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NUTRITIONAL COMPOSITION AND EFFECTIVE DEGRADABILITY OF FOUR FORAGE TREES GROWN FOR PROTEIN SUPPLEMENTATION

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ABSTRACT: *The chemical composition and ruminal degradability of dry matter (DM) and nitrogen (N) of four browse legumes (Gliricidia sepium, C. calothyrsus, A. angustissima and Leucaena. pallida) were evaluated. The in sacco degradability of protein and DM of the four browse legumes were determined using four mature Friesian- Holstein rumen-cannulated steers (440=20kg) live weight. The objective of this study was to evaluate the usefulness of the browse legumes using the nylon bag technique. Nylon bags with 3g samples of dried ground legumes (3mm screen) were incubated in the rumen. The incubation times were 0, 6, 12, 48, 72 and 120 hours in four cannulated Friesian-Holstein steers. The browse legumes were analysed for nutritive value in terms of dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), Ash, condensed tannin (CT), calcium (Ca^{2+}) and Phosphorus (P). Dry matter degradability was significantly different ($P<0.05$) and Gliricidia was highest, followed by L. pallida then A. angustissima and C. calothyrsus in descending order. Crude protein degradability was significantly ($P<0.05$) lower than that of DM and was highest in L. pallida, G. sepium, A. angustissima and finally C. calothyrsus at the bottom. Effective degradability of DM in the rumen of the steers was highest with G. sepium (880g/kg DM at rumen outflow rate of 0.02/h) and least with C. calothyrsus (504g/kg DM) ($P<0.001$). Effective degradability of nitrogen was highest with L. pallida (645g/kg DM at outflow rate of 0.02/h) and least with C. calothyrsus (103g/kg DM) ($P<0.001$). The degradability profiles of these browse indicated that they can be used as alternative protein supplements.*

Key words: *Nylon Bag Technique, Degradability, Effective Degradability, Ruminal, Rumen Cannulated Steers, Rumen Outflow Rates*

ORIGINAL ARTICLE

INTRODUCTION

Seasonal fluctuation in crude protein (CP) content of tropical grasses from 15% in December to 3% in May affects livestock production (Francis and Sibanda, 2001; Ngongoni et al., 2006). Nitrogen is the most limiting element in agricultural production, and its deficiency reduces the productivity of crops, pasture and animals. Multipurpose tree legumes are increasingly recognized for their capacity to enhance the productivity and sustainability of tropical agricultural systems, both in developed and developing countries of the world (Abia et al., 2006; Ngongoni et al., 2006; Mupangwa et al., 2003). Tree legumes can provide fuel wood, nutrient-rich mulch, erosion control and land stabilization as well as other products such as food and fencing materials for farmers (Abia et al., 2006; Mapiye et al., 2006). Tree legumes are usually long- lived, drought tolerant and require less maintenance, and therefore enhance the sustainability of farming systems. However, one of their major uses is as a source of protein-rich high quality forage in ruminants (Mupangwa et al., 2002; Abia et al., 2006). In developing countries, forage legumes contribute high protein herbage to supplement low protein crop residues and other mature poor quality feeds (Matizha, 2000; Mlay et al., 2005; Ajayi et al., 2007).

Nutritionally, the forage and browse legumes are superior forage in protein and mineral content (particularly calcium, magnesium, copper and cobalt.) even though their nutritive value falls slightly with age (Baloyi et al., 1997). Protein supplementation from these sources could enhance carbohydrate fermentation in ruminant feeding on low quality roughage (Ngongoni et al., 2007) and hence increase their digestibility (Cabrita et al., 2005) as well as voluntary feed intake (Mupangwa et al., 2002; Lazzarini et al., 2009). However, full knowledge of the nutritive value and the content of anti-nutritional factors of these browse legumes is an essential prerequisite to properly balance their use in ruminant animal nutrition. The legumes can provide the ruminants with rumen degradable nitrogen and undegradable digestible dietary nitrogen, which pass the rumen undegraded to the intestines

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(Mupangwa et al., 2003). The objective of the research is to address the nutritive value and degree of degradation using the nylon bag technique Mehrez and Ørskov (1977).

