Improving Microbial Protein Synthesis in the Rumen of Sheep Fed Fresh Tofu Waste by Crude Tannin Extract of *Acacia mangium*

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(Diterima 3 September 2012; disetujui 25 September 2012)

ABSTRAK

WINA, E., D. YULISTIANI, SUSANA I.W.R. and B. TANGENDJAJA. 2012. Peningkatan sintesis protein mikroba dalam rumen domba yang diberi ampas tahu segar dengan ekstrak tanin kasar dari *Acacia mangium. JITV* 17(3): 207-214.

Tanin dapat dijumpai di dalam tanaman tropis dan keberadaan tanin dapat mengurangi nilai nutrisi pakan. Namun, tanin dapat memberi pengaruh yang menguntungkan karena melindungi protein dari degradasi rumen. Ampas tahu tersedia banyak di Negara dimana tahu diproduksi secara luas termasuk di Indonesia. Percobaan pakan dilakukan pada domba untuk mempelajari pengaruh tanin terhadap sintesis protein mikroba dan kondisi fermentasi rumen, kecernaan dan retensi N pakan yang mengandung ampas tahu. Lima belas ekor domba digunakan dan terbagi dalam 3 perlakuan, yaitu: 1) Perlakuan kontrol, pakan tanpa tanin, 2) Perlakuan pakan + tanin dan 3) Perlakuan pakan + tanin + *polyethylene glycol* (PEG). Domba diletakkan di kandang metabolis. Mereka diberi pakan perlakuan selama 14 hari dan kemudian, 7 hari berikutnya, feses dan urin dikumpulkan. Parameter rumen (pH, amonia, SCFA dan protozoa) dan sintesis protein mikroba, kecernaan, retensi nitrogen diamati. Hasil menunjukkan bahwa konsentrasi tanin sebesar 4% dalam pakan akan menekan produksi amonia dalam rumen domba baik sebelum dan 3 jam sesudah diberi pakan pada pagi hari. Total asam lemak terbang dan asam lemak terbang individu tidak berbeda antar perlakuan. Tanin sebesar 4% tidak mempengaruhi konsumsi harian. Tanin mengurangi kecernaan bahan kering, bahan organik dan protein tetapi hanya secara signifikan menekan kecernaan ADF ($P < 0,05$) dan cenderung menekan kecernaan NDF ($P < 0,1$). Penambahan PEG mengikat tanin dan menghilangkan sifat negatif tanin. Dalam percobaan ini, tanin mempunyai sifat positif dalam meningkatkan sintesis protein mikroba yang mungkin dapat meningkatkan produksi domba.

Kata Kunci: Tanin, *Acacia mangium*, *Polyethylene Glycol*, Ampas Tahu, Kecernaan

ABSTRACT

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Tannin can be found in many tropical plants and the presence of tannin may reduce the nutritional value of feed. However, it can give beneficial effect as it protects the protein from its excessive degradation in the rumen. Tofu waste is abundant in a country, including Indonesia, where tofu is produced widely. A feeding trial on sheep to study the effect of tannin on microbial protein synthesis and condition of rumen fermentation, digestibility and N retention of feed containing tofu waste was conducted. Fifteen sheep were allocated to one of 3 treatments, i.e: 1) control treatment, feed without tannin, 2) feed + tannin treatment and 3) feed + tannin + polyethylene glycol (PEG) treatment. The sheep was placed in the individual metabolism cage. They were fed with treatment feed for 14 days and then, the following 7 days, faeces and urine were collected. At the end of the treatment, rumen fluid was taken from each sheep before and after feeding. Rumen parameter (pH, ammonia, SCFA, protozoa) and microbial protein synthesis, digestibility, nitrogen retention were observed. Results showed that inclusion of 4% tannin in the feed depressed ammonia production in the rumen of sheep before and 3 hours after morning feeding. Total SCFA and individual SCFA were not different among treatments. Tannin did not affect daily feed intake. Tannin lowered the dry matter, organic matter and protein digestibilities but it significantly reduced ADF digestibility ($P < 0.05$) and tended to reduce NDF digestibility ($P < 0.1$). , tannin increased the microbial protein synthesis. PEG addition bound tannin and alleviate the negative effect of tannin.

