Kasetsart J. (Nat. Sci.) 41 : 282 - 287 (2007)

Beta-carotene, Mimosine and Quality of Leucaena Silage Kept at Different Duration

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ABSTARCT

Leucaena leucocephala leaves (LL) were ensiled by mixing with 20% rice bran and 20% water (fresh LL basis). The material was kept for 21, 51, 81 and 111 days in vacuumed double layer plastic bags, each containing 26 kg. Five bags were randomly taken at each interval for quality evaluation by organoleptic test as well as by organic acid and chemical analysis. It was found that the ensiling period did not have much influence on most of the chemical compositions. All samples of leucaena leaf silage (LLS) had pH of 4.4-4.5 and 35.22-35.65% DM (dry matter). The compositions on DM basis were 21.49-22.29% CP (crude protein), 7.76-8.22% EE (ether extract), 31.18-33.68% NDF (neutral detergent fiber), 2.0-2.9% acetate and 6.9-9.7% lactate (DM basis). DM loss was 10.35-12.32% which was in the normal range for good quality silage. The most interesting points were the increment of β-carotene after ensiling from 88.50 to 99.92-120.25 mg/kg DM while mimosine content decreased over 90% (from 1.79 to 0.12-0.16% of DM) which were superior to a drying method. It indicated that LLS is a good alternative for preserving LL and for reduction of mimosine.

Key words: leucaena, β-carotene, mimosine, silage, organic acids

INTRODUCTION

Leucaena leucocephala is a legume plant, commonly found in Thailand and many other tropical countries. The nutritive value is comparable to alfalfa. The leaf contains around 24% CP and 116-161 mg β-carotene/kg DM (Lamchoun, 1998). It is widely used in animal feed for monogastrics and ruminants as a source of CP, vitamins and minerals. In addition, it also provides pigment for skin and egg yolk. However, there is a limitation of using leucaena leaves (LL) as animal feed because of its high mimosine content. This toxic substance is a non-protein amino acid. The chemical name is β-N-(3-hydroxy-4-pyridone)-α-amino propionic acid. After ingestion it converts to 3-hydroxy-4(1H)-pyridone (DHP), that can induce goiter (Jone, 1994). And since the structure of mimosine is similar to that of tyrosine, it becomes an antagonist to this amino acid and inhibits protein synthesis. Therefore, it reduces growth and production performance. In addition, it interferes with B_6 activity which is necessary for cystathionine synthetase and cystathionase in converting methionine to cystine, thus causes hair loss (Liener,

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Received date : 15/08/06 Accepted date : 05/02/07

1989). In monogastrics, the inclusion of LL above 5-10% of the ration depresses growth rate and induces alopecia, cataract, paralysis, infertility, low production efficiency and finally death (Norton, 1994).

In Thailand, mimosine content in LL is 1-5% and in commercial LL meal is 1-2% (Panja, 1983; Pharkwiwat, 1984). Ruminants in South East Asia can tolerate higher mimosine level due to gram negative rod shape rumen microbes "*Synergists jonesii*" which can degrade mimosine and its derivative DHP into non toxic substances (Jone, 1994). However, these microbes are not found in monogastrics, therefore it is necessary to reduce this toxic substance before use. The most popular method is drying under the sun. Although other methods such as soaking in ferrous sulfate or in running water or the supplement of amino acids, Fe, Al and Zn are reported to be effective (Panja, 1983; Pharkwiwat, 1984; Wee and Wang; 1987), there are some practical limitations.

Ensiling may be an appropriate method for preservation and toxic reduction because LL is surplus in wet season during which drying is rather difficult. Hongo *et al.* (1986) and Sunagawa *et al*. (1989) reported that around 90% of mimosine is destroyed after 14-21 days of ensiling. Since LL is a legume, it contains low soluble carbohydrate and high buffering capacity. Therefore silage additives such as rice bran (RB) should be added (Thepprapakarn, 2001).

However, the report on mimosine residue and β-carotene content in leucaena leaf silage (LLS) is still limited, therefore this study aimed to determine the quality and chemical composition of LLS during 111 days of ensiling.

MATERIALS AND METHODS

Fresh LL leaves with green petioles are chopped into 2-5 cm length and mixed with rice bran and water at the ratio of 100:20:20 (w/w). Then the mixture was filled in 2 layer plastic bags (26 kg each), vacuumed, closed tightly and kept for 21, 51, 81 and 111 days. At the end of each period, 5 bags were randomly opened and the samples were taken for Proximate and Detergent analysis (AOAC, 1984; Georing and Van Soest, 1970). Physical quality of the silage was evaluated by organoleptic test (Gross, 1982). The pH was determined according to Bal *et al*. (1997). The concentrations of lactic, acetic and butyric acids were analysed by distillation (Zimmer, 1966). Mimosine and β-carotene content were analysed by the modified methods of Hegarty *et al*. (1964).

