Assessment of nutritional profile of ensiled guava leaves (*Psidium guajava* l.) as off-season feed for ruminants in the tropics

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Abstract

This research aimed to investigate the nutritional potential of ensiled guava leaves (Psidium guajava L.) as an alternative livestock feed during the off- season in the tropics- a period characterised by scarce and low- quality forage, which has resulted in poor animal performance, particularly in ruminants. Three studies were conducted. The first study involved ensiling guava leaves with different additives: groundnut cake (GLGNC), palm kernel cake (GLPKC), and without additives (GLNAD). The second study determined the chemical composition of ensiled guava leaves, while the third assessed the in vitro gas production parameters and characteristics of ensiled guava leaves over a 24- hour incubation period to predict the metabolizable energy (ME), organic matter digestibility (OMD), short- chain fatty acids (SCFA), soluble degradable fraction (a), insoluble degradable fraction, potential degradability (a + b), and rate of degradation (c). The results revealed that temperature and pH values ranged from 29. 0 to 30.0. 0 ° C and 4. 4.2 to 5. 5.0 for GLGNC and GLNAD, respectively. The physical qualities of all silages were firm, pleasant, and adequate. Significant differences were observed in the proximate composition; dry matter was highest (87. 03%) in GLNAD. Crude protein levels were highest (22. 34%) in GLGNC and lowest (18. 13%) in GLNAD. The highest (48. 32%) values for Neutral Detergent Fibre (NDF) were recorded in GLNAD, while the lowest (41.63. 63%) values were recorded for GLGNC. All macro and micro minerals examined were similar. Metabolizable Energy (ME), Organic Matter Digestibility (OMD), and Short Chain Fatty Acids (SCFA) values ranged from 3. 30 to 4. 70 MJ/Kg DM, 37. 74% to 43. 53%, and 0. 14 to 0. 0.22 mmol in GLNAD and GLGNC, respectively. The same trend was observed for a, b, a + b, and c fractions, ranging from 3. 10 to 4. 73 ml/200mg DM, 13. 00 to 18. 56 mL/200mg DM, 16. 10 to 23. 29 mL/200mg DM, and 0. 10 to 0. 21 ml/hr in GLNAD and GLGNC, respectively. Total gas produced was highest (17. 87 mL/200mg DM) in GLGNC and lowest (12. 83 mL/200mg DM) in GLNAD. The volume of methane gas produced was highest (4. 33 ml/200mg DM) in GLGNC and lowest (2. 42 mL/200mg DM) in GLNAD. It can be concluded that ensiled guava leaves with groundnut cake (GLGNC) demonstrated better potential as an alternative resource for ruminants, particularly during the dry season.

Keywords: Guava leaves, *in vitro* gas technique, ruminant, silage

Running Title: Ensiled Guava Leaves (*Psidium guajava*) as Ruminant Feed in the Tropics.

Évaluation du profil nutritionnel des feuilles de goyave ensilées (Psidium guajava L.) comme aliment de contre-saison pour les ruminants sous les tropiques

