

## In vitro gas production of maize residues fractions grown on soils treated with organic and inorganic fertilizers

<sup>1</sup>Sosina, A. O., <sup>2</sup>Inyang, U. A., <sup>3</sup>Adewuyi, P. A. <sup>1</sup>Babayemi, O. J.



<sup>1</sup>Department of Animal Science, University of Ibadan, Ibadan

<sup>2</sup>Department of Animal Science, University of Uyo, Akwa Ibom, Nigeria

<sup>3</sup>School of Agriculture and Environmental Science, University of Gambia, Gambia.

Corresponding author: [dayososina@gmail.com](mailto:dayososina@gmail.com): +2348038270701

### Abstract

*There are concerns that fertilizer application may influence the nutritional composition of crop residues used for livestock production. The study investigated the in vitro gas production characteristics of maize residue fractions treated with different fertilizer sources.*

*Fresh samples of fertilizer sources treated maize fractions from on-farm experimental plots were oven dried and analyzed for Crude Protein (CP, %), Neural Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) using the Near-Infrared Reflectance Spectroscopy. maize fractions obtained were grain, stover, leaf, cob, and husk while fertilizer sources were compost organic manure, 20-10-10 N.P.K. inorganic fertilizer and no fertilizer as control. Gas production of fertilizer-treated maize fractions was recorded at 24-hour intervals. The experiment was laid out in a randomized complete block design with three replicates. Data were analyzed using analysis of variance (ANOVA). Results showed that control significantly influenced cob CP (12.69 %), and N (2.03 %), leaf CP (15.63 %) and N (2.53%) while compost organic fertilizer significantly influenced stover NDF (70.09) and ADF (45.07). Also, control had influence on y (mL/h-1), a (mL3), a+b, and b (mL-1) values (17.40, 4.67, 29.067 and 24.40), respectively. Grain had higher y (36.00), a (4.20) and b (73.56). The total volume of gas produced from compost organic manure and inorganic fertilizer-treated maize residues were low. Gas production and degradation rate were more efficient in grains and cob compared with other fractions.*

**Keywords:** Crop residues, Ecological area, Degradability, Digestibility, Fertilizers, Smart climate Agriculture.

## Production De Gaz In Vitro Des Fractions De Résidus De Maïs Traités Avec Des Engrais Organiques Et Inorganiques

### Résumé



*Des préoccupations existent quant au fait que l'application d'engrais peut influencer la composition nutritionnelle des résidus de cultures utilisés pour la production animale. L'étude a investigué les caractéristiques de production de gaz in vitro des fractions de résidus de maïs traitées avec différentes sources d'engrais.*

*Des échantillons frais de fractions de maïs traitées avec des sources d'engrais provenant de parcelles expérimentales à la ferme ont été séchés au four et analysés pour les protéines brutes (CP, %), les fibres au détergent neutre (NDF) et les fibres au détergent acide (ADF) en utilisant la spectroscopie proche infrarouge. Les fractions de maïs obtenues étaient le grain, la tige, la feuille, l'épi et la balle tandis que les sources d'engrais étaient le compost de fumier organique, l'engrais inorganique NPK 20-10-10 et aucun engrais comme témoin. La production de gaz des fractions de maïs traitées avec des engrais a été enregistrée à des intervalles de 24 heures. L'expérience a été mise en place dans un dispositif en blocs complets randomisés avec trois répétitions. Les données ont été analysées à l'aide de l'analyse de variance (ANOVA).*

*Les résultats ont montré que le témoin a significativement influencé le CP de l'épi (12,69 %) et le N (2,03 %), le CP des feuilles (15,63 %) et le N (2,53 %) tandis que l'engrais organique compost*

*a significativement influencé le NDF de la tige (70,09) et l'ADF (45,07). Aussi, le témoin a eu une influence sur les valeurs de y (mL/h-1), a (mL3), a+b, et b (mL-1) (17,40, 4,67, 29,067 et 24,40), respectivement. Le grain avait des valeurs plus élevées de y (36,00), a (4,20) et b (73,56). Le volume total de gaz produit à partir des résidus de maïs traités avec de l'engrais organique compost et de l'engrais inorganique était faible.*

*La production de gaz et le taux de dégradation étaient plus efficaces dans les grains et les épis par rapport aux autres fractions.*

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**Mots-clés** : Résidus de cultures, Zone écologique, Dégradabilité, Digestibilité, Engrais, Agriculture climatique intelligente.

## **Introduction**

In recent times the use of fertilizer became a practice among farmers to increase crop production efficiency and maximize income. Supplementary soil fertility increases both the crop/grain and biomass yield necessary for ruminants. Over time, the use of various sources of fertilizer to increase maize fruit and foliage productions has become trendy among rural farmers in a crop-livestock production system. Despite this, there is limited information on the availability of nutrients from different fertilizer-treated residues when fed to ruminants.

Fermentation kinetics of forages consumed by ruminants is dependent on the rate of passage and the degradation rate (Makkar and Becker, 1997). The rate and extent of dry matter fermentation in the rumen are crucial determinants of the nutrients utilized by ruminants (Getachew *et al.*, 2004). The relevance of evaluating the nutritional value of forage makes an important contribution to the protein and energy intake of grazing cattle (Blummel *et al.*, 1999). Traditionally, the energetic value of forage consumed by grazing cattle is estimated from in vitro organic matter digestibility or in situ organic matter degradability obtained after 48 h incubation in the rumen (Babayemi *et al.*, 2010). The in vitro gas production technique can also evaluate the nutritive value of forages and estimate the rate and extent of DM degradation indirectly using the gas produced during fermentation (Menke and

Steingass, 1988, Njidda, 2011, Kintl *et al* 2023). The in vitro gas production (IVGP) technique (Menke and Steingass, 1988) is used widely in animal nutrition for feed evaluation and to study the kinetics of microbial fermentation processes in the digestive tract.

