# *IN VITRO* AND METHANE GAS PRODUCTION POTENTIALS OF FRESH, PRESERVED AND CONCENTRATE DIETS CONTAINING GRADED LEVELS OF *Enterolobium cyclocarpum* LEAVES

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## ABSTRACT

*Presently in Nigeria, Enterolobium cyclocarpum (EC) leaves has not gained popularity amongst ruminant animal farmers. Hence this study was carried out to assess its nutritive components as regards preservation methods and combinations. Six diets were formulated for study 1 to be: T1 – 100% EC leaves, T2 – 100% ensiled EC leaves, T3 – 100% sun dried EC leaves, T4 – 75% ensiled + 25% sun dried EC leaves, T5 – 50% ensiled + 50% sun dried EC leaves and T6 – 25% ensiled + 75% sundried EC leaves. For study 2, concentrate basal diet was supplemented with four levels of EC leaves as follows: Diet 1 – 0% EC leaves, Diet 2 – 7.50% EC leaves, Diet 3 – 15% EC leaves and Diet 4 – 22.50% EC leaves. There was significant difference in crude protein content with T4 being least (14.53%) and T1 (22.50%) the highest (p<0.05). The phytochemicals were affected (p<0.05) in ensiled leaves for tannins, phytates and hydrocyanides (74.41 mg/100g, 2.51 mg/100g and 4.50 mg/100g respectively). Gas volume was highest (p<0.05) for T6 (18.67 ml/200mg DM) and least was recorded for T1 (12.00 ml/200mg DM) in study 1. Methane ranged from 10 ml (T5) to 13.33 ml (T2). Dry matter degradability was highest (p<0.05) for T2 (68.03 g/100g) but least in T1 (44.10 g/100g). In study 2, gas volume ranged from 17.67 ml/200mg DM (D2) to 19.33 ml/200mg DM (D4). Methane ranged from 12.67 ml/200mg DM (D2) to 14.00 ml (D4). Dry matter degradability (g/100g) ranged from 66.77 (D4) to 70.23 (D1). The studies showed that the preserved leaves of Enterolobium cyclocarpum at 25%+75% (ensiled+dried) and a 15% concentrate mix could serve as a possible source of supplements in ruminant diets especially during the off season.*

**Keywords**: *Enterolobium cyclocarpum*, phytochemicals, in vitro gas production, methane, dry matter degradability

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## INTRODUCTION

The subsistence of ruminant animals on grasses cannot be guaranteed especially during the dry season, when grasses or forages have become highly lignified or non-existent for ruminants to thrive on, as evident in Nigeria. Hence, to fill the void created by this, browse plants come in handy though having its drawback (Babayemi, 2006; Ekanem *et al*., 2020). Browse species have been noted in literature to be rich in protein (Fasae *et al*., 2010 and Garcia-Montes de Oca *et al*., 2011) and in addition serve as live fences, shelter, curative purposes among other uses (Sosa, 2000). *Enterolobium cyclocarpum* (EC) is one amongst other browse species such as *Gliricidia sepium, Leucaena leucocephala, Moringa oleifera, Lablab purpureus* etc found in Nigeria. *Enterolobium cyclocarpum* foliage are not too palatable to ruminants and this is predicated on the possession of elevated levels of phytochemicals (such as tannins, saponins) though having a crude protein value range of 15.59 – 18.60 %, 8.16 – 48.20% for crude fibre while ether extract and ash were within the range of 2.21 – 11.00% and 4.90 – 11.80% respectively (Babayemi, 2006; Galindo *et al*., 2014).

However, preservation of forages has been noted to be efficacious in diminishing the levels of phytochemicals/plant secondary metabolites (Ifut *et al*., 2015 and Ekanem *et al*., 2020). Forages can be ensiled, sun cured and toasted (Idowu *et al*., 2013). In assessing the potentiality of forages, in-vitro gas production technique can be utilized since it is rapid and cost friendly (Babayemi *et al*., 2004).

The objective of this research was to assay the *in vitro* total gas and methane production potentials of fresh (untreated) and treated (sun-dried and ensiled) *Enterolobium cyclocarpum* leaves.

