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THE EFFECT OF *Acacia villosa* SUPPLEMENTATION AND ITS COMBINATION WITH *Gliricidia maculata* IN A RATION CONTAINING NATIVE GRASS ON *in vitro* FERMENTABILITY AND DIGESTIBILITY

Anita S. Tjakradidjaja[1,2], Komang G. Wiryawan[1,2], Sinar Jenny S. Togatorop[2], I. G. Ayu Widiani D. Rahmawati[2]

Abstract

These experiments were conducted to study the effect of *Acacia villosa* supplementation and its combination with *Gliricidia maculata* in a ration containing native grass on *in vitro* fermentability and digestibility. Rumen fluids of naturally adapted and gradually adapted sheep were used as inocula in these studies. On the basis of *in vitro* studies, *A. villosa* can be used as a protein supplement as high as 100% in combination with native grass; however, this level should be adjusted to the response of animal to acacia feeding. Combination between *A. villosa* and *G. maculata* at a ratio of 10 to 30% with 60% native grass produced the best combination. Adaptation to acacia becomes an important factor as it improved the ability of gradually adapted sheep to digest acacia at the same extent as naturally adapted sheep.

Key words: Protein supplement, *Acacia villosa*, *Gliricidia maculata*

Introduction

In Indonesia, grass is usually given to the animal as a single feed. Such practice causes low productions obtained from the animals due to insufficiency of nutrient quality and quantity to meet the animal requirements. Therefore, it is necessary to provide protein supplements such as browse legumes to improve animal production (Chriya *et al.*, 1997; Kaitho *et al.*, 1998).

Acacia spp. is one of browse legumes that can be used as protein supplements especially during dry season (Elseed *et al.*, 2002). Although nutrient compositions varied among species of *Acacia*, these species contained high amount of crude protein (17-22%) and minerals (Ca = 1.2-4.9%; K=0.4-3.2%; Mg=0.13-1.49%) with low concentrations of NDF (19-30.4), ADF (13.3-25.7%) and lignin (1.8-4.9%) in comparison to nutrient compositions of grasses (Elseed *et al.*, 2002; Abdulrazak *et al.*, 2001; Blair *et al.*, 1988). However, *Acacia* spp. also contained some

[1]Centre for Life Sciences Study, Research Institute – Bogor Agricultural University – Kampus Dramaga – Bogor 16680
[2]Department of Animal Nutrition & Feed Sciences, Faculty of Animal Science – Bogor Agricultural University – Kampus Dramaga – Bogor 16680

antinutrients and toxins such as tannin, saponin and non-protein amino acid (Wina and Tangendjaja, 2000; Evans *et al.*, 1993). Total condensed tannin was found in high concentrations in *Acacia* spp. ranging from 1.3 to 5.7 % in samples collected at the late dry period (Elseed *et al.*, 2002). These concentrations reduced nutrient degradability and digestibility affecting their use as protein supplements for the animals (Elseed *et al.*, 2002; Kaitho *et al.*, 1998; Chriya *et al.*, 1997).

One alternative method to overcome negative effects of tannin is by combining species of *Acacia* with other browse legumes or protein supplements that contain none or low concentrations of tannins such as *Gliricidia sepium* and *Senna siamea* (Jackson *et al.*, 1996). This combination is expected to dilute tannins that are present in *Acacia* sp.; such study has also been applied to reduce the negative effects of tannin of *Flemingia macrophyla* which was combined with *Crotylia argentea* in a ration containing *Brachiaria dictyoneura* (Fassler and Lascano, 1995). Therefore, these experiments were developed to study the effect of acacia supplementation and its combination with *Gliricidia maculata* in a ration containing native grass on *in vitro* fermentability and digestibility using rumen fluids of sheep that were naturally and gradually adapted to acacia feeding as sources of inocula.

Materials and Methods

Materials

In the first experiment, *A. villosa* was used as a protein supplement and combined with native grass at a level of 0, 25, 50, 75 and 100%. In the second experiment, the ratio between native grass and combined legumes was 60:40%; *A. villosa* was combined at a ratio of 0, 10, 20, 30 and 40% with *G. maculata*. Rumen fluids of naturally adapted and gradually adapted sheep were used as inocula in both experiments.

Variables measured

Variables measured in both *in vitro* experiments included ammonia (NH_3) and VFA concentrations, total bacterial population, protozoal number, and dry matter (DM) and organic matter (OM) digestibilities.