Mahesh and Mohoni 2013, Kint *et al.* 2023 also suggested that the in vitro gas production technique should be considered for estimating Metabolizable Energy (ME) in tropical feedstuffs. Getachew *et al.* (2004); Htet *et al.*, 2017; Samarappuli and Berti, 2018 described that the gas produced in the straw comprises two phases; one being the soluble phase (rapid gas production) contributed by the soluble portion of the straw and the other contributed by the insoluble fibrous portion of cell-wall.

Further, Menke and Steingass (1988) described the third phase of gas production, which is contributed by microbial turnover. As cell wall components (NDF and ADF) are known to have a negative correlation with gas production (Kintl *et al* 2022, 2023), and thus readily available soluble carbohydrate fractions found in fungal treated substrates are expected to produce more gas (Fievez *et al.*, 2005, Adefemi and Ayoade 2017) and short-chain fatty acids (SCFA), with an increased ME contents (Getachew *et al.*, 2004; Shrivastava *et al.*, 2011; Mahesh and Mohini, 2013). Forage maize contains Crude Protein (%) in the

range of 7.5-8.5, Crude Fiber (%) 32-34, while the fat content (%) is usually 1-2.5 with Ash (%) range of 7-9 and nitrogen-free extract range of 50-60 % (Sosina, 2017, Kinlt *et al.* 2020). It is evident from the nutritional profile of forage maize that it does not contain a sufficient quantity of crude protein which is the most important quality parameter of forage as compared to forage legumes which contain crude protein of 20-24% (Mahesh and Mohoni 2013, Kint *et al.* 2023).

Since feeding of maize crop residues is a common practice in the rural areas for raising ruminants in a crop-livestock production system (Sosina 2017). Thus, the study determines the effect of fertilizer sources on gas production, effect of different residue fractions on gas production and the interaction of these components (maize residue fractions and fertilizer sources) on gas production

**Materials and methods**

The study was conducted at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The area has an altitude of approximately 1,500m above sea level, an annual mean temperature of about 20°C and annual rainfall varying from 1,200 to 1,400mm. The rainfall is bimodal, falling in two seasons, the long rains lasting from March to June and short rains from October to December.

Experimental layout and management: A maize variety, early maturing 75 days SUWAN yellow, common to farmers in the area was planted at a spacing of 75 cm ×

50cm inter and intra-row, respectively with two seed/hole resulting in a population of 5,333 plants/100m<sup>2</sup>. A commercial organic fertilizer (Compost manure) was applied at 30-60 Kg N/ha while commercial inorganic fertilizer (20-10-10 N.P.K. fertilizer) was applied at 40-60Kg /ha at 2-3 weeks after planting; and top-dressed with urea (75Kg/ha) at 4 and 6 weeks after planting. Other good agronomic procedures (GAP) for maize production were appropriately followed after planting.

Collection and processing of maize residue: The identified wet samples of the maize fractions- stover, cob, leaf and husk-harvested at day 75 for the tested fertilizer sources were air-dried for a day to a constant weight. The air-dried samples were then weighed and then oven dried at 70°C for a day while the differential was the Dry matter (DM) weight. The oven-dried MR samples were milled using electrical miller into 1mm particle size. The milled feed resources were packed into an airtight sample sac for further chemical analysis using Near-infrared reflectance spectroscopy (NIRS). Matured maize plant from small plot adoption technique (SPAT) comprising of a fractional representation of the whole farmer’s plot of about 5m×5m spacing was used for the study. Maize plants samples treated with different fertilizer sources i.e. Compost Organic manure (T1), 20-10-10 N.P.K. inorganic fertilizer (T2) and no fertilizer as control (T3) with three (3) replicates each as shown in Table 1.

**Table 1: Nutrient composition of fertilizer sources**

<b>FERTILIZER SOURCES</b>	<b>N (%)</b>	<b>P (%)</b>	<b>K (%)</b>
Organic (T <sub>1</sub> )	3.51	1.12	2.01
Inorganic (T <sub>2</sub> )	20.02	10.02	10.03
Control (T <sub>3</sub> )	1.02	0.23	0.13
SEM	0.67	0.17	0.10

Adapted from Makinde and Ayoola (2010)

Note: T1 (commercial compost manure), T2 (20-10-10 N.P.K. fertilizer) and T3 (No fertilizer application as control).

Determination of the chemical composition of the maize residue (MR) fractions treated with different fertilizer sources: The maize residue (MR) were ground to pass through a 1mm mesh and scanned with NIRS. Feed samples were analyzed with the mixed feed global calibrations model using the software package Win ISI (FOSS, Denmark model NIRS 5000) at International Livestock Research Institute (ILRI), Ibadan, Nigeria. Crude protein, ether extract, ash content, crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), In vitro organic matter (IVO) and starch ASIS were analyzed using NIRS.

