Bot. Bull. Academia Sinica 26: 67-81 (1985)

DETOXIFICATION OF LEUCAENA LEAVES AS FEED BY ENSILAGE[1]

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(Received July 27, 1984; Accepted December 29, 1984)

Abstract

Leucaena leaves (LL) mixed with corn powder (CP) at ratios of 10:0, 9:1, 8:2, or mixed with rice straw at a ratio of 8:2 were ensiled for 1, 2, or 3 months. Mimosine content decreased during ensilage in samples of all treatments while samples of LL:CP=8:2 ensiled for 3 months contained the lowest amount of mimosine. Samples of (dried fresh LL or LL ensiled for 2 months) : CP=8:2 were used to make various diets. These 3 diets together with a normal diet were used for feeding experiments of rats to evaluate effectiveness of ensilage. General composition analyses (including metabolic energy), fatty acid composition, and amino acid analysis (including % essential amino acids and protein efficiency ratio) of the samples did not change during ensilage to an extent which could explain the improved performance of rats fed ensiled diets. Rats fed 1/3 (diet with dried fresh LL:CP=8:2)+2/3 normal diet showed significantly lower ($P<0.05$) liveweight gain, feeding efficiency, or protein efficiency ratio. They also showed typical symptoms of mimosine toxicity such as retardation of growth, alopecia and cataract formation etc. All these deleterious effects of mimosine in LL can be prevented by a simple, economical process-ensilage.

Key words: Detoxification; leucaena; mimosine; rats; ensilage.

Introduction

Leucaena is a nitrogen-fixing, sub-tropical tree or shrub with a rather rapid rate of growth. So it has been proposed and tried as a suitable vehicle for tropical reforestation (Middleton, 1980). In some parts of the tropics, such as the Philippines and India, it has already been heavily planted (Gupta, 1980; Parera, 1980). Leucaena is a versatile tropical tree legume (Brewbaker and Hutton, 1979). It has been tried for fuelwood production (Curran, 1980; Jones, 1981; Pathak and Patil,

[1] Paper No. 286 of the Scientific Journal Series, Institute of Botany, Academia Sinica. The work was supported by the Council for Agricultural Planning and Development, R. O. C.
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1980), manure (Bottenberg, 1981; Gill and Patil, 1982), nursing tree (Curran, 1980; Granert, 1980), wood production (Van Den Beldt and Brewbaker, 1980; Hu and Kiang, 1982; Tang, 1981), intercropping (Das and Reddy, 1982; Swift, 1982), forage production (Bray, 1982; Shih and Hu, 1981). However, the presence of the toxic amino acid analogue, mimosine, in both seeds and leaves; and tannins in leaves of leucaena causes some problems for utilization of leucaena seeds and leaves (Cheeke and Telek, 1980; Chen and Lai, 1981; Holmes, 1980; Jones, 1979; Jones and Megarrity, 1981). Cautions and pretreatments are necessary in order to use leucaena seeds and/or leaves safely as forage, feed, or material in food industry (Acamovic and Felix D'Mello, 1981; Gonzalez Vargas and Wyllie, 1982; Holmes, 1980; Lesniak and Liu, 1981; Matsumoto, 1951). A brief report describing ensilage as a process to decrease mimosine content of a mixture of leaf, twig, and stem of leucaena has been published (Rosas *et al.*, 1980). In this report, we present a detailed study of ensilage as an effective way to lower mimosine content of leucaena leaves and thus alleviate the adverse effects of mimosine demonstrated by feeding experiments of rats. Changes of qualitative and quantitative properties of components with biochemical and nutritional importance during ensilage are also included.

Materials and Methods

Materials

Leaves of leucaena (Salvador type K28) were collected from Pingtung. Corn powder (CP) was bought from local market at Pingtung City. All chemical reagents used were of analytical grade. Control diet of rats (Rodent Laboratory Chow 5001) was a product of LabChows (U.S.A.) with ingredients as follows: crude protein not less than 23.4%; crude fat not less than 4.5%; crude fiber not more than 5.0%; ash not more than 7.3%; added minerals not more than 2.5%; nitrogen free extract about 49.8%; water 7.5%.

