Can *Moringa oleifera* Be Used as a Protein Supplement for Ruminants?

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ABSTRACT : The possibility of using *Moringa oleifera* as a ruminant protein supplement was investigated by comparison between nutritive and anti-nutritive value of its different morphological parts with that of conventionally used *Leucaena leucocephala* leaf meal (LL). Parameters determined were chemical composition, rumen degradable protein (RDP), acid detergent insoluble protein (ADIP), pepsin soluble protein (PESP), non-protein nitrogen (NPN) total soluble protein (TSP) and protein potentially digested in the intestine (PDI). Total phenols (TP) and total extractable tannins (TET) were also evaluated as anti-nutritive factors. *In vitro* gas production characteristics were measured and organic matter digestibility (OMD) was estimated basing on 24 h-gas production. Crude protein content ranged from 265-308 g/kg DM in *M. oleifera* leaves (MOL) and seed cake (MOC) respectively. *Leucaena leucocephala* and *Moringa oleifera* soft twigs and leaves (MOLSTL) had CP content of 236 and 195 g/kg DM while *Moringa oleifera* soft twigs alone (MOST) and *Moringa oleifera* bucks (MOB) had 160, 114 and 69.3 g/kg DM respectively. RDP was highest in (MOC) (181 g/kg DM) followed by (MOL) (177 g/kg DM) and was lowest in MOB (40 g/kg DM). The proportion of the protein that was not available to the animal (ADIP) was ($p<0.05$) higher in MOL and MOC (72 and 73 g/kg DM) respectively and lowest in LL (29 g/kg DM). The PDI was high in LL (74 g/kg DM) followed by MOC (55 g/kg DM) then MOL (16 g/kg DM). PESP was highest ($p<0.05$) in MOC followed by MOL then LL (273, 200 and 163 g/kg DM respectively). MOC exhibited highest NPN content (116 g/kg DM) and was lowest in MOB (18 g/kg DM) ($p<0.05$). Highly ($p<0.05$) TSP was observed in MOC and MOL (308 and 265 g/kg DM respectively) followed by LL (236 g/kg DM). MOL had negligible TET (20 g/kg DM) when compared with about 70 g/kg DM in LL. Highly ($p<0.05$) b and a+b values were observed for MOLSTL (602 and 691 g/kg DM respectively) followed by MOL (490 and 538 g/kg DM). Highest c value was observed in MOSTL followed by MOC and MOL (0.064, 0.056 and 0.053 rate/hour) respectively. OMD was highest ($p<0.05$) for MOSTL followed by MOC and then MOL (579, 579 and 562 g/kg DM respectively). LL exhibited lower ($p<0.05$) OMD (467 g/kg DM). It was concluded from this study that the high crude protein content in MOL and MOLST could be well utilized by ruminant animals and increase animal performance however, high proportion of unavailable protein to the lower gut of animals and high rumen degradable protein due to negligible tannin content render it a relatively poor protein supplement for ruminants. MOC can be a best alternative protein supplement to leaves and leaves and soft twigs for ruminants. *(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 42-47)*

INTRODUCTION

In the tropics the scarcity for animal feed is one of the major constraints to increased livestock production particularly during the dry season. Farmers rely on crop residues and low quality standing hay to feed their animals. Crop residues are low in nitrogen and high in lignocellulose, which leads to low digestibility and low voluntary intake. Consequently the energy and nitrogen intake of animals raised on such feeds can not sustain adequate levels of performance (Schiene and Ibrahim, 1989).

Studies had shown that multipurpose trees can be used as cheap protein supplements which can improve digestibility, voluntary intake and general performance of animals fed low quality feeds (Kakengi et al., 2001). Multipurpose trees such as *Leucaena leucocephala* are widely used and are known to increase animal performance in many areas of the world. However, the use of *L.*

leucocephala is hampered by its susceptibility to psyllid (*Heteropsal*a *cubana*) attack. The psyllid attack has been reported to reduce its availability particularly during the dry season. *Gliricidia sepium* another multipurpose with potential as a good source of N. Its is however, limited by its low acceptability and higher contents of toxic compounds and odour (Norton, 1995). *Moringa oleifera Lam* (*syns. Moringa pterygosperm*, family Moringaceae) is a multipurpose tree, which was thought, could substitute *L. Leucocephala* as it possesses useful characteristics as multipurpose tree species. Its leaves and green fresh pods are used as vegetables by humans and are rich in carotene and ascorbic acid with a good profile of amino acids (Makkar and Becker, 1996). It is also used as livestock feed and its twigs are reported to be very palatable to ruminants and have appreciable crude protein levels (Sutherland et al., 1990; Sarwatt et al., 2002; Kimoro, 2002).

