

Digestibility and Ruminal Fermentation Characteristic of Native Grass Silage Supplemented with Different Levels of *Leucaena leucocephala*

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ABSTRACT

This study was conducted to determine the digestibility of native grass silage supplemented with different levels of *Leucaena leucocephala*. The study was designed using completely randomized design with 3 levels supplementation of *Leucaena* (10, 20, and 30%) and each treatment was done in 6 replications. All of the silage treatments were inoculated with 4% *Lactobacillus plantarum*, and stored in mini-silos for 21 days. The results showed that supplementing native grass with 20% *Leucaena* resulted in the greatest ($P < 0.05$) dry matter and organic matter digestibilities. However, the greatest digestibility of crude protein obtained at 10% *Leucaena* supplementation and that digestibility decreased linearly with the increasing levels of supplementation ($P < 0.05$). Although there were no effects was detected due to *Leucaena* supplementation on culture rumen pH and VFA concentration, a significant decrease of $\text{NH}_3\text{-N}$ concentration and significant increase of rumen microbial protein were noticed ($P < 0.05$) as the level of *Leucaena* supplementation was increased. These data indicate that supplementing native grass with 30% *Leucaena* is the best treatment in term of microbial protein yield, although nutrient digestibilities were lower than the other treatments.

Keywords: Native grass, *Leucaena leucocephala*, Supplementation, *Lactobacillus plantarum*, silage.

INTRODUCTION

Forage is one of the most important factors in supporting the productivity of ruminant livestock. Adequacy of feed is a relatively common problem in developing countries including Indonesia. The feedstuff crisis associated with protein content is also a serious problem, especially in areas where long dry seasons. Native grass has a low quality especially indicated by low protein content; Especially insufficient to meet ruminants' needs during the growth and fattening period of 13% (NRC, 1996). This condition requires an appropriate solution so that the inequality of nutrient availability and adequacy can be overcome by the preservation process through silage process and supplementation efforts with high protein containing materials; One of which is supplementation with *Leucaena leucocephala*.

Leucaena leucocephala plays an important role as feed ruminants and is known as the provider of the cheapest protein. The use of *Leucaena* in making native grass silage is expected to enlarge the effect of a supplementary effect either from nutrient quality or from the quality of silage digestibility in the rumen. Souza et al. (2014) showed the feeding of silage with the addition of legumes in the beef cattle yielded the same performance and digestibility compared with the maize silage. This study aims to determine the best level of *Leucaena* supplementation in making native grass silage as ruminant feed in terms of digestibility and rumen fermentation parameters *in vitro*.

MATERIALS AND METHODS

Materials

The material of this research is in the form of Native grass and *Leucaena* which were taken from PIAT-UGM area. The timing of the native grass cutting used an approached by observing the vegetative phase (before/close to flowering). Inoculant *L. plantarum* uses BINOSIL-01 commercial products produced by the Biotechnology Research Center-LIPI Indonesia. The silage process was conducted on a laboratory scale using glass jars as the mini-silos.

Methods

About 700 g of the mixed native grass and *Leucaena* according to the treatment level (10, 20, and 30%) have been carried out until the water content of about 65%, besides, the cut size is about 3 – 6 cm were used as a silage making material. The treatments were as follows: LLS10 = Native grass + *Leucaena* 10%, LLS20 = Native grass + *Leucaena* 20%, LLS30 = Native grass + *Leucaena leucocephala* 30%. The whole silage treatments were given 4% of Inoculant *L. plantarum*, and 5% of molasses (v/w) (Yusuf, 2009). After that, the silages were fermented for 21 days.

After harvesting (21 days), a sample of silage was taken about 200 g for drying at 55^oC oven and grounded using Wiley mill with 1 mm sieve size and analyzed its chemical composition following (AOAC, 2005) (presented in Table 1). A subgroup of the sample about 0.5 g was weighed for testing of the digestibility and the fermentation *in vitro*.