Experimental Design

There were 4 leucaena-leaf (LL) diets and 3 ensilage periods. So all together 12 treatments were obtained. Four replicate samples (bottles) were taken for each treatment and triplicate samples were taken for each bottle. Two independent determinations of mimosine content were carried out for water extract of each sample tested.

Ensilage

Leucaena leaves (LL) were cut into pieces 2-3 cm long and then 4 groups of

diet were made. Group A, LL only; group B, LL:CP=9:1 (w/w); group C, LL:CP=8:2; group D, LL: dried rice straw=8:2. Plastic bottles (diameter: 7 cm; height: 11 cm) with screw caps were used as containers. Adhesive tapes were used to wrap the peripheral of caps to ensure anaerobic fermentation. Period of ensilage: 1 month, 2 months, or 3 months. Samples were used immediately for various analyses or feed once the bottle was opened at the end of ensilage.

Determinations of Mimosine Content in Leucaena Leaves

This followed mainly the procedure of Brewbaker and Kaye (1981) which was applied to air-dried leaf samples (one g dry weight needed).

Reagent solutions: A. 0.1 N HCl (one liter needed for each ten samples); B. 1 liter 0.1 N HCl with 1.5 g activated carbon, keep in suspension during use with magnetic stirrer; C. Diluent solution of 1 g (Na_2-EDTA·$2H_2O$) in 4 liters water; D. 60% $FeCl_3$ solution, obtained by dissolving 4 g ($FeCl_3$) in 500 ml of 0.1 N HCl.

Collect and assay samples: 1. Samples of 10 g were collected and dried at or below 40°C in an oven until no further weight loss occurred and then ground to powder. High temperatures should be advoided. 2. Weigh out 1.0 g of dried LL powder in volumetric flask and filled to 100 ml with reagent A. The suspension was homogenized with a Polytron (made in Swiss) for 1 min. (Bottles can be stored at this stage at room temperature). 3. A 10-ml aliquot of the homogenate was transferred to a test tube immersed in a boiling water bath. Added 15 ml reagent B, and boiled for 15 min. (Tubes were covered with aluminum foil). Longer boiling was required for very woody samples. 4. The boiled sample solution, after cooling, was filtered through #2 Whatman paper. A 2-ml aliquot of the filtrate was taken, and 5 ml reagent C plus 1 ml reagent D was added. Samples were kept in darkness for 15 min prior to analysis for full color development. 5. Read absorbance at 535 nm, correcting against a blank achieved by diluting a duplicate 2-ml samples of filtrate with 5 ml reagent C plus 1 ml water (in lieu of reagent D).

Determine mimosine values: 1. Prepare a calibration curve using solutions containing between 0.0025 and 0.025% mimosine in 0.1 N HCl, treating 2-ml aliquots as in steps 4 and 5 above. 2. Determine absorbance of sample (corrected against blank); apply to curve from step 1; mimosine in %=250 A_{535}.

General Composition Analysis

This was done according to Horwitz (1975). Crude total protein was calculated by multipling amounts of total nitrogen by 6.25. Carbohydrate=100—water—(crude protein)—(crude fat)—ash (%, by weight). Calculation of metabolic energy was done according to Atwater system (Watt and Merrill, 1963). Energy coefficients of protein, fat, and carbohydrate were 4.27, 9.02, and 3.87 Kcal/g, respectively.

Amino Acid Analysis

This was done with the help from staff of Northern Instrument Service Center of National Science Council. The amino acid analyzer used was a Dionex-D-300 Component System (U.S.A.). Separation of amino acids was achieved by a single column. Color reagent was ninhydrin. Derivatives of proline was detected at 430 nm and those of the other amino acids at 570 nm. Samples were dissolved in 6 N HCl and sealed in a N_2-flushed tube. Hydrolysis of protein was carried out at 110°C for 24 h. After hydrolysis, HCl was repelled by evaporation. The dried samples were dissolved with 0.2 N sodium citrate (pH 3.25) and were ready for amino acid analysis.

