

## Using Treated *Leucaena* (*Leucaena leucocephala*) Leaves as Supplements to Thai Brahman Cattle Giving a Basal Diet of Rice Straw

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**Abstract:** Four male Thai Brahman cattle (261±3.2 kg; 24-36 months old) were fed rice straw as a basal diet and *Leucaena* treated in one of three methods: untreated (control), *Leucaena* treated with NaOH solution, *Leucaena*+PEG (Poly Ethylene Glycol). Using *Leucaena* treated with NaOH solution as a supplement improved ( $p<0.05$ ) fibre digestion but N-balance reduced ( $p<0.05$ ). Whilst N-balance, N-digestibility and fibre increased ( $p<0.05$ ) but Dry Matter (DM) digestibility decreased ( $p<0.05$ ) when animals supplemented with *Leucaena*+PEG. The effect of Condensed Tannins (CT) in *Leucaena* depends on the treatment of *Leucaena*, NaOH solution and PEG. Using NaOH solution treatment is potentially beneficial for improving fibre digestion but N-balance was reduced. However, adding PEG beneficial for improving fibre digestion, N-balance and N-digestion but DM digestion decreased.

**Key words:** Brahman cattle, *Leucaena*, NaOH, Poly Ethylene Glycol (PEG), rain tree pod, rice straw, Thailand

### INTRODUCTION

Mimosine is a toxic non-protein amino acid occurring in genus of *Leucaena* (Brewbaker and Hylin, 1965) that toxic substance is easily solved by inoculating *Synergistes jonesii* (Palmer *et al.*, 2010; Jones and Megarritty, 1986; Jones and Lowry, 1984). The high condensed tannins contents in *Leucaena* are much problematic substances, its usually contained 2-6% (Hughes, 1998).

The formation of tannin-protein complexes in *Leucaena* consequently used as feed in ruminants has been shown to protect dietary protein degradation in the rumen (Reed, 1995) and subsequently ammonia-N concentration limits (Waghorn, 2008) and microbial activity in the rumen were depressed (Scalbert, 1991). There are however, two ways which have been attempted to be deactivated tannins and other secondary compounds in *Leucaena*; the addition of chemicals with a high affinity for tannins such as polyvinylpyrrolidone and Polyethylene Glycol (PEG), a synthetic polymer to

which tannins have a greater binding affinity than protein (Waghorn *et al.*, 1994) and the use of alkaline treatments such as NaOH solution or ash solution (Price *et al.*, 1979). Thus, the present study was to determine the effects of the differently treated *Leucaena* and whether it was suitable to be a protein supplement on whole tract digestibility of nutrients, N-balance, microbial production in the rumen and some blood metabolites in Thai Brahman cattle fed rice straw as a basal diet.

### MATERIALS AND METHODS

**Animals, experimental design and diets:** Four male Thai Brahman cattle weighing (261±3.2 kg; 24-36 months old) purchased from the Tabkwang Livestock Research and Breeding Centre, Department of Livestock Development, Saraburi province (150 km from Bangkok) and were used in this study. The experiment was conducted at Animal House, Department of Animal Husbandry, Faculty of Veterinary Science, Nakhonpathom (50 km from Bangkok) where animals were housed in individual pens. The

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experimental design was a 3×4 incomplete latin square design and animals were allocated to 3 supplements. About 3 supplemental diets consisting of (kg on fresh weight); 500 g sun-dried *Leucaena* Leaves (LL), 445 g oven dried RTP, 50 g cassava meal and 5 g premix (untreated LL) were used as the control, 500 g LL treated with NaOH (LL + NaOH), 445 g oven dried RTP, 50 g cassava meal and 5 g premix, 500 g LL, 445 g oven dried RTP, 50 g PEG and 5 g premix (LL+PEG).

Sun-dried *Leucaena* rain tree pods were used at present purchasing from a farmer in Nakhon Pathom province while rice straw was purchased from Department of Animal Husbandry, Faculty of Veterinary Science. The *Leucaena* was chopped into 6-8 mm pieces and sun-dried for 12-18 h.

