IN VITRO DEGRADATION OF *ACACIA VILLOSA* BY RUMINAL MICROBES OF ADAPTED AND NON-ADAPTED SHEEP TO ACACIA FEEDING

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Abstrak

Akasia berpotensi digunakan sebagai suplemen pakan karena kandungan proteinnya cukup tinggi (13-32% dari bahan kering), tetapi penggunaannya dibatasi oleh adanya antinutrisi dan toksin. Salah satu pendekatan yang dapat dilakukan untuk mengurangi pengaruh negatif dari antinutrisi dan toksin tersebut adalah dengan menggunakan mikroba rumen dari ternak yang secara alami sudah mengkonsumsi akasia. Tujuan penelitian ini adalah untuk mengetahui kemampuan mikroba rumen dari ternak yang sudah diadaptasi maupun yang belum diadaptasi dengan pakan akasia dalam mendegradasi leguminosa Acacia villosa, Calliandra calothyrsus dan Leucaena leucocephala. Hasil penelitian menunjukkan bahwa ada perbedaan antara kemampuan mikroba rumen yang diadaptasi dengan akasia dibandingkan dengan yang tidak diadaptasi dalam mendegradasi ketiga leguminosa tersebut. Akasia dan kaliandra kurang dapat dicerna dibandingkan dengan lamtoro. Hal ini kemungkinan disebabkan adanya perbedaan kandungan antinutrisi (tanin) yang berbeda diantara ketiga leguminosa tersebut. Dari hasil penelitian ini dapat disimpulkan bahwa ternak yang diadaptasi dengan pakan akasia mempunyai mikroba rumen yang toleran terhadap antinutrisi tanin yang ada pada akasia dan membuka peluang dilakukannya isolasi terhadap mikroba tersebut.

Kata kunci: akasia, adaptasi, antinutrisi, mikroba rumen

Introduction

Acacia sp. can be used as supplements to improve ruminant production in Indonesia and other tropical areas due to its high concentration of protein (13-32 % DM basis) (Praptiwi, 1985; Tangendjaja and Wina, 2000; Norton, 2000). However, its utilization is limited by the presence of antinutritional and toxic compounds such as tannins, toxic non-protein amino acids (2-amino-4-acetylaminobutyric acid-ADAB; 2,4-diaminobutyric acid-DABA; and oxalylalbizzine), and mimosine (Ahn *et al.*, 1989; Evans *et al.*, 1993).

Ruminants that naturally adapt to feed containing the antinutrients are able to digest those legumes due to its rumen microorganisms have evolved to tolerate the toxins. One approach to overcome the negative effects of antinutrients is by using those rumen microbes. This approach was evaluated in this experiment to obtain informations about the ability of rumen microbes of the adapted and non-adapted sheep to acacia feeding to degrade *Acacia villosa*, *Calliandra calothyrsus* and *Leucaena leucocephala*.

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Research Method

In Vitro Digestibility Trial

Each legume leaves (1 g of dry-ground weight) was incubated in three types of media containing McDougall solution (12 ml) and each type of rumen fluid (8 ml) from different sheep. Rumen fluid was obtained from the rumen of sheep naturally fed acacia from Kupang (Kupang), sheep adapted to acacia feeding from Balitnak-Ciawi (Ciawi) and non-adapted sheep from IPB-Darmaga (Darmaga). These mixtures were flushed with CO_2 to maintain anaerobic condition, and then incubated at 39° C. For degradability study, the incubation was conducted for 0, 3 and 6 h; ammonia concentration and VFA production in supernatants were measured, respectively using microdiffusion Conway and steam distillation. Dry matter (DM) and organic matter (OM) digestibilities were determined following a two-stage procedure of Tilley and Terry (1963); for these measurements, incubation was carried out for 2 x 24 h.

Statistical Analysis

A factorial 3x3 randomised complete block design with three replications was applied in the experiment of ammonia and VFA productions with sources of rumen fluids and legumes, respectively as factor I and II. A similar experimental design was also applied in DM and OM digestibility study in which factor I was sources of rumen fluids and factor II was legumes. To determine the effects of treatment, data were analysed with analysis of variance (Steel and Torrie, 1995).

Results and Discussion

Rumen fluid from three different animals differed in their abilities to degrade the legumes, but the effects were influenced by species of legumes and the incubation period. The rumen fluid from the sheep naturally eat acacia (Kupang) degraded all the legumes at a similar extent throughout the incubation period. Acacia was degraded at a lower extent than calliandra and leucaena by the rumen fluid of adapted sheep from Ciawi and by the rumen liquour of non-adapted sheep from Darmaga.

