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Effects of *Tephrosia candida* DC Leaf and its Mixtures with Guinea Grass on *in vitro* Fermentation Changes as Feed for Ruminants in Nigeria

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Abstract: Ruminants in the tropics are slow growing, arising from low quality feed. The use of indigenous legume trees and Guinea grass is a good strategy for an improved livestock performance. The nutrient composition and secondary metabolites of differently year of established *Tephrosia candida* leaf were determined. The mixtures of *Tephrosia candida* leaf and Guinea grass as treatments A (100% Tephrosia + 0% Guinea grass), B (75% Tephrosia + 25 Guinea grass), C (50% Tephrosia + 50% Guinea grass), D (25% Tephrosia + 75% Guinea grass) and E (0% Tephrosia + 100% Guinea grass) were incubated for in vitro gas production for 24 hours. The total gas (ml/200 mg DM) at post incubation was measured. Methane (mmol/200 mg DM) was evaluated by introducing 10 M NaOH into the content. Metabolizable energy (MJ/Kg DM) and organic matter digestibility (%) were calculated. Results showed that the crude protein (g/100 g DM) ranged between 19.33 and 23.18 while neutral detergent fibre (g/100 g DM) ranged from 25 – 32. The Tephrosia forages contained condensed tannin and steroids. The inclusion levels of the legume apparently increased the total gas production (range 1 – 6), metabolizable energy (range 2.99 – 4.75), organic matter digestibility (range 21.46 – 33.80) and methane (29.30 – 234). It is concluded that Tephrosia candida forage may be a good combination with Guinea grass for livestock production but may be higher than 50% inclusion in order to minimize energy loss through methane.

Key words: Forage, secondary metabolites, incubation fermentation, digestibility, methane

Introduction

One of the major sustainable pastures in the tropics is Guinea grass. Guinea grass grows well in the humid tropics of Nigeria. The grass is low in crude protein and insufficiently available in the dry season (Bamikole and Babayemi, 2004), suggesting a need for supplementation. Supplementations of Guinea grass with browse trees were reported (Isah *et al*., 1999) to improve the performance of ruminants. The multipurpose trees such as *Leucaena leucocephala*, although relished by ruminants is high in mimosine (antinutritional factor), which limits its utilization by the animals (Arigbede *et al*., 2003). Other browse trees also exist, which could be suitable as forage for ruminants, being high in biomass production and available when needed. *Tephrosia candida* is a perennial shrub and the leaf is a source of protein for ruminants (Babayemi *et al*., 2003a).The seed was reported to contain high amount of crude protein (Babayemi *et al*., 2004a). Among other tropical seeds for the production of volatile fatty acids and as protein by-pass, *Tephrosia candida* seed showed superiority due to the presence of condensed tannin (Fennah, 1942; Babayemi *et al*., 2004b; Fievez *et al*., 2005). There are more than 300 species of Tephrosia and many of them have been implicated to be deleterious against insects (Simmonds *et al*., 1990), suggesting their potentials as feed for ruminants.

In the recent times, in vitro fermentation techniques (Fievez *et al*., 2005) has been employed to predict the nutritive value of tropical browse seeds. Although gases produced during rumen fermentation are colossal waste products and of no nutritive value to the ruminants, but gas production tests are used routinely in feed research as gas volumes are related to both the extent and rate of substrate degradation (e.g. Blummel *et al*., 1997). In vitro methods for evaluation of feeds are an important tool as they allow quick assessment of nutritional value and potential deleterious activity of any anti-nutritional compound present in the material. The present study seeks to evaluate the effects of chemical composition, secondary metabolite and *in vitro* gas production characteristics of *Tephrosia candida* leaf and Guinea grass.

Materials and Methods

Sample collection: Three months old *Tephrosia candida* leaves regrowths were collected differently in 2004 from 1, 2, 3, 4 and 5 year old established plots from the Teaching and Research Farm, University of Ibadan. The location is 7°27'N and 3°45'E at an altitude of between 200 and 300 m above sea level; mean temperature of 25 – 29°C with an average annual rainfall of about 1250 mm. The soils are much drained and belong to the alfisol (Rhodic kandiustalf). Dry matter of the samples was determined immediately after collection at 105°C and thereafter, ground to pass through 1 mm sieve.

