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Apparent Digestibility, Nitrogen Balance, Ruminal Microbial Nitrogen Production and Blood Metabolites in Thai Brahman Cattle Fed a Basal Diet of Rice Straw and Supplemented with Some Tropical Protein-rich Trees

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ABSTRACT : The effects of four types of tropical protein-rich trees on nutrient digestibility, nitrogen (N) balance, urinary purine derivative (PD) excretion and blood metabolites in four Thai Brahman cattle (290±2.5 kg) were studied. The animals were fed twice daily, with each feeding consisting of 1 kg (fresh weight) rice straw and one of the four dietary supplements: i) 1.98 kg oven-dried rain tree pods (RTP) and 20 g premix (RTPP), ii) 980 g RTP and 1 kg sun-dried leucaena leaves and 20 g premix (LLRT), iii) 980 g RTP and 1 kg sun-dried cassia leaves and 20 g premix (CLRT) and iv) 980 g RTP and 1 kg sun-dried mulberry leaves and 20 g premix (MLRT). The apparent dry matter (DM) and organic matter (OM) digestibilities were higher ($p<0.05$) in cattle fed the CLRT supplement than in those fed the other supplements, whilst the apparent digestibility of neutral detergent fibre (NDF) was higher ($p<0.05$) in cattle fed the CLRT and MLRT supplements than in those fed the other supplements. The N-balance of cattle fed LLRT and CLRT supplements was higher ($p<0.05$) than in cattle fed RTPP and MLRT supplements, whilst the apparent digestibility of N was highest ($p<0.05$) in cattle fed RTPP supplement, compared to the other supplements. Allantoin and PD excretion in the urine, and the ratios of allantoin/DOMI and PD/DOMI were higher ($p<0.05$) in cattle fed RTPP and MLRT than for those fed LLRT and CLRT supplements. Plasma β-hydroxy butyrate (β-HBA) and insulin concentrations were higher ($p<0.05$) in cattle fed RTPP supplement than in those fed the other supplements. The study demonstrated the value of using local multipurpose trees (MPTs) to improve Brahman cattle feeding systems in the tropics. (**Key Words :** Brahman Cattle, Cassia, Leucaena, Mulberry, Rain Tree Pod, Rice Straw)

INTRODUCTION

There are several species of multipurpose trees (MPTs) in tropical areas and some of them may be suitable for improving livestock production systems. A few *in vivo* studies have shown that the pod of the rain tree (*Samanea saman*) can be used as an energy and protein supplement for animal production systems in tropical climates, due to it being highly digestible and containing high total sugar (18-30%) and protein (16-30%) (Jetana et al., 2007; 2008). In Thailand, *Leucaena leucocephala* is used as a protein supplement for improvement of beef cattle and dairy cow production. Usually, the leucaena is cut and sun-dried for use in cut and carry feeding systems. Sun-drying helps to alleviate the high anti-nutritional compounds found in the plant and restricting intake to less than 30-35% of total dry matter intake (DMI) also minimises the effects of these compounds on animal performance (Hammond, 1995; Yami et al., 2000). Mulberry (*Morus alba*) leaves are highly palatable and digestible (70-90%) to herbivorous animals and can also be fed to monogastrics (Nulchuen et al., 2003). Protein content in leaves and young stems, with a good essential amino acid profile, varies from 15-28% depending on variety (Sánchez, 2000). Mineral content is high and no anti-nutritional factors or toxic compounds have been identified (Sánchez, 2000). *Cassia siamea* Lam. (Fabaceae) is a medium sized legume tree in the tropics. Its leaves contain high levels of protein (FAO, 1999). Cutting and drying (cut and carry; sun-dried) these leaves, as is

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commonly done with leucaena, and mixing with other ingredients may be another useful way improve livestock production.

Thus, the present study was undertaken to determine which of these MPTs are suitable to be fed as a protein supplement to Thai Brahman cattle fed a basal diet of rice straw. The objectives of the experiments reported in this paper were to determine and compare the effects of supplementation with either the leaves of three MPTs or rain tree pods on whole tract apparent digestibility of DM, OM and fibre, N balance, ruminal microbial production and some blood metabolite values.