## MATERIALS AND METHODS

***Study Area, Leaves collection and Processing:*** The leaves of *Enterolobium cyclocarpum* (EC) were harvested from the Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom state, Nigeria. Uyo is located between latitudes $4^0$59' and $5^0$04' N and longitudes $7^0$52 and $8^0$00' E. Uyo is found in the tropical rainforest of Nigeria. Rainfall goes to as high as 3200 mm and low as 800 mm a year (Uniuyo Meteorological station, 2021). The laboratory research was carried out at the Department of Animal Science, University of Benin, Benin, Edo state. Upon collecting the leaves, they were divided into three portions. One portion went for phytochemical analyses; second one was ensiled and the third sun dried. Thereafter, all preserved leaves were also subjected to analyses. The fresh and treated EC leaves were used to formulate six (6) sole and combined experimental diets as follows:

T1 = 100 % fresh EC leaves (100 % FENT)
T2 = 100 % ensiled EC leaves (100 % ENENT)
T3 = 100 % sun dried EC leaves (100 % DENT)
T4 = 75 % ensiled + 25 % sun dried EC *l*eaves (75 % ENENT)
T5 = 50 % ensiled + 50 % sun dried EC leaves (50 % ENENT)
T6 = 25 % ensiled + 75 % sun dried EC leaves (25 % ENENT)

### *Phytochemical Determination*

The leaves were analyzed for tannin, saponin, oxalate, phytate and hydrocyanide as described in Ekanem *et al*. (2020). Where tannins were determined according to Folin-Dennis Spectrophotometric method (Pearson, 1976) and saponin determined by Double solvent extraction gravimetrical method (Haborne, 1973). Phytate and ammonia nitrogen were analyzed by the procedures of McCance and Widdowson (1953) and Nesslers colorimeter method of AOAC (1990). The concentrate diets supplemented with EC leaves are as presented in Table 1

### *In Vitro Gas Production*

Rumen fluid was obtained from West African Dwarf goats through suction tube via the oesophagus before morning feeding. The animals were fed 60:40, forage to concentrate ratio. Incubation was carried out as described by Menke and Steingass (1988) in 120ml calibrated syringes in three batches at 39°C. The inoculums (30 ml) containing cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g $Na_2HPO_4$ + 0.57g KCl + 0.47g NaCl + 0.12g $MgSO_4.7H_2O$ + 0.16g $CaCl_2$. $2H_2O$) was added to 200mg sample in the syringe in a ratio (1:4 v/v) under continuous flushing with $CO_2$. The syringes containing only inoculum served as the blank while the bags containing only the substrate served as the control. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs.

After 24 hours of incubation, 4 ml of NaOH (10M) was added to estimate the amount of methane produced (Fievez *et al.*, 2005). The average volume of gas produced from the blanks was deducted from the total volume of gas produced.

**Table 1: Ingredient composition of concentrate diets**

| INGREDIENTS | DIET 1 (0%) | DIET 2 (7.5%) | DIET 3 (15%) | DIET 4 (22.5%) |
|---|---|---|---|---|
| Fresh EC | 0.00 | 7.50 | 15.00 | 22.50 |
| Wheat offal | 60.00 | 60.00 | 60.00 | 60.00 |
| BDG | 10.00 | 10.00 | 10.00 | 10.00 |
| PKC | 27.00 | 19.50 | 12.00 | 4.50 |
| Bone meal | 2.00 | 2.00 | 2.00 | 2.00 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| V/TM | 0.50 | 0.50 | 0.50 | 0.50 |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| *Calculated nutrient composition:* | | | | |
| % Crude protein | 14.75 | 14.82 | 14.89 | 14.97 |

EC = *Enterolobium cyclocarpum* leaves. PKC = Palm kernel cake, V/TM = Vitamin/trace mineral premix

To determine post *in vitro* fermentation characteristics, the sealed dacron bags containing the sample were taken out from the syringes, washed with water until the water becomes clear and oven dried at 100°C to constant weight and the dry matter determined expressed as the percentage of the original sample weight. Dry matter degradability (DMD) was calculated using the formulae:

$$DMD\ \% = \frac{\text{Wt. of sample before incubation} - \text{Wt. of sample after incubation}}{\text{Wt. of sample before incubation}} \times \frac{100}{1}$$

