The Nutritional Value of Mulberry Leaves and their Use as Supplement to Growing Sheep Fed Ammoniated Rice Straw

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Introduction

The use of crop residues as basal diets for fattening cattle and lambs has been promoted in China during the last decade with a lot of success (Dolberg and Finlayson 1995; Guo and Yang 1996). Farmers still generally use as supplement high levels of concentrates, including cereal grains and oilseed meals. It is important to find alternative supplements (Preston, 1995). With growing lambs, Liu *et al.* (1998) observed that the growth rate was dramatically increased when the ammonia bicarbonate rice straw (ABRS) diet was supplemented with small amount of rape seed meal (RSM), and that the benefits of ammoniation were least when high level of RSM was used.

Sericulture, based on mulberry (*M. alba*) leaves, is an important farming activity in China, with over 10^6ha planted in China and 10^5ha in Zhejiang Province. Yield of fresh leaves is in the order of 15 to 22 t/ha/year. Silkworm production is not always profitable, since depends on silk price and world trade. Alternative ways of using mulberry foliage would be welcomed by farmers when income from sericulture is low. Mulberry leaves are relished by sheep and goats and have high nutritive value with protein content of about 20 % of dry matter (DM) (FAO, 1998). Roothaert (1999) observed that dairy heifers had higher voluntary intake, and thus higher potential of milk production, when consuming mulberry fodder than with cassava tree (*Manihot glaziovii*) and leucaena *(Leucaena diversifolia*). Mulberry leaves could be considered an appropriate supplement for sheep fed a basal diet of ammoniated straw, replacing partially or totally the oilseed meals, which could then be used in monogastric diets. However, there is little information on this subject.

The objectives of the present study were: (1) to evaluate the nutritional value of mulberry leaves collected from clones at different stages of maturity (Experiment1); and (2) to determine the effect of mulberry leaves as supplement, substituting RSM, to growing lambs fed ABRS (Experiment2). The results of Experiment1 has been published in Livestock Research for Rural Development (Yao *et al*, 2000).

Materials and Methods

Experiment 1. Evaluation of the nutritional value of mulberry leaves.

Sampling of mulberry leaves.

Mulberry clones were: Tuantou Heyebai (TH), Husang No.9 (HS), Tongxiangqing (TX) and Nongsang No.8 (NS). Leaves were sampled six times in 1998, three in the spring: 28 April, 14 May and 29 May; and three in autumn: 28 August, 30 September and 30 October. Samples were taken in the morning, weighed immediately and oven-dried at 65^{0}C. The sub-samples were milled to pass a 2mm-sieve for further nutritional evaluation.

Nutritional evaluation

All samples were analysed for crude (CP) and true protein (TP) following AOAC (1990); for neutral detergent fibre (NDF) of Van Soest *et al*. (1992). Amino acid (AA) contents were determined using an AA analyser (Knauer, Germany). The nutritional value of mulberry leaves was evaluated with *in vitro* gas production (GP) of Menke and Steingass (1988) with calibrated glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschieβ, Germany). Samples for GP determination were ground with a hammer mill to pass a 1mm-screen. About 200mg (DM) samples were introduced into syringes with rumen liquor collected from two rumen fistulated Huzhou sheep, fed an ammoniated rice straw diet (75% ABRS, 5% RSM and 25% concentrate mixture). The GP data were then fitted to the equation $GP=a+b(1-\exp(-ct))$ (Ørskov, 1985), where *a*, *b* and *c* are constants and *GP* is the gas production from the substrate at time t.

Statistical analysis

Results for each season were analysed according to a 3 x 4 factorial design. The difference of means was tested using Duncan's new multiple range test (Steel and Torrie, 1980).

Experiment 2. Effect of mulberry leaves to replace rapeseed meal on performance of growing sheep fed ammoniated rice straw.

Experimental feeds

The ABRS was prepared by the 'stack method'. Fertiliser grade ammonium bicarbonate (17% N) was used and the weight proportion of straw:bicarbonate:water was 100:10:30 (Liu *et al.*, 1991). The straw was treated under the ambient temperature (15-

20^0C) for 30 days. Treated straw was exposed to the air for a maximum 24h before feeding it to allow free ammonia to escape.

Mulberry leaves (*M. alba*) were collected at Tongxiang Silkwork Breeding Farm. All leaves were harvested in Autumn (late October) and air-dried before storage.

