

## Evaluation of Nutritive Value of *Pleurotus tuber-regium* Biodegraded Maize Cob using in Vitro Gas Production and Fermentation Characteristics

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**ABSTRACT:** This study investigated the nutritive value of *Pleurotus tuber-regium* biodegraded maize cob using in vitro gas production and fermentation characteristics. *Pleurotus tuber-regium* degraded maize cob (PTRMC) was used to formulate four experimental diets (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>) at 0%, 15%, 30%, and 45% inclusion levels in a completely randomized design. The chemical, mineral, and phytochemical compositions of the diets were analyzed. To determine the in vitro gas generation and fermentation characteristics of the diets, they were incubated for 24 hours (0, 3, 6, 9,... to 24 h) in three replicates, with incubations repeated twice to make six replicates per treatment. The results of the study revealed that increasing PTRMC levels in the diets significantly (p<0.05) improved the feed's nutritional profile. Crude protein content significantly (p<0.05) increased with an increase in PTRMC, with D<sub>3</sub> and D<sub>4</sub> being significantly (p<0.05) higher than D<sub>1</sub> and D<sub>2</sub>. Conversely, neutral detergent fibre and acid detergent fibre decreased (p<0.05) significantly with an increase PTRMC levels in the diets. Mineral content significantly (p<0.05) improved with increasing PTRMC. The phytochemical contents were observed to significantly (p<0.05) decrease with an increase in PTRMC in the diets. The in vitro digestibility results of diets D<sub>3</sub> and D<sub>4</sub> were significantly (p<0.05) improved, and the methane production was reduced (p<0.05) significantly with the increase in PTRMC in the diets. In vitro gas production, short-chain fatty acids, organic matter digestibility, and in vitro dry matter digestibility increased (p<0.05) significantly with higher PTRMC levels in the diets, suggesting enhanced rumen fermentation and energy availability. Overall, this study highlights the potential of PTRMC as alternative feedstuff to improve ruminant animal feed quality and digestibility.

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### INTRODUCTION

Feeding ruminants in the tropics during the dry season has always been difficult due to a consistent lack of feed supplies, both in terms of quantity and quality. This has led to declining animal health and low productivity, which has made the use of substitute feed sources like crop residues and agricultural by-products necessary (a lignocellulosic biomass) (Shrivatava *et al.* 2014; Koura *et al.*, 2015). Using these agricultural by products could help bridge the gap in feed supplies during the dry season, protect natural resources, and ensure the sustainability of the ecosystem (Duque-Acevedo *et al.*, 2000). Utilizing the vast amounts of biomass waste that are not edible to humans, such as maize cob, produced through the food chain, can be valued as co-products in the ruminants feed. (Rakita *et al.*, 2021).

Solid State Fermentation (SSF) technology has made significant advancements in bioprocessing, including food, pharmaceutical, textile, biochemical, and bioenergy industries. Solid state fermentation is processed through fungi, bacteria or yeast (Pandey, 2003; Soccol and Vandenberghe, 2003). Biological treatment has fascinated an interest of researchers and it has become a widely studied solution in recent times (McAllister *et al.*, 2003; Sujani and Seresinhe, 2015).

Use of live animals in feed evaluation studies is limited by costs involved in acquiring and managing fistulated animals. Manipulation of live animals for *in vivo* methods of feed evaluation has implications on animal welfare, in addition to the high costs involved (Mohamed and Chaudhry, 2008). Mohamed and Chaudhry (2008) stressed the need for *in vitro* methods of digestibility

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evaluation that do not necessitate the use of fistulated cattle. *In vitro* digestibility studies rely on using mixed rumen microorganisms (buffered rumen fluid) as inoculum of choice to recreate rumen conditions (Menke *et al.*, 1979; Makar, 2004). This is because rumen inoculum has been found to yield results which mimic those from *in vivo* studies (Gizzi *et al.*, 1998; Brown *et al.*, 2002).

The main objective of the study was to evaluate the nutritive value of *Pleurotus tuber-regium* biodegraded maize cob using In vitro gas production and fermentation characteristics as diets for goats.