Calculation of Nutritional Parameters

This included percentage of essential amino acids (% EAA) and protein efficiency ratio (PER).

$$\% \text{ EAA} = (\%\text{Leu} + \%\text{Ile} + \%\text{Lys} + \%\text{Met} + \%\text{Phe} + \%\text{Thr} + \%\text{Try} + \%\text{Val}).$$

Protein efficiency ratio was calculated by three equations according to Alsmeyer *et al.* (1974).

$$\text{PER} = -0.684 + 0.456\ (\text{Leu}) - 0.047\ (\text{Pro}) \quad \ldots\ldots(1)$$
$$\text{PER} = -0.468 + 0.454\ (\text{Leu}) - 0.105\ (\text{Tyr}) \quad \ldots\ldots(2)$$
$$\text{PER} = -1.816 + 0.435\ (\text{Met}) + 0.780\ (\text{Leu}) + 0.211\ (\text{His}) - 0.944\ (\text{Tyr}) \quad \ldots\ldots(3)$$

Fatty Acid Composition

Separation of lipid from aqueous phase of test samples was carried out by "Folch extraction procedure of lipids" and fatty acid composition was determined by separation and quantitation of methyl esters of fatty acids on gas chromatographic column (Metcalfe and Schmitz, 1961; Metcalfe *et al.*, 1966). The instrument used was a Varian Model 3700. Conditions of experiment were carrier gas N_2, 40 ml/min; H_2, 0.5 kg/cm^2; air, 1 kg/cm^2; injection temperature, 230°C. Temperature program: initial temperature 150°C, 4 min; final temperature 210°C; 2°C/min.

Ensilage for Feeding Experiments of Rats

Same as described above except that dried LL-corn powder was fixed at 8:2 and time period was 2 months. After being ensiled, samples were dried at 40°C. Dried samples were used as feeds for rats.

Feeding Experiments of Rats

Male young Long Evens rats were purchased from Animal Center of Medical School of National Taiwan University and divided into 4 groups. Each group

contained 6 rats and was fed with different diets. Changes of liveweight and consumption of feed of each rat were recorded at the end of every week to calculate feeding efficiency (liveweight gain/food intake ×100%) and protein efficiency ratio (liveweight gain/protein consumed) of each rat. After 6 weeks, rats were killed. Liver, kidney, and spleen of each rat were taken out and weighed. Photographs of healthy and sick rats were also taken for ease of comparison of the effects of various diets.

Results

Decrease of Mimosine Content During Ensilage

Table 1 presents data showing decrease of mimosine content in samples of various treatments during ensilage. Under the best condition mimosine content dropped to 49% of control samples (1.78/3.61=0.49). Samples of treatment with LL:CP of 8:2 gave the best results. Although decrease of mimosine content during ensilage was observed for all samples without exception, the decrease was not linear with storage time. Manipulations of data of Table 1 are shown in Tables 2, 3, and 4. In Table 2, we can see that both treatment and duration of storage of ensilage of leucaena leaves were very significant sources of variance. There was also a very significant interaction between treatment and duration. If we combined all 4 treatments together and took the average, then there were very significant differences between 3 durations of ensilage (Table 3). In a similar manner, if we combined all 3 durations of ensilage together and took the average, then a significant difference was observed between treatment T_2 and T_4. A very significant difference was found between treatment T_1 and T_3; T_2 and T_3; T_3 and T_4.

In Table 4, it can be seen the combination that decreased the mimosine content to the lowest level was T_3-D_3 (samples with LL:CP of 8:2 ensiled for 3 months).

Table 1. *Change of mimosine content (%) during ensilage of leucaena leaves*[1]

Silage source[2]	Duration		
	D_1 (1 month)	D_2 (2 months) $n=24$	D_3 (3 months)
LL:CP 10:0	2.24±0.24	2.83±0.34	2.89±1.06
LL:CP 9:1	2.41±0.13	3.03±0.21	2.76±0.29
LL:CP 8:2	1.78±0.20	2.33±0.15	1.77±0.18
LL:RS 8:2	2.13±0.25	2.81±0.35	2.58±0.04

[1] LL: leucaena leaves; CP: corn powder; RS: rice straw. Figures in second, third, and fourth rows have already been converted to mimosine content (%, on dry weight basis) in recipe with LL: CP=10:0.