The digestibility values of the dry matter, organic matter, protein, and the digestibility of crude fiber were determined based on the *in vitro* methods. Rumen fluid as inoculum was obtained from two Bali cows fistulated with fed *Pennisetum purpureoides* and concentrate (with a proportion 60:40). The silage digestibility analysis was performed using stage I of the 2-stage *in vitro* method procedure as described in Tilley and Terry, (1963) was done in 6 replications for each nutrient digestibility test. To determine the NH₃ rumen following Chaney and Marbach (1962), VFA following Filípek dan Dvořák (2009), and to determine the Microbial proteins synthesis following the procedure by Plumer (1987).

Data Analysis

The data obtained were tabulated and analyzed by a one-way ANOVA for all parameters in the digestibility and ruminal characteristic fermentation. To reveal the difference among parameters, Duncan's multiple range test was applied as presented by Steel and Torrie (1993).

Table 1. Chemical composition of native grass silage supplemented with different levels of *Leucaena leucocephala*

Chemical composition (% DM)	Treatment		
	LS10	LS20	LS30
DM	22.94	23.33	23.81
OM	86.44	87.29	87.88
CP	12.29	13.82	15.09
CF	25.40	24.76	24.19
EE	2.38	2.10	2.06
ETN*	46.38	46.61	46.53
TDN*	51.79	52.28	52.61

LS10 = native grass + *Leucaena* 10%, LS20 = Native grass + *Leucaena* 20%, LS30 = Native grass + *Leucaena* 30%.

DM = dry matter, OM = organic matter, CP = crude protein, CF = crude fiber. * was calculation according to Harris *et al.* (1973) *cit.* Hartadi *et al.* (2005).

RESULT AND DISCUSSION

Ruminal digestibility of native grass silage

The *In vitro* dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD) are shown in Table 2.

Table 2. Digestibility of *native grass* silage supplemented with different levels of *Leucaena leucocephala*

Variable	Level of <i>Leucaena leucocephala</i> supplementation			P-value
	LLS10	LLS20	LLS30	
IVDMD	49.51 ^a ±1.11	52.24 ^b ±1.17	48.72 ^a ±1,26	0.000
IVOMD	47.43 ^{ab} ±1.73	49.06 ^b ±1.63	46.22 ^a ±1,96	0.045
IVCPD	35.04 ^c ±0.73	30.70 ^b ±0.73	22.94 ^a ±0,81	0.000
IVCFD	56.14±1.85	53.74±2.51	57.29±1.95	0.194

LLS10 = native grass + *Leucaena* 10%, LLS20 = native grass + *Leucaena* 20%, LLS30 = native grass + *Leucaena* 30%.

IVDMD= *in vitro* dry matter digestibility, IVOMD= *in vitro* organic matter digestibility, IVCPD= *in vitro* crude protein digestibility, IVCFD= *in vitro* crude fiber digestibility. Means in the same row with different superscripts differ significantly (P<0,05).

Table 2 shows that elevated levels of *Leucaena* supplementation from 10% (LLS10) to 20% (LLS20) showed a significant increase in DMD (49.51% vs. 52.24%; P<0.05); however, DMD values were seen Decreased at 30% supplementation level (LLS30) (48.72%). The increasing DMD in the treatment of LLS20 compared with the treatment of LLS10 was due to an increase in DM content and a decrease of CF content in silage treatment (Table 1). Increased DM content indirectly increases the portion of digestible nutrients, as Barros-Rodríguez *et al.* (2015) reported that the addition of 20 – 40% significantly increased the digestibility of NDF under *In sacco* conditions., and the decrease in CF content directly decreases the portion of the digestibility factor. Qu *et al.* (2013) suggest higher CP content and lower NDF content in legumes compared to grass may have an effect on digestion. Furthermore, with higher CP content and lower NDF and ADF concentrations of mixed corn and lablab silage increased significantly higher DM digestibility than single corn silage. In contrast to the treatment LLS30 which produces a low DMD, it was thought to be caused by a decrease accumulation of tannin contained by silage treatment as a consequence of increased levels of supplementation. The higher tannin content the feed degradation is lower. Tan *et al.* (2011) demonstrated in *in vitro* testing increased levels of condensed tannins on *Leucaena* from level 10 – 30 mg / 500 mg DM linearly decrease the DMD *Leucaena*.

