

Research Article

White Kabesak (*Acacia leucophloea* RoxB) Leaves Utilization in Concentrate on Fermentation Products and *In Vitro* Gas Production

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ABSTRACT

This study aimed to evaluate gas production and *in vitro* fermentation products from feed containing leaves of white kabesak (*Acacia leucophloea* Roxb. Willd). Feed was composed of a ratio of 60% natural grasses and 40% concentrate. The treatments were used levels of white kabesak leaves in concentrates i.e. 0, 10, 20, 30 and 40% (v/v) in the dry matter (DM) basis as treatments, R₀, R₁, R₂, R₃ and R₄ treatments, respectively. The results showed that inclusion of *A. leucophloea* leaves in concentrate increased organic matter and crude fiber contents but decreased the crude protein content. Increasing level of *A. leucophloea* leaves in concentrate decreased gas production (ml/500 mg DM) from 198.29 (R₀) to 139.93 (R₄). The gas production rate (ml/hour) was relatively constant between 0.034 to 0.036 on R₀ - R₃ and 0.028 on R₄. Gas production at 48 hours incubation (ml/500 mg DM) decreased from 153.38 (R₀) to 103.23 (R₄). The NH₃ concentrations ranged from 6.17-7.31 mg/100 ml and the total VFA was 83.07-91.96 mM. The lowest C₂ / C₃ ratio was in R₂ (2.63). The highest IVDMD was 50.18-67.14% in R₀ and the lowest IVOMD was 55.04-71.35% R₄. The use of *Acacia leucophloea* leaves at level 20% in concentrates as supplements was more efficient in reducing gas production and *in vitro* fermentation products.

Keywords: *Acacia leucophloea*, Concentrate, Gas production, *In vitro*, Fermentation products

Introduction

Low quality of feed nutrients is often reported to be the main factor affecting the productivity of ruminant animals in the tropics, especially in eastern Indonesia including Timor. Legumes supplementation provides protein and thus can increase animal production. The high CP content in these species is an advantage to rumen microbes that depend on dietary source of nitrogen to build up their body protein [1]. The tree species are important due to their high protein content, their contribution with easy fermentation carbohydrates and fiber of better degradability, as well as their positive effect on the use of nitrogen (N) within rumen [2]. Previous study reported that Bali cattle maintained only on low quality hay during dry season had a decreased in body weight [3], as a result of exten-

sive system where the animals are left to graze in the pasture without any nutrition management from farmers.

Legumes supplementation is one of the strategies to increase animal production since it is high in crude protein and it is mainly available in Timor. In addition, legumes supplementation will reduce the cost production compared to the use of commercial concentrates. Bunglavan and Dutta (2013) stated that concentrates fed to animals resulted in inefficiency of its utilization in the rumen as well as it is expensive and thus should be reduced or prevented [4]. Forages have supplied the most economical feeds for ruminants and with the increasing price of grain feeds, forage has become much more important as a feed resource, since it cuts down feed cost, thereby reducing the total

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cost of animal production [5].

Acacia leucophloea Roxb. Willd. (*A. leucophloea*) with the local name "kabetesak putih" is a legume tree that has adapted to the environment in Timor and has been used to feed cattle and goats in the area. The crude protein content of *A. leucophloea* found in the leaves was reported ranges from 15-17% [6], and has the ability to support natural grass as a basal diet for ruminants. The use of *A. leucophloea* as ruminant feed, however, has not been intensively utilised by farmers in Timor and thus evaluation about its potential is still limited. Although *A. leucophloea* has been used as a supplement for ruminants but only based on the experience and without nutritional considerations. Therefore, the purpose of this study was to determine the potential of *A. leucophloea* in concentrate as a supplement on fermentation and *in vitro* gas production.

Material and Methods

Leaves of *Acacia leucophloea*

The *A. leucophloea* leaves used in this study were collected from *A. leucophloea* trees around Kupang city, East Nusa Tenggara province. The leaves were then sundried for 3-4 days until air dry and ground into flour before used for chemical analysis. The nutrient content of *A. leucophloea* leaves were 90.22% DM, 6.68% ash, 14.72% CP, 30.40% fibre, 0.01% EE, NFE 48.19%.