The rain tree pods were oven dried at 75°C for 72 h after purchasing then immediately ground (<4 mm) and stored in air-tight storage containers. The sun-dried *Leucaena* was divided into three portions, the 1st portion used for untreated sun-dried *Leucaena* supplement and the 2nd portion was sun-dried *Leucaena* treated with NaOH solution to reduce its tannins content and the 3rd portion was mixed with 1 g PEG MW 4000: 10 g sun-dried *Leucaena*. The PEG was purchased from Asian Chemicals Ltd, Chachoengsao province, Thailand.

The procedure of *Leucaena* treated with NaOH in brief, 20 kg of chopped sun-dried *Leucaena* was soaked in plastic containers containing pH 12 of NaOH solution (100 g NaOH dissolved in 30 L of tap water). After 18 h of soaking, the soaked *Leucaena* was taken out and washed with tap water until the washing water was clear.

Then, it was sun-dried before keeping it in gunny bags for storing and ready for use. Each of the animal was fed (fresh weight) twice daily (07:00 and 17:00 h) with 2 kg of the respective supplements and 1 kg rice straw. Drinking water was freely available at all times. The supplements offered per day per animal are shown in Table 1.

The experiments were carried out in three 21 days periods each comprising 14 days dietary adaptation and 7 days experimentation. On day 1 and 21 of each period,

the animals were weighed before the morning feed (06:00 h) and the animals were transferred (2 days before the collection period) into individual metabolism crates fitted with containers for urine and faeces collection.

The studies carried out were whole tract digestibility of nutrients, N-balance, urinary PD excretion and some blood metabolite values. All animals were cared for according to the guide for the Use of Laboratory of Animals for Scientific Purposes [Chulalongkorn University Animal Care and Use Committee (CU-ACUC)]. Whole tract apparently digestibility was determined by total collection from days 15-21. About 10% representative aliquots of the supplements and the daily offered rice straw (no rice straw and supplement refusal for each animal throughout the three experimental periods) and the faecal outputs samples were collected and stored at -20°C. At the end of each sampling period, samples from each animal were bulked and then oven dried at 65°C for 72 h, prior to analysis for Dry Matter (DM), ash, Nitrogen (N) and Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) contents.

Daily total urine output was collected into a plastic bag containing 100 mL of 20% (v/v) H<sub>2</sub>SO<sub>4</sub> in order to maintain a final pH<3. The urine was collected at 24 h intervals for 7 days (days 15-21). The volume of acidified urine was immediately recorded; two sub-samples taken: 20 mL was diluted five times then stored at -20°C prior to PD analysis and 50 mL of the original acidified urine was kept at -20°C for determination of total N. Blood samples were collected from the jugular vein at 0 h and at 3, 6 and 9 h after the morning feed (day 21, 06:00 h). Blood samples were drawn using a 2 way blood collection needle (Vacurette® Austria, Model 18 G×1½) and transferred into two heparinised vacutainers (9 mL tube<sup>-1</sup>) and one tube containing sodium fluoride and potassium oxalate (for analysis of non-esterified fatty acids). The tubes were immediately centrifuged at 2500×g for 25 min, individual plasma was stored in tubes (3-3.5 mL tube<sup>-1</sup>) at -20°C for analysis.

**Analytical procedures:** Dry matter in the feed and the faecal samples was determined by drying them to a constant weight in an oven at 105°C for 48 h. The ash in the feed and the faecal samples was determined by burning them to a constant weight in a muffle furnace at 550°C for 8 h. The Organic Matter (OM) was calculated as the difference between DM and ash contents. The N content in the feed and the faecal samples was measured by the micro Kjeldahl method (AOAC, 2000 method No. 995.04). NDF and ADF was analyzed according to Van Soest *et al.* (1991). Total phenolic compounds, tannin compounds and the condensed tannins in feed samples

Table 1: Daily dietary supplements (kg/animal on fed basis)

