# The Formation of 'Ruminal Bypass Protein' (*In Vitro*) by Adding Tannins Isolated from *Calliandra calothyrsus* Leaves or Formaldehyde

ELIZABETH WINA[1] and DINDIN ABDUROHMAN[2]

[1] *Balai Penelitian Ternak, PO Box 221, Bogor 16002*
[2] *Fakultas MIPA Jurusan Kimia, Institut Pertanian Bogor*

(Diterima dewan redaksi 9 September 2005)

**ABSTRAK**

WINA, E. dan D. ABDUROHMAN. 2005. Pembentukan protein 'lolos cerna rumen' (*in vitro*) dengan penambahan isolat tanin dari daun kaliandra atau formaldehida. *JITV* 10(4): 274-280.

Protein 'lolos cerna rumen' berguna untuk meningkatkan jumlah protein yang masuk ke dalam abomasum. Salah satu teknik membuat protein 'lolos cerna rumen' adalah dengan membentuk senyawa komplek protein dengan tanin atau formaldehida. Ada dua percobaan yang dilakukan. Percobaan pertama bertujuan untuk mengetahui jumlah tanin yang optimum untuk menurunkan kecernaan bahan kering sumber protein. Senyawa tanin diisolasi dari daun kaliandra (*Calliandra calothyrsus*), kemudian isolat tanin ditambahkan sebanyak masing-masing 0,10, 20, 30, 40, 50 mg ke dalam tabung *in vitro* yang berisi 0,5 g sumber protein (daun gamal, bungkil kedelai atau kasein) dan diinkubasi selama 48 jam. Hasil percobaan menunjukkan bahwa penambahan tannin yang baik adalah 60 mg/g untuk daun gamal atau kasein dan 80 mg/g untuk bungkil kedelai. Percobaan ke dua bertujuan untuk membandingkan kemampuan tanin dan formaldehida sebagai pembentuk komplek dengan protein. Komplek tanin-protein atau formaldehida-protein yang terbentuk diinkubasi dengan cairan rumen selama 48 jam dan dilanjutkan dengan inkubasi dengan larutan pepsin-HCl selama 24 jam (total waktu inkubasi 72 jam). Setelah diinkubasi baik sampai 48 atau 72 jam, kecernaan bahan kering maupun protein untuk senyawa komplek tannin-protein selalu jauh lebih tinggi dibandingkan dengan senyawa komplek formaldehida-protein. Jumlah protein 'lolos cerna rumen' hampir sama antara kedua senyawa pengkomplek tersebut dengan daun gamal (34,4 g/100 g protein untuk tanin-daun gamal vs 32,1 g/100 g untuk formaldehida-daun gamal). Jumlah protein 'lolos cerna rumen' dari komplek tanin-bungkil kedelai (27,9 g/100 g bungkil) hanya separuh dari komplek formaldehida-bungkil kedelai (54,1 g/100 g bungkil) sedangkan komplek tanin-kasein mudah pecah di dalam rumen sehingga jumlah protein lolos cerna rumen sangat kecil. Disimpulkan bahwa kemampuan tanin membentuk protein lolos cerna tergantung dari jenis protein yang digunakan dan lebih rendah dari kemampuan formaldehida. Uji lanjut dari komplek tanin-protein sebagai bahan pakan ternak perlu dilakukan.

