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*Original Article*

# *In vitro* ruminal and post-ruminal nutrients degradation due to varying proportions of *Leucaena leucocephala* added with corn oil in the ration

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**Abstract**

This study evaluated the effects of *Leucaena leucocephala* and corn oil supplemented to diet on *in vitro* ruminal and post-ruminal nutrient degradation using a two-stage *in vitro* technique. The four dietary treatments consisted of Pennisetum purpureum and concentrate in a 75:25 ratio with increasing levels of L. leucocephala (L0= 0%, L25= 25%, L50= 50%, and L75= 75%, DM basis) with three levels of corn oil (CO) (CO1= 0%, CO2= 1%, and CO3= 2% of the substrate) resulting in 12 treatment combinations that were randomly assigned to a 4×3 factorial design. The L75 and CO3 treatment decreased ruminal and total dry matter and organic matter degradation ($p<0.001$). Leucaena supplementation also increased ruminal and post ruminal protein degradation (rdN and pdN) while CO decreased the rdN and the pdN. To conclude, inclusion of Leucaena to the diet by 50% improved nutrients degradation, particularly ruminal and post-ruminal N degradation, while CO at 1% level negatively affected nutrient degradation in the rumen.

**Keywords**: legume supplementation, oil source, rumen fermentation, ruminal undegradable protein, tannins

## 1. Introduction

Protein deficiency experienced by ruminant animals in Indonesia cannot be avoided since low-quality roughage is the main source of feedstuff. Hence, supplementation of a high-protein resource such as Leucaena (*Leucaena leucocephala*) and Gliricidia sepium that can modulate rumen fermentation is advantageous (Barros-Rodríguez *et al.*, 2014; Yuliana, Laconi, Jayanegara, Achmadi, & Samsudin, 2019). Leucaena leaves have been considered effective legumes for improving feed quality of ruminants, due to a high protein concentration and degradability in the rumen (Ahmed, Jusoh, Alimon, Ebrahimi, & Samsudin, 2018). However, an excessive amount of Leucaena in the ration adversely impacts the ruminants because of nitrogen and secondary metabolite compound toxicity, especially of tannins (Ahmed *et al.*, 2018; Barros-Rodríguez *et al.*, 2014).

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The presence of tannins in Leucaena also contributes to altering metabolism that leads to further effects on ruminal and post-ruminal nutrient degradation. Tannin, particularly condensed tannin (CT), in most relevant studies has been shown to protect protein fraction and improve protein efficiency (Pathak, Dutta, Pattanaik, Chaturvedi, & Sharma, 2017). The binding activity of CT, however, depends not only on its concentration but also on its molecular weight and monomeric composition (Saminathan *et al.*. 2014), along with species and environmental conditions (Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000). In the same species of plant, the activity could also differ among varieties (Jackson, Barry, Lascano, & Palmer, 1996) and this might affect the use of nitrogen as a source of amino acids in the rumen and intestine.

Amino acid supply of ruminant animals derives jointly from rumen undegradable protein and microbial protein synthesized in the rumen. Feed solubility and the rate of passage of digesta determine the amount of protein that escapes from rumen degradation (Kang, Wanapat, Pakdee, Pilajun, & Cherdthong, 2012). Accordingly, a strategy to improve post-ruminal protein degradability has been

developed in order to minimize inefficiency in the use of nitrogen in the rumen by exploring the potential of tanninferous legumes. Several studies on various sources of tropical tannin-containing plants to increase post-ruminal protein digestibility that can be absorbed in the intestine (Cortés *et al.*, 2009). Knowles, Pabon, Hess, and Carulla (2017), however, have revealed contrary results as the tannins might have a negative effect in nutrient post ruminal degradation *in vitro*. To our knowledge, the evidence of binding activity of tannin-containing Leucaena prior to protein polymer is unclear.

