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In vitro fermentation of shed leaves of selected trees as forage for ruminants

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Abstract

Shed leaves from deciduous plants are wastes which sometimes constitute environmental pollution if not properly disposed of. The chemical composition, *in vitro* gas production parameters and characteristics of shed leaves from browse plants; *Tamarindus indica* (Ti), *Terminalia catappa* (Tc), *Bambusa vulgaris* (Bv), *Anacardium occidentale* (Ao) and *Cola nitida* (Cn) were investigated. Shed leaves of each plant were picked, cleaned and pulverized. Chemical composition and *in vitro* gas production analyses were carried out. Results revealed that chemical composition of shed leaves differed significantly ($p<0.05$). Dry matter ranged from 91.82 to 93.10%. Crude protein content was highest in Bv (10.90%) and lowest in Tc (4.89%), ash content was highest in Tc (16.13%) and lowest in Bv (15.83%). Neutral detergent fibre was highest in Tc (61.53%) and lowest in Ti (52.92%). Calcium content ranged from 0.229 to 0.297 g/100g DM across the treatments. *In vitro* gas production, metabolizable energy, organic matter digestibility, short-chain fatty acids and methane gas of shed leaves 24 hours post-incubation ranged from 6.67 to 10.67 mL/200mg DM, 3.46 to 4.34 MJ/kg DM, 24.06 to 30.30%, 0.02 to 0.19 mmol and 2.5 to 4.5 mL/200mg DM, respectively. Bv leaves recorded the highest values (10.00 and 10.67 mL/200mg DM) for the insoluble degradable fraction (b) and potential degradability (a+b) respectively. The lowest (6.33 and 6.67 mL/200mg DM) values for 'b' and 'a+b' fractions were obtained in *Tc* leaves respectively, while the rate of degradation 'c' was not significantly different across all leaves. Conclusively, shed leaves can be used as dry season forage for ruminants.

Introduction

The ruminant production systems are challenged by an all-year-round feed supply. The lush green forages that serve as major nutrient sources to ruminants during the wet season are always scarce and become lignified during the dry season thereby making the dry matter and nutrient composition drop below the nutrient requirements of these animals (Akinwande *et al.*, 2019). The available grains and feed crops are always in high demand resulting in great competition between man, livestock and industries. However, several unconventional feed resources are abundant and sometimes constitute environmental pollutants in modern society (Olaoye *et al.*, 2015). Shed leaves from trees are abundant in the dry season when feed supply to livestock production is lower in quantity. *Tamarindus indica* (tamarind), *Terminalia catappa* (almond), *Bambusa vulgaris* (bamboo), *Anacardium Occidentale* (cashew) and *Cola nitida* (kolanut) are important trees that grow well in many parts of Nigeria. The leaves of tree fodders are considered nutritious feed due to their high proteins, vitamins and minerals (Azim *et al.*, 2011).

In vitro gas production technique has proven to be the most reliable, simple and efficient way of evaluating fodder trees and shrubs for their potential in the animal industry (Theodorou *et al.*, 1994). This study aimed to assess the nutritive value of shed leaves of some predominant plants as forage for ruminant production using the *in vitro* gas production technique.

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Materials and Methods

Sample collection

Trees of *Tamarindus indica, Terminalia catappa, Bambusa vulgaris, Anacardium occidentale* and *Cola nitida* within the environment of Tai Solarin University of Education, Ijebu-Ode, were marked randomly for Shed leaves collection. The location is 7º21'N and 3º 45'S at an altitude of between 200 and 300 m above sea level. The mean temperature is 25-29ºC with an average rainfall of about 1250mm. Approximately 2 to 3kg of shed leaves from each tree were picked at the base of the tree roots. They were cleaned by dusting with a clean soft cloth, and air-dried for 3 days at room temperature of 25ºC until constant weight is attained. The dried leaves were pulverized (ground to fine powder) to 2mm through the use of a hammer mill. Two (2) kg of each sample was packaged in an air-tight container and taken to the laboratory for chemical analysis and *in vitro* gas fermentation assessment.