Characteristics	Supplements (kg/animal on fed basis)		
	Untreated LL	LL+NaOH	LL+PEG
<b>Ingredients (g kg<sup>-1</sup>)</b>			
<i>Leucaena</i>	2.00	-	2.00
<i>Leucaena</i> treated NaOH	-	2.00	-
Rain Tree Pod (RTP)	1.78	1.78	1.78
Cassava meal	0.20	0.20	-
Poly Ethylene Glycol (PEG)	-	-	0.20
Pre-mixes <sup>†</sup>	0.02	0.02	0.02

LL = *Leucaena* Leaves; NaOH = Sodium Hydroxide; PEG = Poly Ethylene Glycol; Pre-mixes contained (g kg<sup>-1</sup> DM basis): vitamin A 40,000,000 unit, vitamin D<sub>3</sub> 4,000,000 unit, vitamin E 40,000 Unit, vitamin B<sub>12</sub> 0.02 g, Mn 160 g, Fe 240 g, Zn 100 g, Cu 20 g, Se 0.5 g, Co<sub>2</sub> g and I 5 g

were determined following Makkar (2000). Total non structural carbohydrates in the feed were determined according to Nelson's reducing sugar procedure (Hodge and Hofreiter, 1962).

Allantoin and uric acid in urine samples was determined by an ultraviolet spectrophotometer Genesys 10 UV according to the procedure of IAEA-TECDOC-945 (1997). Purine derivatives in the urine were measured as allantoin and uric acid and expressed in terms of mmol PD per day.

PD in the urine were measured as allantoin and uric acid and expressed in terms of mmol per day. The concentrations of glucose, urea nitrogen, insulin, Non Estrified Fatty Acid (NEFA) and  $\beta$ -hydroxybutyrate ( $\beta$ -HBA) in plasma were measured using the available commercial kits (Glucose liquicolor and urea liquiUV, Human GmbH-D 65205 Wiesbaden, Germany; Coat-A-Count<sup>®</sup>-Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA; Wako Pure Chemical Ind. Osaka, Japan; Randox Laboratories Ltd. Ardmore, Diamond road, Crumlin, Co. Antrim, UK, BT29 4QY, respectively).

**Statistical analysis:** The means of each parameter were established in accordance with the Analysis of Variance (ANOVA) using the procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by a Least Significant Difference method (LSD).

## RESULTS

The rice straw was used as a basal diet, it contained 887 g DM kg<sup>-1</sup> and on g kg<sup>-1</sup> DM basis; containing 0.81 g N 0.812 g NDF, 700 g ADF, 150 g ADL and 15.0 Mega Joules (MJ) gross energy (Table 2).

The contents of crude protein, phenolic compounds, tannins compounds, condensed tannins, total sugar, reducing sugar, sucrose and GE in the untreated LL and LL+PEG supplements were similar but they were higher than the values in LL+NaOH supplement (Table 3). The coefficient of DM digestibility was lower ( $p < 0.05$ ) in Brahman cattle consumed LL+PEG supplement than in those fed untreated LL supplement however there was no difference between animals fed untreated LL and LL+NaOH supplemental diets.

None of supplements affected the coefficient of OM digestibility but NDF and ADF digestibilities were lower ( $p < 0.05$ ) in animals supplemented with untreated LL than in those with the other supplements but there was no difference between animals fed untreated LL and LL+NaOH supplemental diets (Table 4). In Table 5, it is shown that the N intake was similar in Brahman cattle fed untreated LL and LL+PEG supplements but higher ( $p < 0.05$ ) than in those fed LL+NaOH supplement. The