Animals and design

Forty five growing lambs of the Huzhou breed (a local prolific breed) with initial weight of 16-18kg, were divided into 5 equal groups, considering sex and weight, and randomly allocated to the following treatments:

A: 100g RSM
B: 75g RSM + 60g mulberry leaves
C: 50g RSM + 120g mulberry leaves
D: 25g RSM + 180g mulberry leaves
E: 240g mulberry leaves

Supplementation with RSM at 100 g/d was based on the result in a previous study (Liu *et al*., 1998). All five supplementary treatments were calculated to have similar CP content. All animals had ABRS, mineralized salt block and water *ad libitum* and 100g/head/d of ground corn.

Feeding trial

The experimental period lasted 75d (15d for adaptation). The lambs were weighed, before the morning feeding, every 15d.Feed intake was recorded daily. Feed samples were periodically taken for CP and NDF analysis. The ruminal degradation of DM and CP for RSM and mulberry leaves was measured using the nylon bag technique of Ørskov (1985) as by Liu *et al*. (1997). Data of disappearance rate were fitted to the model of Ørskov (1985): $p=a+b(1-\exp(-ct))$, where p is the disappearance rate at time t (hr), a is the rapidly digestible fraction in the rumen, and b is the fraction slowly digested at rate c ($c>0$).

In vitro gas production test

GP, as described above, was measured to compare the nutritional value of different diets. In order to analyze the associate effect on GP parameters, only the mixtures of RSM and mulberry leaves at different ratio were also incubated. The proportions of both supplements for different combinations (PA, PB, PC, PD and PE) were based on their ratios in the corresponding diets (A, B, C, D and E) in feeding trial.

Statistical analysis

The results were analyzed by one way analysis of variance. The difference of means for the five treatments was tested by using Duncan's new multiple range test (Steel and Torrie, 1980).

Results and Discussion

Composition of mulberry leaves

The chemical composition of mulberry leaves is shown in Table 1.There were no significant differences (P>0.05) among mulberry clones in DM, CP, TP and NDF (for spring leaves). Only the clone Tuantou Heyebai had lower NDF (P<0.05) in the autumn. Sampling time (maturation stage) had great effects on chemical compositions. For spring leaves, contents of CP and TP were slightly higher at mid stage than at early or late stage, whereas the CP content of autumn leaves decreased significantly (P<0.05) with maturation stage. The NDF content increased with maturation stage (P<0.05) in both seasons.

Table 1. Chemical composition of mulberry leaves in spring and autumn

Sample	Dry Matter (%)	Crude Protein (%DM)	True Protein (%CP)	NDF (%DM)
Spring leaves:				
Date				
28 April 98	25.1	21.1ab	87.5	34.6Bc
14 May 98	25.2	21.9a	90.4	38.9Aa
29 May 98	26.6	20.0b	86.3	42.9Aa
Clone				
Tuantou Heyebai	24.4	21.6	86.7	39.5
Husang No. 9	24.1	20.9	89.6	37.5
Tongxiangqing	23.7	20.9	88.3	39.7
Nongsang No. 8	23.6	20.8	88.5	38.5
SE	0.10	0.17	1.16	0.59
Autumn leaves				
Date				
28 August 98	25.9C	22.3A	86.9	36.7Bc
30 September 98	29.9B	21.4A	85.7	40.4Bb
30 October 98	33.8	18.9B	84.7	47.2Aa
Clone				
Tuantou Heyebai	30.4	21.9	84.4	38.9b
Husang No. 9	29.7	20.3	86.4	40.8ab
Tongxiangqing	29.6	19.6	87.5	42.6a
Nongsang No. 8	29.8	21.7	84.9	43.4a
SE	0.42	0.23	0.57	0.46

[A,B,C] Means with different superscripts within mulberry trains or sampling times differ (P<0.01)

[a,b,c] Means with different superscripts within mulberry trains or sampling times differ (P<0.05)

Little seasonal differences were found in contents of CP and TP of mulberry leaves. Average CP contents were 21.1 and 20.9% of DM, and the TP accounted for 88.2 and 85.8% of CP in spring and autumn, respectively. However the NDF content was lower for mulberry leaves in spring (38.8%DM) than for those in autumn (41.4%).

Excepting few amino acids, no significant difference (P>0.05) was observed in individual AA content among clones (data no included). There were little differences in total, essential or non-essential AA among clones. AA content tended to increase with the time, but the differences showed no statistical significance.

