UTILIZATION OF TANNIN CONTAINING SHRUB LEGUMES FOR SMALL RUMINANT PRODUCTION IN INDONESIA

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ABSTRACT

Shrub legumes have been well established in Indonesia and some of which contain significant levels of tannin. Several methods of tannin analysis have been conducted and correlated with its biological property of tannin. Total phenolics and total tannin measured by Folin Ciocalteau have a high correlation with the biological assay of tannin using gas *in vitro* method. Tannin values measured by the above methods negatively correlated with protein digestibility. Several feeding experiments on tannin containing shrub legumes using *Calliandra calothyrsus* as a model plant have been conducted. Feeding in fresh condition or silage form is the best way of feeding tannin containing forages. Several methods were tested to deactivate tannin include PEG addition, soaking in water or alkaline solution. Starch addition is one of the alternatives to mask the effect of tannin as it improved the performance of sheep fed tannin-containing legumes. Cofeeding with other leguminous leaves is another technique to utilize tannin in shrub legumes as protein binding agent. Several experiments showed that tannin could reduce the worm burden in ruminant although its utilization as anthelminthic agent in Indonesia is still limited. In conclusion, tannin containing shrub legumes have several beneficial effects to improve small ruminant production.

Key words: Tannin, legumes, protein binding, anthelmintic agent, small ruminant

ABSTRAK

PEMANFAATAN TANAMAN LEGUMES YANG MENGANDUNG TANIN UNTUK PRODUKSI TERNAK RUMINANSIA KECIL DI INDONESIA

Tanaman legum sudah banyak ditanam di Indonesia dan beberapa jenis tanaman ini mengandung senyawa tanin yang cukup tinggi. Beberapa metode analisis tanin telah dilakukan dan dikorelasikan dengan sifat biologis tanin dan ternyata total fenol dan total tanin yang diukur dengan Folin Ciocalteau mempunyai korelasi yang tinggi dengan sifat biologis yang diukur dengan metode *in vitro* gas. Nilai tanin yang diukur secara kimia mempunyai korelasi negatif dengan nilai kecernaan protein. Beberapa eksperimen pemberian legum yang mengandung tanin seperti *Calliandra calothyrsus* sebagai model telah dilakukan. Pemberian dalam bentuk segar atau silase adalah cara yang terbaik untuk memberikan tanaman yang mengandung tanin. Beberapa metode telah diuji untuk menonaktifkan tanin termasuk pemberian PEG, perendaman dalam air atau larutan basa. Penambahan tepung pati merupakan salah satu alternatif untuk menutupi pengaruh tanin karena dapat meningkatkan performans domba yang diberi legum mengandung tanin. *Cofeeding* adalah teknik lain untuk memanfaatkan tanin dalam legum sebagai agen pengikat protein. Beberapa percobaan memperlihatkan bahwa tanin dapat menekan populasi cacing pada ternak ruminan walaupun pemanfaatan sebagai anthelmintik di Indonesia masih terbatas. Disimpulkan bahwa legum yang mengandung tanin mempunyai beberapa keuntungan untuk meningkatkan produksi ternak ruminansia kecil.

Kata kunci: Tanin, legum, pengikat protein, agen antelmintik, ruminansia kecil

INTRODUCTION

Indonesia dominates the small ruminant sector in Southeast Asia, accounting for more than three-quarters of total sheep and goat numbers. According to the Indonesian statistical book on livestock, the population of sheep and goat in 2009 was 10.47 millions and 15.63 millions, respectively (DGLVS, 2009). Sheep are raised in Java while goats in Java and other islands. They are mainly kept by small farmers and fed mixed forages mainly native grasses or agricultural by-products such as sweet potatoes vines, corn leaves etc. Lack of available feed is a common problem during dry season especially in the eastern part of Indonesia. Legume trees offer a good supplementary feed to those low quality grasses.

Most of shrub legumes in Indonesia were originated from Central or South America and have become naturalized since their introduction some decades ago. The large cultivation of some legumes was conducted by the Forestry people for reforestation in forest buffering zone or for preventing land erosion (TANGENDJAJA *et al*., 1992).

Shrub legumes are important sources of feed as most of them contain high protein level and produce high biomass. However, they also contain secondary compounds, which can be beneficial or detrimental to the animal (LOWRY *et al*., 1992). Tannins are one of the secondary compounds, which have been identified in many leguminous trees. There are two types of tannin, i.e hydrolysable and condensed tannins. Hydrolysable tannin can be degraded in the rumen while condensed tannin is resistant to rumen degradation (MAKKAR and BECKER, 1996). There have been a lot of research on tannins and their effects on animal production, animal and human health and on the environment. Many methods have been developed to analyze tannin content in plant materials, which include its chemical or biological properties. This paper describes several experiments from laboratory analysis, several feeding experiments conducted at Indonesian Research Institute for Animal Production (Balitnak), technology to overcome negative properties of tannin and practical use of tannin containing shrub legumes in Indonesia and more opportunity to utilize tannin in the animal diet.

