Effects of Cassava Leaf Meal on the Rumen Environment of Local Yellow Cattle Fed Urea-Treated Paddy Straw

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ABSTRACT : An experiment was conducted as a Latin square design with four rumen fistulated local yellow cattle with a mean live weight of 230 kg. The treatments were: (CLM_0) urea-treated rice straw *ad libitum* plus 1 kg cassava root meal (basal diet), (CLM_{500}) basal diet plus 500 g cassava leaf meal, (CLM_{1000}) basal diet plus 1,000 g cassava leaf meal, and (CLM_{1500}) basal diet plus 1,500 g cassava leaf meal. The results showed that there were differences in dry matter intake of urea-treated rice straw between treatments ($p<0.05$). The highest total dry matter intake was observed for treatment CLM_{1500}, with 2.62 kg DM/100 kg LWt/day, followed by treatments CLM_{1000}, CLM_{500} and CLM_0, with 2.42, 2.00 and 1.86 kg DM/100 kg LWt/day, respectively. The ruminal ammonia concentration on treatment CLM_{1500} was greater than on treatments CLM_{1000}, CLM_{500} and CLM_0. There were non-significant differences in the ruminal pH among the treatments. The *in sacco* degradability of cassava leaf meal and cassava root meal was high, and on average 75 and 85% respectively of the DM had disappeared after 24 h of incubation. Degradation rate of urea treated rice straw was 64% after 72 h of incubation. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1102-1108*)

Key Words : Cattle, Cassava Leaf Supplement, Rice Straw, Dry Matter Intake, Rumen Environment, Feed Degradation

INTRODUCTION

In Southeast Asia rice straw is the predominant dry season feed for ruminants despite its low nutritive value (Wanapat, 1984). It is deficient in readily fermentable energy, nitrogen, minerals and vitamins, and can not provide for optimum microbial growth in the rumen or tissue development of the host. As a result, growth rates and milk production are generally low and often only about 10% of the genetic potential of the animal (Leng, 1995).

Chemical and physical treatment of rice straw has been widely practiced as a method of improving intake and digestibility (Sundstol and Coxworth, 1984). Ammoniation using urea has received major attention as an appropriate system for developing countries (Owen and Jayasuriya, 1989).

Further improvement in performance may be achieved by supplementing treated rice straw with fresh or dried forage. A good candidate for supplementation is cassava forage, which contains more than 20% crude protein (Reed et al., 1982), in a form which by-passes the rumen, since it is bound in a tannin-protein complex.

Cassava forage has been shown to be an excellent source of protein, as a direct supplement or in concentrate mixtures (Wanapat, 1995). In the Dominican Republic, fresh cassava leaves as the only source of forage in a diet of molasses-urea, supported good growth rates (>800 g/day) in fattening cattle (Ffoulkes et al., 1978; Ffoulkes and Preston, 1978; Ffoulkes and Preston, 1979). The integral cassava plant has been used for dairy cow feeding as a supplement to pasture (Garcia et al., 1994; Garcia and Herrera, 1998).

The hypothesis behind this study is that dry cassava leaf meal, together with urea treated rice straw, will provide sufficient protein and energy for the growth of young cattle. The objectives of the study were: to examine the effects of different levels of cassava leaf meal on ruminal ammonia concentration, ruminal pH and ruminal microflora population in heifers fed diets based on urea-treated rice straw and cassava root meal; and to study the rate and extent of rumen degradability of the feeds on the different rations.

MATERIALS AND METHODS

Location

The experiment was carried out at the experimental farm of the College of Agriculture and Forestry, Ho Chi Minh City, Vietnam. The mean air temperature was 28.2℃ and the mean relative humidity was 76.5%.

Animals

Four local yellow cattle (20-24 months of age and 230 kg live weight on average) were used for the experiment, and were fitted with permanent rumen cannula 3 months before the commencement of the

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experiment.

Housing

The animals were placed in individual stalls in a barn with open walls. Clean and fresh water was available *ad libitum* during the whole experiment.

Experimental design

The treatments were arranged in a Latin square. Each treatment period lasted for 30 days. The first two weeks of each period were for adaptation of the heifers and of the rumen microflora to the new diets. Data on daily feed intake were taken during 7 days of the third week. Feed samples for analysis were taken before feeding during the last 3 days of the same week, *in sacco* degradability during the following 3 days of the fourth week, and rumen samples during the last 2 days of each period.

Diet and treatments

The basal diet consisted of urea-treated rice straw offered *ad libitum* and supplied once daily at about 07:30 h, together with a supplement (1 kg DM/animal/day) which consisted of cassava root meal and 20 g of a mixture of salt and minerals. The straw was treated with 50 g urea per 1,000 g DM of straw, wrapped in airtight plastic film and stored for 3 weeks before feeding. All the cassava root meal was bought on the market at one occasion. In addition 0, 500, 1,000 and 1,500 g DM/day of cassava leaf meal were offered separately on treatments CLM_0, CLM_{500}, CLM_{1000} and CLM_{1500}, respectively. The cassava leaves were collected at the same time from one field after harvesting the roots. The leaves were air dried and ground. The animals had access to the feeds for the whole day.

Measurements

Dry matter intake: All the feeds were weighed before feeding and supplied separately to the heifers. Straw was offered *ad libitum*. The cassava root meal and the amount of cassava leaf meal were supplied according to the treatments throughout the experimental period. Refused feeds were weighed each morning during 7 days of the third week. The feeds were also sampled at these occasions and analyzed for calculation of daily dry matter and organic matter intake, according to the procedures of AOAC (1990).