MATERIALS AND METHODS

Feed samples

Four legume samples of *A. angustissima, C. calothyrsus, G. sepium* and *L. pallida* were grown at the University of Zimbabwe Farm. The altitude is approximately 1300m above sea level. The area is situated at 18°N and 30°E with an annual average rainfall of 800mm. The legumes samples for degradability studies were harvested from the farm, taking the leaves only. The legume samples were dried under shade in the Bio-assay Laboratory in the faculty of Agriculture, Department of Animal Science. During the air drying, the browse leaves were turned frequently to ensure even drying for six days. The sample were milled through a 3mm screen and packed in plastic bags awaiting chemical composition analysis and *in sacco* nutritive evaluation.

Rumen degradability studies

Animals and feeding: The animals were handled in accordance to animal welfare regulation of the country. Four mature Holstein-Friesian steer-weighing 440±20kg surgically fitted with a rubber rumen cannula of 8.5 cm diameter were used to determine the degradability profile of browse legumes using the nylon bag technique Bhargava and Orskov (1987). The steers were not housed in individual pens but allowed to move freely in the Bio-assay Laboratory of the Department of Animal Science. The animals were dosed with a broad spectrum anthelmintic (Systamex, CAPS Veterinary PVT Ltd, Harare, Zimbabwe). The steers were feeding *ad libitum* their usual hay and were supplemented with 8kg concentrates of 3:1 maize to cotton cake with 11% crude protein so as to increase proteolytic microbes. The supplement was given to the animals three weeks before the beginning of the degradability studies. This was done for microbial stimulation as well as for adaptation. Fresh water was always available from automatic drinkers.

Rumen in sacco incubation of the samples

The rumen dry matter (DM) and protein degradability of the feed samples was determined using the nylon bag technique as described by Bhargava and Orskov (1987). Three grams of feed was weighed into each of the eight nylon bags of (9×13) cm and pore size of 49μm (Polymon, Switzerland) for each incubation time and were incubated in the rumen of four fistulated Holstein-Friesian steer. The nylon bags were put around a cork (8 bags per cork and 2 per each sample) and six corks were aligned along a flexible vinyl tube, 40cm long and 6cm outer diameter with a weight on the bottom part. Around the cork bags were tied with a nylon string and the bags were suspended in the rumen of each steer by attaching one end of the vinyl tube onto the cannula cap using nylon thread 25cm long. A cork with 8 bags representing the four samples was withdrawn from each animal at 6, 12, 24, 48, 72 and 120 hours Bhargava and Ørskov (1987). The nylon bags were then washed under running tap water to remove rumen debris from the bags and then machine washed for 30 minutes. The zero hour time of DM loss was determined by soaking weighed nylon bags containing the samples in cold water for one hour, followed by washing under running tap water and then machine wash for 30 minutes. The samples were then treated in a stomacher before analysis for 5 minutes per bag to reduce microbial contamination from the feed residues left adherent to the bags after rumen incubation. The residues in the nylon bags were dried in an oven at 60°C for 24 hours, weighed and thereafter analysed for nitrogen content.

Chemical analysis

Nitrogen content in the feed and in the residues was analysed by Kjeldahl method according to A.O.A.C (1990). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were analysed according to Goering and van Soest (1970). Total ash was obtained by igniting a dried sample in a muffle furnace at 600°C for 24 hours and calcium and phosphorous was determined by the EDTA method and spectrophotometer method, respectively. Condensed tannins were determined in the four legumes according to the method of Porter (1986).

Data and statistical analysis

Degradability of dry matter and protein of the different feed samples was calculated from the disappearance of DM or nitrogen (N) from the bags after washing (zero samples) and incubation into the rumen. The degradability characteristics of DM and crude protein (CP) of each feed was calculated according to the model of Ørskov and McDonald (1979) or McDonald (1981) equations respectively

$$P = a+b\,(1-e^{-ct}) \quad (1) \quad \text{Ørskov and McDonald (1979)}$$

$$P = a+b\,(1-e^{-c\,(t-tl)}) \quad (2) \quad \text{McDonald (1981)}$$

Where; P = the proportion degraded at time **t**, **a** = water soluble proportion, **b** = not water soluble but potentially rumen degradable proportion, **c** = degradation rate of b proportion, **t** = incubation time, **e** = exponential constant, **a + b** = potential degradable fraction and **tl** = time lag. Effective degradability (ED) of DM and N was calculated using assumed outflow rates of 0.02, 0.05 and 0.08 per hour according to Ørskov and McDonald (1979).

$$ED= a + (bc/c+k) \quad (3)$$

Where; **ED** = effective degradability and **a, b** and **c** are constants as described in equation 1 and 2 above and k = is the passage rate. The potential degradability was calculated as (a+b). The effective degradability of DM OM