Résumé

Cette recherche visait à étudier le potentiel nutritionnel des feuilles de goyave ensilées (Psidium guajava L.) comme aliment alternatif pour le bétail pendant la contre-saison sous les tropiques, une période caractérisée par une pénurie et une qualité médiocre du fourrage, ce qui entraîne de mauvaises performances animales, en particulier chez les ruminants. Trois études ont été menées. La première portait sur l'ensilage de feuilles de goyave avec différents additifs : tourteaux d'arachide (GLGNC), tourteaux de *palmiste (GLPKC) et sans additifs (GLNAD). Français La deuxième étude a déterminé la composition chimique des feuilles de goyave ensilées, tandis que la troisième a évalué les paramètres de production de gaz in vitro et les caractéristiques des feuilles de goyave ensilées sur une période d'incubation de 24 heures pour prédire l'énergie métabolisable (EM), la digestibilité de la matière organique (OMD), les acides gras à chaîne courte (AGCC), la fraction soluble dégradable (a), la fraction insoluble dégradable, la dégradabilité potentielle (a + b) et le taux de dégradation (c). Les résultats ont révélé que les valeurs de température et de pH variaient de 29,0 à 30,0,0 °C et de 4,4,2 à 5,5,0 pour GLGNC et GLNAD, respectivement. Les qualités physiques de tous les ensilages étaient fermes, agréables et adéquates. Des différences significatives ont été observées dans la composition immédiate ; la matière sèche était la plus élevée (87,03 %) dans GLNAD. Les niveaux de protéines brutes étaient les plus élevés (22,34 %) dans GLGNC et les plus bas (18,13 %) dans GLNAD. Français Les valeurs les plus élevées (48,32 %) pour les fibres de détergent neutre (NDF) ont été enregistrées dans GLNAD, tandis que les valeurs les plus faibles (41,63,63 %) ont été enregistrées pour GLGNC. Tous les macro et micro minéraux examinés étaient similaires. Les valeurs d'énergie métabolisable (EM), de digestibilité de la matière organique (OMD) et d'acides gras à chaîne courte (AGCC) variaient de 3,30 à 4,70 MJ/kg de matière sèche, de 37,74 % à 43,53 % et de 0,14 à 0,0,22 mmol dans GLNAD et GLGNC, respectivement. Français La même tendance a été observée pour les fractions a, b, a + b et c, allant de 3,10 à 4,73 ml/200 mg DM, 13,00 à 18,56 ml/200 mg DM, 16,10 à 23,29 ml/200 mg DM et 0,10 à 0,21 ml/h dans GLNAD et GLGNC, respectivement. Le gaz total produit était le plus élevé (17,87 ml/200 mg DM) dans GLGNC et le plus bas (12,83 ml/200 mg DM) dans GLNAD. Le volume de méthane produit était le plus élevé (4,33 ml/200 mg DM) dans GLGNC et le plus bas (2,42 ml/200 mg DM) dans GLNAD. On peut conclure que les feuilles de goyavier ensilées avec tourteau d'arachide (GLGNC) présentent un meilleur potentiel comme ressource alternative pour les ruminants, notamment en saison sèche.*

Mots-clés: feuilles de goyavier, technique de gaz in vitro, ruminant, ensilage

Introduction

Ruminant animals largely depend on natural forage for their growth and production. Forage is the fundamental basis of ruminant production, and it constitutes the majority of their diets, as the cheapest and common source of nutrients for livestock (Katoch, 2023). Providing adequate animal feed, therefore, becomes an inevitable task year-round.

In southern Nigeria, the rainy season plays a crucial role in the country's agricultural sector. Grasses grow freely on fertile grounds during the wet season, which typically begins in March or April and lasts until October, followed by a dry period from November to March, usually characterised by hot temperatures, low humidity, and minimal rainfall. Grasses become increasingly dry and low in nutrients as the year advances towards the dry season. This eventually led to a reduction in animal nutrient intake, thereby hampering animal growth and productivity (Davis, 2024). Aditya *et al*. (2013) also opined that most natural forages grow freely along the road sides and on idle agricultural land in the wet season for animal grazing, but later become very scarce during the dry season. Furthermore, there are some other contributing factors to the feed shortage for livestock, such as a reduction in the grazing lands due to expansion in human population, poor soil fertility due to over-cultivation and land degradation caused by erosion. Unreliable and seasonal fluctuations in rainfall, which limit both the amount of feed gathered and the nutrient content provided by the feed, are another limiting factor (Abdullahi *et al*, 2020). Therefore, the sustainable intensification of livestock production systems, such as improving the feed quality, introducing improved forages that deliver economic ecosystem services and environmental welfare of the animals, has become a critical global research priority Mamphogoro *et al.*, 2024.

Alternative feed resources that are not used frequently, such as leaves from the guava tree (*Psidium guajava*), can be harnessed as an improved forage for farm animals.

