

Full Length Research Paper

## ***In sacco* degradation of *Tephrosia candida* and *Leucaena leucocephala* in mixtures with *Panicum maximum* using fistulated West African dwarf sheep**

J.A. Odedire<sup>1\*</sup>, O. J. Babayemi<sup>2</sup> and T. O. Ososanya<sup>2</sup>

<sup>1</sup>Department of Animal Sciences, Obafemi Awolowo University, Ile – Ife, Nigeria.

<sup>2</sup>Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

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An experiment was conducted to compare the utilization of *Tephrosia candida* and *Leucaena leucocephala* in mixtures with *Panicum maximum* as feed for small ruminants, using the artificial bag technique of feed evaluation. Three West African dwarf (WAD) sheep with rumen cannula were used for the experiment. *T. candida* was formulated into diets with *P. maximum* as diets A, B and C while *L. leucocephala* was formulated into diets with *P. maximum* as diets D, E and F in the ratio 3:1, 1:1 and 1:3 respectively for both legumes. The degradation characteristics indicated *L. leucocephala* – based diets as being more ( $P < 0.05$ ) degradable in the rumen than the *T. candida* – based diets, with diet D having the highest potential degradability (a+b) value.

**Key words:** Artificial bag technique, degradation characteristics, feed, small ruminants.

### INTRODUCTION

Browse plants are promising forages for livestock productivity in the tropics, as the plant productions are favoured by the climate of the area. As traditional farmers are getting more awareness about the strategic use of browse trees as total replacement for conventional protein source as supplement to common perennial grasses such as *Panicum maximum* for dry and late rainy seasons, there is need to source for more species. The age long *Leucaena leucocephala* and *Gliricidia sepium* are suitable browse legumes because of being relished by ruminants, ease of establishment and high re-coppicing ability. However, there is restriction in feeding leucaena and gliricidia to ruminants due to the respective presence of mimosine and coumarin contents (Stewart, 1996; Babayemi and Bamikole, 2006a). A plantation of leucaena or gliricidia left uncoppiced has the tendency of overgrowth which can become a nuisance to the arable crop farmer under the multicropping system that is prevalent in this part of

the world. The necessity to prune these plants periodically may impact adversely on the labour demand of the farmer for other arable crops. *Tephrosia candida* is a rapidly growing perennial shrub that is not known to pose threat to companion crops even if left uncoppiced for a long time. The use of *T. candida* as a browse plant for livestock feeding is not common. Notwithstanding, the leguminous shrub is among the over 300 species of *Tephrosia* well distributed in the tropics (Babayemi et al., 2003). The establishment of *T. candida* with some grass species and nutrient composition were comparable with leucaena (Odedire and Babayemi, 2007). In a separate study, *T. candida* was reported to compare favourably with leucaena as forage for livestock (Odedire and Babayemi 2008). In an earlier *in vitro* gas production study, Babayemi and Bamikole (2006b) reported better predicted values of organic matter digestibility, metabolizable energy and short chain fatty acids for candida

\*Corresponding author. [oadeolu@oauife.edu.ng](mailto:oadeolu@oauife.edu.ng) or [oadeolu@yahoo.com](mailto:oadeolu@yahoo.com).

leaf in combination with Guinea grass than sole grass. Information on the nutritive value of *T. candida* as browse plant is scarce.

One of the established methods of assessing the nutritive potential of feeds is by employing the technique of rumen degradation of the feed. The use of *in sacco* feed evaluation method (Ørskov and MacDonald, 1979) has the unique advantage of not only furnishing information on the extent of nutrient release of a feed, but also providing a rapid estimate of the rate at which such nutrient is released (Ørskov et al., 1980). The method has proved to be a reliable means of predicting the digestibility of feedstuffs in the rumen (Ariel et al., 1998). The present study was undertaken to assess the *in sacco* degradability of *T. candida* and *L. leucocephala* when fed as mixtures with *Panicum maximum*, in the rumen of WAD sheep.