[2] Mimosine content (%) of dried fresh LL was 3.61±0.23 ($n=10$).

Table 2. *Variance analysis of ensilage of leucaena leaves*[1]

Source of variation	Degree of freedom	Sum of square	Mean Square	F
Replication (R)	11	4.421	0.402	
Treatment (T)	3	9.835	3.278	18.832**
Ea	33	5.745	0.174	
Duration (D)	3	56.113	18.704	119.369**
TD	9	5.046	0.561	3.578**
Eb	132	20.683	0.157	
Total	191			

[1] This table contains data of control group (LL without being ensiled).
**: $P<0.01$

Table 3. *Differences between treatments or duration of ensilage of leucaena leaves*

Duration[2]	Treatment[1]			
	T_1	T_2	T_3	T_4
D_1		0.084	0.692**	0.146
D_2	0.601**		0.776**	0.231*
D_3	0.362**	0.248**		0.546**

[1] T_1, T_2, T_3, and T_4: Silage with LL:CP=10:0, LL:CP=9:1, LL:CP=8:2, and LL:RS=8:2, respectively. (refer to Table 1)
[2] D_1, D_2, and D_3: Ensiled for 1 month, 2 months, and 3 months, respectively (refer to Table 1)
*, **: $P<0.05$ and $P<0.01$, respectively.

Table 4. *Interaction effect of duration (D) and treatment (T) on ensilage of leucaena leaves*

Treatment	Duration				
	D_0	D_1	D_2	D_3	
T_1	−0.1415	−0.0438	−0.0650	0.2500	0.0000
T_2	−0.2042	0.0695	0.0763	0.0583**	0.0000
T_3	0.3775	0.0162	−0.0380	−0.3560	0.0000
T_4	−0.0320	−0.0423	0.0265	0.0475**	0.0000
	0.0000	0.0000	0.0000	0.0000	

*, **: $P<0.05$ and $P<0.01$, respectively.

Changes of General Composition of Ensiled Leucaena Leaves During Storage

Table 5 shows composition changes (%) of ensiled leucaena leaves during storage. There was not much change of both water and ash for samples of all 4 treatments. There was a general tendency for decrease of crude protein in samples of all 4 treatments. Same phenomenon was observed in both crude fat and crude fiber. The only component which showed a tendency to increase during ensilage was nitrogen-free extract (NFE).

Change of Fatty Acid Composition During Ensilage of Leucaena Leaves

There was not much change of fatty acid composition during ensilage of leucaena leaf meal (Table 6). Higher percentage of linolenic acid (Vitamine F, an essential fatty acid for most mammal) than salad oil (Table 6) or silage of sweet potato root chips (our unpublished data) was found to be a characteristic property of fatty acid composition of leucaena leaves or silage of leucaena leaf meal.

Amino Acid Composition of Fresh and Ensiled Leucaena Leaf Meal

This was shown in Table 7. The ratio of LL to CP of samples was 8:2. Amounts of phenylalanine, arginine, isoleucine, tyrosine, proline, and methionine increased while those of lysine, histidine, valine, aspartic acid, threonine, alanine, and glutamic acid decreased after ensilage. No obvious changes of serine, glycine, and leucine were observed. No comparison of half cystine could be made due to lack of data from fresh samples. Percentage of EAA for fresh and ensiled samples was 10.94 and 11.38, respectively. Calculated PER's of fresh and ensiled samples

Table 5. *Composition changes of ensiled leucaena leaves during storage*

Weight ratio[1]	Storage time (month)	Moisture	Crude protein	Crude fat	Crude fiber	Ash	Nitrogen free extract
		% of fresh weight					
10:0	1	70.6	5.82	0.91	8.26	1.41	13.1
	2	71.5	4.87	0.64	8.12	1.42	12.9
	3	68.4	3.91	0.52	7.66	1.44	18.1
9:1	1	66.8	5.34	1.11	8.12	1.39	17.2
	2	64.2	4.91	0.89	8.41	1.36	20.2
	3	63.7	3.76	0.66	7.01	1.30	23.6
8:2	1	60.2	5.28	1.42	7.64	1.38	24.2
	2	60.1	4.96	1.37	7.01	1.41	25.1
	3	57.6	4.02	1.06	6.42	1.31	29.6
8:2[2]	1	59.1	5.72	1.02	13.8	3.08	17.3
	2	56.0	5.01	0.91	12.1	3.24	22.8
	3	60.2	4.85	0.65	10.6	2.93	20.9

[1] Weight ratio=leucaena leaves: corn powder.
[2] Using rice straw instead of corn powder.