The guava tree (*Psidium guajava*) is a unique and traditional plant with diverse medicinal and nutritive properties. (Fischer and Melgarejo, 2021). It is grown and utilised as an important fruit tree in many countries. The guava is an arboreal or shrubby plant of the evergreen type, and in some cases, deciduous (Fischer and Melgarejo, 2021). The guava tree is a versatile species, found above sea level up to an altitude of 2000m in tropical areas. It is among the most common drought-resistant plants in tropical and subtropical regions, adapted to diverse ecological conditions: warm, semi-warm, dry, semi-dry, and temperate climates (Fischer and Melgarejo, 2021). The Guava tropical fruit tree crop grows on a wide range of soils, withstands acidic soils, and is shade-tolerant. (CABI, 2013; Eco crops, 2015). The leaves of the guava tree are considered one of the most abundant and fresh leaves available all year round, even during the dry season (Akinbode *et al.*, 2021). It is cheap to get, and not exposed to competition between humans and animals (Chatterjee *et al.*, 2014). It is usually discarded as waste after falling from the guava tree and constitutes an unnecessary nuisance in places where they are being cultivated. Feeding guava leaves to farm animals has been a long-time tradition in some parts of Africa. The works of Chatterjee *et al.* (2014) revealed that it contains good nutrients, with 8-15% protein contributing to the rapid growth of farm animals.

In vitro gas production methods for the evaluation of different feeds, such as guava leaves (*Psidium guajava*), for farm animals has gained wide acceptance due to its ease of adoption, it is less animal dependent, repeatability, more appropriate for characterizing soluble or small particulate feeds, and they can be automated, thus reducing the labour, reduced funding from *in vivo* evaluation of feeds, and more accurate (Aditya, *et al* 2013; Lyu *et al.*, 2019).

Silage-making has great potential to solve the seasonal shortage of feed for ruminants in Nigeria by preserving excess forage produced during the wet season for use during the dry period (Olorunnisomo and Adesina, 2014).

This study therefore, assessed the nutritional attributes of guava leaves and their reliability as a feed resource for ruminants, especially during the critical dry season, using *in vitro* gas production technique.

Materials and Methods

Fifteen Guava (*Psidium guajava*) plants of about 3 to 4 years of age, around the environment of Lead City University, Ibadan, Oyo State, bearing the coordinates of Latitude 7.327° N and Longitude 3.880 E, were randomly marked for leave sample collection. From previously marked guava plants, 2-4kg of guava leaves were harvested from each guava tree.

Silage Making

The collected guava leaves were then chopped and ensiled with different additives and left to ferment for 42 days, in the following order, namely:

- (i) 80% Guava Leaves +20% Palm kernel Cake (PKC) =GLPKC
- (ii) 80% Guava Leaves + 20%Groundnut Cake (GNC) = GLGNC
- (iii) 100% Guava Leaves with No Additive = GLNAD

The guava leaves (*Psidium guajava*) and additives were mixed in a ratio of 4:1 (4 parts of leaves and 1 part of additives), i.e 80% of guava leaf and 20% of additives in each treatment. There were also guava leaves, 100% with no additives. The three (3) treatments were placed in separate buckets that contains cellophane bag following the above stated treatments, to mimic a silo. Each of the mixtures was packed into a large polythene bag, placed inside a 65-litre capacity plastic basin and contained 30kg of silage material. Compaction of materials was thorough by standing on the bag repeatedly to eliminate any air pockets, to ensure proper fermentation activity. Subsequently, a 20kg sandbag weight was placed on each bucket to weigh down the contents ensure anaerobic fermentation and to prevent attack from rodents. The process was replicated thrice and fermentation was for 42days under the shed.

Silage characteristics procedure

pH*:* 2-gram samples were measured into a 15 ml beaker, and 20 ml of distilled water was added. Then, it was placed on a burner and allowed to boil for 5 minutes at 100 °C. The liquid content was then decanted and allowed to cool. The pH meter was standardized using a buffer solution, and it was then dipped into the decanted liquid to determine the pH, according to Akinwande (2011)

Temperature*:* Immediately the silage was opened, a thermometer was dipped into the silage to measure the temperature of the ensiled guava leaves (Akinwande, 2011).

Aroma*:* The smell of the silage was perceived as either pleasant or unpleasant.

Texture*:* Some samples were taken by hand to feel the texture, if firm, wet, or watery.

Analysis of proximate components and fibre fractions

Samples of 3 g each were taken from about five different depths in each silo to make a total of 15gms mixed and dried in the oven, first at 65 ^{0}C for enzyme inactivation, later at 80 ^{0}C to constant weight for dry matter determination. Samples were later milled and stored in sample bottles. Later, crude protein (CP), ether extract (EE), and Ash, following the conventional methods, were also analysed. (AOAC, 2023). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the method described by Van Soest *et al*. (1995).