Chemical composition and tannin content of tannin containing shrub legumes

Considerable information on chemical composition of shrub legumes is now available (LOWRY *et al.,* 1992). Most common shrub legumes that have been widely grown in different places in Indonesia contain high level of protein (17 – 29%), but they also contain tannins at the level of 1.2 to 9% (LOWRY *et al.,* 1992). The tannin values in the same legumes may be different in different publications as there are many methods to analyse tannins in the forage materials (MAKKAR, 2003a). Therefore, care should be taken when comparing tannin concentrations from different publications, as not only the methods but also the standard used may be different. Table 1 shows 6 different methods of analysis were applied to measure tannin content in 3 legumes. These methods measured tannin content and biological activity of tannin and detailed protocols were described by MAKKAR (2003a).

For tannin content, the measurement included total tannin or condensed tannin while for biological activity, the measurement included its ability to precipitate Bovine Serum Albumin (BSA = protein) or the ability to decrease gas production in the *in vitro* fermentation. This tannin activity was expressed as the percent increase in gas production after polyethylene glycol (PEG) addition in the *in vitro* fermentation method (MAKKAR, 2003a). The IAEA tannin project in comparing several methods of tannin analysis concluded that only total tannin value measured by PVPP–Folin Ciocalteau method and condensed tannin measured by Butanol–HCl method were highly correlated with the biological activity of tannin. Therefore, it can be suggested that the first screening for tannin level from forage materials can be done by total tannin method (PVPP–Folin Ciocalteau) followed by gas *in vitro* fermentation with and without PEG addition. However, more detailed study on the fractionation of phenolic compounds is required to elucidate the mechanism of tannin effect. There is also very few information on hydrolysable tannins in forage legumes and study on their role in animal production is warranted.

Feeding of tannin containing shrub legumes to small ruminants

Several studies on tannin effect on animal performance have been conducted in different countries and the results showed that tannin negatively correlated with the nitrogen or protein digestibility. MAKKAR (2005) summarized tannin values measured by PVPP–Folin, Bu-HCl, radiolabelled BSA methods and percent increase in gas production which negatively correlated with protein digestibility. A similar study on the effect of tannin from 3 different legumes was conducted at Balitnak, Ciawi. It also showed that increasing tannin level in the consumed diet with higher inclusion of legumes decreased protein digestibility in a dose manner (Figure 1) but it did not negatively affect the intake of legumes containing diets.

Table 1. Tannin content in shrub legumes measured by several methods

	Tannin content			Biological activity		
	Total tannin		Condensed tanin	BSA precipitation		*In vitro*
	PVPP – folin	$FeCl_3$	Bu – HCl	Ponceau – S	Diffusion	PEG effect
	mg/gDM	mg/gDM	mg/gDM	mgBSA/gDM	mg/gDM	%
C. calothyrsus	132.5b	50.6c	37.4b	181.15b	10.9a	70.5a
A. villosa	163.9c	32.1b	12.6a	102.39a	16.5b	233.3b
L. diversifolia	94.4a	24.3a	12.1a	107.75a	13ab	61.1a

Different letter in the same column showed significantly different (P < 0.05)

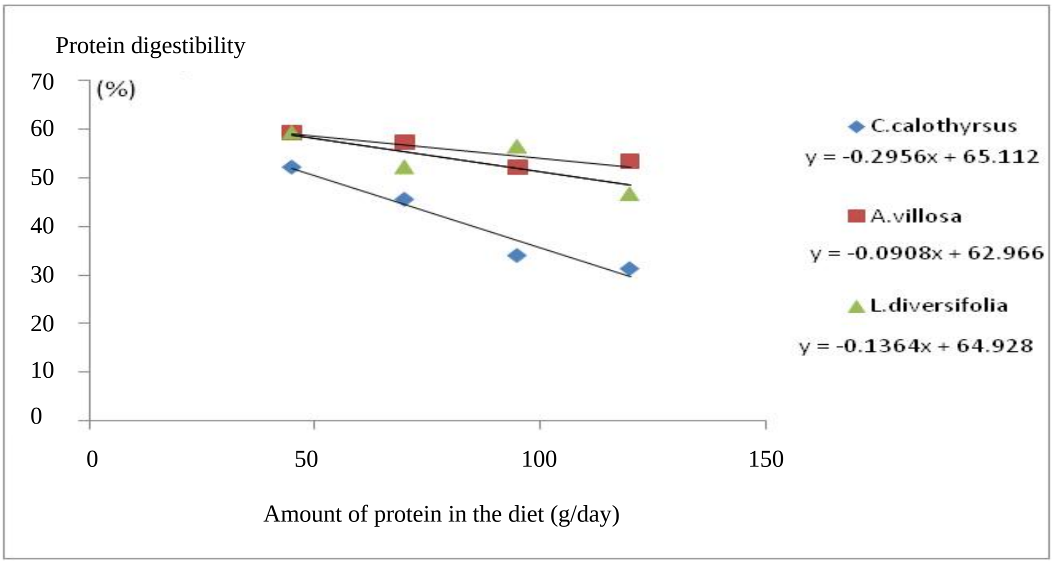

Figure 1. Protein digestibility of diets containing mixed grasses and oven dried legumes at different levels protein

