THE USE OF POLYETHYLENE GLYCOL (PEG) TO INCREASE FERMENTABILITY OF *Acacia angustissima* AND *Acacia villosa*

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ABSTRACT

This experiment was carried out with an aim for increasing fermentability and digestibility of *Acacia angustissima* and *A. villosa* by using polyethylene glycol (PEG) at different levels. The levels of PEG supplemented to each legume were 0, 5 and 10% of dry weight sample. An increase in PEG level improved protein degradation of *Acacia* spp. without affecting VFA production and microbial population. However, the addition of PEG decreased DM and OM digestibilities although the effect on OM digestibility was not significant. The present result has not yet been able to demonstrate an optimum level of PEG as a supplement for reducing tannin effects in both *Acacia* spp. This study also indicated that protein of *A. angustissima* was more degraded in the rumen than that of *A. villosa*, but nutrients of this species of *Acacia* was more digested in the post-ruminal digestive tract than those of *A. angustissima*.

Keywords : Acacia angustissima, A. villosa, polyethylene glycol

INTRODUCTION

Legumes and grasses are important feed sources in tropical and dry areas (Topps, 1992). The legumes are used as protein supplements to improve nutrient quality supplied by grass as a main feed in increasing animal production (Kaitho *et al.*, 1998[a]; Kaitho *et al.*, 1998[b]; Saarisalo *et al.*, 1999).

Acacia spp. can be used as protein supplements due to its high protein content (22-32%) and quality, its faster growth, its ability to adapt to the environment, and its ability to grow in poor quality of soil and its high leaf production (Praptiwi, 1985; Gutteridge, 1994, Wina and Tangendjaja, 2000; Soebroto and Priyosukmana, 1985). However, *Acacia* spp. varied in their protein solubilities and degradabilities in the rumen, as well as their digestibilities in the post-ruminal digestive tract (Kaitho *et al.*, 1998[b]). These variations were influenced by the presence of tannins and other antinutrients and their effects were determined by their concentrations (Ahn *et al.*, 1989; Provenza, 1995; Kaitho *et al.*, 1998[b]; Evans *et al.*, 1993, Wina and Tangendjaja, 2000; Praptiwi, 1985). High concentrations of tannin decreased feed palatability, reducing feed intake, reduced protein degradation in the rumen and decreasing protein availability in the lower digestive tract (Barry and Blaney, 1987; Butler and Rogler, 1992).

Supplementation with polyethylene glycol (PEG) is an alternative method for overcoming tannin problems due to its ability to exchange with protein from protein-tannin complex releasing protein (Jones and Mangan, 1977). The use of PEG was determined by factors such as tannin concentration in plant species and solubility or molecular weight of PEG, etc. (Makkar, 2000; Silanikove *et al.*, 1996[a]; Silanikove *et al.*, 1997) causing differences in the levels of PEG supplemented for a particular plant species. Therefore, this experiment was conducted with an aim for increasing fermentability and digestibility of *Acacia angustissima* and *Acacia villosa* with PEG supplementation at different levels.

MATERIALS AND METHODS

Materials

Leaf samples of *A. villosa* were collected from the field laboratory of Faculty of Animal Science – Bogor Agricultural University – Dramaga – Bogor Those of *A. angustissima* were obtained from the farm station of Research Institute for Animal Production – Ciawi – Bogor. PEG4000 was used at a level of 0,5 and 10% of dry weight of *Acacia* spp. Rumen fluid of naturally adapted sheep was originally obtained from Kupang that has been stored at –80 °C. That of gradually adapted sheep was obtained from Research Institute for Animal Production Ciawi – Bogor; this rumen fluid was also stored at -80 °C. Rumen fluid of non-adapted sheep was collected using a stomach tube from the rumen of sheep kept in field laboratory of Faculty of Animal Science – Bogor Agricultural University – Dramaga – Bogor.