The Fermentation Efficiency (FE) and effect of methane reduction ($CH_4$red %) were calculated using the following formulas

$$\text{Fermentation Efficiency (FE)} = \frac{\text{Dry Matter Digestibility (mg/kg)}}{\text{Total Gas Volume (mL/g)}}$$

$$CH_4\text{red (\%)} = \frac{\text{Average } CH_4 \text{ of the control} - CH_4 \text{ of treated sample}}{\text{Average } CH_4 \text{ of the control}} \times \frac{100}{1}$$

Other post incubation parameters such as metabolizable energy (ME), Organic matter digestibility (OMD) and short chain fatty acids (SCFA) were estimated using the equations below:

$ME = 2.20 + 0.136GV + 0.057CP + 0.00029\ CF$ (Menke and Steingass, 1988).
$OMD = 14.88 + 0.88\ GV + 0.45\ CP + 0.651\ XA$ (Menke and Steingass, 1988).
$SCFA = 0.0239\ GV – 0.0601$ (Getachew *et al.*, 1999),
where, GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash of the incubated samples respectively.

***Statistical Analysis:*** Data obtained were subjected to analysis of variance and the means were separated using Duncan multiple range F-test of SAS (2000).

## RESULTS AND DISCUSSION

***Phytochemicals:*** The phytochemicals found in the preserved and fresh EC leaves are presented in Table 2. For all the phytochemicals analyzed there were significant differences for all the treatments. Tannin was highest for 75% ENENT (158.11 mg/100g) and least recorded for ENENT (100% fresh leaves) (74.41 mg/100g). Saponin was highest for 50% ENENT (4.49 mg/100g) and the least was found in DENT (3.18

mg/100g). Ensiling of the EC leaves elicited a reduction in oxalate content (234.10 – 315.14 mg/100g) when compared to fresh foliage (450.21 mg/100g). Sole ensiling of EC leaves caused a reduction in phytate and HCN content when compared to fresh EC leaves (2.51 and 4.50 mg/100g vs 3.60 and 6.10 mg/100g respectively). However, combination of the preserving EC leaves resulted in an increased in the content for phytate and HCN (80.84 – 89.90 and 10.27 – 19.96 mg/100g respectively).

The preservation of EC leaves did not totally eradicate the phytochemicals, nonetheless, it elicited a reduction in some phytochemicals. This assertion is in agreement with the conclusion of Heckendon *et al.* (2006) that in spite of drying the leaves of Sainfoin browse plants, the hay still had bioactive properties in significant amount compared to fresh leaves. Babayemi (2006) detected saponin and steroids but tannins were not seen in *Enterolobium* leaves qualitatively. Babayemi *et al.* (2010) however detected saponin and tannin in ensiled cassava wastes and *Albizia saman* pods.

Table 2: Phytochemical composition (mg/100g) of fresh and preserved *Enterolobium* leaves

| Parameters | FENT | ENENT | DENT | 75ENENT | 50ENENT | 25ENENT | SEM |
|---|---|---|---|---|---|---|---|
| Tannin | $93.40^{c}$ | $74.41^{e}$ | $75.97^{d}$ | $158.11^{a}$ | $74.59^{e}$ | $130.73^{b}$ | 7.81 |
| Saponin | $3.92^{c}$ | $4.30^{b}$ | $3.18^{f}$ | $3.80^{d}$ | $4.49^{a}$ | $3.64^{e}$ | 0.10 |
| Oxalate | $450.21^{a}$ | $315.14^{b}$ | $288.13^{bc}$ | $276.12^{bcd}$ | $234.10^{d}$ | $253.30^{cd}$ | 17.76 |
| Phytate | $3.60^{e}$ | $2.51^{f}$ | $3.96^{d}$ | $89.90^{a}$ | $81.48^{b}$ | $80.84^{c}$ | 9.81 |
| HCN | $6.10^{e}$ | $4.50^{f}$ | $11.70^{c}$ | $15.90^{b}$ | $10.27^{d}$ | $19.96^{a}$ | 1.29 |

