

***In Vitro* Protein Digestibility and Fermentability of Mulberry (*Morus alba*)- Leucaena Foliage Mixed Feed**

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ABSTRAK

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Penelitian dilakukan untuk mengevaluasi pengaruh campuran hijauan murbei dengan leucaena terhadap kecernaan protein dan produksi VFA (asam lemak volatile) yang diukur secara *in vitro* produksi gas. Hijauan murbei dicampur dengan dua varietas leucaena (*Leucaena leucocephala* hibrida dan *Leucaena leucocephala* lokal) pada tiga aras (0, 25 dan 50%). Penelitian dilakukan menggunakan rancangan acak lengkap. Tepung hijauan murbei, leucaena dan campuran hijauan murbei-leucaena diinkubasi dalam gelas syringe selama 24 jam. Parameter yang diamati adalah produksi gas, kecernaan sejati *in vitro* bahan kering (IVTDMD), kecernaan N *in vitro* (IVND), dan produksi VFA. Hasil penelitian menunjukkan bahwa suplementasi hijauan leucaena pada hijauan murbei menurunkan produksi gas, kecernaan bahan organik, dan kecernaan protein dalam rumen buffer. Campuran hijauan murbei dengan leucaena hibrida pada rasio 50% menghasilkan kecernaan protein terendah dalam rumen buffer dibandingkan dengan perlakuan yang lain. Namun demikian terjadi peningkatan kecernaan protein dalam pepsin HCl yang merupakan indikator prakiraan kecernaan protein dalam usus. Total produksi gas terjadi penurunan pada campuran hijauan murbei leucaena yang diikuti dengan penurunan produksi total VFA. Suplementasi leucaena hibrida pada murbei dengan rasio 1 : 1 paling efektif untuk menurunkan kecernaan protein ruminal dan meningkatkan kecernaan protein yang diinkubasi pada pepsin HCl. Dapat disimpulkan bahwa pencampuran leucaena hybrid mampu memproteksi degradasi protein murbei di dalam rumen.

Kata Kunci: Murbei, Leucaena, Kecernaan Protein, *In Vitro*

ABSTRACT

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This experiment was carried out to determine the effect of mulberry-leucaena foliage mixed feed on protein digestibility and VFA production using an *in vitro* gas production study. Mulberry was mixed with one of 2 leucaena varieties (*Leucaena leucocephala* hybrid and *Leucaena leucocephala* local) at 3 levels (0, 25 and 50%). Study was conducted in completely randomized design. Mulberry foliage, leucaena and mixtures of mulberry-leucaena were incubated for 24 hours in glass syringes. Parameter recorded were gas production, *in vitro* true dry matter digestibility (IVTDMD), *in vitro* N digestibility (IVND) and VFA production. Results of the study showed that supplementation of leucaena to mulberry decreased IVOMD, gas production and protein digestibility in the rumen buffered medium. The mixture of mulberry and leucaena hybrid at ratio 50% resulted in the lowest IVND than other treatment. However it increased protein digestibility in acid pepsin incubation as an estimate of protein availability in intestine. Gas production decreased in mulberry leucaena mixtures was followed by decreased total VFA production. Tannin derived from leucaena hybrid supplementation to mulberry at ratio 1 : 1 was most effective to decrease protein digestion in the rumen and to increase protein digestibility in acid pepsin incubation. In conclusion mixing of hybrid leucaena hybrid with mulberry foliage was able to protect protein degradation from mulberry in the rumen.

Key Words: Mulberry, Leucaena, Protein Digestibility, *In Vitro*

INTRODUCTION

The key of successful and sustainable ruminant farming in tropical conditions is dependent on the attempt to formulate reliable cheap feeding strategy based on resource availability. This is due to the scarcity and fluctuation in quantity and quality of year round supply of conventional feeds. The situation is

exacerbated by the increase human population and decrease land availability for forage crop production that cause ruminants feeding depends on crop residue and agricultural by products with "low nutritional quality". One of the methods to maximize the utilization of fibrous agricultural residues is by supplementing essential nutrients in the basal feed to correct the nutrients imbalances. Several

