

Effect of Polyethylene Glycol (PEG) on Blood Parameters of Sheep Given *Leucaena pallida* Leaves Base Diet

(Pengaruh Penambahan Polyethylene Glycol (PEG) terhadap Parameter Darah Ternak Domba yang Mendapatkan Ransum Dasar Daun *Leucaena Pallida*)

Rusdi

*Feed and Nutrition Section
Faculty of Agriculture, Tadulako University, Palu*

ABSTRAK : Penelitian ini dilakukan untuk mengevaluasi kemampuan PEG dalam mengurangi pengaruh negatif dari pemberian daun *Leucaena pallida* pada ternak domba melalui pengamatan parameter darah. Sebanyak 12 ekor domba ditempatkan secara acak pada dua perlakuan dengan 6 ulangan. Domba diberi pakan dasar daun *Leucaena pallida* dengan tambahan PEG atau tanpa PEG selama empat minggu melalui *automatic feeders*. Data parameter darah antar perlakuan dianalisa dengan uji t. Hasil penelitian menunjukkan bahwa PEG secara konsisten meningkatkan kecernaan makanan terkonsumsi dalam saluran pencernaan domba yang ditunjukkan dengan meningkatnya konsentrasi urea darah, glukosa darah dan β -hydroxybutyrat darah. Dengan demikian dapat dikatakan bahwa penambahan PEG ke dalam ransum yang mengandung tannin berpotensi untuk meningkatkan nilai hayati pakan.

Kata Kunci : Polyethylene glycol, darah, tanin

Introduction

Leucaena pallida is a leguminous tropical multipurpose tree (MPT), which is resistant to the psyllid (*Heteropsylla cubana*) attack and it could survive with a periodical defoliation. Leaves and edible stem have crude protein content ranging from 27.50-35.10 g/kg dry matter (DM) (Dalzell *et al.*, 1998), with 0.7 g/kg DM calcium (Ca), 0.48 g/kg DM phosphorus (P) and 0.68 g/kg DM sulfur (S). However, it contains several antinutritional factors (ANFs) that hamper its use as a feedstuff. One of ANFs is condensed tannin (CT) of 9.30 g/kg DM (Dalzell *et al.*, 1998), or even 20 g/kg DM (Rusdi, 2004). Gobius (2001) reported that *Leucaena pallida* leaves reduced nitrogen balance of sheep as more protein (nitrogen) were voided to the faeces. A similar trend was reported by McNeill *et al.* (1998), even though they found a higher DM intake compared to the others *Leucaena* species introduced.

An alternative method to possibly enhance microbial adaptation is utilization of complexing agents like polyethylene glycol (PEG). PEG binds more tannin than protein and displaces protein from pre-formed tannin-protein complexes (Mangan, 1988). PEG improves nitrogen degradation in the rumen and then therefore, elevates blood urea (Silanikove *et al.*, 1996). The observation reported here was to evaluate the inclusion of PEG to alleviate the detrimental

effects of tannin on sheep receiving *Leucaena pallida* leaves by evaluating the blood parameters.

Research Methods

Leucaena pallida Leaves

The *Leucaena pallida* leaves were generated from cultivated pasture in Spring-Summer season. The trees of *Leucaena pallida* were cut off about 1 meter above the ground and then air dried in an air flow ventilated bunker to reduce water content of 10% or less. The dry materials were stripped to separate leaves from thick stem which were then discarded. Edible leaves (fine stems and leaves) were further coarsely sieved to separate leaf materials from fine stems and then stored in sealed drums until needed.

Animals and Housing

Twelve wether lambs of Border Leicester/Merino/Dorset cross (27.40±2.20 kg) of 5 months old were used as experimental animals. The animals were housed in the individual metabolism crates equipped with individual drinkers and feeders. They were fed pangola grass (*Digitaria decumbens*) hay for 3 days prior to the experimental period when they were fed the experimental diets for 4 weeks. The diets were placed on the a continuous feeder during experimental period.