M. oleifera is native in Himalaya but is currently spread almost world-wide. However, there is scanty information worldwide on its potential as an animal feed. Studies by Sarwatt et al. (2002) on supplementation of *M. oleifera* to poor quality hay fed to growing Small East African Goats (SEAG) showed the existence of a negative nitrogen

balance to goats supplemented with 25 and 50% *M. oleifera* leaf meal. The preliminary results of effect of supplementing crop leftovers with equal amount of *Leucaena* leaf meal (LLM), *Grilicidia* leaf meal (GLM) and *M. oleifera* leaf meal (MOOL) show that goats fed on MOOL were outperformed in terms of growth rate by those fed on the other multipurpose trees. This is perplexing because MOOL has relatively higher crude protein (Sutherland et al., 1990; Makkar and Becker, 1997; Sarwatt et al., 2002) and low antnutritional factors (Makkar and Becker, 1997) yields low animal performance? Therefore, the purpose of this study was to evaluate and compare nutritive value of different morphological components of *M. oleifera* with *L. leucocephala* leaf meal in Tanzania and give recommendation on its use as a ruminant protein supplement.

MATERIALS AND METHODS

Sampling procedures

The sampling of the leaves, leaves and soft twigs, soft twigs, buck and seeds were collected by hand from an hectare of a well-established plot of *M. oleifera* tree plant. The *M. oleifera* trees have variable height ranging from 5-7 metres (18 months of age) with interspacing of 3×3 metres. Separation of the plant samples was done by fractionating them into desirable components. Buck of the plant was taken at a height from about 50 cm from the ground to 100 cm up the stem of the tree. The seeds were taken out of the pod sun dried and pressed to obtain the cake using a normal oil-seed pressing machine.

The samples of *M. oleifera* plant and leaves of *L. leucocephala* were freeze dried at -4°C, coarsely ground and packed in air tight containers ready for nutritive value evaluation.

Buffer soluble crude proteins and rumen degradability of proteins

Buffer soluble crude protein was measured by incubating 0.5±0.005 g of the sample in 40 ml borate phosphate buffer, pH 8.9, at 39°C for 24 h using a shaking water-bath. The mixture was filtered through a Whatman No.54 filter paper with a mild vacuum. The residue obtained was subjected to Kjeldahl protein determination procedure. Crude protein solubilized by the protease (Sigma Chemical Co.) was taken as a measure of rumen degradable crude protein (RDCP) (Krichna-moorthy et al., 1983).

The protein bound to ADF (acid detergent insoluble protein; ADIP) was considered to be unavailable to animals. Therefore, the difference between total crude protein and ADIP is the nitrogen available in the rumen and intestine, thus, the protein potentially digestible in the intestine (PDI)

is: total CP-(ADIP+RDCP).

Pepsin soluble nitrogen was determined by incubating 0.5 g of the sample with 0.5 g pepsin (Wako chemical industries Ltd. WTM 1606) and 50 ml of 0.1 M HCL at 39°C. After 20 h of incubation, the mixture was filtered through nitrogen free filter paper. The residue on the filter paper was made HCL-free by several washings with distilled water until the filtrate was of neutral pH. Nitrogen in the filter paper and the residue was determined using the Kjeldahl method. Pepsin soluble nitrogen was calculated by subtracting total nitrogen from that of the residue. The results were expressed on percentage basis.

Non protein nitrogen (NPN) determination

The NPN for each sample was determined according to the method of Licitra et al. (1996). About 0.5 g of the sample were weighed into Erlenmeyer flask and then added 50 ml of distilled water and allowed standing for 30 minutes. Ten mls of 10% tri-chloroacetic acid (TCA) was added and allowed to stand again for about 25 minutes and then filtered on a Whatman filter No. 54 and washing twice with (TCA) solution. The filter paper and the residue were transferred to Kjeldah flask for nitrogen determination.