## MATERIALS AND METHODS

The experiment was carried out at the International Livestock Research Institute (ILRI), situated within the premises of the International Institute for Tropical Agriculture (IITA), Ibadan (7° 30' N; 3° 54' E), Nigeria. Samples of *T. candida* and *L. leucocephala* were obtained after one year of establishment at the Teaching and Research Farm (TRF) of the University of Ibadan, Nigeria. *P. maximum* was harvested from a six week regrowth from TRF. All harvested samples were immediately taken to the laboratory for dry matter (DM) determination. This was done by placing the freshly harvested samples in an oven at 65°C until constant weight was obtained. After DM determination, the samples were ground into fine particle sizes using laboratory hammer mill with a pore size of 2 mm. Ground samples of *T. candida* and *L. leucocephala* in mixtures with *P. maximum* were formulated using different levels as follows: Treatment A = 75% *T. candida* + 25% *P. maximum*; Treatment B = 50% *T. candida* + 50% *P. maximum*; Treatment C = 25% *T. candida* + 75% *P. maximum*; Treatment D = 75% *L. leucocephala* + 25% *P. maximum*; Treatment E = 50% *L. leucocephala* + 50% *P. maximum*; Treatment F = 25% *L. leucocephala* + 75% *P. maximum*.

### Animal management

Three mature WAD sheep with permanent ruminal cannula were used for the experiment. The sheep were housed in individual pens on wood shavings as bedding. The animals were fed on a mixture of 60% *P. maximum*, 30% *Stylosanthes guianensis* and 10% wheat bran. The animals had access to clean water, salt lick and exercise daily. The area around the cannulae were cleaned after samples' withdrawal from the rumen and disinfected regularly.

### Incubation of feed samples

Dry matter and crude protein degradations were determined using the nylon bag technique (Ørskov et al., 1980). 2.0 g of samples were weighed into 9 x 18 cm nylon bags of pore size 41 microns (Polymon, Switzerland). Bags were incubated in replicates for 12, 48, 60, 72 and 96 h in each of the three rumen – cannulated sheep. At the end of the incubation period, the samples were withdrawn from the rumen and kept in a bucket of cool water to prevent further fermentation and to wash off feed particles adhering outside the bags. The bags were then washed under running cold tap water until the rinsing water was clean and clear, and dried at 65°C for 48

h. Zero-time washing losses were estimated by soaking the feed samples in duplicates, in warm water at about 37°C for one hour followed by washing under running cold tap water and drying in oven at 65°C until a constant weight was obtained. Dry matter loss was calculated from the residue weighed after oven drying. The residues were reground to pass a 1 mm screen for nitrogen determination. Rumen degradation was determined by measuring loss of dry matter from the 2.0 g samples suspended in nylon bags in the rumen for the different lengths of time in the cannulated sheep as described by Mehrez and Ørskov (1977). Rumen degradation characteristics of dry matter and crude protein were calculated according to the non-linear model of Ørskov and McDonald (1979):

$$P = a + b(1 - e^{-ct}),$$

Where, P = potential degradability (the actual degradation after time 't'); a = water soluble fraction; b = insoluble but degradable fraction; c = degradation constant of the 'b' fraction; t = time of degradation; a + b = potential extent of degradation.

### Chemical composition

Crude protein, crude fibre, ash and ether extract were determined according to the method of AOAC (1990) and fibre analysis was carried out using the method of Van Soest et al. (1991) as modified by Nahm (1992).

### Statistical analysis

The experimental design was completely randomized. Data obtained were subjected to the analysis of variance procedure of SAS (1999). Significant means were separated using the Duncan multiple range F-test of the same package. Experimental model:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where,  $Y_{ij}$  = individual observation;  $\mu$  = general mean of the population;  $\alpha_i$  = treatment effect;  $\epsilon_{ij}$  = composite error effect.

