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IN VITRO RUMINAL PROTEIN DEGRADABILITY OF LEAVES FROM THREE TREE SPECIES HARVESTED AT TWO CUTTING INTERVALS

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ABSTRACT: *In vitro ruminal protein degradation characteristics of protein supplements represent an accurate measure of the quality of protein for ruminant animals. As such, crude protein disappearance of Gliricidia sepium, Leucaena leucocephala and Trichanthera gigantea leaves, which are potential sources of supplemental protein for ruminants, was determined using the ANKOM in vitro ruminal degradability technique. Dry matter (DM) and crude protein (CP) disappearance were measured after 0, 2, 4, 6, 12, 24, 36, 48 and 72 h of incubation. Degradation kinetics were described using the Ørskov and McDonald equation* $y = a + b\left(1 - e^{-cx}\right)$*. The degradable part of the insoluble DM fraction (b) was highest (P<0.05) in G. sepium leaves (27%) at the 12 week cutting interval. Effective dry matter degradability (EDMD) was highest (P < 0.05) in the leaves of G. sepium (74.9%) at the 12-week cutting interval. CP washing losses was highest (P < 0.05) in the leaves of L. leucocephala (46.8%) and lowest in T. gigantea leaves (16.3%) at the 6-week cutting interval. Crude protein disappearance was highest (P<0.05) in the leaves of G. sepium and lowest in T. gigantea leaves at both the 6 and 12-week cutting intervals after incubation at 48 h. It is concluded that in vitro ruminal protein degradability is more pronounced in the leaves of G. sepium and L. leucocephala. Approximately 50% of their protein is degraded in the rumen suggesting that they would be useful as sources of readily available nitrogen for rumen microbes challenged with low nitrogen, fibrous basal diets. Trichanthera gigantea leaves have higher levels of rumen undegradable protein suggesting that they can be used to supply by-pass protein for animal.*

Key words: *In Vitro Rumen Degradability, Protein Quality, Effective Degradability, Harvesting Frequency, Tree Forages*

ORIGINAL ARTICLE

INTRODUCTION

Protein-rich forages are critical to ruminant livestock production particularly in developing countries where the quantity and quality of available basal diets fluctuates wildly in response to seasonal rainfall patterns. Tree forages can be used as protein supplements to these diets. As supplements, they supply ruminal microorganisms with a readily available source of nitrogen (N) that enables them to breakdown basal diets efficiently (Mcleod and Minson, 1969; Getachew et al., 1994). Livestock producers are interested in the quality and quantity of protein that these supplements supply. The extent of protein degradation in the rumen gives a measure of the available nitrogen to microorganisms and by-pass protein to the small intestine (Promkot and Wanapat, 2003). Protein quality for ruminants can be determined through the use of rumen degradability characteristics of the protein, especially the ratio of rapidly degradable (soluble) protein to rumen undegradable protein (Crawford et al., 1978). This is because for high producing ruminants, microbial protein alone may be inadequate to meet protein requirements without by-pass (rumen undegradable) protein supply. Proteins with a large rumen soluble N fraction will supply a ruminant animal with little by-pass protein (Crawford et al., 1978). On the other hand, a protein with large rumen undegradable protein fraction will be unable to supply sufficient N to rumen microbes resulting in reduced fermentation and hence poor utilization of the basal diet. Ruminal degradability techniques are therefore useful for characterizing forage protein in terms of its susceptibility to ruminal breakdown. Orskov and McDonald (1979), De Boer et al. (1986) and Chumpawadee et al. (2005), among other scholars, have presented the *in sacco* nylon bag technique as one of the most popular ways of evaluating the extent and pattern of degradability of feed protein in the rumen. Indeed, rate of disappearance, rapidly fermentable fraction, effective degradability and