Variables

Ammonia (NH_3) and VFA concentrations, total bacterial population and protozoal number, as well as dry matter (DM) and organic matter (OM) digestibilities were measured as variables in this experiment

Inoculum preparation

Stored rumen fluids were thawed and sub cultured in BHI medium. BHI medium also contained 5 g of *A. villosa* per 100 ml. The cultures were incubated anaerobically at 39 °C for 24 h. The cultures were then used as inocula in *in vitro* fermentability and digestibility studies.

In vitro fermentability study

The study was carried out following a two stage fermentations of Tilley and Terry method (1963). Cultures contained 1 g of *Acacia* sp.-PEG mixture, 12 ml of artificial saliva (McDougall) solution, 6 ml of autoclaved rumen fluid of sheep and 2 ml of fresh inoculum of each type of rumen fluid. These mixtures were incubated anaerobically in a shaker-bath at 39 °C for 4 h. After 4 h, sample (0.05 ml) was taken from each fermentation tube and used for total bacterial counting; 1 ml of sample was also collected and utilized for protozoal counting. The mixtures were then mixed with 0.2 ml saturated $HgCl_2$ to stop fermentation and the mixtures were then centrifuged at 10,000 rpm for 10 min. Filtrates were not used, but supernatants were used for analyzing NH_3 and VFA concentrations. A micro-diffusion Conway method was used to analyse NH_3 concentration; VFA concentration was determined following a steam-distillation method (General Laboratory Procedure, Department of Dairy Science University of Wisconsin, 1966).

Counting bacterial and protozoal populations

Bacterial population was counted using serial dilution method described by Ogimoto and Imai (1981). Each sample was diluted serially with dilution solution, the diluted sample was then inoculated into solid BHI medium. Inoculated media were incubated anaerobically at 39 °C for 24-48 h. Colonies that grew on solid medium were counted, and bacterial population was calculated after correcting with dilution factor. Protozoal numbers were counted using a counting chamber under a microscope: this procedure was carried out after mixing sample with formal saline solution (4% formaline in 0.9% NaCl solution) at a ratio 1:1.

In vitro digestibility study

The above fermentation procedure was also applied to determine *in vitro* DM and OM digestibilities (Tilley and Terry, 1963), but incubation was carried out for 24 h. After stopping microbial fermentation with 0.2 ml of saturated-$HgCl_2$, the mixtures were centrifuged at 10,000 rpm for 10 min. Supernatants were discarded, filtrates were mixed with 20 ml pepsin-HCl (0.2% w/v). These mixtures were then incubated aerobically in a shaker bath at 39 °C for 24 h which were then filtered through a Whatman filter paper No. 41 using a vacuum pump and hot water. The residues were dried in an oven at 105 °C for 24 h to determine moisture-content. After weighing dried residues, the residues were then dried in an oven at 600 °C for 10 h to analyse ash content. DM digestibility (%) was calculated using this formula : [{DM sample weight – (DM residue weight - DM blank weight)}/ DM sample weight] x 100%. This same formula was also used to calculate OM digestibility (%) after replacing DM weight with OM weight.

Table 1. Ammonia Concentrations of *Acacia* Species as Affected by PEG Supplementation

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	Means ± SE*
		mM/g BK			
A.angustissima	0	8.33 ± 1.20	5.66 ± 0.08	7.89 ± 1.93	7.29 ± 1.07[d]
	5	9.76 ± 1.28	15.77 ± 1.23	9.01 ± 3.09	11.51 ± 1.87[cd]
	10	16.20 ± 0.73	12.13 ± 2.04	15.44 ±2.40	14.59 ± 1.72[c]
	Means±SE	11.43 ± 1.07	11.19 ± 1.12	10.78 ± 2.47	11.13 ± 1.55[a]
A.villosa	0	3.51 ± 1.11	4.15 ± 1.09	4.43 ± 1.16	4.03 ± 1.12[d]
	5	7.11 ± 1.17	4.35 ± 0.27	8.54 ± 0.66	6.67 ± 0.70[cd]
	10	12.91 ± 1.69	9.88 ± 2.81	11.36 ± 1.08	11.38 ± 1.86[c]
	Means±SE	7.84 ± 1.32	6.13 ± 1.39	8.11 ± 0.97	7.36 ± 1.23[b]
Means ± SE		9.64 ± 1.20	8.66 ± 1.25	9.44 ± 1.72	9.25 ± 1.35