Mineral Analysis

Ten minerals were analysed. Ensiled guava leaves (15gms) with HNO_3 / $HClO_3$ mixtures (nitric acid and perchloric acid) (20:5 v/v). The digest was made up to 100 mL in a standard volumetric flask with deionized water. Ca, Na, K, Fe, Cu, Mn, Zn, Mg, and Pb in the digest were determined with the atomic absorption spectrophotometer model 420. (Gallenkemp and Co. Ltd). Phosphorus in the digest was estimated with vanadomolybdate solution. The colour so developed was read with a spectrophotometer at 420 m/u.

In vitro gas fermentation procedure

The silage was left to ferment for 42 days, and it was then opened and taken to the laboratory for *in vitro* gas production analysis. Rumen fluid was obtained from three West African Dwarf female goats through the suction tubes before the morning feed. The animals were fed concentrate consisting of 40 % corn bran, 35% wheat offal, 20% palm kernel cake, 4% oyster shell, 0.5% salt, and 0.5% growers' premix for three (3) days before collecting rumen liquor. Incubation was as reported by (Menke and Steingass, 1988) using 120ml calibrated syringes in three batch incubation at 39^{0}C while 30ml inoculums was introduced into 200mg samples in the syringes containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$ + Na_2HPO_4 + KCL + NaCl + $MgSO_4$ $7H_20$ + $CaCL_2$ $2H_2O$) (1:2, v/v), under continuous flushing with CO_2.

Gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hours of incubation, after which 4 ml of NaOH (10M) was introduced to estimate the volume of methane produced. The average of the volumes of gas produced from the blanks was deduced from the volume of gas produced per sample.

The volume of gas produced at intervals was plotted against the incubation time, and from the graph, the gas production characteristics were estimated using the equation

Y=a+b (1-e-ct) described by Orskov and McDonald (1979). Were

- Y = volume of gas produced at time ‘t’
- a = intercept (gas produced from insoluble fraction)
- c = gas production rate constant for the insoluble fraction (b)
- t = incubation time
- Metabolisable energy and (ME, MJ/Kg DM) and organic matter digestibility (OMD%) were estimated as established by (Menke and Steingass, 1988), and short chain fatty acids (SCFA, umoL) were calculated as reported by (Getachew *et al.*, 1999)
- ME = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF
- OMD = 14.88 + 0.889GV + 0.45CP + 0.651XA
- SCFA = 0.0239*GV – 0.0601

Where GV, CP, CF, and XA are net gas productions (ml/200mg DM), crude protein, crude fibre, and ash of the incubated samples, respectively.

Statistical Analysis

Data obtained were subjected to analysis of variance procedure (ANOVA) of SAS (2021). Significant treatment means were separated by Duncan’s multiple range test of the same package.

Results and Discussion

Table 1 shows the quality characteristics of ensiled guava leaves.

The pH of ensiled guava (*Psidium guajava*) leaves was 4.2 in GLGNC and 5.0 in GLNAD. All the results obtained fell within the range of 3.5-5.5, which is considered good for silages in the tropics (Obua, 2018). Although the pH obtained from all three silages was within the safe pH, it is worth noting that the lower the acidity of a silage, the lower the activity of plant enzymes, which inhibits the growth of unwanted anaerobic bacteria (Moore *et al.*, 2020). Therefore, ensiled guava leaves with additives are more stable for animal use than ensiled guava leaves without additives. The temperature of fermenting forage, varying from 29-30 ^{0}C, is presumed to produce excellent silage because most lactobacillus species are proven to be active at this temperature range (Yamamoto *et al.*, 2011; Muck, 2012). Poor silages due to excessive heat sometimes contain mycotoxins that have been implicated as causes of abortions, reduced intake, poor reproduction, and low milk production (Ma *et al.*, 2017). There is no “rule of thumb” for describing the degree of spoilage required to cause decreases in animal performance (Moore *et al.*, 2020). The brownish-green colour observed in GLGNC and GLPKC may be due to the color of the additives, the green coloration observed still has a close semblance to that of the original guava leaves, which implies that silages were well preserved (Falola *et al*, 2013). The same colour variations were reported for moringa leaves ensiled with different levels of cassava peel (Olorunnisomo and Adesina, 2014). The aroma and texture of all ensiled guava leaves were found to conform to those of a good silage (Falola *et al.*, 2013; Olorunnisomo and Adesina, 2014). All silages were equally firm. Ensiling a firmer crop helps suppress the growth of Clostridium and other undesired bacteria, while promoting the production of Lactic acid bacteria. (Moore *et al.*, 2020).

