THE EFFECT OF TANNIN DEGRADING BACTERIAL INOCULATION ON THE PERFORMANCE OF CALLIANDRA (*Calliandra calothyrsus*) FED GOATS

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ABSTRACT

Calliandra (*Calliandra calothyrsus*) contains tannin that has a detrimental effect to animal performance through the binding of protein and other nutrients in the ration. In order to reduce the negative effect of tannin, tannin degrading bacterial inoculation study was conducted. Eight goats were randomly divided into two groups of four animals each. One group was treated with single inoculation of 10^8 CFU tannin degrading bacteria at the morning feeding and the other group was control (inoculated with medium). Results of the experiment indicated that the inoculated goats had higher feed consumption, body weight gain, tannin tolerant bacteria, proteolytic bacteria and total bacteria, but had similar values of rumen pH, dry matter digestibility, rumen N-NH_3 concentration and population of protozoa. Based on the experimental results, it can be concluded that inoculation of tannin degrading bacteria could improve the performance of calliandra-fed goats.

Key words: Calliandra, Tannin, Bacteria, Inoculation, Goat

INTRODUCTION

Calliandra (*Calliandra calothyrsus*) is one of the potential shrub legumes that can be used as animal feed. This is due to its high nutritive content such as protein (± 24%) and neutral detergent fiber (24 - 34%). Besides, calliandra is able to grow in some different soils and climates. Calliandra also has high productivity (10 ton/ha) (Tangendjaja *et al.*, 1992).

One limiting factor of calliandra utilization as animal feed is its high tannin content. Tannins are complex high molecular weight phenolic polymers that bind with protein, therefore reducing protein digestibility and inhibiting digestive enzymes (Haslam, 1979).

Some animals have developed mechanisms for overcoming negative effects of tannins. Rats synthesize proline-rich saliva protein that binds with tannins, preventing interactions with dietary protein. Some feral goats browsing tannin-containing Acacia have been investigated to have microbial populations that are resistant to tannin (Brooker *et al.*, 1994).

We have isolated one species of bacterium that is tolerant to high concentration of tannins (up to 3% by weight). This bacterium can not utilize tannins as sole energy source, but may provide dietary protein either by acid hydrolysis of tannin-protein complexes or by the increased microbial biomass resulting from microbial growth.

In vitro studies showed that this bacterium significantly improved dry and organic matter digestibility of calliandra (Wiryawan *et al.*, 1997). Based on the *in vitro* result, this bacterium was then used as inoculum in this experiment to study the effect of the inoculation on the performance of calliandra-fed goats.

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MATERIALS AND METHODS

Animals and Feed

Eight goats with average body weight of 22.4 ± 2.3 kg were used in this experiment. The animals were randomly divided into two groups of four animals each. One group was inoculated with tannin degrading bacteria and the other group was as control (inoculated with medium)). All goats were fed elephant grass *ad libitum* for four weeks prior to inoculation and they had never been fed calliandra previously. At the experimental trial, the ration of all goats was abruptly changed from all grass to all calliandra. Calliandra was given *ad libitum*. One hour after calliandra feeding, the inoculation was carried out.

Bacterial Inoculum

The origin of the bacterium. Bacterium used in this experiment was isolated from goat adapted to calliandra. This bacterium is able to grow in 3% tannic acid-containing medium and shows clearing zone when cultured in tannin-agar medium. This bacterium was selected because it exhibited high digestibility of dry and organic matter of calliandra *in vitro* (Wiryawan *et al.*, 1997).

Inoculum preparation. One ml of stock bacteria was inoculated into 500 ml Brain Heart Infusion (BHI) medium, containing (per 100 ml): 3.7 g BHI powder, 0.5 g yeast extract, 2.0 g casein, 1.0 ml hemin 0.05% and 0.02 ml vitamin K_1 and incubated for 24 hours at 39 °C (Ogimoto and Imai, 1984). The next day, the culture was centrifuged at 5000 rpm for ten minutes. The supernatant was discarded and the pellet was dissolved in 50 ml of BHI medium. This cultures was then inoculated into the animals using stomach tube one hour after calliandra feeding, whereas control group was inoculated with 50 ml BHI medium.

Parameters measured. The parameters observed in this experiment were: feed consumption, body weight gain, rumen pH, dry matter digestibility, concentration of ruminal N-NH_3 and Volatile Fatty Acids (VFA), population of tannin tolerant bacteria, proteolytic bacteria, total bacteria and total protozoa.

Statistical analysis. The data collected were statistically analyzed using a completely randomized design (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Consumption, Digestibility and Body Weight Gain

Although there is a tendency that inoculated goats consumed more calliandra than control, but statistically, both values were not significantly different (Table 1). Similar result was obtained for dry matter and protein digestibility. Both groups of animals showed no differences in dry matter digestibility. However compared to previous experiment conducted by Tangendjaja *et al.* (1992), dry matter digestibility recorded in this experiment were higher (55 % vs 47 %). This could be influenced by different species

Table 1. The effect of inoculation on consumption, digestibility, and body weight gain

Parameters	Time (day)	Control	Inoculated
DM consumption (g/d)		860.71	895.13
Digestibility of			
Dry matter (%)		55.4	54.4
Protein (%)		52.54	51.98
Body weight gain (g/d)	0 - 20	- 28	- 26
	21 - 30	32	48*
	31 - 40	10	16*

*Different superscript in the same rows means significantly different (P<.05)

of animal used in the experiment.