Furthermore, the prime importance in optimizing the yield of end-products of rumen fermentation is the key strategy to develop protein-energy balance in the metabolism pathway. Fat supplementation is an important strategy to increase the density of energy in the diet (Patra, 2013) and improve ruminant performance (Wanapat, Mapato, Pilajun, & Toburan, 2011). Corn oil as vegetable oil has potential to improve the energy utilization in the rumen. However, excessive use of fat adversely affects microbial fermentation by interfering with the ruminal microbial ecosystem. Oil supplementation has also become a common strategy to improve the fatty acid profile in animal products (Castagnino *et al.*, 2015). Prior to corn oil addition, effect of corn oil in the diet on nutrient degradation was also tested. Therefore, this study was aimed to evaluate the interaction effects of levels of Leucaena and corn oil on in the substrate ruminal and post-ruminal nutrients degradation *in vitro*.

## 2. Materials and Methods

### 2.1 Sample preparation and experimental design

Forage sample consisted of *Leucaena leucocephala* leave and *Pennisetum purpureum* was randomly collected during the dry season from the research farm of Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta (7°46'07.3"S, 110°23'10.6"E) in the vegetative phase. Forages were cut to 3-5 cm in length and packed in bags, then immediately dried at 55°C for 72 h, then ground and sieved into 2 mm particle size for chemical analysis and *in vitro* assays. A 1 kg bottle of corn oil (PT. Tropicana Slim) was purchased from commercial market, Yogyakarta, Indonesia. The diet was formulated from forage and concentrate in ratio 75:25 (w/w). The forage (75%) consists of Leucaena foliage and Napier grass in four combinations by ratio. Experimental design was set in the following factors; one control experimental diet consists of Napier grass and concentrate without Leucaena, and then with increasing levels of Leucaena (25%, 50%, and 75%, respectively) each combined with three levels of corn oil (0%, 1%, and 2% of the substrate, respectively), resulting in 12 dietary treatments. Corn oil and ethanol solution (1: 10) was added to the tube according to Wu *et al.* (2015) to provide 1% and 2% of corn oil in the McDougall solution. The concentrate was formulated from mixed cassava flour, wheat pollard, and macro minerals formula. Table 1 summarizes the diet compositions and the design of experiments.

Table 1. Nutrient compositions of the dietary treatments

| Diet component (% DM) | Proportion of Leucaena (%) | | | |
|---|---|---|---|---|
| | L0 | L25 | L50 | L75 |
| *Pennisetum purpureum* | 75.0 | 50.0 | 25.0 | 0.0 |
| *Leucaena leucocephala* | 0.00 | 25.0 | 50.0 | 75.0 |
| Cassava bran | 10.0 | 10.0 | 10.0 | 10.0 |
| Wheat pollard | 13.0 | 13.0 | 13.0 | 13.0 |
| Mineral mix * | 2.00 | 2.00 | 2.00 | 2.00 |
| Total | 100 | 100 | 100 | 100 |
| Nutrient chemical composition (%) | | | | |
| Dry matter (DM) | 35.5 | 36.1 | 36.6 | 37.2 |
| Organic matter (OM) | 83.6 | 85.5 | 87.4 | 89.9 |
| Ash | 16.4 | 14.5 | 12.6 | 10.1 |
| Crude protein (CP) | 10.9 | 12.6 | 14.3 | 15.9 |
| Neutral Detergent Fiber (NDF)* | 54.02 | 46.9 | 39.9 | 32.8 |
| Acid Detergent Fiber (ADF)* | 31.3 | 27.5 | 23.6 | 19.8 |
| Non-Fibrous Carbohydrate (NFC) | 15.3 | 22.2 | 28.9 | 36.3 |
| Extract ether (EE) | 3.29 | 3.80 | 4.31 | 4.82 |
| Nitrogen-free extract | 43.4 | 46.7 | 49.9 | 54.8 |
| Total phenolic (TP) | 0.931 | 3.58 | 7.69 | 10.7 |
| Total tannins (TT) | 0.283 | 2.16 | 4.16 | 6.54 |
| Hydrolysable tannin (HT) | ND | 1.27 | 2.59 | 4.09 |
| Condensed tannin (CT) | ND | 0.811 | 1.64 | 2.33 |

