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EFFECTS OF SUPPLEMENTING NATURAL PASTURE HAY WITH FIVE *Calliandra calothyrsus* PROVENANCES ON THE INTAKE, DIGESTIBILITY, NITROGEN BALANCE AND EXCRETION OF PURINE DERIVATIVES BY GOATS

[EFECTO DE LA SUPLEMENTACIÓN DE HENO DE GRAMA NATIVA CON CINCO ACCESIONES DE *Calliandra calothyrsus* SOBRE EL CONSUMO, DIGESTIBILIDAD, BALANCE DE NITRÓGENO Y EXCRECIÓN DE DERIVADOS DE PURINA DE CABRAS]

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SUMMARY

Forty indigenous castrated goats with a body weight of 20.03 ± 0.97 kg were used in a nitrogen balance trial to investigate the effects of supplementing natural pasture hay with five provenances of *C. calothyrsus* on the intake, digestibility, nitrogen balance and excretion of purine derivatives by goats. The goats were assigned to five treatment diets with each provenance at two levels (100g/day and 200g/day) of supplementation in a completely randomized design. Provenance and level of supplementation had a significant effect (P<0.05) on intake of basal diet, supplement, total dry matter and total organic matter intake, while there was no interaction between the provenance and the level of supplement. The nitrogen balance was similar in provenances OFI 9/89 and 12/91 at 100 g/d supplementation, while at 200 g/d supplementation the nitrogen balance was similar between provenances OFI 9/89 and 10/91 and between OFI 12/91 and 62/91. The microbial protein yield was similar between provenances OFI 9/89, OFI 10/91 and 62/91 at 100 g/d supplementation, while at 200 g/d supplementation provenance OFI 9/89 was similar to OFI 12/91 and OFI 23/91 similar to OFI 62/91. On the basis of total dry matter intake at 100 g/day supplementation OFI 23/91 had the highest intake with OFI 10/91 having the lowest. This trend seemed to be reversed at 200 g/day supplementation with OFI 10/91 having the highest intake and OFI 23/91 having the least intake. Provenance OFI 12/91 had the highest microbial protein yield while OFI 23/91 had the lowest. While this study clearly shows that the five provenances had different effects in goat nitrogen metabolism no clear trend of superiority could be established.

Key words: *Calliandra calothyrsus,* provenances, intake, nitrogen balance, native pasture hay

RESUMEN

Cuarenta cabras criollas (castradas) con un peso de 20.03 ± 0.97 kg fueron empleados en una prueba de balance de nitrógeno para investigar los efectos de suplementar una grama nativa con cinco accesiones de *C. calothyrsus* sobre el consumo, digestibilidad, balance de nitrógeno y la excreción de derivados de purina. Las cabras fueron asignadas a cinco dietas experimentales con cada accesiones a dos niveles de inclusión (100 g/d y 200 g/d). Se observó un efecto de accesión y nivel de inclusión (P<0.05) sobre consumo de la dieta basal, suplemento y consumo total de materia seca y materia orgánica. El balance de nitrógeno fue similar para las accesiones OFI 9/89 y 12/91 al nivel de 100 g/d, mientras que al nivel de 200 g/d, el balance de nitrógeno fue similar entre OFI 9/89 y 10/91 y, entre OFI 12/91 y 62/91. El suministro de proteína microbial fue similar entre las accesiones OFI 9/89, OFI 10/91 y 62/91 a 100 g/d, mientras que a 200 g/d OFI 9/89 fue similar a OFI 12/91 y, OFI 23/91 fue similar a OFI 62/91. Con base al consumo total de materia seca a 100 g/d OFI 23/91 tuvo el mayor consumo y OFI 10/91 el menor. La tendencia opuesta se encontró a 200 g/d donde OFI 10/91 obtuvo el mayor consumo y OFI 23/91 el menor. La accesión OFI 12/91 tuvo el mayor suministro de proteína microbial y OFI 23/91 el menor. El estudio mostró que las diferentes accesiones tuvieron diferentes efectos en el metabolismo del nitrógeno de las cabras pero no se pudo establecer una clara tendencia de superioridad para alguna accesión.