• Means within column with different superscripts differ significantly at (P<0.01)

Table 2. Effect of PEG Addition to *Acacia* Species on VFA Concentration

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	*Means ± SE*
		mM/g BK			
A.angustissima	0	60.23 ± 12.38	42.78 ± 6.33	55.88 ± 12.59	52.96 ± 10.44
	5	81.64 ± 17.16	52.45 ± 41.53	58.42 ± 8.38	64.17 ± 22.36
	10	57.88 ± 10.94	60.89 ± 10.41	56.41 ± 16.36	58.39 ± 12.57
	Means±SE	66.58 ± 13.49	52.04 ± 19.52	56.90 ± 12.44	58.51 ± 15.12
A.villosa	0	59.47 ± 22.47	63.56 ± 23.16	58.51 ± 14.54	60.51 ± 20.16
	5	62.55 ± 13.48	63.33 ± 20.31	49.73 ± 9.91	58.54 ± 14.57
	10	82.20 ± 34.47	54.90 ± 12.42	71.48 ± 33.69	69.53 ± 26.86
	Means±SE	68.07 ± 23.47	60.60 ± 18.63	59.91 ± 19.38	62.86 ± 20.53
Means ± SE		67.33 ± 18.53	56.32 ± 19.03	58.40 ± 15.91	60.68 ± 17.83

Statistical analysis

The experiment was conducted following a factorial randomised block design (2x3). Rumen fluids of naturally adapted, gradually adapted and non-adapted sheep were used as blocks (three blocks) with three sub samples within each block. Factor A was species of *Acacia* spp. (*A. angustissima* and *A. villosa*), and factor B was levels of PEG supplemented to each *Acacia* spp. (0, 5 and 10% of dry weight of sample). Analysis of variance (ANOVA) was used to analyse all data, and contrast and polynomial orthogonals were used to examine differences among treatments (Steel and Torrie, 1981).

RESULT AND DISCUSSION

Ammonia concentration (Table 1) differed among *Acacia* species (P<0.01) with *A. villosa* produced a lesser ammonia concentration than *A. angustissima* (P<0.01). PEG supplementation increased ammonia concentration from 5.66 ± 0.61 mM/g DM at 0% to 9.09 ± 0.99 and 12.99 ± 0.85 mM/g DM, respectively, at 5 and 10% (P<0.01). This effect of PEG supplementation followed a

Table 3. Effect of PEG Supplementation to *Acacia* Species on Bacterial Numbers

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	*Means ± SE*
			x 10^3 cfu/ml		
A.angustissima	0	0.01 ± 0.0	0.2 ± 0.1	0	0.1 ± 0.04
	5	21.0 ± 19.0	56.0 ± 55.0	0.9 ± 0.6	26.0 ± 25.0
	10	240.0 ± 120.0	3800.0 ± 2100	1600.0 ± 930	1900.0 ± 1100
	Means±SE	87.0 ± 46.3	1285.4 ± 718.4	534.2 ± 10.2	642.0 ± 375.0
A.villosa	0	490.0 ± 260.0	11.0 ± 7.0	170.0 ± 96.0	220.5 ± 120.0
	5	12.0 ± 11.0	0.02 ± 0.01	390.0 ± 290.0	134.0 ± 100.3
	10	310.0 ± 300.0	110.0 ± 55.0	790.0 ± 490.0	403.3 ± 281.7
	Means±SE	270.7 ± 190.3	40.3 ± 20.7	450.0 ± 292.0	252.5 ± 167.0
Means ± SE		180.0 ± 120.0	660.0 ± 380.0	490.0 ± 310.0	450.0 ± 270.0

linear pattern : Y=-7.60+1.36X (R^2=0.997). There were no significant effects of interaction between *Acacia* spp. and PEG levels, and inoculum sources.