Experimental Design and Diets

Twelve animals were randomly allocated to one of two groups of treatments with 6 replicates. The treatments were diets either with or without PEG. Basal diet was dried *Leucaena pallida* leaves. The leaves were added with protein supplement to stimulate intake. Protein and PEG supplements provided 150 g of crude protein (CP) and 75 g/kg DM of *Leucaena pallida* leaves, respectively. The diet composition is presented in Table 1.

Table 1. Composition of the experimental diets

Ingredients	+PEG	-PEG
Pallida leaf (g)	1020.00	1020.00
Protein supplements (g):		
Fish meal	263.60	263.60
PEG (g)	75.00	0

Intake and Liveweight

Adaptation to the new environment was given for 3 days. The next 7 days were allowed for adaptation to the experimental diets. Feed intakes were recorded daily from day 11 to day 17 of collecting period. Feed offered and feed refusals were recorded and sampled each day and later were analyzed for DM and other chemical components. Initial liveweight was recorded using an electronic scale before the sheep were allocated to the treatment groups. The sheep were weighed weekly during the 4-week feeding period just before the morning feeding.

Blood Sampling

Blood samples were collected from jugular vein at the end of experimental period. Approximately 8 mL of blood was collected onto 10 mL syringes and then transferred into each of two tubes, containing lithium heparin or sodium fluoroxalate. Lithium heparin blood was for urea and β -hydroxybutyrate determination and sodium fluoroxalate blood was for glucose analysis. Blood samples were stored at 5°C and directly sent to the laboratory for analysis.

Chemical and Statistical Analysis

Feed samples were analyzed for dry matter (DM), ash and organic matter (OM). DM was estimated as the residue remaining after samples were dried at 65°C for 48 h. Ash and organic matter were determined by

incineration of samples in a muffle furnace at 550°C for about 5 h, the loss in weight represented the OM content. Chemical blood analysis was done by the Pathology Laboratory of School of Veterinary Science, University of Queensland, Australia.

Analysis of tannin content of the diets was carried out using the Butanol/HCl method by Dalzell and Kerven (1998), in which pure condensed tannin (CT) of *Leucaena pallida* was used as a standard. Tannin was categorized into free, protein bound, fibre bound and total tannins; detail of the analysis has been presented by Rusdi (2004). The data were analysed by T-test analyses (Steel and Torrie, 1980).

Results and Discussion

Results

Chemical composition of *Leucaena pallida* leaves and diets for animals is shown in Table 2. The condensed tannin content of *Leucaena pallida* leaves was higher than actual diet fed to all sheep. PEG influenced blood parameters by consistently increasing the concentration of blood urea ($P < 0.01$) and blood glucose ($P < 0.05$). PEG also tended to increase the beta-hydroxybutyrate ($P > 0.05$; Table 3). Furthermore, PEG stimulated a higher dry matter intake ($P > 0.05$), and significantly increased liveweight gain ($P < 0.05$).

Discussion

Makkar *et al.* (1995) have demonstrated that PEG was the most effective absorbent to bind tannin and followed by polyvinyl pyrrolidone (PVP) and polyvinyl polypyrrolidone (PVPP). PEG has widely been used to bind and inactivate tannin in plant material fed to animals (McNeill *et al.*, 1998; Landau *et al.*, 2000; Gibious, 2001).

The significant findings in the present study demonstrated that PEG elevates the blood urea (Table 3). This finding was supported by Silanikove *et al.* (1996) who reported that an increase in blood urea as PEG were included in the diet, while Bhatta *et al.* (2002) reported an elevation of blood urea when PEG were drenched on kids browsing *Prosopis cineraria*. In contrast, Silanikove *et al.* (1997) reported that giving PEG to goats to bind dietary tannins from oak leaves has no effects on blood urea levels compared to un-supplemented animals. This contradiction could be due to the efficacy of PEG itself as affected by the.