Analysis of NH_3 and VFA concentrations

NH_3 and VFA concentrations were analysed after fermentation procedure (Tilley and Terry, 1963) was carried out. Cultures containing 1 g sample, 12 ml artificial saliva (McDougall) solution, 6 ml autoclaved rumen fluid, and 2 ml inoculum of each rumen fluid were used in Experiment 1 and 2. These cultures were incubated anaerobically in a shaker-bath at 39 °C for 4 h. Samples for counting bacterial population (0.05 ml) and protozoal number (1 ml) were taken from each mixture before the addition of 0.2 ml saturated $HgCl_2$ to stop microbial fermentation.

Cultures were subsequently centrifuged at 10,000 rpm for 10 min. Filtrates were discarded, but supernatants were used for analyzing NH_3 and VFA concentrations. NH_3 concentration was determined using a micro-diffusion Conway method; VFA concentration was analysed following a steam-distillation method (General Laboratory Procedure, Department of Dairy Science – University of Wisconsin, 1966).

Determination of in *vitro* DM and OM digestibilities

In vitro DM and OM digestibilities were determined following a two-stage digestion method of Tilley and Terry (1963). The same procedure of fermentation as described above was also conducted with the incubation was carried out for 24 h. After stopping microbial fermentation by adding saturated $HgCl_2$, the mixtures were centrifuged at 10,000 rpm for 10 min. Supernatants were discarded; filtrates were kept and mixed with 20 ml pepsin-HCl (0.2% w/v). Aerobic incubation was carried out in a shaker bath at 39 °C for 24 h. After filtering the mixtures through a Whatman filter paper No. 41 with vacuum pump and hot water, residues were dried in an oven at 105 °C for 24 h to analyse moisture content. The residues were then dried in an oven at 600 °C for 10 h to determine ash content. DM and OM digestibilities were determined based on the following formula: [{DM or OM sample weight – (DM or OM residue weight – DM or OM blank weight)}/ DM or OM sample weight] x 100%.

Counting microbial population

Serial dilution method described by Ogimoto and Imai (1981) was used for counting bacterial population. After mixing each sample with dilution solution, diluted mixture was inoculated into sterile-solid BHI medium in a Hungate tube. The media were then incubated at 39 °C for 24 h. Colonies grew on the media were counted and total bacterial population was calculated after correcting with dilution factor. Counting protozoal number was carried out after mixing each sample with formal-saline solution in a ratio of 1:1. Each mixture was put into a counting chamber that was placed under a microscope; protozoal number was then counted using a counter.

Statistical analysis

A randomised block design was applied in all experiment with three replications and three sub samples using media containing rumen fluid as blocks. In Experiment 1, five treatments were applied in which native grass was supplemented with *A.villosa* at a level of 0, 25, 50, 75 and 100%. In Experiment 2, ratios between *A. villosa* and *G. maculata* at 0, 10, 20, 30 and 40% were used as treatments. The data were examined with analysis of variance (ANOVA), and differences among treatments were determined using contrast orthogonal (Steel and Torrie, 1981).

Results and Discussions

Substitution native grass with *A. villosa* at different levels (Table 1) affected NH_3 concentration and bacterial population at ($P<0.01$), protozoal population, DM and OM digestibilities at ($P<0.05$). However, the treatment did not cause differences in VFA concentration. The highest NH_3 concentration was obtained when *A. villosa* was added at 0%, then decreased linearly ($Y=12.26-0.037X$, $R^2=0.96$) as the level of *A. villosa* increased ($P<0.01$). A similar pattern was also observed in the reduction of bacterial population ($Y=3x10^{12}-3x10^{10}X$, $R^2=0.82$) and of protozoal population ($Y=43700-258X$, $R^2=0.92$). A reverse situation was observed in DM and OM digestibilities which were increased by an increase in *A. villosa* level following a linear pattern : $Y=23.81+0.12X$ ($R^2=0.87$) for DM digestibility, and $Y=19.65+0.33X$ ($R^2=0.90$) for OM digestibility.

Table 1. Effects of *A. villosa* supplementation at different levels on variables measured in Experiment 1 using rumen fluids from sheep with different adaptations to acacia feeding

Variables	*Levels of A. villosa (%)**				
	0	25	50	75	100
NH_3 concentration (mM/g DM)	12.59 ± 0.26^{A}	11.38 ± 0.54^{Ba}	10.36 ± 0.40^{Ba}	9.45 ± 0.39^{Bb}	9.59 ± 0.07^{Bb}
VFA concentration (mM/g DM)	62.25 ± 8.29	66.54 ± 0.03	59.09 ± 0.78	62.34 ± 7.45	62.39 ± 0.89
Bacterial population ($x10^{11}$ cfu/ml)	37.33 ± 26.00^{A}	17.22 ± 0.53^{B}	5.56 ± 2.22^{C}	1.67 ± 0.56^{C}	1.67 ± 0.56^{C}
Protozoal population ($x10^{3}$ cell/ml)	$9.37 + 0.43^{A}$	6.50 ± 1.22^{Ba}	6.20 ± 0.13^{Ba}	5.13 ± 0.33^{Bb}	3.60 ± 0.20^{Bb}
DM digestibility (%)	25.86 ± 0.74^{B}	25.77 ± 0.74^{B}	27.80 ± 3.38^{B}	32.16 ± 2.39^{A}	37.85 ± 0.56^{A}
OM digestibility (%)	21.72 ± 0.32^{B}	21.69 ± 1.16^{B}	24.36 ± 3.45^{B}	29.21 ± 1.58^{A}	34.64 ± 2.35^{A}