In vitro gas fermentation Procedure

Rumen fluid was obtained from three West African Dwarf female sheep through sunction tube 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% grower premix for three days prior to the collection of rumen liquor. Incubation was as described by Menke and Steingass (1988) using 120 ml calibrated syringes in three batch incubation at 39^{0}C. 30 ml inoculums was introduced into 200 mg samples in the syringes containing cheese cloth strained rumen liquor and buffer ($NaHC0_3$ + Na_2HP0_4 + KCl + NaCl + $MgS0_4$. $7H_20$ + $CaCl_2$. $2H_20$) (1:2, v/v) under continuous flushing with $C0_2$. Gas production was measured at 3 hourly for 24 hours, after 24h of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced. The average of the volume of gas produced from the blanks was deducted 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) where: 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, metabolizable energy (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) was calculated as described 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 /200 mg DM), crude protein, crude fibre and ash of the incubated samples respectively.

Analyses of the chemical and mineral compositions

Dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash contents of samples of shed leaves were determined as described by AOAC (2012). The fibre fractions comprising Neutral detergent fibre (NDF) Acid detergent fibre (ADF) and Acid detergent lignin (ADL) were determined as described by Van Soest *et al*. (1994).

A total of eight minerals were analyzed. The leaves were first digested with nitric acid/perchloric acid mixtures (ratio= 4/1, v/v). Each digest was made up to 100 mL in a standard volumetric flask with deionised water. The calcium (Ca), potassium (K), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and magnesium (Mg) in the digest were determined with an atomic absorption spectrophotometer (Model: 420, Gallenkamp and Co. Ltd). Phosphorous (P) was determined colorimetrically using the phosphovanado-molybdate method of AOAC (2012). The colour, so developed was read in an atomic absorption spectrophotometer at 420 nm.

Statistical analysis

Data obtained were subjected to the analysis of variance (ANOVA) procedure of SAS (2012). Treatment means were separated using Duncan's Multiple Range Tests and the significance was determined at P<0.05.

Results and Discussion

Table 1 shows the chemical composition of shed leaves from the selected trees (*Tamarindus indica, Terminalia catappa, Bambusa vulgaris, Anacardium occidentale* and *Cola nitida*). The dry matter (DM) ranged from 91.82 to 93.10% across the treatments.

Table 1: Chemical composition of shed leaves from selected trees

Parameter	Shed leaves (%)					SEM
	Tamarindus indica	*Anacardium occidentale*	*Terminalia catappa*	*Bambusa vulgaris*	*Cola nitida*	
DM	92.66[b]	92.17[d]	91.82[e]	93.10[a]	92.37[c]	0.03
CP	9.40[b]	6.10[d]	4.89[e]	10.90[a]	6.68[c]	0.04
CF	20.81[e]	21.00[d]	25.15[a]	24.68[b]	21.37[c]	0.01
EE	1.34[c]	1.29[d]	1.47[a]	1.27[d]	1.42[b]	0.01
Ash	4.18[e]	5.23[d]	16.13[a]	15.83[b]	9.34[c]	0.01
NFE	64.28[b]	66.37[a]	52.37[d]	47.32[e]	61.20	0.05
NDF	52.92[e]	54.12[d]	61.53[a]	60.12[b]	59.63[c]	0.03
ADF	33.13[e]	37.13[d]	48.95[a]	47.47[b]	40.12[c]	0.01
ADL	16.48[e]	18.08[d]	24.12[a]	22.79[b]	20.47[c]	0.01

Means on the same row with different superscripts letters are significantly different (p<0.05). DM= Dry matter, CP = Crude protein, EE = Ether extract, NFE = Nitrogen free extract, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ADL = Acid detergent lignin, SEM = standard error of mean.

The dry matter obtained in this study agreeswith the findings of Abiola-Olagunju *et al*. (2017), who reported a value of 90.42% DM in *Terminalia catappa* shed leaf. Crude protein content in shed leaves was highest (10.90%) in *Bambusa vulgaris* and lowest (4.89%) for *Terminalia catappa*. These values are lower than the 20.19% CP reported for *Terminalia catappa* shed leaf (Abiola-Olagunju *et al*., 2017). However, the CP content obtained in *Bambusa vulgaris* and *Tamarindus indica* leaves, were above the 7% CP needed to meet the maintenance requirement of ruminants (Norton, 2003). Meanwhile, the CP in shed leaves of *Terminalia catappa*, *Anacardium occidentale* and *Cola nitida* were below the recommended 7% CP. Hence, feeding animals with these leaves will require protein supplementation which can improve protein status of the animals, soyabean meal or cottonseed meal are examples of protein supplements. The high ash contents for *Bambusa vulgaris* and *Terminalia catappa* leaves (15.83 and 16.13 %) respectively, are an indication of the mineral content being supplied adequately to the animals.