**Kata Kunci:** Protein Lolos Cerna Rumen, *Calliandra calothyrsus*, Tanin, Formaldehida

**ABSTRACT**

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

'Ruminal bypass protein' is one of the strategies to increase the amount of protein, which enters abomasum and hence, increases ruminant productivity. One of the strategies to obtain "ruminal bypass protein" is by complexing tannin or formaldehyde with protein. Two experiments were conducted. The first experiment aimed to obtain the optimum level of added tannins to decrease the dry matter digestibility of protein sources. Tannins were isolated from *Calliandra calothyrsus* leaves. Tannins were added at the level of 0, 10, 20, 30, 40 and 50 mg to each *in vitro* tube containing 0.5 g of protein source (gliricidia leaves, soybean meal or casein) and the tubes were incubated for 48 hours. The result showed that the optimum level of tannin was 60 mg/g of gliricidia leaves or casein and 80 mg/g of soybean meal. The second experiment aimed to compare the ability of tannin to formaldehyde to form complex with protein. One set of tubes containing tannin-protein or formaldehyde-protein complex was incubated with rumen liquor for 48 h and another set was incubated with rumen liquor (48h) and followed by pepsin-HCl for 24 h (total incubation time: 72 h). After incubation at 48 h or 72 h, the dry matter or protein digestibility of tannin-protein complex was much higher than those of formaldehyde-protein complex. The amount of ruminal bypass protein was almost similar between the two agents to complex gliricidia leaves (34.4 and 32.1 g/100g for tannin-gliricidia and formaldehyde-gliricidia, respectively). Tannin-soybean meal interaction produced 27.9 g/100 g of 'ruminal bypass protein', which was half of that from reaction between formaldehyde and soybean meal (54.1 g/100 g). Tannin-casein complex was easily broken down in the rumen so that 'ruminal bypass protein' from this complex was very small. It can be concluded that the ability of tannin to form 'ruminal bypass protein' depends on the type of protein source but is the same with the ability of formaldehyde to bind forage protein (*Gliricidia sepium*). Further feeding evaluation of these tannin-protein complexes is warranted.

**Key Words:** Ruminal Bypass protein, *Calliandra calothyrsus*, Tannin, Formaldehyde

## INTRODUCTION

Limited protein supply is a common phenomenon in the village feeding system especially during the drought season. It has been known that protein degradation in the rumen is rather "a wasteful process" as more than 60% of protein that enter rumen will be degraded easily and become ammonia (MACRAE and ULYATT, 1974; MIN *et al*., 2000). Even though the ammonia is used for microbial protein synthesis and some are absorbed through the rumen wall, the excess of ammonia can not be utilized by the animal and will be excreted in the urine. Many strategies have been developed to overcome limited protein supply to ruminant and one of them is by protecting the feed protein from its degradation in the rumen and, hence, increasing the amount of protein that enters the abomasum. This type of protein is called "ruminal bypass protein", which can be obtained by physical treatment such as heat, or chemical treatments such as using lignosulfonate (BRODERICK *et al.*, 1991), formaldehyde (CAJA *et al.*, 1977) or tannins (MAKKAR, 2003; SUHARTATI, 2005).

Tannins are widely found as secondary compound in many plants and are considered as anti nutritional factor since they decrease the digestibility of protein (MAKKAR, 2003). The binding property of tannin to protein may be beneficial to protect protein from its degradation in the rumen if the binding could be broken in the intestine and the released protein could be used for the animal. *Calliandra calothyrsus,* which is a widespread leguminous plant in some parts of Asia and Africa, is a good source of supplement for growing sheep (WINA and TANGENDJAJA, 2000) or ewes (SUTAMA *et al.,* 1994). But, it contains a high level of condensed tannin as well as of protein (PALMER *et al.*, 2000; LASCANO *et al.*, 2003). Total condensed tannin content in *C. calothyrsus* measured by Butanol-HCl was 21% and by protein precipitation method was 11% (PALMER *et al.,* 2000). The extraction of tannin from *C. calothyrsus* has been studied by SUSANA *et al*. (1994).

Beside soybean meal and casein as protein source, *Gliricidia sepium* which is a leguminous plant, is also potential as a protein source as it has a high soluble protein content with hardly any free tannins.

Formaldehyde is a common reagent, used to complex protein (FRIEDMAN and BRODERICK, 1977), however, the use of chemicals such as formaldehyde for animal feed is not favourable. Natural products now have more attention than chemicals to be used in the animal feed. No information is available on comparing the ability to bind protein between formaldehyde and tannin.

The aim of the present study was to utilize tannins, isolated from *C. calothyrsus,* to bind protein from casein, soybean meal and *G. sepium* and to compare the ability of tannin to that of formadehyde to bind protein.

## MATERIALS AND METHOD

### Materials

Crude tannins were isolated from *C. calothyrsus* leaves. *G. sepium* leaves are harvested from Balai Penelitian Ternak farm. Soybean meal and casein were available commercially.