*Means with small letters differed significantly at ($P<0.05$), and those with capital letters differed significantly at ($P<0.01$)

All variables measured in Experiment 1 (Table 2) were not influenced by differences in inocula used indicating that microbes from the rumen fluids of gradually adapted sheep had similar abilities to ferment and to digest ration containing native grass and *A. villosa* to those from the rumen fluids of naturally adapted sheep.

Table 2. Effects of inocula from rumen fluids of sheep with different adaptations to acacia feeding on variables measured in Experiment 1

Variables	Sources of rumen fluids	
	Naturally adapted sheep	Gradually adapted sheep
NH_3 concentration (mM/g DM)	10.88 ± 0.55	10.43 ± 0.68
VFA concentration (mM/g DM)	62.21 ± 2.36	61.89 ± 3.22
Bacterial population ($x10^{11}$ cfu/ml)	28.22 ± 21.20	17.33 ± 11.83
Protozoal population ($x10^4$ cell/ml)	6.45 ± 0.89	5.87 ± 1.07
DM digestibility (%)	31.32 ± 2.14	28.43 ± 2.68
OM digestibility (%)	26.40 ± 5.39	26.12 ± 6.27

These results indicated that protein of *A. villosa* was less degraded than that of native grass as it was shown by their ammonia concentrations. Ammonia concentrations available from protein of *A. villosa* may not be sufficient for bacterial and protozoal growths in comparison to native grass. On the other hand, nutrients (DM and OM) of *A. villosa* were digested at a greater extent than those of native grass. Substitution of native grass with *A. villosa* at 25, 50 and 75% produced results that were in the range of those obtained with 0% and those with 100% *A. villosa*. Differences in protein degradability of *A. villosa* from that of native grass could be due to differences in their characteristics of protein although *A. villosa* contain a higher amount of protein than native grass (29.60 *vs* 10.12% DM) (Batubara, 1992; Bansi, 2001). A low protein degradability of *A. villosa* could also be affected by the presence of tannin in a high concentration compared to native grass that did not contain tannin (Bansi, 2001; Abdulrazak *et al.*, 2001). On the other hand, native grass had greater amounts of fibrous compounds (NDF=78.87 *vs* 27.32%; ADF=41.46 *vs* 21.50%; ADL=6.04 *vs* 7.77%) than *A. villosa* that influenced their digestibilities in the lower-digestive tract (Batubara, 1992). Therefore, *A. villosa* can be used as a protein supplement in a ration containing native grass as high as 100%; however, this level should be adjusted to the responses of animals to acacia's antinutrients and toxins.

The results in Experiment 2 (Table 3) indicate that treatment affected NH_3 concentration ($P<0.05$), VFA production ($P<0.05$), protozoal population ($P<0.01$) and OM digestibilities ($P<0.05$); however, bacterial population and DM digestibility were not influenced by the treatments. An increase in NH_3 concentration from 9.85 at 0% *A. villosa* to 12.61 mM at 10% *A. villosa* with 30% *G. maculata* was observed; these NH_3 concentrations were then decreased following a quadratic trend ($Y=10.728+0.014X-0.003X^2$, $R^2=0.598$) with an increase in *A. villosa* levels. A cubical pattern of treatment effects was observed in VFA concentration ($Y=63.689-4.563X-0.240X^2-0.004X^3$, $R^2=0.95$) and protozoal number ($Y=3307.5+627.03X-41.955X^2+0.6388X^3$, $R^2=0.90$). Treatment effects on OM digestibility followed a quadratic trend: $Y=26.09-0.708X+0.014X^2$ ($R^2=0.54$). A combination between 0% *A. villosa* and 40% *G. maculata* had the highest DM and OM digestibilities; on the

other hand, the lowest DM and OM digestibilities were found in ration containing 20% *A. villosa* and 20% *G. maculata*, and that containing 40% *A. villosa* and 0% *G. maculata.*