*In vitro Gas Production Techniques:*

Evaluation of the nutritive value of feeds was done with the use of gas production measuring technique. The efficient utilization of roughages had made the technique very popular as it has the advantage in the understanding of the rumen fermentation kinetics. Several gas measuring techniques and in vitro gas methods are in use. The in vitro gas method based on syringes (Menke *et al.*, 1979) appears to be most suitable for use in developing countries. Gas measurements provide useful data on digestion kinetics of both soluble and insoluble fractions of feedstuff. Preparation of the buffer and rumen liquor was carried out as described by Menke *et al.*, (1979). The substrate was placed in calibrated gas tight plastic syringes fitted with a piston. The syringes were incubated in an incubator at  $39 \pm 1^\circ\text{C}$ . Rumen liquor was collected from West African Dwarf (WAD) goats using a suction tube before morning feeding. The liquor was sieved with a four layered cheese cloth and mixed with a buffer solution (9.8g NaHCO<sub>3</sub> + 2.77 (Na<sub>2</sub>

HPO<sub>4</sub> + 0.57gKCl + 0.47gNaCl + 0.12MgSO<sub>4</sub>.7H<sub>2</sub>O + CaCl<sub>2</sub>.H<sub>2</sub>O per 1,000ml) in a ratio 1:2 v/v. A 200g DM of each sample with 30ml of rumen liquor and buffer were placed in each syringe and incubated in triplicates under continuous flushing with carbon dioxide. A blank containing rumen liquor and buffer alone was incubated along with others.

The reading of the blank was subtracted from that of the other syringes. Gas production was recorded at 3, 6, 9,12,15,18 and 21 and 24 hour. After 24 hr of incubation, 4 ml of NaOH (10M) was incubated into inoculums as reported by Fievez *et al.*, (2005) to estimate the amount of methane produced. The value of gas produced at intervals was plotted against the curve using the equation:

$$Y = a + b(1 - e^{-ct}) \text{ (}\ddot{\text{O}}\text{rskov and McDonald, 1979)}$$

Where Y= Volume of gas produced at time t; a = Gas produced from the soluble fractions (mL<sub>3</sub>), b = gas produced from the insoluble but degradable fraction (mL-1); t = incubation time (hrs); (a+b) = potential gas production

ME (MJ/KgDM) = 2.20 + 0.136GP + 0.057CP + 0.002496 CF; OMD (%) = 14.88+ 0.889 GP + 0.45 CP + 0.0651XA  
SCFA (μmol) = 0.0239 GV – 0.0601; Where XA: Ash %; GP: The Net gas production in mL from 200 mg dried sample after 24 hrs of incubation; GV: Gas volume (mL).

**Statistical analysis**

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates Data obtained were subjected to analysis of variance (ANOVA) and the means separated by

Duncan method using Statistical Analysis System (SAS) package.

**Results**

Chemical composition of maize residues fractions: The chemical compositions of the maize residues (i.e. cob, husk, leaf, stover

and grain) treated with fertilizers are shown in Table 2. The observed differences among the maize residue fractions with respect to fat content, starch ASIS, ash, CP, NDF, ADF and ADL, ME, and IVO (*in vitro* organic matter) were considered.

**Table 2: Chemical composition of maize residues fractions**

fertilizer sources		Fat	Fibre	StarchASIS	dm%	ash%	ndm%	cpdm%	ndfdm%	adfdm%
Cob	organic (T <sub>1</sub> )	0.90 <sup>b</sup>	10.83 <sup>c</sup>	7.22 <sup>c</sup>	89.86 <sup>b</sup>	5.34 <sup>a</sup>	1.77 <sup>b</sup>	11.04 <sup>b</sup>	45.89 <sup>b</sup>	18.01 <sup>a</sup>
	In-organic (T <sub>2</sub> )	3.24 <sup>a</sup>	14.58 <sup>a</sup>	44.61 <sup>a</sup>	94.35 <sup>a</sup>	0.12 <sup>b</sup>	1.74 <sup>b</sup>	10.88 <sup>c</sup>	46.59 <sup>a</sup>	14.48 <sup>b</sup>
	Control (T <sub>3</sub> )	3.18 <sup>a</sup>	13.25 <sup>b</sup>	33.27 <sup>b</sup>	93.78 <sup>a</sup>	0.95 <sup>b</sup>	2.03 <sup>a</sup>	12.69 <sup>a</sup>	46.16 <sup>a</sup>	17.82 <sup>a</sup>
	SEM	0.86	2.12	4.68	7.82	1.76	0.67	1.78	1.58	10.67
Husk	sources	Fat	Fibre	StarchASIS	dm%	ash%	ndm%	cpdm%	ndfdm%	adfdm%
	organic (T <sub>1</sub> )	0.64	17.14 <sup>b</sup>	8.02 <sup>b</sup>	92.34 <sup>b</sup>	2.73 <sup>b</sup>	1.06	6.65	65.33 <sup>b</sup>	30.72 <sup>b</sup>
	In organic (T <sub>2</sub> )	0.39	19.36 <sup>a</sup>	11.03 <sup>a</sup>	93.20 <sup>a</sup>	3.93 <sup>a</sup>	1.06	6.64	68.53 <sup>a</sup>	33.45 <sup>a</sup>
	Control (T <sub>3</sub> )	0.29	17.74 <sup>b</sup>	7.62 <sup>b</sup>	92.70 <sup>b</sup>	2.50 <sup>b</sup>	0.99	6.18	61.52 <sup>c</sup>	29.22 <sup>c</sup>
Leaf	SEM	0.97	6.87	2.78	5.37	1.68	0.45	1.56	8.69	12.65
	sources	Fat	Fibre	StarchASIS	dm%	ash%	ndm%	cpdm%	ndfdm%	adfdm%
	Organic (T <sub>1</sub> )	1.81	15.26 <sup>b</sup>	6.69 <sup>b</sup>	92.84	9.97 <sup>c</sup>	2.43 <sup>a</sup>	15.19 <sup>a</sup>	53.69 <sup>b</sup>	29.93
	In organic (T <sub>2</sub> )	1.69	16.25 <sup>a</sup>	10.10 <sup>a</sup>	92.78	10.53 <sup>b</sup>	1.86 <sup>b</sup>	11.63 <sup>b</sup>	57.23 <sup>a</sup>	32.14
Stover	Control (T <sub>3</sub> )	2.04	14.54 <sup>c</sup>	6.66 <sup>b</sup>	92.78	16.48 <sup>a</sup>	2.50 <sup>a</sup>	15.63 <sup>a</sup>	48.99 <sup>c</sup>	31.35
	SEM	0.97	5.47	1.98	3.78	2.8	0.74	1.87	7.74	10.64
	sources	Fat	Fibre	StarchASIS	dm%	ash%	ndm%	cpdm%	ndfdm%	adfdm%
	organic (T <sub>1</sub> )	0.58	21.05	2.74 <sup>c</sup>	94.71	1.81	0.77	4.79	70.09 <sup>a</sup>	45.07 <sup>a</sup>
grain	In organic (T <sub>2</sub> )	1.70	20.79	8.25 <sup>a</sup>	93.70	2.36	0.80	5.00	66.03 <sup>b</sup>	38.84 <sup>b</sup>
	Control (T <sub>3</sub> )	1.27	20.41	6.43 <sup>b</sup>	93.31	2.51	0.85	5.34	59.34 <sup>c</sup>	35.22 <sup>c</sup>
	SEM	0.89	4.45	1.43	1.25	1.23	0.34	1.28	8.92	10.28
	sources	Fat	Fibre	StarchASIS	dm%	ash%	ndm%	cpdm%	ndfdm%	adfdm%
grain	Organic (T <sub>1</sub> )	3.76 <sup>a</sup>	23.23 <sup>a</sup>	1.95 <sup>c</sup>	92.96 <sup>b</sup>	4.78 <sup>b</sup>	1.61 <sup>b</sup>	10.08 <sup>b</sup>	60.07 <sup>b</sup>	31.05 <sup>b</sup>
	In organic (T <sub>2</sub> )	1.25 <sup>c</sup>	19.23 <sup>b</sup>	7.66 <sup>a</sup>	92.91 <sup>c</sup>	4.53 <sup>c</sup>	1.37 <sup>c</sup>	8.56 <sup>c</sup>	55.82 <sup>c</sup>	29.34 <sup>c</sup>
	Control (T <sub>3</sub> )	2.34 <sup>b</sup>	17.23 <sup>c</sup>	5.57 <sup>b</sup>	93.17 <sup>a</sup>	6.33 <sup>a</sup>	1.69 <sup>a</sup>	10.53 <sup>a</sup>	60.07 <sup>a</sup>	31.05 <sup>a</sup>
	SEM	1.80	19.90	5.06	1.45	1.57	0.78	1.34	8.87	9.19