Table 2: Chemical composition in ingredients of diets

Characteristics	Ingredients of diets (g kg <sup>-1</sup> )			
	RTP	Untreated LL	LL+NaOH	Rice straw
Dry Matter (DM)	894.00	922.00	924.00	887.00
Ash	52.40	62.20	57.90	98.60
Nitrogen (N)	20.10	22.90	23.20	8.10
Crude protein(N $\times$ 6.25)	125.00	143.00	146.00	50.60
Neutral Detergent Fibre (NDF)	371.00	632.00	844.00	812.00
Acid Detergent Fibre (ADF)	287.00	412.00	660.00	556.00
Acid Detergent Lignin (ADL)	52.00	165.00	140.00	86.00
Phenolic compounds	50.30	86.00	64.80	1.37
Condensed Tannins (CT)	35.40	42.80	24.80	0.04
Total sugar	204.00	65.40	11.70	12.20
Reducing sugar	110.00	33.00	9.70	14.20
Sucrose	94.10	32.40	0.00	0.00
Gross energy (kcal g <sup>-1</sup> )	4.79	4.78	4.34	3.58

LL = Leucaena Leaves; NaOH = Sodium hydroxide; PEG = Poly Ethylene Glycol

Table 3: Chemical composition in supplements (kg/day/animal)

Characteristics	Method of treatments		
	Untreated LL	LL+NaOH	LL+PEG
<b>Chemical analysis (g kg<sup>-1</sup>)</b>			
DM	899.00	895.00	914.00
Ash	56.80	64.50	86.20
NDF	498.00	486.00	503.00
ADF	334.00	458.00	337.00
ADL	106.00	93.00	107.00
Nitrogen (N)	21.30	21.40	26.20
Crude protein (N $\times$ 6.25)	133.00	134.00	164.00
Phenolic compounds	219.00	10.80	19.40
CT	75.40	4.63	7.18
Total sugar	190.00	85.60	111.00
Reducing sugar	102.00	49.20	59.00
Sucrose	81.50	32.40	52.40
Gross energy (kcal g <sup>-1</sup> )	4.28	4.08	4.28

LL = Leucaena Leaves; NaOH = Sodium hydroxide; PEG = Poly Ethylene Glycol; DM = Dry Matter; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; ADL = Acid Detergent Fibre; CT = Condensed Tannins

Table 4: Intakes of dry matter, organic matter and neutral detergent fibre, acid detergent fibre and the digestibility coefficients of DM, OM, NDF, ADF in Thai, Brahman cattle fed rice straw as a basal diet of rice straw supplemented with different treated Leucaena

Characteristics	Methods of treatment			
	Untreated LL	LL+NaOH	LL+PEG	SEM
Animals (number)	4.00	4.00	4.00	-
BW (kg)	252.00	259.00	254.00	-
BW <sup>0.75</sup> (kg)	63.30	65.50	65.00	-
<b>Intakes (g kg<sup>-1</sup> BW<sup>0.75</sup> day<sup>-1</sup>)</b>				
DM	64.90	63.30	64.30	1.46
OM	62.20	60.80	61.70	1.40
NDF	43.90	47.40	45.10	1.02
ADF	37.20	41.70	38.60	1.05
<b>The digestion coefficients (decimal)</b>				
DM	0.54 <sup>a</sup>	0.52 <sup>ab</sup>	0.50 <sup>b</sup>	0.02
OM	0.58	0.57	0.55	0.02
NDF	0.48 <sup>b</sup>	0.59 <sup>a</sup>	0.57 <sup>a</sup>	0.06
ADF	0.39 <sup>b</sup>	0.42 <sup>ab</sup>	0.46 <sup>a</sup>	0.03

LL = Leucaena Leaves; NaOH = Sodium Hydroxide; PEG = Poly Ethylene Glycol; DM = Dry Matter; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; SEM = Standard Error of Mean; means within the same row with the different superscripts are significantly ( $p < 0.05$ ) different

Table 5: Nitrogen balance and N digestibility in Thai, Brahman cattle fed rice straw as a basal diet of rice straw supplemented with different treated *Leucaena*