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potential degradability of feed protein can be estimated successfully using the *in sacco* nylon-bag technique (Getachew et al., 1998). Cone et al. (2002) also indicated that the *in sacco* nylon-bag technique is the standard method for estimating the volume of protein escaping rumen fermentation in protein evaluation systems for ruminants. However, the *in sacco/in situ* approach lacks capacity to evaluate a large number of forage samples, requiring a large number of fistulated animals whose rumens can be used to incubate feed samples in nylon bags. This study used an *in vitro* rumen fermentation system based on the Daisy^{II} Incubator (ANKOM TECHNOLOGY, MACEDON NEW YORK) to estimate ruminal degradability of tree leaves. The Daisy^{II} Incubator employs ANKOM filter bag technology and simulates and simplifies the *in sacco* rumen degradability technique. A large number of forage samples (100 +) can be processed in one batch. *Gliricidia sepium, L. leucocephala* and *T. gigantea* are three protein-rich forages grown in Trinidad and Tobago. Researchers have since recognize their importance as livestock feeds and are currently evaluating them at various levels. *Leucaena leucocephala* originated from Central America and Mexico and it belongs to the Mimosaceae family (Batson et al., 1987; Shelton and Brewbaker, 1994; Garcia et al., 1996). The shrub thrives well in alluvial and heavy clay soils. However, it has been found growing in saline soils (Batson et al., 1987). *Gliricidia sepium* is native to Mesoamerica and it's a member of the Fabaceae family (Simons and Stewart, 1994). Though recognized as essential forage in many parts, its use has been limited by palatability and toxicity concerns (Simons and Stewart, 1994). *Trichanthera gigantea* is native to Columbia and it belongs to the Acanthaceae family (McDade, 1983). The tree is adapted to the humid tropics and it is capable of thriving in acid (pH 4.5) and poor soils where there is good drainage.

There is a paucity of information as it relates to the ruminal degradability characteristics of these forages at different harvesting stages. Hoffman et al. (1993) reported that maturity stage of forage trees can influence DM and CP degradation fractions and degradation rates. Such information can be used to make informed decisions on how to incorporate the tree leaves into the diets of animals. This study, therefore, seeks to determine the *in vitro* ruminal dry matter and protein degradation parameters for *G. sepium, L. leucocephala* and *T. gigantea* leaves harvested at 6 and 12-week cutting intervals.

MATERIALS AND METHODS

Study site

Leaf samples were obtained from established tree species at the University of the West Indies Field Station (UFS). The UFS (Lat 10° 38' N Lon 61° 23' W) has a relatively flat topography with an altitude of 15.2 meters above mean sea level. Average annual rainfall is 1782.9mm with an average monthly temperature of 27 °C. The soil type is river estate loam. The soil is free draining with a pH range of 5.0 – 6.2.

Sample preparations

Fresh leaf materials (leaves with petioles) were harvested from forage tree species (*L. leucocephala, G. sepium,* and *T. gigantea*) that were trimmed to a height of 1 meter at UFS. Harvesting was done in the morning manually by cutting branches at a distance of 1 m from the growing tip for (*G. sepium*) and 0.5 m for (*T. gigantea* and *L. leucocephala*), 6 and 8 weeks after the trees had been trimmed to a 1 meter height. Leaves from six individual trees for each species were harvested, weighed and stored into brown paper bags separately. Leaf samples were immediately transported to the laboratory and oven dried to a constant weight at 65 °C. The dried samples were then milled to pass through a 1mm sieve using a Wiley Mill (GLEN CRESTON LTD, MIDDLESEX, UK) and kept in separate brown paper bags pending chemical analysis and *in vitro* ruminal fermentation.

Chemical analyses

Chemical analyses were carried out as part of an earlier study (Edwards et al., 2012). Dry matter, organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, soluble and insoluble condensed tannin content of leaves were determined. The chemical composition of the leaves is presented in Table 1 below to further describe the substrates fermented in this study.

