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Nutritional, *In vitro* gas production and fermentation characteristics of *Pleurotus tuber-regium* degraded cassava root sievate based diets for goat

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***Target Audience:** Animal scientists, livestock and ruminant farmers*

Abstract

The study was carried out to evaluate the nutritional, in vitro gas production and fermentation characteristics of Pleurotus tuber-regium degraded cassava root sievate (PTRCRS) based diets for goats. Four experimental diets (T_1, T_2, T_3, and T_4) were formulated with 0, 20, 40 and 60% inclusion levels of PTRCRS in a completely randomized design. The diets were analyzed for chemical, mineral and anti-nutritional factors. The diets were incubated (0-24 h) in three replicates and incubations run at two consecutive times to make six replicates per treatment for estimation of the in vitro gas production and fermentation characteristics. Crude protein and ash were significantly ($p<0.05$) high in T_3 and T_4. Nitrogen free extract was significantly ($p<0.05$) higher in T_1 in comparison with T_3 and T_4. Neutral detergent fibre and acid detergent fibre showed significant ($p<0.05$) lower values with increasing levels of PTRCRS. The mineral compositions were not significantly ($p>0.05$) affected by the biodegradation. Hydrogen cyanide, oxalate and saponins levels were significantly ($p<0.05$) reduced in T_4. In vitro gas production volumes were significantly ($p<0.05$) higher for T_3 and T_4 at 6 and 24 h incubation time. Methane was significantly ($p<0.05$) reduced with increased levels of PTRCRS in the diets. In vitro gas production, short chain fatty acid, metabolizable energy and organic matter digestibility were higher ($p<0.05$) in T_2, T_3 and T_4 groups compared to the control (T_1) group. It was therefore concluded that biodegradation of cassava root sievate with Pleurotus tuber-regium enhanced its nutritive value, in vitro gas production and fermentation characteristics.

***Keywords:** Cassava root sievate, agro by-products, feed enhancement, biodegradation, in vitro digestibility, white rot fungi*

Description of Problem

Ruminants in Nigeria are undernourished, particularly during the dry seasons, due to inadequate fodder and poor nutritional value of available agricultural by-products to meet nutritional needs of ruminants. . Nigeria produces a large amount of cassava root sievate each year. Cassava root sievate has become a valuable agro by-product in Nigeria, obtained from local cassava root processing for *fufu*. Cassava root sievate is a source of energy in the ruminant feeding system, and can be used as a main diet or as a supplement (1). Cassava root sievate is characterized by high lignin content, which can block hydrolytic enzymes from reaching the cellulose and hemicellulose and thus limits rumen degradation by the rumen microbes. Pre-treatments are being tested in feed technology to improve the digestibility or feeding value of the complete product so that it may be fed efficiently to ruminants. (2) found that the lignocellulose potential of the cellulose is encrusted by lignin within the lignocellulose matrix. They believe that a combination of solid-state fermentation (SSF) technology and the capacity of an appropriate fungus to selectively degrade lignin will allow lignocellulose-based biotechnologies to be implemented on an industrial scale. The amount of lignin in lignocellulosic (fibrous crop residues) is known to affect digestibility. Physical, chemical, and biological methods of delignification can be employed to improve the digestibility of lignocellulosics (3). White-rot fungi, such as *Pleurotus tuber-regium*, can degrade fibrous crop residues and other agro by-products, thereby increasing the digestibility and nutritional value of lignocellulose. White rot fungi are able to bio-convert a wide variety of lignocellulosic materials due to the secretion of extra- cellular enzymes (4). *Pleurotus* species have been demonstrated to be more efficient than other edible white-rot fungi (5) in bioconversion of lignocellulosic materials. *Pleurotus* fungi have been shown to remove indigestible cell wall components and boost the dry mater digestibility (DMD) of straw (6). Recent reports (7; 8; 9), have shown that the bio-converted materials have higher feeding values than non-bio converted and can be used as ruminant feed supplement.