Digestibility of a diet was influenced by microbial digestion in the rumen and in the lower gut. Tannin affected digestibility of a diet especially protein digestibility as tannin binds protein and produced tannin-protein complex. RAKHMANI *et al.* (2005) found that different fractions of tannin affected feed digestion at different part of the digestive tract. Oligomers, flavanols and flavonol glycosides fractions isolated from tannin *Calliandra calothyrsus* depressed the microbial digestion in the rumen while polymeric proanthocyanidins fractions increased the nitrogen loss from animals by forming an insoluble complex with protein and fibre. Therefore, it was found that the nitrogen in faeces from sheep fed tannin containing forages would be higher than that from sheep fed non tannin containing forages (Table 2).

Drying tannin containing forages would cause stronger binding of tannin to protein and fibre, hence, reduce the digestibility. As a sole diet, the intake of oven dried calliandra was much lower than the fresh calliandra (Table 2). An increase of bound tannin and a decrease of extractable tannin were reported as a result of oven drying (aerobic drying) (PALMER *et al.*, 2000). Not only oven drying, wilting process (one day wilting) also reduced the protein digestibility and slightly depressed the intake and as a result, the average daily gain of sheep fed wilted calliandra was lower than those fed fresh calliandra.

Table 2. Effect of fresh, oven dried and wilted calliandra on dry matter intake protein digestibility, Nitrogen in feces and daily gain (ADG) of sheep

Experiments	Intake DM (g/day)	ADG (g/day)	CPD (%)	N in feces (%)	References
Exp 1 (n = 8, BW = 9.5kg)*					WINA *et al.* (1993)
Fresh Calliandra 100%	423a		39.1a	4.74a	
Oven dried Calliandra	263b		18.9b	5.23b	
Exp. 2 (n = 30, BW = 15kg)					WINA *et al.* (1997)
Fresh calliandra	812		41.45		
Wilted calliandra	720		35.52		
0% Calliandra	693	65.9b		1.45a	
15% fresh Calliandra	725	65.9b		1.83b	
30% fresh Calliandra	783	77.8c		2.03c	
15% 1day–wilted Calliandra	684	57.5ab		1.97cb	
30% 1day–wilted Calliandra	759	54.0a		2.26d	

* Exp: experiment; n: number of animal; BW: body weight; DM: dry matter; ADG: average daily gain, N: nitrogen

Feeding fresh calliandra to sheep resulted in an increased average daily gain (ADG), however, this gain was only significantly different to control (0% calliandra) when the level of fresh calliandra was 30% of the diet on DM basis (Exp 2, 3 in Table 2). However, dry matter digestibility (DMD) decreased in a dose manner when calliandra was included in the diet. (Exp 3 Table 2).

In Experiment 4 (Table 3), the nitrogen from urea or sulphur supplementation from ammonium sulphate did not give any positive effect when calliandra was included in the feed. The result suggested that additional nitrogen or sulphur was not required when calliandra was fed. The level of nitrogen contributed from calliandra in the rumen was sufficient for the microbial protein synthesis in the rumen although tannin in calliandra might bind some protein and caused less degradation of protein in the rumen. Beside fresh calliandra, feeding calliandra silage is another alternative way during drought season. The intake and digestibility were not affected by calliandra silage. The most significant increase in ADG (64.6% higher) occurred when the animal was supplied with additional starch/ energy source (dried powder cassava) since the intake and the digestibility increased.

Overcoming the negative effect of tannin in tannin containing shrub legumes

High tannin level in the tannin containing shrub legumes should be reduced to prevent the negative effect of tannin in those plants when fed to the animals. Polyethylene glycol (PEG) has been used extensively as a tannin-binding agent to inhibit their biological effect (MAKKAR, 2003b). This was not only proved *in vitro* but also in the *in vivo* experiments. Spraying polyethylene solution to tannin containing legumes (ratio PEG : CT = 2:1) could reduce the tannin level to 50% lower and improved ADG of sheep by 37% (BEN SALEM *et al.*, 1999a). Spraying 4% PEG solution to calliandra forages (12 g PEG in 1kg DM calliandra) improved the nutritional quality of this forage as it was shown by higher daily gain of sheep fed PEG-treated calliandra (Table 4). Higher intake of tannin containing forage has been reported when PEG was added into the diet (MANTZ, 2007). Higher nitrogen retention and higher amount of microbial protein entering the duodenum were found as the result of PEG addition. Spraying with PEG is difficult to be applied in the field, therefore, PEG is suggested to be mixed in the feed blocks (BEN SALEM *et al.*, 1999b). However, as the price of PEG is quite expensive, its application should be considered carefully.