### MATERIALS AND METHODS

#### Processing of experimental feed

Maize cobs from Oba Super-11 were sourced. Maize cobs are by product of maize. The inoculation was conducted at the Tissue Culture Laboratory of National Root Crops Research Institute, Umudike, Abia State. The inoculation room was thoroughly cleaned. Thereafter, the milled maize cob were wetted with water at the rate of 1.0 kg maize cob to 1.0 litre of water and thoroughly mixed to enable complete wetting. 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 maize cob at the rate of 1.0 kg spores to 3.0 kg maize cob. The ends of the polyethene sheets were brought together and sealed. After 45 days, the mass of composted maize 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 experimental diets designated as D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> were formulated from non-biodegraded maize cob (Table 1).

**Table 1: Gross composition of experimental diets**

Ingredients (%)	Diet1	Diet2	Diet3	Diet4
Non-Biodegraded maize cob	45.00	30.00	15.00	0.00
Biodegraded maize cob	0.00	15.00	30.00	45.00
Brewers dried grain	25.00	25.00	25.00	25.00
Palm kernel meal	18.00	18.00	18.00	18.00
Soya bean meal	8.00	8.00	8.00	8.00
Bone meal	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50
Premix*	0.50	0.50	0.05	0.50
Total	100	100	100	100

#### In vitro gas production method

In-vitro gas production of biodegraded and non-biodegraded maize cob 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 maize cob 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<sub>3</sub> + 2.77 g NaHPO<sub>4</sub> + 0.57g KCl + 0.47g NaCl + 2.16 MgSO<sub>3</sub>. 7H<sub>2</sub>O + 16 CaCl<sub>2</sub>. 2H<sub>2</sub>O (1:4 v/v) under continuous flushing with CO<sub>2</sub> (to minimize changes in microbial population and to avoid O<sub>2</sub> 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 method of (Manke and Steingass 1988) 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 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 to prevent escape of gas. Incubation was carried out at 39±1oC and the volume of gas production was measured at every 3 hourly for 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 (Fievez, 2005), Metabolizable energy (ME), organic matter digestibility (OMD), dry matter digestibility (DMD) and short chain fatty acids (SCFA) were estimated according to the methods of to (Manke and Steingass 1988). 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 (Babayemi and Bamikole, 2006).

Triplicate sample of the *Pleurotus tuber-regium* degraded maize cob 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 (AOAC, 2000). The fibre fractions such as neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods of (Van Soest, 1991).

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Gross Energy determination: The gross energy was calculated using the formula according to (Nehring and Haelien, 1973). Data obtained were analyzed using analysis of variance (ANOVA) as described by (SAS, 2008). Significant means were separated using the Duncan multiple new range test.

### RESULTS AND DISCUSSION

**Table 2: Chemical composition of experimental diet**

PARAMETERS(%)	DIET 1	DIET 2	DIET 3	DIET 4	SEM
Dry matter	87.46	87.40	88.49	88.20	0.24
Crude protein	11.35 <sup>c</sup>	12.83 <sup>b</sup>	14.05 <sup>a</sup>	14.36 <sup>a</sup>	0.39
Crude fibre	24.00 <sup>a</sup>	21.39 <sup>b</sup>	21.62 <sup>b</sup>	18.89 <sup>c</sup>	0.62
Ether extract	2.54	2.80	2.44	2.66	0.07
Ash	5.38 <sup>c</sup>	6.55 <sup>b</sup>	7.87 <sup>a</sup>	8.46 <sup>a</sup>	0.43
Organic matter	83.41	78.85	80.62	79.74	0.71
Nitrogen free extract	44.16	43.82	42.51	43.83	0.57
Neutral detergent fibre	75.78 <sup>a</sup>	67.25 <sup>b</sup>	64.02 <sup>b</sup>	55.36 <sup>c</sup>	2.39
Acid detergent fibre	50.00 <sup>a</sup>	46.01 <sup>b</sup>	40.50 <sup>c</sup>	29.42 <sup>d</sup>	4.07
Gross energy	322.00 <sup>b</sup>	386.14 <sup>a</sup>	378.39 <sup>a</sup>	374.54 <sup>a</sup>	12.61

<sup>a-d</sup> means within the same row with different superscripts are significantly different ( $p < 0.05$ )

