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Effects of Different Levels of Rain Tree (*Samanea saman*) Pods in Meal Concentrate on *In Vitro* Fermentation by a Gas Production Technique

Sareena Semae[1], Phongtorn Kongmun[2], Chanvit Vajrabukka[2], Somchai Chanpongsang[3] and Somkiert Prasanphanich[2,*]

ABSTRACT

The effect was studied of various levels of rain tree pods (RTPs) in meal concentrate on *in vitro* fermentation using a gas production technique. RTPs were used at levels of 0 (control), 20, 40, 60, 80 and 100% in the meal concentrate. The gas production was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 66 and 72 hr of incubation. *In vitro* true digestibility (IVTD), total volatile fatty acids (TVFAs), acetate (C_2), propionate (C_3), butyrate (C_4) and ammonia nitrogen (NH_3-N) were investigated. The gas production at 4 to 8 hr was significantly different ($P < 0.05$) among treatments. The IVTD levels in the control diet and with 20, 40 and 60% RTP levels were significantly higher ($P < 0.05$) than for the 80 and 100% levels of RTP. The C_2 level in the control diet was the lowest but levels of C_3 and C_4 were higher than in the other groups. The level of NH_3-N in the control diet and with levels of 20, 40 and 60% RTP levels were significantly higher ($P < 0.05$) than at the 80 and 100% RTP levels. This study revealed that RTPs could be an alternative feedstuff for ruminants and possibly replace meal concentrate up to 60% without any negative effect based on the *in vitro* study.

Keywords: Rain tree pods, feedstuff, digestibility, gas production technique, meal concentrate

INTRODUCTION

In the dry season, the forage mass and quality available for animal feed are both low (Jetana *et al.*, 2010). However, some tropical plants, such as leguminous trees, could be used as feed and some research has reported that ruminants can supplement their diet by browsing leguminous plants and pods with a positive growth response in the animals being reported (Babayemi and Bamikole, 2006). *Samanea saman* (Jacq.) Merr. is normally distributed in tropical regions including throughout Thailand and is generally known as the rain tree (Durr, 2001). It is cultivated as an ornamental shade tree, yielding dark brown and large leathery pods, which are a valuable supplement to goats and other ruminants (Stewart and Dunsdon, 2000; Jetana *et al.*, 2010). It is reported to be highly digestible, having high total sugar (10.00–17.30%) and protein (15.31–18.00%) content, is low in cost and is non toxic (Hosamani, 2005; Jetana *et al.*, 2010). However,

1 Graduate student, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10990, Thailand.

2 Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10990, Thailand.

3 Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

* Corresponding Author, e-mail: agrskp@ku.ac.th.

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some reports found that the pods comprised toxic substances such as tannins (Idowu *et al.*, 2006). Tannins can inhibit digestive enzymes, directly suppress rumen microbial growth and bind to the feed and hence inhibit its degradation in the rumen, though some animals are thought to be able to adapt to high tannin diets by producing proline-rich saliva proteins to bind the tannin and rumen microbes may also adapt to resist and degrade tannins (Hagerman and Butler, 1991).

A few studies have shown that these pods can be used as an energy and protein supplement for animal production systems in tropical climates. Rain tree pods (RTP) could substitute for 20% of the meal concentrate without affecting growth performance. However, substitution of concentrate with this pod at 30% was found to reduce goat weight gain (Thomas *et al.*, 1976). Regardless, goats fed with RTP could increase their dry matter intake (DMI), crude protein digestibility and total digestible nutrient (Hosamani *et al.*, 2005), but Thomas *et al.* (1976) found there was no significant change in the intake of nutrients in goats. Moreover, in heifers, substitution at 10 to 20% did not reduce the DMI and growth performance (Thole *et al.*, 1992). These studies generally showed that the pods had good potential as a feedstuff for animals. However, the benefits of pods as a feedstuff for livestock are less clearly understood. Hence, this study aimed to determine the potential of RTPs as a meal concentrate replacement by studying the *in vitro* fermentation using a gas production technique.