a, b, c means in the same column with different superscript differ significantly (P<0.05) N-Nitrogen, C.P-Crude Protein, NDF- Neutral Detergent Fiber, ADF-Acid Detergent Fiber, ME-Metabolizable Energy, IVO- *In vitro* organic matter.

Fat (%) in maize cob treated with T<sub>1</sub> (3.24) and T<sub>2</sub> (3.18) were similar (P>0.05) but significantly higher (P<0.05) than fat in maize treated with the control treatment. Fiber (%) in maize cob treated with T<sub>2</sub> (14.58) was significantly higher than fiber in T<sub>1</sub> (10.83) and T<sub>3</sub> (13.25) treated cobs, maize cob treated with T<sub>3</sub> had higher fiber % than T<sub>1</sub>. Ash (%) in maize cob treated with T<sub>2</sub> (0.12) and T<sub>3</sub> (0.95) were similar (P>0.05) but significantly higher (P<0.05) than fiber in maize treated with compost organic manure (5.34). NDF (%) in maize cob treated with T<sub>2</sub> (46.59) and T<sub>3</sub> (46.16) were similar (P>0.05) but significantly lower (P<0.05) than NDF in maize treated with the compost organic manure. ME and IVO in maize cob treated with T<sub>1</sub> (10.72, 72.10) and T<sub>2</sub> (10.58, 70.03) were similar (P>0.05) but significantly lower (P<0.05) than ME and IVO in maize cob with control treatment.

Fiber (%) and Starch ASIS in maize husk treated with T<sub>1</sub> (17.14, 8.02) and T<sub>3</sub> (17.74, 7.62) were similar (P>0.05) but significantly higher (P<0.05) than fiber in maize husk treated with N.P.K. inorganic fertilizer (19.36, 11.03). While the NDF, ADF AND ADL (%) values with T<sub>2</sub> (68.53, 33.45 and 3.16) and T<sub>1</sub> (65.33, 30.72 and 1.75) were similar (P>0.05) but significantly higher (P<0.05) than maize husk treated with control. Also, the ME (MJ/Kg) and IVO (%) values with T<sub>1</sub> (9.20, 60.98) and T<sub>3</sub> (9.36, 61.75) were similar (P>0.05) but significantly higher (P<0.05) than husk in maize with N.P.K. inorganic fertilizer.

Ash (%), NDM (%) and CPDM (%) in maize leaf treated with T<sub>3</sub> (16.48, 2.50 and 15.63) and T<sub>2</sub> (10.53, 1.86 and 11.63) were similar (P>0.05) but significantly higher (P<0.05) than Ash, NDM AND CPDM in maize leaf with compost organic manure. While Fiber (%) and NDFDM (%) with T<sub>2</sub>

(16.25, 57.23) were similar (P>0.05) but significantly higher (P<0.05) than fiber and NDFDM of maize leaf of the control treatment. The ME (MJ/Kg) and IVOMD (%) were similar (P>0.05) but significantly higher (P<0.05) than ME and IVO in maize leaf with the control treatment.

