METHODS TO INCREASE INTAKE OF GLIRICIDIA LEAVES (*Gliricidia sepium*) BY SHEEP

I W. KARDA
Fakultas Peternakan, Universitas Mataram, 83125 Nusa Tenggara Barat

SUMMARY

Three trials were conducted to investigate the intake of oven-dried gliricidia by sheep, namely trial 1, addition of polyethylene glycol (PEG), trial 2, pretreatments, and trial 3 addition of additives**.** In the first trial, six rumen fistulated sheep were used to compare two dietary treatments in a change over design to study whether infusion of polyethelene glycol (PEG) into the rumen might increase intakes of gliricidia leaf as tannin in the leaf was believed to limit its intake. In the second trial, four sheep were used to compare four dietary treatments in a latin square design to study whether reheating or freezing the already oven-dried gliricidia leaf might increase its intake by the animals. On the other hand, the third trial was aimed to supplement the sheep with various supplements which are believed to be able to increase the taste of the leaf by the animals. These supplements were wheat millrun, molases, grass hay, cottonseed meal, palm kernel meal, or barley grain. For this reason, ten sheep were used to compare seven dietary treatments in a randomised complete block design. Differences between means were examined by analysis of variance using the General Linear Model procedure of the Statistical Analysis System. The results showed that neither administration of PEG into the rumen nor pretreatments (reheating or freezing) increased intake of gliricidia leaf by sheep. However, only mixing gliricidia with molasses increased the intake of the leaf by sheep over the control diet (gliricidia alone) over the six hours feeding period (43 vs 74 g DM).

Keywords: Gliricidia leaf, PEG, pretreatments, addition of additives

METODE UNTUK MENINGKATKAN KONSUMSI DAUN GAMAL (*Gliricidia sepium*) KERING OVEN OLEH TERNAK DOMBA

RINGKASAN

Tiga jenis penelitian dilaksanakan untuk mempelajari cara meningkatkan konsumsi daun gamal oleh ternak domba. Percobaan 1 bertujuan untuk mempelajari apakah pemasukan (PEG) ke dalam rumen melalui lubang fistula domba dapat meningkatkaan konsumsi daun gamal atau tidak sebab ditengarai bahwa tanin dalam daun gamal mungkin berperan sebagai faktor pembatas konsumsi pakan. Untuk itu, percobaan ini menggunakan enam ekor domba yang difistula rumennya dan dialokasikan ke dalam dua perlakuan, yaitu pemasukan PEG atau air suling ke dalam rumen domba dengan rancangan penelitian *change over*. Percobaan 2 bertujuan untuk mempelajari apakah pemanasan kembali atau pembekuan daun gamal yang telah dioven sebelumnya dapat meningkatkan konsumsinya. Untuk itu, empat ekor domba digunakan dalam penelitian ini, yang dialokasikan ke dalam empat jenis perlakuan, yaitu daun gamal oven (DGO), DGO yang dipanaskan kembali pada suhu 70 ^{0}C atau 100 ^{0}C, atau DGO yang dibekukan pada –15 ^{0}C semalam, dengan rancangan penelitian bujur sangkar latin. Percoban 3 dimaksudkan untuk mempelajari apakah penambahan bahan-bahan seperti menir wheat, molases, rumput kering, tepung biji kapas, bungkil kelapa sawit atau biji barley giling pada daun gamal dapat meningkatkan konsumsinya. Pada percobaan 3 ini, digunakan 10 ekor domba yang dialokasikan ke dalam 7 macam perlakuan dengan rancangan penelitian *randomised complete block*. Analisis data pada penelitian ini menggunakan analisis variansi dengan mengunakan prosedur dari General Linear Model dari SAS. Kedua perlakuan pada percobaan 1 dan 2, yaitu baik pemasukan PEG maupun pemanasan kembali DGO atau pembekuan tidak dapat meningkatkan konsumsi daun gamal oleh ternak domba. Meskipun demikian, hanya penambahan molases (tetes tebu) pada percobaan 3 dapat meningkatkaan konsumsi daun gamal selama 6 jam periode pemberian pakan dibandingkan dengan kontrol (43 vs 74 g bahan kering).