In order to determine the nutritional value of supplements, *in vivo* approaches should be preferably used. *In vivo* feed evaluation, on the other hand, is widely acknowledged to be time-consuming, difficult, expensive, and requires significant amount of feed, making it unsuitable for large-scale feed evaluation. As a result, *in vitro* procedures such as incubating diets with rumen microbes or using a gas method to estimate what would happen *in vivo* are often used. The *in vitro* gas production technique, as revised by (10), is commonly used to assess the nutritional values of feed ingested by ruminants. The *in vitro* gas method is based on measured connections between feed *in vitro* digestibility and *in vitro* gas production, as well as the chemical content of the feed (10). The main objective of the study was to evaluate the nutritional, *in vitro* gas production and fermentation characteristics of *Pleurotus tuber-regium* degraded cassava root sievate based diets for goats

Materials and Methods

Experimental site

The experiment was conducted at the Department of Animal Science Laboratory, University of Benin, Edo State, Nigeria. The lab lies between latitude 6.50" N of the equator and longitude 5.40 and 6E of Greenwich meridian, with mean annual temperature of 33.54°C. The area has an average annual rainfall and relative humidity of 2000mm and 72.5% respectively.

Processing of experimental feed

The cassava root sievate (CRS) from TME419 variety were sourced from Akawa, Nneato, Umunneochi L. G. A. Abia State, Nigeria. The cassava root sievate is a by-product of cassava root. They were 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 sievates (wastes) 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 sizes and to create a greater surface area for microbial activity.

The inoculation was conducted at the Tissue Culture Laboratory of National Root Crops Research Institute, Umudike, Abia State. 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. The tubers of *Pleurotus tuber-regium* (PTR) 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 a wetted CSR at the rate of 1.0 kg spores to 3.0 kg CRS. The ends of the polyethene 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.

The experimental diets designated as T_1, T_2, T_3, T_4 were formulated from non-biodegraded cassava root sievate, brewers dried grain, palm kernel meal, soyabean meal, bone meal, salt and premix to contain 0, 20, 40 and 60% biodegraded cassava root sievate (Table 1).

Table 1. Gross composition of *Pleurotus Tuber-regium* degraded cassava root sievate based diets

Ingredients (%)	T_1	T_2	T_3	T_4
Non-Biodegraded cassava root sievate	60.0	40.0	20.0	0.0
Biodegraded cassava root sievate	0.0	20.0	40.0	60.0
Brewers dried grain	15.0	15.0	15.0	15.0
Palm kernel meal	14.0	14.0	14.0	14.0
Soya bean meal	7.0	7.0	7.0	7.0
Bone meal	3.0	3.0	3.0	3.0
Salt	0.5	0.5	0.5	0.5
Premix*	0.5	0.5	0.5	0.5
Total	100	100	100	100

Vitamin and mineral premix contributed the following to each kilogram of diet: vitamin A 500 IU, vitamin D 1500 IU, vitamin E 3 IU, vitamin K 2 mg, riboflavin 3 mg, pantothenic acid 6 mg, niacin 15 mg, vitamin B12 0.8 mg, choline 3 mg, folic acid 4 mg, manganese 8 mg, zinc 0.5g, iodine 1.0 mg, Co 1.2 mg.

In vitro gas production method

In-vitro gas production of biodegraded and non-biodegraded cassava root sievate meals were done by collecting rumen fluid from five WAD goats through a suction tube after the goats were fed for 14 days with *Pleurotus tuber-regium* degraded cassava root sievate based diets. The rumen liquor was collected between 07:00 – 08:00 hr before feeding the WAD goats into thermos flask.

Preparation of the buffer solution and rumen liquor-buffer solution: The buffer solution prepared was McDougall's solution (g/litre) which consisted of 9.8 $NaHCO_3$ + 2.77 g NaHPO4 + 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. The rumen liquor and buffer solution were mixed at the ratio of 1:4 (v/v) for the incubation.