Table 1: Quality characteristics of ensiled guava leaves

Parameters	GLGNC	GLPKC	GLNAD
TEMP ^{0}C	29.0	29.0	30.0
pH	4.2	4.6	5.0
Aroma	Pleasant	Pleasant	Pleasant
Texture	Firm	Firm	Firm
Colour	Brownish green	Brownish green	Dark green

*GLGNC- guava leaf ensiled with groundnut cake; GLPKC- guava leaf ensiled with palm kernel; GLNAD guava leaf ensiled without additives

The chemical composition of ensiled guava (Psidium guajava) leaves is presented in Table 2

All parameters measured differed significantly among treatment means (p<0.05). The highest (87.03 %) dry matter was recorded for GLNAD, while the lowest (85.00 %) was recorded for GLGNC. Moisture content and the buffering capacity of the original materials directly correlate with the outcome of the silage produced. This finding aligns with the results of Falola *et al.* (2013), who reported dry matter loss in silage. This result is similar to the findings of Ogunbosoye and Babayemi (2010), in their study on the potential values of some non-leguminous browse plants as dry-season feed for ruminants in Nigeria. The crude protein content of ensiled Guava leaves was highest (22.34%) in GLNAD and the lowest (18.13%) value was recorded in GLGNC. This is expected because outcomes from the findings of various researchers have reported that ensiling with legumes has a good impact on the final crude protein and other proximate components of ensiled materials (Priabudiman and Sukaryana, 2022). This illustrates why GLGNC and GLPKC had better preferences in crude protein values than GLNAD values. Conversely, the range of values reported here for CP is lower than 21.10 - 21.21% reported by Priabudiman and Sukaryana (2022) in cassava leaves silage fermented with rice bran and palm kernel cake. The highest (37.10%) crude fibre was recorded for GLNAD, while the lowest (32.20%) value was recorded for GLGNC. This result is similar to the findings of Falola *et al.* (2013). These variations can be attributed to the effects of these additives on the ensiling process. The ether extract content was highest (1.00%) in GLNAD, while GLGNC recorded the lowest (0.05%) value. The same trend was observed for ash content, with GLNAD recording the highest (4.03%) and GLGNC recording the lowest (3.90%) value. It was also observed that all parameters for fibre fractions differed significantly (p<0.05). Neutral detergent fibre (NDF) (41.63 and 48.32%) acid detergent fibre (ADF) 26.74 and 32.63%, and acid detergent lignin (ADL)12.30 and 13.00% for GLNAD and GLGNC, respectively. Values of NDF and ADF reported here are lower than 46.48 - 57.94% and 33.63 – 45.67%, respectively, obtained elsewhere for Spondia mombin leaves reported by Ikusika *et al.* (2023). Farmers Entrance (2025) stated that fibre is essential in livestock feed as it provides roughage, which assists digestion, while the NDF of the diet controls the voluntary feed intake of animals. Although a high content of ADF and NDF is beneficial in feed for ruminants, excessively high ADF content is negatively related to digestibility (Ismartoyo *et al.*, 2022). It has been established that both NDF and ADF have an inverse relationship with voluntary feed intake (Ismartoyo *et al.*, 2022). Therefore, the values of NDF, ADF, and ADL obtained in this study could imply that *Psidium guajava* leaves are suitable for ruminants, especially ensiled GLGNC and GLPKC, which recorded lower values than GLNAD in all the parameters. It is also essential to note that the overall objective of silage making is not to alter the chemical composition of that silage, but rather to make forage available for the animal at all times (Falola *et al.*, 2013)