Characteristics	Methods of treatment			SEM
	Untreated LL	LL+NaOH	LL+PEG	
Intakes N (g kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	1.26 <sup>a</sup>	1.00 <sup>b</sup>	1.24 <sup>a</sup>	0.03
N in urine (mg kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	108.00 <sup>b</sup>	88.50 <sup>c</sup>	145.00 <sup>a</sup>	0.78
N-faeces (mg kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	638.00 <sup>ab</sup>	690.00 <sup>a</sup>	576.00 <sup>b</sup>	3.14
N-balance (mg kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	517.00 <sup>a</sup>	225.00 <sup>b</sup>	521.00 <sup>a</sup>	3.28
N digestibility (g kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	495.00 <sup>b</sup>	311.00 <sup>c</sup>	537.00 <sup>a</sup>	25.50

LL = *Leucaena* Leaves; NaOH = sodium hydroxide; PEG = Poly Ethylene Glycol; N = Nitrogen; SEM = Standard Error of Mean; means within the same row with the different superscripts are significantly ( $p < 0.05$ ) different

Table 6: Urinary purine derivatives excretion and the ratios of PD to DOMI and blood metabolites in Thai, Brahman cattle fed rice straw as a basal diet of rice straw supplemented with different treated *Leucaena*

Characteristics	Methods of treatment			SEM
	Untreated LL	LL+NaOH	LL+PEG	
Urinary purine derivatives excretion				
Allantoin (mmol kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	1.46	1.40	1.31	0.17
Uric acid (mmol kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	0.36 <sup>a</sup>	0.33 <sup>a</sup>	0.14 <sup>b</sup>	0.07
PD (mmol kg <sup>-1</sup> BW <sup>0.75</sup> day <sup>-1</sup> )	1.82	1.73	1.45	0.22
Allantoin/DOMI (mmol kg <sup>-1</sup> )	41.80	40.90	40.00	4.64
PD/DOMI (mmol kg <sup>-1</sup> )	52.40	50.70	44.20	6.17
Microbial N efficiency (gN/DOMR)	63.30	54.60	53.30	13.50
Urea-N (mg dL <sup>-1</sup> )	23.5.0 <sup>a</sup>	18.00 <sup>b</sup>	17.80 <sup>b</sup>	5.93
Glucose (mg dL <sup>-1</sup> )	75.00	72.90	75.90	2.22
NEFA (μmol L <sup>-1</sup> )	85.8.0 <sup>ab</sup>	106.00 <sup>a</sup>	71.80 <sup>b</sup>	23.10
β-HBA (μmol L <sup>-1</sup> )	581.00 <sup>b</sup>	885.00 <sup>a</sup>	625.00 <sup>b</sup>	97.00
Insulin (μi.u. mL <sup>-1</sup> )	9.73 <sup>b</sup>	9.38 <sup>b</sup>	12.60 <sup>a</sup>	0.84

LL = *Leucaena* Leaves; NaOH = Sodium Hydroxide; PEG = Poly Ethylene Glycol; NEFA = Non-Esterified Fatty Acid; β-HBA; β-hydroxy butyrate; SEM = Standard Error of Mean; PD = Purine Derivatives; DOMI = Digestible Organic Matter Intake; microbial N efficiency (gN/DOMR) = the results of calculated microbial supply are expressed as a g microbial N per kg Digestible Organic Matter in the Rumen (DOMR) (Chen and Gomez, 1995); Means within the same row with the different superscripts are significantly ( $p < 0.05$ ) different

urine-N was lower ( $p < 0.05$ ) in cattle fed LL+NaOH supplement than in those fed untreated LL and LL PEG supplements. However, the urine-N was greater ( $p < 0.05$ ) in animals fed LL+PEG than that in those fed untreated LL supplement. The faecal-N output was lower ( $p < 0.05$ ) in animals fed LL+PEG supplement than in those fed LL+NaOH supplements, no difference was found in between animals fed untreated LL and LL+NaOH supplements. The N-balance was lower ( $p < 0.05$ ) in animals consumed LL+NaOH supplement than in those consumed untreated LL and LL+PEG supplements. The urinary allantoin, total PD excretions, the efficiency of microbial nitrogen in the rumen, the ratios of allantoin/DOMI and PD/DOMI were not different ( $p < 0.05$ ) among cattle fed all *Leucaena* supplements (Table 6). The