Kata kunci: Daun gamal, PEG, praperlakuan, penambahan bahan suplemen

INTRODUCTION

Lowry (1990) suggested that the real constraint to gliricidia feed value for ruminants lies in its palatability. It is suggested by this author that animals often refuse gliricidia leaf on the basis of smell and even reject it without tasting it. This led him to conclude that the problem lies with volatile compounds released from the leaf surface. Apart from volatile compounds, low palatability may be related to other deleterious factors such tannins, essential oils or other aroniatic compounds which are frequently present in many tree leaves (Kumar and Vaithiyanathan 1990*).*

The benefits of using polyethylene glycol (PEG) supplements in increasing the utilisation of iferous forages by both sheep and goats have been reported by many authors. For example, mulga (*Acacia aneura*) fed to sheep (Pritchard *et al*., 1992); carob (*Ceratonia siliqua*) fed to sheep (Silanikove *et al*., 1994) or oak (*Quercus calliprinos*), pistacia (*Pistacia lentiscus*) and carob (*Ceratonia siliqua*) fed to goats (Silanikove *et al*., 1996). More recently, Ahn *et al*. (1997) found better utilisation of barley straw by sheep supplemented with oven-dried gliricidia leaves compared to fresh materials. This led them to suggest that drying may enhance the formation of bypass protein and destroy some of the anti-nutritional factors in fresh gliricidia. Drying was also reported to reduce tannin contents of 12 browses examined Ahn *et al*. (1989). Altervatively, pretreatments such as wilting is also suggested by many authors (eg. Nitis, 1986; Hawkins *et al*., 1990) as a means of increasing gliricidia acceptability.

Other approaches such as addition of molasses and salt (B. Lowry, pers. comm.), and accustomisation of the animal by prolonged exposure and /or penning with adapted animals (Simons and Stewart, 1994) are also considered to be important in influencing gliricidiaacceptability by animals. This trial examined the acceptability by sheep of *Gliricidia sepium* by manipulating it through supplementing with polyethylene glycol, subjecting it to pretreatments, or addition of additive.

MATERIALS AND METHODS

Gliricidia leaves (local cultivar) were harvested from the plant grown at the University of Queensland, Mount Catton Research Farm. Sufficient leaves including rachis were obtained and oven-dried (50 ^{0}C for 48 h) for the feeding trials described below. The Retalhuleu cultivar was subsequently available, and sufficient leaves including rachis were also obtained for chemical analyisis.

Trial 1 : Effects of polyethylene glycol on intakes of gliricidia by sheep.

Animals and dietary treatments.

Six rumen fistulated sheep (34 ± 0.8 kg, mean± SD) were used to compare two dietary treatments in a change-over design. Local cultivar of gliricidia leaf was fed alone *ad lib.*,or gliricidia ad *lib.* with intra-rurninal administration of polyethylene glycol (PEG, NW 4000, BDH Ltd, England) at the rate of 1.7 g PEG/g condensed tannin consumed (Barry and Forss, 1978). PEG was dissolved in 100 mL water before administration into the rumen, while the same volume of water without PEG was administered into the rumen of the unsupplemented sheep. PEG and water were administered at 0730 and 1200 h daily.

Feeding routines and measurements

Before the commencement of the trial, all sheep were familiarized with oven-dried gliricidia offered 2 h/d for 7 days. During the experiment, the leaf was offered alone *ad lib.* every third day for a total of 4 intake measurements. All sheep were offered Rhodes grass cv. Callide hay *ad* lib. on the days when intake of leaf was not measured. Fresh water and mineral blocks *ad lib.* were also provided throughout the trial. The amount of leaves consumed was calculated by difference. On the last day of intake measurements, rumen fluid samples were taken at 30 minutes before and 6 h after administering the first dose of PEG for rumen ammonia-N determination.

Trial 2 : Effects of gliricidia pretreatments on intakes by sheep.

Animals and dietary treatments.