supplementation strategies have been developed using commercial concentrate, this supplementation could improve crop residues digestibility, intake and animal performance. However, although the above method is effective, the using of concentrate by small farmers is limited by cost particularly when it is imported. The high cost of imported concentrate led to seeking for alternative locally available forages for ruminants feed. This effort has practical implication because it is easy to adopt by small scale farmers. Tree foliages, shrubs and agro-industrial by-products are important in animal production in the tropics because they do not compete with human food and can provide significant protein supplements (Makkar 2003). Recently, there is increasing interest on the use Mulberry as ruminants feed due to its biomass production potency, palatability and nutritive value. Supplementation of pelleted mulberry leaves was able to improve rumen fermentation and nutrient digestibility of cattle fed on rice straw basal diet (Huyen et al. 2012), recently Yulistiani et al. (2015) also reported that in urea treated rice straw basal diet, mulberry supplementation can replace urea and rice bran as energy and protein sources. However, protein degradability of mulberry was very high. After 24 hours incubation, the protein of mulberry was degraded more than 80% in the rumen regardless of dietary treatments of the sheep (Yulistiani et al. 2008). Gemeda & Hassen (2015) also reported that, *in vitro* incubation of mulberry (*Morus alba*) produced highest NH₃N among the tropical browse plants from South Africa, indicating that protein in mulberry was highly degradable in the rumen resulting in the loss of valuable essential amino acid sources for host animals.

There are many methods to reduce protein feed degradability in the rumen Saddul et al. (2004) reported that heating of mulberry foliage could reduce its protein degradability. Compared to other treatments, Protein protection using tannin is better and give positive response, Getachew et al. (2008) reported addition of quebraco tannin or tannin acid ranges from 5-15% reduced *in vitro* rumen degradability of protein in alfalfa forage.

Leucaena, one of leguminous trees can be used as tannin source to protect protein degradability of soy bean meal (Cortés et al. 2009). Hybrid *Leucaena* developed in Malaysia has been reported to have high content of condensed tannin (13%) (Khamseekhiew 2006) and moderate amount (2.0-4.5% DM) of condensed tannin has a beneficial effect on protein metabolism in ruminants and give better nutritional value of tree fodder species (Camacho et al. 2010). Condensed tannin decreased rumen degradation of dietary protein and increased absorption of amino acids in the small intestine (Barry & McNabb 1999). The mixture of mulberry, *Leucaena* and *Tectona grandis*

leaves could result in increase rumen escape protein (Anbarasu et al. 2004), which can be utilized by the host animals. The objectives of this study were to evaluate the digestibility and protein degradability in mixture of *Leucaena* and mulberry by *in vitro* gas production technique and to determine the best ratio of *Leucaena* to mulberry in reducing protein degradation in the rumen.

MATERIALS AND METHODS

Feed

Mulberry foliage

Mulberry grown at the experimental plot of the Department of Animal Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, was harvested after about 5-7 weeks re-growth. Foliage was air-dried under shed for 3 days, chopped then ground using hammer mill and passed through a 1mm sieve.

Leucaena foliage

The foliage was harvested at 8 weeks of re-growth (from previous cutting), oven-dried at 45°C for 2 days, chopped, ground using hammer mill and passed through a 1mm sieve. *Leucaena leucocephala* local variety and *Leucaena leucocephala* hybrid variety contained condensed tannin (CT) 9.6 and 13.1% respectively (Khamseekhiew 2006), while mulberry foliage contained CT 0.16% (Saddul 2005).

Preparation of diets

Mulberry foliage was mixed with each of two varieties of *Leucaena* (hybrid or local) at 2 levels: 25% and 50% on DM basis as shown in Table 1 and the nutrient composition of the diets presented in Table 2.

In vitro OM digestibility

A 24 hours gas production test was carried out to determine truly degradable fermented substrates (*in vitro* true dry matter degradability/IVTDMD). In this incubation, 500 mg sample was incubated in 40 ml of medium. The medium was prepared according to (Makkar et al. 1997). Each diets treatment was incubated in 4 syringes as replication. Gas volume was recorded at 2, 4, 6, 8, 10 and 24 h incubation. After terminating the incubation, five ml of supernatant from each syringes was taken for volatile fatty acid (VFA) analysis, prior the residue in the syringe was transferred into a 600 ml spoutless beaker. The syringe was washed with a total of 70 ml of NDS solution. The procedure of

