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In vitro gas fermentation assessment of *Persea americana* leaf and acceptability by WAD Sheep

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Abstract

Ruminants in the tropics are slow growing, resulting from low quality feed. The use of indigenous multipurpose plants is a good strategy for an improved livestock performance. Chemical composition and Coefficient of preference of green, yellow and brown leaves of *Persea americana* (PA) by West African Dwarf Sheep were determined. *In vitro* gas production (IVGP) of green, yellow and brown leaves of PA were carried out over a period of 24 hr incubation. Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were predicted. 10 M NaOH was introduced into the inoculums after 24 hrs from which methane (CH_4) production was measured. Results indicated that chemical composition ranged significantly ($p<0.05$) among the different forms of PA leaves. Dry matter ranged between 88.75 and 91.22% in green and brown leaf respectively, same trend was observed for neutral detergent fibre, it ranged from 59.48 to 60.01% in green and brown leaf respectively, while the crude protein ranged between 23.59 and 25.85% in brown and green leaf respectively.

The CoP ranged between 1.58 and 2.01 in brown and green leaf respectively. The IVGP, ME, OMD, SCFA and methane production were not significant ($p>0.05$). Same trend was observed for all the *in vitro* gas fermentation characteristics with the green leaf recording the highest value, while the lowest values were obtained in the brown leaf. They ranged between 6.25 and 8.50 ml/200mg DM; 3.73 and 3.96 MJ/Kg DM; 38.66 and 41.67 %; 0.18 and 0.22 µmol; 2 and 4 ml/200mg DM in brown and green leaf respectively.

The result of this study showed that all forms of *Persea americana* leaf have potential as prospective forage for ruminant production in the tropics

Key words: coefficient of preference, *in vitro* gas production, nutritive value, *Persea americana* leaves

Introduction

Browse plants, besides grasses constitutes one of the cheapest source of feed for ruminants (Okah and Anita, 2016). These plants are important for animal production owing to their potentially good nutritive value. Browses have been used as feed by both domestic and wild life (Ukanwoko and Ironkwe 2013). They provide year round evergreen fodder for browsing

animals and supply vitamins and frequently mineral element mostly lacking in grasses (Ahamefule *et al*., 2006). It is therefore necessary to research into ways of managing browse plants to balance forage quality and quantity. *In vitro* fermentation has been used to evaluate digestibility and nutritional value of feed as it is cheaper, less laborious and most importantly, allows experimental conditions more accurately than the *in vivo* methods (Ajayi and Babayemi, 2008). It allows a large number of feed samples to be to be handled simultaneously. It is based on the quantification of substrate degraded and gas produced in rumen fermentation system based on syringes.

This study was therefore designed to assess the nutritive value and acceptability of Persea Americana leaf by West African Dwarf Sheep in the Humid tropics.

Material and Methods

Collection of samples

Twenty matured *Persea americana* trees within the environment of Government Science and Technical college Ijebu-Ode were marked randomly for collection of samples. Green and yellow leaves are plucked from each *Persea americana* tree, while brown leaves were picked from the floor around the root of each plant. Reasonable numbers of each leaf type were collected and taken to the laboratory for analysis.

Acceptability study

The acceptability study was carried out at the sheep and goat unit of the Department of Agricultural Science, Tai Solarin University of Education, Ijagun. Ijebu-Ode. Ogun State. Three different forms of *Persea americana* leaves namely: green, yellow and brown leaves were used for the study. Fifteen adult West African Dwarf Sheep housed in group pen were used in the cafeteria feed preference study that lasted for two weeks, including one week of adaptation. The green and yellow leaves were harvested fresh each day, while the brown leaves were picked from the fallen ones around the base of *Persea americana* trees, 4 kg each of the leaves were introduced on cafeteria basis to the animals in three different containers. The positioning of the leaves was changed daily to prevent bias by the animals taking a particular part of the pen as the position for a particular form of leaf. The amount consumed was monitored for eight hours daily and the quantity consumed for each form of leaf was recorded. The animals were then released for grazing. Feed preference was determined from coefficient of preference (CoP) value calculated from the ratio between the intakes of each individual feed sample divided by the average intake of three feed samples (Mako, 2009). On this basis, a feed was taken to be relatively preferred if the CoP value is greater than unity.

$$\text{CoP} = \frac{\textit{intake of individual forage offered}}{\textit{mean intake of all the forage offered}}$$

***In vitro* gas production**

Rumen fluid was obtained from three West African Dwarf female goats 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% growers premix for three days prior to the collection of rumen liquor. Incubation was as reported (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 $C0_2$

The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24, 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 (Menke and Steingass 1988) and short chain fatty acids (SCFA, *u*mol) was calculated as reported (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.

Statistical analysis

Data obtained were analyzed and subjected to analysis of variance procedure (ANOVA) of SAS (2012). Significant treatment means were separated by Duncan's multiple range test of the same package.