The NDF, ADF and ADL contents were highest in *Terminalia catappa* (61.53, 48.95 and 24.12%) and lowest in *Tamarindus indica* (52.92, 33.13 and 16.48%). The NDF obtained for *Anacardium occidentale* in this study was higher than the 51.41% reported by Mako *et al.* (2020) in the shed leaf of *Anacardium occidentale*, but agrees with the value of 57.94% reported for *Spondia mombin* shed leaf elsewhere (Ikusika *et al.*, 2023). Neutral detergent fibre measures most of the structural components in plant cells (i.e. lignin, hemicellulose, cellulose). When a forage is high in NDF content, it negatively correlates with feed intake (Shi *et al.,* 2023). Observed NDF values in all the shed leaves are within the range of 55-60% NDF content which could enhance feed intake and optimal performance of animals (Akinwande *et al*., 2019)

Mineral composition of shed leaf

Minerals play a key role in physiological, structural and regulatory functions within the animal body. The macro and micro mineral contents of shed leaves (Table 2) are significantly different (p<0.05). Calcium ranged from 0.229 to 0.297g/100g DM in *Tamarindus indica* (tamarind) and *Cola nitida (*kolanut leaf), respectively. The values obtained here for macro and micro minerals investigated are in agreement with the values reported for *Spondias mombin* leaves (Ikusika *et al*., 2023). The micro minerals analyzed all follow the same pattern as macro minerals with *Cola nitida* (kolanut) leaf recording the highest values for all minerals analyzed, while *Tamarindus indica* (tamarind) recorded the lowest values. The values obtained for macro and micro mineral content of shed leaves in this study are within the recommended level that meets the requirements of sheep and goats for proper functioning of the body system (NRC 2002).

Table 2: Mineral composition of shed leaves from selected trees

Parameter	Shed leaves					SEM
	Tamarindus indica	*Anacardium occidentale*	*Terminalia catappa*	*Bambusa vulgaris*	*Cola nitida*	
Macro-minerals (g/100g DM)						
Calcium	0.229^{e}	0.236^{d}	0.291^{b}	0.248^{c}	0.297^{a}	0.001
Phosphorus	0.333^{d}	0.345^{c}	0.348^{c}	0.355^{b}	0.385^{a}	0.001
Potassium	0.830^{d}	0.831^{d}	0.846^{c}	0.859^{b}	0.904^{a}	0.001
Magnesium	0.242^{d}	0.250^{c}	0.252^{c}	0.259^{b}	0.305^{a}	0.001
Micro-minerals (mg/kg)						
Manganese	38.85^{d}	41.55^{c}	48.00^{e}	43.05^{b}	53.50^{a}	0.099
Zinc	56.00^{d}	56.60^{c}	49.50^{e}	57.50^{b}	63.00^{a}	0.075
Iron	118.9^{e}	121.6^{d}	122.9^{c}	123.6^{b}	133.8^{a}	0.095
Copper	14.05^{d}	14.25^{d}	16.40^{b}	15.00^{c}	18.70^{a}	0.061

Means on the same row with different superscripts letters are significantly different (p<0.05). SEM = standard error of mean

Table 3 shows the *in vitro* fermentation characteristics of shed leaves at 3 hourly for 24 hours incubation period. Fraction ‘a’ indicates the amount of gas produced from the soluble fraction of the feed samples. This is the fraction that the rumen microbe ferment first to obtain energy. There was no significant (p<0.05) difference in the volume of gas produced from the soluble fraction across the treatments. The highest value (1.00 ml/200mg DM) was recorded for *Tamarindus indica* (tamarind) leaf, while the lowest (0.33 ml/200mg DM) was obtained for *Terminalia catappa* (almond) leaf. This result is lower than the range of values (1.67–3.67 ml/200mg DM) reported by Bamigboye and Oluwarinde (2017) for forages used as feed resources for ruminants, likewise for the value (2.50 ml/200mg DM) in shed leaf of *Persea americana* (Mako *et al*., 2018). The ‘b’ fraction indicates the fraction that is insoluble but degradable.The ‘b’ fraction varied significantly (p<0.05) among the treatment means, with the highest value (10.00 ml/200mg DM) being recorded for *Bambusa vulgaris* leaf, while the lowest value (6.33 ml/200mg DM) was obtained in *Terminalia catappa* leaf. These values are higher than those reported (7.00 ml and 6.94 ml) for *Anacardium occidentale* shed leaf (Mako *et al*., 2020) and *Persea americana* shed leaf (Mako *et al*., 2018) respectively. The ‘a+b’ fraction indicates the potential degradability of the shed leaves investigated. It followed the same trend with the ‘b’ fraction with the highest value (10.67 ml/200mg DM) being recorded for *Bambusa vulgaris* leaf while the lowest value (6.67 ml/200mgDm) was obtained in *Terminalia catappa* leaf. These values are comparable and in agreement with values recorded for *Persea americana* leaf (Mako *et al*., 2018) but lower to the values of 9.00 – 22.67 ml reported by Bamigboye and Oluwarinde (2017) for forages in rangeland. The rate of degradation ‘c’ for shed leaves was not significantly different and ranged from 0.03 - 0.08 ml/hr in *Anacardium occidentale* and *Cola nitida* leaf respectively. These values are lower than values reported for *Persea americana* leaves (Mako *et al*., 2018), but similar to values reported by (Bamigboye and Oluwarinde, 2017).