Key Words: Tannin, *Acacia Mangium*, Polyethylene Glycol, Tofu waste, Digestibility

INTRODUCTION

Tannin is commonly found in many tropical plants or forages. As a secondary compound, tannin is known as a plant protector against the predator attack. Tannin has astrigent taste that may reduce the intake of leaves/forages containing tannin. Tannin is often used as skin hides and has been extracted from bark and is produced commercially. The common source of tannin for livestock is legume forages. Commercially, tannin has been produced and extracted from bark of *Acacia mearsnii* or *Acacia mimosa* and is used as skin hides.

Tannin, extracted from the bark of *Acacia mangium* with 50% acetone or 50% methanol gave the highest yield and also showed the highest biological activity of tannin on *in vitro* fermentation (WINA *et al*., 2010). Further experiment showed that increasing tannin level more than 4% in the *in vitro* fermentation drastically reduced the dry matter digestibility of dried tofu waste and also reduced the by pass rumen fraction.

Tofu waste, unlike soybean meal, is a cheap good protein source and readily available in Asia. Tofu is made from soybean by extraction and precipitation to produce a semi-solid product rich in protein. During the tofu preparation, the extracted protein, may or may not be heated, and is separated by filtration. The residues (waste products), have very high water content, (82-90%), but in dried form, it has high protein (22.1-23.6% DM) and fiber contents, (WINA *et al*., 2008). It has been used as animal feed. However, the protein is very soluble and easily degraded in the rumen. Therefore, effort to protect the protein of tofu waste may be beneficial for the ruminant.

Addition of quebracho tannin (1-25 g/100g) or tannic acid depressed the nitrogen degradation rate of soybean meal in the rumen and reduced the fraction that easily degraded (FRUCTOS *et al.*, 2000, HERVAS *et al*., 2000). *In vitro* fermentation of tofu waste in the presence of *Calliandra calothyrsus* containing high tannin showed a linear reduction of protein digestibility of tofu waste with increasing level of *C. calothyrsus* (WINA *et al*., 2008). By this property, tannin could give beneficial effect as it improved bypass protein. Utilization of tannin from banana trunk to protect feed protein gave a positive effect on animal performance (MATHIUS *et al.*, 2004). Beside giving positive effect on animal performance, tannin may be used as a methane reducing agent. JAYANEGARA *et al*. (2009; 2010) studied that several tropical plants containing tannin had the ability to reduce methane in the *in vitro* fermentation.

This experiment aimed to evaluate the efficacy of tannin extracted from *Acacia mangium* to improve rumen fermentation and microbial protein synthesis and reduce methane production in the rumen without reducing digestibility.

MATERIALS AND METHODS

Crude tannin was extracted from the bark of *Acacia mangium* according to the method of WINA *et al*. (2010). The experiment was arranged in a completely randomized design using fifteen male sheep allocated in one of 3 dietary treatments. All sheep were fed with king grass and tofu waste (1 : 1 DM). The treatments were: 1) Tofu waste without tannin; 2) Tofu waste with tannin. Each day before feeding, tannin extract was mixed with dried tofu waste at the level of 4%, 3) Tofu waste with tannin + polyethylene glycol. Each day before feeding, tannin extract and polyethylene glycol (PEG) was mixed with dried tofu waste at the level of 4% and 8%, respectively.

The feeding trial was started with 2 weeks of adaptation period and one week of faeces and urine collection. Faeces was collected and weighed daily. Subsample of faeces was dried to obtain dry matter of faeces. Offered and residue of feed were weighed every day. Proximate analyses of offered feed, feed residue and faeces were conducted, then dry matter, organic matter and protein and fibre (NDF, ADF) digestibilities were calculated.

Urine was collected daily in a plastic pile containing 100 ml of 2N sulphuric acid. Urine was diluted to 2 litre daily. Subsample of urine was taken and after one week, the same volume of subsamples was combined and poured into a bottle for each sheep. Urine was analysed for its Nitrogen content by Kjeldahl followed by auto analyser, for allantoin content according to the method of CHEN and GOMEZ (1992) to estimate microbial protein syntesis.

Rumen liquor was taken from each sheep in the morning before feeding and 3 hours after feeding. The pH of rumen liquor was measured and the ammonia content in rumen liquor were determined. Short chain fatty acid concentration in rumen liquor was analysed using gas chromatography. Methane production in the rumen was estimated using the formula: $CH_4 = 0.45\ C_2 - 0.275\ C_3 + 0.40\ C_4$ (MOSS *et al*., 2000).