Proximate composition: Nitrogen (N) content of the forages was determined by the standard Kjeldahl method (AOAC, 1990) and the amount of crude protein was calculated (N x 6.25). Organic matter was obtained by ash determination using muffle furnace. Neutral detergent fibre and acid detergent fibre were prepared as reported (Van Soest *et al.*, 1991).

Qualitative determination of secondary metabolites: The secondary metabolites in the leaves of *Tephrosia candida* were determined (Babayemi *et al.,* 2004a). Briefly, 2g of each milled sample were weighed in duplicates into extraction bottles, using two extraction solvents, (methanol and petroleum ether). Methanol-water (MW) was prepared using 9:1 (v/v). Equal amount (30ml) of each solvent was added into the sample and the mixture was agitated using the mechanical Shaker (Gathehamp, Germany) at 2,000 rpm for 90 minutes. The agitated solution was immediately filtered and rinsed using separating funnel. Two layers were distinctly formed; lower (MW) and upper (Petroleum ether) fractions, were separated into 50 ml flask each. Out of the MW fraction, 1-67ml of each sample was dispensed into 9 ml distilled water. 1 ml of it was pipette into calibrated test-tubes (duplicates). The content was shaken for 30 seconds the agitated test-tubes was allowed to stand for 15 minutes, after which the height of the foam (mm) in the test-tube was measured.:5 mm or less = Negative; 5 – 9 mm = Low saponin; 10 – 14 mm = Medium saponin; 15 mm or more = High saponin.
1 ml of MW fraction was again pipette into two bottles each, after which the prepared solutions of 1% $FeCl_3$ (w/v) at 1 ml were dispensed into the two bottles. A characteristic colour change was used in detecting the presence of hydrolyzable or condensed tannin (phenols). No change = No phenols or tannins; Dark blue = Water-soluble or hydrolyzable tannin; Dark green = Condensed tannins. From the petroleum ether fractions 10 ml was measured into test-tubes (n = 2) using a calibrated measuring cylinder. This was later evaporated under warm water, after which 0.5 ml chloroform, 0.25 ml acetic acid anhydride and 0.125 ml concentrated H_2SO_4 were added. The solution was later shaken together for 30 seconds and the colour reaction was inferred: Blue or green = Steroids; Pink, Red or purple = Triterperoids; Light yellow = Saturated steroids or triterperoids

***In vitro* gas production:** Tephrosia samples were collected from 1 and 2 years established plots, pooled and sub-sampled for further analysis. The treatments were A (50% *Tephrosia candida* + 50% Guinea grass), B (100% Guinea grass), C (25% *Tephrosia candida* + 75% Guinea grass), D (75% *Tephrosia candida* + 25% Guinea grass) and E (100% *Tephrosia candida*). Rumen fluid was obtained from three West African dwarf female goats through suction tube before the morning feed. The animals were fed with 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.
Incubation was as reported (Menke and Steingass, 1988) using 100 ml calibrated syringes in three batch incubation at 39°C. Into 200 mg sample in the syringe was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$+ Na_2HPO_4+KCl+NaCl+$MgSO_4.7H_2O$+$CaCl_2.2H_2O$) (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 and after 24 h of incubation, 4 ml of NaOH (10 M) was introduced into the incubated samples as reported (Fievez *et al.*, 2005) 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 the 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 Ørskov 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).

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 (SAS, 1995) package.

Results

Shown in Table 1 is the proximate composition of Tephrosia candida established over a period of five years and that of the grass. All the parameters varied from one to five year old established plots. The dry matter increased while crude protein decreased with the year of establishment. Neutral detergent fibre, ether extracts and ash contents did not have a consistent trend. The ash content decreased in the two year established plots and increased again in three year cultivated plots. There was a consistent decline of ash composition after the three year old established Tephrosia plots. Also presented in Table 2 are the secondary metabolites of the shrub. Irrespective of the period of existence, the foliages collected showed no saponin content. The Tephrosia samples indicated the presence of condensed tannins and steroids.