MATERIAL AND METHODS

Animals, diets and experimental design

Four male, Brahman cattle with an average weight of 290±2.5 kg (24-30 month old) were purchased from the Tab-Kwang Livestock Research and Breeding Centre, Department of Livestock Development, Saraburi Province (150 km from Bangkok) and were used in this study. The animals were transported to the Animal House, Department of Animal Husbandry, Faculty of Veterinary Science, Nakorn-pathom (50 km from Bangkok), where they were housed in individual pens. The experimental design was a double 4×4 complete Latin square design (4 supplements and 4 animals). The animals were allocated to one of four supplemental diets consisting of (kg/d/animal): i) 3.96 kg oven dried rain tree pods (RTP) and 40 g premix (RTPP), ii) 1.96 g oven-dried rain tree pods and 2 kg sun-dried leucaena leaves and 40 g premix (LLRT), iii) 1.96 g oven-dried rain tree pods and 2 kg sun-dried cassia leaves and 40 g premix (CLRT) and iv) 1.96 g oven-dried rain tree pods and 2 kg sun-dried mulberry leaves and 40 g premix (MLRT).

The leucaena and cassia leaves, and the rain tree pods were purchased in one amount, and enough for the whole experiment from the farmer at Kasetsert University, Kampangseng Campus, Nakhonpathom Province (80 km from Bangkok); the mulberry leaves were purchased from a farmer in Ratchaburi Province (100 km from Bangkok). The leucaena, cassia and mulberry leaves were chopped into 6-8 mm pieces and dried by the sun 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. Rice straw was purchased from the Department of Animal Husbandry, Faculty of Veterinary Science, and Chulalongkorn University.

Each of the Brahman cattle was fed (as fed basis) twice daily with 2 kg of the respective supplements and 1 kg rice straw. The experiment consisted of four 21-d periods; each comprising 14 d dietary adaptation and 7 d sample collection. On day 1 and 21 of each period, the animals were weighed before the morning feed (06:00 h), and on day 12 of each period, the animals were transferred to individual metabolism crates for collection of urine and faeces (days 15-21).

Whole tract *in vivo* digestibility measurements

Whole tract *in vivo* digestibility was determined by collecting all the faeces from days 15-21 of the sample collection period and faecal samples were stored at -20°C. No refused feed was found throughout the experiment. 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 DM, ash, N and NDF content.

Urine collection

Daily total urine output was collected into a plastic bag containing 100 ml of 20% (v/v) H_2SO_4, in order to maintain a final pH below 3. The urine was collected at 24-h intervals for 7 d (days 15-21). The volume of the acidified urine was immediately measured and, two sub-samples taken; i) 20 ml was diluted 5 times, then stored at -20°C prior to PD analysis and ii) 50 ml of the original acidified urine was kept at -20°C for determination of total N.

Blood collection

On day 21 of each experimental period, blood samples were taken from the jugular vein at 0 h and at 3, 6 and 9 h after the morning feed. The samples were drawn using a 2-way blood collection needle (Vacuette® Austria, Model 18 G×1 1/2) and transferred into two heparinised vacutainers (9 ml/tube) and one tube containing sodium fluoride and potassium oxalate (for analysis of non-esterified fatty acids). The tubes were gently inverted a couple of times and immediately centrifuged at 2,500 g for 25 min. Individual plasma samples were stored in tubes (3-3.5 ml/tube) at -20°C for analysis.

Analytical methods

The DM content of the feed and faecal samples was determined by drying 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 combustion in a muffle furnace at 550°C for 8 h. The OM was calculated by subtracting the ash from the DM content. The N content in the feed, the urine and the faecal samples was determined by the micro Kjeldahl method (AOAC, 2000 method no.995.04). NDF was analysed according to Van Soest et al. (1991; structure A). Gross energy (GE) was determined by using an Auto Bomb Calorimeter LECO model AC-350 (Corporation, USA).

Total phenolic compounds, condensed tannins and total sugar determinations

The content of total phenolic compounds and condensed

tannins in RTP, leucaena, cassia and mulberry leaves, and rice straw were assayed by Folin-Ciocalteu 2 N (Sigma® Aldrich) and butanol-HCl reagent according to the procedures of Makkar (2000). Total non-structural carbohydrates in the feed were determined according to Nelson's reducing sugar procedure (Hodge and Hofreiter, 1962).

Purine derivatives determination

Allantoin was determined according to the method described by Young and Conway (1942) which is based on the Rimini-Schryver reaction. Determination of uric acid was carried out by its reaction with uricase using a commercial kit (Uric acid Liquicolor, Human GMbH-D 65205). Purine derivatives in the urine were measured as allantoin and uric acid and expressed in terms of mmol/d.