Nutritional value of mulberry leaves

The nutritional value of mulberry leaves based on the GP test is presented in Table 2. In spring, clone Nongsang No.8 had higher nutritive value (P<0.05). There was little difference between clones in the GP_{24} and potential GP for autumn leaves, though rate of GP was slightly higher for clones Nongsang and Tongxiangqing. The estimated organic matter digestibility (OMD) (Menke and Steingass, 1988) showed a similar tendency as GP parameters (Table 2). Similar to CP content (Table 1), mid-spring samples tended to have a higher GP (P<0.05) than early or late sampling, while for autumn leaves the GP in late season was lower (P<0.05), with little difference between those at early and middle season.

In General the nutritional value of spring leaves was much higher than autumn leaves. The spring mulberry leaves with OMD of 65.6~71.3% are comparable to some leguminous hays such as alfalfa and vetch (FAO, 1998). According to the farmers' practice, the twigs of mulberry trees must be cut and modified in late May or early June in order assure autumn leaves for the silkworm. These twigs and leaves may be dried and stored for winter use.

Table 2. *In vitro* gas production (GP) parameters and estimated organic matter digestibility (OMD) of mulberry leaves

Sample	GP at 24h (ml)	Potential GP (ml)	Rate of GP (%h^{-1})	OMD (%)
Spring leaves:				
Date				
28 April 98	43.7ab	47.8ab	9.70	69.2ab
14 May 98	46.9a	52.2a	8.79	71.3a
29 May 98	38.5b	43.3b	9.25ab	65.6b
Clone				
Tuantou Heyebai	41.9ab	45.7ab	7.26	69.1ab
Husang No. 9	43.1ab	47.4ab	9.02	68.8ab
Tongxiangqing	39.6b	43.9b	8.90	65.9b
Nongsang No. 8	47.6a	52.7a	9.50	71.9a
SE	0.94	1.04	0.11	0.74
Autumn leaves				
Date				
28 August 98	32.5a	38.7	7.15A	61.4Aa
30 September 98	31.7ab	38.3	6.69AB	60.3ABa
30 October 98	28.7b	35.4	6.18B	56.3Bb
Clone				
Tuantou Heyebai	31.0	37.8	6.57ab	60.0
Husang No. 9	30.8	38.0	6.16b	58.8
Tongxiangqing	30.8	36.7	7.01a	58.4
Nongsang No. 8	31.3	37.4	6.95a	60.1
SE	0.55	0.60	0.11	0.47

[A,B,C] Means with different superscripts within mulberry trains or sampling times differ (P<0.01)

[a,b,c] Means with different superscripts within mulberry trains or sampling times differ (P<0.05)

Dry matter intake

CP and NDF contents for mulberry leaves used in feeding trial were 23.0 and 43.7% DM, and the corresponding values for RSM were 42.3 and 51.1% DM, and for ABRS, 13.3 and 63.9% DM, respectively. The rumen degradation results are shown in Table 3. The rates of disappearance of DM were higher for mulberry leaves than for RSM, but those of CP were lower. CP fractions degrading rapidly (*a*) or slowly (*b*) were much lower for mulberry leaves than for RSM. Rumen escape protein was higher in mulberry leaves than in RSM.

Table 3. Constants of dry matter and crude protein of the equation $p=a+b(1-\exp(-ct))$ together with 48h rumen degradability (D_{48}) of the rapeseed meal and mulberry leaves

	Rapeseed meal		Mulberry leaves	
	Dry matter	Crude protein	Dry matter	Crude protein
a (%)	19.2	32.6	20.5	19.5
b (%)	47.9	67.4	53.9	50.2
c (%/hr)	2.70	2.07	3.10	2.57
se	2.07	2.50	0.65	0.84
a+*b* (%)	67.1	86.5	74.4	69.7
ED[a] with passage rate at				
2.00 %/hr	46.7	66.9	53.3	47.7
4.00 %/hr	38.5	55.6	44.0	39.1
D_{48} (%)	54.5	76.2	62.1	54.9

[a] ED = effective degradability

Animals consumed all rapeseed meal, ground corn and mulberry leaves offered. The intake of ABRS was slightly increased when supplementing with of mulberry leaves, and hence total intake increased with the increases in mulberry leaves (Table 4). Total DM intakes were 451, 455, 495, 540 and 590 g/d for lambs on diets A, B, C, D and E, respectively. Basal ammoniated straw accounted for more than 50% of total diets in all treatments, and intake from forage exceeded 85 % of total diets when the

RSM was fully substituted by mulberry leaves (diet E). Intake of the basal diet is usually decreased due to a substitution effect when forage is supplemented to a straw-based diet. Tharmaraj *et al*. (1989) observed a decline in the DM intake of both ammoniated and untreated RS when supplemented with *Gliricidia*, a leguminous tree. Liu *et al*. (1997) found that inclusion of milk vetch silage at a level higher than 23 % of diets reduced the intake of ammoniated rice straw by growing heifers. In the present study the mulberry leaves accounted for 11, 21, 29 and 35 % of dietary DM intake in diets B, C, D and E, but the ABRS intake was even slightly increased with the rising levels of mulberry leaves.