Four sheep (61± 3.7 kg; mean ± SD) were used to compare four dietary treatments in a latin square design. The dietary treatments were oven-dried (50^0 C for 48 h) local cultivar gliricidia leaf, or this leaf redried either at 70 ^{0}C or 100 ^{0} C, or the dried leaf frozen at -15 ^{0}C overnight before the following morning feeding.

Feeding routines and measurements

All sheep were familiarised with the leaf for 4 days before the trial began, Test meals of 100 g leaf were offered at 0700 h for 6 h, and the amount of the test meal consumed was calculated by difference. Rates of intake of gliricidia leaf were measured over 1 h and 6h. postfeeding. After this period all sheep were offered 1000 g Rhodes cv. Callide hay, and the hay residues were removed at 1700 h. The sheep also received fresh water and multimineral blocks *ad lib*. throughout the trial. The trial lasted for four days.

Trial 3 : Effects of addition of additives on intake of gliricidia by sheep.

AnimaIs and dietary treatments.

Ten sheep (46 ± 13.7 kg; mean ± SD) were used to compare seven dietary treatments in a randomised complete block design in which sheep constituted the block. Local cultivar gliricidia leaf was either fed alone, or mixed (100 g leaf and 50 g additive, both as-fed) with wheat millrun, molasses, grass hay, cottonseed meal, palm kernel meal, or barley grain. Additives were also fed alone to examine their rates of intakes using the same sheep.

Feeding routines and measurements.

All sheep were familiarized with oven-dried gliricidia leaf for one week before the commencement of the trial. In the first phase of the trial which lasted for seven days, the test meals were 100 g leaf and 50 g additive (both as-fed). Preliminary feeding showed that leaves fed with additives were selected against by sheep. To overcome this the leaves ere crushed and intimately mixed with the additives. Meals were offered at 0700 h for six hours. The amount of the test forage consumed was calculated by difference. Rates of intake of gliricidia leaf were measured over 1 h and 6 h postfeeding and consumption of gliricidia leaf was estimated by assuming that selection for or against dietary components had been prevented. Each day after the test feeds, the sheep were offered 800 g Rhodes grass cv. Callide hay. The hay residues were removed at 1700 h. The sheep also received fresh water and multimineral blocks *ad lib.* throughout the trial. In the second phase, rates of intake of individual additives were measured using the same sheep for a period of six days.

Chemical analysis.

Gliricidia leaves were analyzed for dry matter (DM, 100^0 C for 24 h). Condensed tannin was determined by a modification of the butanol-HCI technique of Perez-Maldonado (1994). Rumen ammonia-N concentration was determined by steam distillation using MgO and $CaCl_2$ (AOAC, 1970). Cournarin and dihydrocournarin were determined by gas chromatography and followed by identification using mass spectra.

Statistical analysis.

Differences between means were examined by analysis of variance using the general linear model procedure of the Statistical Analysis Systems (SAS, Institute Inc., 1988). Significant differences between the treatment groups were determined by least significant difference (LSD) and were declared at $P<0.05$.

RESULTS

The chemical compositions of gliricidia leaf are presented in Table 1. Dry matter contents of oven-dried gliricidia leaves increased following redrying either at 70 or 100^0 C. Nitrogen and condensed tannin contents were similar among treatments, except the condensed tannin level of oven-dried leaves following redrying at 100^0 C was lower compared to the other treatments. pretreatments reduced cournarin contents, but did not change dihydrocoumarin levels.

Retalhuleu gliricidia cultivar contained much higher levels of cournarin, but its dihydrocourmarin content was less than one-third of that dihydrocournarin in the local gliricidia cultivar.

Table 1. The chemical composition of gliricidia leaf as affected by pretreatments.

	Treatments				
Constituents	gliricidial [1] (G)	G. redried at 70^0C	G.redried at 100^0C	G. frozen at -15^0 C overnight	Retalhuleu [2]
Dry matter (%)	88.7	96.3	97.5	86.4	94.3
N (% DM)	3.02	3.05	3.59	3.32	3.06
Condensed tannin (%DM)	7.0	6.8	5.2	7.1	2.6
Coumarin (mg/kg DM)	142	69	79	56	7158
Dihydrocournarin (mg/kg) DM)	1152	1170	1213	939	343

[1]Gliricidia oven-dded (50 ^{0}C for 48 h) [2] Retalhuleu oven-dried (50 ^{0}C for 48 h).