## RESULTS

The chemical and fibre compositions of the experimental diets are shown in Tables 1 and 2 respectively. In the tables, variations in the values of the parameters under study were significant ( $P < 0.05$ ). The highest CP value (15.96 %) was obtained in diet D followed by that of A (14.75 %) and the least for diet C (9.52 %). The NDF value ranged from 39.70% in diet D to 46.90% in diet C. Lignin contents varied from 4.60% in diet D to 5.60% in diet C while ash content was least for diet F with a value of 6.00% but highest (9.00 %) in diet A.

Figure 1 shows the mean dry matter disappearance of the diets of different combinations of grass/legume mixture. All the diets showed increasing trend of dry matter release as the period of incubation progressed. Diet D was observed to be highest (80.6 %) in the DM loss throughout the incubation periods. Diet F had the least DM loss until 24 h, after which there was a drastic loss from 49.4 to 71.6%. Diet E showed a consistent rate of disappearance, increasing from 37.5% at 0 h to 71.2% at 72 h period. Table 3 shows the dry matter degradation

**Table 1.** Chemical composition (g/100 g DM) of the experimental diets containing different portions of grass and legume mixtures.

Diet	Crude protein	Ether extract	Ash
A	14.75 <sup>b</sup>	10.50 <sup>b</sup>	9.00 <sup>a</sup>
B	11.33 <sup>c</sup>	9.00 <sup>c</sup>	8.00 <sup>b</sup>
C	9.52 <sup>d</sup>	7.50 <sup>d</sup>	7.00 <sup>c</sup>
D	15.96 <sup>a</sup>	11.50 <sup>a</sup>	8.50 <sup>ab</sup>
E	12.03 <sup>c</sup>	8.50 <sup>c</sup>	8.50 <sup>ab</sup>
F	11.25 <sup>c</sup>	7.50 <sup>d</sup>	6.00 <sup>d</sup>
SEM	0.305	0.242	0.181

a, b, c, d Means in the same column with different superscripts differ significantly ( $P < 0.05$ ). A, 75% *Tephrosia candida* + 25% *Panicum maximum*; B, 50% *Tephrosia candida* + 50% *Panicum maximum*; C, 25% *Tephrosia candida* + 75% *Panicum maximum*; D, 75% *Leucaena leucocephala* + 25% *Panicum maximum*; E, 50% *Leucaena leucocephala* + 50% *Panicum maximum*; F, 25% *Leucaena leucocephala* + 75% *Panicum maximum*.

**Table 2.** Fibre components (g/100 g DM) of the experimental diets containing different proportions of grass and legume mixtures.

Diet	Neutral detergent fibre	Acid detergent fibre	Lignin	Cellulose	Hemicellulose
A	40.60 <sup>d</sup>	35.80 <sup>abc</sup>	4.90 <sup>bc</sup>	30.90 <sup>b</sup>	4.80 <sup>c</sup>
B	43.20 <sup>bc</sup>	36.30 <sup>ab</sup>	5.20 <sup>ab</sup>	31.10 <sup>b</sup>	6.90 <sup>b</sup>
C	46.90 <sup>a</sup>	36.90 <sup>a</sup>	5.60 <sup>a</sup>	31.30 <sup>ab</sup>	10.00 <sup>a</sup>
D	39.70 <sup>d</sup>	34.60 <sup>c</sup>	4.60 <sup>c</sup>	30.00 <sup>c</sup>	5.10 <sup>c</sup>
E	42.30 <sup>c</sup>	35.20 <sup>bc</sup>	4.90 <sup>bc</sup>	30.30 <sup>c</sup>	7.10 <sup>b</sup>
F	44.10 <sup>b</sup>	36.80 <sup>a</sup>	5.00 <sup>bc</sup>	31.80 <sup>a</sup>	7.30 <sup>b</sup>
SEM	0.406	0.377	0.150	0.190	0.185

a, b, c, d Means in the same column with different superscripts differ significantly ( $P < 0.05$ ). A, 75% *Tephrosia candida* + 25% *Panicum maximum*; B, 50% *Tephrosia candida* + 50% *Panicum maximum*; C, 25% *Tephrosia candida* + 75% *Panicum maximum*; D, 75% *Leucaena leucocephala* + 25% *Panicum maximum*; E, 50% *Leucaena leucocephala* + 50% *Panicum maximum*; F, 25% *Leucaena leucocephala* + 75% *Panicum maximum*.