### *The isolation of crude tannins from* C. calothyrsus *leaves*

Fresh *C. calothyrsus* leaves (0.5 g) were ground with dry ice and then 20 ml of 70% (v/v) aqueous acetone containing 0.1 g/100 ml ascorbic acid was added. The mixture was centrifuged at 3000 rpm at 4$^{o}$C for 10 min. The supernatant was separated and the extraction was repeated two times. The combined supernatant was evaporated by a rotary evaporator to get rid of acetone. The aqueous fraction was extracted by diethyl ether 3 times. The diethyl ether was removed and the aqueous fraction was evaporated shortly to get rid of the remaining diethyl ether and then, was freeze dried to obtain: Crude tannin extracts (TERRILL *et al*., 1992).

### In vitro *experiment*

Two experiments were conducted. The first experiment aimed to obtain the optimum level of added tannins to decrease the dry matter digestibility of protein sources. In the first experiment, 0.5 g of protein source (freeze dried gliricidia leaves, milled soybean meal or casein,) was weighed into an *in vitro* tube. Crude tannins were added at the level of 0, 10, 20, 30, 40 and 50 mg to each tube. Forty ml of Menke buffer (MENKE *et al*., 1979) and 10 ml of rumen liquor were added to the tube. Rumen liquor was taken from sheep fed elephant grass. Gas $CO_2$ was flushed into the tubes and then, the tubes were incubated in the water bath at 39$^{o}$C for 48 h. The content of the tube was filtered after 48 hours of incubation and the residue was then dried in the oven to obtain the dry weight of the residue.

The second experiment aimed to compare the ability of tannin to formaldehyde to form complex with protein *in vitro*. In the second experiment, there are two sets of *in vitro* tubes were prepared. One set was incubated until 48 h and another set was incubated with rumen liquor for 48 h and followed by 24 h incubation with pepsin-HCl (total incubation 72h) (TILLEY and TERRY, 1963). The tubes contained 0.5 g of protein source (gliricidia leaves, soybean meal or casein) and tannins were added into the tube at the level that was obtained from the first experiment. In a separate tube, instead of tannins, 37% formaldehyde solution was added at the level of 2 g/100 g protein source (BARRY, 1976).

Formaldehyde was used in this experiment as a positive control.

The content of the tube was filtered after 48 or 72 h of incubation and the residue was then dried in the oven. The dry matter of residue was obtained after weighing and crude protein of the residue was determined by Kjeldahl method.

The solution that passed the filtration was collected and was taken for ammonia analysis (CONWAY and BRYNE, 1933).

### *Calculation*

The 'ruminal by pass protein' (g/100 g substrate) = the amount of protein digested by ruminal microbes and pepsin-hydrochloric acid (total incubation time = 72 h)- the amount of protein digested by ruminal microbes (48 h of incubation).

## RESULTS AND DISCUSSION

The addition of tannin isolate to a protein source in the *in vitro* incubation led to a reduction of dry matter digestibility of the protein source in a dose dependent manner (Table 1). However, the decline of dry matter digestibility was in a different rate with a different protein source; casein had the highest reduction rate (slope = - 0.139), followed by soybean meal (SBM) (slope = - 0.105) and *G. sepium* had the lowest (slope = -0.0975). It means that the addition of tannin negatively affected dry matter digestibility of casein more than that of SBM or *G. sepium*. Digestion of a feed or plant forage in the rumen can be attributed to the combined processes of solubilization and degradation by rumen microorganisms. The presence of tannin in the rumen fermentation negatively affected the digestibility by reducing degradability by rumen microorganism and solubilization of protein (MIN *et al*., 2000). Free tannin will react quickly with soluble protein, therefore, the higher amount of soluble protein in casein than in SBM or *G. sepium* would react more to free tannin and led to a higher reduction rate on digestibility. Protective role of tannins against rumen degradation was more evident for N than for DM (FRUTOS *et al*., 2000). Therefore, the protein digestibility was negatively affected by tannin; and this was expressed by the decreased ammonia production in a dose dependent manner when the level of tannin was increasing (Figure 1). The result suggests that the protein was protected from its degradation and hence, ammonia production was decreased.