Table 2 shows the chemical composition of the experimental diets that containing varying levels of *Pleurotus tuber-regium* degraded maize. Significant ( $p < 0.05$ ) differences were observed in crude protein, crude fibre, ash, NDF, and ADF of the treatment diets. The CP of the treatment diets increased with incremental levels of PTRMC in the diets. However, CP values of D<sub>3</sub> and D<sub>4</sub> were significantly ( $p < 0.05$ ) higher compared to D<sub>1</sub> and D<sub>2</sub>. The treatment diets' crude protein contents ranged from (11.35 to 14.36), exceeding the ARC (1980) recommended minimum requirement of 7% for ruminant maintenance and the Norton et al, (1994) which indicated 8% as requirement for optimal ruminal function. The increase in crude protein observed in the diets with increase in *Pleurotus tuber-regium* biodegraded maize cob is as a result of the *Pleurotus tuber-regium* breaking down tough components of the maize cob like cellulose and lignin (Akinfemi, 2011; Fasiku, S.A. 2021). In the process, it enriches the cob with microbial protein, which is readily digestible by animals. As PTRMC levels in the diets increased, the ash values for the treated diets increased ( $p < 0.05$ ) significantly.

**Table 3: Mineral composition of Experimental Diets**

PARAMETERS	DIET1(0%)	DIET2	DIET3	DIET4	SEM
CALCIUM	1.31 <sup>b</sup>	2.19 <sup>a</sup>	2.30 <sup>a</sup>	2.36 <sup>a</sup>	0.12
PHOSPHORUS	0.61 <sup>c</sup>	0.87 <sup>b</sup>	0.93 <sup>b</sup>	1.36 <sup>a</sup>	0.09
POTASSIUM	2.11 <sup>b</sup>	3.00 <sup>a</sup>	3.25 <sup>a</sup>	3.30 <sup>a</sup>	0.23
SODIUM	0.49 <sup>b</sup>	0.07 <sup>a</sup>	0.80 <sup>a</sup>	0.59 <sup>b</sup>	0.55
MAGNESIUM	0.11	0.12	0.11	0.11	0.01

<sup>a-c</sup> means within the same row with different superscripts are significantly different ( $p < 0.05$ )

The mineral composition (Table 3) showed all parameters except Magnesium were significantly ( $P < 0.05$ ) influenced by the treatment diets. Diet 4 which has the highest level of PTRMC had the highest level of calcium, phosphorus and potassium compared to Diet 1 which serves as a control. The values of calcium and phosphorus were above 0.6 g/d recommendation for a 10 kg goat for all the treatment diets. The calcium and phosphorus values reported in this study were well above 0.3 % 0.25 % respectively recommended by (McDowell, 1985) for ruminant in warm wet climate.

**Table 4: Phytochemicals compositions of the experimental diets**

PARAMETERS (%)	DIET 1	DIET 2	DIET 3	DIET 4	SEM
Saponins	1.91 <sup>a</sup>	1.13 <sup>b</sup>	1.07 <sup>b</sup>	0.75 <sup>c</sup>	0.13
Tannins	0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.00
Phytates	0.62 <sup>a</sup>	0.30 <sup>b</sup>	0.33 <sup>b</sup>	0.25 <sup>b</sup>	0.05
Oxalates	0.03 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.00
Hydrogen cyanide	0.05 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.01
Alkaloids	0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.00

<sup>a-</sup> means within the same row with different superscripts are significantly different ( $p < 0.05$ )

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The phytochemical compositions of the experimental diets are presented in Table 4. The values of all the parameters were significantly ( $p < 0.05$ ) reduced as the PTRMC increases in the diets. The saponins, tannins and phytates, oxalates, hydrogen cyanide and alkaloids were significantly ( $p < 0.05$ ) influenced. The effect of all the phytochemicals depends largely on the amount present in the feed and quantity taken by the animals (Villalba *et al.*, 2017; Tedeschi, 2021). The reduction in the level of phytochemicals may be attributed to the effect of biodegradation of maize cobs with PTR in the diets.

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

PARAMETERS (mL/200 mg DM)	DIET 1	DIET2	DIET3	DIET4	SEM
3HOURS	6.67	7.33	7.91	8.17	0.45
6HOURS	14.00 <sup>c</sup>	16.67 <sup>b</sup>	17.2 <sup>b</sup>	29.17 <sup>a</sup>	0.91
9HOURS	23.3 <sup>c</sup>	27.67 <sup>b</sup>	30.12 <sup>ab</sup>	35.30 <sup>a</sup>	1.66
12HOURS	25.33 <sup>c</sup>	30.93 <sup>b</sup>	34.30 <sup>ab</sup>	39.76 <sup>a</sup>	1.66
15HOURS	28.67 <sup>c</sup>	35.98 <sup>b</sup>	38.56 <sup>ab</sup>	41.55 <sup>a</sup>	2.00
18HOURS	31.67 <sup>d</sup>	35.33 <sup>c</sup>	40.24 <sup>b</sup>	43.28 <sup>a</sup>	2.20
21HOURS	33.67 <sup>c</sup>	38.67 <sup>b</sup>	41.77 <sup>b</sup>	45.30 <sup>a</sup>	2.62
24HOURS	36.20 <sup>d</sup>	40.00 <sup>c</sup>	44.37 <sup>b</sup>	49.41 <sup>a</sup>	2.95