In vitro ruminal dry matter and crude protein degradation

The Daisy^{II} Incubator (ANKOM TECHNOLOGY, MACEDON NEW YORK) was used to measure kinetics of DM and CP degradation of *G. sepium, L. leucocephala* and *T. gigantea* leaves. Milled leaf substrates (0.5 g) were weighed into filter bags (F 57) that had been pre-rinsed in acetone. Heat sealed bags were placed in the Daisy^{II} Incubator digestion jars. Sealed blank bags were included to enable the calculation of the blank bag correction factor. About 1 600 ml of ANKOM buffer (ANKOM TECHNOLOGY, MACEDON NEW YORK) was added to each digestion jar. Digestion jars with bags and buffer solution were placed into the Daisy^{II} Incubator set at 39°C and allowed to equilibrate for 30 minutes. Ruminal fluid was collected at 8:00 am. The donor was a crossbred Holstein heifer that was offered tanner grass, *G. sepium, L. leucocephala, T. gigantea* leaves and dairy concentrate (MASTER MIX FEEDS LTD, TRINIDAD). Rumen digesta from multiple sites within the rumen was sampled by hand and the rumen fluid squeezed into a prewarmed thermos flask. It was then transported to the laboratory, blended and strained through two layers of warm cheese cloth. The strained rumen fluid was held under carbon dioxide at 39 °C. Digestion jars were removed from the incubator, one at a time, and 400 ml of rumen fluid inoculum was added to each jar. Inoculated digestion jars were purged with CO_2 for 30 seconds after which they were sealed and returned into the

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incubator. All bagged samples were placed in the jars at the start of the incubation period and were than sequentially withdrawn at 2, 4, 6, 12, 24, 36, 48 and 72 h. After each withdrawal, bags were thoroughly rinsed with cold tap water until the water was clear. Time 0 h samples were not incubated but were washed in cold water to determine solubility at time 0 h. After rinsing, bags were placed in the ANKOM^{200} Fiber Analyzer and the procedure for NDF determination was followed, that is, samples were refluxed with neutral detergent solution for 1hr according to Van Soest et al. (1991).

Calculations

In vitro ruminal DM degradability was determined using the following formula:

$$\%\ IVTD\ (DM\ basis) = 100 - (W3 - (W1\ x\ C1))\ x\ 100/(W2\ x\ DM)$$

Where: W1 = Bag tare weight, W2 = Sample weight, W3 = Final bag weight after *In vitro* and sequential ND treatment, C1 = Blank bag correction factor (final oven-dried weight÷ original blank bag weight).

In vitro ruminal CP disappearance was calculated by subtracting the CP content of the degraded residue at each incubation time from the CP content of samples before degradation.

The DM and CP degradation data were fitted, using Datafit 9 (OAKDALE ENGINEERING) to the exponential equation (Ørskov and Mc Donald, 1979): $Y = a + b\left(1 - e^{-cx}\right)$

Where, *y* is the disappearance of DM or CP during time t; *a* is the rapidly soluble fraction (washing losses); *b* is the degradable part of the insoluble fraction; *c* is the rate of degradation of fraction b; and *t* is time of incubation. Potential degradability was calculated as *a+b*. The effective degradability of DM (EDDM) was calculated using the equation below, after assuming a ruminal fractional outflow rate (r) of 2 %/h at maintenance feeding levels.

$$EDDM = a + (bc)/(c + r)$$

where: r is the estimated rate of outflow from the rumen and a, b, and c are the parameters described in the Ørskov and McDonald exponential equation above.

Statistical Analysis

Data of DM and CP disappearance, degradation kinetics were analyzed using the general linear model (GLM) procedure of MINITAB (version 15) according to the following model:

Y = μ + D + F + D*F + e

where: Y = dependent variable, μ = overall mean, F = species effect (*G. sepium, L. leucocephala* and *T. gigantea*), D = cutting interval effect (6, 12-week), F*D = species*cutting interval effect and e = residual error.