**Table 1.** Dry matter digestibility of casein, soybean meal and gliricidia leaves at different levels of tannin isolate at 48 h of *in vitro* incubation

| Level of tannin isolate (mg/g protein source) | Dry matter digestibility (%) | | |
|---|---|---|---|
| | Casein | Soybean meal | *G. sepium* leaves |
| 0 | 93.27[a] | 77.43[a] | 49.63[a] |
| 20 | 88.20[b] | 77.70[a] | 49.13[a] |
| 40 | 84.47[c] | 74.53[b] | 48.23[a] |
| 60 | 80.67[d] | 71.23[c] | 43.96[b] |
| 80 | 80.27[d] | 69.68[d] | 42.03[b] |
| 100 | 79.33[d] | 68.20[d] | 41.10[b] |

Different letter on the same column indicates significantly different (P<0.05)

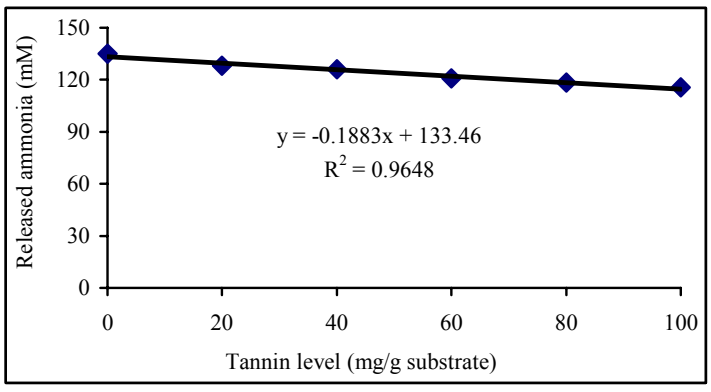


**Figure 1.** The effect of tannin isolate (0-100mg/g substrate) on ammonia concentration (mM) at 48 h of *in vitro* fermentation of casein

According to the previous report, the minimum concentration of condensed tannin needed to inhibit proteolysis in the *in vitro* fermentation is 80 mg/g protein (JONES and MANGAN, 1977) or 100 mg/g protein (TANNER *et al.,* 1994). The results in Table 1 showed that there was no further significant difference in DM digestibility of casein with the addition of tannin above 60 mg/g. Therefore, it was decided that the level of tannin used for casein for the second experiment was 60 mg/g casein. DM digestibility of soybean meal and *G. sepium* was not significantly different when the levels of tannin were above 80 and 60 mg/g substrate, respectively (Table 1). The levels of tannin, therefore, used for soybean meal and *G. sepium* were 80 and 60 mg/g substrate, respectively. The same amount of mimosa tannin was used to protect protein in *G. sepium* was reported by PURBOYO (2005). His result showed that 60 mg/g mimosa tannin was the optimum level to reduce protein solubility (40% lower than control) and protein degradation of *G. sepium* in the rumen (32% lower than control). The amount of tannin required to bind protein is dependent on the type of protein and also the type of tannin. Tannins interact with protein by hydrogen bonding and hydrophobic interactions. Protein with high proline content has a high affinity for tannin. The tertiary structure of protein, collagen-type helix or random coil has a higher affinity for tannin than the compactly folded protein (HAGERMAN and BUTLER, 1981).

Dry matter (DM) and crude protein (CP) digestibilities of casein, SBM and *G. sepium* leaves reacted with tannin or formaldehyde in the second experiment are presented in Figure 2 and 3. In the rumen fermentation (48 h incubation), both DM and CP digestibilities of casein, SBM and *G. sepium* leaves were much higher when reacted with tannin than with formaldehyde. High digestibility of DM or CP at 72 h of incubation also occurred in the presence of tannin, which indicates that tannin at the maximum level of 80 mg/g protein source did not affect the intestinal (pepsin) digestion of tannin-treated protein. This experiment is in agreement with previous reports that quebracho tannin or tannic acid at the level of 100 mg/g SBM did not affect intestinal digestion of SBM protein (HERVAS *et al*., 2000; FRUTOS *et al.,* 2000). Both DM and CP digestibilities at 48 and 72 h of incubations, however, were lower in the presence of formaldehyde than that of tannin suggesting that formaldehyde (2 g/100 g protein source) protects protein not only from the degradation in the rumen but also partly from the hydrolysis by pepsin in the lower gut.