Blood metabolites determination

Plasma glucose concentrations were measured after deproteinisation by an enzymatic colorimetric test using a commercial kit (Glucose liquicolor, Human GMbH-D 65205 Wiesbaden, Germany). Plasma urea nitrogen (PUN) was measured by a fully enzymatic method using a commercial kit (Urea liquiUV, Human GMbH-D 65205 Wiesbaden, Germany). Plasma insulin concentrations were determined by radioimmunoassay, according to the recommend procedures supplied by the kit manufacturer (Coat-A-Count®-Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma non-esterified fatty acid (NEFA) concentration was analysed using a commercial diagnostic kit (No. 279-75401, Wako Pure Chemical Ind. Osaka, Japan). Plasma β-HBA was analysed using a commercial diagnostic kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, UK, BT29 4QY).

Digestibility measurements of nutrients

According to MAFF (1983), the digestibility coefficients of nutrients can be calculated as:

$$\text{Digestibility coefficient of the DM} = \frac{\text{Intake DM (g/d) - Faecal DM (g/d)}}{\text{Intake DM (g/d)}}$$

Using the same procedure (by replacing DM with OM, NDF and N), the digestibility coefficients of OM, NDF and N were calculated.

N-balance measurement

Nitrogen balance calculations were also carried out using the simple relationship now accepted as a basis for nutrient balance research in all mammals (Maynard and Loosli, 1969) :

$$N_{\text{animal products}} = N_{\text{feed}} - N_{\text{excreted}}$$

and modified in the present study as:

N-balance (g/d) = Intake N (g/d)-faecal N (g/d)-urine N (g/d)

Statistical analysis

The means of each parameter measured in this study were analysed by Analysis of Variance (ANOVA) using the procedures of the Statistical Analysis System Institute (SAS 1998). The differences between means were compared by a least significant difference method (LSD).

RESULTS

The chemical composition of the diets

The ingredients (DM basis) and chemical composition of the feeds are presented in Table 1. The rice straw was used as the basal diet and three different sun-dried leaves of MPTs mixed with RTP were used as protein supplements. The rice straw contained 887 g DM/kg and on g/kg DM basis, it contained 0.81 g N, 812 g NDF and 15.0 mega joules (MJ) GE. The GE of rice straw and supplements was measured in a bomb calorimeter as MJ/kg DM of feed. The DM content of all supplements was similar (885-895 g/kg fresh weight), and the N contents of RTPP, LLRT, CLRT and MLRT supplemental diets were quite similar (19.9-21.4 g/kg DM). However, the content of condensed tannins varied widely among the different MPTs, ranging from 56.0, 75.4, 79.0 and 95.6 g/kg DM in MLRT, LLRT, RTPP and CLRT, respectively. Similarly, the sucrose content also varied among the different types of MPTs, ranging from 80.9, 81.5, 95.4 and 151 g/kg DM in CLRT, LLRT, MLRT and RTPP, respectively. However, the GE of RTPP, LLRT, CLRT and MLRT supplemental diets were quite similar, within 19.6-19.9 MJ/kg DM.

Whole tract *in vivo* digestibility

As shown in Table 2, the apparent whole tract digestibility of DM and OM was higher ($p<0.05$) in cattle consuming the CLRT supplement (0.55, 0.58) than in those supplemented with RTPP (0.52, 0.54), LLRT (0.52, 0.55) and MLRT (0.51, 0.54). NDF digestibility was lower ($p<0.05$) in cattle supplemented with RTPP (0.30) than in those fed LLRT supplement (0.41); with no difference in NDF digestibility in cattle fed either CLRT (0.50) or MLRT (0.48) supplements.

Nitrogen balances

As shown in Table 3, N intake was similar in cattle fed LLRT, CLRT and MLRT supplements (1.238, 1.253 and 1.249 g N/kg $BW^{0.75}$/d, respectively), but was lower in animals fed the RTPP supplement(1.184 g N/kg $BW^{0.75}$/d).