Chemical analysis

The dry matter of the sample was determined by oven-dried at 105°C overnight and the ash content was determined by burning the feed sample in the furnace at 600°C for 8 hours. The chemical analysis was performed following procedures of AOAC [7]. Van Soest analysis was used for determining acid detergent fiber (ADF) and neutral detergent fiber (NDF) [8]. Condensed tannin, total tannin and total phenol analysis was following the method of the Vanillin-HCl [9].

In vitro gas production

An amount of five hundred milligrams of feed samples (60% grass: 40% concentrate) were weighed and put into a 100-ml syringe (Fortuna, Häberle laborotechnik, Germany) with a piston that had been smeared with vaseline and the tip of the piston connected with silicone rubber and then covered with a plastic clip. The concentrate con-

sists of 5% soybean meal, 8% coconut cake, 15% rice bran, 12% finely ground corn. The dietary treatments were: 40% concentrate: 0% leaves *A. leucophloea* (R0); 30% concentrate: 10% leaves *A. leucophloea* (R1); 20% concentrate: 20% leaves *A. leucophloea* (R2); 30% concentrate: 10% leaves *A. leucophloea* (R3) and 40% leaves *A. leucophloea*: 0% concentrate (R4).

Rumen fluid was collected from a cross-breed FH cow fed corn forage (CP 9.37%) as much as 10-12 kg and concentrates (PAP milk, CP 16%), and water was provided ad libitum. The rumen liquid was collected in a pre-warmed thermos flask and filtered through 4-layers of gauze. The rumen liquid was mixed with a buffer solution in a ratio of 1 : 4 (v/v). The buffer solution used consisted of NaHCO₃ + Na₂HPO₄ + KCl + NaCl + MgSO₄.7H₂O + CaCl₂.2H₂O. The mixture was kept stirred under CO₂ at 39°C, by using a magnetic stirrer fitted with a hot plate, under continuous flushing with CO₂. A portion (50 ml) of the buffer mixture with rumen fluid was transferred into the syringe containing the feed sample and then incubated in a water bath (Ehret brand) at 39°C for 48 hours.

The method used for *in vitro* gas production was based on the techniques described by Makkar *et al.* [10]. Gas production was recorded at 0, 2, 4, 8, 12, 24- and 48-hours post incubation using a syringe equipped with a needle. Shaken was performed every 1 hour after incubation. The rate and production of gas are determined for each treatment according to the modified model of the equation of Ørskov and McDonald [11] as follows:

$$Y = b(1 - e^{-ct})$$

Where:

- Y : the volume of gas production at the time "t"
- b : potential gas production (ml/g DM) of insoluble fractions
- c : constant gas production rate (h⁻¹) for insoluble fractions (b)
- t : incubation time

After 48 hours incubation, the syringe was removed and cooled in a container of cold water for 30 minutes. The contents of the syringe were removed and put in a fermenter tube. The pH values were measured using a digital pH meter (Schott-Handylab 1). Sub samples of mixed rumen buffer

Table 1. Feed ingredients and chemical composition of experimental diets

Chemical composition*	Treatments								
	R ₀	R ₁	R ₂	R ₃	R ₄				
Dry matter (%)	89.77	89.89	89.84	89.88	90.06				
Organic matter (%)	87.89	87.86	88.19	88.47	88.5				
Crude protein (%)	12.14	12.13	12.25	12.38	11.72				
Crude fiber (%)	27.9	28.32	28.64	31.21	34.54				
<i>A. leucophloea</i> leaves	Total tannin**	Condensed tannin**	Total phenol**	NDF	ADF	Cellulose	Hemi-cellulose	Lignin	Silica
	0.97	0.49	3.52	46.8	34.8	23.55	11.98	9.83	0.45

Note:

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R₀ = 40% concentrate: 0% leaves *A. leucophloea*, R₁ = 30% concentrate: 10% leaves *A. leucophloea*; R₂ = 20% concentrate: 20% leaves *A. leucophloea*; R₃ = 30% concentrate: 10% leaves *A. leucophloea* and R₄ = 40% leaves *A. leucophloea*: 0% concentrate.