Soaking in lime solution reduced the tannin content in acacia (WINA *et al.*, 2005) as a result of high pH solution which mediates the oxidation of tannin structure to quinone (MAKKAR and BECKER, 1996). The alkaline condition might also make cell wall/ fibre swollen and, hence, fibre was easily degraded by the rumen microbes. However, in the feeding trial, lime treated calliandra or acacia neither improved the digestibility of feed nor the performance of sheep (Exp 5 Table 5, WINA *et al.*, 2005; ALAM *et al.*, 2005). In contrast, the alkaline condition of ash solution improved CP and NDF digestibility significantly. Higher protein was retained when feeding sheep with ash-treated *Acacia cyanophylla* forages compared to that with untreated Acacia (BEN SALEM *et al.*, 2005a). Further evaluation to see the effect of ash treated forages on animal performance is warranted since wood ash solution may be an alternative and cost-effective way to deactivate tannin in the village area where people used fire wood for cooking their food.

Table 3. Feeding fresh calliandra to substitute elephant grass to sheep

Experiments	DM Intake (g/day)	ADG (g/day)	DMD (%)	References
Exp 3 (n = 18, BW = 11kg)*				WINA (*unpublished*)
0% Calliandra	435a	44.0a	61.77a	
15% fresh Calliandra	492a	59.1a	58.28b	
30% fresh Calliandra	572b	77.2b	57.05b	
Exp. 4 (n = 35, BW = 15kg)				WINA *et al.* (1995)
0% Calliandra	457a	44.4a	54.83b	
40% fresh Calliandra	677c	61.8b	46.97a	
40% fresh Calliandra +urea	637bc	64.2b	43.13a	
40% fresh Calliandra + urea+ ammonium sulphate	543ab	60.2b	47.58a	
40% fresh Calliandra +cassava	621bc	73.2c	56.37b	

* Exp: experiment; n: number of animals; BW: body weight; DMD: dry matter digestibility

Table 4. Effect of detannification (PEG, soaking in water or quick lime solution) on the intake, ADG of sheep and digestibility of feed

Experiments	DM Intake (g/day)	ADG (g/day)	DMD (%)	References
Exp 5				
30% Calliandra	666	72.9a	49.6a	WINA *et al.* (1994)
30% Calliandra + PEG	716	86.7b	56.8b	
30% Calliandra + Quick Lime	711	69.1a	45.9a	
Exp 6				
30% fresh Acacia	557	38.9a	49.5a	WINA *et al.* (2005)
30% H_2O-soaked Acacia	615	44.7a	51.1a	
30%H_2O-soaked Acacia + cassava	719	71.0b	57.8b	

In experiment 4 (Table 3) and experiment 6 (Table 4), the addition of cassava flour improved the ADG of sheep significantly. Cassava flour or starch is a source of energy, which is required for increasing the microbial synthesis (GETACHEW *et al.*, 2000) in tannin containing feed. Starch supplementation also increased feed intake and microbial N production *in vivo* (HINDRICHSEN *et al.,* 2004). In our experiment, it may also help in masking the effect of tannins, which suggest that cassava addition could be used as a strategy to reduce or overcome tannin effect rather than the removal of tannins. Cassava is an abundant energy source available year-round in many tropical countries and is easy to be used by the farmers for feeding their animals. Beside cassava, rice bran is another cheap energy source for animal feed in rice producing countries.

Utilization of tannin in tannin containing shrub legumes as a protein binding agent

Table 5 shows that tannin isolated from *Calliandra calothyrsus* could be very useful as a binding agent for forage protein (*Gliricidia sepium*) as it gave the same reactivity as formaldehyde to produce the same amount of bypass protein *in vitro* (34.4 vs 32.1 g/100g *G. sepium*). The amount of ruminal bypass protein is measured as the difference between the amount of protein digested in stage 2 – protein digested in stage 1 of Tilley and Terry *in vitro* method.

Table 5. The difference in digestible CP of casein, soybean meal and *G. sepium* leaves between 72 and 48 hours of *in vitro* incubation in the presence of tannin or formaldehyde

Binding agent	Casein	Soybean meal	*Gliricidia sepium* leaves
	g/100 g substrate		
Tannin isolate	5.3	27.9	34.4
Formaldehyde	81.4	54.1	32.1

Source: WINA and ABDUROHMAN (2005)

Cofeeding of fresh calliandra with another legume (*Gliricidia sepium*) to goat gave a better ADG than feeding Gliricidia alone (Exp 7 Table 6). It was assumed that tannin in Calliandra might protect the soluble protein of gliricidia to form a complex of tannin-protein. This complex, then flowed to the duodenum and at low pH in the duodenum, the binding of tannin to protein was released. The protein could be hydrolysed to amino acid and be absorbed and utilized for animal growth (MAKKAR, 2003b).