a-f Means on the same row with different superscripts significantly differ ($p<0.05$); FENT – 100% Fresh EC leaves; ENENT – 100% Ensiled EC leaves; DENT – 100% Sundried EC leaves; 75 ENENT – 75% Ensiled + 25% Sundried EC leaves; 50 ENENT - 50% Ensiled + 50% Sundried EC leaves; 25 ENENT - 25% Ensiled + 75% Sundried EC leaves; EC – *Enterolobium cyclocarpum*; HCN – Hydrocyanide.

***In Vitro total gas and methane gas production:***

The post *in-vitro* fermentation characteristics of fresh and preserved EC leaves are shown in Table 3. There were significant differences ($p<0.05$) in total gas volume (Gv), percentage methane ($CH_4$) to Gv, dry matter digestibility (DMD), short chain fatty acid (SCFA), metabolizable energy (ME) and organic matter digestibility (OMD). Total gas volume was significantly high ($p<0.05$) in the 25% ensiled leaves combined with the 75% sun dried EC leaves (18.67%). Moderate total gas volume was produced by ENENT (16.00ml/200g DM), DENT (16.33ml/200mg DM), ENENT 75 (15.00 ml/200mgDM) and ENENT 50 (14.00ml/200mg DM). The lowest Gv (12.00 ml/200mg DM) was recorded for fresh EC leaves (FENT). High total gas production indicates that majority of substrate has gone into gas production thereby reducing the production of VFA and other beneficial end products.

Similarly, low gas production can be due to inadequate fermentation of substrate or the fact that fermentation has taken place in the favour of VFA rather than gas. Methane gas volume was highest in ENENT (13.33ml) and lowest in ENENT 50 (10.00ml). Methane reduction of 11.08 to 35.73% was obtained in this study. Dry matter and OM digestibility were generally low, possibly due to the presence of secondary metabolites such as tannin and saponin in the leaves (Stewart, 2018). The high production in Gv, DMD, SCFA and OMD for processed leaves against fresh ones can be alluded to reduction in secondary metabolites as described by Babayemi and Bamikole (2009) in preserved cocoyam. The values obtained in this study was lower than those reported by Babayemi (2006) on *Enterolobium* leaves, of 31.33mL, 7.66 MJ/kg DM, 51.42% and 0.69umol for Gv, ME, OMD and SCFA respectively, even the methane gas produced. Figure 1 presents the gas volume and methane gas produced.