The incubation was carried out according to (10); using 240 ml calibrated transparent plastic syringes with 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 in order to completely eliminate air in the inoculum. The silicon tube fitted to the syringe was tightened by a metal clip to prevent escape of gas. Incubation was carried out at $39 \pm 1^{o}C$ and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 hrs.

At the end of 24 hours of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced according to the methods described by (11), Metabolizable energy (ME), organic matter digestibility (OMD), dry matter digestibility (DMD) and short chain fatty acids (SCFA) were estimated according to the methods of (10). The average of the volume of gas produced from the blanks were deducted from the volume of gas produced per sample.

ME (MJ/Kg DM) = 2.20 + 0.136GV + 0.057CP + 0.0029 CF (12)

OMD (%) = 14.88 + 0.889GV + 0.45 CP + 0.651XA according to (12)

SCFA = 0.0239 V – 0.0601 according to (13)

% IVDMD = 1 – [(Residue + filter paper) – filter paper] – blank / (sample weight)

Where; Blank = (Blank + filter paper) – filter paper; GV = Total gas volume; CP = crude protein; CF = crude fibre; XA = ash

Triplicate sample of the *Pleurotus tuber-regium* degraded cassava root sievate based diets 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 (14). The fibre fractions such as neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods of (15).

Gross Energy determination: The gross energy was calculated using the formula

$T = 5.72Z_1 + 9.50Z_2 + 4.79Z_3 + 4.03Z_4 \pm 0.9\%$; according to (16). where; T = Gross energy; Z_I = Crude protein; Z_2 = Crude fat; Z_3 = Crude fibre; Z_4 = Nitrogen free extract.

Data obtained were analyzed using analysis of variance (ANOVA) as described by (17). Significant means were separated using the Duncan multiple new range test.

Results and Discussion

The chemical composition of the

experimental diets containing graded levels of *Pleurotus tuber regium* degraded cassava root sievate is presented in Table 2. Crude protein, ash NFE, NDF and ADF showed significant ($p<0.05$) difference while DM, CF, EE and gross energy (GE) were not influenced ($p>0.05$) significantly by the treatment diets. The CP of the treatment diets increased with incremental levels of PTRCRS in the diets. However, CP values of T_3 and T_4 were significantly ($P<0.05$) higher compared to T_1 and T_2. The crude protein content of all the diets were above the acceptable 7% CP for ruminant maintenance as recommended by (18) and 8% suggested by (19) for ruminal function. The higher CP observed in the treatment diets could balance for imbalance of amino acids produced during protein degradation. Ash values for the treatment diets increased ($p<0.05$) with increasing of PTRCRS in the diets. The observation in this study could be attributed to mycelia biomass of the fungus during bioconversion. The variations in the NFE values could be attributed to the influence of graded levels of PTRCRS on the diets. Nitrogen free extract contains both starches and some proportion of hemicellulose and lignin (20). There was decreasing ($p<0.05$) trend in NDF and ADF with increasing level of *Pleurotus tuber regium* degraded cassava root sievate in the diets. The decrease in NDF was nevertheless not too fast as to cause any challenge as (21) noted that in order to maintain optimum roughage digestion, ruminant diets should contain a minimum 20 % NDF on DM basis. The neutral detergent fibre fraction is made up of hemicellulose, cellulose and lignin resulting in improved fibre that is effective in stimulating rumen motility. The reduction in the ADF highlighted the nutritional superiority of the biological treatment. In earlier study, (21) reported that diets with lower values of ADF are of good nutritional quality. Perhaps, the lower NDF and ADF values in PTRCRS containing diets indicated that *Pleurotus tuber regium* solubilized and utilized the cell walls as carbon sources and hence altered the ratio of insoluble to soluble carbohydrates in the diets, compared with the control diet containing non-biodegraded cassava root sievate.

The mineral composition (Table 3) though not significantly ($p<0.05$) influenced by the treatment diets were well above 0.6 g/d recommendation for a 10 kg goat by (22) for calcium and phosphorus. The calcium and phosphorus values reported in this study were well above 0.3 % 0.25 % respectively recommended by (23) for ruminant in warm wet climate.