NDFDM (%), ADFDM (%) and ADLDM (%) in maize stover treated with T<sub>1</sub> (70.09, 45.07, 6.22) and T<sub>2</sub> (66.00, 38.84, 5.59) were similar (P>0.05) but significantly higher (P<0.05) than NDF, ADF and ADL in maize stover with the control treatment. While the ME (MJ/Kg) and IVOMD (%) in maize stover treated with T<sub>3</sub> (8.35, 54.49) and T<sub>2</sub> (8.02, 52.28) were similar (P>0.05) but significantly higher (P<0.05) than ME and IVO in maize stover with the N.P.K. inorganic fertilizer.

The ash (%), NDM (%), CPDM (%), NDFDM (%), ADFDM (%), ADLMD (%) and ME (MJ/Kg) in maize grain treated with T<sub>3</sub> (6.33, 1.69, 10.53, 60.07, 31.05, 3.84 and 8.96) and T<sub>1</sub> (4.78, 1.61, 10.08, 60.07, 31.05, 3.84 and 8.96) were similar (P>0.05) but significantly higher (P<0.05) than these values in maize grain with N.P.K. inorganic fertilizer inorganic.

***In vitro gas production and fermentation characteristics of maize residues fractions treated with different fertilizer sources are shown in Table 3.***

**Table 3: Main effect of maize residues treated with fertilizer on In vitro gas characteristics**

PARAMETERS	y (mL)	t(hrs)	a (ml/200 mg DM)	a + b(ml <sup>3</sup> )	b(ml <sup>3</sup> )	c(mlh <sup>-1</sup> )
<b>Fertilizer</b>						
Control (t <sub>3</sub> )	17.40 <sup>a</sup>	14.73	4.67 <sup>a</sup>	29.067 <sup>a</sup>	24.40 <sup>a</sup>	0.05
Inorganic (t <sub>2</sub> )	11.33 <sup>b</sup>	12.80	2.67 <sup>b</sup>	22.53 <sup>b</sup>	19.87 <sup>b</sup>	0.05
Organic (t <sub>1</sub> )	9.33 <sup>b</sup>	12.80	1.87 <sup>b</sup>	22.67 <sup>b</sup>	20.80 <sup>b</sup>	0.04
<b>Maize residue fraction</b>						
Grain	36.00 <sup>a</sup>	15.33	4.20 <sup>ab</sup>	77.78 <sup>a</sup>	73.56 <sup>a</sup>	0.03
Cob	12.89 <sup>b</sup>	13.67	5.11 <sup>a</sup>	20.44 <sup>b</sup>	15.33 <sup>b</sup>	0.07
Chaff	5.56 <sup>c</sup>	12.33	1.78 <sup>b</sup>	10.22 <sup>c</sup>	8.44 <sup>c</sup>	0.06
Leaf	4.78 <sup>c</sup>	10.89	2.44 <sup>b</sup>	8.44 <sup>c</sup>	5.56 <sup>c</sup>	0.06
Stover	4.22 <sup>c</sup>	15.00	1.78 <sup>b</sup>	7.33 <sup>c</sup>	5.56 <sup>c</sup>	0.04
<b>P-VALUE</b>						
Fertilizer type	<0.01	0.4576	0.01	<0.01	0.01	0.01
Maize fraction	<0.01	0.2804	0.02	<0.01	<0.01	0.30
Fertilizer, maize fraction	0.01	0.6518	0.10	<0.01	<0.01	0.56

a,b,c: means on the same column with different super superscript are significantly different(p<0.05), a=zero time which ideally reflects the fermentation of soluble fraction, b=extent of gas production from insoluble but degradable fraction, a+b=potential extent of gas production, c=rate of gas production at time (t), Y=Volume of gas produced at time (t)

Significant influence of fertilizer was observed on all in vitro gas parameters observed except for t (hrs). The T<sub>1</sub> fertilizer had significantly higher (P<0.05) volume of gas produced at 24hrs (17.40 mL), rate of gas production (0.054), time at fermentation of soluble fraction (14.73), extent of gas production from insoluble but degradable fraction (24.40), potential extent of gas production (29.067) and zero time which ideally reflects the fermentation of soluble fraction (4.67) compared to other fertilizer sources while higher values of monitored

gas parameters were obtained for grain among the maize residue fractions, followed by cob. Maize chaff, leaf, and stover however had similar (P>0.05) but lower values compared to grain and cob.

Interactions within fertilizer types and maize fractions were very significant influenced by the gas production and the potential extent of gas production (as in Table 4). Fertilizer application had a significant influence on the total gas production volume after incubation for 24 hours and the amount of soluble and insoluble carbohydrates in the maize residue

fractions. No Fertilizer had significant influenced Cob N, CP, NDF and ADL value (2.03, 12.67, 46.16 and 17.82). Maize grain produced the highest rate of gas production

at 24 hrs (0.038 mL/h-1) while the lowest value was recorded for cob (0.072mL/h-1). The highest gas production was obtained in T<sub>1</sub> while the least in T<sub>2</sub>.