Intakes of gliricidia leaf by sheep (n = 6) over an adaptation-period of 7 days were low (64.7 ± 19.6 g DM/d, mean ± SD). Administration of polyethylene glycol into the rumen did not increase intake of gliricidia leaf compared to unsupplemented sheep (P<0.05, Table 2). Similarly, rumen ammonia-N concentration was not affected by administration of PEG into the rumen (P>0.05). Similarly, intakes of gliricidia leaf were not altered by pretreatments (P.>0.05, Table 3).

Table 2. Intake of gliricidia leaf by sheep and rumen ammonia-N concentration as influenced by administration of polyethylene glycol into therumen.

Parameters	Treatments		
	Control	PEG	Lsd (P<0.05)
Intake (g DM/d)	283.7[a]	271.2[a]	211
Rumen ammonia-N (mg/L)			
30 mins before feeding	13.0[a]	9.5[a]	12.7
6 h after feeding	25.73[a]	25.0[a]	27.5

[aa] Means with similar notations are not different (P>0.05).

Table 3. Amounts of gliricidia leaf eaten by sheep as affected by pretreatments.

	Treatments				
Intake (g DM)	gliricidia[1]	gliricidia redried at 70 'C	gliricidia at 100^0C	gliricidia frozen at -15^0C overnight	Lsd (P<0.05)

In the first hour	39^{a}	22^{a}	25^{a}	34^{a}	30
Over 6 h	47^{a}	33^{a}	31^{a}	46^{a}	43

[1]Gliricidia oven-dried at 50 OC for 48 h. " Means, within rows with similar notations are not different (P>0.05).

None of the additives in the third trial increased intake of gliricidia leaf in the first hour of eding. In contrast, palm kernel extract reduced intake of gliricidia compared to other treatments, except for the additive grass hay (P<0.05). However, if intake was measured over 6 h only additive molasses increased intake of the leaf significantly compared to the control treatment (Table 4). When additives were fed alone, ground barley grain and cottonseed were eaten the fastest while palm kernel meal and grass hay were eaten most slowly.

Table 4. Intake of gliricidia leaf and of additives by sheep.

	Additives							
Intake of gliricidia (g DM)	none	wheat millrun	molasses	grass hay	cotton seed meal	palm kernel meal	ground wheat grain	Lsd (P<0.05)
In first h	34^{bc}	41^{bc}	49^{bc}	28^{ab}	51^{b}	8^{a}	56^{c}	25
Over 6 h	43^{ab}	49^{ac}	74^{c}	42^{ab}	64^{bc}	30^{a}	63^{bc}	29
Additive Intake (gDM/mins)	-	10^{b}	8^{b}	2^{a}	21^{c}	0.5^{a}	26^{d}	5

[abc] within rows, means with dissimilar notations are significantly different (P<0.05).

DISCUSSION

Several authors have reported that gliricidia leaf contains plant secondary metabolites such as condensed tannins (Ahn *et* al., 1989; Ash, 1990; Osei *et* al., 1990; Jackson *et al.,* 1996), cournarin and melilotic acid (Griffiths, 1962; Wina *et al.,* 1993), and a cyanogenic glycoside (Manidool, 1985). However, lower values of condensed tannins in gliricidia leaf were reported by these authors compared to the values found in this trial. High variability in condensed tannin contents was also reported by Castillo *et al.* (1997) in several lines of *Leucaena leucocephala* when samples were taken in December 1992 and in July 1993 (3.4 and 6.6 % of DM, respectively). It is suggested that season, soil fertility status, and methods of sampling are important factors in determining condensed tannin levels (Kumar and Vaithiyanathan, 1990; Thapa *et al.*, 1997; DaIzell. and Shelton, 1997).