characteristics of the legume/grass diets. The least ( $P < 0.05$ ) soluble fraction 'a' of the dry matter component (27.6 %) was recorded for diet C while the highest (39.4 %) was obtained for diet D. The 'b' fraction of the diets ranged from 29.8% in diet A to 46.7% in diet D. The differences among the 'b' values of diets D, E, and C were not significant ( $P > 0.05$ ) despite the variations in their values, but the 'b' value of diet F (41.0 %) was lower ( $P < 0.05$ ) in comparison to that of D and E. However, the difference in the 'b' values of diets F and C was not significant ( $P > 0.05$ ). Significant differences ( $P < 0.05$ ) were observed in the 'a + b' values of the diets. The values ranged from 69.1% in diet A, to 86.1% in diet D. The difference between the 'a + b' fractions of diets D and E were not significant. The 'a + b' values obtained for diets A, B, C, and F were not significantly different ( $P > 0.05$ ) from each other, but different ( $P < 0.05$ ) from diets D and E. The degradation constant of the insoluble but fermentable fraction of the diets (c) ranged from 0.03 to 0.09 but the differences in the 'c' values of the various diets were not significant ( $P > 0.05$ ). Lag time observed for the diets' degradation was least ( $P < 0.05$ ) in diet D

(4.93 h) and highest in diet B (9.13 h).

Mean crude protein disappearance of the diets showed increasing trend over the period of incubation with diet D exhibiting the highest rate (Figure 2). Table 4 shows the crude protein degradability of the diets. There were significant differences ( $P < 0.05$ ) in all the degradation characteristics observed among the diets. The water soluble fraction (a) was highest ( $P < 0.05$ ) in diet D (9.22%) and least in diet C (3.13%). The 'b' fraction ranged from 4.44% in diet A to 6.98% in diet B. The 'a + b' fraction of the diets ranged from 8.82 (diet A) to 15.81 % (diet D). The degradation constant (c) of the diets differed significantly ( $P < 0.05$ ) with the least value recorded for diet C (0.015% h<sup>-1</sup>) and the highest obtained for diet A (0.047% h<sup>-1</sup>).

## DISCUSSION

The crude protein content of the diets were sufficient for meeting the requirement of the ruminants as the values obtained were above the critical levels indicated by different authors (ARC, 1980; NRC, 1981; Norton, 1994a)

