of the substrate by rumen microbes in the *in vitro* system. PTRCRS tended to produce the highest volume of *in vitro* gas at any time, but the highest gas production was at 24 hrs. This entails that more carbohydrate fermentation took place at 24 hrs incubation period. The volume of gas 48.67-63.00 mL/200 mg DM produced at 24 hrs reported in this study is higher than 25.37-34.82 mL/200 mg DM reported by Olfaz *et al.*[40] for olive, mulberry and sour orange leaves, but compared with 43.67 and 54.33 mL/200 mg DM for cassava peels and yam peels as reported by Akinfemi *et al.*[24]. The 64.00 mL/200 mg DM *in vitro* gas volume produced at 24 hrs by *Pleurotus tuber regium* degraded cassava root sievate explain the ability of the fungi in degrading lignin and hemicellulose by ensuring a high level of carbohydrate fermentation within the time.

The result of *in vitro* digestibility characteristics of non-biodegraded and biodegraded cassava root sievate is shown in Table 5. Organic matter digestibility (OMD), *In vitro* dry Matter Digestibility (IVDMD), short-chain fatty acids (SCFA) and metabolisable energy (ME) showed significant ($p<0.05$) differences across the treatment groups. Methane values obtained in this study ranged from 20.67-24.00 mL/200 mg DM. The amount of methane produced by livestock is dependent on the body size, age, digestive system, quality and quantity of feed intake. The non-significant ($p>0.05$) effect reported in this study for methane may be attributed to the similarities in age, size and breed of the goats. The results showed high values for OMD in all fungi treated groups with the highest values occurring in PTRCRS treated group. OMD has been reported Sommart *et al.*[39], to have a positive correlation with gas volume. The reduced CF values of the *Pleurotus* degraded CRS and increased CP may be attributed to the improvement in OMD, hence, high NDF, ADF and ADL values in feedstuffs results to lower fibre degradation. Chumpawadee *et al.*[41] attributed high OMD to high nutrient uptake and enhanced rumen microbes. The variations in IVDMD may be due to differences in CF and fibre fraction contents. The higher IVDMD observed for PTRCRS may be due to the higher gas production observed for this substrate. Digestibility has been reported to be synonymous with *in vitro* gas production, with a high positive correlation obtained between gas production and dry matter digestibility Ansah *et al.*[42], Fievez *et al.*[15]. In addition, the significant ($p<0.05$) difference for IVDMD suggested possible differences in microbial species and activity. The microbial population is dependent on the type of diet fed Aderinboye *et al.*[43] and since donors were sustained on different diets, microbial species were expected to vary. The varied range of 1.10-1.47 UM obtained for SCFA in this present study is higher than 0.56-0.94 UM reported by Akinfemi *et al.*[4] for sorghum stover degraded substrate using *Pleurotus ostreatus* and *Pleurotus pulmonarius* and 0.1789-0.4179 µmol reported by Barde *et al.*[20] for cassava peel treated with *Pleurotus ostreatus, Pleurotus tuber-regium*, *Pleurotus eryngii* and *Lentinus.* The variations may be attributed to differences in the substrate, duration of fermentation, fungi species used and

volume of gas produced. Getachew *et al*.[17] reported a close association between SCFA and gas production *in vitro*, which is an indicator of energy available to the animal. The higher values obtained for the biodegraded group as against the non-biodegraded may indicate higher energy availability to the animals. The higher ($p<0.05$) ME observed for the fungi treated groups is an indication that there is an enhancement in the available energy values of the substrates and thus, the potential of being incorporated in conventional diets. previously, Datsomor *et al*.[5] observed a positive relationship between total gas production, ME, SCFA and OMD.

CONCLUSION

Pleurotus tuber regium (PTR) was the most effective fungi for biodegradation of cassava root sievate (CRS) and enhanced the nutritive value of the agro-waste by improving the crude protein and mineral contents, reducing the crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin. The biodegradation of CRS using different fungi spp. (*Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus eryngii* and *Pleurotus ostreatus*) on CRS resulted in a higher calcium value in comparison with NBCRS. Hydrogen cyanide, tannins and saponins were significantly reduced by the biodegradation of CRS. The *in vitro* gas production and *in vitro* digestibility characteristics of non-biodegraded and biodegraded CRS showed that PTRCRS produced the highest volume of *in vitro* gas at 6, 12 and 24 hrs incubation periods. OMD, IVDMD, SCFA and ME were generally better in the biodegraded CRS when compared with NBCRSM. PTRCRS specifically yielded better OMD, IVDMD and SCFA among the biodegraded CRS and NBCRSM.

SIGNIFICANCE STATEMENT

This study discovered that biodegradation of cassava root sievate enhanced its nutritive value that can be beneficial to ruminant researchers in ensuring a regular supply of feed. This study will help researchers to uncover the biological means of improving agro-waste. Thus a new theory on optimization of solid-state fermentation may be achieved.

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