Determination of anti-nutritional factors

Phenolics and tannin composition were determined from a 200 mg sample extracted with 10 ml 70% acetone in water bath maintained at 130 cycles per second for 90 minutes (Makkar, 2000) to let phenolics present in plant materials dissolve to liquid phase. Then centrifuged at 3,000 revolutions for 20 minutes at 4°C to recover phenolics and tannins in the supernatant. The collected supernatant was used for various assays. Total extractable phenolics was assayed calorimetrically by Folin-Ciocalteu assay (Jolkumen-Tiito, 1985). Folin-Ciocalteu assay coupled with tannin binding agent, polyvinyl polypyrolidone was used to estimate total extractable tannins gravimetrically (Makkar et al., 1993).

In vitro degradability

The *in vitro* degradation of *M. oleifera* leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST), buck (MOB), seed cake (MOC*)* and *L. leucocephala* leaves were determined from gas production measurements using the method of Menke and Steingass (1988). Triplicate dry samples (200±2 mg) milled to pass through 1 mm aperture were incubated into 20 ml of artificial saliva (containing a buffer solution, macro element, micro-element, reducing solution and resazurine) and 10 ml of rumen liquor. Gas volume (ml/200 mg sample DM) was read as the displacement of the syringe plunger at 6, 12, 24, 48, 72, 96 and 120 h. Rumen liquor used in this experiment was

Table 1. Chemical composition (g/kg DM) of *M. oleifera* leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST), backs (MOB), seed cake (MOC) and *L. leucocephala* (LL) harvested in Tanzania

Feedstuff	MOL	MOLST	MOST	MOB	MOC	LL
DM	883	882	881	892	897	8,897
CP	265	195	114	69	308	236
NDF	111	237	432	501	277	272
ADF	79	376	227	418	222	189
ADL	13	81	38	104	110	76
Hemicellulose	32	138	204	94	83	83
Cellulose	66	295	189	109	314	114
Ca	122	116	77	174	33	158
P	79	108	71	39	171	36

withdrawn on the last day of every period from all three sheep used in the trial. Corrected cumulative gas production data was fitted into the same model described as G=a+b (1-e^{-ct}), where G=corrected cumulative gas production after t hours; a+b=potential gas production; b=asymptote of gas production; and c=fraction rate of gas production.

Forage organic matter digestibility

In vitro organic matter digestibility (IOMD) was estimated using the equation of Menke and Staingass (1988) and Makkar and Becker (1996) based on 24 h gas production (Gv) and crude protein content (CP % DM): OMD (%) = 14.88+0.889 Gv+0.45 CP.

Preparation and collection of rumen fluid

The rumen fluid was obtained from 3 healthy mature Japanese corridale female sheep fitted with permanent rumen cannulae (70 mm). Animals were fed twice a day with a diet constitutes of 3 parts of Timothy hay and 1 part of concentrates (2 parts of wheat bran and rolled barley) to meet their body requirements (ARC, 1990). Mineral premix block and calcium carbonate were supplied to maintain a stable rumen environment. Rumen fluid was collected early in the morning before feeding, that is 8-14 h after previous feeding to maintain rumen fluid condition. The collected rumen fluid was handled and flushed with CO_2 to maintain anaerobic condition.

Chemical analysis

All forage components were milled through a 1 mm screen before being analysed. All feeds were analysed for DM, OM, CP and ash in accordance with the AOAC (1980) method. The ADF and NDF values were analysed according to Van Soest (1994).

Statistical analysis

All data were processed and analysed using a simple student's T-test of SAS (1999) program, and the means of all samples were separated for comparison using the least

significant difference at 5% level (p<0.05). The following model was used:

$$Y_{ij} = \mu + M_i + S_j + e_{ij}$$

Where; Y_{ij} = dependent variable in question
μ = general mean
M_i = the effect of ith morphological component
S_j = the effect of jth legume specie
e_{ij} = error term

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of different morphological components of *M. oleifera* and *L. leucocephala* are presented in Table 1. Between the morphological parts of *M. oleifera* the seed cake (MOC) had substantially higher CP content followed by leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST) and back (MOB) which exhibited the lowest CP content. More or less the reverse trend was true for the fibre fractions. Between species *M. oleifera* cake (MOC) and leaves (MOL) had higher CP values than *L. leucocephala*. The CP values obtained from MOL and MOB were comparable to those reported by Makkar and Becker, (1996) and Sarwatt et al. (2002) however, CP value for MOST was slightly higher than that observed by the former author, the difference could probably be attributed to difference on the stage of growth of the plant. The CP value for *L. leucocephala* was within the normal range reported elsewhere (Norton et al., 1990; Kakengi et al., 2001).