Palabras clave: *Calliandra calothyrsus,* accesiones, consume, balance de nitrógeno, gramíneas nativas.

INTRODUCTION

The evaluation and selection of shrub legumes as feed resources for livestock is an ongoing exercise in Zimbabwe. *Calliandra calothyrsus* is one of the multipurpose tree species with potential to alleviate dry season livestock fodder shortages. However, it is known to have high tannin content, which negatively affect digestibility and voluntary feed intake (Dzowela, Hove, Topps and Mafongoya, 1995). Norton and Ahn (1997) reported high condensed tannin concentrations of between 80 and 120 g / kg dry matter, which have also been implicated in the low availability of nitrogen for ruminant use. *C. calothyrsus* is adapted to a wide range of environments and produces large amounts of foliage of high nitrogen content acceptable to cattle and goats (Palmer and Ibrahim, 1996). In Zimbabwe six provenances, namely, Oxford Forestry Institute (OFI) 9/89, OFI 10/91, OFI 11/91, OFI 12/91, OFI 23/91, and OFI 62/91 have been identified as having good browse and seed production characteristics. The nitrogen content ranges from 27.15 g/kg dry matter to 32.51 g/kg DM, while potential nitrogen degradability is between 782.2 g/kg N and 888.2 g/kg N (Abia, 2000).

The proanthocyanidin structure, which was measured using high performance liquid chromatography, was shown to be different for two provenances of *C. calothyrsus* (CIAT 22316 and CIAT 22310) (Lascano, Avila and Stewart, 2003). The dry matter intake, digestibility, total nitrogen flow to the duodenum and nitrogen apparently absorbed in the small intestine of sheep were also different between CIAT 22316 and CIAT 22310 (Lascano *et al*., 2003). The differences in nitrogen utilization were attributed to different tannin structures rather than to tannin concentrations (Lascano *et al*., 2003). The structure of tannins in tropical legumes has an effect on the utilization of nitrogen by ruminants. Studies with temperate legumes have shown that tannins protect protein from being degraded in the rumen and by so doing increase protein flow to the small intestine and amino acid absorption (Barry and Manley, 1984; Waghorn, John, Jones and Shelton., 1987). Condensed tannins are decreased by drying (Ahn, Robertson, Elliott, Gutteridge and Ford, 1989), and supplements of dried *C. calothyrsus* increase both straw intake and nitrogen retention in sheep more effectively than fresh supplements (Ahn, Elliot and Norton, 1997).

This study investigated the effects of supplementing a basal diet of natural pasture hay, consisting mainly of *Hyparrhenia filipendula, Hyperthelia dissoluta* and *Heteropogon contortus*, with five provenances of air-dried *C. calothyrsus* on the intake, digestibility, nitrogen balance and excretion of purine derivatives by goats.

MATERIALS AND METHOD

Animals and Management

Forty indigenous castrated goats with a body weight of 20.03 ± 0.97 kg were used in a nitrogen balance trial. The goats were drenched against endo- parasites before the start of the experiment using valbazin supplied by CIBA Geigy Co Ltd (Switzerland). The goats were kept in individual metabolism crates that facilitated separate collection of urine and faeces for a fourteen days adaptation and seven days total collection period. The trial was carried out at Grasslands Research Station in Marondera which is 75 km east of Harare.

Feeding material

The five *C. calothyrsus* provenances feeding material was harvested from three year old plants established on red clay loam soils without fertilizer. The forage contained a significant fraction of flowers and pods at the time of harvesting. Leaves were separated from stems and only leaves, flowers and pods were fed to the goats. The chemical composition of the material fed is given in Table 1.

Experimental Design and Treatments

The goats were assigned to five treatment diets each with two levels of supplementation in a completely randomized design as shown in Table 2. Each experimental animal received the supplement at 08:00 h and then 800g of native pasture hay (consisting mainly of *Hyparrhenia filipendula, Hyperthelia dissoluta* and *Heteropogon contortus*) at 12:00 h every day. Water was offered every three hours during daytime in plastic buckets.