The anti-nutritional compositions of the experimental diets are presented in Table 4. Hydrogen cyanide (HCN), oxalates and saponins values were significantly ($p<0.05$) reduced in the PTRCRS diets, while tannins and phytates were not significantly ($p>0.05$) influenced. The effect of all the anti-nutritional factors depends largely on the amount present in the feed and quantity taken by the animals. The reduction in the level of HCN may be attributed to the PTRCRS in the diets. (24) and (25) attributed the reduction in cyanogenic glycosides during hydrolysis to the ability of the *Pleurotus spp.* to secrete amylase, xylanase and linamarase enzymes to form hydrolytic complex bound to the cyanide compound. Oxalate is a dicarboxylic acid anion present as insoluble salts of potassium, sodium and ammonium or as calcium. The reduction observed in this study for oxalate may be attributed to the activities of the PTR on the CRS during the solid-state fermentation. *Pleurotus spp.* have been reportedly used in the degradation of anti-nutrients due to tannase, phytase and oxalate oxidase which abound in the fungus (26; 7). The significant ($p<0.05$) reductions of saponins in this study may be attributed to effect of the test ingredient. (27) noted that decrease in various anti nutrient levels could be due to the production of various enzymes during the vegetative and reproductive phases of the fungi.

Table 2: Chemical composition of experimental diets

Parameters (%)	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	SEM
Dry matter	91.33	91.90	91.55	91.84	0.83
Crude protein	9.28^{b}	9.82^{b}	14.12^{a}	16.06^{a}	1.11
Crude fibre	14.57	13.35	11.52	9.62	1.00
Ether extract	0.84	0.72	0.72	1.02	0.19
Ash	8.26^{c}	12.17^{b}	12.71^{ab}	13.03^{a}	0.73
Organic matter	83.08	79.73	80.7	78.81	0.95
Nitrogen free extract	58.39^{a}	55.85^{ab}	52.49^{b}	52.12^{b}	1.04
Neutral detergent fibre	55.57^{a}	49.30^{b}	34.44^{c}	31.18^{c}	3.84
Acid detergent fibre	49.50^{a}	39.15^{b}	29.78^{c}	21.91^{d}	3.92
Gross Energy (Kcal/g)	3.66	3.51	3.54	3.59	0.77

$^{a-d}$ Means within the same row with different superscripts are significantly different (P<0.05)

Table 3: Mineral composition of experimental diets

Parameters (%)	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	SEM
Calcium	1.74	1.73	1.75	1.77	0.03
Phosphorus	0.89	0.95	0.93	0.95	0.01
Potassium	0.32	0.29	0.31	0.34	0.02
Magnesium	0.12	0.15	0.13	0.13	0.01
Sodium	0.36	0.39	0.42	0.50	0.03
Iron	0.47	0.43	0.40	0.48	0.03

Means within the same row with same superscripts are similar (P>0.05)

Table 4: Anti-nutritional compositions of experimental diets

Parameters (%)	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	SEM
Hydrogen cyanide (mg/kg)	2.25^{a}	1.67^{ab}	1.23^{ab}	0.56^{c}	0.27
Tannins	2.05	1.55	0.90	0.53	0.32
Phytates	0.80	0.54	0.87	0.59	0.13
Oxalate	0.32^{a}	0.29^{ab}	0.19^{ab}	0.17^{b}	0.03
Saponins	0.03^{ab}	0.04^{a}	0.03^{ab}	0.02^{b}	0.00

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

The *in vitro* gas production over the incubation period of 24 h is shown in Table 5. The volume of *in vitro* gas produced at different incubation time by diets containing graded levels of PTRCRS were significantly (p<0.05) influenced at 6, 9, 12, 18, 21 and 24 hours. This suggests differences in carbohydrate fermentation among the diets at these hours. From *in vitro* gas production for incubation period of 24 hrs, T_1 maintained the lowest *in vitro* gas production. This suggested that lower carbohydrate fermentation occurred in this treatment in comparison with the treatment (T_2, T_3 and T_4) diets. This is in agreement with the results of (8) for non-fungi treated maize husk. The lower gas production for T_1 may be attributed to higher content of NDF and ADF that protected the carbohydrate from degradation by the rumen microbes. This perhaps agreed with the reports of (28) that *in vitro* gas volume is a good parameter to predict digestibility, end product of fermentation and microbial protein synthesis of the substrates by rumen microbes.

