Evaluation of Mulberry (*Morus alba*) as Potential Feed Supplement for Ruminants: The Effect of Plant Maturity on *In situ* Disappearance and *In vitro* Intestinal Digestibility of Plant Fractions

D. Saddul, Z. A. Jelan*, J. B. Liang and R. A. Halim[1]

Dept. of Animal Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT : The *in situ* nylon bag degradation and *in vitro* intestinal digestibility of dry matter (DM), and crude protein (CP) of mulberry (*Morus alba*) plant fractions was studied at four harvest stages, 3 (W3), 5 (W5), 7 (W7) and 9 (W9) weeks. Degradability of DM and CP of the whole plant and stem fractions declined significantly ($p<0.01$) with advancing plant maturity in the order W3>W5 and W7>W9 and W3>W5>W7>W9, respectively. The degradation of DM and CP of the leaf fraction was also influenced by plant maturity but no trend was observed. The degradation of DM and CP of the whole plant and leaves increased rapidly during the first 48 and 24 h of incubation, respectively, when maximum degradation was reached. *In vitro* intestinal digestibility of CP was more influenced by the residence time in the rumen than by plant maturity. This study showed that mulberry is suitable as a supplement, particularly to low-quality roughages, in providing a source of rapidly available nitrogen to the rumen microbes, hence improving the roughage degradability and intake. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1569-1574)***

Key Words : Maturity, Mulberry, Nylon Bag, Nutrient Degradation, Low-quality Roughages

INTRODUCTION

Fodder trees have been shown to have tremendous potential as protein supplements (Devendra, 1993; Leng et al., 1993; Thorne et al., 1999) to provide soluble nitrogen, by-pass protein, whilst providing a source of energy and minerals, which are required by the rumen microorganisms. The multipurpose shrub, mulberry (*Morus alba*), offers a promising alternative as protein supplement (Sanchez 2002; Singh and Makkar, 2002) to low-quality basal diets, given the high contents of crude protein (CP) (Sanchez, 2002), minerals and metabolisable energy (ME) as well as its low fibre content (Sanchez, 2002; Saddul et al., 2003). Mulberry has been shown to have positive effects on rumen functions and body metabolism and is suitable for improving the efficiency of utilization of low-quality fodder (Yao et al., 2000; Liu et al., 2002; Singh and Makkar, 2002). Research on mulberry as a protein source in ruminant diets focused mainly on the utilization of the leaves but information on the whole plant is sparse. A first study recently conducted in Malaysia to evaluate its potential productivity under local conditions has been conclusive with respect to the nutritional composition of the whole plant as well as the leaves (Saddul et al., 2003).

This present study aims at evaluating the effects of plant maturity on the degradability characteristics of mulberry with reference to its eventual utilisation as a protein supplement to low-quality fibrous diets.

* Corresponding Author: Z. A Jelan. Tel: +60-603-89468298, Fax: +60-603-89432954, E-mail: aznam@agri.upm.edu.my

[1] Department of Crop Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

Received December 19, 2003; Accepted July 12, 2005

MATERIALS AND METHODS

Animals and diets

Three Brahman×Kedah-Kelantan bulls (body weight: 275±20 kg) fitted with rumen cannula were used to determine the *in situ* disappearance of mulberry plant fractions. The animals, housed in individual pens (3×5 m), were maintained on *Pennisetum purpureum* (Napier grass) and mulberry (70:30) at a dry matter (DM) intake of 1.5% of body weight. They were allowed a 14-day adaptation period prior to the start of the study. The feed was offered in two equal portions at 8.3 0 h and 16.30 h and drinking water was freely accessible.

Sample preparation

Mulberry whole plant, sampled at four harvest stages, 3 (W3), 5 (W5), 7 (W7) and 9 (W9) weeks, respectively, was separated into leaf (with petiole) and stem fractions and oven-dried at 60°C for 72 h. The samples were ground to pass through a 2-mm sieve for chemical analysis and nylon bag study.