Table 1. Chemical analysis of the ingredients and experimental diets (g/kg DM)

	Rain tree pod (RTP)	Sun-dried leucaena leaves (LL)	Sun-dried cassia leaves (CL)	Sun-dried mulberry leaves (ML)	Rice straw
DM	894	922	914	894	887
Ash	52.4	62.2	77.6	49.3	98.6
N	20.1	22.9	21.9	23.0	8.10
Crude protein (N×6.25)	125	143	137	144	50.6
NDF	371	632	608	595	812
Phenolic compounds	194	219	137	96.2	63.0
Condensed tannins	79.0	72.5	113	33.7	13.8
Total sugar	338	48.1	41.3	121	0
Reducing sugar	172	34.5	29.0	79.6	0
Sucrose	151	13.6	12.4	41.5	0
Gross energy (MJ/kg)	20.0	20.0	19.5	20.0	15.0

Ingredients and chemical analysis of supplements (g/kg DM)				
Supplements	RTPP	LLRT	CLRT	MLRT
Rain tree pod	990	490	490	490
Sun-dried leucaena leaves	-	500	-	-
Sun-dried cassia leaves	-	-	500	-
Sun-dried mulberry leaves	-	-	-	500
Premix [1]	10	10	10	10
Chemical analysis (g/kg DM)				
DM	885	899	895	882
Ash	51.9	56.8	64.5	50.4
NDF	367	498	486	479
N	19.9	21.3	20.8	21.4
Crude protein (N×6.25)	124	133	130	134
Phenolic compounds	194	219	165	144
Condensed tannins	79.0	75.4	95.6	56.0
Total sugar	335	190	187	226
Reducing sugar	172	102	99.6	125
Sucrose	151	81.5	80.9	95.4
Gross energy (MJ/kg DM)	19.9	19.9	19.6	19.8

[1] Contained (g/kg DM basis): vitamin A 40,000,000 unit, vitamin D_3 4,000,000 unit, vitamin E 40,000 Unit, vitamin B_{12} 0.02 g, Mn 160 g, Fe 240 g, Zn 100 g, Cu 20g, Se 0.5 g, Co 2 g and I 5 g.

Table 2. Intakes of DM, OM and NDF and the digestibility coefficients of DM, OM and NDF in Brahman cattle fed a basal diet of rice straw supplemented with some tropical protein-rich trees

	Body weight (kg)	$BW^{0.75}$ (kg)	Intake (g/kg $BW^{0.75}$)			Coefficient of digestibility (decimal)		
			DM	OM	NDF	DM	OM	NDF
RTPP	286±3.2	69.6±0.6	77.3^{a}	66.1^{a}	56.0^{a}	0.52^{b}	0.54^{b}	0.30^{c}
LLRT	294±5.7	70.9±1.0	77.1^{a}	67.8^{a}	52.3^{a}	0.52^{b}	0.55^{b}	0.41^{b}
CLRT	287±5.6	69.6±1.0	78.0^{a}	68.4^{a}	54.9^{a}	0.55^{a}	0.58^{a}	0.50^{a}
MLRT	294±5.3	70.8±1.0	77.4^{a}	66.6^{a}	52.2^{a}	0.51^{b}	0.54^{b}	0.48^{a}
SED [1]			1.49	1.29	2.90	0.02	0.02	0.03

[1] Standard error of mean.
Values within the same column with different superscripts are significantly ($p<0.05$) different.

Table 3. Effect of diets on N intake, urinary N, faecal N, N-balance and N digestibility in Brahman cattle fed a basal diet of rice straw supplemented with some tropical protein-rich trees

	N-intake (g/kg $BW^{0.75}$/d)	Faecal-N (mg/kg $BW^{0.75}$/d)	Urinary N (mg/kg $BW^{0.75}$/d)	N-balance (mg/kg $BW^{0.75}$/d)	N digestibility (g/kg $BW^{0.75}$/d)
RTPP	1.184[a]	898[a]	139[ab]	147[c]	759[a]
LLRT	1.238[a]	733[b]	127[b]	378[a]	594[b]
CLRT	1.253[a]	634[c]	156[a]	463[a]	507[c]
MLRT	1.249[a]	767[b]	119[b]	362[b]	616[b]
SED[1]	0.02	30.4	11.9	32.8	23.2

[1] Standard error of mean.
Values within the same column with different superscripts are significantly ($p<0.05$) different.