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and N was calculated using the assumed fractional outflow rate k of 0.02 and 0.05 per hour according to the equation of Ørskov and McDonald (1979). The experimental design used is a completely randomized design using the model:

$$Y_{ijk} = \mu + A_i + T_j + I_k + \bar{e}_{ijk} \quad (4)$$

Where; μ = overall mean, A_i = fixed effects of animal, T_j = forage species, I_k = incubation period and $\bar{e}_{ijk}$ = error effects respectively. Analysis of variance (ANOVA) test was carried out on the disappearance of DM, OM, and CP value using Statistical Analysis System (SAS) (1998). The mean values so obtained were fitted to the equation of McDonald and Ørskov (1979) using SAS Institute (1998)

RESULTS

Chemical composition of browse legumes used

The chemical composition of the four browse legumes is presented in the Table 1. The crude protein (CP) contents were significantly different ($p < 0.05$) with *G. sepium* having the highest followed by *A. angustissima*, *C. calothyrsus*, and finally *L. pallida* in that order. The species were significantly different ($p < 0.05$) in Ash content, ADF and condensed tannins. The *C. calothyrsus* has the highest tannins of 102g/kg DM, which higher than other browse species. The highest Neutral detergent fibre (NDF) and acid detergent fibre (ADF) was found in A. angustissima and the lowest is in *G. sepium.* There was no significant ($p>0.05$) difference in dry matter (DM) for all the treatment diets. Organic matter (OM) was significantly different ($p < 0.05$) between treatments and *G. sepium* has 817g /kg DM' *A. angustissima* has 884g/kg DM, *C. calothyrsus* has 870g/kg DM and *L. pallida* has 840g/kg DM.

Table 1 - Chemical composition (g/kg/DM) of browse legumes used in the degradability study

Diet	DM	OM	CP	ADF	NDF	CT	P	Ca^{2+}	Ash
Gs	924	817[b]	305[a]	248[c]	214	26[c]	2.8[a]	30[a]	107[a]
Aa	931	884[a]	265[b]	339[a]	257	64[b]	1.3[b]	17[b]	46[c]
Cc	912	870[a]	227[c]	293[b]	222	102[a]	1.3[b]	24[ab]	42[c]
lp	909	840[b]	218[c]	295[b]	231	55[b]	2.9[a]	21[ab]	69[b]
Significance	ns	***	***	***	ns	***	***	***	***
LSD	35	27	23	36	46	17	0.3	11	19
Grand mean	919	853	254	294	231	62	2.1	23	66

DM dry matter, Organic matter (OM), crude protein (CP), acid detergent fibre (ADF), Neutral detergent fibre (NDF), condensed tannin (CT), phosphorus (P), calcium Ca; *G. sepium (Gs), A. angustissima (Aa), C. calothyrsus (Cc), and L. pallida (Lp);* Means with same [abcd] superscripts in column are not significantly different (P>0.05); LSD = least significant different; Significant level =0.05***; ns= not significantly different

Degradability of dry matter

The mean rumen degradability constants **a, b, c** and effective degradability constants **P** for DM and CP are given in table 2 and 3 respectively. The rapidly degradable fraction (a) for DM was significantly different ($p<0.001$). The following order was observed and the highest was in *G. sepium* (422g/kg), *L. pallida* (427g/kg), with *A. angustissima* (410g/kg) and lowest in *C. calothyrsus* (337g/kg). There was no significant difference ($P>0.05$) in the slowly degradable dry matter (SDDM) content of *G. sepium* and *L. pallida* but the SDDM content of *A. angustissima* was greater $p < 0.05$ than that of *C. calothyrsus*. *G. sepium* had a significantly $p < 0.001$ higher rate of degradation than that of *L. pallida, A. angustissima* and *C. calothyrsus* respectively. However, *L. pallida* and *A. angustissima* did not show any difference in the rate of degradation ($p < 0.05$). The mean rates of degradation were 0.235, 0.037, 0.016 and 0.034/hr respectively, for *G. sepium, A. angustissima, C. calothyrsus* and *L. pallida.*

Table 2 - Dry matter and effective DM degradability (g /kg) of the four treatments

Browse legumes	a	b	c	a + b	ED k = 0.02	ED k = 0.05
Gs	422[a]	497[a]	0.235[a]	919[a]	880[a]	832[a]
Aa	410[a]	430[b]	0.037[b]	840[b]	689[c]	593[c]
Cc	347[b]	354[c]	0.016[c]	701[c]	504[d]	433[d]
Lp	427[a]	476[a]	0.034[b]	903[ab]	726[b]	620[b]
S.e	43.9	28.2	0.06	73.2	54.1	67.3