Rumen degradability

Nylon bags (average pore size of 45 μm; size 6×12 cm), containing about 5 g of sample were prepared in duplicate and inserted in the rumen of each animal before the morning feeding. The bags were retrieved at 4, 8, 12, 24, 48, 60 and 72 h post-incubation, immediately immersed in cold water to inhibit further microbial action, then washed under running tap water and kept at -20°C overnight. The frozen

Table 1. Chemical composition of mulberry at four harvest stages (% DM)

	W3	W5	W7	W9	SEM
Whole plant					
DM	25.2^{c}	30.9^{b}	30.3^{b}	34.4^{a}	0.4
CP	31.2^{a}	30.0^{b}	21.8^{c}	17.9^{d}	2.1
OM	91.9^{b}	90.5^{c}	92.6^{b}	94.2^{a}	0.6
NDF	26.8^{d}	32.1^{c}	36.4^{b}	54.4^{a}	3.9
ADF	21.8^{d}	27.7^{c}	30.3^{b}	45.1^{a}	3.2
Leaf					
DM	24.9^{c}	31.8^{ab}	30.4^{b}	32.4^{a}	0.4
CP	35.9^{a}	32.2^{b}	28.4^{c}	30.9^{d}	1.0
OM	89.4^{b}	88.3^{c}	90.7^{a}	90.2^{ab}	0.4
NDF	28.9^{a}	31.2^{a}	25.6^{b}	31.4^{a}	0.9
ADF	15.9^{b}	24.9^{a}	21.1^{a}	16.4^{b}	1.5
Stem					
DM	30.9^{b}	24.1^{c}	23.7^{c}	36.3^{a}	0.8
CP	16.8^{a}	10.2^{b}	7.9^{c}	7.4^{d}	1.4
OM	88.6^{d}	93.3^{c}	94.8^{b}	96.0^{a}	1.1
NDF	58.0^{c}	64.4^{b}	69.5^{a}	70.1^{a}	1.9
ADF	44.2^{d}	48.2^{c}	51.9^{b}	55.3^{a}	1.7

Means with different superscripts in a row differ significantly ($p<0.01$). SEM: Standard error pooled across harvest stages.

bags were thawed, washed with cold water in a domestic washing machine for 3×5 min and dried in a forced-air oven at 60°C for 72 h. Washing losses were determined by soaking 4 bags containing about 5 g of sample in tap water for 20 minutes and by treating them in the same way as the incubated bags but without incubation in the rumen. The dried residues were pooled by replicates for chemical analysis.

Intestinal digestibility

Intestinal digestibility of whole plant and leaf fractions of mulberry was determined by the three-step *in vitro* procedure of Calsamiglia and Stern (1995), modified according to McNiven et al. (2002). A separate set of samples was incubated in duplicate nylon bags for 12 and 16 h to obtain ruminal residues for the determination of intestinal digestibility. In brief, about 0.3 g of ruminal residues was weighed into mobile bags (3.5×5 cm) and incubated for 24 h in a buffered pepsin-pancreatin solution in a shaking water bath maintained at 39°C. At the end of the incubation, the bags were washed thoroughly with distilled water, dried in oven at 60°C for 72 h and weighed to obtain the DM loss. The dried residues were analysed for Kjeldahl-N.

Calculation of degradability parameters

The degradation constants for DM and CP were calculated from the non-linear model proposed by Ørskov and McDonald (1979) using the Neway Excel Programme (Chen, 1995): $p = a+b\ (1-e^{-ct})$, where, p is the amount of nutrient degraded (%) at time *t*, a is the rapidly soluble fraction (%), b is the rumen-insoluble but potentially degradable fraction (%), c is the rate of degradation of the *b* fraction (h^{-1}) and *t* is the time of incubation (h). The effective degradability (ED) of DM and CP was estimated from the equation: ED = a+[(b×c)/(c+k)], where, k is the estimated rate of solid outflow from the rumen. Potential degradability (PD) was estimated as (a+b).

Chemical analysis

The feed samples were analysed for DM, organic matter (OM) and Kjeldahl-N according to the procedures of AOAC (1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by the method of Van Soest et al. (1991). The residues were analysed for DM and Kjeldahl-N.