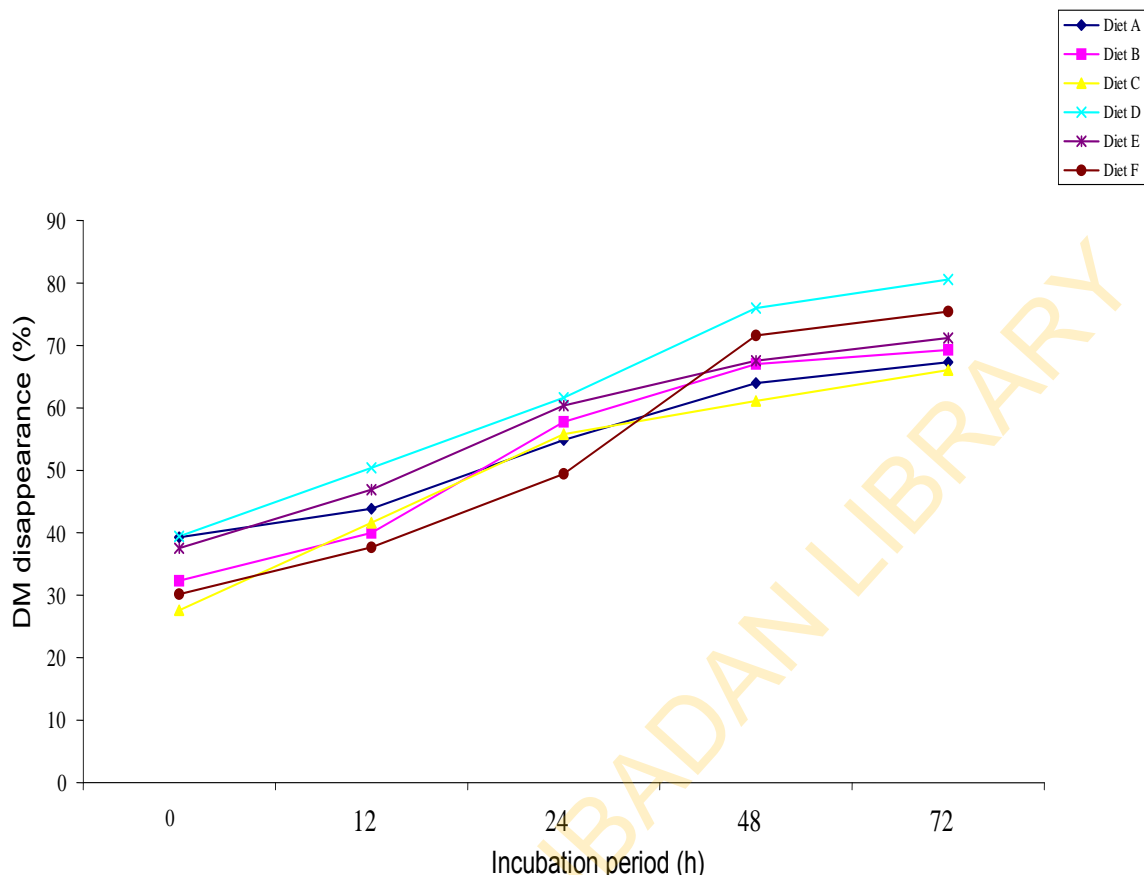


Figure 1. Mean Dry matter disappearance of diets of different combinations of grass /legume mixtures.

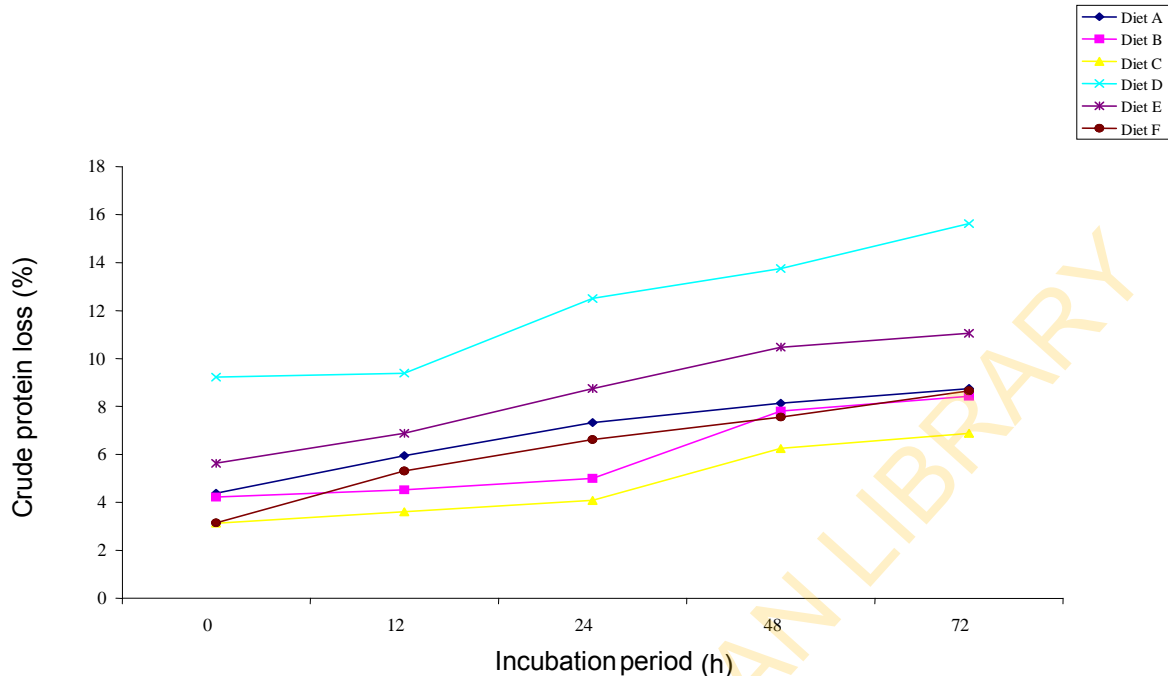
Table 3. Dry matter degradability of diets of different combinations of grass/ legume mixtures in the rumen of WAD sheep