[abcd] figures with the same superscripts in column are not significantly different (P>0.05); a= quickly degradable fraction; b = slowly degradable fraction; c = rate constant; ED = effective degradability at outflow rate (k) of 0.02/h and 0.05/h; S.e = standard error; *G. sepium (Gs), A. angustissima (Aa), C. calothyrsus (Cc), and L. pallida (Lp)*

Crude protein (CP) degradability

The *in situ* CP degradation constants and effective CP degradability's of the four browse legumes are given in Table 3. The quickly degradable CP (QDCP) fractions of the legumes were significantly different. *G. sepium* (320g/kg) had higher ($p<0.001$) QDCP content than *A. angustissima* (117g/kg), *C. calothyrsus (111gkg) and L. pallida (84g/kg).* There was no significant difference ($p>0.05$) between *A. angustissima* and *C. calothyrsus.* The slowly degradable crude protein (SDCP) contents were also dependent on the levels of tannins and ADF. *G. sepium*

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and *L. pallida* had significantly different (p>0.05) SDCP values, which were higher than that of *A. angustissima.* There was significant (p<0.05) difference in SDCP between *A. angustissima and C. calothyrsus.*

Table 3 - *CP and effective crude protein degradability (g/kg) of the four browse legumes*

Browse legumes	a	b	c	a + b	ED k = 0.02	ED k = 0.05
Gs	320^{a}	564^{b}	0.016^{a}	884^{a}	571^{b}	456^{b}
Aa	117^{b}	57^{c}	1.308c	174^{b}	173^{c}	172^{c}
Cc	111^{b}	-8^{d}	0.590^{c}	103^{c}	103^{d}	104^{d}
Lp	84c	643^{a}	0.036^{b}	727^{a}	645^{a}	554^{a}
S.e	32	125	0.260	157	148	139

^{abcd} Figures with the same superscripts in column are not significant (p > 0.05). a = quickly degradable fraction; b = slowly degradable fraction; c = rate of constant; ED = effective degradability at outflow rate (k) of 0.02 and 0.05 per hour. S.e = standard error; *G. sepium (Gs), A. angustissima (Aa), C. calothyrsus (Cc), and L. pallida (Lp)*

DISCUSSION

Chemical composition

The crude protein content of all the treatment legumes studied in this project were in excess of the proposed as the minimum required for lactation (120g CP/kg DM) and growth (113g CP/kg) in ruminants (Francis and Sibanda, 2001). The values are slightly lower from those reported in other studies especially for *C. Calothyrsus* (Abia et al 2006). However, the results suggest that the studied legumes may be good for protein supplement in both lactating and growing ruminants. There are species differences in CP content of the studied legumes and thus could be attributed to different species differences Mupangwa, et al, (2003) and the differences in maturity of the harvested leaves (Baloyi et al., 2008). *G. sepium* leaves had a higher crude protein than others and the results were comparable to those obtained by Rekha et al. (2004) and Archimede et al. (2009).

CP and Dry matter degradability

A. angustissima and *C. calothyrsus* had lower QDDM and QDCP content than *G. sepium* and *L. pallida.* This could be attributed to higher levels of tannins which bind to microbes. Therefore from the study *G. sepium* and *L. pallida* can provide rumen microbes with adequate volatile fatty acid (VFA) and ammonia for host ruminant energy and microbial multiplication. The results for *G. Sepium* conform to those found by Gonzalez et al., (2003) and Keopasenht et al. (2004). The lower QDDM and QDCP content in *C. Calothyrsus* and *A. acacia* may be associated with increase in ADF content. These browse with low QDDM and QDCP may not be used in high producing ruminant as they may fall supply adequate degradable energy and nitrogen for volatile fatty acid production and ammonia respectively. The results show that *C. Calothyrsus* and *A. Angustissima* may not support ruminants at high fractional outflow rates. Higher levels of tannin in these two browses than other also may have also caused a decrease in degradability of forage because tannins cause precipitation of microbes and enzymes which are supposed to cause break down of the molecules as resulted by Makkar (2003).