Table 2: Chemical composition (%) of ensiled Guava leaves

Parameters	GLGNC	GLPKC	GLNAD	SEM
Dry matter	85.00^{c}	85.61^{b}	87.03^{a}	0.50
Crude protein	22.34^{a}	20.01^{b}	18.13^{c}	0.20
Crude fibre	32.20^{c}	34.00^{b}	37.10^{a}	0.20
Ether extract	0.50^{c}	0.70^{b}	1.00^{a}	0.01
Ash	3.90^{b}	3.93^{bc}	4.03^{a}	0.01
Neutral detergent fibre	41.63^{c}	44.35^{b}	48.32^{a}	1.50
Acid detergent fibre	26.74^{c}	29.28^{b}	32.63^{a}	1.15
Acid detergent lignin	12.30^{c}	12.78^{b}	13.00^{a}	0.20

a,b, c means on the same column with different superscript differed significantly ($p<0.05$). GLGNC guava leaf ensiled with groundnut cake; GLPKC= guava leaf ensiled with palm kernel; GLNAD= guava leaf ensiled without additive

Macro and Micro (%) mineral content of ensiled guava (Psidium guajava) leaves

The results of **macro and micro minerals** of guava leaves ensiled with different additives are presented in Table 3. It was observed that no significant differences were observed for both macro and micro minerals; however, guava leaves ensiled with GNC additives recorded the highest values for both macro and micro minerals, followed by guava leaves ensiled with PKC, and the lowest values were recorded for guava leaves ensiled with no additives. However, the values obtained here are within the recommended safe limit that will enhance the optimum functioning of an animal body system (NRC 2021). Fermentation has been reported to improve micronutrient availability (Motarjemi 2002). The high values obtained for GLGNC and GLPKC in this experiment align with the findings of Gürsoy *et al*. (2024), who reported that legume additives in silages have the potential for improving the final silage characteristics. Therefore, GLGNC and GLPKC show more potential as good feed for ruminants in the dry season when grasses are scarce for proper growth and supplementation.

Table 3: Macro and micro mineral content of ensiled Guava leaves

	Micro (%)					Micro (Mg/kg)				
Parameters	Ca	P	Na	K	Mg	Fe	Mn	Zn	Cu	Pb
GLGNC	0.90	0.11	0.92	0.06	0.31	189.1	29.61	13.70	59.00	0.002
GLPKC	0.73	0.05	0.60	0.01	0.20	170.0	29.10	13.20	57.32	0.001
GLNAD	0.79	0.08	0.80	0.03	0.25	180.6	29.45	13.42	57.90	0.001
SEM	0.52	0.20	0.55	0.22	0.50	9.3	2.5	1.00	3.55	0.10

*GLGNC guava leaf ensiled with groundnut cake; GLPKC guava leaf ensiled with palm kernel; GLNAD guava leaf ensiled without additives

Table 4 shows the *in vitro* gas production parameters of ensiled guava leaves estimated from gas production. Significant ($p<0.05$) differences were observed among the treatment means for metabolizable energy ME (3.30 – 4.70 MJ/Kg DM) organic matter digestibility OMD (37.43 – 43.43%) and short-chain fatty acid SCFA (0.14 – 0.22 mmol) in GLNAD and GLGNC. This result is comparable and in agreement with the value range of 3.64 – 5.50 MJ/Kg DM, 39.83 – 51.27%, and 0.17 – 0.50 mmol reported for *Anacardium occidentale* leaf (Mako *et al.*, 2020). Chemical composition in combination with *in vitro* digestibility and ME content can be considered useful indicators for preliminary evaluation of the potential nutritive value of forages (Kafilzadeh and Heidary, 2013)

Table 4: *In vitro* gas production parameters of ensiled Guava leaves

Parameters	ME (MJ/Kg DM)	OMD (%)	SCFAMmol
GLGNC	4.70[a]	43.53[a]	0.22[a]
GLPKC	4.10[b]	40.00[b]	0.18[b]
GLNAD	3.30[c]	37.74[c]	0.14[c]
SEM	0.20	0.50	0.01

a, b, c means on the same column with different superscript differed significantly ($p<0.05$). GLGNC guava leaf ensiled with groundnut cake; GLPKC guava leaf ensiled with palm kernel; GLNAD guava leaf ensiled without additive

In vitro gas production characteristics of ensiled guava leaves (Psidium guajava)