In this study, PEG supplementation at different levels increased ammonia concentration in both species of *Acacia* meaning that tannin could be one of major antinutrients affecting protein degradation of *Acacia* spp. An increase in ammonia concentration with PEG supplementation showed that PEG was able to bind tannins from tannin-protein complexes (Jones and Mangan, 1977; Silanikove *et al.*, 1996[a]). This resulted in an increase in protein availability in the rumen which was subsequently degraded by rumen microbes (Odenyo *et al.*, 1997; Saarisalo *et al.*, 1999). This study demonstrates that protein of *A. angustissima* was more degraded by rumen microbes than that of *A. villosa* as indicated by a greater ammonia concentration of *A. angustissima* than that of *A. villosa*. This result also confirms other studies that indicate differences in protein degradation occur among species of *Acacia*. The different could be related to their protein quantity and quality, and their tannin concentrations (Kaitho *et al.*, 1998[b]; Saarisalo *et al.*, 1999) consequently affecting differences in the level of PEG used as a supplement and in the effect of PEG.

Differences in *Acacia* species did not cause differences in VFA concentrations (Table 2) demonstrating that nutrients other than protein may be fermented at a similar extent. VFA concentrations were slightly increased with PEG supplementations from 56.74 ± 5.86 mM/g DM at 0% to 61.35 ± 7.80 and 63.96 ± 8.00 mM/g DM,

Table 4. Effect of PEG addition to *Acacia* species on protozoal numbers

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	*Means ± SE**
			x 10^3 cell/ml		
A angustissima	0	4.7 ± 1.3	0.7 ± 0.7	2.0 ± 1.2	2.4 ± 1.1[b]
	5	0.7 ± 0.6	8.0 ± 1.2	0.7 ± 0.6	3.1 ± 0.8[ab]
	10	6.7 ± 1.3	8.0 ± 1.2	4.7 ± 1.3	6.4 ± 1.3[a]
	Means±SE	4.0 ± 1.1	5.6 ± 1.0	2.4 ± 1.0	4.0 ± 1.0
A.villosa	0	3.3 ± 0.7	4.0 ± 0	1.3 ± 0.7	2.9 ± 0.4[b]
	5	4.0 ± 1.1	8.7 ± 2.7	4.7 ± 2.4	5.8 ± 2.1[ab]
	10	8.0 ± 0	8.0 ± 0	6.0 ± 1.2	7.3 ± 0.4[a]
	Means±SE	5.1 ± 0.6	6.9 ± 0.9	4.0 ± 1.4	5.3 ± 1.0
Means ± SE		4.5 ± 0.8	6.2 ± 0.9	3.2 ± 1.2	4.7 ± 1.0

* Means within column with different superscripts differ significantly at (P<0.05)

Table 5. Average *in vitro* DM Digestibility of *Acacia* Species Supplemented with PEG

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	Means ± SE*
		% DM			
A.angustissima	0	27.17 ± 1.36	25.30 ± 1.75	27.01 ± 1.94	26.49 ± 1.68[a]
	5	27.10 ± 2.98	22.17 ± 1.15	21.23 ± 1.47	23.50 ± 1.87[ab]
	10	29.63 ± 5.39	20.67 ± 1.16	20.54 ± 1.14	23.61 ± 2.57[b]
	Means±SE	27.97 ± 3.23	22.71 ± 1.35	22.93 ± 1.52	24.53 ± 2.04
A.villosa	0	31.44 ± 7.61	26.08 ± 2.85	26.41 ± 2.53	27.98 ± 4.33[a]
	5	24.58 ± 3.63	24.32 ± 1.58	24.90 ± 4.51	24.60 ± 3.12[ab]
	10	14.58 ± 2.99	18.48 ± 2.59	21.46 ± 3.19	18.17 ± 2.93[b]
	Means±SE	23.53 ± 4.74	22.96 ± 2.34	24.26 ± 3.41	23.58 ± 3.46
Means ± SE		25.75 ± 3.99	22.84 ± 1.85	23.59 ± 2.46	24.06 ± 2.75