* 1 kg of mineral mix comprised: 150 g calcium, 50 g phosphorus, 25 g magnesium, 5 mg cobalt, 250 mg copper, 10 mg selenium, 50 g Sulphur, 4 g zink

** Calculated from individual NDF and ADF for each feedstuff

L0= diet without Leucaena inclusion; L25= diet + 25% Leucaena; L50= diet + 50% Leucaena, L75= diet + 75% Leucaena (DM basis)

### 2.2 *In vitro* incubation procedure

The *in vitro* procedure of Tilley and Terry (1963) as modified by Knowles *et al.* (2017) involving two digestion phases was followed to determine DM, OM, and CP degradation. In the first stage, a 500 mg diet sample was incubated (in 5 replicates) under anaerobic conditions with rumen liquor and McDougall solution in the ratio of 1:4 for 48 h at 39°C. Blanks and standard hay were included in each incubation run. After 48 h of incubation, a set of tubes were filtered, and the substrate from this phase was used to estimate ruminal degradation for DM (rdDM), OM (rdOM), and N (rdN). Another five remaining tubes were further incubated for 48 h with HCl/ pepsin to estimate total *in vitro* degradation for DM (tdDM), OM (tdOM), and N (tdN). Post-ruminal nutrient degradation was estimated as difference between ruminal degradation and total degradation.

Ruminal fluid was obtained from two rumen-fistulated Bali cattle (*Bos indicus*) weighing 250-300 kg. The cattle were fed with fresh Napier grass and concentrate (70:30 w/w) at 3% DM of body weight (BW) per day. After 7 days of adaptation phase, rumen fluid was collected and filtered into a pre-warmed thermos before morning feeding, from various location within the rumen. The rumen fluid sample was immediately transported to the laboratory and was filtered through a four layer polyester material (PeCAP, pore size 355 µm; B & SH Thompson, Ville Mont-Royal, QC, Canada) to be used together with McDougall formula in the tubes.

### 2.3 Determination of in vitro ruminal and post-ruminal nutrient degradation

Ruminal and post-ruminal nutrient degradation were measured according to Knowles *et al.* (2017), a modification of the procedure of Tilley and Terry (1963), in which the dry matter, organic matter, and N degradation were calculated from initial and final weights of samples' DM, OM, and N, after correction using standard hay and blank sample. Briefly, after 48 h of incubation, the residue remaining in the bottle was filtrated through crucibles filter. The residue was dried at 105ºC until constant weight and the result was used for determination of ruminal DM and OM degradability. Furthermore, the difference between ruminal nutrient degradation and total nutrient degradation was taken to be the value of post-ruminal nutrient degradation. The apparent ruminal and total DM, OM, and N degradations are calculated from similar formulas, with the difference in the incubation times (48 and 96 h).

### 2.4 Laboratory analysis

The individual feedstuff and diet samples were analyzed according to Association of Official Analytical Chemists (AOAC, 1995) for DM (method 973.18), OM (method 942.05), and CP (method 984.13), while extract ether was using the method by Kamal (1997) as this method is recognized and commonly used in Indonesia. The NDF and ADF fractions were determined using the method described by Van Soest, Robertson, and Lewis (1991). The analysis was conducted in duplicate. The total phenolic compounds (TP), total tannins (TT), and condensed tannin (CT) of Leucaena leaves were measured according to Makkar (2003). Prior to nutrient degradability measurement, the residues from 48 h and 96 h incubation times, respectively, were filtered and oven-dried at 105°C for 12 h to determine the DM residue and further combusted at 550°C for 2 h to determine the OM residue. The CP contents of the residues were obtained from Kjeldahl analysis (Knowles *et al.* 2017).