Table1. Chemical composition of five provenances of *Calliandra calothyrsus* fed to goats

Provenance	DM (g)	g/kg DM						
		OM	N	ADF	NDF	ADIN	NDIN	PA
9/89	882.8	943.6	27.2	205	400	0.026	0.027	29.3
10/91	895.0	955.6	28.5	235	410	0.024	0.032	46.2
12/91	882.5	943.2	31.6	260	405	0.024	0.032	46.2
23/91	890.0	949.4	28.2	240	465	0.021	0.025	54.1
62/91	883.6	944.5	32.5	200	405	0.026	0.028	50.7

DM = dry matter, OM = organic matter, N = nitrogen, ADF = acid detergent fibre, NDF = neutral detergent fibre, ADIN = acid detergent insoluble nitrogen, NDIN = neutral detergent insoluble nitrogen, PA = proanthocyanidins. (Source: Abia, 2000).

Table 2: Treatment diets fed to indigenous goats in a nitrogen balance trial

Treatment	Composition
Diet 1	NPH + 100g OFI 9/89
Diet 2	NPH + 200g OFI 9/89
Diet 3	NPH + 100g OFI 10/91
Diet 4	NPH + 200g OFI 10/91
Diet 5	NPH + 100g OFI 12/91
Diet 6	NPH + 200g OFI 12/91
Diet 7	NPH + 100g OFI 23/91
Diet 8	NPH + 200g OFI 23/91
Diet 9	NPH + 100g OFI 62/91
Diet 10	NPH + 200g OFI 62/91

OFI = Oxford Forestry Institute,
NPH = natural pasture hay

Measurements and collections

The total collection period lasted for seven days, following an adaptation period of fourteen days, during which feed offered, refusals, urine and faeces collected were weighed and recorded at 08:00 hours everyday. Urine and faeces were collected separately using sieve collectors positioned in such a way that faecal pellets rolled to a metal tray and urine was collected into a plastic jar which contained 25 mls of 10% v/v sulphuric acid. Only 10% of total daily urine collected was kept for each individual animal at - 20ºC until required for laboratory analysis. Another 10% from the remaining urine was further diluted ten times to prevent allantoin evaporation and kept at - 20ºC in the cold room awaiting allantoin analysis to determine microbial nitrogen yield. Daily faecal samples were also collected in plastic bags weighed and stored at - 20ºC awaiting chemical analysis. All sample bags were clearly labeled.

Chemical Analysis

The dry matter content of feed, refusals and faeces was determined by drying the samples at 60ºC for 48 hours and then ash by incineration for 6 hours at 550ºC. Total nitrogen in the samples was determined by the Kjedahl method (AOAC, 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using the method of Goering and Van Soest (1970). Allantoin analysis was done according to the procedures of Chen and Gomes (1992).

Statistical Analysis

Analysis of variance was carried out to determine the treatment effects using the Proc GLM procedure of SAS (SAS, 1998). The model was:

$$Yijk = \mu + Pi + L_J + (PxL)_{ij} + B1(Lwt)_{ijk} + eijk$$

Where:
Yijk = observed variable e.g. dry matter intake
μ = overal mean,
Pi = provenance effect
L_j = supplement level (100 g or 200 g)
$(PxL)_{ij}$ = interaction of provenance and level of supplement
$B1(Lwt)_{ijk}$ = live weight covariate
eijk = residual error.

A comparison of means was done using Tukey's studentized range test of SAS (1998).

Microbial protein yield

Microbial protein yield (MPY) was calculated using the formula:

$$MPY\ (g/d) = [(X\ mmol) \times 70] / (0.83 \times 0.116 \times 1000)$$

Where:
X = D/0.84
D = allantoin excretion (mmol/day)

Intake

Intake was calculated as the difference between feed offered and refusals corrected for dry matter content of feeds and refusals on a daily basis.