Table 1. List of experimental diets

Type of diet	Ratio (%)			Calculated CT content of mixed feed (%)
	Mulberry	<i>Leucaena</i> hybrid	<i>Leucaena</i> local	
Mulberry (M)	100	0	0	0.16
<i>Leucaena leucocephala</i> hybrid (LH)	0	100	0	9.60
<i>Leucaena leucocephala</i> local (LL)	0	0	100	13.10
M/LH (1 : 1)	50	50	0	6.45
M/LL (1 : 1)	50	0	50	4.81
M/LH (3 : 1)	75	25	0	3.13
M/LL (3 : 1)	75	0	25	2.46

LL: *Leucaena leucocephala* local variety; LH: *Leucaena leucocephala* hybrid variety; M: Mulberry

Table 2. Nutrient composition of experimental feeds

Feed ingredients	Chemical composition (% DM)				
	DM	OM	CP	NDF	ADF
Mulberry	92.2	91.7	18.9	41.5	25.4
<i>Leucaena leucocephala</i> hybrid (LH)	93.6	95.7	21.5	45.1	28.3
<i>Leucaena leucocephala</i> local (LL)	93.0	95.1	21.3	44.5	29.0
M/LH (1 : 1)	92.3	93.7	20.2	43.3	26.8
M/LL (1 : 1)	92.6	93.4	20.1	43.0	27.2
M/LH (3 : 1)	93.1	74.1	25.4	38.2	19.0
M/LL (3 : 1)	92.4	92.5	19.5	42.2	26.3

M/LH (1 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%

M/LH (3 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%

M/LL (1 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%

M/LL (3 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%

(Van Soest et al. 1991) was then applied by refluxing the incubation residue for 1.0 h and filtering the undigested matter on pre-tared filter crucibles. True digested of dry matter was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment. Rumen fluid was obtained from rumen-cannulated cattle maintained on roughage concentrate diet at the ratio of 60 : 40 DM.

Determination of *in vitro* N digestibility

Determination of *in vitro* N digestibility (IVND) was conducted on the samples using a modified Tilley & Terry (1963) two-stage digestion method (Palmer & Jones 2000). 0.5 g DM of each sample was weighed into calibrated glass syringes, followed by addition of 40 ml of rumen fluid: buffer (1 : 4). There were 2 sets of 4 replicates of each treatment together with four

blanks in separate tubes that received no sample were incubated. The syringes were incubated in water bath at 39°C for 24 h. Then the content of the syringes was centrifuged for 20 min at 2500 g. Supernatant of 3 ml was taken for VFA analysis. The remaining supernatant was decanted and 40 ml of distilled water was added. The suspension was thoroughly mixed using a vortex mixer and then centrifuged again for 20 min, the supernatant again discarded, and the process repeated 3 times. Each tube from one set of 4 replicates then added with 40 ml of acid pepsin (2 g of 1 : 10,000 pepsin in 1 l of 0.1 M HCl), thoroughly mixed then incubated at 39°C for 24 h. After incubation, the mixture was centrifuged for 20 min, the supernatant discarded, and the residue dried at 65°C for 48 h prior to weighing. Another one set of 4 replicates after being centrifuged was oven dried at 65°C for 48 h prior to weighing. The calculation of IVND in the rumen medium (X)

$$X = \frac{A - (B-D)}{A} \times 100$$

Whereas calculation of total IVND (Y) was:

$$Y = \frac{A - (C-D)}{A} \times 100$$

A = N in sample before incubated

B = N in residue after samples incubated in the rumen buffered medium

C = N residue after samples incubated in acid pepsin solution

D = N residue of the blank after incubation

$$P = \frac{R}{A} \times 100$$

Where:

P = The IVND in the acid pepsin

R = total IVND (X) - IVND in the rumen (Y)

The predicted N digestibility was also calculated from the incubation of individual feeds which then extrapolated, this calculation was done to see the synergistic effect of mulberry and leucaena mixture.

Chemical analyses

The feed samples were analyzed for DM, OM and CP according to the procedures of AOAC (2000). ADF and NDF were determined using the method of Van Soest et al. (1991).

The volatile fatty acid (VFA) and molar proportions of acetic, propionic and butyric acids of supernatant from incubated samples were determined by gas chromatography (Model G1540N, Agilent Technologies, USA) fitted with a flame ionization detector (FID) and a packed column 5% Thermon-3000, Shincarbon A 60/80. Nitrogen was used as the carrier gas at 40 ml/min and the oven temperature was maintained at 220°C. Injection and FID temperature were fixed at 260°C.