MATERIALS AND METHODS

Experimental design

The experiment was established as a completely randomized design with three replicates. The complete feed consisted of meal concentrate and roughage; feed was divided into two portions with 40% as meal concentrate (including RTPs combined with commercial meal concentrate) and 60% as roughage. The 40% meal concentrate was standardized to 100% and then the portions of meal concentrate and RTPs were calculated according to the treatments. The dietary treatments were 0, 20, 40, 60, 80 and 100% DM of RTP substituted in meal concentrate. Pangola hay (*Digitaria eriantha*) was used as the roughage source. All substrates were dried at 65 °C until a constant weight was recorded and then were ground to pass through a 1 mm sieve.

Chemical composition analysis

The substrates of RTP, hay and meal concentrate were analyzed for their dry matter (DM), crude protein (CP), ether extract (EE) and ash according to Association of Official Analytical Chemists (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the methods of Van Soest *et al.* (1991).

Preparation of inocula

Rumen fluid was obtained from two slaughtered cows at an abattoir in Prathum Thani province according to Chaudhry (2008). The rumen liquor was collected into a thermos flask that had been pre-warmed to 39 °C. Then, the inocula were prepared following the procedures of Menke and Steingass (1988). The ratio of artificial saliva to rumen fluid was 2:1. The substrate mixtures were individually weighed to approximately 200 mg and carefully dropped into each serum bottle and the actual weight recorded. After weighing, the bottles were put in an incubator at 39 °C. Thereafter, 30 mL of inoculum solution was added to each bottle. Then, they were incubated at 39 °C until the gas volume was recorded.

Recording gas production

During incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 66 and 72 hr. The cumulative gas production was fitted to the model of Ørskov and McDonald (1979) as shown in Equation 1:

$$Y = a + b(1 - e^{-ct}) \quad (1)$$

where Y is the volume of gas (mL per 200mg DM) produced at time (t), a is the gas production from the immediately soluble fraction (milliliters), c is the gas production rate constant for the insoluble fraction (milliliters per hour), a + b is the potential gas production (milliliters) and t is the incubation time (hours).

Determination of *in vitro* true digestibility

At 48 hr post incubation, the samples were determined for *in vitro* true digestibility (IVTD) according to Van Soest and Robertson (1985). The sample from the whole treatment was transferred quantitatively to a spoutless beaker by repeated washing with 100 mL neutral detergent solution. Then, the sample was dried at 100 °C for 5 hr and the actual weight was recorded. The DM content of the residues was weighed and the IVTD calculated using Equation 2:

(IVTD) =

(DM of feed taken for incubation – NDF residue) /

(DM of feed taken for incubation) × 100 (2)

Measurement of volatile fatty acid and ammonia nitrogen

At 12 hr post incubation, the samples were analyzed for volatile fatty acids (VFAs) and ammonia nitrogen (NH_3-N). First, 5 mL of H_2SO_4 (1M) was added to each sample. The mixture was centrifuged at 16,000×*g* for 15 min and the supernatant was removed and stored at -20 °C prior to analysis of the VFAs and NH_3-N. The samples were analyzed for VFAs with acetate, propionate and butyrate using high performance liquid chromatography according to Samuel *et al.* (1997). NH_3-N was analyzed according to Bremner and Keeney (1965).

Statistical analysis

Data obtained were subjected to analysis of variance using the SAS package (SAS, 1996). Where significant differences occurred, the means were separated using Duncan's multiple range test at a level of $P < 0.05$.

RESULTS AND DISCUSSION

The chemical composition of the substrates is presented in Table 1. Diets containing high levels of RTPs had a slightly higher ADF but a lower NDF. The CP contents of each feed ranged from 16.08 to 16.76%. The compositions were similar to those reported by Thole *et al*. (1992) and Hosamani *et al.* (2005). In the current study, the pods contained 18.8% of CP which was

Table 1 Chemical composition in meal concentrate (dry matter basis) using different rain tree pod (RTP) levels.