The result of the *in vitro* digestibility of the experimental diets is presented in Table 6. All the indices evaluated were significantly (P<0.05) influenced except *in vitro* dry matter digestibility (IVDMD). Methane volume decrease with increasing levels of PTRCRS in the diets. The observation in this study further indicated the inclusion of PTRCRS in the diets of goats improved the quality of the diets. This is in conformity with the reports of (3) that enteric methane emissions are highest when animals are fed poor quality diets. (29) in earlier study also observed linear reduction in methane from fungal treated wheat straws. PTR bioconversion of NDF and ADF may have resulted in the improvement in the quality of the diets as a result of cell wall degradation and overall improvement in carbohydrates digestibility. The IVGP (ml/200 mgDM) values obtained in this study ranged from 44.12 to 55.67 ml/200 mgDM. The PTR bioconversion of the CRS enhanced the quality of the diets, which further enhanced the fermentation and ensured more gas production, as gas production is positively correlated with degradable carbohydrate (30) attributed higher IVGP in fungi treated feed resources to the hydrolytic ability of the fungi species, nature of carbohydrate and potency of the rumen liquor used for incubation. The SCFA of the different diets were highest (p<0.05) in T_3 and T_4 and lowest in T_1. The higher value recorded for T_3 and T_4 may be ascribed to the higher gas production, which indicates higher available energy. In addition, the higher SCFA for T_3 and T_4 is in conformity with the metabolizable energy (ME) values of those diets. This indicates that up to 80% of goats and any other ruminant maintenance energy could be met when fed with these diets (31). (32) submitted that *in vitro* gas production system helps to better quantify nutrient utilization and its accuracy in describing digestibility in animals and a number of experiments has validated this. The ME values reported in this study showed that, the ME of the experimental diets fell within the recommended ME values of 6 to 13 MJ/kg DM reported by (33) for small ruminants. This is an indication that, the energy requirements of the experimental goats were met. The *in vitro* OMD followed a similar pattern as the IVGP, SCFA and ME, thus indicating increased microbial activities. Similarly, the high IVOMD for the treatment diets may be attributed to lower fibre fractions and increased CP values of the diets. The result of this present study corroborates the findings of (34) who attributed high fermentable nitrogen and readily degradable cell wall fractions to the increase in the substrates available cellulolytic microbes with a consequential increase in digestibility.

Table 5: Volume of *in-vitro* gas produced at different incubation time by the experimental diets

Parameters (%)	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	SEM
3 Hrs	7.74	8.98	7.79	8.23	0.23
6 Hrs	9.64^{b}	10.84^{b}	13.04^{a}	14.77^{a}	0.80
9 Hrs	22.35^{b}	24.17^{ab}	27.26^{a}	26.65^{a}	0.82
12 Hrs	26.89^{b}	27.22^{ab}	29.18^{a}	28.83^{ab}	0.43
15 Hrs	31.67	29.84	33.14	34.80	1.20
18 Hrs	34.73^{b}	39.18^{ab}	37.92^{ab}	42.22^{a}	1.10
21 Hrs	38.30^{b}	43.62^{ab}	47.97^{a}	44.63^{ab}	1.48
24 Hrs	44.12^{c}	48.45^{b}	55.67^{a}	54.33^{a}	1.78