(0.6 ml) were added 3 ml of methaphosphoric acid solution, then centrifuged at 9000 rpm for 15 minutes, and the supernatant was stored at -20°C before VFA analysis by using gas chromatography. The amount of 1 ml of sub-sample was prepared and analysed for NH₃ concentration by using the Conway method [12]. Samples were removed from the syringe and filtered using Whatman paper 41 Φ 0.11 mm which was previously heated in a 105°C oven for 12 hours, then cooled in an exicator and weighed. The dish containing feed residue sample was mixed with a neutral detergent solution and then rinsed and dried. The DM digestibility and OM digestibility values were obtained by subtracting DM and OM residues from their initial DM and OM amounts before incubation, respectively.

Statistical analysis

Data obtained from this study were analysed using analysis of variance (ANOVA) to compare fermentation products (total NH₃, total VFA and partial VFA, DM digestibility and OM digestibility as well as in vitro gas production using the General Linear Model (GLM) of Statistics for Windows (1993). The difference between treatments was followed by Duncan test [13].

Results and Discussions

Feed chemical analysis

The nutrient content of the feed in each treat-

ment showed an iso-protein, where the crude protein of each treatment was compiled to meet CP 12%. Increasing levels of *A. leucophloea* leaves in feed increased the organic matter and crude fiber content but tends to decrease the crude protein content. The proximate analysis results and Van Soest of *A. leucophloea* leaves are presented in Table 1. Total tannin, condensed tannin and total phenol were low compared to other legume plants. The content of condensed tannin (CT) in *Acacia tortilis* (22.52%), *Acacia galpinii* (22.72%), *Acacia sieberiana* (1.68%), and *Acacia hebeclada* (1.56%) [14]. The content of secondary compounds in each legume plant is varied.

Gas production

Increasing the level of *A. leucophloea* leaves in the treatments had significantly affect ($P < 0.05$) gas production values (Table 2). The highest gas production was achieved by treatment R₀ (198.29 ml) and the lowest one was in R₄ (139.93 ml). The potential yield of gas produced indicated that inclusion of *A. leucophloea* leaves in treated feed had higher feed quality than when feed without *A. leucophloea* leaves. Increasing the level of *A. leucophloea* leaves increased the crude fiber content as well as the tannin content indirectly which causes the components of organic matter being difficult to be digested and resulting in decreases of gas production. This is in line with findings of Jayanegara and Sofyan [15], that high amounts of

Table 2. Mean values of gas production (b), potential gas production rate of insoluble fractions (c), and gas production 48 h incubation (y) of *A. leucophloea* treatment

Variables	Treatments				
	R ₀	R ₁	R ₂	R ₃	R ₄
b: Gas production (ml/500 mg DM)	198.29±35.0 ^b	157.71±8.3 ^a	150.61±5.6 ^a	141.84±5.1 ^a	139.93±18.2 ^a
c: Gas production rate of insoluble fractions (ml/h)	0.035±0.003	0.034±0.004	0.036±0.005	0.036±0.004	0.028±0.007
y: gas production 48 h incubation (ml/500 mg DM)	153.38±6.3 ^b	119.05±0.93 ^a	117.82±5.40 ^a	105.82±2.87 ^a	103.23±5.03 ^a

Note:

abc = values within a row with different superscripts differ: $P < 0.05$

R₀ = 40% concentrate: 0% leaves *A. leucophloea*, R₁ = 30% concentrate: 10% leaves *A. leucophloea*; R₂ = 20% concentrate: 20% leaves *A. leucophloea*; R₃ = 30% concentrate: 10% leaves *A. leucophloea* and R₄ = 40% leaves *A. leucophloea*: 0% concentrate

tannin in plants can decrease the ability of rumen microbes to degrade carbohydrates and proteins and inhibited enzyme activity. Amnifard *et al.* [16], reported that the b value of soybean meal incubated in rumen fluid containing corn silage (control) was significantly higher than if incubated in rumen fluid containing *khinyuk* leaves (*Pistachio khinyuk*) by 30% (132.36 ml/300 mg DM vs 116.2 ml/300 mg DM).