RESULTS AND DISCUSSION

Chemical composition of ingredient and LLS

Chemical compositions of LL, RB and LLS ensiled with RB are shown in table 1. Crude protein of fresh LL in this experiment is higher than that reported by Göhl (1975) but lower than that reported by Cheva-Isarakul (1982) who reported 21.0 and 26.0% CP, respectively. It might be due to the age of plant, the ratio of leaves, stems and pods as well as season and cultivation condition. NDF and ADF were similar to those

Table 1 Chemical compositions (DM basis) of fresh leucaena leaves (LL), rice bran (RB) and leucaena silage (LLS).

	DM	OM	CP	EE	Ash	NDF[1/]	ADF[1/]	ADL	NFC	pH
		(% DM)								
LL	30.49	92.01	23.50	3.08	7.99	39.14	23.70	8.67	26.29	6.03
RB	91.01	90.72	12.70	10.55	9.28	19.75	9.71	1.52	47.72	-
LL[2/]	37.07	91.89	22.82	7.95	8.11	34.76	16.54	5.01	26.36	5.08

[1/] ash free [2/] ensiled with 20% rice bran

reported by Halim (1992).

LLS had lower CP, ADF and ADL but higher EE and ash than those of fresh LL. This might be due to the inclusion of RB as a silage additive.

Physical and chemical property of LLS

The quality of LLS, as evaluated by organoleptic test, was good to fairly good (Table 2). Although the precision of this test method may not be high due to the experience and the sensitivity of test panels, it is practical and popular since it needs no equipment. It was found that the scent of lactic acid in LLS was milder than that in corn silage. The odour of RB was also noticed but no smell of fungi or rotting was detected. The odour of LLS was similar to that of tea leaf silage which is a local product for human consumption in Northern Thailand.

The colour of LLS was darker than that of good quality grass silages. It might be owing to the loss of Mg in chlorophyll when reacted with organic acids and became phaeophytin which has brown color (Watson and Nash, 1960). In addition, legume leaves have more pigments than grass, therefore legume silage had darker colour than grass silage. However, the colour intensity also depends on other factors such as oxygen amount in silo and temperature during ensiling. LLS in this study had good texture and had no mold. Only small amount of fungi was found at the opening point of some bags. It might be due to oxygen remaining at this point after closing these bags.

Organic acids of LLS ensilaged at different ages, determined by distillation techniques, are shown in Table 3. Although lactic acid of the silage kept for 81 days was significantly higher than that of the others, no significant differentce was found on quality scores because light smell of butyric acid was also noticed. All samples were considered good grade silage even though pH were higher than 3.7-4.2 which is generally found in good quality grass silage. It is owing to the fact that leucaena is leguminous plant, therefore it contains high buffering capacity, thus inhibits pH change. However, pH and acid content

Table 2 Quality of leucaena silage ensiled at different durations.

	Ensiling period (days)			
	21	51	81	111
Odor	11.06^{ab}	10.10^{a}	10.92^{a}	11.90^{b}
Color	2.00^{b}	1.68^{a}	1.92^{b}	1.96^{b}
Texture	3.40	3.06	3.60	3.82
Score	**16.48^{b}**	**14.84^{a}**	**16.48^{b}**	**17.68^{b}**

Values in a row with different superscripts differ significantly ($p<0.05$)

Score: 16-20 = grade 1 (good – very good), 10-15 = grade 2 (fairy – good), 5-9 = grade 3 (fair), 0-4 = grade 4 (poor)

Table 3 Organic acids and pH of leucaena silage at different ensiling periods.

Ensiling period	pH	Acids (% of DM)			Acid (mEq/100 gDM)			Score
(days)		Acetic	Butyric	Lactic	Acetic	Butyric	Lactic	
21	4.5	2.00	0.00	6.86^{a}	33.35	0.00	76.11^{a}	94.00
51	4.4	2.10	0.00	7.62^{a}	34.95	0.14	85.89^{a}	92.10
81	4.4	2.79	0.00	9.65^{b}	46.53	0.21	107.09^{b}	84.80
111	4.4	2.88	0.00	8.09^{a}	47.97	0.23	89.82^{a}	85.30

Values in a column with different superscripts differ significantly ($p<0.05$)

Score: 81-100 = (very good), 61-80 = (good), 41-60 = (fair), <40 = (bad)

of all LLS samples in this study were in the normal ranges of good quality silage according to the report by Watson and Nash (1960); i.e. ≤65% moisture, pH < 4.8, lactate 3-14% and butyrate < 0.2% (DM basis).

Chemical compositions of LLS at different ensiling periods are shown in Table 4. It was found that the length of ensiling period had no influence on DM loss and most of the chemical compositions. The loss of DM of 10.35-12.32% found in this experiment was in a normal range. McDonald *et al*. (1991) reported that the unavoidable loss of silage due to the action of plant enzymes, microbial enzymes, plant respiration and ensiling technique were 1-2, 2-4 and 2-5%, respectively.