Digestibility

Digestibility was calculated as the portion of dry matter intake (DMI) not recovered in faeces.

Nitrogen balance

Nitrogen balance (NB) was calculated as the amount of average daily nitrogen intake (NI) not excreted in faeces (FN) and urine (UN).

NB = NI (g/d) – (FN + UN) (g/d)

RESULTS

Intake

The dry matter intake of the basal diet of natural pasture hay and supplements of five *C. calothyrsus* provenances by goats is shown in Table 3.

Provenance and level of supplement had a significant effect (P<0.05) on intake of basal diet, supplement, total dry matter and total organic matter intake, while there was no interaction between the provenance and the level of supplement. The supplement intakes at 100 grams supplementation level were 91% (OFI 12/91), 87% (OFI 9/89), 86% (OFI 23/91), 85% (OFI 10/91) and 83% (OFI 62/91) while at 200 grams supplementation level it was 89% (OFI 12/91), 82% (OFI 23/91), 80% (OFI 62/91), 74% (OFI 9/89) and 72% (OFI 10/91). The supplement intake was highest with OFI 12/91 at both levels of supplementation. Supplement intake was lower at 200g supplementation than at 100 g supplementation.

Digestibility

The digestibility of five *C. calothyrsus* provenances given to goats as supplements to natural pasture hay are shown in Table 4.

There were significant (P<0.05) differences in digestible dry matter, organic matter and digestible nitrogen intake with provenance type and level of supplementation, while there was no interaction between provenance type and level of supplementation. The digestible dry matter and digestible nitrogen intakes at 100 grams supplementation were highest with OFI 12/91.

Table 3: Dry matter intake (g/day) of goats given 100g or 200g of air-dried leaves of five *C. calothyrsus* provenances as supplements to a basal diet of natural pasture hay

Provenance	Level	A	B	C	D	E	F	G	H	I
OFI 9/89	100	473.2^a	86.5^a	560^a	546.5^a	329.8^a	340.0^a	0.691^a	2.17^a	164.3^a
	200	432.0^d	148.7^e	582^e	481.2^e	305.8^e	314.7^e	1.410^e	4.06^e	177.5^e
OFI 10/91	100	419.4^b	84.9^a	505^b	500.2^b	289.9^b	299.9^b	0.667^a	2.45^b	251.5^b
	200	471.9^e	144.8^e	616^d	513.2^d	313.3^d	322.8^d	0.829^d	4.98^d	309.9^d
OFI 12/91	100	455.8^c	91.3^b	547^c	536.0^c	322.5^c	336.5^a	0.687^a	2.52^b	251.8^b
	200	418.0^b	177.8^d	595^f	498.2^f	302.7^e	313.1^e	1.358^c	5.84^c	273.8^c
OFI 23/91	100	496.6^d	86.2^a	582^d	568.2^d	337.9^d	348.8^c	0.804^b	2.33^c	317.5^c
	200	382.6^a	164.1^c	546^b	452.6^b	267.4^c	276.7^f	1.376^c	4.63^b	297.7^b
OFI 62/91	100	441.8^c	83.3^a	524^e	535.1^e	298.1^e	308.8^d	0.84^b	2.31^c	264.4^d
	200	471.1^e	159.0^c	619^d	516.2^d	310.3^d	320.3^b	1.49^e	4.60^b	314.3^a
s.e.										
Provenance		29.66*	13.76*	33.40*	28.95*	17.91*	18.26*	0.20*	0.48*	16.11*
Level		18.72*	8.69*	21.10*	18.27*	11.30*	11.53*	0.13*	0.31*	10.20*
(P x L)		42.03	19.51	47.30	41.02	25.38	25.88	0.29	0.68	22.83