Results and Discussion

Table 1 shows the *in vitro* gas production parameters and characteristics of green, yellow and brown leaf of *Persea americana* estimated from gas production. No significant differences was observed among the different forms of leaves for metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA). They ranged from 3.73- 3.96 MJ/Kg DM; 38.66 – 41.77% and 0.18 – 0.22 µmol in brown and green leaf respectively. These results are comparable and in agreement with the values of 4.74 MJ/Kg DM; 38.03 % and 0.22 µmol reported for *Spondia mombin* leaf (Omoniyi *et al.*, 2013), but lower and at variance with the report elsewhere for *Spondia mombin* leaf (Ogunbosoye and Babayemi, 2010). Chemical composition in combination with in vitro digestibility and ME content can be considered useful indicators for preliminary evaluation of potential nutritive value of forages (Kafilzadeh and Heidary, 2013).

Same trend was observed for in vitro gas production characteristics: insoluble degradable fraction (b), potential degradability (a+b) and rate of degradation (c), except for soluble degradable fraction (a) that varied significantly ($p<0.05$) among the leaf forms. They ranged from 6.94 – 7.78ml; 10.15 – 11.78 ml; 0.10 – 0.22ml/h and 2.50 – 4.00 ml in brown and green leaf respectively. These results are lower and at variance with the report of Ogunbosoye and Babayemi (2010) for *Spondia mombin* leaves. The 'b' fraction becomes the major source of gas generated during the course of fermentation. The lower 'b' values obtained in this study are indications of the fibrous nature of the incubated samples (Mako, 2009). The 'a+b' fraction indicates the extent of degradation of the leaves. It follows the same trend with the 'b' fraction.The values of ME, OMD and SCFA obtained here is an indication that animals will be able to obtain energy from the leaves.

Total gas produced ranged from 6.25 and 8.50 ml/200mg DM in the brown and green leaf respectively (Table 2). No significant variation was observed. Gas production is an indication of degradability of samples (Arifuddin *et al.*, 2017). The degradation observed in the samples

is an indication that *Persea americana* leaf can be used as feed supplement for ruminants in the tropics. Same trend was observed for methane produced (Table 1), it ranged between 2.0 and 4.0 ml/200 mg DM in brown and green leaf respectively. Methane production indicates an energy loss to the ruminant and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi and Bamikole, 2006)

Table 1: *In vitro* gas production characteristics and parameters of different forms of *Persea americana* leaf

	In vitro gas production parameters					*In vitro* gas production characteristics			
Leaves	ME	SCFA	OMD	TIVGP	CH_4	a	b	C	a+b
Green	3.96	0.22	41.67	8.50	4.00	4.00a	7.78	0.22	11.78
Yellow	3.81	0.20	39.76	6.75	3.00	4.00[a]	7.65	0.13	10.94
Brown	3.73	0.18	38.66	6.25	2.00	2.50[b]	6.94	0.10	10.15
SEM	0.24	0.04	1.55	1.05	1.02	0.83	1.61	1.21	1.74

[a,b]= means on the same column with different superscript differed significantly ($p<0.05$)
a (ml/200 mg DM)= soluble degradable fraction; b (ml/200 mg DM)= insoluble degradable fraction; a+b (ml/200 mg DM) = Potential degradability; c (ml/h)= rate of degradation; ME= Metabolizable energy (MJ/Kg DM); SCFA (µmol)= Short chain fatty acid; OMD (%) = Organic matter digestibility; TIVGP (ml/200 mg DM)= total in vitro gas production; CH_4 (ml/200 mg DM)= Methane; SEM = standard error of mean

The preference level of animals fed the green, yellow and brown *Persea americana* leaves are on display in Table 2. A coefficient of preference (CoP) value higher than unity was taken to be preferred or accepted while the converse was true for a CoP value less than unity. For this reason, all the leaf forms were accepted by the animals, the green leaf was mostly preferred with CoP of 2.01. Furthermore, the NDF of a forage or sample is a good indicator of how much forage/sample an animal will consume. As the NDF content of forages increases, forage intake and net energy decreases (Tadele, 2015).

Table 2: Preference of experimental animals introduced to green, yellow and brown leaf of *Persea americana*

Form	Mean daily (kg DM) Consumption of all animals	Coefficient Of preference	Ranking
Green	3.23	2.01	1
Yellow	2.05	1.60	2
Brown	1.93	1.58	3

Conclusions and Outlook

In vitro digestibility and acceptability can be considered as useful indicators for preliminary evaluation of the likely nutritve value of this browse plant. It can therefore be concluded that *Persea americana* leaf has potential as a forage for ruminants in the humid tropics, especially during the off season. Feeding trial should be initiated on different specie of livestock (cattle, sheep and goat) using this plant.

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