Table 1 - The effect of species and cutting interval (weeks) on the chemical composition (g/kg DM) of *Gliricidia sepium, Leucaena leucocephala* and *Trichanthera gigantea* at UFS (Edwards et al., 2012)

Item / Species	Cutting interval	Chemical components[1]								
		DM	OM	CP	ADIN	ADF	NDF	ADL	SCT	ICT
G. sepium	6	895^{a}	915^{a}	284^{a}	34^{a}	405^{a}	582^{a}	22^{a}	0^{a}	0
	12	911^{a}	907^{a}	257^{a}	27^{a}	438^{a}	577^{a}	26^{a}	0^{a}	0
L. leucocephala	6	907^{b}	918^{a}	318^{b}	37^{b}	539^{b}	609^{b}	33^{b}	0.2^{b}	0
	12	921^{b}	913^{a}	268^{a}	34^{b}	491^{b}	597^{b}	32^{b}	0.2^{b}	0
T. gigantea	6	859^{c}	739^{b}	226^{c}	38^{b}	549^{b}	622^{b}	25^{c}	0^{ac}	0
	12	877^{c}	737^{b}	185^{b}	30^{c}	541^{c}	648^{c}	26^{ac}	0^{ac}	0
Species		***	***	***	***	***	***	***	***	NS
Cutting interval		**	NS	***	**	NS	NS	NS	NS	NS
Species*Cutting interval		NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b,c} Means within a column with different superscripts differ significantly (P<0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant. [1]Chemical components: DM = dry matter, OM = Organic matter, CP = Crude protein, ADIN = Acid detergent insoluble nitrogen, ADF = Acid detergent fibre, NDF = Neutral detergent fibre, ADL = Acid detergent lignin, SCT = Soluble condensed tannins, ICT = Insoluble condensed tannins.

RESULTS

In vitro ruminal DM degradability

Dry matter disappearance (DMD) data are presented in Table 2. Dry matter washing losses were highest (P<0.05) in *L. leucocephala* leaves (74%) and lowest in *T. gigantea* leaves (59%) at the 6 week cutting interval. A similar ranking of species with regards to DM washing losses was also observed at the 12-week cutting interval. Dry matter disappearance after 36 h of incubation was lowest (P<0.05) in *T. gigantea* leaves at the 6-(67.8) and 12-week-(67%) at cutting interval. At 48 h incubation, DMD was highest (P<0.05) in the leaves of *G. sepium* (83%) and lowest in *T. gigantea* leaves (68%) at the 6 week harvesting interval (Table 2). Similarly, DMD was highest (P<0.05) in the leaves of *G. sepium* (77.5%) and lowest in *T. gigantea* leaves (67%) at the 12 week harvesting interval. The rapidly soluble DM fraction (*a* fraction), the degradable part of the insoluble DM fraction (*b* fraction), rate of DM degradation of fraction *b* (*c*) and potential DM degradation (*a+b*) are presented in Table 3. The rapidly soluble DM fraction (a) was highest (P<0.05) in *L. leucocephala* leaves (73%) and lowest in the leaves of *T. gigantea* (59 %) at the 6 week harvesting interval (Table 3). Similarly, the rapidly soluble DM fraction (a) was highest (P<0.05) in *L. leucocephala* leaves (64.9%) and lowest in the leaves of *T. gigantea* (56.4%) at the 12-week harvesting interval

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(Table 3). The degradable part of the insoluble DM fraction (*b*) was highest (P<0.05) in *L. leucocephala* leaves (17.7%) at the 6 week cutting interval. The *b* fraction was highest (P<0.05) in *G. sepium* leaves (27%) at the 12 week cutting interval. The rate of DM degradation of fraction *b* (*c*) was lowest (P<0.05) in the leaves of *G. sepium* (2%/h) at the 12 week cutting interval. Potential DM degradation (*a+b*) was highest (P<0.05) in *L. leucocephala* leaves at 6-week (90.7%) and 12-week (77.4%) cutting intervals (Table 3). Effective dry matter degradability (EDMD) was highest (P<0.05) in the leaves of *G. sepium* (74.9%) at the 12-week cutting interval (Table 3).