Chemical composition (%DM)	Percentage of RTP replacement					
	control	20	40	60	80	100
Organic matter (OM)	92.03	93.72	93.30	92.87	92.45	94.15
Crude protein (CP)	16.76	16.25	16.72	16.40	16.08	16.36
Ether extract (EE)	2.67	1.45	2.43	2.18	1.94	1.69
Ash	7.97	6.28	6.70	7.13	7.55	5.85
Neutral detergent fiber (NDF)	51.30	47.57	48.50	49.43	50.37	46.63
Acid detergent fiber (ADF)	31.50	37.53	31.02	34.51	38.01	64.04
Acid detergent lignin (ADL)	6.69	5.79	6.01	6.24	6.46	5.57
Nitrogen free extract (NFE)	53.49	52.28	50.52	50.71	51.01	52.26

DM = Dry matter. %NFE = %DM – (%CP+%CF+%EE+%Ash)

lower than the 24.50% reported by Babayemi *et al.* (2010). In the current study, NDF, ADF and lignin had lower values than those reported by Babayemi *et al.* (2010). The nutritional value of the pods depends on the soil, pod age and the age of the tree producing the pod (Durr, 2001).

The cumulative gas production for each treatment was plotted as a cumulative gas curve (Figure 1) and the values for the estimated parameters obtained from the kinetics of gas production are shown in Table 2. The gas volumes at 4, 6, and 8 hr after incubation were significantly different among treatments. In each period the highest cumulative gas value was found for the 40% pod replacement level. The cumulative gas production was lowest in the control group. However, the cumulative gas production levels from 10 to 48 hr were not significantly different. The 40% pod replacement substrates showed the highest degradability during the first 8 hr of incubation.

The highest potential levels of gas production at 72 hr ranged from 67.3 to 70.0 mL per 200mg DM. The intercept value (a) for the different treatments representing the gas production from soluble fractions ranged from 1.74 to 4.58 and was significantly different among treatments, whereas the gas production from the insoluble fraction (b), the potential extent of gas production (a + b) and the rate constants of gas production for the insoluble fraction (c) were not significantly different. These results may have been due to the residual fractions being less readily available to the microbes in the rumen (Hall, 2000). The low values of the estimated parameters can also be attributed to the high fiber content prevalent in the hay which was used in this study. Moreover, gas production is a good estimate of feed degradation which in turn is a good parameter to predict digestibility, the fermentation of end products and microbial protein synthesis of microbes *in vitro* (Bergman, 1990).

The effects of replacing concentrate with RTPs on the *in vitro* true digestibility (IVTD), volatile fatty acids (VFAs) as acetate (C_2), propionate (C_3), butyrate (C_4) and ammonia

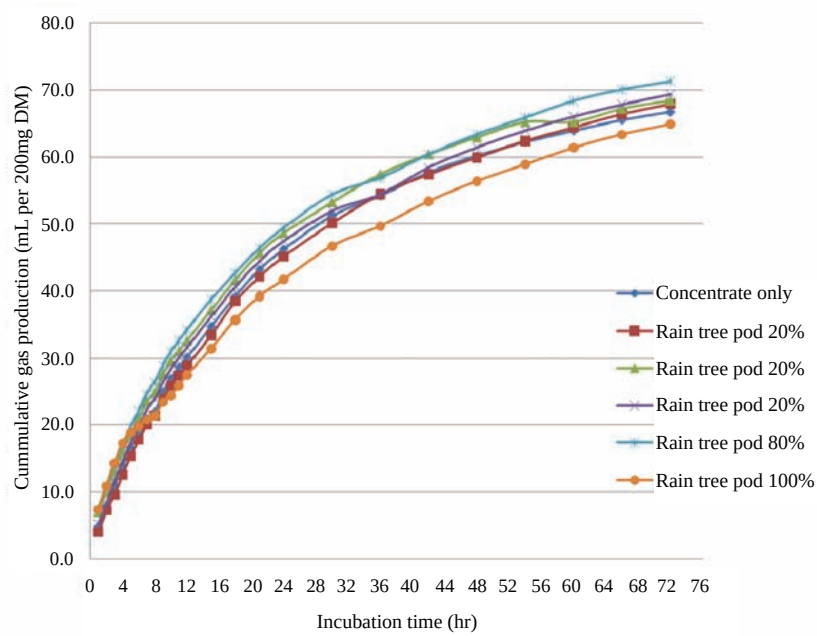

Figure 1 Cumulative gas production of different levels of rain tree pod replacement for each treatment at different times of incubation