Table 5 shows the *in vitro* production characteristics of ensiled *Psidium guajava* leaves. It was observed that all parameters measured differed significantly ($p<0.05$). The value of soluble degradable fraction 'a' of *Psidium guajava* leaves ensiled with groundnut cake (GLGNC) recorded the highest (4.73mL/200mg of DM) value, while samples ensiled with no additive recorded the lowest (3.10mL/200 mg DM) value. These values were higher than 2.5-4.0mL/200mg DM, reported by Mako *et al.* (2018) for *Psidium guajava* leaves. The soluble degradable fraction a' indicates the amount of gas produced from soluble degradable fraction of the samples. This is the fraction that rumen microbes first ferment to obtain energy for their body metabolism. The highest (18.56mL) insoluble degradable fraction 'b' of the ensiled guava leaves was obtained in GLGNC, while GLNAD recorded the lowest (13.00mL) value. Mako *et al.* (2020) study on the nutritional potential of Anacardium occidentale (Cashew) leaf for ruminants in the humid tropics supports this report. The 'b' fraction is the fraction that is fermentable at a slower rate compared to the soluble fraction. It is the fraction that the rumen microbe ferments after the rapidly fermented fraction has been depleted. The lower the values obtained for insoluble degradable fraction 'b', the higher the fibrous nature of the incubated samples**.** (Mako, 2009). The 'a+b' and 'c' followed the same trend as 'a' and 'b'. It was observed that the highest (23.29 mL) value of 'a+b' and 'c' (0.21mL/hr) was obtained in GLGNC, while GLNAD recorded the lowest (16.10 and 0.10mL/hr) values, respectively. These values agree with the range of 13.26 - 23.34 ml/200mg and 0.08 – 1.19 mL/hr values for a+b and c reported by Mako *et al.* (2020). However, the results revealed that the additives enhanced the rate of degradation. The relatively low fibre content in the silage can facilitate the colonization of the feed by the microbial rumen population, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1995).

Table 5: *In vitro* gas production characteristics of ensiled guava leaves

Parameters	a (mL)	b (mL)	a+b (mL)	c (mL/h)
GLGNC	4.73[a]	18.56[a]	23.29[a]	0.21[a]
GLPKC	4.00[b]	16.10[b]	20.10[b]	0.15[b]
GLNAD	3.10[c]	13.00[c]	16.10[c]	0.10[c]
SEM	0.20	0.50	1.50	0.01

a, b, c means on the same column with different superscript differed significantly ($p<0.05$). GLGNC guava leaf ensiled with groundnut cake; GLPKC guava leaf ensiled with palm kernel; GLNAD guava leaf ensiled without additive

Figure 1 shows the gas production parameters of ensiled *Pisidium guajava* leaves. The total gas produced ranged from 2.00- 4.63mL within the first 3hrs of incubation, and after 24hrs, the total gas produced ranged from 12.83- 17.87mL in GLNAD and GLGNC, respectively. It was observed that the net volume of gas produced increased progressively as the incubation time progressed. Gas production indicates the extent of degradability and fermentability of feeds and microbial synthesis (Mako *et al.*, 2018). Rajan *et al.* (2018) reported that the kinetics of gas production could be affected by carbohydrate fraction. The highest gas production was obtained in GLGNC, followed by GLPKC could be related to the low fibre fraction content. This corroborates the findings of Mako et *al.* (2020), who reported that gas production was negatively related to neutral detergent fibre (NDF) content and positively correlated to crude protein content. Mako *et al.* (2020) also reported that, though gas production is a nutritionally wasteful product, it provides an important basis for predicting ME, OMD, and SCFA.