Table 3. Effects of combination between *A. villosa* and *G. maculata* on variables measured in Experiment 2 using rumen fluids from sheep with different adaptations to acacia feeding

Variables	*Ratio between A.villosa and G. maculata (%)**				
	0:40	10:30	20:20	30:10	40:0
NH_3 concentration (mM/g DM)	19.85 ± 1.25^{Ab}	12.61 ± 0.85^{Aa}	9.13 ± 0.11^{B}	7.72 ± 0.92^{B}	7.54 ± 0.13^{B}
VFA concentration (mM/g DM)	64.27 ± 3.36^{A}	36.19 ± 2.09^{Bb}	43.68 ± 1.31^{Ba}	45.32 ± 0.89^{Ba}	40.37 ± 2.97^{Bb}
Bacterial population ($x10^{11}$ cfu/ml)	16.70 ± 0.40	21.10 ± 15.40	6.31 ± 3.99	4.09 ± 1.63	7.40 ± 7.31
Protozoal population ($x10^{3}$ cell/ml)	3.17 ± 0.50^{Ba}	6.58 ± 0.92^{A}	3.33 ± 1.00^{Ba}	2.17 ± 0.17^{Bb}	2.00 ± 0.33^{Bb}
DM digestibility (%)	27.67 ± 0.34	26.68 ± 2.48	21.46 ± 1.98	22.90 ± 0.91	22.91 ± 054
OM digestibility (%)	25.09 ± 0.50^{a}	23.95 ± 2.53^{a}	13.11 ± 1.96^{c}	20.27 ± 1.06^{b}	20.15 ± 0.70^{b}

*Means with small letters differed significantly at ($P<0.05$), and those with capital letters differed significantly at ($P<0.01$)

The results in Experiment 2 also showed that rumen fluids of sheep differed in their adaptations to acacia feeding did not cause any differences in all variables measured in Experiment 2 (Table 4). These results confirm the results in Experiment 1 and were in agreements with those found by Wina and Tangendjaja (2000), and Odenyo *et al.* (1999[a]). Therefore, gradual adaptation to acacia feeding could develop the ability of rumen microbes to degrade *A. villosa.*

Table 4. Effects of inocula from rumen fluids of sheep with different adaptations to acacia feeding on variables measured in Experiment 2

Variables	Sources of rumen fluids	
	Naturally adapted sheep	Gradually adapted sheep
NH_3 concentration (mM/g DM)	9.98 ± 1.03	8.76 ± 0.86
VFA concentration (mM/g DM)	46.65 ± 3.79	45.29 ± 5.90
Bacterial population ($x10^{11}$ cfu/ml)	6.96 ± 2.90	15.30 ± 6.00
Protozoal population ($x10^{4}$ cell/ml)	4.03 ± 0.95	2.87 ± 0.72
DM digestibility (%)	24.58 ± 0.87	24.07 ± 1.80
OM digestibility (%)	20.85 ± 1.68	20.92 ± 2.67

When *A. villosa* was combined with *G. maculata*, proteins and other nutrients of *A. villosa* at 40% were also less degraded and fermented in the rumen compared to *G. maculata* at 40%. Its DM and OM were also digested in a lower extent in the lower digestive tract than those of *G. maculata*. These results demonstrate that *A. villosa* could not be used as a protein supplement as good as *G. maculata*. These could be due to differences in quantity and quality of nutrients, and in type and concentration of antinutrients/toxins (Blair *et al.*, 1988; Jackson *et al.*, 1996; Kaitho *et al.*, 1998; Odenyo *et al.*, 1999[b], Wina *et al.*, 2001; Abdulrazak *et al.*, 2001). Combination between those legumes may reduce negative effects of antinutrients in *A. villosa*. However, the amount of *A. villosa* used was quite small compared to *G. maculata* (10% *vs* 30%) because nutrient degradability and fermentability in the rumen, as well as its digestibility in the post-ruminal digestive tract decreased as the level of *A. villosa* increased. The amount of *A. villosa* should be increased since this legume has an ability to survive in poor condition and could be the only source of protein supplements during dry period (Elseed *et al.*, 2002). Therefore, other methods to improve its utilization should be explored such as combination with other possible legumes, or combine treatment between PEG supplementation and legume supplementation, etc.

Conclusion

A. villosa can be used as a protein supplement in a ration containing native grass as high as 100%; however, this level should be adjusted to the response of animal to *Acacia* feeding. A combination between *A. villosa* and *G. maculata* at a ratio of 10:30% with 60% of native grass in a diet produced the best combination based on variables measured in this experiment. Adaptation to acacia becomes an important factor as it improved the ability of gradually adapted sheep to digest acacia as good as that of naturally adapted sheep.

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