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COUMARIN IN *GLIRICIDIA SEPIUM*: THE EFFECT OF AGE AND CUTTING TIME AND ITS EFFECT ON INTAKE BY SHEEP

Elizabeth Wina , Ida Heliati and B. Tangendjaja[1]

ABSTRACT

Coumarin is one of phenolic compounds found in *Gliricidia sepium*. Its characteristic smell caused low palatability on ruminant that has not been adapted to it. The experiment chose 3 provenances of Gliricidia collected by Oxford Forestry Institute GS1137.51, 1137.39 and 1137.48 represented Gliricidia with low, medium and high coumarin content, respectively. Three replicated on each type were observed and each replicate consisted of 6 trees planted in row (1x1 m distance) and only leaves from 4 trees inside the row that being collected, freeze dried and analyzed for its coumarin content by high performance liquid chromatography. Observation on age up to 10 weeks after the tree being cut to 75 cm height above ground showed that the highest coumarin content was on the leaves of third and fourth weeks old and decreased as the leaves matured. This occurred on the medium and high coumarin type provenances. The low coumarin type provenance showed a different pattern. Cutting date during dry season caused generally lower coumarin content in all types than that during rainy season (379.6, 5079.4, 9965.8 vs 889.1, 6635.9, 11254.4 ppm for low, medium and high type, respectively). Experiment on intake to four sheep for each type showed that sheep consumed medium and high coumarin type Gliricidia in lower amount than low type on the first three days since Gliricidia being offered. The consumption of all types got almost the same after that. The average intake of low, medium and high type gliricidia was 323.3, 278.7 and 295.5 g/day, respectively. More than 72% of coumarin intake was not recovered in urine or feces. In conclusion, coumarin level was affected by age of the leaves and cutting date and its effect on intake was only at the first three days of feeding trial.

Key words : Coumarin, Gliricidia, Intake, Sheep

INTRODUCTION

Coumarin is a secondary compound that has been found in several plants (Benson *et al.*, 1981; Pritchard *et al.*, 1983; Smith &van Houter, 1987). Serious haemorrhagic cases were reported on cattle or horses when coumarin in mouldy sweet clover were changed to dicoumarol (Benson *et al.*, 1981; Pritchard *et al.*, 1983). However, on fresh or dried leaves, there was no such report. There was also no report of a toxicity problem on *Gliricidia sepium, which* contained coumarin. Previous experiment showed that there are big variation of coumarin contents from hundred ppm to 20,000 ppm in 26 provenance of Gliricidia sepium collected from Central and South America by Oxford Forestry Institute (Tangendjaja & Wina, 1995). Coumarin is a major compound in the volatile fraction of *Gliricidia sepium*, therefore it is responsible for the strong characteristic smell of gliricidia leaves (Sutikno & Supriyati, 1995). Low palatability of gliricidia leaves was assumed due to the strong smell. Wilting process would help reduce the smell and increase the intake.

In this experiment, coumarin level was observed during growth and during harvesting at different times. Its effect on gliricidia intake by sheep was also reported.

[1] Research Institute for Animal Production, P.O. Box 221 Bogor, Indonesia

MATERIALS AND METHODS

Effect of age

There were 26 provenances of Gliricidia collected by Oxford Forestry Institute that grew in three replicates at Ciawi, West Java with an altitude of 300 m height above sea level and a rainfall of 2700 mm per year. The provenances that were chosen in this experiment were GS 1137.39, GS 1137.48 and GS 1137.51. Three replicated on each type were observed and each replicate consisted of 6 trees planted in a row (1x1 m distance) and only leaves from 4 trees inside the row that being collected. The experiment was started after the trees were harvested and cut to 75 cm height above the ground. After two weeks, a small plastic card tagged the new opened leaves. The tagging was done to the new leaves on the same branch every week till tenth weeks. At the end of experiment, the leaves were harvested and were separated based on their ages. All the leaves were freeze dried and ground. The free coumarin was extracted by methanol and was analyzed by High Performance of Liquid Chromatography.

Effect of cutting time

This experiment used the same provenances as previous experiment. The harvest times were four times every 12 weeks and started from May 1992. The leaves that harvested were the new leaves till the sixth leaves on the same branch. One replicate consisted of a mixture of leaves from four trees. The leaves were freeze dried and ground. The coumarin analysis was done as previous experiment.

Effect of coumarin to intake of Gliricidia on sheep

This experiment was done in two periods that used 2 sheep for each treatment and each period. The treatments were three different provenances of Gliricidia, which was the same as previous experiment. The sheep were placed in the metabolism cages and the sheep never have any experience of consuming Gliricidia. Without any adaptation period, the sheep was fed with Gliricidia for 5 days. The offer and the residual feed were weighed. Feces and urine were collected on the second day till the fifth day. Samples of offer and residual feed and feces were freeze dried and ground. Coumarin analysis was done on those samples and urine.