Faecal-N excretion was lower ($p<0.05$) in cattle supplemented with CLRT (634 mg N/kg $BW^{0.75}$/d) than in those fed LLRT and MLRT supplements (733 and 767 mg N/kg $BW^{0.75}$/d, respectively), and the faecal-N of animals supplemented with RTPP (898 mg N/kg $BW^{0.75}$/d) was higher ($p<0.02$) than in animals fed the other supplements (634-767 mg N/kg $BW^{0.75}$/d). The urine-N of cattle supplemented with CLRT (156 mg N/kg $BW^{0.75}$/d) was higher ($p<0.05$) than in those supplemented with LLRT and MLRT (127 and 119 mg N/kg $BW^{0.75}$/d, respectively); however, the urine-N of animals fed RTPP (139 mg N/kg $BW^{0.75}$/d) and CLRT supplements was not different. The N-balance of cattle that consumed the RTPP supplement (147 mg N/kg $BW^{0.75}$/d) was lower ($p<0.05$) than in those that consumed LLRT, CLRT and MLRT supplements (378, 463 and 362 g N/kg $BW^{0.75}$/d, respectively). N-balance was not different between animals fed LLRT and CLRT, but was higher than for those supplemented with MLRT. There was no difference in N digestibility in animals supplemented with LLRT and MLRT (594 and 616 g N/kg $BW^{0.75}$/d, respectively), and these were higher than for those supplemented with CLRT (507 g N/kg $BW^{0.75}$/d). However, the N digestibility of animals supplemented with RTPP (759 g N/kg $BW^{0.75}$/d) was higher ($p<0.05$) than in those supplemented with LLRT, CLRT and MLRT.

Urinary purine derivatives and the ratios of allantoin/ DOMI and PD/DOMI

Urinary allantain excretion of Brahman cattle supplemented with CLRT (0.593 mmol/kg $BW^{0.75}$/d) was lower ($p<0.05$) than in those supplemented with RTPP and MLRT (0.818 and 0.790 mmol/kg $BW^{0.75}$/d, respectively). Urinary allantoin excretion of cattle supplemented with LLRT (0.654 mmol/kg $BW^{0.75}$/d) and CLRT (0.593 mmol/kg $BW^{0.75}$/d) was not different (see Table 4). Urinary uric acid excretion was higher ($p<0.05$) in cattle supplemented with RTPP (0.141 mmol/kg $BW^{0.75}$/d) than in those supplemented with LLRT and CLRT (0.081 and 0.068 mmol/kg $BW^{0.75}$/d, respectively), but no differences were detected in the urinary uric acid excretion among cattle supplemented with LLRT, CLRT and MLRT (0.081, 0.068 and 0.103 mmol/kg $BW^{0.75}$/d, respectively). However, the urinary PD excretion of cattle supplemented with RTPP (0.960 mmol/kg $BW^{0.75}$/d) was higher ($p<0.05$) than in those supplemented with LLRT and CLRT (0.735 and 0.661 mmol/kg $BW^{0.75}$/d, respectively). There was no difference in urinary PD excretion between cattle supplemented with LLRT and CLRT. The urinary PD excretion of cattle supplemented with MLRT (0.894 mmol/kg $BW^{0.75}$/d) was higher ($p<0.05$) than in those supplemented with LLRT and CLRT (0.735 and 0.661 mmol/kg $BW^{0.75}$/d, respectively). The ratios of allantoin/DOMI (23.1 mmol/kg DOMI/d) of cattle supplemented with RTPP and MLRT were higher ($p<0.05$) than for those supplemented with LLRT and CLRT (17.9 and 15.3 mmol/kg DOMI/d, respectively), but the ratio of allantoin/DOMI of cattle fed either LLRT or CLRT supplements was not different. The ratios of PD/DOMI (27.0, 26.1 mmol/kg DOMI/d) were higher ($p<0.05$) in

Table 4. Effect of diets on allantoin, uric acid and purine derivatives and the ratios of allantoin/DOMI and PD/DOMI in Brahman cattle fed a basal diet of rice straw supplemented with some tropical protein-rich trees

	Allantoin (mmol/kg $BW^{0.75}$ /d)	Uric acid (mmol/kg $BW^{0.75}$ /d)	PD (mmol/kg $BW^{0.75}$ /d)	Allantoin/DOMI (mmol/kg/d)	PD/DOMI (mmol/kg/d)
RTPP	0.818[a]	0.141[a]	0.960[a]	23.1[a]	27.0[a]
LLRT	0.654[bc]	0.081[b]	0.735[bc]	17.9[b]	20.0[b]
CLRT	0.593[c]	0.068[b]	0.661[c]	15.3[b]	17.1[b]
MLRT	0.790[ab]	0.103[ab]	0.894[ab]	23.1[a]	26.1[a]
SED[1]	0.070	0.023	0.084	2.07	2.44