Another *in vitro* experiment showed that tannin in calliandra could slow down the degradation of soybean meal and tofu waste, hence, increased the rumen undegraded protein to the duodenum. Similar result was also reported by CORTES *et al.* (2009).

Feeding a mixture of calliandra with tofu waste to sheep resulted in a better ADG compared to feeding only tofu waste (Exp 8 Table 6). Feeding tannin-protein complex may give two beneficial effects since it reduced excess protein degradation in the rumen and increase amino acid supply to the duodenum (FRUTOS *et al.,* 2000).

There was no difference of ADG or digestibility when feeding of calliandra one hour earlier than feeding tofu waste/soybean meal compared to cofeeding of calliandra and tofu waste/soybean meal. In contrast, BEN SALEM *et al.* (2005b) reported that feeding of acacia 1 hour earlier than feeding soybean meal gave a significantly higher ADG compared to cofeeding of acacia and soybean meal. The work on feeding strategy of tannin containing shrub legumes with other protein source or non tannin forages, therefore requires further evaluation.

Effect of long term feeding of tannin containing shrub legumes

One of the advantages of feeding tannin containing forages is increasing the apparent intestinal absorption of essential amino acids especially essential branched-chain amino acids (BCAA; ROY *et al.*, 2004). BCAA is required for milk protein synthesis, suggesting that tannin-containing legumes may have potential as forages for dairy cows or for lactating ewes. Feeding 30% of fresh calliandra to pregnant ewes (5 months feeding) significantly reduced the body weight loss of the ewes, reduced the interval post partum-estrus and improved the ADG of lamb (Table 7).

Grazing tannin containing forages (willow, *Lotus corniculatus*) using alternate system with non tannin forages resulted in a lower body weight loss of the ewes and an increased reproduction rate (PITTA *et al.*, 2005; RAMIREZ-RESTREPO and BARRY, 2005; MCWILLIAMS *et al.*, 2005). It can be concluded that long term feeding of tannin containing shrub legumes gains positive effect on both ewes and lambs performances.

Table 6. Utilization of tannin as protein binding agent (*in vivo* experiment)

Experiments	DM intake (g/day)	ADG (g/day)	CPD (%)
Exp 7 (n + 30, BW = 15kg)*			
30% fresh calliandra	701a	54.57	44.0b
30% fresh gliricidia	603b	50.29	55.38a
15% calliandra + 15% gliricidia	740a	61.14	52.93a
Exp 8 (n = 35, BW = 21kg)			DMD(%)
Tofu waste	702b	68.51	70.39a
Calliandra 1 hour earlier than tofu waste	742b	83.57	64.48b
Calliandra + tofu waste cofeeding	767ab	78.01	67.25ab
Calliandra 1 hour earlier than soybean meal	855ab	79.95	67.97a
Calliandra + soybean meal cofeeding	843ab	79.64	68.51a

* Exp: experiment; n: number of animals; BW: body weight; CPD: crude protein digestibility

Other application of tannin containing shrub legumes

In recent years, there is a growing interest on the use of tannin as anthelmintic agent. It has been known that worm problem is a big issue in small ruminant production, especially in the village or grazing system. It has been reported that there is a growing problem of residue and resistance to anthelmintic drug. Thus there is merit in the development of alternative systems of control of such parasites. Some high tannin containing forages have been shown to improve the resilience of animals to gastrointestinal (G1) parasites. The effects may be directly due to the presence of condensed tannins that bind nematodes or indirectly due to improvement in protein nutrition, which enhances the

Table 7. Effect of fresh Calliandra on the reproductive and productive performances of ewes and the performance of lamb

Parameter	Without Calliandra	30% Calliandra
Ewes, body weight kg (n = 20)		
Initial weight	22.6	22.3
At lambing	27.3	29.1
At weaning	24.6a	27.1b
Daily consumption (g/day)		
During pregnancy	975	1015
During lactation	1017	1287
Interval post partum-estrus (day)	62.7a	51.0b
Ovulation rate	1.7	1.8
Lamb		
Birthweight (kg)	1.8	1.8
ADG (g/day)	53.7a	89.2b
Weaning weight (kg)	6.7a	9.1b

Values with different superscript within the same row were significantly different ($P < 0.05$)

Source: SUTAMA *et al.* (1994)

immune response. It was suggested that the level of tannin in plant for reducing fecal egg count (FEC) is within the range of 45 to 55g/kg DM (MIN and HART, 2003). MIN *et al.* (2005) reported that grazing some tannin containing forages for 2 weeks reduced the faecal egg count of does. Therefore, tannin reduced FEC in both sheep and goat. However, only intestinal worm was reduced by Quebracho tannin (8% in the diet) while the abomasal worm remained unaffected (ATHANASIADOU *et al.,* 2001). To understand the mechanism how tannin controls GI parasites, CRESWELL (2007) has studied in more detailed on the effects of tannin containing legumes as anthelmintic agents for ruminant.