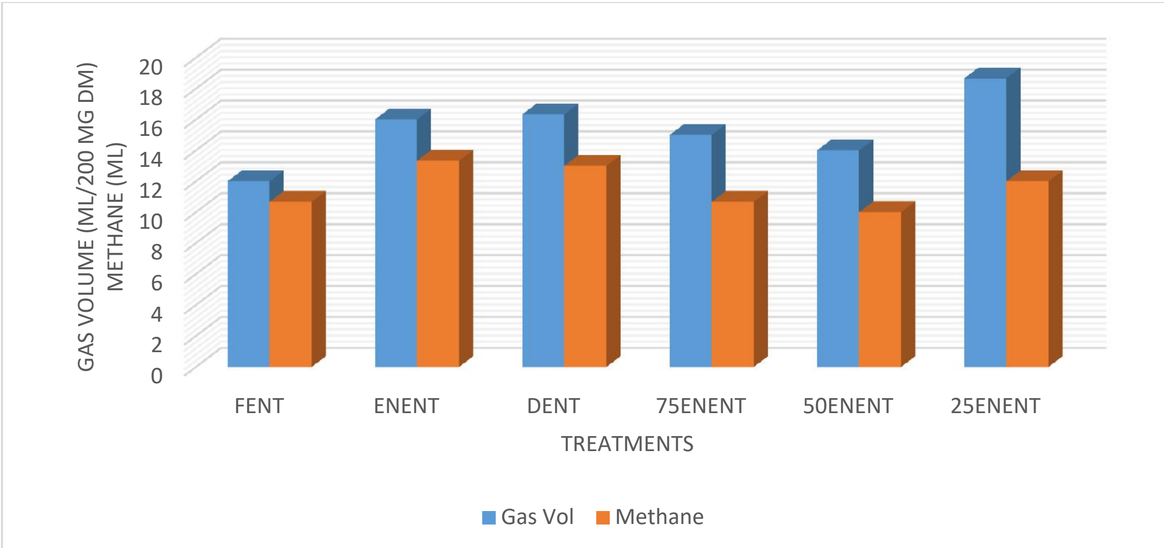


**Figure 1: Gas volume and methane gas produced.**

**Table 3: Post *in vitro* incubation (24hrs) of fresh and preserved *Enterolobium cyclocarpum* leaves**

| Parameters | FENT | ENENT | DENT | ENENT 75 | ENENT 50 | ENENT 25 | SEM |
|---|---|---|---|---|---|---|---|
| Gv (ml/200mg DM) | $12.00^{b}$ | $16.00^{ab}$ | $16.33^{ab}$ | $15.00^{ab}$ | $14.00^{ab}$ | $18.67^{a}$ | 0.70 |
| $CH_4$(ml) | 10.67 | 13.33 | 13.00 | 10.67 | 10.00 | 12.00 | 0.67 |
| CH4/Gv (%) | $88.90^{a}$ | $83.00^{b}$ | $79.60^{c}$ | $71.10^{e}$ | $71.40^{d}$ | $64.27^{f}$ | 2.01 |
| DMD (g/100g) | $44.10^{b}$ | $68.03^{a}$ | $66.53^{ab}$ | $60.37^{ab}$ | $56.13^{ab}$ | $64.73^{ab}$ | 3.07 |
| FE | 0.04 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 |
| $CH_4$red (%) | 11.08 | 16.69 | 20.39 | 28.87 | 28.57 | 35.73 | 3.29 |
| SCFA /200mgDM | $0.23^{e}$ | $0.32^{b}$ | $0.33^{b}$ | $0.30^{c}$ | $0.27^{d}$ | $0.39^{a}$ | 0.01 |
| ME(MJ/kgdm) | $5.09^{d}$ | $5.21^{c}$ | $5.44^{b}$ | $5.07^{e}$ | $5.03^{f}$ | $5.76^{a}$ | 0.06 |
| OMD (%) | $38.23^{f}$ | $40.53^{c}$ | $42.45^{b}$ | $38.77^{d}$ | $38.48^{e}$ | $43.31^{a}$ | 0.48 |

$^{a-f}$ Means on the same row with different superscripts are significantly different (p<0.05);

FENT = 100% Fresh *Enterolobium cyclocarpum* leaves; ENENT = 100% Ensiled *Enterolobium cyclocarpum* leaves; DENT = 100% Sundried *Enterolobium cyclocarpum* leaves; ENENT 75 = 75% Ensiled + 25% Sundried *Enterolobium cyclocarpum* leaves; ENENT 50 = 50% Ensiled + 50% Sundried *Enterolobium cyclocarpum* leaves; ENENT 25 = 25% Ensiled + 75% Sundried *Enterolobium cyclocarpum* leaves. SEM = Standard error of mean. Gv = Net gas production, $CH_4$ = Methane gas, $CH_4$GV = Methane/total gas volume, DMD = Dry matter degradability, FE = Fermentation Efficiency, $CH_4$red = Methane reduction, SCFA = Short chain fatty acid, ME = Metabolisable energy, OMD = Organic matter digestibility

Table 4 shows the post *in vitro* fermentation characteristics of different levels of EC leaves in combination with concentrate diets. There were significant differences (P<0.05) in percentage $CH_4$/Gv, SCFA, ME and OMD. Although not significantly different across treatment (P>0.05), methane production was low in Diet 3 (12.67 ml) thereby indicating a reduction in methane gas by 33.32%. Dry matter digestibility was reduced in diets containing EC rich in tannin and saponin. Saponin is known to deter activities of microbes and tannins make feed unavailable for degradation in the rumen (Babayemi, 2006; Babayemi *et al.,* 2010). Organic matter digestibility was generally low due to the presence of plant secondary metabolites such as tannin and saponin (Stewart, 2018).