nitrogen (NH_3-N) are shown in Table 3. The results showed that the IVTD was significantly highest in the control followed by the levels of 20, 40 and 60% of RTP replacement (74.31, 73.25, 73.30 and 72.30%, respectively). The replacement levels of 80 and 100% had the lowest IVTD values (62.29 and 65.09%, respectively). The lowest IVTD may have been due to the presence of tannins although there was also a high crude protein level. It is generally known that tannins have anti-nutritional

Table 2 Gas volume and kinetic parameter values from fermentation with rain tree pod (RTP) replacement in meal concentrate

Incubation time (hr)	Percentage of RTP replacement in meal concentrate						SEM
	control	20	40	60	80	100	
Gas volume (mL per 200 mg DM)							
2	7.63	7.93	10.63	8.47	10.00	10.00	0.77
4	13.73^{c}	13.37^{c}	16.57^{a}	14.70^{abc}	16.13^{ab}	16.33^{ab}	0.79
6	18.63^{b}	18.27^{b}	21.70^{a}	19.77^{ab}	21.27^{a}	20.07^{ab}	0.73
8	21.97^{b}	22.17^{b}	25.67^{a}	23.67^{ab}	25.27^{a}	23.10^{ab}	0.82
10	27.33	26.57	30.23	27.90	29.60	27.10	0.93
12	30.63	29.67	33.30	31.20	32.73	30.23	0.95
24	46.87	45.57	49.03	47.20	48.20	45.03	1.04
48	61.20	59.83	63.23	61.57	62.60	59.67	0.98
72	67.77	67.27	69.97	69.47	70.50	67.93	1.02
Gas production parameter							
a (mL)	1.74^{c}	2.08^{bc}	4.03^{ab}	2.81^{bc}	3.97^{ab}	4.58^{a}	0.64
b (mL)	67.28	66.53	66.40	67.57	67.00	65.04	0.88
a+b (mL)	69.02	68.61	70.43	70.38	70.98	69.62	0.77
c (hr^{-1})	0.05	0.04	0.05	0.05	0.05	0.04	0.00

DM = Dry matter, SEM = standard error of the mean.

$^{a, b, c}$ = Means with different lowercase superscripts in a row differ significantly ($P < 0.05$)

Table 3 Comparison of *in vitro* true digestibility (IVTD), volatile fatty acids (VFAs) and ammonia nitrogen of different levels of RTP (rain tree pod) replacement in meal concentrate.

Parameter	Percentage of RTP replacement in meal concentrate						SEM
	Control	20	40	60	80	100	
IVTD (%)	74.31^{a}	73.25^{a}	73.30^{a}	72.30^{a}	62.29^{b}	65.09^{b}	2.11
TVFAs ($mM.L^{-1}$)	29.39^{b}	29.35^{b}	39.31^{a}	39.90^{a}	25.27^{b}	33.48^{ab}	0.98
Acetate (%)	67.08^{c}	69.44^{b}	70.13^{ab}	71.24^{a}	70.40^{ab}	70.02^{ab}	0.44
Propionate (%)	22.38^{a}	21.60^{b}	21.10^{c}	20.90^{c}	21.74^{b}	21.87^{b}	0.15
Butyrate (%)	10.54^{a}	8.96^{b}	8.76^{b}	7.86^{c}	7.85^{c}	8.12^{bc}	0.31
C2:C3 (%)	2.99^{c}	3.22^{b}	3.33^{ab}	3.41^{a}	3.24^{b}	3.21^{b}	0.14
Ammonia nitrogen ($mg.dL^{-1}$)	20.41^{a}	19.67^{a}	20.41^{a}	20.42^{a}	16.95^{b}	16.65^{b}	0.33

SEM = Standard error of the mean.