Statistical analysis

Analysis of variance (ANOVA) using a randomized block design (Gomez and Gomez, 1984) was carried out on the degradation parameters a, b, c, ED and PD for DM and CP of mulberry plant fractions using the GLM procedure of SAS (1990), with harvest stage as treatment effects and the animals as blocks.

RESULTS AND DISCUSSION

The chemical composition of the mulberry plant fractions is given in Table 1. The DM, OM, NDF and ADF contents of all plant fractions increased significantly ($p<0.01$) with advancing plant maturity, whereas the CP content declined significantly. This is consistent with findings from previous studies (Saddul et al., 2003). The leaf to stem ratio at W3, W5, W7 and W9 was respectively 5.2, 1.9, 1.7 and 0.88 and declined significantly ($p<0.01$) with advancing maturity as reported by Saddul et al. (2004).

The degradation of DM and CP decreased significantly ($p<0.01$) with plant maturity at harvest in the following order: W3>W5 and W7>W9 (Tables 2 and 3). There were significant plant maturity-incubation time interactions ($p<0.05$) for DM and CP. Overall, nutrient degradation increased significantly from 4 to 48 h of incubation ($p<0.01$), following which, nutrient loss till 72 h of incubation was not significant.

DM disappearance

Degradability of DM of mulberry plant fractions decreased significantly ($p<0.01$) with increasing intervals between harvests, with more pronounced effects on the whole plant and stem fraction, compared to the leaves. Dry matter disappearance of all plant fractions increased significantly from 4 to 48 h of incubation at all harvest stages, after which, DM losses were not significant till 72 h. Overall DM degradability of whole plant, leaf and stem

Table 2. DM degradability characteristics of mulberry plant fractions at four maturity stages

Parameter	Harvest stage					
	W3	W5	W7	W9	Overall	SEM
Whole plant						
a (%)	43.8^{a}	35.9^{b}	35.2^{b}	29.4^{c}	36.1	1.9
b (%)	48.5^{a}	52.5^{a}	48.7^{a}	41.9^{b}	47.9	1.6
PD (%)	92.3^{a}	88.4^{ab}	83.9^{b}	71.4^{c}	83.9	0.38
ED^{2} (%)	69.3^{a}	60.4^{b}	59.6^{b}	50.3^{c}	59.9	2.6
c (fraction^{-1})	0.076	0.059	0.078	0.077	0.073	0.004
Leaf						
a (%)	48.3^{a}	50.3^{a}	52.2^{a}	44.6^{b}	44.2	3.0
b (%)	47.3^{b}	45.2^{b}	44.0^{b}	50.8^{a}	51.4	3.0
PD (%)	95.6	95.5	96.2	95.4	95.6	0.17
ED (%)	73.8	73.2	74.2	71.3	71.3	1.3
c (fraction^{-1})	0.113^{a}	0.084^{b}	0.089^{b}	0.084^{b}	0.093	0.047
Stem						
a (%)	33.6^{a}	26.1^{b}	23.3^{c}	21.8^{d}	26.2	1.7
b (%)	41.7^{a}	36.3^{b}	33.4^{c}	30.2^{d}	35.4	1.6
PD (%)	75.3^{a}	62.5^{b}	56.7^{c}	51.9^{d}	61.6	3.3
ED (%)	61.6^{a}	46.2^{b}	41.6^{c}	37.9^{d}	46.8	3.4
c (fraction^{-1})	0.172^{a}	0.085^{b}	0.072^{b}	0.082^{b}	0.103	0.015

Means with different superscripts in a row differ significantly ($p<0.05$).
SEM: standard error of means.
PD[1]: Potential degradability.
ED[2]: Effective degradability at an outflow rate of 0.05 h^{-1}.
a and b are respectively the rapidly soluble and rumen-insoluble but potentially degradable fractions.
c is the rate of degradation of the b fraction.