Table 6. *Change of fatty acid composition during ensilage of leucaena leaves*[1]

Fatty acid	Duration (month)			
	D_0	D_1	D_2	D_3
	% (by weight)			
Palmitic acid (16:0)	15.4	15.8	14.0	14.4
Stearic acid (18:0)	trace	trace	trace	trace
Oleic acid (18:1)	19.8	19.9	21.5	21.9
Linoleic acid (18:2)	51.1	49.4	49.3	47.6
Linolenic acid (18:3)	13.6	14.7	15.0	15.9

[1] LL:CP=8:2.
[2] Salad oil had a fatty composition (%) of: palmitic acid, 9.4; stearic acid, 4.1; oleic acid, 28.5; linoleic acid, 55.0; linolenic acid, 3.0.

Table 7. *Amino acid composition of fresh and ensiled leucaena leaf meal*[1]

Amino acid	Fresh	Ensiled (2 months)
	g/100 g crude protein	
Lys	1.37[2]	1.23[2]
His	0.78	0.70
Val	1.77	1.38
Asp	3.41	2.96
Thr	0.93	0.81
Ser	1.18	1.16
Phe	1.15	1.38
Gly	1.98	2.00
Ala	2.75	2.45
1/2 CyssCy	—	0.14
Arg	0.84	1.05
ILE	2.42	3.23
Leu	2.76	2.75
Tyr	0.86	1.07
Pro	1.35	2.13
Glu	3.98	3.50
Met	0.54	0.60

[1] LL:CP=8:2.
[2] This value has been corrected for the availability of lysine according to Kan *et al.* (1977).

are presented in Table 8. PER of samples decreased after ensilage no matter equation 1, 2, or 3 was used.

General Composition of Various Diets

Not many differences were detected between various diets as far as general composition was concerned (Table 9). Diet of group A contained higher percentage of crude protein and ash than those of groups B, C, and D. Percentages of crude protein, crude fat, crude fiber, ash, and nitrogen-free extract of diets of groups B, C, and D were quite similar. As far as metabolic energy was concerned, diets of all four groups provided quite similar values.

Nutritional Evaluation of Rats Fed Control or Various LL Diets

Liveweight of rats fed control or various LL diets is shown in Table 10 and Fig. 1. The results were quite clearcut. Rats of group A, fed control diet, grew the best while those of group B, fed 1/3 (dried fresh LL:CP=8:2)+2/3 normal diet,

Table 8. *Protein efficiency ratio of fresh and ensiled leucaena leaf meals calculated from their amino acid composition*

Equation[1]	Calculated PER	
	Fresh	Ensiled (2 months)
1	0.51	0.47
2	0.70	0.67
3	− 0.076	− 0.27

[1] According to Alsmeyer *et al.* (1974)

Table 9. *General composition of various diets*

Group	Water (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Nitrogen free extract (%)	Energy (Kcal/100g)
A	10.0	23.4	4.5	5.0	8.5	49.8	333
B[a]	10.0	21.7	4.1	9.3	6.7	51.1	327
C	10.0	21.1	4.0	8.6	6.8	52.1	328
D	10.0	21.8	4.2	7.8	7.2	51.6	331

Group A: Control, normal diet.
Group B: (diet with dried fresh LL:CP=8:2)/normal diet=1:2.
Group C: (diet with ensiled LL:CP=8:2)/normal diet=1:2.
Group D: (diet with ensiled LL:CP=8:2)/normal diet=1:3.
[a] Mimosine contents (%, by dry weight) of fresh LL and ensiled LL (2 months) were 3.45 and 1.61, respectively, which were comparable in LL only recipe.