Table 1: Chemical composition (g/100g DM) of *Tephrosia candida* leaf and Guinea grass

Content	*Tephrosia candida*					Grass
	1 year	2 year	3 year	4 year	5 year	
Dry matter	25.56	28.17	29.94	32.86	34.63	30.71
Crude protein	23.18	21.52	21.42	20.12	19.53	7.35
Neutral detergent fibre	25.00	32.00	28.00	30.00	26.00	69.00
Ether extracts	12	16	16	11	11	3.19
Ash	7.5	4.5	10	9	6.5	7.12

Table 2: Effect of year of establishment on secondary metabolite of *Tephrosia candida*

Forage	Secondary metabolite					
	Saponin		Phenols		Steroids/triterpenoids	
	Fm. ht.[1]	comment	colour change	Comment	col. chan.[3]	Comment
1 year	0	negative	Light green	con. tan.[2]	Dark green	Steroids
2 year	0	negative	Dark green	con. tan.[2]	light green	Steroids
3 year	0	negative	Dark green	con. tan.[2]	dark green	Steroids
4 year	0	negative	Dark green	con. tan.[2]	light green	Steroids
5 year	0	negative	Dark green	con. tan.[2]	light green	Steroids

[1]fm. ht.= foam height, [2]con. tan.=condensed tannin, [3]col. chan.=colour change

Table 3 contains the potential gas production, gas production from the insoluble fraction, volume of gas produced and time of production. In vitro gas production characteristics varied significantly ($P < 0.05$) among the fermented feedstuffs. There was apparent decrease in the value of the parameters with a decreasing amount of *Tephrosia candida*. More and less gas was produced at 100% and 0% level of *Tephrosia candida* respectively. Methane production, metabolizable energy and organic matter digestibility of *Tephrosia candida* and Guinea grass mixtures are in Table 4. The amount of methane (CH_4), the organic matter digestibility (OMD) and the metabolizable energy (ME) of the feeds varied significantly among the treatment means. It was observed that as the percentage inclusion of Guinea grass was reducing, there was concomitant production of CH_4. There was also apparent increase in ME and OMD as the level of Tephrosia was increasing in the mixture.

Discussion

In the developing countries, where investment on research is minimal and are unable to afford the expensive facilities, the use of spot test (Babayemi *et al.*, 2004a) to determine the presence of secondary metabolites and that of syringes (Fievez *et al.*, 2005) to evaluate the nutritive value of feeds are adequate. The variations observed in the nutrient compositions of *Tephrosia candida* by the year of establishment, suggested the effect of age as an essential factor that determines the nutritive value of forage. The crude proteins obtained at present were higher than those reported for *Tephrosia bracteolata* and *Tephrosia linearis* (Ogungbesan, 2004). High protein in the diet and especially in the forage should be desired as it largely determines the intake and digestibility (Babayemi *et al.*, 2003b). The neutral detergent fibre (NDF) was within the range of 24 – 61% and 17 – 61% documented for forages by Topps (1992) and Budi and Wina (1995) respectively. The high crude protein and the medium content of NDF in Tephrosia candida probably present the plant as a complete diet for ruminants. The ash content obtained in the present study was relatively low when compared to 11% obtained elsewhere (Anugwa *et al.*, 2000). Since ash content signifies the mineral levels, it then implies that when supplying the Tephrosia candida foliage to ruminants, the diets may have to be fortified with mineral supplements. The value for ether extract (EE) was higher than those established by Ogungbesan (2004) but lower than that of Tephrosia candida seeds reported (Babayemi *et al.*, 2004a). The low EE in the present study connotes that the forage is low in energy and therefore, must be supplemented with high energy sources.

The Tephrosia candida foliage showed the presence of condensed tannins and steroids. Year of establishment did not affect the presence of these anti-nutritional factors. Tannins are beneficial to ruminants as it forms complex with protein in the rumen, thereby escaping the degradation by proteolytic enzymes. Saponin was not detected in the foliage. Tannins do not co-exist with saponin, because the surfactant properties of saponins negate the anti-digestibility effects of tannins (Freeland *et al.*, 1985).