Table 4. Dry matter intake and growth performance of lambs offered different diets.

Parameter	**Experimental diets (g Mulberry : g RSM)**					
	0:100	**60:75**	**120:50**	**180:25**	**240:0**	
No. of animals (head)	9	9	9	9	9	
Feed intake (g DM/d)						
Rapeseed meal	88	66	44	22	0	
Mulberry leave	0	52.3	104.5	156.6	209.0	
Ground corn	86	86	86	86	86	
Ammoniated rice straw	277.1	250.5	260.6	275.8	295.6	
Total intake	451.1	454.8	495.1	540.4	590.6	
Liveweight						
Initial weight (kg)	18.0	17.7	17.8	16.2	18.4	0.5
Growth rate (g/d)	58±4 [a]	47±8 [b]	40±6 [c]	46±5 [b]	55±3 [a]	1
Feed efficiency (kg/kg)						
Total intake/gain	7.78	9.67	12.37	11.75	10.74	
Concentrate/gain	3.00	3.23	3.25	2.35	1.56	
Feed cost/kg gain (Yuan) #	4.56	5.39	6.25	5.38	4.47	

Price (Yuan/kg): Ammoniated rice straw 0.20; RSM 1.20; corn 1.20, mulberry leaves 0.40 1 USD = 8.25 Yuan.

[a,b,c] Means with different superscripts differ (P<0.05).

Performance of lambs

Lamb growth rates are shown in Table 4. The growth rate in diet with only RSM was comparable to that obtained in a previous trial (63g/d) by Liu *et al*. (1998), who supplemented ABRS diet with 100g RSM and 100g rice bran. The animals supplemented with mulberry leaves only grew the same as those with RSM, but the growth rates were lower ($P<0.05$) when both supplements were given together. There was not difference among sex and groupings (Figure 1). Among the limited information on mulberry leaves as animal feeds, Leng (1997) mentioned that dairy cows achieved up to 18 liters of milk/day on forage supplemented with fresh mulberry foliage. In his perspective, the production rate on high intakes of tree foliages such as mulberry may be as good as those of cattle on ammoniated straw and supplemented with 1-1.5 kg/d of cottonseed meal.

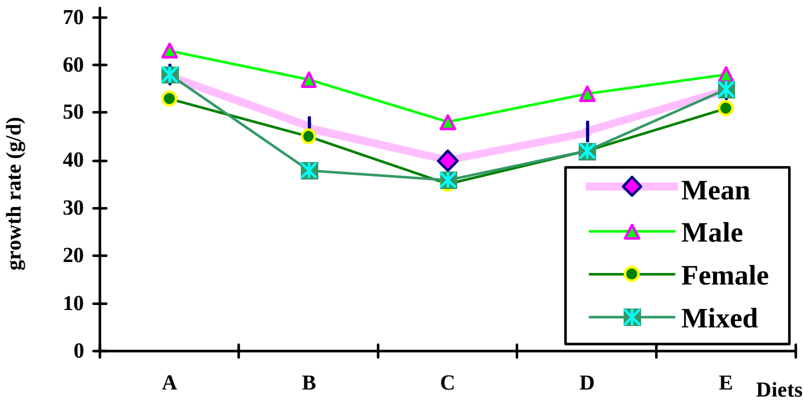

Figure 1. Growth rate of lambs offered ammoniated rice straw diets supplemented with different combinations of rapeseed meal and mulberry leaves

While feed efficiency was higher when receiving only RSM, concentrate consumption per kg of weight gain was lower when higher level of mulberry leaves was supplemented. Compared to other treatments, feed cost per kg gain was lower in the diets with only RSM or mulberry, the lowest in the latter one. When mulberry leaves was used as the supplement to substitute for RSM, there was a benefit of Yuan 0.09 /day (1 US$=8.25 Yuan), equivalent to Yuan 9.00 for the usual fattening period of 100d.