Protein degradability

In vitro rumen crude protein degradability (RDCP) at 24 h of incubation was higher (p<0.05) in MOC and MOL. MOLST, MOST and MOB, which had low RDCP (Table 2). However, when the RDCP was expressed as a percentage of the total CP content, MOST had the highest RDCP proportion followed by MOL, MOLST, MOC and MOB with 77.2, 66.8, 66.2, 58.4 and 58% respectively. LL had the lowest percentage RDCP (55.9). After subtracting NPN from the RDCP, the rumen degradable true protein (RDTP) was higher (p<0.05) in MOL (58.5%) and lowest in LL (19.9%). When ADIP values were presented as percentage of total CP were higher (p<0.05) in MOB (36.2%) followed by MOL, MOLST, MOC, MOST and LL (Table 2). This amount was then considered to be that which was unavailable to animals while 17.9, 7.2, 6.0, 5.8 and 1.8% MOC, MOLST, MOL, MOB and MOST respectively was the potentially digestible in the intestine or post-rumen. On the other hand 31.8 and 28% of the total CP in LL was found observed to be potentially digestible post-ruminally

Table 2. Mean (±SE) comparison of crude protein (CP), rumen degradable crude protein (RDCP), acid detergent insoluble protein (ADIP), protein potentially digestible in the intestine (PDI), buffer soluble crude protein (BSCP), non protein nitrogen (NPN) and pepsin soluble crude protein (PECP) contained (g/kg DM) in *M. oleifera* leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST), backs (MOB) and seed cake (MOC) with *L. leucocephala* (LL) harvested in Tanzania

Sample	CP (a)	RDCP (b)	ADIP (c)	PDI (a-(b+c))	NPN* (d)	RDTP** (e)	PSCP
MOL	265[b]±1.4 (66.8)	177[a]±1.9 (27.2)	72[a]±1.0 (6.0)	16[d]±0.5 (8.3)	22[c]±0.1 (58.5)	155[a]±0.1 (75.5)	200[a]±1.3
MOLST	195[d]±0.2 (66.2)	129[b]±0.1 (26.7)	52[b]±1.1 (7.2)	14[d]±1.0 (13.3)	26[c]±0.3 (52.8)	103[b]±0.1 (70.8)	138[c]±0.4
MOST	114[f]±0.2 (77.2)	88[d]±0.2 (21.1)	24[d]±0.1 (1.8)	2[e]±0.1 (19.3)	22[c]±0.5 (57.9)	66[d]±0.4 (57.9)	66[d]±0.4
MOB	69[g]±0.1 (58.0)	40[e]±0.1 (36.2)	25[d]±0.2 (5.8)	4[e]±0.1 (26.1)	18[c]±1.1 (31.9)	22[f]±0.1 (1.4)	1[e]±0.1
MOC	308[a]±0.1 (58.4)	180[a]±0.9 (23.7)	73[a]±0.1 (17.9)	55[b]±1.2 (37.7)	116[a]±0.5 (20.8)	64[d]±0.2 (88.6)	273[b]±0.1
LL	236[c]±1.3 (55.9)	132[b]±0.2 (12.3)	29[c]±0.2 (31.8)	75[a]±1.3 (36.0)	85[b]±0.3 (19.9)	47[e]±0.2 (69.1)	163[b]±1.0

* Expressed as CP (N×6.25).
** e=b-d; as NPN (d) is considered to be completely digestible in the rumen.
Values in the parentheses are the percentage of the total protein.

(p<0.05) (Table 2).