Presented on Table 4 are the *in vitro* fermentation parameters of shed leaves. Metabolizable energy (ME) of shed leaves ranged from 3.46 to 4.34 MJ/kg DM, these values are lower than the range of values (10.9 to 13.6 MJ/kg DM) obtained for dominant weed species in north central Nigeria (Akinfemi and Mako, 2012). The obtained ME in this study is also lower than the values (5.02 - 6.58 MJ/kg DM) reported in forages from rangeland (Bamigboye and Oluwarinde, 2017). The organic matter digestibility (OMD) and short chain fatty acid (SCFA) ranged from 24.06 to 30.30% and

Table 3: *In vitro* gas fermentation characteristics of shed leaves

Shed leaves	a	b	a+b	c
Tamarindus indica	1.00	8.00[b]	9.00[b]	0.06
Anacardium occidentale	0.67	6.67[d]	7.33[cd]	0.08
Terminalia catappa	0.33	6.33[e]	6.67[d]	0.05
Bambusa vulgaris	0.67	10.00a	10.67[a]	0.07
Cola nitida	1.00	7.33[c]	8.33[bc]	0.03
SEM	0.21	0.33	0.27	1.03

a,b,c,d = Means on the same column with different superscripts are significantly different ($p<0.05$), 'a'= soluble fraction, 'b'=insoluble fraction, 'a+b'=potential degradability, 'c'=rate of degradation, SEM = standard error of means

Table 4: *In vitro* gas fermentation parameters of shed leaves

Shed leaves	ME	OMD	SCFA	CH_4
Tamarindus indica	4.02[b]	27.38[b]	0.02[b]	4.0[b]
Anacardium occidentale	3.61[cd]	24.49[d]	0.12[cd]	3.5[c]
Terminalia catappa	3.46[d]	24.06[d]	0.10[d]	2.5[e]
Bambusa vulgaris	4.34[a]	30.30[a]	0.19[a]	4.5[a]
Cola nitida	3.78[c]	25.90[c]	0.14[bc]	3.0[d]
SEM	0.04	0.24	0.01	0.01

a,b,c.d = means on the same column with different superscript are significantly ($p<0.05$) different. ME=metabolizable energy, OMD=organic matter digestibility, SCFA=short chain fatty acid, CH_4=methane SEM = standard error of means

0.02 to 0.19 mmol, respectively, being higher in shed leaves of *Bambusa vulgaris* and lower in *Terminalia catappa*. These values are lower than values obtained by Akinfemi and Mako (2012) for dominant weeds, but compared with the values (38.78 % and 0.18 mmol) reported for *Spondias mombin* leaf (Ikusika *et al*., 2023). These parameters (ME, OMD and SCFA) are also sources of energy for animals. It indicates that these leaves will supply energy to animals consuming them. Methane production indicates energy loss to the ruminants, and many tropical feedstuffs have been implicated in increased methanogenesis (Babayemi and Bamikole, 2006) as an integrated part of carbohydrate metabolism (Demeyer and Van Nevel 1975). Significant differences were observed in methane production among the shed leaves. Methane produced ranged from 2.5 to 4.5 ml/200mg DM, being highest in shed leaves of *Bambusa vulgaris* and lowest for *Terminalia catappa*. Feedstuffs that show high capacity for gas production are synonymous with high methane gas production (Mako *et al.*, 2013). This could be attributed to the high methane production obtained for *Bambusa vulgaris* and *Tamarindus indica* leaves.