RESULTS AND DISCUSSION

Rumen fermentation

Differences of rumen fermentation among treatments were observed through the analysis of pH, ammonia content, short chain fatty acid concentration and estimated methane production. Table 1 shows that pH rumen was similar among treatments (6.76) and slightly lower for all treatments 3 hours after feeding, but there was no significant difference due to treatments. Addition of tannin or tannin plus PEG did not affect pH condition of rumen and pH condition was in the normal range for rumen microbes to grow in the rumen and favourable for fibrolytic microbes to grow as they cannot survive in the low pH of rumen (acidic) condition.

Ammonia content in rumen liquor decreased significantly when tannin was mixed with tofu waste (Table 1). As tannin bound protein of tofu waste, it protected protein from its degradation in the rumen causing less degradation of protein to ammonia, hence, reduced ammonia production in the rumen. Ammonia production in the rumen 3 hours after feeding remained lower than control in the presence of tannin. This result

Table 1. Rumen condition of sheep fed with tofu waste, with and without tannin and with tannin +PEG before and 3 hours after feeding

Rumen	Tofu waste	Tofu waste + tannin	Tofu waste + tannin + Polyethylene glycol	Sem
pH before feeding	6.80	6.80	6.70	0.09
pH 3 hours after feeding	6.40	6.60	6.20	0.11
NH3 before feeding (mM)	16.30[a]	13.70[b]	14.50[ab]	0.59
NH3 3 hours after feeding (mM)	16.80[a]	12.40[b]	17.10[a]	1.36
Total SCFA before feeding (mmol/L)	66.70	83.00	96.00	10.8
Total SCFA 3 hours after feeding (mmol/L)	148.50	101.40	136.10	20.0
Molar proportions before feeding (mol%)				
Acetic acid	76.33	72.41	73.54	2.70
Propionic acid	13.34	16.49	15.85	1.79
n-butyric acid	6.81	8.04	7.77	0.77
Iso-butyric acid	1.62	1.43	1.15	0.12
n valeric acid	0.24	0.33	0.17	0.20
Iso-valeric acid	1.77	1.30	1.52	0.34
Ratio acetic / propionic acid	5.73	4.74	4.66	0.59
Molar proportions 3 hours after feeding (mol%)				
Acetic acid	71.81	69.69	66.37	3.48
Propionic acid	20.72	21.06	23.63	2.23
n-butyric acid	5.58	7.54	8.16	1.47
Iso-butyric acid	0.70	0.85	0.65	0.11
n valeric acid	0.61	0.30	0.68	0.12
Iso-valeric acid	0.57	0.56	0.51	0.09
Ratio acetic/propionic acid	3.47	3.30	2.83	0.45
Methane before feeding (mmol/L)	22.24	26.06	30.65	3.87
Methane after 3 hours feeding (mmol/L)	43.07	29.65	36.90	6.67

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

is in agreement with the previous report that tannin reduced the rate and extent of degradation of forage protein which then lower the ammonia production in the rumen (PATRA and SAXENA, 2011). PUCHALA *et al*. (2005) also reported that ruminal ammonia N concentrations were lower in goats fed condensed tannin containing pasture, *Sericea lespedeza* (3.7 mg/dL) than crab grass/ tall fescue (9.9 mg/dL) that might have been due to the reduced protein breakdown in the rumen.

Addition of PEG improved ammonia production as PEG bound tannin and prevented tannin to bind protein of tofu waste. Unbound protein of tofu waste, then, could be easily degraded by rumen microbes to ammonia, causing higher ammonia production in the rumen.

Beside ammonia, branched short chain fatty acid, isobutyrate and isovalerate were also the result of protein degradation. Although it was not significantly different, the molar proportion of isobutyrate and isovalerate in the rumen of sheep before feeding was lower in tannin addition group than control treatment but was similar to control treatment 3 hours after feeding. Ammonia production in tannin group remained lower than control treatment even before and after feeding of tannin.

Total short chain fatty acid (SCFA) concentration in the rumen before feeding did not show any difference among treatments but there was a slight increase in the tannin and tannin + PEG treatment ($P > 0.05$, Table 1). Three hours after feeding, increased SCFA concentration was produced in all treatments indicating fermentation process was on going and SCFA were produced.

After feeding, SCFA production in tannin treatment was lower than control or tannin+PEG treatment, but it was not significantly different among treatments. Tannin at the level of 4% in this experiment was still tolerable; however, higher level of tannin in the rumen would give a negative effect not only to protein degradation but also to fibre degradation as it may depress the growth or activity of fibrolytic microbes (MCMAHON *et al.*, 2000). The total number of cellulolytic bacteria, including populations of *Fibrobacter succinogenes* and *Ruminococcus* spp., was significantly lower in sheep supplemented with calliandra and these populations increased when animals were treated with PEG (MCSWEENEY *et al*., 2001).