Besides its anthelmintic activity, some studies show that tannin also can be used to inhibit the growth of *E. coli* in the aerobic condition by generating H_2O_2 (SMITH *et al*., 2003) or inhibit the growth of other pathogenic bacteria such as *Salmonella*, *Campylobacter* spp., *Clostridium* (SINGH *et al*., 2003). Therefore, the study on the use of tannin powder for treating diarrhea for young ruminants or monogastric animals is warranted.

With regard to global warming issue, several studies have been conducted to reduce methane emissions from ruminant. Ruminant is blamed to be the highest producer of methane since ruminant produces methane in the rumen and also in the hindgut. Faeces is a good source of methane (biogas) when kept anaerobically. Tannin has been reported to reduce methane production in the rumen. JAYANEGARA *et al.* (2009) found that *in vitro* tannin bioassay method is best correlated with its potency to reduce methane production. CARULLA *et al.* (2005) reported that feeding *Acacia mearsnii* tannins were able to reduce methane release by 13%. Study using pure culture (TAVENDALE *et al*., 2005) showed that tannin reduced methane production by reducing the methanogenic bacteria. TIEMANN *et al*. (2008) reported that feeding tannin containing legumes, *Calliandra calothyrsus* or *Flemingia macrophylla* to sheep reduced methane production, however, the methane-suppressing effect of CT was resulted from reduced fibre digestion and reduced fermentation activity and not from specific inhibition of methanogens. More studies are required to confirm the mechanism of tannin in reducing methane production.

Practial use of tannin containing shrub legumes in Indonesia

Some farmers have been using local adapted legumes more than 30% of the diet to feed their livestock, however, tannin level in this type of diet was less than 2% (Table 8). Therefore, there are only minor effect of tannin might appear to reduce the protein degradation in the rumen or increase the amino acid supply to the duodenum or reduce the worm burden. The use of higher amount of tannin containing shrub legumes to feed ruminant should be evaluated at local feeding system and promoted among the farmers.

Table 8. Feed composition of village feeding in Kali Gesing (West Java) and Amarasi (East Nusa Tenggara)

Forage	% in the diet	% tannin in the diet
Goats feeding in Kali Gesing, Central Java (high land area)		1.3
Native grass	50.6	
Calliandra calothyrsus	11.7	
Gliricidia sepium	7.9	
Leucaena leucocephala	5.0	
Erythrina subumbrans	8.8	
Artocarpus leaves	11.7	
Cassava leaves	3.5	
Cattle feeding in Amarasi, East Nusa Tenggara (dry area)		0.67
Leucaena leucocephala	54	
Native grasses	17	
Sesbania grandiflora	10	
Legume straw	8	
Banana leaves	5	
Corn leaves	2	
Rice straw	8	
Cassava leaves	1	
Sweet potato leaves	0.4	

Source: TANGENDJAJA *et al.* (2000)

CONCLUSION

Based on the available information, it is concluded that the first screening for tannin level from forage materials can be done by total tannin method (Folin Ciocalteau-PVPP) followed by gas test with PEG addition.

Harvesting and processing of tannin containing shrub legumes before feeding will affect digestibility of these legumes, therefore feeding in the fresh condition is highly recommended for tannin containing shrub legumes. But, when feeding in fresh condition may not be practical for some areas, feeding silage of shrub legumes is another choice.

PEG is still an effective chemical to deactivate tannin. Although soaking in calcium hydroxide or water reduced tannin content, they did not improve the animal performance. It was found that feeding tannin–containing legumes mixed with other non-tannin containing legumes or protein source improved animal performance.

Reduced worm burden, inhibited the growth of pathogenic bacteria, reduced methane production in the rumen are several other beneficial effects of tannin containing shrub legumes on ruminant.

REFERENCES

ALAM, M.R., A.K.M.A. KABIR, M.R. AMIN and D.M. MCNEILL. 2005. The effect of calcium hydroxide treatment on the nutritive and feeding value of *Albizia procera* for growing goats. Anim. Feed Sci. Technol. 122: 135 – 148.

ATHANASIADOU, S., I. KYRIAZAKIS, F. JACKSON and R. COOP. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasit. 99: 205 – 219.

BEN SALEM, H., A. NEFZAOUI, L. BEN SALEM and J.L. TISSERAND. 1999a. Intake, digestibility, urinary excretion of purine derivatives and growth by sheep given fresh, air dried or polyethylene glycol-treated foliage of *Acacia cyanophylla* Lindl. Anim. Feed Sci. Technol. 78: 297 – 311.