• Means within column with different superscripts differ significantly at (P<0.05)

respectively, at 5 and 10%, but this increase was not statistically significant. This may indicate that PEG has a greater effect on protein degradation than on fermentation of other nutrients such as carbohydrate. Interaction between *Acacia* species and PEG levels did not produce significant effects on VFA concentrations. Differences among rumen fluids as inocula did not show any significant effects on VFA concentrations.

Although there were differences in bacterial numbers among *Acacia* species, the different was not statistically significant (Table 3). PEG supplementations and its interaction with *Acacia* species, as well sources of inocula did not produce significant effects on bacterial numbers (Table 3). Bacterial numbers were 112.52 ± 58.12, 80.42 ± 65.31 and 1144.84 ± 462.43 ($x10^4$ cfu/ml) when PEG was supplemented at 0, 5 and 10%.

On the other hand, protozoal numbers were influenced by level of PEG (P<0.05) with the numbers were 2.67 ± 0.46, 4.44 ± 0.95 and 6.89 ± 0.46 ($x10^3$ cell/ml), respectively at 0, 5 and 10% of PEG. Protozoal numbers were not affected by other factors (Table 4).

Although PEG supplementation increased ammonia concentration, the increase in ammonia concentration produced a small effect on bacterial population. Population of these rumen microbes

Table 6. Average *in vitro* OM Digestibility of *Acacia* Species Supplemented with PEG

Acacia species	PEG level (%)	Source of inoculum			
		Naturally adapted sheep	Gradually adapted sheep	Non-adapted sheep	Means ± SE*
		% DM			
A.angustissima	0	15.13 ± 1.46	12.80 ± 1.33	14.54 ± 1.31	14.16 ± 1.37
	5	19.80 ± 4.14	12.53 ± 1.17	11.85 ± 1.23	14.73 ± 2.18
	10	23.06 ± 7.10	10.63 ± 0.95	11.07 ± 1.60	14.92 ± 3.22
	Means±SE	19.33 ± 4.23	11.99 ± 1.15	12.49 ± 1.38	14.60 ± 2.26[b]
A.villosa	0	31.37 ± 6.45	29.67 ± 2.59	31.57 ± 1.79	30.87 ± 3.61
	5	24.44 ± 2.47	29.47 ± 2.42	29.89 ± 2.89	27.93 ± 2.59
	10	22.89 ± 1.49	22.75 ± 2.43	26.72 ± 1.90	24.12 ± 1.94
	Means±SE	26.23 ± 3.47	27.30 ± 2.48	29.39 ± 2.19	27.64 ± 2.71[a]
Means ± SE		22.78 ± 3.85	19.64 ± 1.82	20.94 ± 1.79	21.12 ± 2.49

• Means within column with different superscripts differ significantly at (p<0.01)

may be increased, but used by protozoa as protein sources increasing protozoal population. Differences in species of *Acacia* did not cause differences in bacterial population, or differences in protozoal population. Although *Acacia* spp. contained saponin (Wina and Tangendjaja, 2000), differences in species of *Acacia* did not caused differences in protozoal population; saponin concentration of *A. angustissima* may be similar to that of *A. villosa*.