Statistical analysis

The experiment was carried out in a completely randomized design and data were analysed using Anova of SAS package version 9.1 (2002). Means were compared by Duncan's multiple range test.

RESULT AND DISCUSSION

Result

The effect of *Leucaena* mixed with mulberry on gas production, IVDMD and amount of substrate fermented

is shown in Table 3. Gas production and true organic matter digestibility were significantly ($P < 0.05$) lower in mulberry mixed with *Leucaena* than mulberry alone. However, the the amount drry matter being fermented was not significantly decreased when mulberry was mixed with hybrid *Leucaena* at 50% level. Hybrid *Leucaena* had significantly ($P < 0.05$) higher gas production, digestibility and amount of substrate fermented than the local variety of *Leucaena*.

Table 4 shows the effect of mulberry mixed with *Leucaena* on protein digestibility in rumen buffer media, acid pepsin solution and total protein digestibility. Protein degradation in the buffered rumen media was significantly decreased in mixture of mulberry and *Leucaena* compared to mulberry alone. The decrease of protein degradation was highest in mulberry mixed with 50% hybrid *Leucaena* M/LH (1 : 1). The decrease of protein degradation in rumen buffered media caused the increased protein degradation in the acid pepsin incubation. The M/LH (1 : 1) mixture had the highest protein digestion in acid pepsin. However, other mixtures, their protein digestion was significantly lower M/LH (3 : 1) and M/LL (3 : 1) or similar M/LL (1 : 1) to *Leucaena*. Total protein digestibility was significantly ($P < 0.05$) decreased in mulberry mixed *Leucaena* at all supplementation levels. The total protein digestibility of M/LH (3 : 1) and M/LL (3 : 1) was comparable to both *Leucaena* hybrid and local. The protein digestibility of M/LH (1 : 1) and M/LL (1 : 1) were comparable but they were higher than M/LH (3 : 1) and M/LL (3 : 1) mixture.

Figure 1 shows the measured and predicted value of protein digestibility in the rumen from different mixture of mulberry and *Leucaena* at different level. The predicted values were calculated from the incubation of individual feeds which then extrapolated. It shows that the measured value in protein degradation of mulberry and *Leucaena* mixture in buffered rumen media was lower than the predicted value. The highest difference between measured and predicted protein degradation was observed at M/LH (3 : 1) (42%).

Figure 2 shows the measured and predicted values of protein digestibility in acid pepsin from the mixture of mulberry and *Leucaena*. It shows that the measured of protein digestibility was higher than the predicted values. The highest difference between measured and predicted values was in the M/LH (3 : 1) (65%).

The effect of *Leucaena* mixed with mulberry on VFA production is shown in Table 5. The mixture of mulberry and *Leucaena* significantly decreased total VFA compared to mulberry alone. However, the total VFA production was not significantly different between the mixture with different *Leucaena* varieties and ratios. Total VFA production of all mixtures was not significantly different to *Leucaena* variety alone except for M/LL (1 : 1) mixture.

There were no significant differences in the proportion of butyric acid among treatments. The exception was in the M/LH (3 : 1) mixture which had significantly higher value than the hybrid *Leucaena*. Mulberry had significantly higher proportion of iso valeric acid than other diets except for M/LH (3 : 1).

However, the proportion of iso valeric acid of M/LH (3 : 1) was only significantly different to hybrid *Leucaena*. The proportion of Acetic acid, propionic acid and iso butyric acid was comparable ($P>0.05$) among treatments.

Table 3. Estimates of *in vitro* true dry matter digestibility (IVTDMD), fermentable substrate, volume gas production derived from 24 hours *in vitro* fermentation of 500 mg DM of the experimental diets

Treatment	Gas production (ml)	IVTDMD (%)	Substrate fermented (DM, mg)
Mulberry (M)	85.7 ^a	80.5 ^a	402.7 ^a
<i>Leucaena</i> Hybrid (LH)	59.5 ^e	75.8 ^b	378.9 ^b
<i>Leucaena</i> Local (LL)	55.8 ^f	67.3 ^c	336.4 ^c
M/LH (1 : 1)	74.3 ^c	77.5 ^{ab}	387.4 ^{ab}
M/LH (3 : 1)	67.6 ^d	68.8 ^c	344.0 ^c
M/LL (1 : 1)	76.9 ^b	73.7 ^b	368.3 ^b
M/LL (3 : 1)	75.6 ^{bc}	73.6 ^b	368.2 ^b
S.E.M.	0.78	1.46	0.23