Table 3. CP degradability characteristics of plant fractions at four harvest stages

Parameter	Harvest stage					
	W3	W5	W7	W9	Overall	SEM
Whole plant						
a (%)	41.2^{a}	40.5^{ab}	33.9^{b}	35.5^{b}	37.8	1.4
b (%)	58.6	59.8	63.1	59.7	60.1	0.9
PD (%)	99.8^{a}	99.3^{b}	96.9^{ab}	95.2^{b}	97.8	0.7
ED (%)	71.3^{a}	66.6^{b}	65.9^{b}	63.5^{b}	66.8	1.1
c (fraction^{-1})	0.075	0.065	0.073	0.074	0.071	0.002
Leaf						
a (%)	46.1^{a}	36.0^{ab}	46.4^{a}	31.1^{b}	39.9	2.7
b (%)	51.8^{b}	52.5^{b}	53.4^{b}	68.4^{a}	59.0	2.8
PD (%)	97.9	98.5	99.8	99.4	98.9	0.35
ED (%)	71.9^{a}	70.5^{a}	69.8^{a}	63.5^{b}	67.1	1.5
c (fraction^{-1})	0.104^{a}	0.078^{b}	0.078^{b}	0.074^{b}	0.080	0.005
Stem						
a (%)	54.6^{a}	51.2^{b}	48.9^{c}	47.2^{d}	50.5	1.1
b (%)	36.4^{a}	29.2^{b}	26.7^{c}	23.1^{d}	28.8	1.9
PD (%)	90.9^{a}	80.5^{b}	73.9^{c}	72.0^{d}	79.3	2.8
ED (%)	79.8^{a}	67.6^{b}	64.2^{c}	64.3^{c}	68.9	2.4
c (fraction^{-1})	0.199^{a}	0.099^{c}	0.119^{bc}	0.140^{b}	0.139	0.014

Means with different superscripts in a row differ significantly ($p<0.01$).
SEM: Standard error of means.
PD[1]: Potential degradability.
ED[2]: Effective degradability at an outflow rate of 0.05 h^{-1}.

fractions was 66.7, 77.9 and 51.5%, respectively. Reports available on the DM degradability of mulberry (Liu et al., 2002; Shayo, 2002; Singh and Makkar, 2002; Bakshi and Wadhwa, 2004) and they focused mainly on the leaves but in most cases the age of the leaves at harvest were not specified.

The soluble fraction, a, of whole plant DM decreased significantly ($p<0.01$) with advancing plant maturity (Table 2) from 43.8% at W3 to 29.4% at W9. There was, however, no significant influence of plant maturity on the soluble fraction of leaf and stem DM. The average value of the soluble fraction of whole plant, leaf and stem, pooled across harvest stages, was 36.1%, 44.2 and 26.2%, respectively. These results are within the range of values of 20-40% reported by Shayo (1997) for young leaf and bark and old bark, respectively, and 30.4% for whole plant by Sanchez (2002). However, Schmidek et al. (2002) reported a lower *a* value of 31.4% for 90 d old leaves while that reported by Liu et al. (2002) was 20.5%.

The insoluble but potentially fermentable fraction, b of whole plant and stem DM declined significantly ($p<0.01$) with advancing plant maturity (Table 2) whereas that of the leaves declined significantly only after W7. The b value for whole plant obtained in this study was comparable with that reported by Sanchez (2002) and Liu et al. (2002).

Given the significant effects of maturity on the soluble fractions of the whole plant and stem, the potential degradability (PD) declined significantly from 92.3% at W3 to 71.4% at W3 (Table 2). The mean PD of the leaf fraction was 95.6% and was not influenced by maturity. The PD of the whole plant and the leaf reported by other authors (Liu et al., 2002; Sanchez, 2002; Singh and Makkar, 2002) was lower than obtained in this study.

The overall effective degradability (ED) of all plant fractions declined significantly from W3 to W9 ($p<0.01$). Increasing the outflow rate from 0.02 to 0.08 resulted in a significant decrease in the ED of DM for all plant fractions at all harvest stages.

The lag phase was not influenced by plant maturity for all plant fractions and was 3.4, 4.3 and 2.5 h for the whole plant, leaf and stem, respectively. The rate of degradation, c, of whole plant DM was 0.073 and was not influenced by harvest stage. The rate of degradation of the leaf and stem fractions was higher ($p<0.01$) at W3 (0.113 and 0.172, respectively) and this value decreased to 0.084 and 0.082, respectively with advancing maturity to W9.