Table 10. *Liveweight of rats fed control or various leucaena leaf diets*

Weeks	Diet[1]			
	A	B	C	D
	Average±standard deviation, g			
0	75.6± 9.2[a]	75.6± 9.1[a]	75.7± 7.5[a]	75.7± 7.4[a]
1	117.2± 7.2[b]	103.3±10.9[a]	111.7± 9.6[ab]	120.5± 9.8[bc]
2	148.8±19.6[bd]	126.4±10.0[a]	145.4±12.6[b]	157.1±12.9[cd]
3	194.7±23.9[b]	149.9±13.2[a]	180.9±16.2[b]	196.7±15.6[b]
4	230.6±28.4[b]	175.0±13.8[a]	215.1±19.3[b]	229.7±17.3[b]
5	261.4±36.3[bc]	192.1±17.1[a]	236.9±19.8[c]	251.3±17.2[bd]
6	291.2±40.1[a]	204.7±18.4[b]	263.1±24.7[a]	279.7±17.7[a]

[1] Diet A, B, C, and D are the same as in Table 9.
[2] Significant difference existed between pair of values with different alphabets according to Duncan's multiple range test (5% level).

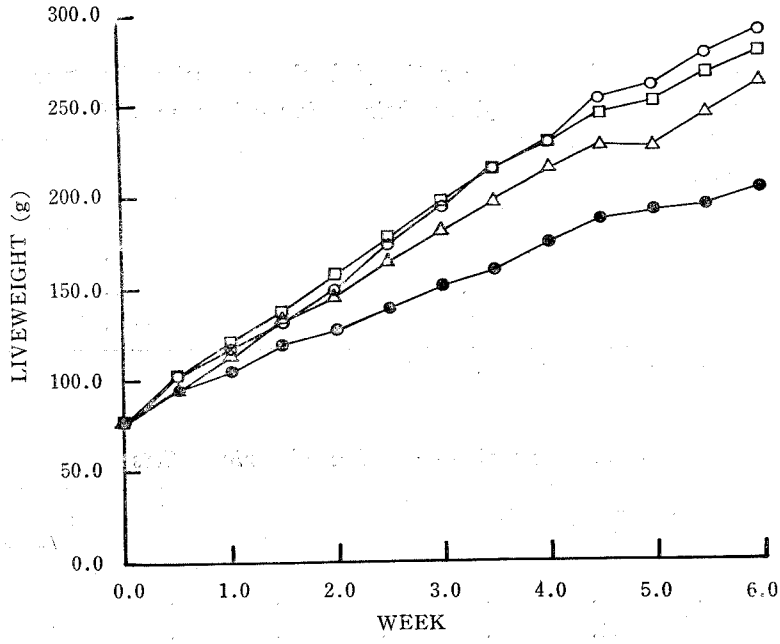

Fig. 1. Liveweight of rats fed control or various leucaena leaf diets. ○—○, diet A; □—□, diet D; △—△, diet C; ●—●, diet B. Groups A, B, C and D are the same as in Table 9.

grew the worst. Ensilage of LL significantly improved the growth performance of rats (results of both group C and group D). Although rats of group A were the heaviest consistently among four groups during 6 weeks of feeding experiment, there was no significant difference between group A, C, and D in the last four

weeks except the 5th week. Fig. 1 shows the effectiveness of ensilage on liveweight gain of rats. Pictures in Fig. 2 showed that in addition to retardation of growth, rats of group B actually looked sick. But rats of both group C and group D showed no symptoms at all.

Cumulative feed intake at the end of the 6th week was found to be: group A, 831.5±110.9 g (n=6); group B, 626.0±50.6 g (n=6); group C, 731.9±108.2 g (n=6); group D, 780.7±48.9 g (n=6).

From data of Table 11, it was clear that rats of group B not only ate least but also performed significantly lowest feeding efficiency during week 1, 2, and 6. Ensilage of LL significantly improved the performance of feeding efficiency of rats.

