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Preliminary Studies on Spent Tea Leaf: *In vitro* Gas Production as Affected by Chemical Composition and Secondary Metabolites

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Abstract: Nutrient composition and qualitative analysis of saponin, tannin and steroids were determined. *In vitro* gas production of the tea leaf (TL) and STL were carried out in 24 h incubation. Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were also predicted. NaOH (10 M) was introduced into the inoculums after 24 h, from which methane (CH_4) production was measured. TL and STL had CP, CF, EE, ash and NDF 16.4 and 18.6%, 14 and 23%, 3.8 and 2.5%, 6.0 and 2.0%, 39.0 and 46.0% respectively. Qualitative evaluation of secondary metabolites showed the two stuffs contained condensed tannins and steroids. Saponin was found and enhanced methanogenesis in STL than TL. The cumulative gas produced at 24 h was 14 and 7 ml/200 mg DM for STL and TL respectively. The ME was similar but varied significantly ($P < 0.05$) in OMD, SCFA and CH_4 productions. The result showed that spent tea leaf had potential to be used as protein and energy supplements for ruminants in the tropics.

Key words: Tea leaf, spent tea leaf, secondary metabolite, gas production, digestibility, methanogenesis

Introduction

Livestock in the tropics are still facing the challenges of poor nutrition, as the available crop residues or by-products are of low nutritive value (Babayemi *et al*., 2004a). Yamamoto *et al*. (1997) reported that tea foliages contained different amino acids, proteins, tannins, polyphenols, suggesting that tea leaf may have ability as protein supplement in animal feed. Notwithstanding, tea leaf also possesses tannins and saponin. Tannin and saponin are an important secondary metabolites in some forages, which may be antinutritional or beneficial to ruminants (Babayemi *et al*., 2004b) The by-product from tea leaf (TL), otherwise known as spent tea leaf (STL) may be used as an alternative feed resources for ruminant animals. On world scale, Nigeria is a medium producer of tea crop, being favoured by the humid condition in the southern part of the country. There are many companies using the TL for production of Coffee, Lipton and other products in Nigeria. They produce large quantities of chaff, which are burnt. Apart from losing the economic value of the waste, a huge amount of capital is expended in disposing it. In some places, the waste constitutes environmental hazards through indiscriminate dumping and incineration. The by-product may be utilized by ruminants, which are capable of converting fibrous feed into human edible food as milk and meat. Although, the gas production from the *in vitro* fermentation is a nutritional waste, it remains one of the reliable means to measure quality of feeds (Fievez *et al*., 2005). This preliminary study was undertaken to access the nutritive value of the TL and the STL through the analysis of their chemical composition and antinutritional components as well as their *in vitro* digestibility and gas production potential.

Materials and Methods

Sample collection: The fresh tea leaf (TL) was obtained from the tea plantations of the Cocoa Research Institute of Nigeria (CRIN) at Ibadan. Spent tea leaf (STL) was collected from a tea making company, located in Taraba State of Nigeria. The materials were immediately oven dried at 65 ºC until a constant weight was obtained for dry matter determination.

Qualitative determination of saponin, phenols and steroids: Saponin, phenols and steroids were determined as reported (Babayemi et al; 2004a). Briefly, 2 g of sample was extracted with 30 ml petroleum ether (PE) and 25 ml methanol water (MW, 9/1, v/v). The mixture was shaken at 250 revolutions per minute for 1.5 h, filtered and separated by a funnel. The lower (MW) and upper layers were emptied into 50 ml volumetric flasks. From the MW fraction, 1.67 ml was dispensed in 9 ml distilled water, filtered and from this; 1 ml was taken into a test tube. The test tube was shaken for 30 seconds and left to stand for 15 minutes. Saponin content was evaluated from the height of the foam layer as negative (< 5 mm), low (5 - 9 mm), medium (10 - 14 mm) and high (> 15 mm). For phenol analysis, 1 ml from the MW fraction was dispensed into five bottles with 1 % $FeCl_3$ (w/v) added at different levels (0.2, 0.4, 0.6, 0.8 and 1 ml respectively). Phenols form complexes with ferric iron, resulting in a blue solution and hence, their presence was scored as: no phenols (no colour change), hydrolyzable (dark-blue) and condensed tannins (dark-green). For steroids, 10 ml from the PE fraction was evaporated in a water bath at 45°C and 0.5 ml chloroform, 0.25 ml acetic anhydride and 0.125 ml conc. H_2SO_4 were added. The mixture was agitated briefly and the colour reaction was accessed as being steroids (blue or green), triterpenoids (read, pink or purple) or saturated steroids (light yellow).