Crude protein disappearance

Overall protein degradability decreased with plant maturity ($p<0.01$) for all plant fractions and was 75.3, 74.1 and 72.5%, for the whole plant, leaf and stem fractions, respectively. Plant maturity significantly ($p<0.01$) decreased the soluble fraction a of all plant fractions, the b fraction of the stem and the c fraction of the leaves and stem. Consequently this resulted in a significant ($p<0.01$) decline in the PD of the whole plant. Liu et al. (2002) reported lower values for the a and b fractions (19.5 and 50.2,

Table 4. DM disappearance (%) in the rumen and *in vitro* intestinal digestibility at four maturity stages

	Rumen incubation time					
	12 h			16 h		
	Ruminal	Intestinal	Total tract	Ruminal	Intestinal	Total tract
Whole plant						
W3	68.7[a]	11.6	80.4[a]	85.5[a]	3.6[b]	89.0[a]
W5	67.4[a]	11.2	78.7[a]	74.8[b]	8.4[a]	83.2[b]
W7	60.3[ab]	9.9	70.3[b]	71.2[b]	5.6[b]	76.7[c]
W9	53.7[b]	8.4	62.1[c]	63.5[c]	4.9[b]	68.4[d]
Mean	62.5	10.3	72.8	73.7	5.6	79.3
SEM	2.1	0.8	1.8	1.8	0.5	1.7
Leaf						
W3	74.3	10.1[ab]	84.4	86.8	5.1	91.9[a]
W5	65.2	13.0[a]	78.2	78.7	7.9	86.7[b]
W7	77.2	8.8[b]	86.1	87.3	4.7	91.9[a]
W9	66.3	12.9[a]	79.2	82.8	6.1	88.9[ab]
Mean	70.8	11.2	81.9	83.9	5.9	89.9
SEM	1.9	0.6	1.4	1.2	0.5	0.8

[1] Percent of total DM disappearing in the rumen (RDDM %), calculated as (DM in sample before rumen incubation-DM in residue after rumen incubation)/DM in sample before rumen incubation×100.

[2] Percent of total DM disappearing after *in vitro* incubation, calculated as (DM in sample before rumen incubation-RDDM-DM in residue after *in vitro* incubation)/DM in sample before rumen incubation×100.

[3] RDDM–*in vitro* disappearance (%).

Means with different superscripts in a column differ significantly ($p<0.05$). SEM: Standard error of means.

Table 5. Ruminal CP disappearance (%) and *in vitro* intestinal digestibility of mulberry fractions at four maturity stages

	Rumen incubation time					
	12 h			16 h		
	Ruminal	Intestinal	Total tract	Ruminal	Intestinal	Total tract
Whole plant						
W3	72.8	22.9	95.8	90.8[a]	7.2[b]	98.1[a]
W5	70.7	23.9	94.6	77.9[c]	18.3[a]	96.2[c]
W7	70.3	25.2	95.5	83.3[b]	13.9[a]	97.3[b]
W9	72.2	23.0	95.3	82.7[bc]	14.4[a]	97.2[b]
Mean	71.5	23.8	95.3	83.7	13.5	97.2
SEM	1.7	1.5	0.29	1.2	1.1	0.18
Leaf						
W3	74.8	19.4	94.2	88.8	8.8	97.6
W5	65.8	26.4	92.3	84.0	11.5	95.5
W7	74.3	21.2	95.5	90.5	7.1	97.6
W9	69.2	24.7	93.9	89.6	7.0	96.6
Mean	71.0	22.9	93.9	88.2	8.6	96.8
SEM	1.6	1.2	0.6	0.9	0.8	0.3

[1] Percent of total CP disappearing in the rumen (RDP %), calculated as (CP in sample before rumen incubation-CP in residue after rumen incubation)/CP in sample before rumen incubation×100.

[2] Percent of total CP disappearing after *in vitro* incubation, calculated as (CP in sample before rumen incubation-RDP-CP in residue after *in vitro* incubation)/CP in sample before rumen incubation×100.

[3] RDP–*in vitro* disappearance (%).