Means with different superscript in the same column are significantly different ($P<0.05$)

S.E.M.: standard error means

M/LH (1 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%

M/LH (3 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%

M/LL (1 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%

M/LL (3 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%

Table 4. Estimates apparent protein digestibility of mulberry, *Leucaena* and mixed mulberry and *Leucaena* after incubation for 24 hours in rumen buffer media followed by acid pepsin digestion

Treatment	Microbial protein digestion (%)	Acid pepsin digestion (%)	Total protein digestion
Mulberry (M)	83.7 ^a	7.6 ^d	91.3 ^a
<i>Leucaena</i> Hybrid (LH)	13.6 ^d	55.9 ^b	69.4 ^c
<i>Leucaena</i> Local (LL)	15.7 ^{cd}	53.6 ^b	69.4 ^c
M/LH (1 : 1)	13.6 ^d	60.2 ^a	73.8 ^b
M/LH (3 : 1)	18.8 ^{bc}	48.8 ^c	67.6 ^c
M/LL (1 : 1)	21.0 ^b	55.4 ^b	76.4 ^b
M/LL (3 : 1)	21.7 ^b	46.5 ^c	68.2 ^c
S.E.M. it is better to put std for each value.	1.37	0.86	0.93

Means with different superscript in the same column are significantly different ($P<0.05$)

S.E.M. = standard error means

M/LH (1 : 1) = mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%

M/LH (3 : 1) = mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%

M/LL (1 : 1) = mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%

M/LL (3 : 1) = mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%

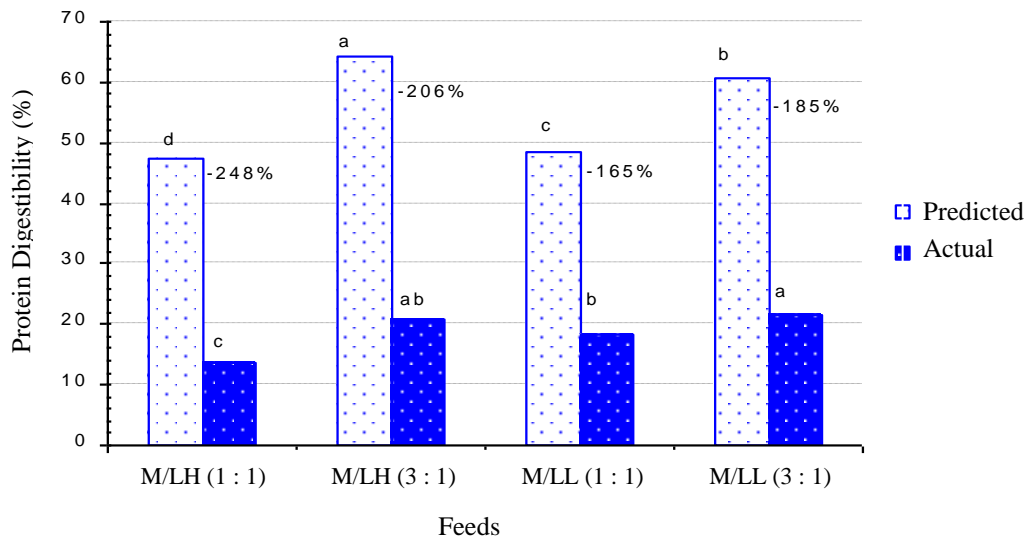


Figure 1. Protein degradability in rumen buffered media extrapolated from incubation of individual feed and measured by incubation of two feeds in different combination M/LH (1 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%; M/LH (3 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%; M/LL (1 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%; M/LL (3 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%).