***In vitro* gas production:** Rumen fluid was obtained from three West African dwarf female goats through suction tube before the morning feed. The animals were fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% Guinea grass. Incubation was as reported (Menke and Steingass, 1988) using 120 ml calibrated syringes in three batches at 39°C. To 200 mg sample in the syringe was added 30 ml inoculum containing cheese cloth strained rumen liquor and buffer (9.8 g $NaHCO_3$ + 2.77 g Na_2HPO_4 + 0.57 g KCl + 0.47 g NaCl + 0.12 g $MgSO_4.7H_2O$ + 0.16 g/litre $CaCl_2.2H_2O$) (1:4, v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h. After 24 h of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

The volume of the gas produced was plotted against the incubation time, and the gas production characteristics were estimated using the equation $Y = a + b (1 – e^{-ct})$ described by Qrskov and McDonald (1979), where Y = volume of gas produced at time't', a = intercept (gas produced from the soluble fraction), b = gas production from the 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) 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 + 0.651 XA; SCFA = 0.0239*Gv – 0.0601; where Gv, CP, CF and XA are net gas production (ml/200 mg DM), crude protein, crude fibre and ash of the incubated samples respectively.

Chemical composition: Crude protein, ether extracts and ash were determined according to the conventional methods (AOAC, 1995). Neutral detergent fibre (NDF) was also determined using the method described by Van Soest *et al.* (1991).

Statistical analysis: Data obtained were subjected to analysis of variance (ANOVA) and mean separations where there were significant differences was by Duncan multiple range F-test using Statistical Analysis System (1988) package.

Table 1: Proximate composition and neutral detergent fibre (g/100 g DM) of tea leaf and spent tea leaf

Composition	Tea leaf	Spent tea leaf
Dry matter	73.5	91.8
Crude protein	16.4	18.6
Crude fibre	14.0	23.0
Ether extract	4.5	2.5
Ash	6.0	2.0
Neutral detergent fibre	39.0	46.0

Results

Chemical analysis: In Table 1, the results for proximate composition and fibre analysis are presented and in Table 2 is that of qualitative determination of saponin, phenols and steroids. The STL was higher in CP, CF and NDF than the TL. Both the TL and the STL showed the presence of condensed tannins and steroids. Although the qualitative analysis for saponin in those leaves was judged to be negative, the indication for saponin presence was higher in the TL than the STL.

***In vitro* gas production test:** Fig. 1 shows the *in vitro* gas production pattern of TL and STL over a period of 24 h. Potential gas production (b) (ml 200 mg DM) apparently varied ($P < 0.05$) between samples from 6.0 ml (TL) and 18.0 ml (STL). The gas production rate (c) (mlh^{-1}) also differed significantly ($P<0.05$). The fractional rate ranged from 0.04 ml h^{-1} in the ST to 0.05 ml h^{-1} in STL There was no significant difference ($P > 0.05$) among the samples in the total time (t h^{-1}) taken to produce the final volume (ml 200 mg DM) of the gas. In Table 4 are metabolizable energy (ME) (MJ/Kg DM), organic matter digestibility (OMD) (%), short chain fatty acids (SCFA) and methane (CH_4) (ml) values of TL and STL. The ME of the TL and STL were similar ($P > 0.05$). There were significant variations ($P < 0.05$) in OMD of the two leaves. Spent tea leaf was significantly ($P < 0.05$) degraded than the TL. The proportion of short chain fatty acids (SCFA) was significant higher ($P < 0.05$) in STL than TL. However methane production was significantly enhanced in STL than that of TL.