The differences in RDCP and PDI between MOL, MOLST and LL could probably be attributed to differences in concentration of tannin or other anti-nutritive compounds as well as protein (Salawu et al., 1997). By-pass protein in LL could be beneficial in the rumen as tannin protein complexes have been reported to be cleaved at low gastric pH (2.5-3.5) in the abomasum and the high pH (8-9) in the small intestine (Mangan, 1988). If cleavage of such protein complex occurs with tannins in LL leaves, the tannin protein complex formed in the rumen could be of practical advantage to the animal if the protein is released for digestion in the small intestine (Barry and Manley, 1986; Kumar and D'Mello, 1995). However, not all concentrations of tannins in the plant can be beneficial to animal. For example condensed tannin concentration of 5% is reported to be advantageous to animal while above this level could be detrimental (Mangan, 1988) by displacing proteins from the pre-formed tannin-protein complex (Jones and Mongan, 1977) or may inhibit microbial endoglucanase activity and the degradation of cellulose (McAllister et al., 1994). Results from this study show that there is a minimum tannin concentration below which a protein content of forage could be beneficial to the ruminant animal. *M. oleifera* leaves and leaves and soft twigs which are the normally supplemented parts to animals were found to have low tannin concentrations (≤2%) hence relatively poor protein supplement to ruminant animals. The tannin concentrations in MOL and MOLST observed in this study slightly differ from those reported by Markkar and Becker (1996). The differences could be due to differences in soil conditions and climate (Barry and Duncan, 1984) from which the samples were taken.

NPN and pepsin soluble proteins

The NPN as a percentage of total nitrogen was (p<0.05) lower in MOL (8.3%) than in MOLST (13.3%) and MOST (19.3%). However, higher NPN percentages were observed in MOC (37.7%) and LL (36.0%). High solubility of protein was observed in MOC where about 89% of its total protein solubirised in acid-pepsin enzyme followed by MOL, MOLST, LL, MOST and MOB which had 75.5, 70.8, 69.1, 57.9 and 1.4% respectively (Table 2). The acid pepsin digestible protein in MOL could be regarded as a potential rumen by pass protein but a limitation of high RDCP render it rather less suitable to ruminants than monogastric animals. In a more recent study (Kaijage, 2002) on substitution of MOLST for the more expensive protein source has shown better poultry performance in terms of growth and production

Gas production characteristics, organic matter digestibility and anti-quality contents

The degradability characteristics in the data for production MOL, MOLST, MOST, MOB, MOC and LL incubated *in vitro* and the estimated organic matter digestibility (OMD), total phenols (TP) and total extractable tannins (TET) are presented in Table 3. Figure 1 shows the *in vitro* gas production curves for MOL, MOLST, MOST, MOB, MOC and LL respectively. High gas productions were prominently higher for all incubation hours for MOLST, MOL and MOC than for LL. MOST had the lowest gas production values followed by MOB (Figure 1). The presence of CP, NDF and TET (Tables 1, 2 and 3) may have affected gas production *in vitro*. For MOL, MOLST and MOC the potential gas production (a+b) and asymptote (b) were significantly influenced by high protein and low

Table 3. Mean (±SEM) (g/kg DM) degradability characteristics, organic matter digestibility (OMD), total phenols (TP) and total extractable tannins (TET) of *M. oleifera* leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST), back (MOB), seed cake (MOC) as compared to *L. leucocephala* (LL) harvested in Tanzania

Sample	Gas production parameters (ml/200 mg DM)			OMD*	TP	ET
	b	a+b	c			
MOL	$490^b \pm 4.4$	$538^b \pm 3.4$	$0.05^a \pm 0.003$	$562^b \pm 2.2$	$31^b \pm 0.01$	$20^b \pm 0.05$
MOLST	$603^a \pm 4.3$	$692^a \pm 4.9$	$0.064^a \pm 0.005$	$579^a \pm 2.5$	$24^b \pm 0.01$	$18^c \pm 0.01$
MOST	$269^e \pm 0.3$	$270^e \pm 0.3$	$0.024^c \pm 0.002$	$306^e \pm 0.5$	$13^c \pm 0.01$	$9^d \pm 0.08$
MOB	$418^d \pm 0.5$	$503^c \pm 1.6$	$0.029^c \pm 0.005$	$420^d \pm 0.1$	$8^c \pm 0.06$	$7^d \pm 0.02$
MOC	$458^c \pm 0.3$	$547^b \pm 0.1$	$0.056^a \pm 0.002$	$579^a \pm 0.2$	$8^c \pm 0.50$	$7^d \pm 0.48$
LL	$423^d \pm 1.0$	$435^d \pm 0.4$	$0.036^b \pm 0.005$	$468^c \pm 2.8$	$85^a \pm 0.26$	$70^a \pm 0.06$