<sup>a-c</sup> means within the same row with different superscripts are significantly different ( $P < 0.05$ )

Table 5 shows the volume of in-vitro gas production over the incubation period of 24 hours. The volume of in vitro gas produced at different incubation time by diets containing graded levels of PTRMC 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 hours, diet 1 maintained the lowest in vitro gas production. This suggested that lower carbohydrate fermentation occurred in this treatment in comparison with the other diets (D2, D3 and D4). This is in agreement with the results of (Akinfemi, 2010) for non-fungi treated maize husk.

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

PARAMETERS	DIET 1	DIET2	DIET3	DIET4	SEM
METHANE (mL/200 mg DM)	24.87 <sup>a</sup>	19.47 <sup>b</sup>	17.13 <sup>bc</sup>	16.07 <sup>c</sup>	0.73
IVGP (mL/200 mg DM)	37.20 <sup>c</sup>	40.00 <sup>bc</sup>	44.37 <sup>ab</sup>	49.41 <sup>a</sup>	2.95
OMD(%)	49.00 <sup>c</sup>	54.28 <sup>bc</sup>	58.73 <sup>ab</sup>	64.51 <sup>a</sup>	2.09
IVDMD(%)	48.25 <sup>ab</sup>	32.33 <sup>b</sup>	46.93 <sup>ab</sup>	57.66 <sup>a</sup>	2.89
SCFA(μmol)	0.56 <sup>c</sup>	0.70 <sup>bc</sup>	0.88 <sup>b</sup>	1.12 <sup>a</sup>	0.70
ME(MJ/Kg DM)	6.95 <sup>c</sup>	7.77 <sup>bc</sup>	8.53 <sup>ab</sup>	9.59 <sup>a</sup>	0.34

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

Table 6 shows the result of the in vitro digestibility of the experimental diets. All the parameters evaluated were significantly ( $P < 0.05$ ) influenced. Methane volume decrease with increasing levels of PTRMC in the diets. The observation in this study further indicated the inclusion of PTRMC in the diets of goats improved the quality of the diets. This is in conformity with the reports of (Yilkal, 2015) that enteric methane emissions are highest when animals are fed poor quality diets. Mahesh, (2012) 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 37.20 (Diet 1) to 49.41 (Diet 4) ml/200 mgDM. Gas production is positively correlated with degradable carbohydrates; the PTR bioconversion of the maize cobs improved the diets' quality, which in turn improved fermentation and nutritional quality. The high IVGP in diets with PTRMC could be attributed to the hydrolytic ability of the fungi species, nature of carbohydrate and potency of the rumen liquor used for incubation (Babayemi, 2007).

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The SCFA of the different diets were highest ( $P < 0.05$ ) in D4 and lowest in D1. The higher value recorded for D4 may be ascribed to the higher gas production, which indicates higher available energy. In addition, the higher SCFA for D4 is in conformity with the metabolizable energy (ME) values of those diets (Jiwuba, *et al.* 2022). This suggests that these diets could provide up to more than 70% of the maintenance energy needed by goats and other ruminants (Bergman, 1990, NRC, 2007).

In vitro gas production system has been reported to be a better method to quantify nutrient utilization, and its accuracy in description of digestibility in ruminant animals has been proved by numerous experiments (Sallam, *et al.*, 2007). 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 (Steele, 2006) for small ruminants.

### CONCLUSION

This study demonstrates that *Pleurotus tuber-regium* degraded maize cob (PTRMC) has significant potential as an alternative feedstuff to ruminants. Incorporation of PTRMC increased the content of crude protein. Furthermore, it resulted in a reduction of neutral detergent fibre and acid detergent fibre, indicators of improved digestibility. These findings suggest that PTRMC can be a valuable tool for improving animal feed quality and potentially enhancing animal performance.

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