**Table 4: Interaction of fertilizer sources and maize residues on *in vitro* gas characteristics**

Residue/ fractions	Sample	Y	t	a	(a+b)	b	C
leaf	Organic (T <sub>1</sub> )	2.12 <sup>c</sup>	15.12 <sup>a</sup>	0.78 <sup>c</sup>	10.89 <sup>a</sup>	10.57 <sup>a</sup>	0.01
	Inorganic (T <sub>2</sub> )	4.24 <sup>b</sup>	6.23 <sup>c</sup>	2.56 <sup>b</sup>	6.46 <sup>b</sup>	4.49 <sup>b</sup>	0.12
	Control (T <sub>3</sub> )	8.35 <sup>a</sup>	9.34 <sup>b</sup>	6.37 <sup>a</sup>	10.97 <sup>a</sup>	4.39 <sup>b</sup>	0.08
stover	Organic (T <sub>1</sub> )	2.45 <sup>c</sup>	18.25 <sup>a</sup>	0.89 <sup>b</sup>	6.89 <sup>b</sup>	6.69 <sup>b</sup>	0.02
	Inorganic (T <sub>2</sub> )	6.13 <sup>a</sup>	18.17 <sup>a</sup>	4.78 <sup>a</sup>	8.47 <sup>a</sup>	4.67 <sup>c</sup>	0.04
	Control (T <sub>3</sub> )	4.37 <sup>b</sup>	15.26 <sup>b</sup>	0.79 <sup>b</sup>	8.52 <sup>a</sup>	8.92 <sup>a</sup>	0.05
Chaff	Organic (T <sub>1</sub> )	8.16 <sup>a</sup>	18.28 <sup>a</sup>	2.78 <sup>b</sup>	12.57 <sup>a</sup>	10.89 <sup>a</sup>	0.05
	Inorganic (T <sub>2</sub> )	8.20 <sup>a</sup>	6.67 <sup>c</sup>	4.89 <sup>a</sup>	12.63 <sup>a</sup>	8.68 <sup>b</sup>	0.12
	Control (T <sub>3</sub> )	4.78 <sup>b</sup>	15.47 <sup>b</sup>	2.68 <sup>b</sup>	6.28 <sup>b</sup>	4.55 <sup>c</sup>	0.05
grain	Organic (T <sub>1</sub> )	30.28 <sup>c</sup>	12.19 <sup>b</sup>	4.56 <sup>b</sup>	82.78 <sup>a</sup>	78.69 <sup>a</sup>	0.03
	Inorganic (T <sub>2</sub> )	32.38 <sup>b</sup>	18.59 <sup>a</sup>	4.48 <sup>b</sup>	74.46 <sup>b</sup>	70.17 <sup>b</sup>	0.03
	Control (T <sub>3</sub> )	48.89 <sup>a</sup>	18.48 <sup>a</sup>	10.29 <sup>a</sup>	80.89 <sup>a</sup>	70.20 <sup>b</sup>	0.04
cob	Organic (T <sub>1</sub> )	8.48 <sup>b</sup>	12.36 <sup>c</sup>	6.68 <sup>b</sup>	10.27 <sup>b</sup>	4.20 <sup>c</sup>	0.06
	Inorganic (T <sub>2</sub> )	8.53 <sup>b</sup>	18.78 <sup>a</sup>	4.48 <sup>c</sup>	12.09 <sup>b</sup>	8.88 <sup>b</sup>	0.04
	Control (T <sub>3</sub> )	20.89 <sup>a</sup>	15.57 <sup>b</sup>	8.89 <sup>a</sup>	44.78 <sup>a</sup>	36.66 <sup>a</sup>	0.03
	SEM	1.35	2.45	0.59	3.20	2.51	0.01

a,b,c: means on the same column with different super superscript are significantly different(p<0.05), a=zero time which ideally reflects the fermentation of soluble fraction, b=extent of gas production from insoluble but degradable fraction, a+b=potential extent of gas production, c=rate of gas production at time (t), Y=Volume of gas produced at time (t)

*In vitro* gas production and fermentation characteristics of maize residues varied significantly among the different fertilizer sources after 24 hours of fermentation. Methane production (mL / 200mg DM) of maize residues presented in Table 3 and ranged 3.19 (stover) to 11.00 (grain). ME (MJ/Kg DM), an indication of energy ranged 3.19 (stover) to 11.00 (grain). There were significant differences (P>0.05) in the

amount of ME from the different maize residues. Table 5 showed the interaction within maize residue (MR) and fertilizers. The highest gas production/volume (GV, ml/ 200mg DM) of 21.07 ml was observed for control and the least gas production with 11.27 in inorganic fertilizer. Grain had higher ME (11.00 MJ/Kg DM) because of the presence of more soluble carbohydrate compared with other residues thus higher

energy produced from rumen degradation while control fertilizer recorded the highest (5.61) and lowest of 4.12 for inorganic fertilizer (Table 5). Organic matter digestibility (OMD, %) ranged 3.67 (stover) to 63.22 (grain) while for fertilizers it ranged 27.89 (inorganic) to 37.96 (control). There were significant differences in the OMD of

the maize residues. Short chain fatty acid (SCFA) is an indication of energy made available to the ruminant host. The SCFA ( $\mu\text{mol}$ ) ranged 0.03 (stover) and 1.45 (grain) while for fertilizers it ranged between 0.21 and 0.44 for inorganic and control, respectively as seen in Tables 5.