BEN SALEM, H., A. NEFZAOUI, L. BEN SALEM and J.L. TISSERAND. 1999b. Different means of administering polyethylene glycol to sheep: Effect on the nutritive value of *Acacia cyanophylla* Lindl. foliage. Anim. Sci. 68: 809 – 818.

BEN SALEM, H., S. ABIDI, H.P.S. MAKKAR and A. NEFZAOUI. 2005a. Wood ash treatment, a cost-effective way to deactivate tannins in *Acacia cyanophylla* Lindl. foliage and to improve digestion by Barbarine sheep. Anim. Feed Sci. Technol. 122: 93 – 108.

BEN SALEM, H., H.P.S. MAKKAR, A. NEFZAOUI, L. HASSAYOUN and S. ABIDI. 2005b. Benefit from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla* Lindl.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. Anim. Feed Sci. Technol. 122: 173 – 186.

CARULLA, J.E., M. KREUZER, A. MACHMULLER and H.D. HESS. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Aus. J. Agric. Res 56: 961 – 970.

CORTÉS, J.E., B. MORENO, M.L. PABÓN., P. AVILA, M. KREUZER, H.D. HESS and J.E. CARULLA. 2009. Effects of purified condensed tannins extracted from Calliandra, Flemingia and Leucaena on ruminal and postruminal degradation of soybean meal as estimated *in vitro*. Anim. Feed Sci. Technol. 151: 194 – 204.

CRESWELL, K.J. 2007. Anthelmintic Effects of Tropical Shrub Legumes in Ruminant Animals. PhD thesis. James Cook University, Australia. 293 p.

DGLVS. 2009. Livestock Statistics. Directorate General of Livestock and Veterinary Services. www.ditjennak.go.id/bank/tabel4.1pdf (February 1, 2010).

FRUTOS, P., G. HERVAS, F.J. GIRALDEZ, M. FERNANDEZ and A.R. MANTECON. 2000. Digestive utilization of quebracho-treated soyabean meals in sheep. J. Agric. Sci. 134: 101 – 108.

GETACHEW, G., H.P.S. MAKKAR and K. BECKER. 2000. Tannins in tropical browses: Effects on *in vitro* microbial fermentation and microbial protein synthesis in media containing different amounts of nitrogen. J. Agric. Food Chem. 48: 3581 – 3588.

HINDRICHSEN, I.K., P.O. OSUJI, A.A. ODENYO, J. MADSEN and T. HVELPLUND. 2004. Effect of supplementation of maize stover with foliage of various tropical multipurpose trees and *Lablab purpureus* on intake, rumen fermentation digesta kinetics and microbial protein supply of sheep. Anim. Feed Sci. Technol. 113: 83 – 96.

JAYANEGARA, A., N. TOGTOKHBAYAR, H.P.S. MAKKAR and K. BECKER. 2009. Tannins determined by various methods as predictors of methane production reduction potential of plants by an *in vitro* rumen fermentation system. Anim. Feed. Sci. Technol. 150: 230 – 237.

LOWRY, J.B., R.J. PETHERAM and B. TANGENDJAJA. 1992. Plants Fed to Village Ruminants in Indonesia. Notes on 136 Species, Their Composition and Significance in Village Farming Systems. ACIAR Technical Reports, Canberra. No 22. 60 p.

MAKKAR, H.P.S. 2003a. Quantification of Tannin in Tree and Shrub Legumes. A Laboratory Manual. Kluwer Academic Publishers, Netherlands.

MAKKAR, H.P.S. 2003b. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rum. Res. 49: 241 – 256.

MAKKAR, H.P.S. 2005. Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage: Achievements, result, implications and future research. Anim. Feed Sci. Technol. 122: 3 – 12.

MAKKAR, H.P.S. and K. BECKER. 1996. Effect of pH, temperature and time on inactivation of tannins and possible implications in detannification studies. J. Agric. Food Chem. 44: 1291 – 1295.

MANTZ, G.K. 2007. Using Polyethylene Glycol to Enhance the Intake of *Sericea lespedeza* by cattle. PhD thesis. Utah State University, Logan, Utah, USA. 198 p.

MCWILLIAM, E.L., T.N. BARRY, N. LOPEZ-VILLALOBOS, P.N. CAMERON and P.D. KEMP. 2005. Effects of willow (Salix) versus poplar (Populus) supplementation on the reproductive performance of ewes grazing low quality drought pasture during mating. Anim. Feed Sci. Technol. 119: 69 – 86.

MIN, B.R. and S.P. HART. 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81(suppl. 2): E102 – E109.

MIN, B.R., S.P. HART, D. MILLER, G.M. TANITA, E. LOETZ and T. SAHLU. 2005. The effect of grazing forage containing condensed tannins in gastro-intestinal parasite infection and milk composition in Angora does. Vet. Parasit. 130: 105 – 113.