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

Table 6: *In vitro* digestibility of the experimental diets

Parameters	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	SEM
Methane (mL/200 mg DM)	24.51^a	23.64^b	23.47^b	22.82^c	0.24
IVGP (mL/200 mg DM)	44.12^c	48.45^b	55.67^a	54.33^a	1.78
IVDMD (%)	52.38	58.28	62.32	61.81	1.83
SCFA (µmol)	0.99^c	1.10^b	1.27^a	1.24^a	0.04
ME (MJ/Kg DM)	8.77^c	9.39^b	10.61^a	10.53^a	0.30
OMD (%)	58.82^c	63.16^b	71.55^a	71.25^a	2.07

IVGP = *In vitro* gas production; IVDMD = *In vitro* dry matter digestibility; SCFA = Short chain fatty acids; ME = Metabolizable energy; OMD = Organic matter digestibility. $^{a-c}$ means within the same row with different superscripts are significantly different ($P<0.05$)

Conclusion and Application

This study showed:

1. That the nutritive value of cassava root sievate containing diets were enhanced through biodegradation using *Pleurotus tuber-regium*.
2. That the addition of biodegraded cassava root sievate in the diets of WAD goats reduced the methane production *in vitro*.
3. That the volume of *in vitro* gas production was improved among the biodegraded cassava root sievate diets.
4. That short chain fatty acids, metabolizable energy and organic matter digestibility were improved among the treatments containing biodegraded cassava root sievate.

References

1. Jiwuba, P. C. Assam, E. M. and Eka, E.C. (2018). The effects of different levels of fufu sieviate meal on the blood chemistry and haematological characteristics of WAD goats. *Sustainability, Agriculture, Food and Environmental Research* 6(1): 1-10.

2. Malherbe S, Cloete TE (2003). Lignocellulose biodegradation: fundamentals and applications: A review. *Environmental Science and Biotechnology*. 1:105-114.

3. Yilkal, T. (2015). Role of white rot fungi as a biological treatment of low-quality animal feeds: Review. *Scholarly Journal of Agricultural Science* 5(7): 247-255.

4. Chang, S. T. and Buswell, J. A. (1996). Mushroom nutriceuticals. *World Journal of Microbiology and biotechnology*, *12*(5), 473-476.

5. Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K. and Tanaka, T. (2005). Evaluation of pre-treatment with *Pleurotus ostreatus* for enzymatic hydrolyses of rice straw. *Journal* of Bioscience and *Bioengineering.* 100(6): 637-643

6. Fazaeli, H., Mahmodzadeh, H., Azizi, A., Jelan, Z. A., Liang, J. B., Rouzbehan, Y., & Osman, A. (2004). Nutritive value of wheat straw treated with Pleurotus fungi. *Asian-Australasian Journal of Animal Sciences*, *17*(12), 1681-1688.

7. Batra, A. and Saxena, R. K. (2005). Potential tannase producers from the genera Aspergillus and Penicillium. *Process Biochemistry*, 40, 1553-1557.

8. 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.

9. Jiwuba, P.C., Ahamefule, F.O., Okah, U., Jiwuba, L.C., Ikwunze, K., Onwujiariri, B.E and Nsidinanya, N. O. (2020). Chemical composition of biodegraded and non-biodegraded cassava root sievate. *Proceedings of 25th Annual Conference of ASAN 2020, Abuja, Nigeria (30-33)*

10. Menke, K. and Steingass, H. (1988). Estimation of the energetic feed value from chemical analysis and in vitro production using rumen fluid. *Animal Research* and *Development,* 28: 7-55.

11. Fievez, V., Babayemi, O.J. and Demeyer, D. (2005). Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production requiring minimal laboratory facilities. *Animal Feed Science* and *Technology,* 123 (1): 197-210. ????

12. Babayemi, O.J and. Bamikole, M.A. (2006). Effect of *Tephrosia candida* DC leaf and its mixtures with Guinea grass on *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition,* 5(1): 14-18.??

