

IN VITRO GAS PRODUCTION OF BIODEGRADED AND NON-BIODEGRADED CASSAVA ROOT SIEVATE

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ABSTRACT

This study was conducted to determine the *in vitro* gas production of biodegraded cassava root sievate and non-biodegraded cassava root sievate. The biodegraded cassava root sievate (CRS) were inoculated with *Pleurotus tuber-regium*, *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii* for 45 days in a completely randomized design. Inoculation was done by carefully lifting the foil covering each tray and quickly broadcasting one gram (1g) portion of *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii* spawn grain onto substrates using a disinfected inoculation spoon. For the *Pleurotus tuber-regium*, the tubers were weighed, washed, dissected to smaller bits and soaked in water for two hour 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 a 4th wetted CSR at the rate of 1.0 kg spores to 3.0 kg CRS. Five treatments (T₁, T₂, T₃, T₄ and T₅) comprising non-biodegraded cassava root sievate (NBCRSM), *Pleurotus tuber-regium* treated cassava root sievate (PTRCRS), *Pleurotus pulmonarius* treated cassava root sievate (PPCRS), *Pleurotus eryngii* cassava root sievate (PECRS) and *Pleurotus ostreatus* treated cassava root sievate (POCRS) respectively. Triplicate sample of air dried biodegraded and non-biodegraded CRS were analysed for *in vitro* gas production at 3, 6, 9, 12, 15, 18, 21 and 24 hours. Results showed that the volume of gas produced at 6, 12, 18, 21 and 24hr were significantly ($p < 0.05$) influenced with PTRCRS showing the best production at different time intervals. It could however be concluded that *Pleurotus tuber-regium* treated cassava root sievate produced the best result and therefore should be recommended to improve the nutritional values of cassava root sievate for enhanced animal production.

Keywords: Solid-state fermentation, white rot fungi, cassava root sievate, biodegradation, alternative feedstuffs.

INTRODUCTION

Cassava root sievate (CRS) is the residue (waste) that results from processing the root of cassava into fufu, a popular West African food. The sievate is the solid waste produced as a consequence of cassava root production and made up of 88.60%, 2.57% and 13.38% dry matter, crude protein and crude fibre respectively (Jiwuba *et al.*, 2018). They 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, due to high lignin content of cassava root sievate, its utilization is impaired in ruminant feeding; hence the need to improve the nutritive value of cassava root sievate through biodegradation (use of white rot fungi). The biodegradation of agricultural waste by 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 with the aim of producing high quality product. Some white-rot fungi are able to decompose free phenolic monomers and to break the bonds with which lignin is cross linked to the polysaccharides in straw thereby enhancing the digestibility (Fazaeli *et al.*, 2006). Fazaeli *et al.* (2002) noted that *Pleurotus* fungi spp can be grown on agro waste and capable of decomposing the structural carbohydrate.

Gas production methods has been used to determine the rate and extent of dry matter (DM) degradation and the effect of the some anti-nutritive factors such as HCN. *In vitro* techniques in feed evaluation has recently gained popularity majorly due to minimal use of animals, less expensive, less time consuming, accuracy, allows more precise maintenance of incubation conditions and simulates the rumen fermentation process making it useful for evaluating the potentials of feedstuffs to supply nutrients to ruminants. The *in vitro* method for feed evaluation has numerous advantages over the *in vivo* method since it utilizes small amounts of test feeds suitable for screening large samples of feed. However, these techniques are more rapid and precise, requiring less substrate than *in situ* procedures, they still require an inoculum to create the fermentative environment (Mould *et al.*, 2005). Small ruminants (sheep and goat) are commonly used as donor animals for *in vitro* trials in Nigeria possibly due to reduced cost of maintenance and lower feed requirements relative to cattle. This study was therefore aimed to evaluate the *in vitro* gas production of biodegraded and non-biodegraded cassava root sievate using West African dwarf goats as donor animal.