[1] Standard error of mean.
Values within the same column with different superscripts are significantly ($p<0.05$) different.

cattle fed RTPP and MLRT supplements than in those supplemented with LLRT and CLRT (20.0 and 17.1 mmol/kg DOMI/d, respectively), but the ratio of PD/DOMI of animals fed LLRT and CLRT supplements was similar.

Blood metabolites

Blood metabolite concentrations are presented in Table 5; diet had no effect ($p>0.05$) on plasma urea-N, glucose and NEFA concentrations. There were also no differences in plasma β-HBA and insulin concentrations in cattle fed LLRT, CLRT and MLRT supplemental diets. However, plasma β-HBA and insulin concentrations were higher ($p<0.05$) in cattle supplemented with RTPP than in those fed LLRT, CLRT and MLRT supplemental diets.

DISCUSSION

In general, carbohydrates are the largest component in tropical forages and contribute 60-70% of the net energy used for maintenance and production in ruminants. Carbohydrates generally can be divided into fibre and non-fibre components. The fibre or structural portion, commonly called neuteral detergent fibre (NDF), includes cellulose, lignin and hemicellulose. The NDF fraction represents the slower digestible fibrous portion of the diet. In the present study, fibre was determined by the NDF analytical method, this may be sufficiently representative of fibre in a dietary study. Due to NDF including both cellulose and hemicellulose, these are major components of the fibre fraction (Beever, 1993). This analytical method also matches with rice straw containing high fibre and low protein, which was used as a basal diet in this study. On the other hand, non-structural or non-fibre carbohydrates (NFC) include starch, sugars and pectin. Non-structural carbohydrates represent the more rapidly digested fractions in the rumen. Glucose, fructose and sucrose make up almost all of the free sugar present in forage plants, and glucose and fructose and sucrose constitute most of the free reducing sugar (Pichard and Alcalde, 1992). Other free sugar such as maltose, melitose, melibiose, raffinose and stachyose occur in low quantities. Glucose and fructose are the principle monosaccharides present in plants, although always in low concentrations (10-30 g/kg DM), while sucrose may constitute the main fraction of reserve carbohydrate, reaching levels of 20-80 g/kg DM (Pichard and Alcalde, 1992). In the present study, NFC determined using reducing sugar seems unsuitable to leucaena, cassia and mulberry leaves, but this analytical method is appropriate for rain tree pods in which sugar is the main carbohydrate component (Staples and Elevitch, 2006). Due to leaves of MTPs not being high in starch content, NFC was determined using reducing sugar content.

Effects on whole *in vivo* digestibility

The digestibilities of DM and OM were highest in cattle fed the CLRT supplemental diet. This could have been due to the rate of supply of degradable N in the rumen synchronising with the available energy supply. However, NDF digestibility decreased in cattle supplemented with RTPP when compared with those fed the other supplements. This possibly arises from RTP, which contained highly available sucrose in the rumen (151 g/kg of DM supplement), more than the other supplements (80.9-95.4 g/DM kg); consequently pH in the rumen could be decreased and cellulolytic microbial activity might be constrained. Hoover (1986) demonstrated that reduced pH in the rumen has a major impact on fibre digestion. This is in agreement with studies (Smith et al., 1973, Stewart, 1977; Mould and Ørskov, 1983) reporting the depression of cellulolytic microbial activities at low rumen pH. Similar observations of a reduction of NDF digestion in the rumen were reported by England and Gill (1985), Huhtanen, (1987) and Khalili and Huhtanen (1991) in cattle fed grass silage-based diets supplemented with sugar. It is possible that in cattle fed RTPP supplement, the reduction in NDF digestion in the whole digestive tract resulted from the increase in passage of NDF from the rumen and the whole digestive tract. This is attributed to the fact that there was a decrease in the rate of digestion and a longer lag time (Khalili and Huhtanen, 1991). This longer lag time may be explained by utilisation of soluble carbohydrates (sugar) before the degradation of fibre by rumen micro-organisms