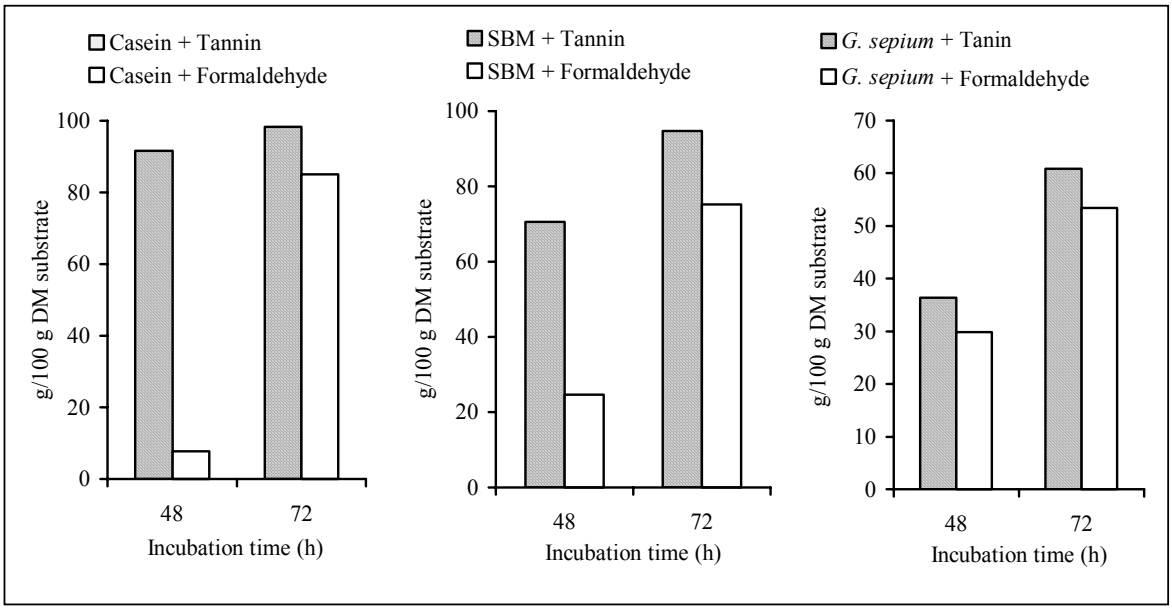


**Figure 2.** *In vitro* dry matter digestibility (g/100 g substrate) of casein, soybean meal (SBM) and *G. sepium* in the presence of tannin or formaldehyde at 48 and 72 h of incubation