Analysis of coumarin

One gram of dried ground sample was extracted with 25 ml of 80% methanol solution for 30 minutes shaking. The supernatant was separated by centrifugation. One milliliter of the supernatant was filtered through a Milipore filter type HA with pore size of 0.45um. The High Performance of Liquid Chromatography (Waters machine) was set up using Alltech Spherisorb Column (250 mm x 4.6 mm ID), 280 nm UV detector and Pharmacia Fine Chemicals recorder. The solvent used was 50% methanol in 2% acetic acid solution with a flow rate of 1.0 ml/min. Concentration of coumarin standard used was 1.6 mg/ml and the volume of standard and samples solution injected into the column was 10 ul.

RESULTS AND DISCUSSION

Effect of age

The three provenances were chosen based on the previous result (Tangendjaja & Wina, 1995). Gliricidia no 1137.51, 1137.39 and 1137.48 represented the low, medium and high coumarin level in the leaves, respectively. In this experiment, coumarin content for these provenances was still consistent as previous result.

Figure 1 shows that the pattern of coumarin content in three different provenances varied. The coumarin level was very low in the first new leaves and reached maximum on the third or fourth weeks leaves and decreased slowly afterwards. This phenomena occurred to the provenances no. 1137.48 and 1137.39 (the high and medium coumarin content, respectively). It is almost similar to mimosine, a secondary compound in *Leucaena leucocephala* (Tangendjaja *et al.*, 1986) or total phenol in Oak leaves

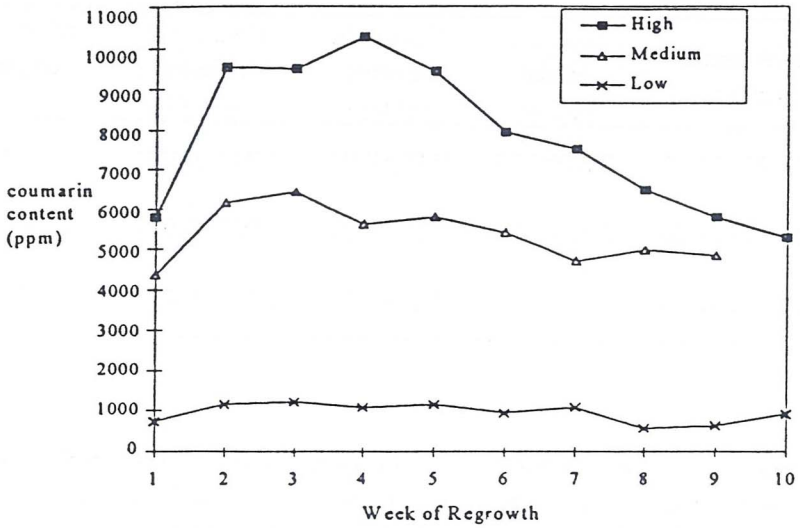

Figure 1. Coumarin content of high, medium and low type Gliricidia during regrowth

Table 1. Coumarin content (ppm) in gliricidia leaves at different cutting times

Provenance No.	Dry season			Rainy season		
	May 1992	August 1992	Average	November 1992	March 1993	Average
GS1137.51 (low type)	506.07	253.08	379.57	758.72	1019.47	889.09
GS1137.39 (medium type)	3524.29	6634.5	5079.39	5392.80	7879.0	6130.9
GS1137.48 (high type)	10074.33	9857.3	9965.81	11982.5	10526.3	11254.4

(Makkar, *et al.*, 1988) which was high in the young leaves and decreased as the leaves matured. Provenance no 1137.51 (low coumarin content) has a bit different pattern. After reached optimum level at the third week leaves, coumarin content remained almost constant up to the tenth week.

In the visual observation, there was an insect attack on the new opened leaves and young stems during dry season and it seems that high level of coumarin in that part could not prevent this attack. This insect attack did not happen to *Calliandra calothyrsus* which contained tannin as its secondary compound. Probably, the effectiveness of coumarin as a self defense mechanism of plant toward certain predator is less than tannin.

Effect of cutting time

Table 1 shows that there was a variation of coumarin in leaves when the leaves were harvested at different time. Generally, in three provenances, the coumarin content was lower at harvesting during dry season than during wet season. Plant cell response to dry season when limited water supply would be an inhibition or decrease of synthesis or a hydrolysis of macromolecules (Brady, 1973). Puffe *et al.* (1986) showed the effect of cutting date on coumarin, shikimic

Table 2. Daily intake of different Gliricidia provenances and coumarin intake and output (in urine and feces)

Provenance	DM Intake (g/day)	Coumarin intake (g/day)	Coumarin in urine (g/day)	Coumarin in feces (g/day)	Metabolised coumarin (%)
GS 1137.51 (low type)	323.26±46.93	0.33±0.08	0.06±0.03	0.004±0.005	72.32±13.39
GS 1137.39 (med- type)	278.67±78.69	2.10±0.70	0.24±0.07	0.008±0.007	82.63±8.70
GS 1137.48 (high type)	295.51±64.08	3.53±0.47	0.42±0.27	0.021±0.012	83.73±13.39

acid and hydroxy cinnamoic acid contents varied within and between species of Lolium, Trifolium, Medicago sp. During dry season, when evaporation is quite high, it may also reduce coumarin content as coumarin is a major compound found in the volatile fraction (Sutikno & Supriyati, 1995).