Table 5. Effect of diets on blood metabolite concentration in Brahman cattle fed a basal diet of rice straw supplemented with some tropical protein-rich trees

	Urea-N (mg/dl)	Glucose (μmol/ml)	NEFA[2] (μmol/L)	β-HBA[3] (μmol/L)	Insulin (μ.i.u./ml)
RTPP	14.4[a]	72.9[a]	67.8[a]	1,378[a]	22.2[a]
LLRT	14.2[a]	77.0[a]	62.5[a]	868[b]	13.7[b]
CLRT	20.9[a]	73.6[a]	73.0[a]	982[b]	16.6[b]
MLRT	15.5[a]	73.8[a]	65.3[a]	1,007[b]	13.6[b]
SED [1]	3.62	2.87	17.1	120	2.35

[1] Standard error of mean. [2] Non-esterified fatty acids. [3] *beta*-hydroxybutyrate.
Values within the same column with different superscripts are significantly ($p<0.05$) different.

(Mertens, 1977).

NDF digestibility was also lower in cattle supplemented with LLRT than in animals fed CLRT and MLRT supplements, though the quantity of N intake in the rumen was not different. This may be due to the content of condensed tannins being higher in the LLRT supplement than in MLRT. It is generally known that tannins have anti-nutritional effects, particularly in the rumen. The main effects of tannins appear to be attributable to their protein-binding capacity. Barry and Duncan (1984) and Barry et al. (1986) reported reduced digestibility of fibre, protein and some other nutrients in the rumen in the presence of tannins in the diet.

Similarly, NDF digestibility was observed in animals fed with CLRT and MLRT supplements, but the intake of condensed tannins in animals fed CLRT and LLRT was not different. This may be due to differences in the types of condensed tannins and phenolic compounds in leucaena and cassia leaves. Generally, tannins are phenolic compounds that precipitate proteins, but not all phenolic compounds precipitate protein (Jansman, 1993). The capacity for protein binding differs with tannins from different sources. This is due to the tendency of tannins to form insoluble complexes with proteins being influenced by many factors such as the characteristics of tannins (molecular weight, structural heterogeneity), the protein source (degree of glycosylation, amino acid composition and molecular weight) and reaction conditions such as pH, temperature, reaction time, and relative concentrations of the reactants (Hagerman and Bulter, 1989).

N-balances

N balance was greater in cattle fed LLRT, CLRT and MLRT supplemental diets than those fed RTPP. This may imply that animals fed LLRT, CLRT and MLRT supplemental diets showed improved synchrony between carbohydrate and N compounds in the rumen, thereby decreasing faecal-N output. This was in agreement with Howard et al. (2007), who reported that a diet containing synchrony between carbohydrate and N compounds generally increased the N-balance.

In the present study, cattle fed LLRT and CLRT supplemental diets containing condensed tannins at about 6.13-7.65% of total DM intake improved the N balance. This differs slightly from other studies (Waghorn et al., 1987; Aerts et al., 1999; Mlambo et al., 2004) which reported that, when the proportions of condensed tannins ranged between 2-5% of total DM intake, beneficial effects on protein metabolism were achieved by reducing the fermentation of forage protein to ammonia in the rumen, increasing the quantity of protein digested in the small intestine and decreasing urinary N excretion. The other beneficial effects of dietary tannins appear to be more important for preventing excessive ruminal degradation of dietary proteins (McNabb et al., 1993) and improving N-balance and N digestibility (Waghorn et al., 1987; 1994; McNabb et al., 1996). Tannins also reduce the risk of bloating by binding proteins which are responsible for ruminal foam formation and decrease the activity of gas-producing microbes in the rumen (Mangan, 1988). Whilst N balance in animals fed LLRT, CLRT and MLRT supplemental diets was comparable, the results showed that N balance was higher in cattle fed with LLRT and CLRT supplemental diets than in those fed MLRT. This effect of the content of condensed tannins in LLRT and CLRT was higher than that in the MLRT supplemental diet.

In contrast, apparent N digestibility increased in cattle fed RTPP when compared with the other supplemental diets. The increased N digestibility may have been due to the high sucrose content in RTPP, consequently urinary N excretion in animals fed RTPP decreased when compared with animals fed the other supplemental diets. The increase in apparent ruminal N digestibility due to sucrose in RTPP was possibly due to decreased loss of ammonia-N passage through the rumen wall and increased hindgut fermentation (high faecal-N output). This was in agreement with Howard et al. (2007) and Owens et al. (2008) who reported that generally the digestibility of N increased when the animal was given a diet containing high sucrose.