13. Getachew, G., Makkar, H.P.S. and Becker, K. (1999). Stoichiometric relationship between short chain fatty acids and in vitro gas production in presence and polythyleneglycol for tannin containing browses. EAAP satellite symposium.

14. AOAC, (2000). Association of Official Analytical Chemists: Official Methods of Analysis. 6th Edition. Washington DC, USA.

15. Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991). Methods of dietary fibre, neutral detergent and non-starch polysaccharides in relation to rate of passage. *Journal of Agricultural Science*, 92: 499-503.

16. Nehring, K. and Haelien, G.W.F. (1973). Feed evaluation and calculation based on net energy. *Journal of* Animal *Science,* 36(5): 949 – 963.

17. SAS. (2008). Statistical Analytical Systems, 9.4 for Windows x64 Based Systems. SAS Institute Inc., Cary, NC 27513, USA.

18. ARC (1980). The nutrient requirement of ruminant livestock. CABI. Farnham Royal, U.K.

19. Norton, B.W.B., Lowry, C. and Sweeney, M.C. (1994). The nutritive value of Leucaena specie. Paper presented at Int'l. Workshop on Leucaena. R.D. Bogor, Indonesia. 20-29 January 1994.

20. Khan, M.A., Mahr-un-nisa, M. and Sarwar, M. (2003). Techniques measuring digestibility for the nutritional evaluation of feeds. *International Journal* of *Agriculture* and *Biology,* 5: 132-141.

21. David, L. (2012). Nutritive value of feeds for beef cattle. Oklahoma Cooperative Extension Fact Sheets.

22. NRC (1981). Nutrient Requirements for goats: Angora, dairy and meat goat in temperate and tropical countries. National Research Council; National Academy of Science Press, Washington DC.

23. McDowell, L.R. (1985). Nutrient requirement of ruminant. In L.R. McDowell (ed.) Nutrition of Grazing Ruminants in warm climates. Academic Press, London pp 21 – 34.

24. Padmaja, G. (1995). Cyanide detoxification in cassava for food and feed uses. **Critical** *Reviews* in *Food Science* and *Nutrition,* 35: 299-339.

25. Aro, S.O. (2008). Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. *African Journal of Biotechnology,* 7: 4789-4797.

26. Cavallazzi, J. R. P., Brito, M. S., Oliveira, M. G. A., Villas-Bôas, S. G., and Kasuya, M. C. M. (2004). Lignocellulolytic enzymes profile of three Lentinula edodes (Berk.) Pegler strains during cultivation on eucalyptus 317 bark-based medium. *Food, Agriculture and Environment*, 2, 291-297.

27. Jacqueline, E.W. and Broerse, B. V. (1996). Assessing the potential in Biotechnology: Building on farmers knowledge. CTA Book edited by Joske B., Bertuis A and Wim, A.

28. Sommart, K., Parker, D.S., Rowlinson, P. and Wanapat, M. (2000). Fermentation characteristic and microbial protein synthesis in a vitro system using cassava, rice straw and dried razi grass as substrates. *Asian-Australasian Journal of Animal Sciences*, 13: 1084-1093.

29. Mahesh, M.S. (2012). Fungal bioremediation of wheat straw to improve the nutritive value and its effect on methane production in ruminants. MVSc thesis submitted to National Dairy Research institute (Deemed University), Karnal, Haryana, India.

30. Blümmel, M., Makkar, H. P. S. and Becker, K. (1997). *In vitro* gas production: a technique revisited. *Journal of Animal Physiology and Animal Nutrition*, 77(1- 5): 24-34.

31. Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiology Review*, 70, 567-590.

32. Sallam, A. Z. M., Robinson, P. H., El-Adawy, M. M. and Hassan, A. A. (2007). *In vitro* fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. *Animal Feed Science and Technology*, 38, 318–330.

33. Steele, M. (2006). Goats. CTA-Macmillan Publishing Ltd., London and Basingstoke. (152).

34. Adebowale, E. A. (1994). Maize residues as ruminant feed resources in Nigeria. *WAR/RMZ* (73), 24-30.