The results of *in vitro* DM and OM digestibilities demonstrated that the rumen fluid of Kupang was more capable ($P<0.05$) in digesting all legumes than the rumen fluid of Ciawi and Darmaga (Table 1). The rumen fluid of sheep from Ciawi and Darmaga digested the legumes at a similar extent. Acacia and calliandra were digested at the same extent, but leucaena had the highest DM and OM digestibilities ($P<0.05$).

Table 1. Dry Matter Digestibility (%) of Legumes Incubated in Three Different Rumen Fluid

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia villosa	49.86 ± 2.03	19.48 ± 5.32	23.43 ± 1.27	30.90±2.88^b
Calliandra calothyrsus	46.40 ± 4.61	25.66 ± 3.81	24.18 ± 2.79	32.08±3.74^b
Leucaena leucocephala	61.64 ± 4.66	43.74 ± 0.94	44.96 ± 2.60	50.11±2.73^a
Mean ± SE	52.63 ± 3.77^a	29.63 ± 3.36^b	30.86 ± 2.22^b	

Different superscript means significantly different ($P<0.05$)

Table 2. Organic Matter Digestibility (%) of Legumes Incubated in Three Different Rumen Fluid

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia villosa	49.13 ± 1.25	19.18 ± 4.65	26.20 ± 5.22	31.50 ± 3.71[b]
Calliandra calothyrsus	45.20 ± 4.32	24.21 ± 1.44	23.05 ± 3.05	30.82 ± 2.94[b]
Leucaena leucocephala	60.98 ± 4.42	42.69 ± 1.12	43.98 ± 2.55	49.22 ± 2.70[a]
Mean ± SE	51.77 ± 3.33[a]	28.69 ± 2.40[b]	31.08 ± 3.61[b]	

Different superscript means significantly different ($P<0.05$)

These results indicated that the rumen microbes from the adapted sheep differed in their capability of digesting acacia and the other legumes from the non-adapted sheep. However, there may also be differences in the degree of resistance to the toxin between the microorganisms from their rumen of naturally eat acacia (Kupang) and those from the rumen of adapted sheep to acacia feeding (Ciawi) causing differences in the legume digestibility (Brooker, 2000; Wiryawan *et al.*, 2000). Differences in the microbial species tolerating legume toxins may also occur as it has been noticed for those resistant to tannin (Brooker *et al.*, 2000; McSweeney *et al.*, 2000; Plumb *et al.*, 2000; Tjakradidjaja *et al.*, 2000).

A similarity in acacia and calliandra digestibilities demonstrated that both legumes contain similar antinutrients such as tannins (Ahn *et al.*, 1989). Tannin is also found in leucaena (McNeill *et al.*, 2000; Norton, 2000). However, this study indicated that the effect on reducing digestibility in leucaena was not as great as those in acacia and calliandra.

At 0 hour, there was no differences in ammonia concentration amongst the treatments and its concentration tended to increase when the legumes were incubated with different rumen fluids (Table 3).

At 3 and 6 hours incubation time, ammonia concentration of cultures using Kupang sheep rumen fluid was quite stable and significantly ($P<0.05$) lower compared to the other source of rumen fluid (Table 4,5). This might be due to the equilibrium between its production and utilization. Meanwhile, ammonia concentration of Ciawi and Darmaga sheep tended to accumulate as incubation progressing.

The three legumes contain high protein, but the present of tannin greatly reduced the hydrolysis of protein because protein was bound by tannin forming stable complex which is difficult to be attacked by protease. As a result NH_3 concentration was low. However, in this regard, tannin has crucial role in slowly releasing ammonia to ascertain that the rumen microbes are not negatively affected by high concentration of NH_3 and keep the NH_3 concentration in normal range. *Acacia villosa* has the highest tannin content therefore NH_3 concentration of culture with acacia substrate was significantly ($P<0.05$) lower compared to the other legumes.

There was variations in VFA productions observed (Table 6, 7, 8). VFA concentrations fluctuated with the incubation period. Rumen fluid of sheep naturally eat acacia degraded the legumes at the same extent, except for acacia. Acacia was less fermented at 3 h incubations by the rumen fluids of adapted sheep (Kupang sheep). This is due to high concentration of tannin in acacia compared to calliandra and leucaena which inhibited protein and carbohydrate fermentation by rumen microbes as it was shown by low dry matter and organic

Table 3. NH_3 Concentration (mM) of Legumes Incubated in Three Different Rumen Fluid at 0 Hour