The effect of coumarin to Gliricidia intake on sheep

Figure 2 shows that in the first three days there was a higher intake of low type Gliricidia compared to the high type, but after that the intake of high type Gliricidia was higher than that of low or medium type ones. It indicates that coumarin in high level that caused very strong smell only affected the consumption at the beginning of the trial. The adaptation was relatively quick for low and medium type as the intake reached plateau after five days of feeding but the intake of high type Gliricidia still increased. Many reports suggested that Gliricidia had low palatability and it required wilting process to increase its palatability, consequently, intake (Mathius, 1992). In this experiment, there was no other alternative for the sheep to consume except Gliricidia leaves. The increase of daily intake also indicated that there was no toxicity in Gliricidia. In contrast, another experiment without any alternative except *Acacia villosa* showed the decrease of daily intake by sheep causing death in a week (Wina, unpublished result). With cafeteria system which sheep was offered with many different provenances of Gliricidia, there was no correlation between coumarin content and the level of intake, which indicates that coumarin did not influence intake of Gliricidia (Stewart, personal communication). There was no significant difference of average of daily intake by sheep among all types of tested Gliricidia (Table 2). This result is in agreement as the result of the experiment at the East Java experimental station which found no difference on gliricidia consumption by cattle fed cafeteria system of five different provenances of gliricidia. (Aryogi *et al.*, unpublished result).

Coumarin analysis in urine and feces (Table 2.) showed that only small amount could be detected which means that most of coumarin (72-84%) disappeared during digestion. Previous *in vitro* experiment showed that coumarin almost completely disappeared in the rumen (Wina *et al.*, 1993). Most of phenolic compounds were metabolized in ruminant digestion tract to be excreted as benzoic acid in the urine and only 20-34% were excreted as the original compound (Martin, 1982). The theory of coumarin being condensed to become dicoumarol in the rumen has not been proved yet. However, coumarin became dicoumarol was reported occurred in mouldy sweet clover but not in fresh or dried leaves (Benson *et al.*, 1981).

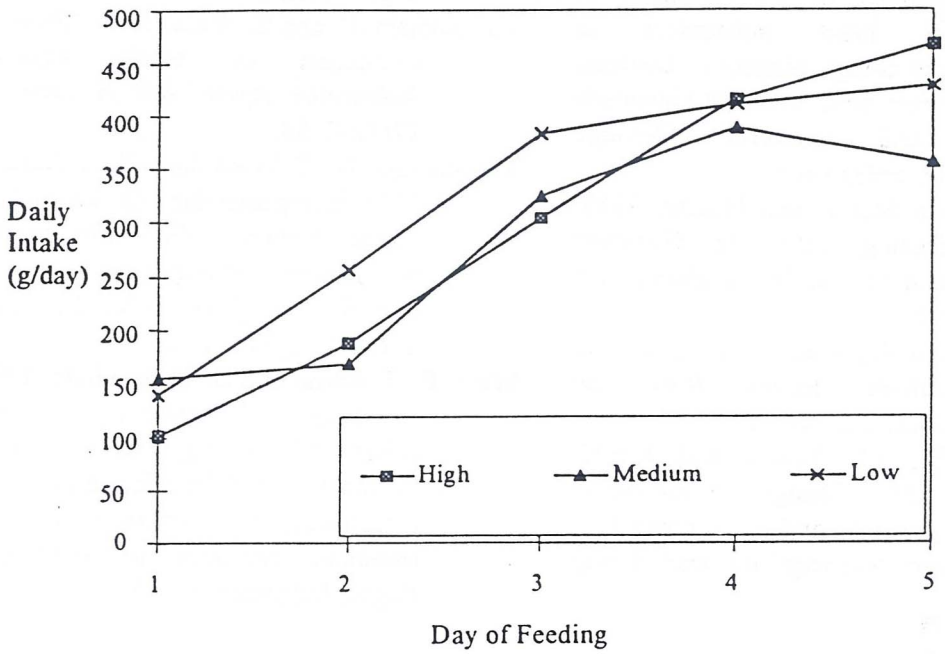

Figure 2. Daily intake of different type of Gliricidia by sheep

CONCLUSION

In conclusion, coumarin level in Gliricidia was affected by age of the leaves and cutting date and its effect on intake was only at the first three days of feeding trial

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