**Table 5: Effects of maize residues and different fertilizer sources on in vitro gas volume and fermentation characteristics**

FERTILIZER	GV	SCFA	OMD	ME	METHANE
organic fertilizer (t <sub>1</sub> )	19.40 <sup>b</sup>	0.41 <sup>b</sup>	37.71 <sup>a</sup>	5.54 <sup>a</sup>	6.00 <sup>a</sup>
inorganic fertilizer (t <sub>2</sub> )	11.27 <sup>c</sup>	0.21 <sup>c</sup>	27.89 <sup>b</sup>	4.12 <sup>b</sup>	5.20 <sup>b</sup>
control (t <sub>3</sub> )	21.07 <sup>a</sup>	0.44 <sup>a</sup>	37.96 <sup>a</sup>	5.61 <sup>a</sup>	5.20 <sup>b</sup>
SEM	2.34	0.04	4.36	1.24	0.78
<b>Maize fractions</b>					
Grain	23.32 <sup>a</sup>	1.45 <sup>a</sup>	63.22 <sup>a</sup>	11.00 <sup>a</sup>	12.34
Cob	7.42 <sup>b</sup>	0.18 <sup>b</sup>	10.22 <sup>b</sup>	4.04 <sup>b</sup>	5.48
Leaf	3.60 <sup>c</sup>	0.03 <sup>c</sup>	4.00 <sup>c</sup>	3.77 <sup>b</sup>	7.28
Husk	2.81 <sup>c</sup>	0.06 <sup>c</sup>	5.11 <sup>c</sup>	3.34 <sup>c</sup>	4.37
Stover	1.97 <sup>c</sup>	0.03 <sup>c</sup>	3.67 <sup>c</sup>	3.19 <sup>c</sup>	4.28
SEM	1.96	1.36	1.37	1.54	1.20
<b>P-value</b>					
Fertilizer	<0.01	<0.01	<0.01	<0.01	<0.01
Maize fraction	<0.01	<0.01	<0.01	<0.01	<0.01
Fertilizer & maize fraction	<0.01	<0.01	<0.01	<0.01	<0.01

a,b,c: means on the same column with different superscripts are significantly different ( $p < 0.05$ ). GV-- Total gas volume (mL), Methane (mL/200g DM), OMD-- Organic matter digestibility (%) ME -- Metabolizable energy (MJ/Kg DM), SCFA-- Short chain fatty acid ( $\mu\text{mL}$ )

The in vitro gas production characteristics of maize residues: Grain, cob, husk/chaff, leaf and stover, treated with fertilizers for ruminant production are presented in Tables 3 and 4. Table 3 indicated in vitro fermentation characteristics varied significantly amongst the incubated maize

residues: grain, cob, chaff, leaf and stover. Values for 'a' which was initial gas produced (mL/200 mg DM) ranged 1.78 and 5.11 ml for chaff and cob, respectively. The parameters a, b, a+b, c, y and t varied significantly among the incubated maize residue. 'a' the initial gas produced at

incubation, 'b' the fermentation of degradable fraction 'c' the rate of gas production, 'a+b' the final gas produced at 24th hour, 'y' the peak of gas production and 't' the corresponding time (as in Table 4). Maize grain produced the highest gas volume at 24th hours (a+b = 77.78 ml/200 mg DM) while the lowest value was recorded for stover (a+b = 7.33 ml). There were no significant differences in the maize residue result for "C".

Maize grain produced the lowest rate of gas production at 24 hrs (0.038 mlh<sup>-1</sup>) while the highest value was recorded for cob (0.072mlh<sup>-1</sup>). The potential gas production from insoluble but degradable fraction (ml<sup>3</sup>) "b" varied from 5.56 (stover) to 73.56 (grain). There were significant differences in the maize residue result for (a+b). While "y" is the volume of gas produced (ml) varied from 4.22 (stover) to 36.00 (grain). There were significant differences in the maize residue result for y. The potential degradability (a+b) depicts the level at which diet could be degraded if it were in the actual rumen of the animal. This largely depends on how much of the fiber fraction (NDF and ADF) have been broken down for microbial activity. The soluble fraction (a) in the feed usually lead to increased much gas production. Insoluble degradation fraction (b) described the fermentation of the insoluble fraction and it can be used to predict feed intake. The Compost organic manure Organic recorded the least 'a' value of 1.87 ml while control had the highest of 4.67 ml. The 'b' value ranged 19.87 ml N.P.K inorganic fertilizer to 24.40 ml for control.

Table 5 shows the effect of fertilizer on maize residues as regards in vitro gas production characteristics. The grain x no fertilizer recorded the highest value of 10 ml for 'a' (soluble fraction) while leaf x

organic, stover x compost organic manure and stover x control had the least of 0.1 ml. For the insoluble fraction 'b' leaf x N.P.K. inorganic fertilizer, leaf x control, stover x N.P.K. inorganic fertilizer, Chaff x control and cob x Compost organic manure had the least value of 4 mL while the highest was seen in grain x compost organic manure (78 mL).

### **Discussions**

Using the NIRS in the effect of fertilizer sources on gas production, gave the opportunity to simulation of digestion, estimating feed quality and application in animal nutrition evaluation of various feedstuff. This technique involves *in vitro* organic matter digestibility to estimate the digestibility of organic matter constituents in feeds particularly for ruminant.

Fertilizer application, a general practice among farmers, is believed to affect the maize plant's growth, yield and residue composition. The result agreed that most of the N, P, K, nutrients from the 20-10-10 N.P.K. inorganic fertilizer applications influenced the digestibility of the grain and the other maize fractions (cob, stover, husk/chaff). The higher value of soluble fractions (a) of (4.20) grain, (5.11) cob attest to the report of Adefemi and Ayoade (2017) and Kintl *et al.*, (2023) that reported similar result with fungi treatment and fertilizer treatment respectively.

However, the control (no fertilizer) produced more gas than the fertilizer-treated maize residue could be due to the high soil fertility. Most farmers engage in good agricultural practices (GAP) thus increasing soil fertility, soil structure and reducing production cost due to fertilizer application (Sosina 2017). Furthermore, increased fertility due to fertilizer application could be responsible for production of insoluble and indigestible component of the maize residue

fraction. Also, it could be traceable to the high lignin content of the maize fractions. The result was similar to the findings of Kintl *et al.*, (2020, 2022, 2023) with fertilizer application with maize and legume silage. The fermentation of the insoluble, but degradable fraction increases with fertilizer sources which agreed with Adefemi and Ayoadé (2017) that used fungi treatment.