In vitro gas production of shed leaves incubated for 24 hours is shown in Figure 1. The rate and extent of gas production can be considered a good indicator of the digestibility and fermentability of feeds and microbial protein synthesis (Elghandour *et al*., 2015). Significant differences ($p<0.05$) were observed in gas production for all the shed leaves from 3 to 24 hr incubation periods. The net volume of gas produced increased progressively with hours of incubation. Total gas produced ranged from 6.67 to 10.67ml/ 200 mg DM in *Bambusa vulgaris and Terminalia catappa* leaves respectively. This result is low compared to the range of values (9.00 to 22.67 ml/200mg DM) reported for *in vitro* rumen fermentation kinetics and nutritional evaluation of several non-conventional plants with potential for ruminant feeding (Maduro Dias *et al*., 2023). Gas production is an indicator of the degradability of samples (Arifuddin *et al.*, 2017). The degradation observed in the shed leaves suggests that these shed leaves can be used as feed supplements for ruminants.

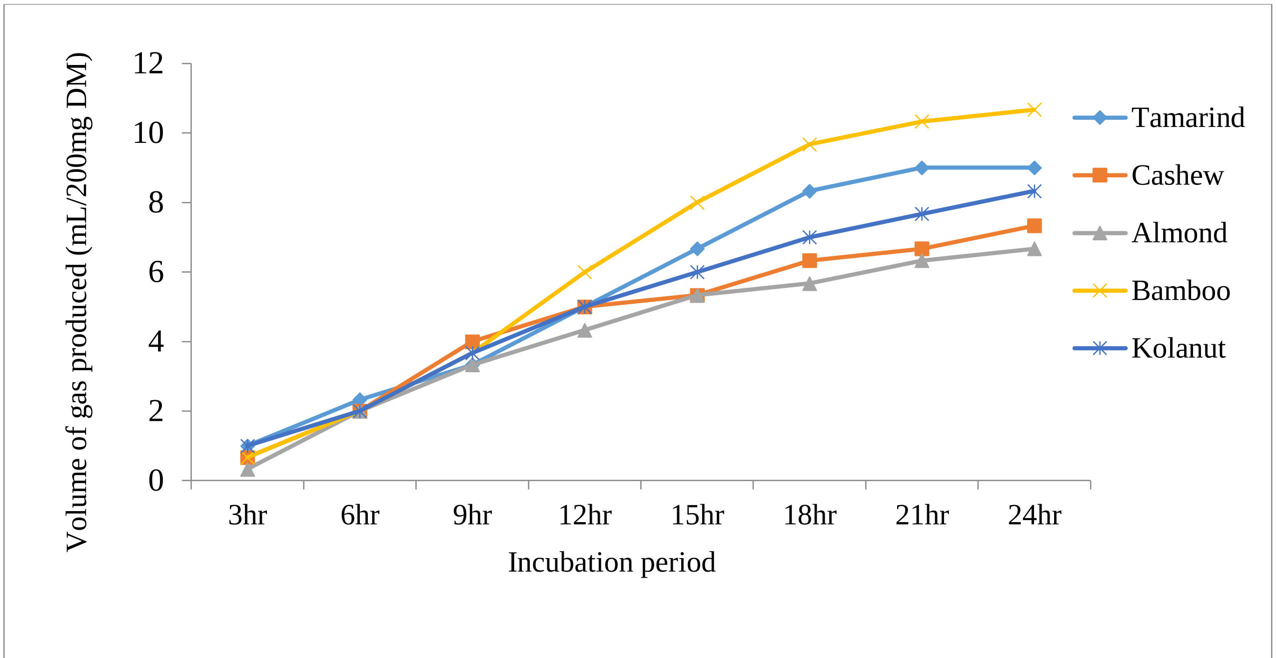

Figure 1: *In vitro* gas production of shed leaves

Conclusion

Based on this study, it can be concluded that the selected shed leaves of *Tamarindus indica*, *Terminalia catappa*, *Bambusa vulgaris*, *Anacardium occidentale* and *Cola nitida* trees can be potential feed supplements for ruminants during the dry season. Feeding trials to assess the performance of ruminant species fed diets incorporated with shed leaves of these selected trees are required.

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