Means without common superscript in columns of a sample are significantly different ($p<0.05$). *Abbreviations*: a, zero time intercept; b, asymptote of gas production; a+b, potential gas production; c, fraction rate of gas production (fraction/hour); SE, standard error of the mean. * OMD=14.88+0.889 Gv+0.45 CP; Gv, 24 h gas production; CP, crude protein content.
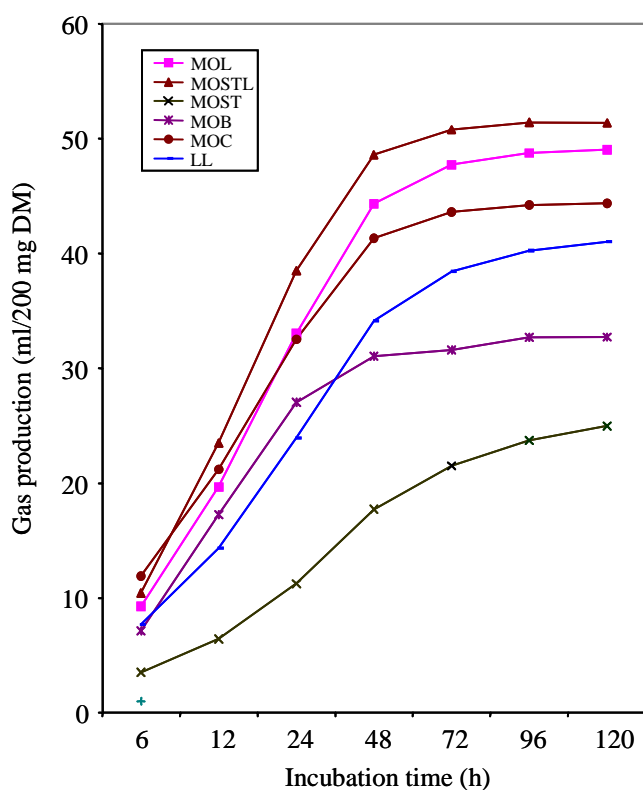

Figure 1. Gas production from *M. oleifera* leaves and soft twigs (MOLST), soft twigs (MOST) and seed cake compared to *L. leucocephala* (LL) incubated in rumen liquor from sheep.

concentration of total extractable tannin contents (Table 3). From this results grading *M. oleifera* morphological parts with reference to LL which was proposed to be used as a standard for tanniniferous tree legumes with desirable nutritive value and fermentation characteristics (McSweeney et al., 1999) MOC found to be a better ruminant supplement than the leaves, leaves and soft twigs and the rest of the parts. However, there are speculations that MOC contains alkaloids, saponins, cyanogenic glucosides and glucosinolates therefore needs to be pre treated before it is fed to animals as it can has deleterious effect when fed. However, literature reveals that

concentrations of such antinutritional factors vary with the variety (Dogra et al., 1975). Seeds of some varieties are consumed by humans after roasting and taste like groundnuts (Ramachandra et al., 1980). Furthermore, the MOC is used to purify water that is consumed by human being, possibly there could be some sub-clinical or clinical effect on internal organs. Thus, necessitate further studies on possibility of MOC grown in Tanzania to having harmful effect to animals before full recommendations on its use as a direct or pre-treated protein supplement to ruminants.

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REFERENCES

AOAC. 1980. Association of Agriculture Chemist Official Methods of analysis. 9th Edition.

ARC. 1990. Agricultural Research Council. The nutrient requirement of ruminant livestock. 4th edition. CAB International Wallingford Oxon, UK. pp. 73-310.

Barry, T. N. and T. R. Manley. 1986. Interrelationship between the concentration of total condenced tannins, free condensed tannins and lignin in *Lotus sp*. And their possible consequence in ruminant nutrition. J. Sci. Food Agric. 37:248-254.

Dogra, P. D., B. P. Singh and S. Tandon. 1975. Vitamin content in Moringa pod vegetable. Curr. Sci. 44:125-127.

Jolkumen-Tiito, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. J. Agric. Food Chem. 33:213-217.

Jones, W. T. and J. L. Monagan. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop) with fraction 1 leaf protein and with sub maxillary mucoprotein and their reversal by polyethelene glycol and pH. J. Sci. Food Agric. 28:126-136.

Kaijage, J. K. 2002. Effect of substituting *Moringa oleifera* leaf meal for cotton seed cake as a protein supplement on feed intake, growth performance and digestibility of low quality hay using sheep weaners. Partial submitted MSc. Thesis.