In molar proportion, tannin reduced the proportion of acetic acid, but increased propionic acid and butyric acid, although the difference was not significantly ($P < 0.05$). Propionic acid is a glucogenic source for livestock and is used more efficient than acetic acid. Production of animal improves when higher production of propionic acid since this acid is used to form "muscle tissue" while propionic and butyric acids are required for milk production. In other words, tannin could be included in a ration to improve meat and milk production.

Calculated methane production 3 hours after feeding decreased with tannin addition although the organic matter digestibility was not significantly reduced. Tannins in *Sericea lespedeza*, *C. calothyrsus* and *Flemingia macrophylla* have been reported to minimize methane emission in goat (ANIMUT *et al*., 2008; TIEMANN *et al*., 2008). The reduction of methane production seems to have been mostly the result of a reduced organic matter and fibre digestion (PUCHALA *et al*., 2005). In this experiment, this mechanism has not yet happened as the tannin addition was not very high. Propionic acid bacteria used more hydrogen to form propionic acid and this would in competition with methanogens that also requires hydrogen to form methane.

Feed consumption and nutrient digestibility

Feeding tannin or tannin + PEG only slightly increased daily consumption of grasses ($P > 0.05$) (Table 2). The result shows that tannin at the level of 4% or with addition of PEG did not cause any negative effect to the consumption of forage or tofu waste. Feed consumption, however, increased in the presence of PEG (MANTZ *et al*., 2009) as PEG might neutralize the negative effect of tannin. Digestibility of dry matter, organic matter, protein and fibre did not significantly different between control and tannin treatment (Table 3).

These values reduced when PEG was added, however, when PEG was considered as inert substance and the weight of PEG was deducted from faeces weight, the digestibility of DM, OM, Protein were not significantly different among treatments. NDF and ADF digestibilities were significantly lower in the presence of tannin compared to control or tannin+PEG treatments. Lower NDF and ADF digestibilities have been reported in feeding tannin containing legumes such as *Calliandra calothyrsus* and *Flemingia macrophylla*. ADF digestion was much more affected by tannin than NDF digestion (TIEMANN *et al*., 2008). This suggests that degradation of cellulose as part of ADF fraction was reduced by tannin addition which could be related to a selective suppression of cellulolytic bacteria by tannin (MCSWEENEY *et al*., 2001) and this suggestion was appropriate with our data.

Nitrogen balance, microbial N supply and efficiency of microbial N synthesis

Feed nitrogen consumptions were not significantly different for all treatments, however, the amount of nitrogen which was excreted through faeces was slightly higher in the presence of tannin although it was not significantly different (Table 4). According to MIN *et al.* (2003), adding tannin at the level of 4% to the ruminant feed is the maximum level of tannin causing higher bypass protein (tannin-protein complex). This bypass protein was expected to be fully degraded by digestive enzymes in the abomasum, however, some of this complex might not be degraded and come out with faeces, hence, contributed to higher weight of faeces in tannin treatment compared to control. Nitrogen in the urine slightly decreased in the presence of tannin and tannin+PEG although it is not significantly different Lower nitrogen content in urine was reported by DAWSON *et al*. (1999) when sheep was fed with quebracho tannin. Partition of nitrogen from urine to faeces because of tannin would be beneficial as nitrogen in faeces was less volatile than that in urine and nitrogen in faeces could be used as good fertilizer. The ratio of retained nitrogen to consumed nitrogen per day was slightly higher although it was not different from control treatment.

Table 2. The effect of tannin and tannin + PEG on daily consumption of tofu waste and grasses by sheep

	Tofu waste	Tofu waste + tannin	Tofu waste + tannin+ Polyethylene glycol	Sem
DM grass (g/day)	249.40	256.70	293.90	
DM tofu waste (g/day)	358.30	358.30	358.30	
Total intake (g/day)				
DM	607.70	614.90	652.10	14.10
OM	589.20	600.10	628.90	13.75
CP	86.31	86.73	87.82	0.52
NDF	356.00	365.20	389.30	11.53
ADF	209.30	215.30	231.30	7.63
Total intake (g/kgW0.75)				
DM	75.70	74.40	78.30	2.40
OM	73.40	72.60	75.60	2.20
CP	10.74	10.49	10.56	0.20
NDF	44.30	44.20	46.80	1.60
ADF	26.10	26.00	27.80	1.00