Means with different superscripts in a column differ significantly ($p<0.05$). SEM: Standard error of means.

respectively) and PD for the leaves (69.7%), without specific mention of the leaf age.

Plant maturity and outflow rate influenced the ED of protein for all plant fractions, but interaction between maturity and outflow rate was non-significant. The mean ED of whole plant, leaf and stem fractions at an outflow rate of 0.05 was 66.8, 67.1 and 68.9%, respectively.

Plant maturity did not influence the lag phase of whole plant (mean 3.9 h) and leaf fraction (mean 6.1 h), while the lag phase for the stem fraction was higher ($p<0.01$) at W3 and W5 (respectively, 3.0 and 3.4 h) than at W7 and W9 (2.1 h). The rate of degradation, c, of whole plant CP was not influenced by plant maturity (mean 0.074 h), but it was decreased significantly ($p<0.05$) for the leaf and stem fractions from W3 (0.104 and 0.199, respectively) to W5.

In vitro intestinal digestibility

Ruminal and intestinal disappearance of DM and CP of

mulberry whole plant and leaf fraction are given in Table 4 and 5. Rumen DM disappearance of whole plant decreased with increased plant maturity, at both incubation times. Leaf DM disappearance was however, not influenced by plant maturity but increased with increased rumen incubation time from 12 to 16 h. Whole plant and leaf CP disappearance was not influenced by plant maturity at 12 h of ruminal incubation, but at 16 h of incubation. CP disappearance of the whole plant declined significantly with advancing maturity while that of the leaves was not influenced. The *in vitro* intestinal digestibility of DM and CP of both the whole plant and leaf decreased with increasing incubation time from 12 to 16 h, but was not influenced by harvest stage. Total tract disappearance of the whole plant DM decreased with advancing maturity at both incubation times, while the effect of plant maturity on total tract DM disappearance of the leaf was significant only at 16 h of rumen incubation. Plant maturity significantly ($p<0.01$) influenced total tract disappearance of CP in the whole plant only after 16 h of ruminal incubation.

CONCLUSION

The results showed the high ruminal degradability of mulberry, which declined significantly with maturity, with more pronounced effects in the whole plant and the stem compared to the leaf fraction. This was brought about by a decline in both the soluble and potentially fermentable fractions. The *in vitro* DM and CP intestinal digestibility of whole plant and leaf was influenced more by the duration of the rumen incubation than by plant maturity. Taking into account the significant effects of plant maturity on the nutritional composition (CP, ADF and NDF), leaf to stem ratio and the degradation pattern of mulberry, it can be concluded that mulberry re-growths are best harvested at 5-week intervals.

The high nutritional value of mulberry as well as the high soluble fraction is a good source of rapidly available nitrogen to the rumen microbes, hence, creating favourable rumen environment as reported by Yao et al. (2000), Liu et al. (2002), Singh and Makkar (2002) and Mgheni et al. (2001). This is a good indicator of the appropriateness of mulberry foliage as a supplement to low-quality basal diets for improving fibre degradation and intake. The high rate of ruminal degradation also suggests that it can be included at high levels in the diet. The high voluntary intake potential of mulberry has been reported by Bakshi and Wadhwa et al. (2004). Diet manipulations aiming at increasing the rumen outflow rate may enhance the bypass protein value of mulberry.

ACKNOWLEDGEMENTS

This research was conducted under the IRPA Grant (No: 01-02-04-0862 EA001) of the Ministry of Science, Technology and Innovation Malaysia. The postgraduate fellowship granted by the Third World Organisation for Women in Science (TWOWS) and the support of the management, particularly Mr. S. Naidu, Director of the Agricultural Research and Extension Unit (AREU) of the Food and Agricultural Council (FARC) for the grant of study leave are gratefully acknowledged.

REFERENCES

AOAC. 1990. Official Methods of Analysis, 15th Ed. Association of Official Analytical Chemists, Washington, DC.

Bakshi, M. P. S. and M. Wadhwa. 2004. Evaluation of forest tree leaves of semi-hilly arid region as livestock feed. Asian-Aust. J. Anim. Sci. 17(6):777-783.