### 2.5 Statistical analysis

Data were subjected to Analysis of Variance using General Linear Model (GLM) Procedure of SAS (v. 9.1.3, SAS Institute Inc., Carry, NC, 2008) for completely randomized design following 4 × 3 factorial arrangement. The statistical model included terms for syringe as random effect, levels of Leucaena, levels of corn oil, and Leucaena × corn oil interactions as fixed effect with following mathematical model:

$$Yijk = \mu + Ai + Lj + Mk + (L \times M)jk + eijk;$$

Where Lj= Leucaena substitution, Mk= CO supplementation, and (L × M)jk= interaction of Leucaena × CO. Treatment means were compared by Tukey's procedure for multiple comparisons of means. Significant effect of treatments was declared when $p<0.05$.

## 3. Results

### 3.1 *In vitro* apparent ruminal nutrient degradation

The effects of Leucaena and corn oil inclusion on apparent ruminal DM, OM, and N degradability are presented in Table 2. There is strong evidence that increasing levels of Leucaena and addition of corn oil greatly affected ruminal DM (rdDM), OM (rdOM), (Table 2) and N degradability (Figure 1) ($p<0.001$). However, there was no interaction between the treatments on these variables ($p>0.05$).

On an average, Leucaena supplementation increased rdDM and rdOM by 4.6% and 8.4%, respectively, at supplementation level of 25% in comparison with control ($p<0.05$; Table 2). However, there was no effect from higher levels of Leucaena. As regards nitrogen degradability, there was a linear increase in L25 and L50 treatments compared with control, while L75 did not give any further effect compared to lower proportions of Leucaena. In contrast, the addition of corn oil barely affected rdDM or rdOM in any treatment ($p<0.05$). Furthermore, the addition of corn oil did not result in marked differences among the treatments ($p>0.05$). The two factors also did not have an interaction effect on rdN ($p>0.05$).

Table 2. Effects of increasing proportions of leucaena and corn oil on *in vitro* ruminal nutrients degradation

| Treatment | | Ruminal nutrients degradation | |
|---|---|---|---|
| Leucaena (L) | Corn oil (CO) | rdDM (%) | rdOM (%) |
| L0 | CO1 | $52.4^{abc}$ | $54.8^{c}$ |
| | CO2 | $51.1^{abc}$ | $53.3^{c}$ |
| | CO3 | $51.9^{abc}$ | $54.5^{c}$ |
| L25 | CO1 | $56.2^{a}$ | $61.1^{ab}$ |
| | CO2 | $53.3^{ab}$ | $57.6^{abc}$ |
| | CO3 | $53.1^{ab}$ | $57.4^{abc}$ |
| L50 | CO1 | $56.1^{a}$ | $62.2^{a}$ |
| | CO2 | $52.0^{abc}$ | $57.5^{abc}$ |
| | CO3 | $51.6^{abc}$ | $57.1^{abc}$ |
| L75 | CO1 | $52.0^{abc}$ | $58.5^{abc}$ |
| | CO2 | $49.5^{bc}$ | $55.6^{bc}$ |
| | CO3 | $48.0^{c}$ | $54.0^{c}$ |
| SEM | | 1.02 | 0.369 |
| *p*-value | | | |
| L | | <0.001 | <0.001 |
| CO | | <0.001 | <0.001 |
| L x CO | | >0.05 | >0.05 |

SEM= standard error of mean; L= p-value for effect of Leucaena; CO= p-value for effect of corn oil; L×CO= p-value for interaction effect L×CO; **= highly significant ($p<0.01$); *= significant ($p<0.05$); ns= not significant ($p>0.05$); L0= forage without Leucaena substitution; L25= forage+ 25% Leucaena substitution; L50= forage+ 50% Leucaena substitution, L75= forage+ 75% Leucaena substitution (on DM basis); CO1= substrate without corn oil; CO2= substrate with 10 mg corn oil addition; CO3= substrate with 20 mg corn oil addition; rdDM= ruminal DM degradability; rdOM= ruminal OM degradability; rdN= ruminal N degradability.

$^{abcd}$ Different superscripts in the same column show significant effects of Leucaena (L), Corn Oil (CO), or the interaction effect L×CO.