Organic matter digestibility of silage treatments showed a similar pattern with a dry matter digestibility. The highest OMD was obtained at LLS20 treatment and showed a significant difference with LLS30 treatment (49.06 vs. 46.22; P <0.05), whereas LLS10 treatment did not show any different response with LLS20 treatment nor with LLS30 treatment resulting in OMD of 47.43%. The higher and different OMD treatment of LLS20 compared to the LLS30 treatment is closely related to the high DMD in the silage treatment. High dry matter digestibility can be sure to produce high OMD. Raharjo *et al.* (2013) suggest OM degradation is closely related to DM degradation since most DM is composed by OM. However, the LS10 treatment did not show any difference with other treatments. As discussed earlier, allegedly it is also associated with the degradation activity restrictions by tannin compounds contained in *Leucaena*.

Leucaena supplementation significantly affected of CPD silage treatment. The treatment of LLS10 yielded the highest CPD and was linearly decreased in the treatment of LLS20 and LLS30 with DCP of 32.64, 28.40, and 21.20%; respectively, $P < 0.05$. The decrease in CPD in conjunction with a confirmed increase in supplementation level at rumen fluid NH_3 concentrations in the study (Table 3), this condition further reinforces the assumption of protective activity of proteins and other nutrient components by tannin compounds derived from *Leucaena*. The results obtained in this study consist with Tan *et al.* (2011) who reported that elevated tannin levels from 10 to 30 mg / 500 mg of DM produced undigested proteins were increasing. Niderkorn *et al.* (2012) also showed that the addition of sainfoin as much as 25 – 100% without the addition of polyethylene glycol (PEG) significantly decreased protein digestibility. However, CF digestibility of native grass silage was not affected by *Leucaena* supplementation.

Ruminal fermentability of native grass silage

Ruminal fermentability characteristics of native grass silage are shown in Table 3.

Table 3. Ruminal fermentability characteristics of native grass silage supplemented with different levels of *Leucaena leucocephala*

Variable	Level of <i>Leucaena leucocephala</i> supplementation			P-value
	LLS10	LLS20	LLS30	
Culture Ph	7.33±0.29	7.32±0.26	7.33±0.21	0.821
Total VFA, <i>Mm</i>	52.49±2.22	54.97±1.00	52.91±2.16	0.296
Individual VFA, <i>Mm</i>				
Acetate (A)	29.48±1.04	31.30±1.27	30.50±1.49	0.292
Propionate (P)	17.35±1.17	17.84±1.08	16.32±0.83	0.485
Butyrate	5.67±0.47	5.83±0.20	5.65±0.41	0.826
A:P Ratio	1.70±0.12	1.76±0.17	1.82±0.12	0.605
$\text{NH}_3\text{-N}$, ml/100 mL	25.59 ^c ±1.20	17.19 ^b ±1.03	13.47 ^a ±1.08	0.000
Microbial N, % DM microbial biomass	7.46 ^a ±0.79	8.27 ^a ±0.76	9.58 ^b ±0.97	0.002