Diet	Rumen degradation characteristic				
	a (%)	b (%)	a + b (%)	c (%h <sup>-1</sup> )	Lag time (hr)
A	39.29 <sup>b</sup>	29.79 <sup>d</sup>	69.08 <sup>b</sup>	0.049	8.63 <sup>ab</sup>
B	32.33 <sup>d</sup>	37.09 <sup>c</sup>	69.42 <sup>b</sup>	0.093	9.13 <sup>a</sup>
C	27.58 <sup>f</sup>	43.77 <sup>ab</sup>	71.35 <sup>b</sup>	0.072	6.33 <sup>ab</sup>
D	39.44 <sup>a</sup>	46.69 <sup>a</sup>	86.13 <sup>a</sup>	0.038	4.93 <sup>b</sup>
E	37.54 <sup>c</sup>	45.90 <sup>a</sup>	83.44 <sup>a</sup>	0.062	6.50 <sup>ab</sup>
F	30.17 <sup>e</sup>	41.03 <sup>b</sup>	71.20 <sup>b</sup>	0.032	7.97 <sup>ab</sup>
SEM	0.001	3.026	3.025	0.017	0.983

a, b, c, d, e, f means in the same column with different superscripts differ significantly ( $P < 0.05$ ). A: 75 % *Tephrosia candida* + 25 % *Panicum maximum*, B: 50 % *Tephrosia candida* + 50 % *Panicum maximum*, C: 25 % *Tephrosia candida* + 75 % *Panicum maximum*, D: 75 % *Leucaena leucocephala* + 25 % *Panicum maximum*, E: 50 % *Leucaena leucocephala* + 50 % *Panicum maximum*, F: 25 % *Leucaena leucocephala* + 75 % *Panicum maximum*.

implying that the voluntary dry matter intake, as well as the rumen efficiency may not be negatively affected as to reduce protein and energy availability to the animal. The NDF and ADF values can be said to be moderate enough to provide the animals with enough grit for good mastication and ruminal function. The digestibility of a feedstuff is defined by the potential degradability of the material, the

rate of degradation of this potentially degradable fraction, and its residence time in the rumen (Ørskov et al., 1980). The highest DM and CP disappearance recorded for diet D implied it to be highly degradable in the rumen. Diets D, E, and F were mixtures of *P. maximum* and *L. leucocephala* while diets A, B, and C were mixtures of *P. maximum* and *T. candida*. Diet D contained a high proportion



**Figure 2.** Mean crude protein disappearance from the experimental diets of different proportions of grass /legume mixtures.