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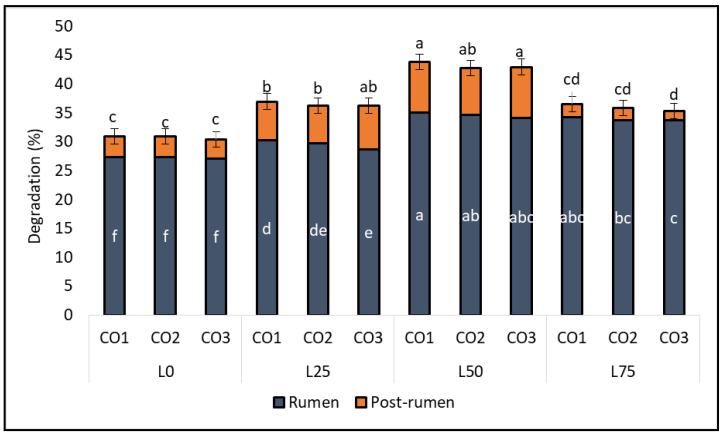


Figure 1. Ruminal and post ruminal nitrogen degradation, L0= forage without Leucaena substitution; L25= forage+ 25% Leucaena substitution; L50= forage+ 50% Leucaena substitution, L75= forage+ 75% Leucaena substitution (on DM basis); CO1= substrate without corn oil; CO2= substrate with 10 mg corn oil addition; CO3= substrate with 20 mg corn oil addition, $^{abcd}$ Different superscripts in the same column indicate significant effect of Leucaena (L), corn Oil (CO), or the interaction L×CO

Table 3. Effects of increasing levels of Leucaena and corn oil on *in vitro* apparent post ruminal and total tract nutrients degradation

| Treatment | | Post-ruminal degradation | | | Total tract degradation | | |
|---|---|---|---|---|---|---|---|
| Leucaena (L) | Corn oil (CO) | pdDM (%) | pdOM (%) | pdN (%) | tdDM (%) | tdOM (%) | tdN (%) |
| L0 | CO1 | 8.22 | 4.21 | $3.58^{c}$ | $60.6^{abc}$ | $59.1^{abc}$ | $31.2^{d}$ |
| | CO2 | 8.10 | 3.66 | $3.48^{c}$ | $59.1^{bc}$ | $56.9^{c}$ | $30.8^{d}$ |
| | CO3 | 7.84 | 4.08 | $3.27^{c}$ | $59.8^{abc}$ | $58.5^{bc}$ | $30.4^{d}$ |
| L25 | CO1 | 9.03 | 3.91 | $6.76^{b}$ | $65.3^{a}$ | $65.0^{ab}$ | $36.9^{b}$ |
| | CO2 | 7.67 | 3.09 | $6.57^{b}$ | $60.9^{abc}$ | $60.7^{abc}$ | $36.3^{bc}$ |
| | CO3 | 8.59 | 3.96 | $7.53^{ab}$ | $61.7^{abc}$ | $61.4^{abc}$ | $36.2^{bc}$ |
| L50 | CO1 | 8.37 | 3.10 | $8.79^{a}$ | $64.5^{ab}$ | $65.3^{a}$ | $43.8^{a}$ |
| | CO2 | 8.70 | 3.95 | $8.04^{ab}$ | $60.7^{abc}$ | $61.4^{abc}$ | $42.7^{a}$ |
| | CO3 | 8.67 | 3.43 | $8.84^{a}$ | $60.2^{abc}$ | $60.5^{abc}$ | $42.9^{a}$ |
| L75 | CO1 | 8.95 | 4.16 | $2.35^{cd}$ | $60.9^{abc}$ | $62.6^{abc}$ | $36.5^{bc}$ |
| | CO2 | 8.33 | 3.78 | $2.08^{cd}$ | $57.8^{c}$ | $59.4^{abc}$ | $35.8^{bc}$ |
| | CO3 | 8.02 | 3.74 | $1.55^{d}$ | $56.0^{c}$ | $57.7^{c}$ | $35.2^{c}$ |
| SEM | | 1.02 | 0.369 | 0.426 | 5.69 | 7.04 | 0.217 |
| *p*-value | | | | | | | |
| L | | >0.05 | >0.05 | <0.001 | <0.001 | <0.001 | <0.001 |
| CO | | >0.05 | <0.001 | >0.05 | <0.001 | <0.001 | <0.001 |
| L x CO | | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