Sokoine University of Agriculture.

Kakengi, A. M. V., M. N. Shem, R. Otsyina and E. Mtengeti. 2001. Performance of grazing cattle in semi+arid areas of Western Tanzania and the marginal productivity of *Leucaena leucocephala* leaf meal supplement. *Agroforestry*, Systems 52:73-82.

Kimoro, B. N. 2002. Potential of selected Mult-purpose trees as feed for growing goats on maize stover basal diet. On going study.

Krishnamoorthy, U., C. J. Sniffen, M. D. Stern and Van Soest, P. J. 1983. Evaluation of the mathematical model of rumen digestion and *in vitro* simulation of rumen proteolysis to estimate rumen undegraded nitrogen content of feedstuffs. Br. J. Nutr. 50:555-568.

Kumar, R. and J. P. F. D'Mello. 1995. Anti-nutritional factors in forage legumes. In: (Ed. J. P. F. D'Mello, C. Devendra), Tropical legumes in animal nutrition, CAB International, Wallingford, UK, pp. 95-133.

Licitra, G., T. M. Hernandez and P. J. VanSoest. 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.

Makkar, H. P. S. and K. Becker. 1996. A bioassay for polyphenols (tannins). Polyphenols communications 96, Bordenaux (France). July 15-18, 1996.

Makkar, H. P. S. and K. Becker. 1997. Nutrients an antiquality factors in different morphological parts of the Moringa oleifera tree. J. Agric. Sci. 128:311-322.

Makkar, H. P. S., M. Blummel and K. Becker. 1993. Formation of complexes between polyvinylpyrroidones or polyethylene glycols and tannins and their implication in gas production and true digestibility *in vitro* techniques. Br. J. Nutr. 73:897-913.

Makkar, H. P. S. 2000. Quantification of tannins in tree foliage. A laboratory mannual for the FAO/IAEA coordinated research project on 'Use of Nuclear and Related Techniques to Develop Simple Tannin Assay for Predicting and Improving the safety and Efficiency of Feeding Rumminants on Tanniniferous Tree Foliage'. Joint FAO/IAEA Vienna, Austria, 2000.

Mangan, J. L. 1988. Nutrition effects of tannins in animal feed. Nutr. Res. Rev. 1:209-231.

McAllister, T. A., H. D. Bae, L. J. Yanke, K. J. Cheng and A. Muir. 1994. Effect of condensed tannins from birdstoof trefoil on endoglucanase activity and the digestion of cellulose filter paper by anaerobic fungi. Can. J. Microbiol. 40:298-305.

Menke, K.H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Develop. 28:7-55.

Norton, B. W. 1995. Browse legumes as supplements. In: Forage tree legumes in Tropical Agriculture (Ed. A. R. C. Gutteridge and H. M. Shelton) CAB Int. Wallingford, UK. pp. 245-257.

Ramachandran, C., K. V. Peter and P. K. Gopalakrishman. 1980. *Drumstic (Moringa oleifera)*: a multipurpose Indian vegetable. Economic botany 34:276-283.

Salawu, M. B., T. Acamovic, C. S. Stewart and F. D. DeB. Hovell. 1997. Assesment of Caliandra colothyrsus: *in sacco* degradation and *in vitro* gas production in the presence of Quebrancho tannins with or without browse plus. Anim. Feed Sci. Technol. 69:219-232.

Sarwatt, S. V., S. S. Kapange and A. M. V. Kakengi. 2002. The effects on intake, digestibility and growth of goats when sunflower seed seed cake is replaced with Moringa oleifera leaves in supplements fed with *Chloris gayana* hay. *Agroforestry*, Kluwer Academic Publisher (In press).

Sarwatt, S. V. 1989. Feed intake, Growth Rate and Digestibility Coeffients of growing sheep fed hay supplemented with Crotalaria orchroleuca. Animal Feed Science Technology 28:51-59.

SAS. 1999. Statistical analysis system. SAS/STAT User's guide. Statistical analysis Institute, Inc. Carry, NC, USA.

Sutherland, J. P., G. K. Folkard and W. D. Grant. 1990. Natural coagulants for appropriate water treatment: a novel approach, Waterlines, April (4), pp. 30-32.

Van Soest, P. 1994. Nutritional ecology of the ruminants. O and B Books. Corrallis, Oregon, p. 374.