The result showed a positive correlation between content of CP and content of ash, which agreed with Adefemi and Ayoadé (2017) that reported same with treated maize husk maybe associated with the intricate network of fungi mycelium embedded in the treated substrate.

Also increased CP may be the result of secretion of certain extracellular enzymes in the maize husk which are proteinous in nature during their breakdown and its subsequent metabolism. Kintl *et al.*, (2023, 2022) reported the production of biogas from silages of mixed culture as compared with the pure maize silage. Also, the result agreed that CF and lignin in the maize residue fractions are poorly degradable and hence useable in fermentation process (Kintl *et al.*, 2023).

The content of ash was statistically significant between all treatments. Contrariwise, Htet *et al.*, (2017) reported that the highest content of ash (7.7%) was recorded in the maize mono-sulphate as compared with the treatment of the mixed cultivars of maize or soy. Kintl *et al.*, (2023) reported maize NDF 58.11 %, Htel *et al.*, (2017) measured 40.1% in the maize monosulphate. Kintl *et al.*, 2020 reported the values of 48.67% for silage. The contents of ADF and CF represent important indicators of the degradability of biomass which enter the process of fermentation (Samarappuli and Berti 2018). In maize, a lower amount of lignin is contained in the ear, leaf and

grain than the plant stalk, stover, cob and chaff (Titei 2022). The result agreed with Kintl *et al.*, (2023) reported high content of starch (%) 19.85 maize leaf but 20.7 in maize silage. The result showed that fertilizer application can influence CP in the maize fraction residues. The result agreed with Kintl *et al.*, (2023) reported biogas yield was positively determined with the higher content of starch in maize silage and negatively with the high content of ash, ADF and lignin.

Also, result indicated the negative action of increased contents of ADF, ash and lignin on the production of biogas were reported by Kintl *et al.* (2022), The difference in gas production observed from these maize residues were due to the different levels of soluble and insoluble fractions contained in the maize residue. The result was consistent with the findings of Sosina (2017), Getachew *et al.*, (2004), Bogoro *et al.*, (1999), who worked on maize residues, maize forages and sorghum offals. Generally, gas production was dependent on a number of factors notably the degradable carbohydrate and the nature of the carbohydrate present (Blummel and Becker, 1997). Nature and levels, presence of anti-nutrition factors has been reported to influence the amount of gas produced during fermentation (Babayemi *et al.*, 2010). High crude protein content of the maize residue has been traceable to enhance rumen microbial multiplication that influences fermentation.

However, others reported that inverse relationship between crude protein and gas production (Blummel *et al.*, 1997) which could be traceable to the high level of rumen by-pass protein. The inverse relationship was also reported between gas production and ash content of forages. This result also agreed with the findings of Akinyemi and

Ayoade (2017) that reported improved fermentation of insoluble fractions leading to higher gas production with fungi-treated maize cobs. Also, the higher gas production in cob might be due the insoluble component trapped in the insoluble fractions arising from both compost organic manure and 20-10-10 N.P.K. inorganic fertilizer applications (Sosina 2017)

The organic component in the degradable material or maize fraction could also be traceable to the original soil fertility. Akinfemi and Ayoade (2017) reported that CF fractions (NDF, ADF, ADL) decreases significantly with fungi treatment decomposing the fiber contents where fungi growth converts parts of cell wall into soluble sugars for energy. But gas rate production (c) constant obtained in all fungi treatment were not significant. The higher the gas volume the more the digestibility.

While considering the interaction of these components (maize residue fractions and fertilizer sources) on gas production, the quantity of gas produced during fermentation reflects the amount of substrate digested and the microbial metabolic pathway (Getachew *et al.*, 1998). Gas production helps to measure digestion rate of soluble and insoluble fractions of roughages (Menke and Steingass, 1988). Gas volume has strong relationship with feed intake and growth rate (Blummel and Becker, 1997).

However, others reported that inverse relationship between crude protein and gas production (Blummel *et al.*, 1997) which could be traceable to the high level of rumen by-pass protein. The inverse relationship was also reported between gas production and ash content of forages. This agreed with the findings of Menke and Steingass (1988) that reported increase in ash and NDF of feedstuff which led to low levels of gas

production. The OMD value was a good measure of the amount of feed which was accessible to the microbes in the rumen. The observed OMD and ME in the present study were low compared with that reported by Blummel *et al.* (1997). Blummel and Orskov (1993) found that “b” value could account for 88% variance in intake. Blummel *et al.* (1997) indicated that intake of feed was mostly explained by the rate of gas production, “C”, which affects the rate of feed passage through the rumen. It had also been reported that fermentation of carbohydrate fraction could be affected by kinetic of gas production. Kinetic of gas production is usually affected by a number of fractions which include: CP of the forage, anti-nutritional factors present in the forage, potency of the rumen liquor, CF level of the forage.

#### **Conclusion**

Apart from maize grains, maize residues can go a long way to support ruminant as a supplementary feed to forage. Fertilizer application though having influence on the total gas production volume after incubation for 24 hours and the amount of soluble and insoluble carbohydrates in the maize residue fractions was still short compared with no fertilizer application. Hence, maize residue fractions treated with compost organic manure and 20-10-10 N.P.K. inorganic fertilizer are of nutritional benefits to ruminants in a crop-livestock integrated production system.

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