SEM= standard error of mean; L= p-value for effect of Leucaena; CO= p-value for effect of corn oil; L×CO= p-value for the interaction effect L×CO; **= highly significant ($p$ <0.01); *= significant ($p$<0.05); ns= not significant ($p$>0.05); L0= forage without Leucaena substitution; L25= forage+ 25% Leucaena substitution; L50= forage+ 50% Leucaena substitution, L75= forage+ 75% Leucaena substitution (on DM basis); CO1= substrate without corn oil; CO2= substrate with 10 mg corn oil addition; CO3= substrate with 20 mg corn oil addition; pdDM= post ruminal DM degradability; pdOM= post ruminal OM degradability; pdN= post ruminal N degradability (bypass protein degradation); tdDM= total DM degradability; tdOM= total OM degradability; tdN= total N degradability

$^{abcd}$ Different superscripts in the same column show significant effects of Leucaena (L), Corn Oil (CO), or the interaction effect L×CO

### *3.2 In vitro* apparent post-ruminal and total tract nutrient degradability

The apparent post-rumen and total tract degradabilities of DM, OM, and CP are presented in Table 3. The apparent post-ruminal nutrient degradability is undigested nutrient in the rumen but is digested in the phase of HCl and pepsin addition. The pdDM and pdOM were not affected by Leucaena, corn oil, or L×CO interactions (p>0.05) while tdDM and tdOM increased with L50 (p<0.05). In a comparison with L75, there was marginal evidence that tdDM and tdOM decreased compared with L50 (p<0.072). The pdN and tdN were affected by levels of Leucaena (p<0.001) but not by corn oil or an interaction between L and CO (p>0.05).

## 4. Discussion

The increase in rdDM and rdOM occurred due to changes in the chemical composition of the diet, mainly the higher protein content and lower fiber fraction, particularly ADF. Consequently, there was a better balance of energy and protein when Leucaena was included in the diet than in the

control diet. Leucaena contains less ADF than Napier grass in the present study (17.9% vs 39.2%, respectively), thus a higher level of Leucaena contributed a lesser fiber content that is undigestible materials, such as lignin and silica (Jiménez-Peralta *et al.*, 2011). Since Leucaena has a lower ADF content, it is highly digestible in the rumen. Barros-Rodríguez *et al.* (2014) suggested that the Leucaena digestibility ranges from 60-70% so that Leucaena supplementation to the level where the condensed tannin (CT) contained has not yet toxic or negative effects, will be able to increase rdDM and rdOM. This was also supported by Yuliana *et al.* (2019), in which the low level of tannin in the diet did not impair ruminal fermentation and digestibility.

The high supply of proteins in the L50 treatment contributed to improved rumen dry matter degradability. However, there was no further increase in the L75 case relative to L25 and L50, possibly because of secondary metabolites and NH3 concentration. For the first reason, L75 contained 2.33% condensed tannin (CT), which is categorized as a high level. Jayanegara, Leiber, and Kreuzer (2012) suggested that the effect of CT on nutrient digestibility in the rumen is negative when the level is higher than 2% of DM. Secondly, excessive $NH_3$ formation in the rumen is likely to impair rumen ecology and environment when the level of degradable protein exceeds its maximum requirement (Patra & Aschenbach, 2018). As regards the increased degradability with Leucaena supplementation, the smaller particle size of Leucaena increased the proportion of soluble carbohydrates that are easily degraded by rumen microbes (Noviandi *et al.* 2014). Phesatcha and Wanapatet (2016) reported that rdDM and rdOM increased with Leucaena supplementation.

Furthermore, unsaturated fatty acids (UFAs) contained in corn oil are thought to be a major factor decreasing rdDM. The UFAs sources are known to have toxic properties against some species of rumen microorganisms, including protozoa (Martin, Rouel, Jouany, Doreau, & Chilliard, 2008), which has an effect on decreasing rdDM and rdOM. Wu *et al.* (2015) reported that linoleic acid (LA) supplementation decreased gas production and nutrient digestibility, and Tan *et al.* (2011) also reported similar results. An increased rdN occurred when inclusion of Leucaena, as effect of increasing CP concentration in the diet.