$^{a, b, c}$ = Means with different lowercase superscripts in a row differ significantly ($P < 0.05$).

effects particularly in the rumen and the main effects of tannins appear to be attributable to their protein-binding capacity (Turner *et al*., 2005). Barry *et al*. (1986) reported that a reduction in the digestibility of fiber, protein and some other nutrients in the rumen was caused by the presence of tannins in the diet.

The fermentation end products of microbial digestion are VFAs which are absorbed from the rumen and serve as a source of energy for ruminants (Sutton *et al*., 1993). The concentrations of C_2, C_3 and C_4 were significantly different among treatments. The group replacement with 60% RTPs produced the highest C_2 concentration (71.24%) but there was no significant difference ($P > 0.05$) between the replacement groups with 40, 80 and 100% pods (70.13, 70.40 and 70.02%, respectively). However, the C_2 concentration was higher than the group replacement with 20% RTPs (69.44%) and the control group (67.08%). These results may be attributed to the high neutral detergent soluble fiber fraction in those groups. The C_2 concentration was lowest in the control group (67.08%), since this group had a low fiber content causing the C_2 concentration to decrease because fiber degradation is related to increased C_2 production (Murphy *et al*., 1982).

The concentration of C_3 in the control group was significantly higher (22.38%) than in the other treatments, perhaps due to the high level of soluble carbohydrate in the meal concentrate. Treatments involving meal replacement with 40 and 60% RTP levels had significantly lower C_3 concentrations than those treatments involving replacement with 20, 80 and 100% RTP levels and this may have been associated with an increase in the readily degradable starch content in the diets with pod supplementation resulting in a higher propionate level and decreased rumen acetate concentrations (Sutton *et al*., 1993). The low C_3 concentration could have an effect on animal productivity. Furthermore, C_3 has been shown to be the major glycogenic fatty acid in ruminants (Preston and Leng, 1987). Thus, high C_3 levels can indicate a high level of soluble fractions in the feed. The value of C_4 in the control group was significantly higher than in the other groups. In fact, C_4 was present in large concentrations. However, the C_2 and C_3 ratio in the control group was significantly lowest. Under optimal rumen fermentation conditions, the C_2 and C_3 ratio should be greater than 2.2:1 (Tagang *et al*., 2010). Thus, in the current study, the C_2 and C_3 ratio in all diets was higher than suggested by the literature.

The values of NH_3-N in the control group and replacement at 20, 40 and 60% RTP levels (20.41, 19.67, 20.41 and 20.42 $mg.dL^{-1}$, respectively) were significantly higher than in the other groups. The control group and replacement at 20, 40 and 60% RTP levels were not significantly different. The NH_3-N concentration in the treatments with replacement with 80 and 100% RTP levels was lower than in the other treatments; this may have been due to the presence of tannins which may inhibit protein digestion (Hosamani *et al*., 2005). In addition, the NH_3-N concentration is related to the protein content in feed because rumen microorganisms require ammonia for the growth and synthesis of microbial proteins (Eschenlauer *et al*., 2002). The extent of this conversion depends on a variety of factors including the solubility of the proteins, their resistance to breakdown and the rate of passage of the feed through the rumen (Eschenlauer *et al*., 2002). Hennessey (1996) suggested the provision of a readily fermentable source of nitrogen for ammonia production to ensure adequate ruminal microbial fermentation.

CONCLUSION

Based on this study it can be concluded that RTPs could be substituted in meal concentrate up to 60% without any negative effect on the IVTD and NH_3-N concentration. However, the C_2 concentration in the group that replaced the RTPs was higher than in the control group, while

the C_3 and C_4 concentrations were low with RTPs replacement. This study showed the potential of the incorporation of RTPs in the diet of ruminants to replace meal concentrate, especially with dairy cattle to improve the milk fat content, since the RTP substitution in meal concentrate increased the C_2 concentration without affecting the digestibility of the feed. However, further investigation of RTP replacement in feeding trials should be performed.

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