MATERIALS AND METHODS

The experiment was conducted at the Department of Animal Science Laboratory, University of Benin, Edo State, Nigeria. The cassava root sievate from TME 419 were sourced from Akawa, Nneato, Umunneochi L. G. A. Abia State. The sievate was gotten after the cassava roots meant for *fufu* production are peeled or not, washed clean and soaked in clean water for 3-5 days to ferment so as to reduce the hydrogen cyanide level and also to soften the roots prior to sieving. Thereafter, the soaked cassava roots were sieved, the sievate (waste) collected and sundried for about 7 days. The sundried cassava root sievate were coarsely milled using a blur mill to reduce their particle size and create a greater surface area for microbial activity.

The inoculation was conducted at the Tissue Culture Laboratory of National Root Crops Research Institute, (NRCRI) Umudike. The inoculation room was thoroughly swept, washed and disinfected using Izal in water at the rate of one litre Izal to four litres 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 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 cassava root sievate. 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 a dealer at Orié Ugbá market, Umuahia, Umuahia North L.G.A. Abia State.

For the *Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus eryngii*, thirty kilograms (30 kg) of CRS were soaked in plastic basin with clean water for five hours (5 hrs) to ensure that the residues absorbed enough water. After draining the water from the substrates, they were allowed to stand for three hours (3 hrs). Thereafter, 500g portions each of CRS were weighed and transferred into three different cleaned, labelled aluminum trays measuring 15cm × 11cm × 4cm. The open end of the aluminum trays were covered with polyethylene films and aluminum foil. The trays and their contents were steam-pasteurized for four (4 hrs) in a 200L 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 (1g) 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, quickly covered and was held firmly with rubber band and cello tape. The trays were arranged in shelves in an incubation room so that fermentation could commence.

For the *Pleurotus tuber-regium*, the tubers were weighed, washed, dissected to smaller bits and soaked in water for two hour 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 a 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 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 were taken out of the inoculation trays from the inoculation room and sun dried by spreading

them thinly on a drying surface to terminate growth of the fungi and to dry the material. The materials were put in sacks and stored until required for use.

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.

The buffer solution prepared was McDougall's solution (g/litre) which consist of 9.8 NaHCO₃ + 2.77g NaHPO₄ + 0.57g KCL + 0.47g NaCl + 2.16 MgSO₃. 7H₂O + 16 CaCl₂. 2H₂O (1:4 v/v) under continuous flushing with CO₂ (to minimize changes in microbial population and to avoid O₂ contamination) was added using another 50 mL plastic calibrated syringe.

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

Incubation was carried out according to Menke and Steingass (1988) using 120ml calibrated transparent plastic syringes with fitted silicon tube. The sample weighing 200mg was carefully dropped into syringes and thereafter, 30ml each of the inoculum containing cheese cloth strained rumen liquor and buffer solution was added. The syringe was trapped and pushed upward by the piston in order to completely eliminate air in the inoculum. The silicon tube fitted to the syringe was tightened by a metal clip so as to prevent 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.

The experimental design used was Completely Randomized Design (CRD). Data obtained were analysed using analysis of variance (ANOVA) as described by SAS (2008). Significant means were separated using the Duncan Multiple New Range Test.

RESULTS AND DISCUSSION

The volume of *in-vitro* gas produced at different incubation time by non-biodegraded and biodegraded cassava root sievate is presented in Table 1. The treatments were significantly ($p<0.05$) influenced at 6, 12, 18, 21 and 24 hours. The significant ($p<0.05$) difference observed in this study is in agreement with the findings of Okpanachi *et al.* (2018). At 3, 9 and 15 hours, the volume of *in vitro* gas produced by non-biodegraded and fungi degraded CRS where not influenced ($p>0.05$). It was observed that the *in vitro* gas produced tend to increase with incubation time (hour) and possibility of more gas beyond 24 hours. The potency of the rumen liquor used for incubation, nature and level of fibre may be responsible to the amount of gas produced during fermentation. Demeyer and Van Nevel (1975) noted that the amount of gas produced is dependent on 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.* (2000) submitted that gas volume is a good index to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. PTRCRS tended to produce highest volume of *in vitro* gas at any time, but the highest gas produce was at 24 hour.