In vitro ruminal protein degradability

In vitro ruminal CP disappearance data are presented in Table 4. CP washing losses was highest (P<0.05) in the leaves of *L. leucocephala* (46.8%) and lowest in *T. gigantea* leaves (16.3%) at the 6-week cutting interval. A similar trend followed where CP washing losses was highest (P<0.05) in the leaves of *L. leucocephala* (43.3%) and lowest in *T. gigantea* leaves (12.4%) at the 12-week cutting interval. Crude protein (CP) degradability at 24 h incubation was lowest (P<0.05) in *T. gigantea* leaves at the 6-(13.7%) and 12-week-(30.9 %) harvesting interval (Table 4).

At 36 h incubation time, CP disappearance was highest (P<0.05) in *G. sepium* leaves at 6-week (48%) and 12-week (49%) harvesting intervals. At the 48 h incubation CP disappearance was highest (P<0.05) in the leaves of *G. sepium* (60%) and lowest in *T. gigantea* leaves (29%) at the 6-week cutting interval. A similar trend followed where CP disappearance was highest (P<0.05) in the leaves of *G. sepium* (50%) and lowest in *T. gigantea* leaves (27%) at the 12-week cutting interval. The convergence criterion for the Ørskov and McDonald nonlinear model was not met for the degradable part of the insoluble CP fraction (*b*) and the rate of CP degradation of fraction *b* (*c*) for all species. As a result mean CP degradability values per incubation time are presented in Table 4.

DISCUSSION

In vitro ruminal DM degradability

Dry matter disappearance increased with increasing incubation time (Table 2). This is consistent with reports by Kirkpatrick and Kennelly (1987) which showed increases in DM and CP disappearance of barley (*Hordeum vulgare*), canola meal and soybean meal and Paya et al. (2008) who recorded increases in DM and CP disappearance of corn grain, soybean meal, wheat bran and alfalfa (*Medicago sativa*) with increasing incubation time. Though not statistically significant, DMD was lower at the 12 week cutting interval for all species. Dry matter disappearance was highest in *G. sepium* leaves and lowest in the leaves of *T. gigantea* at both harvesting intervals suggesting that the DM in *G. sepium* leaves is highly degradable. In addition, this can be due to lower fibre fractions (NDF, ADF) in the leaves of *G. sepium* (Table 1) as *in situ* DM disappearance is positively correlated with reducing sugars and negatively correlated with NDF (Vitti et al., 2003). The lower DM degradability in *T. gigantea* leaves can be attributed to its higher fibre content (NDF, ADF) (Table 1) as acid detergent fibre is negatively correlated with DM degradability (Smith et al. 1991).

Table 2 - *In vitro* ruminal dry matter disappearance (%) of *G. sepium, L. leucocephala* and *T. gigantea* at 6 and 12 weeks cutting intervals

Item / Species	Cutting Interval	Incubation period (h)								
		0	2	4	6	12	24	36	48	72
G. sepium	6	65^{aA}	65^{a}	66^{a}	68^{a}	68^{a}	76^{a}	80^{a}	83^{a}	85^{a}
	12	61^{bA}	56^{b}	65^{a}	66^{a}	67^{a}	73^{a}	76^{b}	77.5^{b}	82^{b}
L. leucocephala	6	74^{aB}	72^{c}	73.5^{b}	75^{c}	75^{b}	77^{a}	79^{ac}	81^{c}	82^{b}
	12	63^{bB}	66.5^{a}	67.4^{c}	69^{a}	69^{ac}	69^{ab}	73^{d}	74^{d}	74.7^{c}
T. gigantea	6	59^{aC}	61^{d}	59.5^{d}	61^{d}	62^{d}	62^{c}	67.8^{e}	68^{e}	75^{c}
	12	58^{aC}	59^{d}	58^{d}	58^{e}	60^{e}	69^{ab}	67^{e}	67^{e}	68.5^{d}
Species		***	NS	***	***	***	NS	***	***	***
Cutting interval		***	NS	*	*	*	NS	*	*	***
Species*Cutting interval		**	NS	NS	NS	NS	NS	NS	NS	NS
SEM		1.3	3.9	1.5	1.6	1.7	4.2	1.9	1.9	1.5

In a column where Species*CI is significant, lowercase superscripts compare CI means within species, while uppercase superscripts compare species for each CI. In columns where Species*CI is not significant, CI means are compared across species. Means within a column with different superscripts (a - e) differ (P < 0.05), *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant.