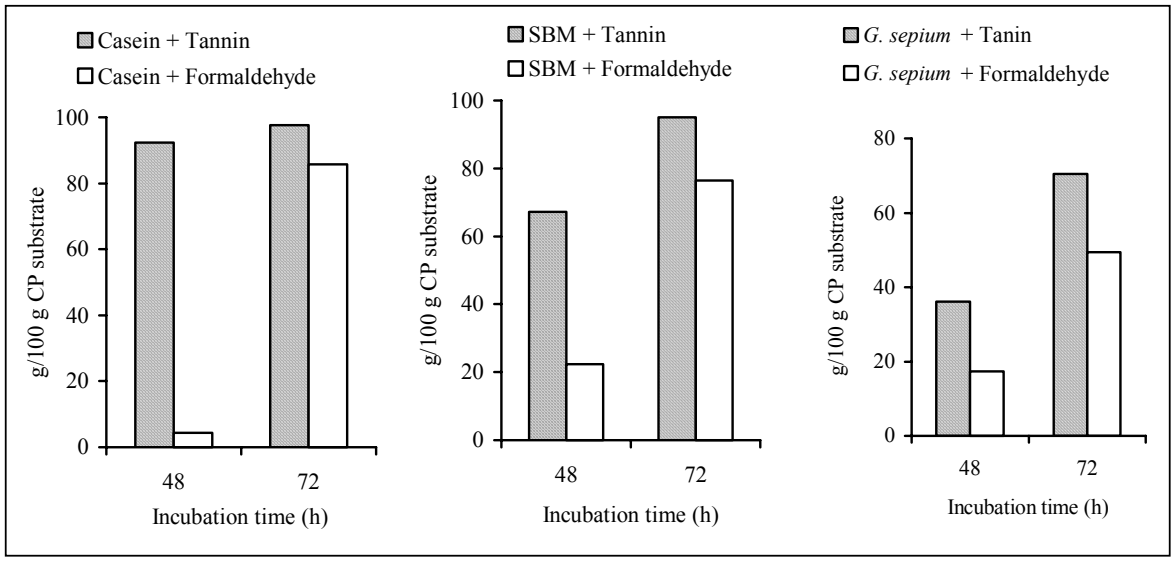


**Figure 3.** *In vitro* crude protein digestibility (g/100 g substrate) of casein, soybean meal (SBM) and *G. sepium* in the presence of tannin or formaldehyde at 48 and 72 h of incubation

**Table 2.** The concentration of ammonia (mM) released into the solution at 48 and 72 h of *in vitro* fermentation of casein, soybean meal, *G. sepium* in the presence of tannin isolate or formaldehyde

| Time of incubation (h) | Ammonia released into the medium (mM) | | | | | |
|---|---|---|---|---|---|---|
| | Casein | | Soybean meal (SBM) | | *G. sepium* leaves | |
| | Tannin | Formaldehyde | Tannin | Formaldehyde | Tannin | Formaldehyde |
| 0 | 11.2 ± 1.1 | 12.8 ± 0.4 | 15.9 ± 1.0 | 12.5 ± 0.3 | 19.6 ± 3.3 | 11.3 ± 0.2 |
| 48 | 110.1 ± 7.9 | 14.9 ± 0.2 | 83.3 ± 1.9 | 13.4 ± 0.2 | 64.6 ± 3.6 | 11.9 ± 0.2 |
| 72 | 122.6 ± 5.7 | 24.4 ± 0.9 | 83.9 ± 3.6 | 18.4 ± 0.2 | 69.3 ± 4.8 | 19.4 ± 0.4 |

Table 2 shows the released of ammonia into the medium after 48 and 72 h of fermentation of casein, SBM and *G. sepium* leaves in the presence of tannin or formaldehyde. The concentration of ammonia in the rumen depends on the degradation rate of protein in the rumen, the synthesis rate of microbial protein, the absorption rate of ammonia through the rumen wall and the microbial lysis in the rumen. The ammonia concentration in the rumen (48 h of incubation) is an important parameter for the synthesis of microbial protein (PRESTON and LENG, 1987), however, the value of ammonia concentration at 72 h of incubation has a low correlation to the protein degradation since protein that enters duodenum is degraded to peptide or amino acids which are then absorbed through the intestinal wall. The concentration of ammonia released into the *in vitro* rumen was much higher in the presence of tannin than that of formaldehyde. It means that in the presence of tannin in the rumen, some proteins were released from the tannin-protein complex and were degraded to ammonia but in the presence of formaldehyde, the protein was well protected from the microbial degradation. This may be because the bonding of tannin-protein was weaker than that of formaldehyde-protein. The higher ammonia in the rumen in the presence of tannin is beneficial as supplementation of non-protein nitrogen, such as urea is not needed to maximize rumen fermentation. In contrast, when the ammonia in the rumen is too low, feeding of formaldehyde-casein requires urea supplementation to obtain a maximum growth (KEMPTON and LENG, 1979).

The reaction of tannin to protein is different from the reaction of formaldehyde to protein. Tannin reacts with protein in hydrogen bonding or hydrophobic interaction (HAGERMAN and BUTLER, 1981) while formaldehyde reacts with terminal amino groups of proteins, followed by a condensation reaction to form a stable methylene linkage between protein chains (FERGUSON, 1975). Both linkages are easily broken by low pH in the duodenum, the animal's amino acid supply, therefore, being increased (BARRY, 1976).