However, it fell within the range reported by Fasae *et al*. (2010) of 40.80% - 74.75% for selected leaves of trees. Higher gas volumes observed here in comparison to Table 3 might be attributed to the inclusion of concentrate in the mixture. This reason also occasioned the increase in all the parameters measured. Medina *et al.* (2003) had alluded that a higher gas volume production results in more substrate disappearance, indicating greater digestibility as seen in Table 4.

**Table 4: Post *in vitro* incubation (24hrs) of concentrate diet containing graded levels of *Enterolobium cyclocarpum* leaves**

| Parameters | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM |
|---|---|---|---|---|---|
| Gv (ml/200mg DM) | 18.67 | 17.67 | 19.00 | 19.33 | 0.06 |
| $CH_4$(ml) | 13.67 | 13.00 | 12.67 | 14.00 | 0.68 |
| $CH_4$/Gv (%) | 73.20$^{b}$ | 73.57$^{a}$ | 66.70$^{d}$ | 72.00$^{c}$ | 0.83 |
| DMD (g/100g) | 70.23 | 68.03 | 68.23 | 66.77 | 1.53 |
| FE | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 |
| $CH_4$red (%) | 26.78 | 26.43 | 33.32 | 27.57 | 4.68 |
| SCFA /200mgDM | 0.39$^{a}$ | 0.36$^{b}$ | 0.39$^{a}$ | 0.40$^{a}$ | 0.01 |
| ME(MJ/KgDm) | 5.72$^{b}$ | 5.56$^{c}$ | 5.72$^{b}$ | 5.83$^{a}$ | 0.03 |
| OMD (%) | 43.12$^{c}$ | 41.73$^{d}$ | 44.46$^{b}$ | 44.82$^{a}$ | 0.37 |

$^{a-d}$ Means on the same row with different superscripts are significantly different ($p<0.05$).
Diet 1 = 0% *Enterolobium cyclocarpum* (EC) leaves inclusion concentrate diet. Diet 2 = 7.50% EC leaves inclusion concentrate diet. Diet 3 = 15% EC leaves inclusion concentrate diet. Diet 4 = 22.50% EC leaves inclusion concentrate diet.
SEM = Standard error of mean. Gv = Net gas production, $CH_4$ = Methane gas, $CH_4$GV = Methane/total gas volume, DMD = Dry matter degradability, FE = Fermentation Efficiency, $CH_4$red = Methane reduction, SCFA = Short chain fatty acid, ME = Metabolisable energy, OMD = Organic matter digestibility.

A diets characteristic such as contents of Crude protein, rumen degradable nitrogen and dietary fibre influences the efficacy of plant extracts to produce gas. High contents of rumen degradable nitrogen could also be implicated in the lowering of gas production as corroborated by Akanmu *et al.* (2020). Moreover, a low effective degradability and slower degradation speed may have accounted for the low gas production seen in this study. The findings of this study on methane reduction agrees with those of Al-Marzooqi *et al*. (2022) and Akanmu *et al.* (2020) that the decrease may be attributed to inhibition of microbes (methanogens) which are associated with a decline in available $H_2$, thereby decreasing methane gas. A reduction in methane production within the range of 28.17 to 30.93% was reported by Chaturvedi *et al.* (2015) for tree leaves (*E. officinalis, Azadirachta indica* and *C. phlomdis*).

## CONCLUSIONS

- The preservation of *Enterolobium cyclocarpum* leaves as hay reduced the concentrations of anti-nutritional factors, especially tannin, saponin and oxalates, while still preserving the bioactive substances in them.
- Ensiling led to reduction in tannin, oxalate, phytate and hydrocyanic acid, better dry matter digestibility and at 25% it caused a better methane reduction.
- The concentrate mix at 15% of EC leaves showed a better potential in methane gas reduction.
- Sun drying of the leaves proved most effective as nutrients were concentrated more in the hay while ensiling elevated saponin levels.

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