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia vilosa	4.73 ± 0.41	7.88 ± 0.32	8.08 ± 0.40	6.90±0.38
Calliandra calothyrsus	6.80 ± 0.50	9.25 ± 0.71	7.62 ± 2.40	7.89±1.20
Leucaena leucocephala	9.70 ± 0.60	7.51 ± 1.54	7.52 ± 1.28	8.24±1.14
Mean ± SE	7.08±0.50	5.59±0.86	7.74±1.36	

Table 4. NH_3 Concentration (mM) of Legumes Incubated in Three Different Rumen Fluid at 3 Hours

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia vilosa	6.26 ± 0.85	6.39 ± 0.15	8.14 ± 0.93	$6.93±0.64^b$
Calliandra calothyrsus	6.44 ± 1.21	11.98 ± 1.43	8.14 ± 0.93	$8.85±1.19^a$
Leucaena leucocephala	7.55 ± 0.43	11.84 ± 0.89	9.36 ± 2.85	$9.58±1.39^a$
Mean ± SE	$6.75±0.83^b$	$10.07±0.82^a$	$8.55±1.57^{ab}$	

Different superscript means significantly different ($P<0.05$)

Table 5. NH_3 Concentration (mM) of Legumes Incubated in Three Different Rumen Fluid at 6 Hours

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia vilosa	6.23 ± 0.42	9.18 ± 1.05	6.12 ± 0.18	$7.18±0.55^b$
Calliandra calothyrsus	9.04 ± 1.35	18.25 ± 1.46	9.56 ± 0.52	$12.28±1.11^a$
Leucaena leucocephala	5.77 ± 0.43	18.63 ± 1.38	11.78 ± 1.62	$12.06±1.14^a$
Mean ± SE	$7.01±0.73^c$	$15.35±1.30^a$	$9.15±0.77^b$	

Different superscript means significantly different ($P<0.05$)

matter digestibility. In addition, acacia also contains saponin which affect the activity of protozoa and consequently reducing VFA production (Tangendjaja and Wina, 2000). At 6 hours incubation as the effect of tannin and saponin to rumen microbes decreasing, the VFA concentration of culture with acacia substrate tended to increase.

Surprisingly, the rumen fluid of Darmaga sheep (non adapted to acacia feeding) seems to be unaffected by the utilization of all legumes. VFA concentration is stable at least for 6 hours incubation. This might be due to feeding system applied in Darmaga in which all animals were fed native grass containing combination of grass and shrubs. Several shrubs might contain tannin and other antinutrient factors that could stimu-

Table 6. VFA Concentration (mM) of Legumes Incubated in Three different rumen fluid at 0 Hour

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia villosa	67.35 ± 14.73	70.81 ± 29.40	85.49 ±22.13	74.55±22.09
Calliandra calothyrsus	42.31 ± 8.24	56.13 ± 6.04	58.72 ± 8.24	52.39± 7.51
Leucaena leucocephala	45.77 ± 6.74	69.94 ± 11.87	45.77 ± 3.11	53.83± 7.24
Mean ± SE	51.81 ± 9.90	65.63 ± 15.77	63.33 ±11.16	

Table 7. VFA Concentration (mM) of Legumes Incubated in Three Different Rumen Fluid at 3 Hours

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia villosa	39.72 ±12.45	49.22 ± 10.79	88.94 ± 22.13	59.29 ± 15.12
Calliandra calothyrsus	67.35 ± 8.33	81.17 ± 14.22	65.63 ± 5.25	71.39 ± 9.27
Leucaena leucocephala	47.49 ± 0.86	63.04 ± 14.14	53.54 ± 6.23	54.69 ± 7.08
Mean ± SE	51.52 ± 7.21	64.48 ± 13.05	69.37 ± 11.20	

Table 8. VFA Concentration (mM) of Legumes Incubated in Three Different Rumen Fluid at 6 Hours

Legumes	Source of Rumen Fluid			
	Kupang	Ciawi	Darmaga	Mean ± SE
Acacia villosa	85.49 ± 11.29	70.81 ± 29.40	85.49 ± 22.13	80.60 ± 20.94
Calliandra calothyrsus	58.72 ± 10.51	56.99 ± 1.50	65.63 ± 5.25	60.45 ± 5.75
Leucaena leucephala	50.08 ± 6.74	69.94 ± 15.54	53.54 ± 3.11	57.85 ± 8.46
Mean ± SE	64.76 ± 9.51	65.91 ± 15.48	68.22 ± 10.16	

late the rumen microbial resistance to those antinutrients.

Conclusion

The rumen fluid of adapted sheep to acacia feeding in Indonesia may possess microbes which are able to digest acacia, but the degree of tolerating acacia antinutrients from the rumen microbes of sheep naturally eat acacia differ from those of adapted sheep. This leads to a possibility of isolating the microbes which are resistance to acacia toxin.

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