urinary uric acid excretion was lower ( $p < 0.05$ ) in animals fed LL+PEG supplement than in those fed untreated LL supplement but there were no differences in urinary uric acid excretion when compared to animals fed the other supplements. As shown in Table 6, the PUN decreased ( $p < 0.05$ ) in Brahman cattle fed untreated LL supplement when compared to animals fed treated *Leucaena* supplements. Levels of glucose concentrations in the plasma were not different in cattle when compared to animals fed all supplements. Plasma NEFA was not different in animals fed untreated LL and LL+NaOH *Leucaena* supplements, it was lower ( $p < 0.05$ ) in animals fed LL+PEG supplement than those fed LL+NaOH supplement.

Plasma of β-HBA concentration was higher ( $p < 0.05$ ) in animals fed LL+NaOH supplement than those fed with untreated LL and LL+PEG supplements. Plasma insulin concentrations was greater ( $p < 0.05$ ) in cattle fed LL+PEG supplements than those fed with untreated LL and LL+NaOH supplements.

## DISCUSSION

The fibre (NDF, ADF) digestion in animals decreased when fed untreated LL, this may be due to the condensed tannins affecting limiting protein degradation in the rumen (RDP), ammonia-N concentration and microbial activity (Waghorn, 2008). This therefore affects productivity, especially of fibre digestion, this may be slowing down the rate of passage from the rumen ( $k_1$ ) may be enhancing rumination and it might be reducing voluntary feed intake. The fibre digestion however improved when animals were fed *Leucaena* treated with NaOH diet, possibly due to the NaOH solution breaking down the lignin bond and the disintegration of fibre reduces particle size and the passage rates increased (Klopferstein, 1978) and sodium salts increased osmotic pressure and rumen washout (Jackson, 1977).

Likewise, the fibre digestion increased in animals when fed LL+PEG diet, possibly with sufficient RDP is available in the rumen due to tannins being deactivated by PEG. The soluble carbohydrates in the rumen may decrease as it is binding with PEG (McSweeney *et al.*, 2001; Brooker *et al.*, 1999). Therefore, this condition can elevate pH in the rumen, this may be suitable to optimise cellulolytic microbial activity. These ideas were supported by Silanikove *et al.* (1996), Waghorn *et al.* (1987) and Barry *et al.* (1986) who found an increase in fibre digestibility of diet containing rich-tannins when PEG was added to it. The N-balance was lower in Brahman cattle fed untreated LL rather than those fed LL+PEG supplement because condensed tannins always increase the N content of faeces and decrease urinary

N output (Waghorn and McNabb, 2003). However, the N-balance in animals fed LL+PEG supplement was enhanced when PEG was added, affecting the decrease of urinary N and the increase of faecal N excretions when compared to animals fed untreated LL and LL+NaOH supplements. This was in agreement with several workers (Barry *et al.*, 1984; McNabb *et al.*, 1993; Waghorn and Shelton, 1997) who reported that the additional PEG in diet containing phenolic compounds usually increases the digestibility and availability of nutrients such as protein, carbohydrates and minerals hence, enhancing the values of N-balance.

The urine-N was higher in animal fed Leucaena treated and LL+PEG supplement than in those fed LL+NaOH diets because the quantity of N intake being slightly higher in those fed LL+PEG diet. However, the values of N excreted in faeces were lower in animals fed LL+PEG supplements than in those supplemented with the other supplemental diet.

This study demonstrated that proteins were bound with tannins before it appeared in the faeces (Makkar *et al.*, 1995) indicating that fewer excess fermentable N sources in the rumen were lost into the urine when animals were fed diets containing tannins (Aerts *et al.*, 1999).

Nevertheless, the urinary N excretions decreased in animals fed untreated LL and LL+NaOH diets but the faecal N increased in animals fed LL+NaOH diet. This may be due to the N attached fibre in animals fed LL+NaOH diet being excreted in the faeces more than in those fed LL+PEG diet. Inactive tannins content in Leucaena leaves in order to increase the efficiency of using protein in the rumen and enhance the ruminal microbial production by treating with NaOH solution or adding of PEG.