The metabolic products of rumen microbes that were excreted in the urine is presented in Table 5. Rumen microbes would be metabolized and excreted in the form of purine derivates which allantoin is the major purine derivate. Total excreted and absorbed purine derivates increased by 106% and 164%, respectively in the presence of tannin and only 49 and 80%, respectively in the presence of tannin+PEG suggesting that supply microbial N to post rumen increased significantly in the presence of tannin. Higher microbial protein produced by sheep fed a diet containing quebracho tannin has been reported by VASTA *et al.* (2010). Two possible causes of the increased microbial protein yield are (i) improved urea utilization occurring via bacterial uptake of urea-N recycled to the rumen in response to lower ruminal ammonia concentration, and (ii) a "bacteria-sparing" effect arising from reduced protozoal numbers by tannin resulting in decreased predation of bacteria (MCMAHON *et al.*, 2000).

This experiment shows that tannin at the level of 4% in the diet containing tofu waste showed a beneficial effect by affecting rumen fermentation through reduced ammonia production and increased microbial N supply and increased efficiency of microbial N synthesis. Microbial protein reaching duodenum is important protein contribution for ruminant as it has relatively good amino acid balance, hence would improve sheep performance. Higher level of tannin in the diet would be detrimental to ruminant and the use of Polyetyhlene glycol (PEG) to bind tannin will be important and useful as PEG will improve digestibility and nitrogen retention.

Table 3. Digestibility of feed containing tofu waste, with and without tannin and with tannin+PEG

Digestibility (%)	weight of PEG was included in the weight of faeces								weight of PEG was excluded in the weight of faeces							
	TW		TW + tan		TW + tan + PEG		Sem		TW		TW + tan		TW + tan+PEG		Sem	
DM	72.5	a	70.7	a	66.9	b	1.01		72.5		70.7		71.8		1.01	
OM	74.9	a	73.2	a	69.6	b	0.93		74.9		73.2		74.1		0.94	
CP	77	a	74.6	ab	70.1	b	1.95	P < 0.1	76.9		74.6		74.5		1.78	
NDF	69.1	a	66	ab	63.4	b	1.07		69.1	a	66.0	b	68.8	a	1.07	P < 0.1
ADF	65.8	a	60.1	b	58.5	b	1.34		65.8	a	60.1	b	64.7	a	1.34	

Different letter on the same row indicates significantly different (P < 0.05); TW = tofu waste; PEG = polyethylene glycol; Tan = tannin

Table 4. Nitrogen balance in sheep fed tofu waste, with and without tannin and with tannin+PEG

Nitrogen balance	Tofu waste	Tofu waste + tannin	Tofu waste + tannin + Polyethylene glycol	Sem
N intake (g/day)	13.81	13.88	14.05	0.08
N faeces (g/day)	3.18	3.53	3.59	0.25
N urine (g/day)	2.61	2.50	1.89	0.27
N excretion (g/day)	5.79	6.03	5.49	0.36
N retained (g/day)	8.02	7.85	8.57	0.38
N retained/ N intake	0.58	0.57	0.61	0.26

Table 5. Purine derivates and microbial N supply and efficiency of microbial N synthesis in sheep fed tofu waste, with and without tannin and with tannin + PEG

Parameter	Tofu waste		Tofu waste + tannin		Tofu waste + tannin + Polyethylene glycol		Sem
Allantoin (mmol/day)	2.09	a	4.22	c	3.12	b	0.23
Excreted purines (mmol/day)	2.61	a	5.38	c	3.90	b	0.29
Absorbed purines (mmol/day)	2.25	a	5.93	c	4.06	b	0.39
Microbial N supply (g/day)	1.63	a	4.32	c	2.95	b	0.28
Effciency of microbial N synthesis (g N/kgDOMI)	3.7	a	9.9	c	6.8	b	0.73

Note: Different letter on the same row indicates significantly different ($P < 0.05$)

CONCLUSION

Adding tannin to tofu waste at the level of 4% did not cause any negative effect on daily consumption or digestibility except NDF and ADF digestibilities. Methane production in the rumen decreased in the presence of tannin indicating a beneficial effect of tannin to the environment. Tannin at the level of 4% in the ration increased microbial protein supply and improved the efficiency of microbial protein synthesis, 2.65 higher than without tannin.

ACKNOWLEDGEMENT

This experiment was funded by the Indonesian Government (RK/N-1/APBN2008). A sincere thank is given to Gunawan and Eka Susilawati who have helped the authors finalized this experiment.

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