A low value of b in R₄ compared to other treatments was due to high crude fiber content as well as high lignin content as reported by Zulkarnain *et al.* [17] that fiber components (cellulose and hemicellulose) affects the values of b in *in vitro* gas production.

Increasing the level of leaf *A. leucophloea* in the treatment had no effect on gas production rate of insoluble fraction (value c), but had increased ($P < 0.05$) gas production after 48 hours incubation (y value). Concentrate treatment without *A. leucophloea* (R₀) had higher gas production of 48 hours incubation than the treatments with the addition of *A. leucophloea* leaves (R₁, R₂, R₃, and R₄). The highest gas production at R₀ was 153.38 ml/500 mg DM and the lowest was in treatment (103.23 ml/500 mg DM). The results shown in treatment R₄ reflect the characteristics of *A. leucophloea* leaves as a single concentrate feed. Makkar *et al.* (2007) indicated that the ability of tannin to interact with feed components, especially protein and crude fiber was largely contributing to the amount of gas production [18]. Sallam *et al.* [19], suggest

that high NDF, ADF and phenolic compounds can limit the *in vitro* fermentation. The relationship between 48 hours incubation and gas production indicated a positive correlation in which the longer the incubation period, the higher the gas production in each treatment level of *A. leucophloea* in feed, as shown in Figure 1.

NH₃, VFA, DM digestibility and OM digestibility Values

The average NH₃, total VFA and partial VFA values, DM digestibility and OM digestibility of feed at different levels of *A. leucophloea* leaves at the 48-hour incubation are listed in Table 3.

Inclusion of of *A. leucophloea* leaves in feed had significantly ($P < 0.05$) affect the NH₃ concentration. The concentration of NH₃ in R₀ was not different from R₂ but higher than R₁, R₃ and R₄. Treatment R₂ was not different from R₁ and R₄, and R₃ was not different from R₄. Inclusion of *A. leucophloea* leaves as a single supplement (R₄) resulted in decreased NH₃ concentration, which may due to lower crude protein content and tannin concentration in R₄ than the other treatments that affect the rumen fermentation. Feed fermentation in the rumen containing tannins resulted in lower NH₃ concentrations than those without tannins content [20, 21].

Feed concentrate with *A. leucophloea* leaves had decreased ($P < 0.01$) total VFA and partial VFA against the C₂/C₃ ratio. The highest total VFA concentration was found at R₀ (91.96 mM)