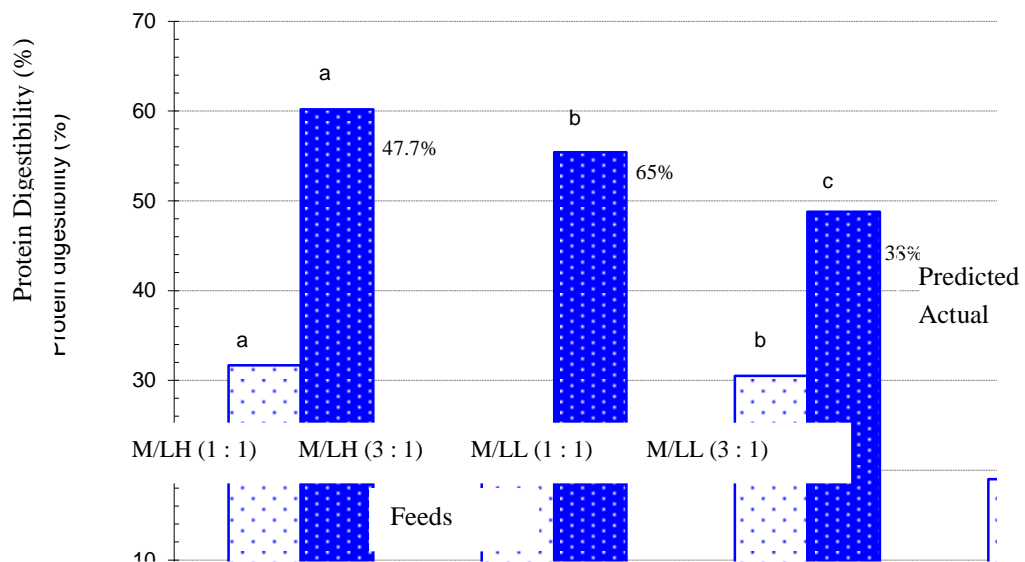


Figure 2. Protein digestibility in acid pepsin solution media extrapolated from incubation of individual feed and measured by incubation of two feeds in different combination M/LH (1 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%; M/LH (3 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%; M/LL (1 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%; M/LL (3 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%.

Discussion

In vitro digestibility

There is a good agreement between the magnitude of IVTOMD to the corresponding gas production. The high the IVTOMD value the higher is the gas production (Makkar 2003). However, in this study though gas production of mulberry was reduced when it was mixed with leucaena, but in mixture mulberry leucaena hybrid 50% (M/LH= 1 : 1) its IVTOMD was not significantly decreased (Table 3). Moreover, in *Leucaena* hybrid, though its gas production was significantly lower than other diets treatment, its IVTOMD was comparable to other diets except for mulberry. This indicates that the levels of tannin presented in the mixture diets did not limit the fermentability of the feeds. Getachew et al. (2000) and Guerrero et al. (2012) also reported that despite the lower gas production of tanniferous browses, its true digestibility was not affected by the presence of tannin. Similarly Norrapoke et al. (2014) also observed in rice straw diet supplemented by plant containing condensed tannin and saponin (mangosteen powder) did not reduce feed digestibility but decreased gas production. This condition was caused by the leaching of tannins from feed during fermentation, contributing to the DM loss but without contributing to the gas production or inhibition of cell soluble (Getachew et al. 1998; Makkar et al. 1997).

Further more, condensed tannin in the feed have more pronounced effect on protein degradability (Tiemann et al. 2008). However, Huang et al. (2010) reported that addition of various levels (2-5%) purified tannin from leucaena hybrid which was similar leucaena variety used in the current study to *Panicum maximum* grass substrate was able to reduce both *in vitro* DMD and total gas production, but the extent of reduction in gas production was higher than in digestibility. The gas decline by 19.68% when tannin was added at 2% and further decline to maximum 33.63% when the tannin addition was increased into 4% DM In this study though the levels of tannin addition (based on tannin content of leucaena) was higher (6.45% and 3.13% at leucaena hybrid mixture at 50% and 25% respectively the reduction of gas production was lower (13.3% and 21% respectively for mixture at 50 and 25%) the difference of gas production due to tannin addition in the current study with previous study reported by Huang et al. (2010) might be caused by the difference of tannin activity in extracted form (Huang et al. 2010) or in intact in the plant materials form such in the current study. Naumann et al. (2015) also reported that at different level of tannin content and different source of tannin in the diet did not affect total gas production. This indicated that effect of tannins on feed fermentation varied. Different tannin from different feedstuff have different inhibitory effect on rumen microbial activity which in turn affect on feed fermentation (Singh et al. 2005; Tiemann et al. 2008).