**Table 3.** The difference in DM and CP digestibility of casein, soybean meal and *G. sepium* leaves between 72 h and 48 h of incubation in the presence of tannin or formaldehyde

| Binding agent | Casein | | Soybean meal | | *G. sepium* leaves | |
|---|---|---|---|---|---|---|
| | DM | CP | DM | CP | DM | CP |
| | g/100 g substrate | | | | | |
| Tannin isolate | 6.7 | 5.3 | 24.3 | 27.9 | 24.4 | 34.4 |
| Formaldehyde | 77.2 | 81.4 | 50.6 | 54.1 | 23.5 | 32.1 |

Table 3 shows the amount of ruminal bypass protein produced by tannin or formaldehyde. The values were calculated from data presented in Figure 2 and 3 and they varied depending on the protein sources. Ruminal bypass protein by tannin was much lower than that by formaldehyde. The protection of tannin to casein at 60 mg/g casein produced a very small amount of ruminal bypass protein (5.3 g/100g). It could be due to the high solubility of casein or the linkage between casein-tannin may be very weak. HAGERMAN and BUTLER (1980) reported that in the presence of high soluble protein, the complex of tannin-casein could redissolve; hence, high ruminal DM or CP degradation occurred. The protection of tannin to SBM was stronger than that to casein but was weaker than the protection of formaldehyde to SBM, therefore, the amount of ruminal bypass protein produced by tannin was half than that produced by formaldehyde (27.9 *vs* 54.1 g/100g SBM). The amount of ruminal bypass protein is almost the same when *G. sepium* leaves were reacted with either tannin or formaldehyde (34.4 *vs* 32.1 g/100g *G. sepium*). This study highlighted that tannin could be very useful as a binding agent for forage protein as it gave the same reactivity as formaldehyde to produce the same bypass protein. WAGHORN and SHELTON (1997) have reported that tannin-containing plants can be used in the *in vitro* fermentation to partially precipitate soluble protein in low tannin-containing plants and MIN *et al.* (2000) showed that condensed tannin from *Lotus corniculatus* was effective at reducing protein degradation from *Trifolium repens* in the rumen. Feeding tannin-forage protein complex may give two beneficial effects since it supply high ammonia in the rumen and more amino acids into the duodenum. Therefore, a feeding trial of this tannin-forage protein complex needs to be pursued especially to animals fed with high roughage and low protein diet. In conclusion, tannin isolated from C. *calothyrsus* can be used as a protein-binding agent and has a similar activity with formaldehyde to bind forage protein (*Gliricidia sepium)* which supply the same amount of ruminal bypass protein to the animal.

## REFERENCES


BARRY, T.N. 1976. The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. *Proc. Nutr. Soc*. 35: 221-229.

BRODERICK, G.A., R.J. WALLACE and E.R. ORSKOV. 1991. Control of rate and extent of protein degradation. *In:* Physiological Aspects of Digestion and Metabolism in Ruminants. T. TSUDA, Y. SASAKI and R. KAWASHIMA (Eds.). Academic Press, New York. pp. 541-592.

CAJA, G., J.F. GALVEZ, A. ARGAMENTERIA and J. CIRIA. 1977. Inhibition of ruminal deamination *in vitro* by formaldehyde treatment of sunflower-seed, soyabean and fish meals: Response curves to protective treatment. *Anim. Feed Sci. Tech*. 2: 267-275.

CONWAY, E.J. and A. BYRNE. 1933. An absorption apparatus for the determination of certain volatile substances. I. The micro-determination of ammonia. *Biochem. J.* 27: 419-429.

FERGUSON, R.A. 1975. The protection of dietary proteins and amino acids against microbial fermentation in the ruminant. *In:* Digestion and Metabolism in the Ruminant. I.W. MCDONALD and A.C.I. WARNER (Eds.) University of New England, Publishing Unit, Armidale, Australia. pp. 448-464.

FRIEDMAN, M. and G.A. BRODERICK. 1977. Protected proteins in ruminant nutrition. *In vitro* evaluation of casein derivatives. *In:* Protein Crosslinking: Nutritional and Medical Consequences. M. FRIEDMAN (Ed.). Plenum Press, New York and London. pp. 545-558.