Table 2. Chemical composition (g/kg DM) of the diets

Composition	<i>L. pallida</i> leaves	+PEG	-PEG
Dry matter	937	942	939
Organic matter	881	855	865
Nitrogen	40	48	50
Condensed tannin			
Free	129	74	112
Protein bound	11	16	12
Fibre bound	10	8	4
Total	150	98	128

Table 3. Blood-urea, blood-glucose and blood- β -hydroxybutyrate (BHB), dry matter intake (DMI) and live weight gain (LWG) of sheep fed *Leucaena pallida* basal diets either with or without PEG¹⁾

Parameters	+PEG	-PEG	Probability
Urea, mmol/L	12.97 ^a	8.45 ^b	0.00
Glucose, mmol/L	4.13 ^a	3.15 ^b	0.05
BHB, mmol/L	0.35	0.30	0.10
DMI, kg/h	1.36 ^a	1.33 ^b	0.36
LWG, g/h	189.39 ^a	133.31 ^b	0.04

^{a,b} Superscripts within the same row indicate significant differences

ratio PEG to tannins in feeds as noted above. These ratios depend not only on the level but also on the activity of tannins. The present phenomenon indicated an improvement of protein degradation in the rumen by the action of PEG, as reported that PEG increase rumen concentration of ammonia when sheep were intra-ruminally infused with PEG on *Lotus pedunculatus* diet (Waghorn *et al.*, 1994), or kids drenched with PEG on *Prosopis cineraria* diet (Bhatta *et al.*, 2002). Initially, mastication process allows the release of tannin from the plant and simultaneously PEG binds the released free tannin. Additionally, physical process in the rumen allows PEG displace protein-tannin complexes to PEG-tannin complexes, consequently proteins are freely available for the microorganism and further degrade the protein to form ammonia as nitrogen source for microbial protein analysis *per se*. Since there is a positive relationship between rumen ammonia and blood urea, then the increase in rumen ammonia will be followed by the increase in blood urea after ammonia being transferred to urea by the liver, regarded as a blood urea recycle process in ruminant animals (Hogan, 1996).

As nitrogen in the form of ammonia is freely available, thus microorganisms are capable to further digest more carbohydrate components of the diets and released more volatile fatty acids (acetic acid,

propionic acid and butyrate acid; VFA), as final products of rumen fermentation of carbohydrates. Makkar *et al.* (1995) reported that inclusion of PEG in tannin-containing feeds resulted in an increase in gas production due to elevation in VFA production without many changes in the molar proportion of VFA as a reflection of the increase in digestibility of the feeds. McSweeney *et al.* (1999) suggested that VFA increased in tannin containing feeds as a response to PEG provides a better indication of fermentable carbohydrate in the rumen. Similarly, Waghorn *et al.* (1994) found that PEG accelerate fermentation rate and therefore elevate the production of VFA. The pathways of glucose synthesis in which volatile fatty acids, particularly propionate acid is regarded as a main precursor for glucose synthesis through gluconeogenesis pathways (Lehninger, 1982), while β -hydroxybutyrate is stage of pathways to be used as energy source in which butyrate is a main precursor. Thus, enhancement of these blood parameters in the present results indicated the improvement of carbohydrate fermentation of ingested feed by inclusion of PEG. The findings agree with the previous studies of Makkar *et al.* (1995) and McSweeney *et al.* (1999).

A plethora studies has been recorded to clearly demonstrate the effectiveness of use PEG in tannin-

containing diets to elevate its feeding value. PEG supplementation has been shown to increase voluntary intake and digestibility of tannin-containing diets, allowing a positive effect on organic matter degradation (Silanikove *et al.*, 2001). Inclusion of PEG in tannin containing forages has been shown to ameliorate its toxicity, increasing intake and body weight in sheep, rats and rabbits (Saarisalo *et al.*, 1999; Smith *et al.*, 2003). So, the ultimate effect of PEG inclusion is to stimulate dry matter intake with subsequent a marked improvement in liveweight gain, which is clearly demonstrated in the current results and also in study of Bhatta *et al.* (2002).

Conclusion

Inclusion of PEG in tannin-containing diet consistently improved digestibility of ingested feed in the digestive tracts by enhancing the concentration of urea, glucose and β -hydroxybutyrate in the blood, which in turn improved the liveweight gain of the animals. Thus, additional PEG is clearly reduces the inhibitory effect of *Leucaena pallida* tannins in the digestive tracts and therefore, improves the feeding value of the forage.

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