Relationship between growth performance of lambs and diet GP

The results of *in vitro* GP test for different diets are summarized in Table 5. The GP for diets with low levels of mulberry (B and C) was lower than other diets. The potential GP (a+b) was significantly higher in diet with only RSM ($P<0.05$). The GP_{48} value showed a similar trend to the potential GP, although the difference between diets was not statistically significant. These results suggest a negative associative effect between rapeseed and mulberry leaves.

Table 5. Parameters of in vitro gas production (GP) for different experimental diets

Parameters	**Experimental diets (g Mulberry : g RSM)**					**SE**
	0:100	**60:75**	**120:50**	**180:25**	**240:0**	
a (ml)	2.5	3.0	2.0	1.7	2.9	0.3
b (ml)	36.4	33.3	32.9	33.6	34.4	3.6
a+b, ml	38.9 ^{a}	36.3 ^{bc}	34.5 ^{c}	35.6 ^{bc}	37.7 ^{ab}	1.4
Rate of GP, %/h	3.56	4.01	4.38	5.33	4.43	0.07
GP_{48}, ml	31.2	30.0	29.4	31.5	31.5	4.5

^{a,b,c} Means with different superscripts differ ($P<0.05$).

Table 6 presents the result of GP test when only mixtures of mulberry leaves and RSM were incubated. The potential GP and rate of GP for mulberry leaves (PE) were higher than for RSM (PA), but the GP parameters was not improved proportionally when mulberry leaves were increased (PB and PC). There was apparently negative associative effect between mulberry leaves and RSM (Figure 2) where the estimated GP values were much lower than the measured ones. This may partially account for the growth rate of lambs offered different diets (Table 4). There may exist some secondary plant compounds in mulberry leaves that exert a detrimental effects on ruminal microbes or reduce the utilization of the dietary nutrients (Leng, 1997). Further study is needed to clarify these aspects.

Table 6. Parameters of *in vitro* gas production of mixtures of mulberry leaves and rapeseed meal

Treatment	**PA**	**PB**	**PC**	**PD**	**PE**
Mulberry:RSM	**0:100**	**41:59**	**68:32**	**86:14**	**100:0**
GP parameters					
a(ml)	3.3	2.9	2.7	1.1	-0.2
b(ml)	29.1	29.5	32.4	38.0	41.7
a+b(ml)	32.4	32.4	35.1	40.1	41.5
c(%/h)	4.22	4.41	5.92	6.15	6.10
GP_{48} (ml)	27.7	28.3	32.7	37.3	39.2

Implications

Mulberry leaves have a high nutritional, in spring higher than in autumn. When used as supplement to ammoniated rice straw diet, mulberry berry may fully substitute for rapeseed meal, but attention should be paid to the negative associate effect between rapeseed meal and mulberry leaves when supplemented together. The benefits resulting from supplementation with mulberry leaves included an increased intake of basal diet, less consumption of concentrate and an increased income. However the growth rate of lambs on the ammoniated straw diets in the present study were not very high regardless of the supplement. The one of the reasons may be that the straw intakes were not high. Further study is needed to investigate the response to the increasing percentage of mulberry leaves in diets for lambs.

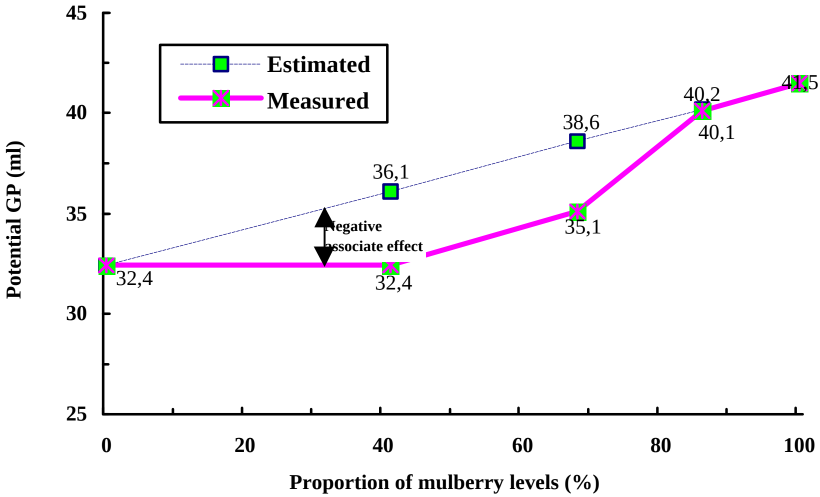

Figure 2. Comparison of measured gas production (potential GP) with the estimated value at different proportions of rapeseed meal and mulberry leaves

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