As a source of protein, Leucaena supplementation linearly increased CP levels of diet, which caused N degradation into $NH_3$-N in the rumen also to increase. Proteins entering rumen will degenerate into amino acids and peptides accumulated into $NH_3$-N as a source of nitrogen for microbes (Pina, Valadares, Tedeschi, Barbosa, & Valadares, 2009). The decreased rdN with L75 is thought to be the result of tannin intervention contained in Leucaena. Toxic effects of tannins occur directly by either CT or HT against bacteria and rumen protozoa. Increasing the inclusion of Leucaena to 75% contributed to increased total tannin and CT concentrations in the diet, whereas in this L75 the respective concentrations were 62.4 g/kg DM of total tannins and 23.5 g/kg DM of CT, respectively. Tannins are known to have the ability to protect polymers, both carbohydrate and protein. However, compared to carbohydrates, tannins have a higher sensitivity to bind proteins (Brinkhaus, Bee, Silacci, Kreuzer, & Dohme-Meier, 2016; Jayanegara *et al.*, 2012).

A decrease in L75 treatment was in agreement with Tan *et al.* (2011) who reported a decrease in ruminal N degradability with increasing levels of CT extracted from Leucaena with minimum doses at 20% DM. Binding activity of CT from Leucaena reduced protein degradation to $NH_3$-N in the rumen, and concomitantly the post ruminal protein availability increased. Huang *et al.* (2010) suggested Leucaena has protein binding characteristics that might reduce protein degradation in the rumen by formation of CT-protein complexes. Protein that escaped from being degraded in the rumen would enhance flow of protein to the intestine. Barros-Rodríguez *et al.* (2014) and Nguyen, Wanapat, Phesatcha, and Kang, (2017) suggested that Leucaena is highly digestible, ranging from 60-70%. Nevertheless, the use of Leucaena at high levels has often adversely affected the animal performance due to the effects of secondary metabolites, especially mimosine and tannins. Jayanegara *et al.* (2012) suggested that the effects of CT on $CH_4$ and nutrient digestibility might be observed when administered at > 2% DM. As regards the results of this study, Leucaena at the level of 75% elevated the total tannins and CT contents to more than 6% and 2% DM, respectively.

Tannins and PUFA give negative effects on nutrient digestibility when used in excessive amounts. Corn oil as PUFA source inhibits nutrient degradation in the rumen by both direct and indirect mechanisms, i.e by suppressing the population of fiber degrading bacteria and by protecting the fiber fraction (Martin *et al.*, 2008). Increasing CP concentration in the diet by inclusion of Leucaena generally led to an increase in $NH_3$-N in the rumen. However, if the accumulation of $NH_3$-N in the rumen exceeds a limit, it would have a toxic effect on rumen microbiome and ruminal fermentation (Patra & Aschenbach, 2018), resulting in a decline in rdN and pdN. It can be observed from the reduced values of rdN, pdN, and tdN with the L75 treatment. The CT contained in L75 might be the reason for N degradability depression. Tannins are known to have the ability to protect nutrient polymers, primarily the protein fraction (Brinkhaus *et al.*, 2016). The presence of tannin activity protecting proteins in the rumen was confirmed in this study, whereas the amount of pdN was higher with L25 and L50 treatments.

## 5. Conclusions

The inclusion of Leucaena in the diet for up to 50% improved nutrient ruminal and post-ruminal degradation, particularly the N degradability. This study also suggested that inclusion of either corn oil or of Leucaena up to 75% DM negatively affected nutrient degradability. This could be associated with the presence of secondary metabolite compounds, especially tannins contained in the Leucaena, and negative effects of high fat content from the corn oil added to the ration. Toxicity of nitrogen from Leucaena can also adversely affect rumen fermentation when its concentration is excessive.

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