**Table 4.** Crude protein degradability of diets of different grass/legume combinations in the rumen of WAD sheep.

Diet	Rumen degradation characteristic				
	a (%)	b (%)	a+b (%)	c (%h <sup>-1</sup> )	Lag time (h)
A	4.38 <sup>c</sup>	4.44 <sup>e</sup>	8.82 <sup>e</sup>	0.047 <sup>a</sup>	2.63 <sup>f</sup>
B	4.22 <sup>d</sup>	6.98 <sup>a</sup>	11.20 <sup>b</sup>	0.016 <sup>e</sup>	11.43 <sup>a</sup>
C	3.13 <sup>f</sup>	6.21 <sup>c</sup>	9.34 <sup>d</sup>	0.015 <sup>e</sup>	8.67 <sup>c</sup>
D	9.22 <sup>a</sup>	6.59 <sup>b</sup>	15.81 <sup>a</sup>	0.042 <sup>c</sup>	10.70 <sup>b</sup>
E	5.63 <sup>b</sup>	5.71 <sup>d</sup>	11.34 <sup>b</sup>	0.045 <sup>b</sup>	6.53 <sup>e</sup>
F	3.15 <sup>e</sup>	6.58 <sup>b</sup>	9.73 <sup>c</sup>	0.022 <sup>d</sup>	7.17 <sup>d</sup>
SEM	0.001	0.026	0.028	0.001	0.129

a, b, c, d Means in the same column with different superscripts differ significantly ( $P < 0.05$ ). A, 75% *Tephrosia candida* + 25% *Panicum maximum*; B, 50% *Tephrosia candida* + 50% *Panicum maximum*; C, 25% *Tephrosia candida* + 75% *Panicum maximum*; D, 75% *Leucaena leucocephala* + 25% *Panicum maximum*; E, 50% *Leucaena leucocephala* + 50% *Panicum maximum*; F, 25% *Leucaena leucocephala* + 75% *Panicum maximum*.

of leucaena (75 %). High rumen degradability has been reported for *L. leucocephala* (Keir et al., 1997). *L. leucocephala* contains hydrolysable tannin that is highly degradable in the rumen, and they have been found not to interfere with protein digestibility (Hagerman et al., 1992; Norton, 1994b). Diets A, B, and C had lower DM and CP disappearances. The lower DM and CP values were as a result of the content of tannins present in *T. candida*, which is the condensed type. Condensed tannins (flavonoids) have been reported to have a more profound digestibility-reducing effect (Barry and McNabb, 1999), forming a less digestible complex with dietary pro-

teins and may bind to inhibit endogenous proteins (Kumar and Singh, 1984). More so, condensed tannins have been reported to depress the rumen fluid and particulate content of the rumen (Hagerman et al., 1992).

An observation of the trend of the dry matter and crude protein degradability characteristics of the diets, revealed a definite pattern in the water soluble fractions which aligned with the previous reports on the influence of tannins. It was noted that the higher the proportion of the legumes in the diet, the more soluble the 'a' fraction. Expectedly, diet D had the highest potential extent of degradation in both the CP and DM degradability. This was in

agreement with the report of Hagerman et al. (1992) and Norton (1994b) that hydrolyzable tannins have been found not to affect protein digestibility. Condensed tannins in *T. candida* have been reported to reduce wasteful protein degradation in the rumen by the formation of a protein–tannin complex (Barry, 1987). Norton (1994) suggested the possibility of condensed tannins to inhibit microbial enzymes in the rumen and decrease the availability of plant proteins for digestion in the intestine. There seemed to be an inverse relationship between the fermentable 'b' fraction of feed and its rate constant 'c'. This was expressed in the results of the DM and CP degradability of the diets. The higher the 'b' fraction of a diet, the lower the rate constant 'c'. The lag time is not reliable as an index of degradation since it is only relevant for a feed that requires an initiation period for the degradation to start, in which case the 'a' value of such feed will read negative (Ørskov et al., 1980).

## Conclusion

The study shows that *T. candida* can be fed to grazing ruminants as a by-pass protein because of its ability to form a protein complex which will be resistant to the activities of rumen microbes, as a result of which it is capable of being delivered into the distal end of the animal's gastrointestinal tract for enzymic digestion.