Calsamiglia, S. and M. P. Stern. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 7:1459-1465.

Chen, S. B. 1995. Neway Excel: An Excel Application Program for Processing Feed Degradability data, Rowett Research Institute, Bucksburn, Aberdeen, UK.

Devendra, C. 1993. Nutritional potential of fodder trees and shrubs as protein sources in ruminant nutrition. In Legume trees and other fodder trees as protein sources for livestock. (Ed. A. Speedy and P. Pugliese). Animal Health and Production Paper No.102. FAO, Rome, Italy. http://www.fao.org/DOCREP/003/T0632E/T0632E02.htm#ch2

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. An International Rice Research Institute Book.

Leng, R. A., S. H. Bird, A. Klieve, B. S. Choo, F. M. Ball, G. Asefa, P. Brumby, V. D. Mudgal, U. B. Chaudry, S. U. Haryono and N. Hendratno. 1993. The potential for tree forage supplements to manipulate rumen protozoa and to enhance protein to energy ratios in ruminants fed on poor quality forages. In Legume trees and other fodder trees as protein sources for livestock. Animal Production and Health Paper No. 102. FAO, Rome, Italy, p. 177.

Liu, J. X., Y. B. Jun Yao, J. Q. Yu, Z. Q. Shi and X. Q. Wang. 2002. Mulberry leaf supplement for sheep fed ammoniated rice straw. In Mulberry for animal production. (Ed. M. D. Sanchez). Animal Health and Production Paper No. 147. FAO, Rome, Italy.

Mgheni, D. M., E. E. Ndemanisho, T. Hvelplund and M. R. Weisbjerg. 2001. Evaluation of the feeding value of two tropical cereal straws, maize stover, rice straw and their botanical fractions by nylon and mobile bag technique. African Journal of Science and Technology Science and Engineering Series (2) (1), pp. 65-71.

Orskov, E. R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted to rate of passage. J. Agric. Sci. Camb. 92:499.

Saddul, D., Z. A. Jelan, J. B. Liang and R. A. Halim. 2003. The production potential of *Morus alba* as an animal feed: The effect of harvest stage on yield, persistence and nutritional properties. In Proceedings of the 24th MSAP Annual Conference, 3-4 August, 2003, Melaka, Malaysia.

Sanchez, M. D. 2002. World distribution and utilization of

mulberry and its potential for animal feeding. In Mulberry for animal production. (M. D. Sanchez). Animal Health and Production Paper No. 147. FAO. Rome, Italy.

SAS®. 1990. User's Guide: Statistics, Version 7 Edition. SAS Institute Inc. Cary, NC.

Schmidek, A., R. Takahashi, A. N. de Medeiro and K. T. de Resende. 2002. Bromatological composition and degradation rate of mulberry in goats. In Mulberry for animal production. (Ed. M. D. Sanchez). Animal Health and Production Paper No. 147. FAO, Rome, Italy. pp. 213-217.

Shayo, C. M. 2002. The potential of mulberry as feed for ruminants in Central Tanzania. In Mulberry for animal production. (M. D. Sanchez). Animal Health and Production Paper No. 147. pp. 130-138.

Shayo, C. M. 1997. Uses, yield and nutritive value of mulberry. Tropical Grasslands, 31, pp. 599-604.

Singh, B. and H. P. S. Makkar. 2002. The potential of mulberry foliage as a feed supplement in India. In: Mulberry for animal production. (Ed. M. D. Sanchez). Animal Health and Production Paper No. 147. pp. 139-155.

Thorne, P. J., D. B. Subba, D. H. Walker, B. Thapa, C. D. Wood and F. L. Sinclair. 1999. The basis of indigenous knowledge of tree fodder quality for improving the use of tree fodder in developing countries. Anim. Feed Sci. Technol. 81:119-131.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991 Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3593.

Yao, J., B. Yan, X. Q. Wang and J. X. Liu. 2000. Nutritional evaluation of mulberry leaves as feeds for ruminants. LRRD, 12(2)2000. http:/ www. cipav.org.co/lrrd/lrrd12/2/yao122.htm