The results of DM digestibility study (Table 5) indicate that DM of both legumes were digested at a similar extent. DM digestibility was affected by PEG level ($P<0.05$); DM digestibility decreased from 27.23 ± 1.35% at 0% to 24.05 ± 1.05 and 22.30 ± 1.35 %, respectively at 5 and 10% of PEG. However, interaction between *Acacia* species and PEG level did not influence DM digestibility. This DM digestibility did not differ among source of inocula used in this study.

PEG supplementation did not cause an increase in DM digestibility indicating that PEG supplementation did not improved digestibility of nutrients other than protein such as structural carbohydrate (Saarisalo *et al.*, 1999; Odenyo *et al.*, 1999[a]; Silanikove *et al.*, 1996[b]). This study also indicates that DM of *A. angustissima* was digested at a similar extent to that of *A. villosa* although both species differed in protein degradation in the rumen. However, the result in DM digestibility is still influenced by the result in OM digestibility of both species to indicate their utilization in the lower alimentary tract.

On the other hand, OM of *A. villosa* was greater than that of *A. angustissima* ($P<0.01$). OM digestibility was reduced by PEG supplementation from 20.51 ± 2.74 % at 0% to 17.22 ± 2.85 and 15.41 ± 2.62% respectively at 5 and 10% PEG. However, this reduction was not statistically significant. Other factors did not affect OM digestibility (Table 6).

An increase in PEG levels did not increased OM digestibilities. Although the effect was not significant, the decrease in OM digestibilities was in accordance with that in DM digestibility. This result was in agreement with other findings that PEG did not improve fibrous digestibility although it increased protein digestibility (Saarisalo *et al.*, 1999; Odenyo *et al.*, 1999[a], Silanikove *et al.*, 1996[b]). An optimum level of PEG supplemented to both species of *Acacia* could not be determined in this study since the effects on variables measured were linear. Therefore, there is still a possibility of increasing PEG levels as supplements in future studies.

Although protein of *A. angustissima* was more degraded by rumen microbes than that of *A. villosa*, their VFA concentrations could not show differences in fermentation of nutrients other than proteins. However, OM of *A. angustissima* was less digested than that of *A. villosa* although there were no significant differences in DM digestibilities of *Acacia* spp. used in this experiment. The protein and other nutrient compounds such as non-structural carbohydrate or fibrous compounds of *A. angustissima* might be available in smaller amounts than the other species, and they might not be digested in the lower gastro-intestinal tract as good as that of *A. villosa*. These results also indicate differences among species or varieties/accessions of plant in their fermentability and digestibility (Kaitho *et al.*, 1998[b]; Saarisalo *et al.*, 1999; Odenyo *et al.*, 1999[b], Abdulrazak *et al.*, 2001).

This study did not demonstrate differences in all variables measured among rumen fluids of naturally adapted, gradually adapted and non-adapted sheep. These results indicate that microbes in the rumen fluids of both gradually adapted and non-adapted sheep have similar ability to degrade *Acacia* species to those of naturally adapted sheep. In the case of non-adapted sheep in this study, these sheep also consumed feeds containing native grass mixed with browse legumes containing tannins; so that natural adaptation may also occur to non-adapted sheep used in this experiment. Therefore, adaptation to Acacia feeding became an important factor for the microbes to tolerate Acacia's antinutrients/toxins and to digest *Acacia* spp.; these results were in agreements with those found by Odenyo *et al.* (1997), and Wina and Tangendjaja (2000).

CONCLUSION

PEG supplementation in this study demonstrates the reduction of the negative effects of

tannins from *A. angustissima* and *A. villosa* on protein degradation. This supplementation improved protein degradation, but did not increase VFA concentration, and DM and OM digestibilities. Optimum level of PEG supplement for each *Acacia* species could not be determined exactly; however, 10% PEG can be used as a supplement for *A. angustissima*. There is a possibility of increasing PEG levels from those used in this experiment for further studies.

Protein of *A. angustissima* was more degraded than that of *A. villosa*, but nutrients of *A. vilosa* was more digested in the post-ruminal digestive tract than those of *A. angustissima*.

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