PALMER, B., R. JONES, E. WINA and B. TANGENDJAJA. 2000. The effect of sample drying conditions on estimates of condensed tannin and fibre content, dry matter digestibility, nitrogen digestibility and PEG binding of *Calliandra calothyrsus*. Anim. Feed Sci. Technol. 87: 29 – 40.

PITTA, D.W., T.N. BARRY, N. LOPEZ-VILLALOBOS and P.D. KEMP. 2005. Effects on ewe reproduction of grazing willow fodder blocks during drought. Anim. Feed Sci. Technol. 120: 217 – 234.

RAHMANI, S., J.D. BROOKER, G.P. JONESM and B. PALMER. 2005. Composition of condensed tannins from *Calliandra calothyrsu*s and correlation with *in sacco* digestibility. Anim. Feed Sci. Technol. 121: 109 – 124.

RAMFREZ-RESTREPO, C.A. and T.N. BARRY. 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. Anim. Feed Sci. Technol. 120: 179 – 201.

ROY, N.C., B.R. SINCLAIR, B. TRELOAR, J.S. PETERS and W.C. MCNABB. 2004. Polyethylene glycol reduces the new flux of the branched-chain amino acids across the mammary gland in ewes fed sulla. J. Anim. Feed Sci. 13(suppll.): 343 – 346.

SINGH, B., T.K. BAHT and B. SINGH. 2003. Potential theraupetic application of some antinutrional plants secondary metabolites. J. Agric. Food Chem. 51: 5579 – 5597.

SMITH, A., J.A. IMLAY and R.I. MACKIE. 2003. Increasing the oxidative stess response allows *E. coli* to overcome inhibitory effects of condensed tannins. Appl. Environ. Microbiol. 69: 3406 – 3411.

SUTAMA, I.K., M. ALI and E. WINA. 1994. The effect of supplementation of Calliandra leaves on reproductive performance Javanese fat-tailed sheep. Ilmu dan Peternakan 7(2): 13 – 16.

TANGENDJAJA, B. and E. WINA. 2000. Tannins and ruminant production in Indonesia. *In:* Tannins in livestock and human nutrition. BROOKER, J.D. (Ed.). Proc. of Int. Workshop, Adelaide, Australia May31-June 2, 1999. Can berra. ACIAR Proc. No. 92. pp. 40 – 43.

TANGENDJAJA, B., E. WINA, T. IBRAHIM and B. PALMER. 1992. *Calliandra calothyrsus* and its utilization (Kaliandra dan pemanfaatannya). Balai Penelitian Ternak dan ACIAR 56 p.

TAVENDALE, M.H., L.P. MEAGHER, D. PACHECO, N. WALKER, G.T. ATTWOOD and S. SIVAKUMARAN. 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol. 123: 403 – 419.

TIEMANN, T.T., C.E. LASCANO, H.R. WETTSTEIN, A.C. MAYER, M. KREUZER and H.D. HESS. 2008. Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. Animal 2: 790 – 799.

WINA, E. and D. ABDUROHMAN. 2005. The formation of ruminal bypass protein' (*in vitro*) by adding tannins isolated from *Calliandra calothyrsus* leaves or formaldehyde to several proteins. JITV 10(4): 274 – 280

WINA, E., B. TANGENDJAJA and DUMARIA. 2008: Effect of *Calliandra calothyrsus* on *in vitro* digestibility of soybean meal and tofu wastes. Vol. 20, Article #98. http://www.lrrd.org/lrrd20/6/wina20098.htm. (February 1, 2010).

WINA, E., B. TANGENDJAJA and E. TAMTOMO. 1993. The effect of drying on the digestibility of *Calliandra calothyrsus*. Ilmu dan Peternakan 6(1): 32 – 36.

WINA, E., B. TANGENDJAJA and GUnawan. 1997. Wilting process to *Calliandra calothyrsus*: Its effect on sheep performance. Proc. National Seminar II on Nutrition and Animal Feed, IPB and AINI, Bogor, Indonesia. pp. 47 – 48.

WINA, E., B. TANGENDJAJA and I.W.R. SUSANA. 2005. Effects of chopping and soaking in water, hydrochloric acidic and calcium hydroxide solutions on the nutritional value of *Acacia villosa* for goats. Anim. Feed Sci. Technol. 122: 79 – 92.

WINA, E., I.G.M. BUDIARSANA., B. TANGENDJAJA and GUNAWAN. 1994. Pengaruh polietilen glikol dan kalsium hidroksida terhadap kecernaan nutrient kaliandra dan performans domba. Ilmu dan Peternakan 8(1): 13 – 17.

WINA, E., M. KAYADU and B. TANGENDJAJA. 1995. Pengaruh urea, ammonium sulfat atau tepung gaplek pada performans domba yang diberi kaliandra segar. Pros. Nasional Seminar Sains dan Teknologi Peternakan. Ciawi – Bogor, 25 – 26 Januari 1995. Balai Penelitian Ternak, Bogor. hlm. 176 – 181.