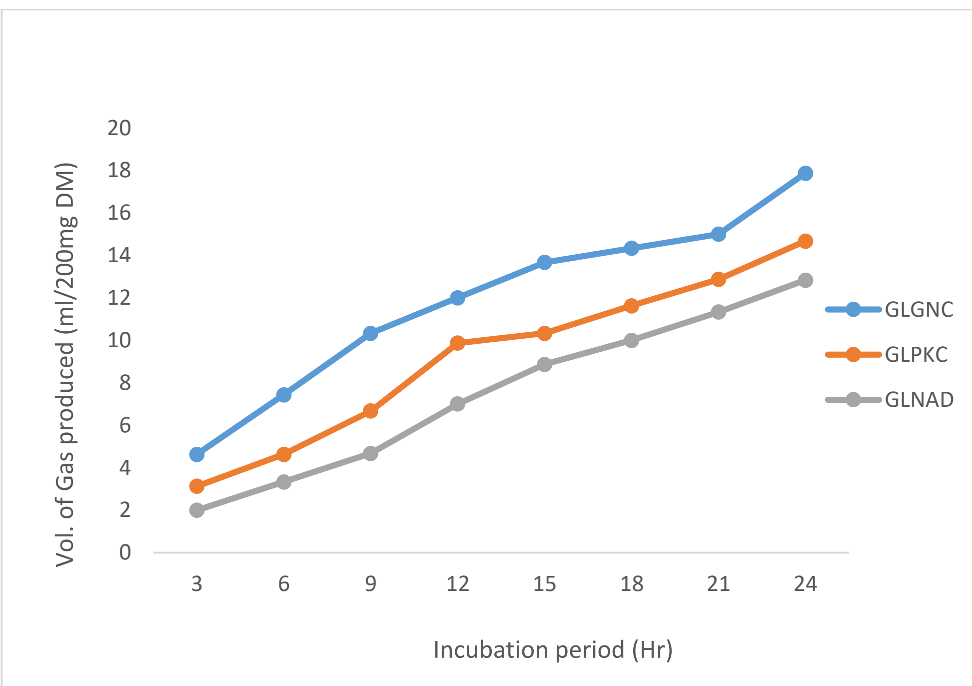

Figure 1: Figure 1: Gas production (mL/200mg DM) of ensiled guava leaves

Methane gas production of ensiled guava leaves

Figure 2 indicates the methane gas production from ensiled *Pisidium guajava* leaves. Ensiled GNGNC produced more methane gas than GLPKC and GLNAD. This is anticipated, since it is a reality that feedstuffs with high gas production also have high methane production (Mako, 2009)

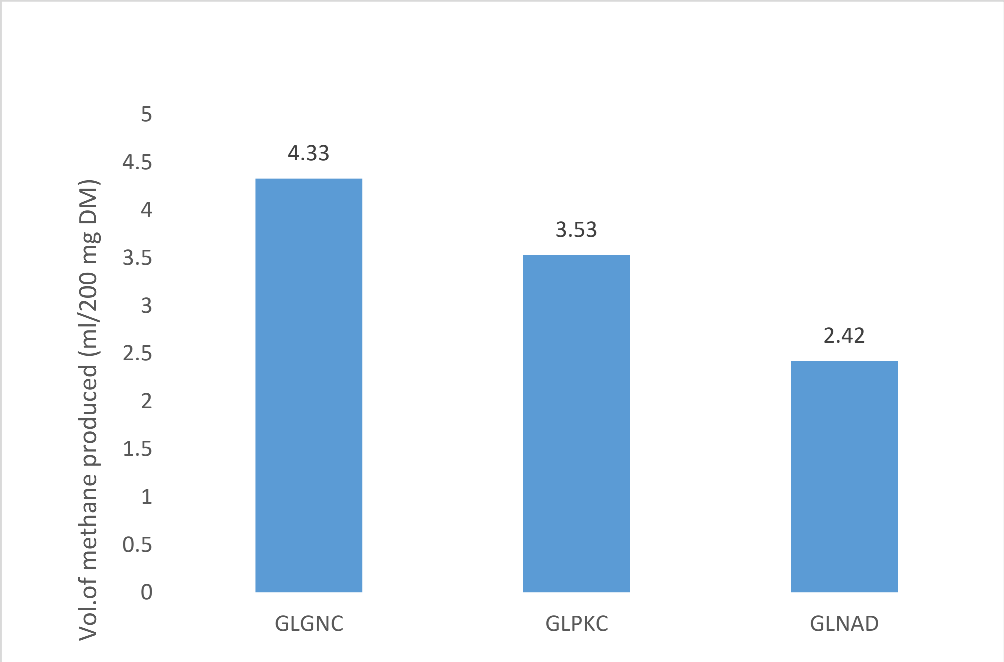

Figure 2: Methane gas production (mL/200mg DM) of fermented guava leaves

Conclusion

Based on the findings from this study, it can therefore be concluded that the high nutritive content of all ensiled *Psidium guajava* leaves silage is an indication that the additives (groundnut cake and palm kernel cakes) could be good feed resources for improved ruminant production. However, it is recommended that *Psidium guajava* leaves silage serve as a potential feed supplement for ruminants in the tropical areas during the off-season. It can be fed directly or incorporated into ruminant feed because of its nutritional value and availability during the dry season.

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