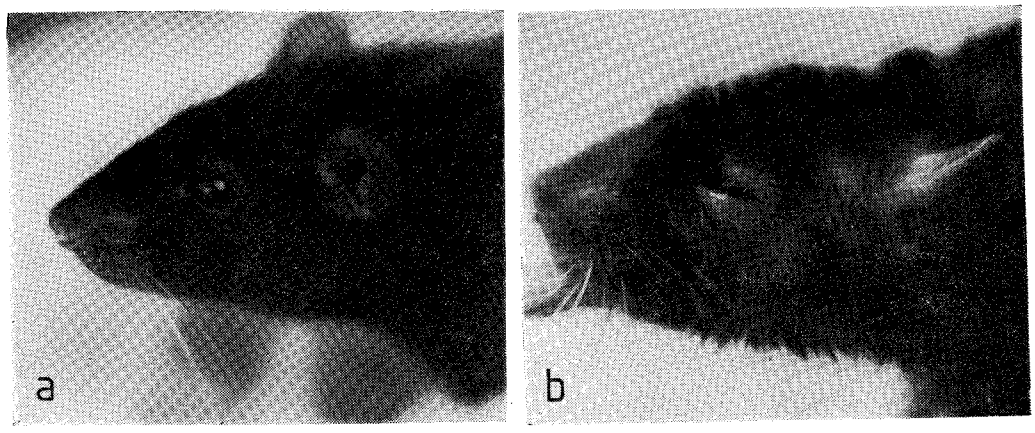

Fig. 2. Photographs of healthy (a) and sick (b) rats. Picture a shows a healthy rat representing those of diet groups A, C and D; picture b shows a sick rat of group B.

Table 11. *Feeding efficiency of rats fed control or various leucaena diets*[1]

Weeks	Diet			
	A	B	C	D
		(%)		
1	41.9± 5.5^b	34.6±1.5^a	39.3±2.9^b	42.0±1.5^b
2	29.5± 6.1^a	13.2±2.1^b	16.3±1.9^c	20.6±1.2^d
3	30.1±11.1^a	22.2±6.4^a	30.1±7.7^a	28.2±2.0^a
4	23.0± 4.7^a	22.6±6.7^a	24.3±3.3^a	24.8±1.5^a
5	17.9± 6.4^a	14.2±3.7^a	14.9±2.4^a	14.1±4.4^a
6	17.9± 3.1^b	11.0±2.4^a	16.5±2.1^b	17.4±5.0^b

[1] Feeding efficiency (FE)=(liveweight gain, g/g feed intake) ×100%.
[2] Composition of diets and statistical manipulation are the same as in Table 10.

Table 12 shows that as far as protein efficiency ratio (PER) is concerned, rats of group B performed as well as rats of the other three groups during the first five weeks. Only in the last week PER of rats of group B was significantly lower than those of other groups.

Weight and weight ratio of organs of rats fed control or various LL diets are presented in Table 13. There was no significant difference between data of all groups.

Table 12. *Protein efficiency ratio of rats fed control or various leucaena diets*[1]

Weeks	Diet[2]			
	A	B	C	D
1	1.79 ± 0.23^a	1.60 ± 0.07^a	1.86 ± 0.12^a	1.59 ± 0.13^a
2	1.26 ± 0.26^a	1.12 ± 0.17^a	1.35 ± 0.13^a	1.17 ± 0.26^a
3	1.29 ± 0.47^a	1.02 ± 0.29^a	1.22 ± 0.11^a	1.09 ± 0.08^a
4	0.99 ± 0.20^a	1.04 ± 0.31^a	1.15 ± 0.16^a	0.96 ± 0.06^a
5	0.77 ± 0.27^a	0.65 ± 0.17^a	0.71 ± 0.12^a	0.55 ± 0.17^a
6	0.77 ± 0.14^b	0.49 ± 0.11^a	0.78 ± 0.10^b	0.68 ± 0.19^b

[1] Protein efficiency ratio (PER): liveweight gain g/g protein consumed.
[2] Composition of diets and statistical manipulation are the same as in Table 9.

Table 13. *Weight (W) and weight ratio (WR, g/100 g body weight) of organs of rats fed control or various leucaena leaf diets*

There was no significant difference between data of all groups. The values are quoted as the mean±s. d.