No significant difference was found on CP content between prior and after ensiling with the exception of the lower CP content of the group kept for 111 days. The unremarkable protein loss was due to the good ensiling condition since the bags were vacuumed, thus only minute amount of oxygen remained in the bags. In addition, moisture level of the ensiling material was optimal (63%) therefore no excess heat was produced. These conditions led to the low dry matter and nutrient loss (Watson and Nash, 1960; McDonald *et al*., 1991).

Furthermore, the low CP loss might be due to the fact that LL has condensed tannin (4-6% DM basis; Balogun *et al*., 1998). This substance is able to inhibit protein degradation by microbial and animal enzymes (Albrecht and Muck, 1991). Moreover, trypsin inhibitor in RB may also inhibit protein degradation. Most (85-90%) of this inhibitor was found in embryo. The other part of RB (without embryo) had 5-10% while polished rice had less than 1% of this inhibitor (Juliano, 1985).

The concentration of ash and that of EE were not affected by ensiling period. Even though the forms of minerals and the pattern of fatty acids may change during ensiling process, their amount should not decrease because no seepage was found due to low moisture content (<65%) of the ensiling materials. Non fiber carbohydrate (NFC) tended to decrease after ensiling due to the conversion of starch to lactic acid (McDonald *et al*., 1991; Jaurena and Pichard, 2001) even though the efficiency may be lower than that of sugar. Hemicellulose tended to decrease and lactic acid increased significantly after ensiling but no change

Table 4 Composition (DM basis) and dry matter loss of leucaena silage in various ensiling periods.

	Ensiling period (days)				
	0	21	51	81	111
DM	37.07	35.56	35.65	35.22	35.65
Dry matter loss (%)	-	10.35	10.92	11.69	12.32
OM	91.89	91.93	91.75	91.57	91.66
CP	22.82^{c}	22.29^{c}	21.56^{ab}	22.22^{bc}	21.49^{a}
EE	7.95	8.03	8.22	7.76	8.02
Ash	8.11	8.07	8.43	8.25	8.34
NFC	26.36^{a}	28.80^{ab}	28.09^{ab}	30.60^{b}	28.47^{ab}
NDF*	34.76^{b}	32.81^{ab}	33.70^{ab}	31.18^{a}	33.68^{ab}
ADF*	16.54^{a}	18.45^{b}	18.37^{b}	18.84^{b}	19.02^{b}
Hemicellulose	18.22^{b}	14.36^{a}	15.33^{ab}	12.34^{a}	14.65^{a}
Cellulose	11.53	11.87	11.86	12.22	12.11
Lignin	5.01^{a}	6.59^{b}	6.51^{b}	6.63^{b}	6.91^{b}

Values in a row with different superscripts differ significantly ($p<0.05$), * ash free

Table 5 β-carotene and mimosine content in leucaena mixed with rice bran and ensiled at different durations.

Ensiling period	β-carotene		Mimosine	
(days)	mg/kg DM	% increment	% DM	% lost
0	88.50[a]	0.00	1.79[b]	0.00
21	99.92[ab]	12.90	0.13[a]	92.74
51	116.29[bc]	31.40	0.14[a]	92.18
81	120.28[c]	35.91	0.16[a]	91.06
111	105.21[abc]	18.88	0.12[a]	93.30

Value in a column with different superscripts differ significantly ($p<0.05$)

was found on cellulose. These results were similar to those reported by Jaurena and Pichard (2001).

Beta-carotene and mimosine content in leucaena silage

β-carotene of LLS increased significantly (Table 5). Even though no clear explanation can be given, the result was in agreement with that reported by Peterson *et al*. (1935; cited by Watson and Nash, 1960) who found the increment of carotene in alfalfa ensiling with acid. However, Hellbery (1945; cited by Watson and Nash, 1960) reported that 11-75% of carotene was loss by oxidation during fermentation. The extent of loss depended on oxygen content and temperature in the silo.

Fermentation decreased 91-93% of mimosine. The length of ensiling had no effect on mimosine loss. The result was in agreement with that reported by Hongo *et al*. (1986) and Sunagawa *et al*. (1989) who found mimosine reduction over 90% in LLS either with or without additives. The reduction of mimosine by ensiling being higher than by sun drying (14.5-51.1% of the original samples) was reported by Panja (1983), Parkwiwat (1984) and Wee and Wang (1987). These results indicated that LLS is an interesting alternative for feed preservation.

CONCLUSION

The ensiling of LL by mixing with 20% rice bran and 20% water (fresh LL weight basis) in airtight containers gave a good quality silage. It can be kept for a long time without deterioration. In addition, it increased β-carotene and decreased mimosine content over 90% which was much better than the preservation and detoxification by sun drying. It is expected to be a good feed for ruminant and monogastric animals.

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