The rapidly soluble DM fraction (*a*) values at the 6 and 12-week cutting intervals for all species were higher than those reported by Kirkpatrick and Kennelly (1987), Promkot and Wanapat (2003), Chumpawadee et al. (2005), Paya et al. (2008) and Ilghami et al. (2008) who used different forage species. The soluble DM fraction (*a)* was highest in *L. leucocephala* leaves which indicates faster initial rate of degradation when compared to the other species. This is attributed to the fact that high soluble fractions make feeds more degradable as microorganisms are able to attach more readily to the soluble fractions (Chumpawadee et al., 2005). The *c* values for all species were similar to those reported by Promkot and Wanapat (2003), Chumpawadee et al. (2005) and Ilghami et al. (2008) but lower than those reported by Paya et al. (2008). Effective dry matter degradability (EDMD) values at 6 and 12-week cutting intervals for all species were higher than those reported by Promkot and Wanapat (2003), Chumpawadee et al. (2005), Paya et al. (2008) and Ilghami et al. (2008). Effective dry matter degradability (EDMD)

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was lowest in the leaves of *T. gigantea* possibly due to its high fibre content (NDF, ADF, ADL) (Table 1). High fibre suggests that less nitrogen would be available for rumen microbes hence reduce degradability due to lower microbial activity. Kamalak et al. (2005) reported that *in situ* DM degradability and estimated parameters were negatively correlated with NDF and ADF but positively correlated with CP content of tumbleweeds (*Gundelia tournefortii*).

***In vitro* ruminal protein degradability**

Crude protein disappearance increased with increasing incubation time in the leaves of *G. sepium* (Table 4). This is supported by Kirkpatrick and Kennelly (1987) who showed increases in DM and CP disappearance of barley (*Hordeum vulgare*), canola meal and soybean meal and Paya et al. (2008) who recorded increases in DM and CP disappearance of corn grain, soybean meal, wheat bran and alfalfa (*Medicago sativa*) with increasing incubation time. Cutting intervals had a minimal influence on the CP disappearance of the species. In a study where tumbleweed (*Gundelia tournefortii*) hays were harvested at three maturity stages, *in situ* DM Disappearance decreased with increasing maturity (Kamalak et al., 2005). Hoffman et al. (1993) reported that maturity stage of alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), rye grass (*Lolium perrene*) and timothy (*Phleum pratense*) affected DM and CP degradation fractions and degradation rates. Crude protein disappearance was highest in the leaves of *G. sepium* and lowest in *T. gigantea* leaves possibly influenced by the nature of the protein. Such data suggest that *T. gigantea* can be used to increase bypass protein or replace readily degradable protein sources in the diet owing to its low degradability by ruminal microbes (Ilghami et al., 2008). The lower degradability of CP in *T. gigantea* can also be attributed to its higher ADIN values (Table 1) in comparison to *G. sepium* suggesting that the majority of its protein may be bound to fibre thus rendering it insoluble and inaccessible by rumen microbes (Kirkpatrick and Kennelly, 1987).

The estimation of degradable part of the insoluble CP fraction (*b*) failed because the convergence criterion for the non-linear model was not met after several iterations using the Datafit (version 9) curve fitting programme (Table 5). This indicates that CP degradation profile did not closely fit the non-linear equation as a result mean degradation values are presented in Table 4. The rate of CP degradation of *b* (*c*) of all species was slower than those reported by Wang et al. (2009) Ximena Valderrama and Rene Anrique (2011). The rate of CP degradation was slowest in *G. sepium* leaves at cutting intervals 6 and 12-week. This may be due to the CP in *G. sepium* leaves having associations with other structural components (fibre) hence lowering the availability to microbial attack (Kohn and Allen, 1995).