Effects on urinary purine derivatives and the ratios of allantoin/DOMI and PD/DOMI

The measurement of PD excretion in urine is a convenient method which usually estimates microbial protein flowing from the rumen. The results of calculated microbial supply are expressed as a g microbial N per kg digestible organic matter intake (DOMI) (see calculations in Chen and Gomez, 1995). The present experiment showed that high condensed tannins in LLRT and CLRT diets depressed microbial production in the rumen (8.53, 6.21 g microbial N/kg DOMI, respectively) when compared to RTPP and MLRT diets (13.8, 13.4 g microbial N/kg DOMI, respectively). The low microbial yield in animals fed diets containing condensed tannins is not surprising, due firstly to less effective protein use for microbial protein synthesis as a result of formation of tannin-protein complexes. Secondly, low metabolisable energy (ME) use for microbial protein synthesis, according to ME values usually being different, depends on the content of condensed tannins, though in the present study gross energy contents determined in all supplemental diets were similar. This is in agreement with Barry and Duncan (1984), who showed that forages containing condensed tannins usually depress ME intake and digestion of OM. Thirdly, the effects of dietary condensed tannins probably inhibit some rumen bacteria (Waghorn, 2008). However, the RTPP supplemental diet

containing high sugar enhanced microbial supply to the small intestine (Chamberlain et al., 1993; Givens and Rulquin, 2004). The high microbial flow from the rumen in cattle fed the MLRT supplement diet may be attributed to the rate of supply of N degradability in the rumen balancing with the available energy supply (Puchala and Kulasak, 1992; Sinclair et al., 1993).

Effects on blood metabolites

None of the supplemental diets affected the concentrations of PUN, but the average value of 16.3 mg urea-N/dl in Brahman cattle fed different supplements generally was similar to the value (17.1 mg urea-N/dl) in animals fed *ad libitum* with rice straw alone as reported by Jetana et al. (2006).

ME intakes probably varied among diets as mentioned above, nevertheless, none of the supplemental diets affected plasma glucose and NEFA concentrations. However, the concentrations of β-HBA in plasma showed the highest level in cattle fed with the RTPP supplemental diet. This may stem from the high content of readily fermentable carbohydrate (sucrose) in rain tree pods; consequently the amount of butyrate in the rumen was increased. Then, butyrate arising from rumen fermentation was largely converted to β-HBA in the rumen epithelium (Bergman, 1990).

However, the concentrations of insulin in plasma were higher in cattle fed the RTPP supplemental diet. This effect of high sugar in RTP, subsequently resulted in the concentration of insulin in the plasma being increased to control glucose metabolism. The range of plasma insulin concentrations of 13.6-22.2 μ.i.u./ml in the present cattle was higher than the range of 8.40-16.6 μ.i.u./ml observed in animals by Jetana et al. (2006; 2009). It is to be expected that the fermentation pattern in the rumen probably is different when animals are being fed RTP. However, glucose concentration of 72.9-77.0 μmol/ml in the plasma of Brahman cattle in the present study was lower than the range of 78.3-110 μmol/ml observed by Jetana et al. (2006; 2009) in Brahman cattle.

In the present study, it was demonstrated that the high sugar content in the rain tree pod has the advantage of increasing the efficiency of microbial growth in the rumen and the appropriate energy and protein source in mulberry leaves enhanced microbial production. In cases where the mimosine content cannot be avoided when leucaena leaves are used as feedstuff for cattle, the benefits of leucaena leaves can be achieved when animals are inoculated with dihydropyridone-degrading bacteria into the rumen (Jones and Megarrity, 1983). However, high tannin contents in leucaena and cassia leaves are troublesome when used as a feed for cattle. Nevertheless, the influence of rain tree pods mixed together with different leaves of MPTs on digestion, N balance and microbial protein production in the rumen has shown it to be useful for livestock production systems in tropical climates. Thus, further study is needed to solve tannin problems in leucaena and cassia leaves and must define anti-nutritional factors and toxic compounds in those materials before they are used as feedstuff.

CONCLUSIONS

The study demonstrated the value of using local multipurpose trees to improve feeding systems for Brahman cattle in the tropics.

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