## REFERENCES

- AOAC (1990). Official methods of analysis. Association of Official Analytical chemists, 15<sup>th</sup> edition, Washington DC, USA.
- ARC (1980). The nutrient requirement of ruminant livestock. CAB Farnham Royal, U.K.
- Ariel A, Mabjeesh SJ, Shabi Z, Bruckental I, Aharoni Y, Zamwel S, Tagari H (1998). *In situ* assessment of degradability of organic matter in the rumen of dairy cows. *J. Dairy Sci.* 81: 1985 – 1990.
- Babayemi OJ, Bamikole MA (2006b). Effects of *Tephrosia candida* DC leaf and its mixtures with Guinea grass on in vitro fermentation changes as feed for ruminants in Nigeria. *Pak. J. Nutr.* 5 (1): 14 – 18
- Babayemi OJ, Bamikole MA (2006a). Nutritive value of *Tephrosia candida* seed in West African dwarf goats. *J. Cent. Eur. Agric.* 7 (4) 731-738.
- Babayemi OJ, Bamikole MA, Oduguwa BO (2003). Intensive feeding effect of *Tephrosia bracteolata* Perr & Guill on West African dwarf goats. *Niger. J. Ecol.* 5: 28- 33.
- Barry NT (1987). Secondary compounds of forages. In: Hackner, J.B. and Ternouth, J.H. (editors). *Nutrition of Herbivores* pp. 91 – 120.
- Barry TN, McNabb WC (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr.* 81: 263 – 272.
- Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArchur C (1992). Tannin chemistry in relation to digestion. *J. Range Manage.* 45: 57-62.
- Keir B, vanLai N, Preston TR, Ørskov ER (1997). Nutritive value of leaves from tropical trees and shrubs: 1. In vitro gas production and in sacco rumen degradability. *Livest. Res. Rural Dev.* 9 (4).
- Kumar R, Singh M (1984). Tannins, their adverse role in ruminant nutrition. *J. Agric. Food Chem.* 32: 447 – 453.
- Mehrez AZ, Ørskov ER (1977). A study of artificial bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci. (Cambridge)* 88: 645 – 650.
- Nahm KH (1992). Practical guide to feed, forage and water analysis (Accurate analysis with minimal equipment). Seoul, Yoo Han Publishing Inc., Korea Republic. pp. 269.
- Norton BW (1994a). Tree legumes as dietary supplements for ruminants. In: Gutteridge, R.C. and H.M. Shelton (editors). *Forage tree legumes in tropical agriculture*. CAB International pp. 192-201.
- Norton BW (1994b). Antinutritive and toxic factors in forage tree legumes. In: Gutteridge, R.C. and H.M. Shelton (editors). *Forage tree legumes in tropical agriculture*. CAB International. pp. 202-215.
- NRC (1981). Nutrient Requirements for goats: Angora, Dairy and Meat goat in temperate and tropical countries. National Research council. National Academy of Science press, Washington DC.
- Odedire JA, Babayemi OJ (2007). Preliminary study on *Tephrosia candida* as forage alternative to *Leucaena leucocephala* for ruminant nutrition in Southwest Nigeria. *Livest. Res. Rural Dev.* 19(9).
- Odedire JA, Babayemi OJ (2008). Comparative studies on the yield and chemical composition of *Panicum maximum* and *Andropogon gayanus* as influenced by *Tephrosia candida* and *Leucaena leucocephala*. *Livest. Res. Rural Dev.* 20(2)
- Ørskov ER, McDonald I (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci. (Cambridge)* 92: 499 – 503.
- Ørskov ER, DeB Hovell FD, Mould F (1980). The use of nylon bag technique for the evaluation of feedstuffs. *Trop. Anim. Prod.* 5: 195 – 213.
- SAS (1999). Statistical Analysis System, SAS/ STAT User's guide, SAS Institute Inc., Cary, North Carolina, U.S.A.
- Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fibre, neutral detergent fibre and non – starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583 – 3597.