LLS10 = native grass + *Leucaena* 10%, LLS20 = native grass + *Leucaena* 20%, LLS30 = native grass + *Leucaena* 30%. Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table 3 illustrated that the *Leucaena* supplementation has no effect on the pH culture, acetate, propionate, butyrate acid concentration, and acetate-propionate ratios (A:P ratio) ($P > 0.05$). Especially for the rumen pH obtained relatively higher than the rumen fluid pH is in the range of 5.5 to 6.9 (Kamra *et al.*, 2005; McDonald *et al.*, 2011). The high pH values obtained in the study thought to be caused by a variety of breeds are used as donor rumen fluid, in this study Bali cattle were used as donor rumen fluid. In this case, the Bali cattle may have a relatively higher pH of rumen among other breeds. Mudita *et al.* (2016) reported rumen pH of Bali cattle about 7.05 to 7.34 with biosupplement and a consortium of organic waste bacteria treatment. Choudhury *et al.* (2015) stated that pH value is one of the varied factors in the rumen. Such variations can be caused by a variety of types and breeds, feed, age and pre-treatment period of adaptation.

The rumen microflora generally has very high proteolytic properties, so that most of the proteins that enter the rumen will be degraded into peptides and amino acids, which in turn will be deaminated into NH_3 (Orskov, 1992). The increasing levels of *Leucaena* supplementation decreased the NH_3 concentration of rumen fluid ($P < 0.05$; Tabel 3). Different and linearly decreases

in NH₃ concentrations in line with increased levels of *Leucaena* supplementation was thought to be caused by; 1) some ammonia have been synthesized by the rumen microbes for cell protein synthesis; This was reinforced by the increased production of N microbial along with the increase of supplementation levels (Table 3), and 2) the limited activity of proteolytic bacteria degradation by tannin compounds as confirmed by the decreasing of CPD (Table 2). The assumption of a tannin protective activity was established although, in our study, there was no testing done on tannin content. However, this opinion supported by Galindo *et al.* (2009) that the proteins of *Leucaena* are relatively less degraded within the rumen due to the presence of total condensed tannins (1.80 - 4% DM). Other researchers also reporting similar results that the decreased NH₃ concentration are in line with the increased use of legumes in the feed (Min *et al.*, 2002; Toral *et al.*, 2016).

The microbial proteins have a very important role in fulfilling the protein needed by the ruminant. Approximately 70 - 90% protein is supplied from rumen microbial cells. The amino acids from the rumen microbial cells of about 80% can be absorbed in the intestinal; therefore, the production of microbial proteins relatively indispensable to support the fulfillment of ruminant proteins (Orskov, 1992). *Leucaena* supplementation has increased the production of rumen microbial protein (P<0,05). Besides, the treatment LLS30 produces the highest microbial protein of 9.58% DM microbial biomass compared with LLS20 and LLS10 treatment that produced the microbial protein of 8,27 and 7,46% DM microbial biomass, respectively (Table 3).

The results obtained in this study correspond to the results reported by Noviandi *et al.* (2014) showed an increase in the level of use of legumes in feed treatment produces improved microbial protein and significantly different (P<0.01). The increased microbial protein in this study allegedly occurred due to 2 possibilities; The first possibility was confirmed as a result of a decrease in the NH₃ concentration of rumen fluid, which means that NH₃ was used by rumen microbes. The second possibility was caused by the activity of tannin compounds contained by *Leucaena* in terms of inhibiting and/or affecting the microbes of the protozoal group thus causing a decrease in protozoan population in rumen fluid. In accordance with Hess *et al.* (2003) reported that the condensed tannins contained by legumes exhibit toxicity to methanogens, most methanogens symbiotic both *ecto* and *endo* with protozoa, the decline of protozoa may affect methanogen populations. Further Patra *et al.* (2012) states sometimes tannins also affect the efficiency of microbial protein synthesis.

CONCLUSIONS

It can be concluded that *Leucaena leucocephala* supplementation of 20% in making *native grass* silage improves nutrient digestibility. However, under the conditions in our study, supplementation of 30% produce the highest microbial N despite lower digestibility than the other treatments.

ACKNOWLEDGMENTS

Thanks to Lembaga Pengelola Dana Pendidikan (LPDP), the Ministry of Finance, The Republic of Indonesia as the main sponsor of this research, either in the financial support and the moral support.

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