No improvement in intake of gliricidia leaf by sheep was observed due to administration of PEG into the rumen. This contrasted with the findings of many authors who have observed an increase in intake of tanniferous forages by either sheep or goats supplemented with PEG. For example, mulga (*Acacia aneura*) fed to sheep (Pritchard *et al*., 1992) ; carob (*Ceratonia siliqua*) fed to sheep (Silanikove et al., 1994) or oak (*Quercus calliprinos*), pistacia (*Pistacia lentiscus*) and carob (*Ceratonia siliqua*) fed to goats (Silanikove *et al*., 1996). According to these authors, condensed tannins in these forages are assumed to have a depressing effect on intakes and on rumen protein degradation due to formation of insoluble tannin-protein complexes. It is known that protein from these complexes can be released by addition of PEG which preferentially binds to tannins (Jones and Mangan, 1977; Oh *et al*., 1980).

From the result of this trial it appears that tannins in gliricidia leaf may not be as important as tannins from other forages in terms of their effects on depressing palatability and voluntary intake by animals (Barry and Duncan, 1984; Barry and Manley, 1984; Barry *et al*., 1986). This was evident from the lack of responses in intake and in rumen ammonia-N concentrations between the control and the PEG supplemented sheep. Norton (1994) has also suggested that the tannin content of gliricidia leaves does not appear to interfere with availability of its protein. A similar lack of response in digestible organic matter intake was also observed by McSweeney *et al*. (1998) in sheep offered buffel grass hay supplemented with 30 % calliandra leaf and 40 g PEG/d. Tannins in different forages may have different nutritional effects on animal metabolism as suggested by many authors (Barry and Manley, 1986; Barry and Manley, 1986 and Wang *et al*., 1994). According to these authors, reactivity of condensed tannins with protein is probably a function of plant condensed tannin concentrations, molecular weights and chemical structures. Other secondary compounds rather than tannins may be responsible for the low palatability of local gliricidia cultivar by sheep.

Pretreatments (reheating and freezing) did not influence dry matter intake, although the coumarin content reduced by 49, 55 or 40 % of the cournarin in the original samples due to redrying at 70^0 C, 100 ^{0}C or freezing at –15 ^{0}C overnight, respectively. On the other hand, the concentration of dihydrocoumarin was similar among the four dietary treatments, levels of 1152, 1171, 1213 and 939 ppm for the oven-dried, redried at 70^0 C, 100 ^{0} C and frozen leaf, respectively were detected. Lack of intake response in a preference test by sheep offered three different wilting states of *Gliricidia sepium was* observed by Sukmawati (1994). It *was* reported that intake of gliricidia leaves was 37.3, 22.6 and 39.7 % of total DM eaten per day for gliricidia wilted for 0, 6 and 24 h, respectively, and the difference *was* not significant. The lack of intake response in our trial may be associated with the similar level of dihydrocoumarin, and redrying or freezing did not affect dihydrocoumarin contents.

Reduced intake of gliricidia leaf when it *was* mixed with palm kernel meal may have been due to the unpalatability of this additive to sheep. The intake rate of this additive when fed alone *was* 0.5 g DM/minute, which *was* much lower compared to the other supplements, especially cottonseed meal and ground barley grain (21 and 26 g DM /minute, respectively). Palm kernel meal is known to contain high levels of copper (Jelan 1990) and feeding this by-prrroduct may induce an aversive response as suggested by Provenza (1996). Mixing gliricidia with molasses increased the intake of leaf by the sheep. This may be due to the liquid of molasses which may enable it to adequately mix with the leaf and mask the smell of volatile compounds in the leaf. This masking effect may be similar to that resulting from adding oil to leaves of *Erythrina fusca* to increase its intake by animals (6 kg oil to 94 kg wilted leaves) as suggested by Preston (1995). This author tentatively suggested that oil may act as a "sink” for volatile secondary compounds which may be responsible for the low palatability. This may not occur when solid supplements such as cottonseed meal are used. It was also noted in other trials that mixing the leaf with cottonseed meal increased intake of the leaf by sheep by only 10% compared to the control diet.

CONCLUSIONS

It is concluded that local gliricidia leaves is not palatable for sheep, and its intake can not be improved by either addition of PEG or redrying and/or fteezing of the previous dried materials. Nevertheless, only mixing the leaf with molasses is able to mask the taste of the leaf leading to improvement in its acceptability.

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