The results indicated that the microbial supply into the intestine was better in animals fed untreated LL diet than in those fed treated Leucaena supplemental diets. This could explain the tannins and carbohydrates in the rumen may be deactivated by PEG (McSweeney *et al.*, 2001; Brooker *et al.*, 1999; Deshpande and Salunkhe, 1982) and latter increasing RDP supply was imbalanced with available energy supply (Sniffen and Ronbinson, 1987) therefore, microbial purine supply to the small intestine was depressed. However, the microbial yield supply to the small intestine decreases in animals fed LL+NaOH diet accordingly the NaOH solution treated Leucaena not only decrease the phenolic compounds and tannins but also reduce N and water soluble carbohydrates contents (Sundstol and Owen, 1984). The treated Leucaena supplemental diets affected the concentrations of PUN but the average value of PUN in animals fed different treated Leucaena was similar to the value in Brahman

cattle fed a basal diet of rice straw and supplemented with some tropical protein-rich trees (Jetana *et al.*, 2010). The PUN was higher in animals fed treated Leucaena diets than in those fed untreated Leucaena diet. It may contain insufficient soluble carbohydrates when Leucaena treated with NaOH solution was used (Klopferstein, 1978). However, the PUN was raised in animals fed untreated Leucaena diet, this indicated that there are imbalances between energy sources and rumen protein degradation in the rumen (Sniffen and Ronbinson, 1987).

None of the supplemental diets affected plasma glucose concentrations, this could be due to all diet having sufficiently soluble carbohydrates, some may come from RTP. Increasing plasma insulin levels in animals fed LL+PEG supplements reflected that insulin must be secreted into the blood for controlling glucose metabolism. However, plasma insulin level in animals fed untreated LL and LL+NaOH supplemental diet did not changed, this may be the fermentation with LL and LL+NaOH supplementation in the rumen sufficiently providing energy sources into the blood circulation therefore, plasma insulin was not raised. Plasma NEFA decreased in animals when fed LL+PEG diet, possibly insufficient energy is available in the rumen due to tannins being deactivated by PEG (McSweeney *et al.*, 2001; Brooker *et al.*, 1999). Decreasing plasma  $\beta$ -HBA in animals fed untreated LL and LL+PEG supplemental diet caused by RDP was limited. This reflected that the butyrate was less in the rumen fermentation therefore, it was less converted to  $\beta$ -HBA in rumen epithelium.

The condensed tannins are potential anti-nutritional agents when Leucaena leaves are used as feedstuff in ruminants. They were deactivated by adding PEG or being treated with NaOH solution resulting in enhanced fibre digestion. At present, the use of NaOH solution treated LL, the result seems to be unsatisfactory according to not only decreased tannins contents but the essential nutrients such as amino acids and mineral, etc., also disappeared during treatment processing. The addition of PEG in diets, containing high phenolic compounds is a simple method therefore it increases fibre digestion. However, the pattern of untreated LL supplement in this study have shown slightly higher production of microbial supply into the small intestines and N-balance than that treated Leucaena supplements even though fibre digestion depressed. In addition, there is no need to purchase special ingredients.

Further, studies only needed for adjusting the proportion between Leucaena and rain tree in order to establish a suitable use as a supplement for improving the quality of cattle feeding system for smallholder farmers.

## CONCLUSION

The effect of CT in *Leucaena* depends on the treatments of *Leucaena*, NaOH solution and PEG. Using NaOH solution treatment is potentially beneficial for improving fibre digestion but N-balance was reduced. However, adding PEG is beneficial for improving fibre digestion, N-balance and N-digestion but DM digestion decreased. Therefore, this has resulted in a substantial research effort to control CT substances in the tropical foliages. This study however, demonstrates that the treatments of *Leucaena* may not be necessary for animals fed *Leucaena* forage together with ground Rain Tree Pod (RTP).

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