The low net gas production (Fig.1) for all the treatments may be due to the high crude protein content of the mixtures. According to Wolin (1960), gas production from

Table 3: *In vitro* gas production characteristics of *Tephrosia candida* and Guinea grass mixtures

Treatment	Gas production characteristics[1]			
	a + b (ml)	b (ml)	y (ml)	C (h^{-1})
100% *T. candida* + 0% Grass	9.0[a]	7.5[a]	6.0[a]	0.070[ab]
75% *T. candida* + 25% Grass	8.0[ab]	6.5[ab]	4.5[ab]	0.040[ab]
50% *T. candida* + 50% Grass	6.5[ab]	5.5[ab]	3.5[ab]	0.076[a]
25% *T. candida* + 75% Grass	5.0[b]	4.0[b]	3.0[ab]	0.039[ab]
0% T. candida + 100% Grass	1.5[c]	0.5[c]	1.0[b]	0.000[b]
Sem	0.67	0.63	0.81	0.133

a,b,c Means on the same column with different superscripts are significantly different (P < 0.05)

Table 4: Methane production (mmol), organic matter digestibility (%) and metabolisable energy (MJ/kg DM) of *Tephrosia candida*/Guinea grass mixtures

Treatments	Fermentation parameter[1]		
	CH_4	ME	OMD
100% T. candida + 0% Grass	234.36[a]	4.75[a]	33.80[a]
75% T. candida + 25% Grass	105.30[ab]	4.43[ab]	31.36[ab]
50% T. candida + 50% Grass	117.18[abc]	4.04[bc]	28.69[bc]
25% T. candida + 75% Grass	97.65[bc]	3.65[c]	26.03[c]
0% T. candida + 100 Grass	29.30[c]	2.99[d]	21.46[d]
SEM	22.48	0.09	0.68

a,b,c Means on the same column with the same subscripts are not significantly (P < 0.05); 1CH_4=methane, ME=metabolisable energy, OMD=organic matter digestibility

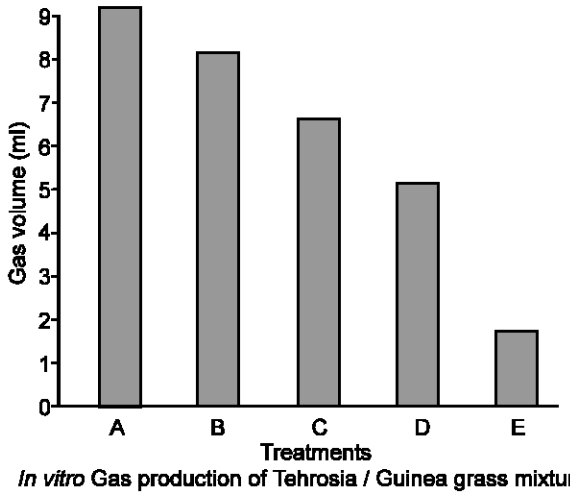

Fig. 1: *In vitro* gas production of Tephrosia candida with Guinea grass mixtures.

protein fermentation is relatively compared to carbohydrate fermentation. A downward trend observed in the amount of gas produced with increasing amount of Guinea grass indicated that the grass probably contained an antinutrient property. Guinea grass is high in crude fibre and this may reduce its digestibility. Digestibility has been described to be synonymous to *in vitro* gas production (Fievez *et al.*, 2005). From the present study, the higher the gas production, the higher was the digestibility. By this observation, there is tendency to nullify the inclusion of Guinea grass. Inclusion of Guinea grass was important, being one of the commonest grasses in the tropics. Apart from being abundant, its inclusion as Tephrosia/Guinea grass mixture consistently reduced the production of methane. Methane production represents a significant energy loss to ruminants and also contributes global warming. Since Tephrosia candida foliage and Guinea grass are two extremes as diet for ruminants, they could be strategically used for optimum performance of ruminants in the tropics. The equal proportions of the two forages had a lower methane production while there were no apparent variations in the metabolisable energy and organic matter digestibility of the mixtures.

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