Diet	Liver		Spleen		Kidney	
	W(g)	WR	W(g)	WR	W(g)	WR
A	13.21±0.65	3.83±0.33	0.51±0.05	0.15±0.24	2.18±0.27	0.64±0.07
B	9.51±0.53	4.09±0.42	0.43±0.07	0.19±0.04	1.54±0.09	0.66±0.07
C	12.22±0.34	4.10±0.13	0.62±0.04	0.21±0.01	1.94±0.08	0.67±0.06
D	11.33±0.50	3.79±0.18	0.52±0.08	0.17±0.04	2.01±0.12	0.66±0.06

Discussion

From data presented above, it is clear that during the ensilage of LL mimosine content showed drastic decrease (Table 1). The decrease ef mimosine content is not linear with time, suggesting that mimosine might be converted during ensilage to other compound(s) through a reversible process. Results of Table 1 and 4 show that as far as disappearance of mimosine is concerned, samples with LL:CP=8:2

ensiled for 3 months was the best. But period of 3 months is a little bit too long, so we decided to use samples ensiled for 2 months for feeding experiment of rats. The results are very satisfactory. Since our results (Table 1) show that samples ensiled for one month contain lower mimosine content than those ensiled for 2 months, practical ensilage of LL may be completed within one month.

Crude protein, crude fat, and crude fiber all decreased while NFE increased during ensilage (Table 5). This suggests that decomposition is very active during ensilage and NFE formed may be carbohydrate molecules of small molecular weight.

In addition to the obvious retardation of growth, significantly lower FE and PER, some rats of group B also developed symptoms typical of mimosine toxicity such as alopecia and cataract formation (Fig. 2) as reported by Lin (1982). All these can be prevented by a simple, economical process-ensilage. Changes of general composition (including metabolic energy), fatty acids, amino acid composition (including % of essential amino acids and protein efficiency ratio) of ensiled leucaena leaves can not account for all we observed. But detoxification of mimosine of LL during ensilage can serve the basis of explaining our data.

One third of ensiled LL in the diet may not be the upper limit of amount of LL that can be used safely. A higher percentage of ensiled LL in the diet is possible.

Work on application of ensiled LL to diets for pigs or cattle is in progress.

Acknowledgements

We thank Miss Hui-Yin Fu for her excellent technical assistance and Dr. Hong-Pang Wu for helping statistical analysis of our data.

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以青貯法除去銀合歡葉子飼料之毒性

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銀合歡葉子和玉米粉以 10:0，9:1，8:2 的比例混合；或者以 8:2 的比例和稻草稈混合。然後青貯一個月，二個月，或三個月。經各種處理的材料其含羞草素（mimosine）的含量均顯著下降，而以銀合歡葉子和玉米粉以 8:2 混合然後青貯三個月者含羞草素的含量下降最多。我們以未經青貯或經青貯二個月的乾燥銀合歡葉子以 8:2 混合玉米粉作爲原料，和市售之標準飼料混合配成 3 種實驗飼料。然後和純粹的市售標準飼料一起餵食大老鼠以便評估青貯過程對銀合歡葉子毒性的解除之有效性。我們同時還做了一般成份分析（包括可代謝之能量），脂肪酸成份分析，氨基酸成份分析（包括不可缺氨基酸之百分比和蛋白質效率比）。結果發現：青貯前後這些項目的變化並不足以說明餵養經青貯之銀合歡葉子之大老鼠爲什麼長得比餵養未青貯者好。所以青貯後含羞草素顯著下降是營養狀況改善的主要原因。大老鼠吃了混合三分之一重量的實驗飼料（新鮮乾燥銀合歡葉子：玉米粉＝8：2）和三分之二重量的市售標準飼料所成的配方六週之後，無論是體重的增加，飼養效率（feeding efficiency），或者是蛋白質效率比（protein efficiency ratio）都顯著地下降（95% 信賴界限）。們同時出現含羞草素中毒的典型症狀，例如：發育不良，脫毛，以及白內障等。銀合歡葉子的毒性它在以青貯葉子取代新鮮葉子的實驗組中就不再出現。所以如果添加的量不超過三分之一的話，青貯過程不失爲一個經濟，容易的方法來消除以銀合歡當飼料時毒性所帶來的困擾。