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.

HAGERMAN, A. and L.G. BUTLER. 1980. Condensed tannin: Purification and characterization of tannin associated proteins. *J. Agric. Food Chem.* 28: 947-952.

HAGERMAN, A. and L.G. BUTLER. 1981. The specificity of proanthocyanidin-protein interactions. *J. Biol. Chem*. 256: 4494-4497.

HERVAS, G., P. FRUTOS, E. SERRANO, A.R. MANTECON and F.J. GIRALDEZ. 2000. Effect of tannic acid on rumen degradation and intestinal digestion of treated soyabean meals in sheep. *J. Agric. Sci.* 135: 305-310.

JONES, W.T. and J.L. MANGAN. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *J. Sci. Food Agric*. 28: 126-136.

KEMPTON, T.J. and R.A. LENG. 1979. Protein nutrition of growing lambs. 1. Responses in growth and rumen function to supplementation of a low-protein cellulosic diet with either urea, casein or formaldehyde treated casein. *Brit. I. Nutr*. 42: 289-302.

LASCANO, C., P. AVILA and J. STEWART. 2003. Intake, digestibility and nitrogen utilization by sheep fed with provenances of *Calliandra calothyrsus* Meissner with different tannin structure. *Arch. Latinoam. Prod. Anim*. 11: 21-28.

MACRAE, J.C. and M.J. ULYATT. 1974. Quantitative digestion of fresh herbage by sheep. The sites of digestion of some nitrogeneous constituents. *J. Agric. Sci.* (Camb.) 82: 309–319.

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

MENKE, K.H., L. RAAB, A. SALEWSKI, H. STEINGASS, D. FRITZ and W. SCHNEIDER. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro. J. Agric. Sci*. (Camb.) 93: 217-222.

MIN, B.R., W.C. MCNABB, T.N. BARRY and J.S. PETERS. 2000. Solubilization and degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39; Rubisco) protein from white clover (*Trifolium repens*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. *J. Agric. Sci.* (Camb.) 134: 305–317.

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.

PURBOYO, S.W. 2005. The effects of mimosa tannin on the protein precipitation in Gliridicia (*Gliricidia sepium*) and protein solubility in the rumen of cattle (*Bos indicus*). *Anim. Prod.* (Universitas Jendral Soedirman, Indonesia) 7: 21-26.

PRESTON, T.R. and R.A. LENG. 1987. Matching ruminant production systems with available resources in the tropics and sub-tropics. Penambul Books. Armidale, Australia.

SUHARTATI, F.M. 2005. Protein Lamtoro leaves (*Leucaena leucocephala*) with tannin, saponin and oil protection and the effect on ruminal undegradable dietary protein (RUDP) and synthesis of rumen microbial protein. *Anim. Prod*. (Universitas Jenderal Soedirman, Indonesia) 7: 52-58.

SUSANA, I.W.R., E. WINA and B. TANGENDJAJA. 1994. Extraction of tannin from Calliandra with various solvents and its interaction with different proteins and pH conditions. *Ilmu dan Peternakan* 8: 38-42.

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

TANNER, G.J., A.E. MOORE and P.J. LARKIN. 1994. Proanthocyanidins inhibit hydrolysis of leaf protein by rumen microflora *in vitro*. *Brit. J. Nutr*. 71: 947-958.

TERRILL, T.H., A.M. ROWAN, G.B. DOUGLAS and T.N. BARRY. 1992. Determination of extractable and bound condensed tannins concentrations in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric.* 58: 321-329.

TILLEY, J.M.A. and R.A. TERRY. 1963. A two stages technique for the *in vitro* digestion of forage crops. *J. Brit. Grassl. Sci.* 18: 104-111.

WAGHORN, G.C. and I.D. SHELTON. 1997. Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. *J. Agric. Sci*. (Camb.) 125: 291-297.

WINA, E. and B. TANGENDJAJA. 2000. The utilization of *Calliandra calothyrsus* as a forage for ruminant in Indonesia. Proc. of a symposium on Seed production and utilization of Calliandra. ICRAF. Bogor-Indonesia. 14-16 Nov 2000. pp. 13-20.