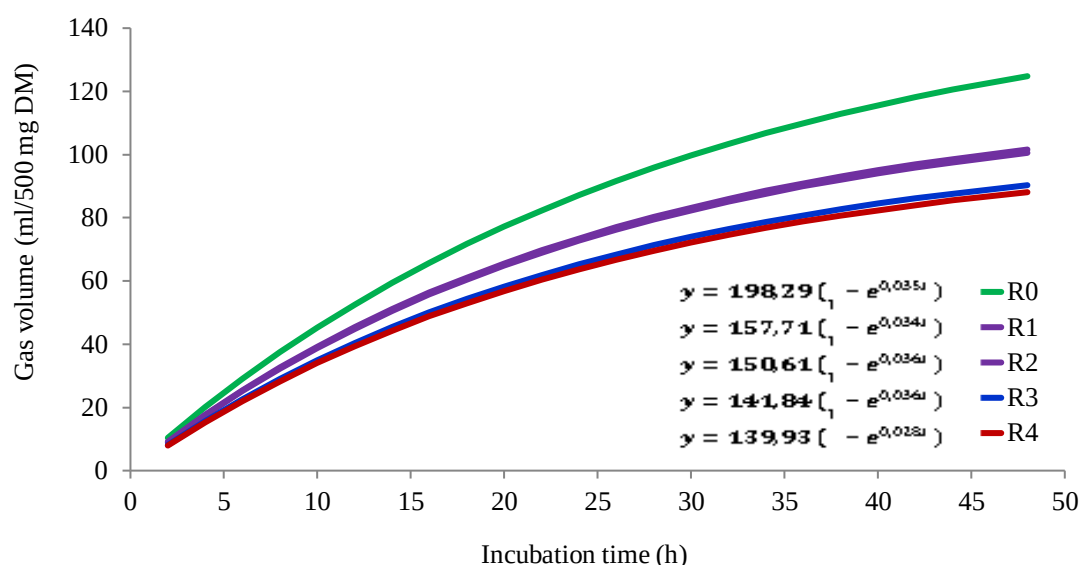


Figure 1. Effect of *A. leucophloea* to concentrate ratio on gas production at different time of incubation. Correlation of gas production and incubation time as follows: R0 (—) $y=198.29(1-e^{-0.035t})$; R1 (—) $y=157.71(1-e^{-0.034t})$; R2 (—) $y=150.61(1-e^{-0.036t})$; R3 (—) $y=141.84(1-e^{-0.036t})$; R4 (—) $y=139.93(1-e^{-0.028t})$

Table 3. Mean values of NH₃, VFA, IVDMD and IVOMD of different level of *A. leucophloea*

Variables	Treatments				
	R ₀	R ₁	R ₂	R ₃	R ₄
NH ₃ (mg/100 ml)	7.31±0.51 ^c	6.80±0.00 ^b	7.06±0.16 ^{bc}	6.17±0.22 ^a	6.29±0.17 ^{ab}
Total VFA (mM)	91.96±1.48 ^b	84.66±3.07 ^a	83.52±6.25 ^a	84.01±0.67 ^a	83.07±0.99 ^a
C2	65.48±0.72 ^b	59.59±0.67 ^b	55.08±6.71 ^a	59.45±0.93 ^{ab}	58.32±1.11 ^a
C3	18.48±0.78 ^a	17.64±1.60 ^a	21.04±0.90 ^b	18.13±0.28 ^a	18.71±0.18 ^a
C4	8.01±0.02 ^b	7.43±0.81 ^b	7.41±0.44 ^b	6.44±0.01 ^a	6.04±0.01 ^a
C2/C3	3.55±0.11 ^b	3.39±0.27 ^b	2.63±0.43 ^a	3.28±0.10 ^b	3.12±0.09 ^b
IVDMD	67.14±0.73 ^c	65.59±0.79 ^c	65.89±0.21 ^c	56.59±0.28 ^b	50.18±1.88 ^a
IVOMD	71.35±1.12 ^b	68.27±2.74 ^b	68.63±1.08 ^b	59.20±2.80 ^a	55.04±3.72 ^a

Note:

abc = values within a row with different superscripts differ: $P < 0.05$; VFA: volatile fatty acid; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility

R₀ = 40% concentrate: 0% leaves *A. leucophloea*, R₁ = 30% concentrate: 10% leaves *A. leucophloea*; R₂ = 20% concentrate: 20% leaves *A. leucophloea*; R₃ = 30% concentrate: 10% leaves *A. leucophloea* and R₄ = 40% leaves *A. leucophloea*: 0% concentrate

and the lowest at R₄ (83.07 mM) but the values obtained were still within the normal range of 70-150 mM [22]. The concentration of total VFA has a positive relationship to gas production where the higher the concentration of VFA the higher the production of gas produced. In the present study, the C₂/C₃ ratio were ranged from 2.63 - 3.55. The lowest C₂/C₃ ratio was in R₂ treatment (2.63) indicating that inclusion 20% of *A. leucophloea* leaves in concentrate was beneficial and effective to rumen microbial in producing energy and illus-

trating high efficiency of fatty acids utilization.

Inclusion of *A. leucophloea* leaves in concentrate had significantly decreased ($P < 0.01$) DM digestibility and OM digestibility. DM digestibility and OM digestibility treatments in R₀ were not different from R₁ and R₂ but were different from R₃ and R₄. These results indicated that digestibility of feed is largely influenced by the crude fiber content but not tannin content. Olivares-Perez *et al.* [23] reported that the ADF content, total phenol and CT had negatively correlated with forage di-

gestibility where the higher the content in the forage, the lower the nutrient digestibility.

Conclusion

A. leucophloea leaves utilization in concentrates as supplements reduces gas production and *in vitro* fermentation products. Inclusion of *A. leucophloea* leaves at 20% in a mixture of concentrates and natural grasses resulted in more efficient gas production and fermentation products

Acknowledgement

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