Table 1. Volume of *in-vitro* gas produced at different incubation time by non-biodegraded and fungi degraded cassava root sievate

Parameters (Hours)	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. ^{a-c} means within the same row with different superscripts are significantly different ($P<0.05$)

This entails that more carbohydrate fermentation took place at 24 hours incubation period. The volume of gas 48.67-63.00 ml/200 mg DM produced at 24 hours reported in this study is higher than 25.37-34.82 ml/200 mg DM reported by Olfaz *et al.* (2018) for olive, mulberry and sour orange leaves, but however, compared with 43.67 and 54.33 ml/200 mg DM for cassava peels and yam peels as reported by Akinfemi *et al.* (2009). The 64.00 ml/200 mg DM *in vitro* gas volume produced at 24 hour by *Pleurotus tuber-regium* degraded cassava root sievate explain the ability of the fungi in degrading lignin and hemi cellulose by ensuring high level of carbohydrate fermentation within the time.

CONCLUSION AND RECOMMENDATION

Biodegradation is a suitable means for improving the nutritional value of cassava root sievate for livestock production. However, *Pleurotus tuber-regium* produced the best bioconversion of cassava root sievate and hence should be recommended to improve nutritional value of cassava root sievate.

REFERENCES

- Akinfemi, A., Babayemi, O.A. and Jonathan, S.G. (2009). Bioconversion of maize husk into value added ruminant feed by using white-rot fungus. *Revista UDO Agrícola*, 9 (4): 972-978.
- Demeyer, D.I. and Nevel, C.J. (1975). Methanogenesis, an integrated part of carbohydrate fermentation and its control. In: Digestion and metabolism in the ruminant, I.W. Mc Donald and A.C.I. Warner, eds., pp. 366–382. The University of New England Publishing Unit, Armidale, Australia
- Fazaeli, H., Azizi, A. and Amile, M. (2006). Nutritive value index of treated wheat straw with *Pleurotus* fungi fed to sheep. *Pak. J. Biol. Sci.* 9 (13):2444-2449.
- Fazaeli, H., Jelani, Z.A., Azizi, A., Rouzbehan, Y., Mirhady, S.A. and Liang, J.B. (2002). Biodegradation of wheat straw by *Pleurotus* fungi: The growth rate on untreated and urea treated wheat straw. *Malaysian J Anim. Sci.* 7(2):39- 49.
- Jiwuba, P. C. Assam, E. M. and Eka, E.C. (2018). The effects of different levels of fufu sievate meal on the blood chemistry and haematological characteristics of WAD goats. *Sustainability, Agri, Food and Environmental Research*, 6(1): 1-10.
- Menke, K. and Steingass, H. (1988). Estimation of the energetic feed value from chemical analysis and *in vitro* production using rumen fluid. *Anim. Res. Dev.* 28: 7-55.
- Mould, F. L., Kliem, K. E., Morgan, R. and Mauricio, R. M. (2005). *In vitro* microbial inoculum: A review of its function and properties. *Animal Feed Science and Technology*, 123(124): 31–50.
- Okpanachi, U., Luka, J.S. and Akpensuen, T.T. (2018). *In vitro* fermentation parameters, volume of gas produced and fermentation characteristics. *FUDMA Journal of Agriculture & Agricultural Technology*, 4(2):212-219.
- Olfaz, M., Kilic, U., Boga, M. and Abdi, A.M. (2018). Determination of the *in vitro* gas production and potential feed value of olive, mulberry and sour orange tree leaves. *Open Life Sci.*, 13: 269–278
- SAS. (2008). Statistical Analytical Systems, 9.4 for Windows x64 Based Systems. SAS Institute Inc., Cary, NC 27513, USA
- Sommart, K., Parker, D.S., Rowlinson, P. and Wanapat, M. (2000). Fermentation Characteristic and Microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried razi grass as substrates. *Asian Aust. J. Anim. Sci.* 13: 1084-1093.