Discussion

The range of crude protein for TL and STL confirmed their high protein content (Yamamoto *et al.*, 1997). The value of CP for STL in the present study was lower than 27.6% reported for green tea waste (Makoto *et al.*, 2004). However, the high amount of crude protein in STL compared favourably to the values reported for some multipurpose trees in Nigeria (Arigbede *et al.*, 2003). Where growing of browse plants are not favoured possibly as a result of the prevailing climatic conditions in some parts of Nigeria, spent tea leaf may be used as

Table 2: Qualitative contents of saponin, phenols and steroids in tea and spent tea leaves

	Saponins		Phenols		Steroids	
Sample	Foam (mm)	comment	Colour change	Comment	Colour change	comment
Tea leaf	3	negative	dark green	con. tan[1]	light green	steroids
Spent leaf	1	negative	dark green	con. tan[1]	light green	steroids

[1]con.tan = condensed tannin

Table 3: *In vitro* gas production characteristics of incubated tea leaf and spent tea leaf

Production features	Leaf		SEm
	Tea	Spent tea	
b	6.0[b]	18.0[a]	1.00
y	7.3[b]	18.0[a]	0.76
t	20.0	18.0	1.0
c	0.040[b]	0.059[a]	0.00026

[a,b]Means on the same column with different superscripts are significantly varied ($P < 0.05$), y = volume of gas produced at time 't', b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

Table 4: Metabolizable energy (MJ/Kg DM), organic matter digestibility (%), short chain fatty acids (μmol) and methane (μmol) production of tea and spent tea leaf incubated for 24 h

Parameters	Fermentation characteristics			
	ME	OMD	SCFA	CH_4
Tea leaf	4.12	32.38[b]	0.10[b]	117.18[b]
Spent leaf	5.23	36.99[a]	0.27[a]	351.54[a]
Sem	0.50	0.49	0.03	0.193

[a,b]Means on the same column with different superscripts are significantly varied ($P < 0.05$). ME = metabolizable energy, OMD = organic matter digestibility, SCFA = short chain fatty acids, CH_4 = methane.

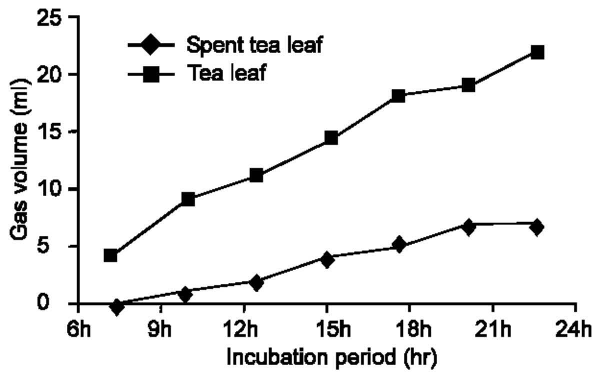

Fig. 1: *In vitro* rumen gas production of tea and spent tea leaves incubated for 24 h

dry season supplement to augment the low crude protein of standing hay or crop residues for ruminants. The qualitative method used for the determination of some secondary metabolites in the present study showed that saponin and tannins were present in both the TL and STL. Judging by the height of foam, TL contained more saponin than STL. The presence of tannins and saponin further enhances the property of the tea waste as a quality feed, being beneficial for ruminant nutrition. Feedstuffs containing saponin had been shown to be defaunating agents (Teferedegne, 2000), and capable of reducing methane production (Babayemi *et al.*, 2004b). The tannin content in the spent leaf is an added advantage as a natural additive in the diet of ruminants. Forages or feeds containing tannins have potential of forming complexes with protein as by-pass protein in the rumen (Barry and McNabb, 1999) by diminishing rumen protein digestibility, thus improving on the availability of protein to ruminants at the lower gut. Tannin forms complex with protein in the rumen as protection against massive proteolysis. Although gas production is a nutritionally wasteful product (Mauricio *et al.*, 1999), but provides a useful basis from which metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) may be predicted. Higher production of gas and the eventual preponderance of SCFA in spent leaf probably showed an increased proportion of acetate and butyrate but may mean a decrease in propionate production (Babayemi *et al.*, 2004b). However, since the spent leaf yielded better SCFA than the tea leaf suggests a potential to make energy available to the ruminants. Spent tea leaf is a waste, being a by-product of the real tea leaf and therefore has an advantage of being used by the livestock farmers in the tea producing areas of the world, especially in Nigeria.

Conclusion: Nutrient composition of spent leaf showed that the chaff might be useful protein supplement in ruminant feeding. Although low in saponin, the tea leaf exhibited the tendency to suppress methanogenesis, being relatively low by the *in vitro* gas production. The enhanced values of metabolizable energy and short chain fatty acids in spent leaf, connotes its ability to meet the energy requirements of tropical ruminants.

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