The SDDM contents of the legumes were above the range of 355 to 457g/kg DM reported for others tropical herbaceous legumes (Mupangwa et al., 2003). *G. sepium* and *L. pallida* maintained a higher SDDM and SDCP content than *C. calothyrsus* and *A. angustissima*. The low degradability of forages with high ADF and tannins is due to reduced penetrative ability to rumen microbes through lignified plant cell walls and precipitations effects of tannins to microbes and enzymes (Makkar et al., 2003, Baloyi et al., 2008). The potential DM degradability values of these legumes are within the range of 485 to 870g/kg (Baloyi et al., 1997) but higher than the range of 532 to 740g/kg reported for other herbaceous tropical legumes (Mupangwa et al., 2003; Baloyi et al., 2008). The decline in potentially degradable DM content of *A. angustissima* and *C. calothyrsus* may be explained by increases in less degradable fractions accumulating in the forages such as lignin and tannins.

The rate of degradation (c) is important in determining effective degradation (a) well as rumen fill Macdonald et al. (2002). The rates of DM degradation were different between legumes. Baloyi et al. (2008) reported a decline in degradation rate of DM in cowpea and silver leaf with increasing in ADF and NDF. The high protein content and the fragility of legume cells walls and high proportion of readily digestible thin walled, non lignified mesophyll tissues of the tropical legumes (Cook et al., 2005) could have resulted in maintenance of high degradation rates of *G. sepium*. The effective DM degradability decreased with increase in ADF and NDF as well as with increase in tannins. *G. sepium* maintained the highest effective degradability with *L. pallida* and *A. angustissima* acacia in the intermediate and *C. calothyrsus* s was the least. These differences could have been caused by the species variation in fibre content and levels of tannins.

The Metabolisable protein system based the evaluation of dietary protein on the concept of quickly (QDP) and slowly (SDP) degradable protein to meet the needs of rumen micro flora and undegradable dietary protein to meet host animal requirements. The QDN fraction in this research was between 84 to 320 g/kg and was lower than the range of 214 to 496 /kg DM reported by Mupangwa et al 2006, Baloyi et al 2008 for other tropical legumes. The differences may be due to species variation and ADF content as well as the level of condensed tannins. Stage of maturity and ratio of stem to leave can also cause this variation as browse legumes tend to have a high stem to leave ratio compared to herbaceous legumes (Mupangwa, 2003). The reduction in QDN content of legumes with

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increasing maturity is due to a decline in the soluble cell contents while cell wall contents increase (Baloyi et al., 2008). The slowly degradable Nitrogen (SDP) content of the forage ranged from –8 to 643 g/kg DM. The variation in SDP of the legumes could be due to difference in stage of maturity. C. calothyrsus *had the lowest value and a negative value of –8g/kg DM. This could be due to microbial attachment to the sample. High levels of tannins could have caused to microbes enzymes and amylase from saliva to stuck to the sample particles. The attached microbes cause an increase in N levels of the residue hence lowering the N degradation of the SDP (Makkar, 2003). The levels of condensed tannins and ADF could be the major causes of variation in degradation and bacteriostatic effects of some tannin.*

Effective degradability is a measure of the proportion of legume DM that can be fermented in the rumen before it passes to the lower digestive tract or post ruminal sites. (MacDonald et al 2002). High tannin levels also cause a decrease in degradability of forage because tannins cause precipitation of microbes and enzymes, which hydrolyze molecules into smaller particle (Makkar, 2003). The higher the tannins may therefore have a beneficial effect (increasing bypass protein or decreasing ammonia loss) or detrimental effect (depressing palatability, decreasing number ammonia, decreasing post-nominal protein absorption) on protein availability (Abia et al., 2006). *G. sepium* and *L. pallida* can be feed to ruminants with high fermentable energy sources as they have high values of **a** and **b** fraction compared to *C. calothyrsus* and *A. angustissima*. In this project the negative values obtained for the quickly degradable fraction could be a result of microbial contamination. The (b) fraction for *C. calothyrsus* was negative, this could be due to its high tannins, which cause, microbes, microbial enzymes and amylase from saliva to stick to food particle to the extent that it was inseparable by stomach. This presented complications in calculating both partial and effective degradability since these are calculated from these constants.

CONCLUSIONS

There is clear evidence that browse legumes can be used with reasonable confidence, as substitutes for protein supplements in at least medium to low producing ruminants and can be suitable for supplementing beef during periods of nutritional bottle-necks. Legumes of *Gliricidia* species and *Leucaena* species are of higher quality and are more suitable supplements where quickly degradable protein supplements are required. Feeding trials of communal environments still need to be done to validate the asserted findings.

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