^{ab}Means in the same column with different superscripts differed significantly (P< 0.05)
(PxL) = provenance x level interaction, A = natural pasture hay intake, B = supplement intake, C = total dry matter intake (basal diet plus supplement), D = total organic matter intake, E = acid detergent fibre intake, F = neutral detergent fibre intake, G = acid detergent insoluble nitrogen intake, H = neutral detergent insoluble nitrogen intake, I = total extractable proanthocyanidins intake
*significant (P<0.05)
s.e. = standard error of the means

Table 4: Digestible DM, OM and N intake of five *C. calothyrsus* provenances given to goats as supplements to natural pasture hay

Provenance	level	DDMI (g/day)	DOMI (g/day)	DNI
OFI 9/89	100	244.2[a]	260.4[a]	2.80[a]
	200	221.4[d]	166.5[d]	3.02[e]
OFI 10/91	100	194.7[b]	222.9[b]	2.30[b]
	200	284.2[c]	223.7[b]	3.63[d]
OFI 12/91	100	249.2[a]	251.6[a]	3.01[c]
	200	279.1[b]	206.5[f]	4.20[f]
OFI 23/91	100	248.1[a]	260.7[a]	2.04[d]
	200	228.2[d]	158.2[e]	2.82[b]
OFI 62/91	100	229.3[c]	280.0[c]	2.04[d]
	200	275.4[b]	206.7[f]	4.43[g]
s.e.				
Provenance		19.61[*]	19.84[*]	0.538[*]
Level		12.38[*]	12.52[*]	0.339[*]
(P x L)		27.79	28.12	0.763

[ab]Means in the same column with different superscripts differed significantly (P< 0.05)
DDMI = digestible dry matter intake, DOMI = digestible organic matter intake, DNI = digestible nitrogen intake
[*]significant (P<0.05)
s.e. = standard error of the means

Nitrogen balance and microbial protein yield

The nitrogen balance and microbial protein yield of goats given five *C. calothyrsus* provenances as supplements to a basal diet of natural pasture hay are shown in Table 5. The nitrogen balance was similar in provenances OFI 9/89 and 12/91 at 100 g/d supplementation, while at 200 g/d supplementation the nitrogen balance was similar between provenances OFI 9/89 and 10/91 and between OFI 12/91 and 62/91. The microbial protein yield was the same between provenance OFI 9/89, OFI 10/91 and 62/91 at 100 g/d supplementation, while at 200 g/d supplementation provenance OFI 9/89 was similar to OFI 12/91 and OFI 23/91 was similar to OFI 62/91. The effect of provenance type and level of supplementation was significant (P<0.05). There was no interaction between provenance type and level of supplementation. The nitrogen balance at 100 grams supplementation was highest with OFI 12/91 while microbial protein yield at 100 g supplementation was highest with OFI 23/91.

Table 5: Nitrogen balance (g/day) and microbial protein yield (g/day) of goats given five *C. calothyrsus* provenances as supplements to a basal diet of natural pasture hay.

Provenance	Level	TNI	FN	UN	NB	MPY
OFI 9/89	100	5.67[a]	2.36[a]	1.15[a]	1.65[a]	2.33[a]
	200	6.31[d]	3.30[d]	0.80[e]	2.22[d]	2.43[e]
OFI 10/91	100	5.54[a]	3.23[b]	1.09[a]	1.21[b]	2.61[a]
	200	6.71[d]	3.07[d]	1.26[d]	2.37[d]	1.65[d]
OFI 12/91	100	5.70[a]	2.69[c]	1.21[a]	1.80[a]	3.76[b]
	200	7.75[c]	3.53[f]	1.02[c]	3.18[c]	2.32[e]
OFI 23/91	100	5.68[a]	3.63[d]	1.11[a]	0.93[c]	3.87[b]
	200	6.66[d]	3.83[d]	0.98[c]	1.85[f]	2.11[c]
OFI 62/91	100	4.43[b]	3.28[b]	0.79[b]	0.40[d]	2.49[a]
	200	7.88[c]	3.41[f]	0.96[c]	3.47[i]	2.06[c]
s.e.						
Provenance		0.363[*]	0.46[*]	0.64[*]	0.559[*]	0.56[*]
Level		0.229[*]	0.29[*]	0.41[*]	0.353[*]	0.35[*]
(P x L)		0.520	0.65	0.91	0.790	0.79