Higher feed consumption of inoculated goats led to a better growth performance. Body weight gain of inoculated goats averaged 38 g/day and significantly higher than that of control (14 g/day). However, in the first twenty days of investigation all goats had body weight reduction. This is due to an abrupt changeover of ration from all grass to all calliandra diet. This result indicated that although the animals are inoculated with tannin degrading bacteria, they still need to be adapted for at least two weeks before given all calliandra diet. After twenty days of inoculation, the inoculated goats recovered quickly and had a compensatory growth of 48 g/day ($P<.05$) compared to control (32 g/day). In the last ten days of observation, the inoculated goats continued to have higher body weight gain compared to that of control. This means that bacterial inoculation could partly deactivate tannin released from calliandra and increased nutrient availability in the rumen for bacterial degradation.

Ruminal $N\text{-}NH_3$ and VFA

In general the concentration of ruminal $N\text{-}NH_3$ ranged from 8 - 13 mM in control and inoculated goats throughout the experiment (Table 2). These values are in the normal range of ruminal $N\text{-}NH_3$ concentration (2.94 - 14.7 mM) (Preston and Leng, 1987). The concentration of $N\text{-}NH_3$ in this experiment was higher than $N\text{-}NH_3$ concentration of goats adapted to calliandra for six months (6 mM) (Sewet, 1997). This was due to total microbial depression in the rumen that consequently reduced $N\text{-}NH_3$ utilization. Statistical analysis shows that there are no significant differences of ruminal $N\text{-}NH_3$ concentration between control and inoculated goats. This might be due to its excessive availability in the rumen. The lowest concentration of ruminal $N\text{-}NH_3$ was recorded three hours after feeding. This indicated that the activity of rumen microbes was very extensive at that time. Therefore the utilization of $N\text{-}NH_3$ for microbial protein synthesis is high, therefore the $N\text{-}NH_3$ level in the rumen was decreased.

Volatile fatty acid concentration in the rumen is influenced by some factors such as rate of production, rate of utilization by rumen microbes and rate of absorption by the host. Result of this experiment shows that VFA values of inoculated animals were consistently higher that that of control animals, although statistically were not different. High VFA values of inoculated animals was due to higher population of total bacteria in the rumen.

Table 2. The effect of inoculation on the concentration of ruminal $N\text{-}NH_3$ and VFA

Parameters	Time (hour)	Control	Inoculated
$N\text{-}NH_3$ concentration (mM)	0	11.68	13.43
	3	8.40	8.82
	6	8.66	10.07
	24	10.22	9.49
	72	11.58	9.44
	240	9.75	10.33
	480	12.62	12.41
VFA concentration (mM)	0	112.76	120.11
	3	110.22	110.07
	6	109.82	109.72
	24	114.72	116.86
	72	106.28	111.78
	240	101.25	111.34
	480	98.08	118.45
	960	106.94	119.90

Table 3. Effect of inoculation on rumen bacterial population (CFU/ml rumen fluid)

Time (hour)	Total Bacteria (10^{10})		Proteolytic (10^{10})		Tannin Tolerant (10^{8})		Protozoa (10^{5})	
	CONT.	INOC.	CONT.	INOC.	CONT.	INOC.	CONT.	INOC.
0	2.76	1.98	1.32	1.36	0	0	0.78	0.63
6	0.90	1.94	0.44	1.20*	0.27	0.57*	0.61	0.59
24	1.06	0.92	0.82	0.96	0.07	0.07	0.70	0.54
240	1.56	2.66	0.92	1.14	0.13	0.37	0.62	0.50
960	2.62	3.06	0.52	0.68	0.13	0.17	0.78	0.42

*Different superscript means significantly different (P<.05) to control

Population of Rumen Microbes. Tannin degrading bacteria were not detected in both groups of animals at zero time. The highest population was recorded six hours after feeding and the inoculated goats had significantly (P<.05) higher tannin degrading bacteria than control animals. Although the population of tannin degrading bacteria was decreasing during forty days observation, the inoculated goats always had higher population compared to control. This result indicated that the inoculated bacteria could survive in the rumen at least for forty days.

Bacterial inoculum is tannin degraders as well as protein degraders (proteolytic), therefore bacterial inoculation increased population of proteolytic bacteria. Statistical analysis shows that at six hours after feeding the population of proteolytic bacteria was significantly (P<.01) higher in inoculated goats compared to control. Population of proteolytic bacteria of inoculated animals remained higher in forty days although statistically it was not different.

Low population of proteolytic bacteria in control goats up to six hours after feeding indicated that during that time there might be an accumulation of tannin in the rumen which bound the available protein, so that the proteolytic bacteria had no access to the protein and consequently the bacteria were washed out of the rumen.

Population of cellulolytic bacteria was not affected by the inoculation of tannin degrading bacteria, as both groups of animals had similar populations during forty days observation. Similar result was obtained for protozoa population in the rumen. Both groups of animals did not show any significant differences in their population. However, there is a tendency that the population was depressed by calliandra feeding. This is not surprising as calliandra contain tannin and saponin that has a negative effect on the growth of protozoa.

In general, population of total bacteria was depressed in the first 24 hours after feeding and slowly recovered in 20 days. Bacterial depression was greater in control animals compared to inoculated goats, although statistically they were not different. Bacterial depression might be caused by restricted nutrition available in the rumen due to its binding by tannin released from calliandra.

CONCLUSION

Inoculation of tannin degrading bacteria improved the performance of calliandra-fed goats through the elevation of feed consumption and body weight gain. The inoculated animals also had higher population of tannin degrading bacteria, proteolytic bacteria, total bacteria and VFA concentration in the rumen. However bacterial inoculation did not affect rumen pH, dry matter digestibility, rumen N-NH_3 concentration and population of protozoa.

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