Table 5. Total VFA production and proportion of VFA of mulberry, *Leucaena* and their mixture after 24 h incubation

Treatment	Total VFA (mM)	Proportion of VFA (%)					
		C2	C3	C4	C5	C4i	C5i
Mulberry (M)	87.8 ^a	66.6	22.3	7.11 ^{ab}	1.23 ^d	1.04	1.92 ^a
<i>Leucaena</i> Hybrid (LH)	48.6 ^c	67.0	22.7	6.24 ^b	1.34 ^{cd}	0.95	1.12 ^c
<i>Leucaena</i> Local (LL)	37.3 ^c	67.0	22.0	6.47 ^{ab}	2.20 ^a	0.90	1.39 ^{bc}
M/LH (1 : 1)	58.3 ^{bc}	67.5	21.3	7.14 ^{ab}	1.59 ^{bcd}	0.97	1.51 ^b
M/LH (3 : 1)	47.6 ^{bc}	65.6	22.1	7.64 ^a	1.75 ^{bc}	1.15	1.74 ^{ab}
M/LL (1 : 1)	66.6 ^b	66.6	21.7	7.23 ^{ab}	1.84 ^{ab}	1.03	1.55 ^b
M/LL (3 : 1)	51.0 ^{bc}	68.2	20.9	7.03 ^{ab}	1.49 ^{bcd}	0.93	1.40 ^{bc}
S.E.M.	6.76	2.11	0.66	0.44	0.15	0.1	0.13

Means with different superscript in the same column are significantly different (P<0.05)

S.E.M. = standard error means;

C2 = acetic acid

C3 = propionic acid

C4i = iso butyric acid

C4 = butyric acid

C5i = iso valeric acid

C5 = valeric acid

M/LH(1:1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 50 : 50%

M/LH (3 : 1): mulberry and *Leucaena leucocephala* hybrid mixture at ratio 75 : 25%

M/LL (1 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 50 : 50%

M/LL (3 : 1): mulberry and *Leucaena leucocephala* local mixture at ratio 75 : 25%

***In vitro* N digestibility**

The mixture of mulberry with 50% hybrid *Leucaena* resulted in the lowest IVND than other treatments. When the level of the *Leucaena* decreased, the N degradability was increased by 50%, indicating that the level of tannin content in *Leucaena* affect the N degradability in the rumen (Table 4). Huang et al. (2010) reported addition of 2% purified tannin from *Leucaea* hybrid reduce degradability by 17.65% of protein Panicum maximum grass, further increased of tannin addition up to 4% did not resulted in further decreased of the protein substrate. Anti nutritional effect of tannin is exerted through reduction of feed protein digestion and a depression of proteolytic enzyme activities (Jones et al. 1994). A reduction in crude protein degradability is the consequence of condensed tannin (CT) binding to dietary constituents and to bacteria. In addition, the CT can reduce attachment of bacteria to plant particle (McAllister et al. 1994). Getachew et al. (2000) reported that IVDN increased when PEG (tannin binding agent) was added to the tannin-rich browse. The increase of the extent of N digestibility by rumen microbial due to PEG addition indicates the amount of protein protected by tannin from microbial degradation. In the present study, the protein degradability of mulberry-*Leucaena* mixture was reduced compared to mulberry alone. The decrease of protein degradability could be due to the effect of tannin from *Leucaena*. Previously Williams et al. (2011) reported that total mixed ration of sainfoin hay based-diet contained 3.8% tannin, rumen ammonia nitrogen content of the diet was lower compared to alfalfa hay based-diet with low tannin content (0.2%), the lower rumen ammonia nitrogen as indication of protein protection by tannin in the rumen. The *in vitro* protein degradability in M/LH (1 : 1) mulberry-*Leucaena* mixture was lower than other mixtures (Table 3). It shows that hybrid *Leucaena* has higher effect in decreasing protein degradability than *Leucaena* local (M/LL). The *Leucaena* varieties used in this study was similar tree reported by Khamseekhiew (2006) and Huang et al. (2010). Khamseekhiew (2006) reported that hybrid *Leucaena* had higher CT content than *Leucaena* local, which caused the lower N degradability in the former. In addition, CT in hybrid *Leucaena* had stronger binding affinity than *Leucaena* local (Huang et al. 2010). The CT which have stronger affinity and have higher molecular weight have higher ptoein binding affinity than those with lower molecular weight (Saminathan et al. 2004). Similar results was also reported by (Kariuki & Norton 2008) that within *Leucaena* genus has different ability in protecting protein. Moreover (Cortés et al. 2009) also observed that effect of tannin in protecting protein beside affected by source of tannin also by ratio of tannin added.