[ab]Means in the same column with different superscripts differed significantly (P< 0.05)
TNI = total nitrogen intake, FN = faecal nitrogen, UN = urine nitrogen, NB = nitrogen balance, MPY = microbial protein yield
[*] significant (P<0.0 5), s.e. = standard error of the means

DISCUSSION

The intake of supplements offered at 100g per day represented an average intake of 16% of total dry matter while 200g per day represented an average intake of 27% of dry matter. The dry matter digestibility at the two levels of supplementation was an average of 43% while the apparent nitrogen digestibility was 48%. Nherera, Ndlovu and Dzowela (1998) reported an apparent nitrogen digestibility of 56.0% at 30% inclusion levels. The low nitrogen digestibility can be attributed to the high condensed tannin content of the *C. calothyrsus* provenances (Abia, 2000). Ahn *et al.* (1989) reported dry matter digestibility of 45.9 % for oven dried *C. calothyrsus* while similarly low values of between 41 and 55.0% were reported by other workers (Salawu, Acamovic, Stewart and Maasdorp, 1997; Merkel, Pond, Burns and Fisher, 1999). Drying of *C. calothyrsus* has been shown to have a negative effect on the voluntary feed intake, with associated low *in sacco* digestibility (Salawu, *et al.,* 1997). On the contrary condensed tannin levels were decreased by drying (Ahn *et al.*, 1989), and supplements of dried *C. calothyrsus* increased both straw intake and nitrogen retention in sheep more effectively than fresh supplements (Ahn *et al.*, 1997)**.** Norton and Ahn (1997) feeding *C. calothyrsus* as a supplement reported that intake was unaffected by drying and the intake of the basal diet was significantly higher when dried supplement was fed.

The provenance type and level of supplementation had an effect on intake of the basal diet of natural pasture hay and on total dry matter intake. The dry matter intake, digestibility, total nitrogen flow to the duodenum and nitrogen apparently absorbed in the small intestine of sheep were also different in work with two provenances CIAT 22316 and CIAT 22310 (Lascano et al., 2003). The differences in nitrogen utilization were attributed to different tannin structures of the different provenances (Lascano *et al*., 2003). Extractable proanthocyanidins intake also differed among the provenances. This could be attributed to different proanthocyanidins content of the provenances which ranged from 29.3 g/kg DM to 56.0 g/kg DM (Abia, 2000). Nitrogen balance values were different between the two levels of supplementation, with goats on the higher supplementation level having higher values. Microbial nitrogen yield was also significantly ($P<0.05$) higher at higher levels of supplementation. The nitrogen balance values for all the five provenances were positive. This was in agreement with the positive values at 160g/day and 320g/day supplementation reported by Hove (2000). The positive nitrogen balance values reported show that the provenances had positive effects on ruminant animal productivity.

On the basis of total dry matter intake at 100 g/day supplementation OFI 23/91 had the highest intake with OFI 10/91 having the lowest. This trend seemed to be reversed at 200 g/day supplementation with OFI 10/91 having the highest intake and OFI 23/91 having the least intake. Provenance OFI 12/91 had the highest microbial protein yield while OFI 23/91 had the lowest.

At 100g supplementation the intake of supplement ranged from 83% to 91% while at 200 g supplementation the supplement intake ranged from 80% to 89%. In all cases the goats did not finish the supplements suggesting that all the *C. calothyrsus* provenances were not highly palatable. Provenance OFI 12/91 had the highest digestible dry matter and nitrogen at 100 g supplementation but not at 200g supplementation. While OFI 12/91 generally showed superiority in most parameters examined at 100g supplementation level no clear trend of provenance performance could be established.

CONCLUSION

This study showed that the five provenances of *C. calothyrsus* had different effects on the metabolism of goats. However, no clear trend of provenance performance could be established.

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