Table 3 - *In vitro* ruminal dry matter degradation parameters of *G. sepium, L. leucocephala* and *T. gigantea* at cutting intervals 6 and 12 week

Species \ Item	CI[1]	Degradation parameters				
		a[2] (%)	b[3] (%)	c[4] (%/h)	a+b[5] (%)	EDDM[6] (%)
G. sepium	6	65[a]	NC[7]	0.02[a]	NC	NC
	12	61[b]	27[a]	0.02[a]	88.3[a]	74.9[b]
L. leucocephala	6	73[c]	17.7[a]	0.01[a]	90.7[a]	79.4[ac]
	12	64.9[d]	12.5[a]	0.05[b]	77.4[a]	72[d]
T. gigantea	6	59[e]	NC	0.00[a]	NC	NC
	12	56.4[f]	NC	0.04[ab]	NC	NC
Species		***	NS	NS	NS	***
CI		**	NS	NS	NS	***
Species*CI		NS	NS	NS	NS	NS
SEM		1.8	2.15	0.02	2.15	1.3

[a-f] Means with different superscripts in a column differ significantly (P<0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant. [2]a = the rapidly soluble fraction; [3]b = the potentially degradable fraction; [4]c = the rate of degradation of fraction b; [5]a+b = potential degradation; [6]EDDM = effective degradability of DM, [1]CI = cutting interval, NC[7] = non convergence

Table 4 - *In vitro* ruminal crude protein disappearance (%) of *G. sepium, L. leucocephala* and *T. gigantea* at 6 and 12 weeks cutting intervals.

Species \ Item	Cutting Interval	Incubation period (h)								
		0	2	4	6	12	24	36	48	72
G. sepium	6	41.8[a]	49.8[a]	38.7[a]	45.4[a]	45[a]	46.2[a]	48[a]	60[a]	59[a]
	12	31.3[b]	40.3[b]	34.8[a]	39.7[a]	42.8[a]	43[a]	49[a]	50[b]	74[b]
L. leucocephala	6	46.8[c]	51.2[a]	52.5[b]	53[b]	47.5[a]	42.7[a]	37[b]	44[c]	42[c]
	12	43.3[c]	44.6[ab]	42.7[c]	41.9[c]	39.8[ab]	44.8[a]	41[b]	43[c]	37[d]
T. gigantea	6	16.3[d]	7.5[c]	9.8[d]	9.8[d]	25.2[c]	13.7[b]	23[c]	29[d]	29[e]
	12	12.4[d]	11.5[c]	8.3[d]	16[e]	22.4[c]	30.9[c]	29[d]	27[d]	32[f]
Species		***	***	***	***	*	**	***	***	***
Cutting interval		NS	NS	NS	NS	NS	NS	NS	*	NS
Species*Cutting interval		NS	NS	NS	NS	NS	NS	NS	NS	NS
SEM		5.7	6	6.2	5.5	6.2	6	5.1	2.2	

[a-f] Means with different superscripts in a column differ significantly (P < 0.05). *P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant.

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CONCLUSION

The results of this study demonstrated that approximately 50% of CP in the leaves of *G. sepium* and *L. leucocephala* could be degraded in the rumen. This indicates that these protein trees can supply a readily available source of N to rumen microbes that have to ferment poor quality grass basal diets. Crude protein disappearance was least in *T. gigantea* leaves which suggest that it can be used supply by-pass protein to the duodenum of the ruminant animal. a feeding strategy where *T. gigantea, G. sepium and L. leucocephala* leaves are combined and offered as protein sources could ensure that both rumen microbial N and by-pass protein requirements are met.

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