The protein degradability of mulberry alone was higher, however when it was mixed with leucaena the protein degradability was reduced though the protein content of this mixture was higher than mulberry (Table 2). The measured protein degradability was lower than the predicted value, indicating that there was an associative effect between mulberry and leucaena when it was incubated together. According Getachew et al. (2005) combination of different feed ingredients incubated together with condition that one feed is not independent to other feed will results in associative effect which can be detected when the response of the combination is not linear. This associative effect caused by the presence of feed ingredients stimulate rumen fermentation and consequently its affect the digestibility of other feed ingredients. Through the positive associative effect, the present study shows that protein digestibility in acid pepsin was higher in the measured values than in the predicted value. Protein availability for digestion in the intestines (acid pepsin) increased from only 8% in mulberry to 82% in M/LH (1 : 1) mulberry-*Leucaena* mixture rate. Although the total protein digestibility decreased from 91.3% in mulberry to 73.8% in M/LH (1 : 1) or 76.4% in M/LL (1 : 1), the protein digestion was available for the absorption in the intestine. The acid pepsin digestible protein could be regarded as a potential rumen by pass protein. McSweeney et al. (1999) reported that in browse (Lucerne) containing no tannin, accumulation of NH₃-N has strong correlation with N degradation. On the other hand, in tannin containing shrub legumes had lower potential N digestibilities in the rumen. However, a large portion is available following the acid pepsin digestion compared to Lucerne, this was due to protein tannin complex would be dissociated post-ruminally. McLeod (1974) as referred by Norton & Ahn (1997) observed that tannin bind protein in the rumen at pH 5.8-6.8 and the linkages are strongly dependent on pH. The linkage being stable at pH 3.5-7.0, but this tannin-protein complexes should be dissociated in the abomasum (pH 2.5-3.5) and in small intestine (pH 7.5-8.5). Therefore protecting protein from digestion in the rumen is the advantage of the presence protein-tannin complex in the diet thereby increasing total supply of feed protein for absorption (Makkar 2003).

***VFA* production**

The higher total VFA production in mulberry than other diets (Table 5) was a results from the high IVTDMD of mulberry (Table 3) this due to VFA is one of the products beside gases and microbial cell from carbohydrates fermentation. Mixing leucaena to mulberry resulted in the decreased of total VFA production. The decrease of total VFA was also reflected in lower IVTDMD and gas production of

mulberry-leucaena mixed diets (Table 3). The decrease of fermentability these diets could be caused by the present of tannin from leucaena. Tannin decrease attachment of fibre digesting microbes to feed particles (McAllister et al. 1994) therefore decrease rate of digestion which in turn decreased VFA production. This is consistent with the previous study that showed the tannin content in tree browse reduced the gas and total VFA production (Getachew et al. 2002; (Khamseekhiew 2006). However, the molar proportion of acetic and propionic, and iso butyric acid was not affected by treatment. On the other hand, the molar proportion of iso-valeric in mulberry-leucaena mixed diet was significantly decreased compared to mulberry. The decreased of iso-valeric which mostly from deamination of some amino acid (Copani et al. 2015) in mixed mulberry leucaena suggest that protein of mulberry was protected by condensed tannin from leucaena. Similar result was also reported by Copani et al. (2015) in mixture of Timothy grass and Sainfonin, and by Niderkorn et al. (2012) in mixture of cocksfoot and sainfonin.

CONCLUSION

Supplementation of *Leucaena* to mulberry decreased *in vitro* true dry matter digestibility (IVDMD) and decreased protein digestibility in the buffered rumen fluid media. *Leucaena* supplementation could increase protein digestibility in acid pepsin incubation as indicator of protein availability in intestine. Hybrid *Leucaena* supplementation to mulberry at ratio 1 : 1 was the most effective level to decrease protein digestion in the rumen and to increase protein digestibility in acid pepsin incubation.

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