1) Chemical composition of feed ingredients and degradability of available feed sources

Feed samples were taken for analyses of crude protein, ether extract, neutral detergent fiber, and acid detergent fiber contents. The crude protein (CP) and ether extract (EE) of the feed samples were determined according to the procedures of AOAC (1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations of feed samples were determined according to the procedure of van Soest et al. (1991). *In sacco* degradation of feed samples was determined during 72 h of each period. The bags were 60×120 mm and made from nylon filter cloth with a pore size of 28 microns (Saatifil PES 28/17), according to the procedure described by Orskov et al. (1980). The sun-dried samples were milled in a grinder (1 mm) and weighed amounts (1.5 g) put in nylon bags. The bags were attached to plastic tubes and incubated in the rumen for 6, 12, 24, 48 and 72 hours. After incubation, the bags were washed by hand under running tap water until the water ran clear, and dried in a microwave oven to a constant weight.

2) Microbial population in the rumen

During 2 days (day 28 and day 29) of each 30 day period, ruminal fluid samples were collected before feeding in the morning and then at intervals of 2 hours over an 8-hour period through a probe placed in a caudal position in the ventral part of the rumen. Protozoa population in the rumen fluid was estimated by diluting 8 ml of ruminal fluid with 16 ml of formaldehyde-saline solution (37% formaldehyde with saline solution 1:9) and counting protozoa under light-microscopy (100× magnification) using a 0.2 mm deep Dollfus counting chamber. Four fields in the counting chamber were filled and protozoa counted, according to the method described by Jouany and Senaud (1979) and Dehority (1993).

3) Bacterial population

Samples of rumen fluid were diluted 1:3 in formol saline solution and again diluted to 1:3 in formol saline solution (1 part of formol 37% and 9 parts of saline 0.9% solution). The formal saline solution-fixed samples were stained with 2.5 g of 4, 6-diamidino-2 phenylindole (DAPI; Sigma) per ml for 30 min. Each sample was filtered onto 0.2-m-pore-size nucleopore filters. Cells were counted at a magnification of × 1,000 with a Nikon epifluorescence microscope equipped with a 100-W Hg lamp and an UV filter set (Navas et al., 1993; Fabrien Joux and Philippe LeBaron, 1997).

4) pH and concentration of ammonia nitrogen in the rumen fluid

The rumen fluid pH was determined immediately after collection, by pH meter. The concentration of ammonia nitrogen in the rumen fluid (NH_3-N) was determined by diluting 15 ml of ruminal fluid with 5 drops of concentrated H_2SO_4 and distilling and titrating of the released ammonia by the standard Kjeldahl procedure (AOAC, 1990).

Table 1. Chemical composition of feed ingredients used in the four periods (mean and SE on DM basis)

	g DM per 100 g fresh sample		g per 100 g of DM									
	X		OM		CP		EE		ADF		NDF	
	(n=28)	SE	(n=28)	SE	(n=12)	SE	(n=12)	SE	(n=12)	SE	(n=12)	SE
URS	69.15	0.25	79.68	0.57	9.67	0.47	1.14	0.02	40.77	0.78	62.11	0.87
CLM	91.72	0.73	91.47	0.48	22.54	0.11	7.57	0.13	18.85	0.17	25.60	0.61
CRM	92.13	0.53	92.28	0.64	1.16	0.01	2.29	0.01	2.34	0.03	3.51	0.07

URS= Urea treated rice straw, CLM= Cassava leaf meal, CRM= Cassava root meal.

Table 2. Daily intakes of dietary ingredients by local yellow cattle (DM/day/cattle)

Item	Diets				SE	P
	CLM_0	CLM_{500}	CLM_{1000}	CLM_{1500}		
URS, kg	3.62[a]	3.67[a]	3.97[b]	4.32[c]	0.12	0.02
CLM, g	0[a]	440[b]	940[c]	1075[d]	0.02	0.001
CRM, g	677	505	660	640	0.04	0.12
Total DM, kg	4.30[a]	4.61[a]	5.57[b]	6.04[b]	0.13	0.001
Total DMI % of LWt (kg/d)	1.86	2.00	2.42	2.62		
URS DMI % of LWt (kg/d)	1.57	1.59	1.72	1.87		
CLM as % of total DMI	0	10	17	18		
Total CP, g	369[a]	468[b]	620[c]	683[c]	0.01	0.001

DMI= Dry matter intake, LWt= Live weight.
Different letters in the same row are significantly different ($p<0.05$).

Statistical analysis: Data were analyzed by ANOVA using General Linear Model and Pairwise comparison in Minitab Statistical Software version 12.21.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of urea treated rice straw, cassava leaf meal, and cassava root meal are shown in table 1. During the experimental period (120 days), there were only small changes in the chemical composition of urea treated rice straw, cassava root meal and cassava leaf meal.

Feed intake

Average daily feed intake is shown in table 2. The total dry matter intake showed a continuous increase with increasing level of cassava leaf supplementation, although the dry matter intake was only significantly different between the two highest levels of cassava leaf meal supplementation, CLM_{1000} and CLM_{1500}, as compared to nil and low supplementation levels, CLM_0 and CLM_{500}. The highest total dry matter intake of 2.62 kg DM/100 kg LWt/day was on the highest level of cassava leaf meal supplementation (CLM_{1500}), followed by treatments CLM_{1000}, CLM_{500} and CLM_0 with 2.42, 2.00, and 1.86 kg DM/100 kg LWt/day, respectively. Cassava leaf meal accounted for 18%, 17%, 10% and 0% of the total dietary DM intake for treatments CLM_{1500}, CLM_{1000}, CLM_{500} and CLM_0, respectively. These results differ from those of Queiroz et al. (1998a), who found no differences in dry matter intake of urea treated corn stover, with or without supplementation of cassava hay. The differences in results between the two studies are probably due to the differences in the chemical and nutritional quality and physical structure of the supplemented cassava feeds. The cassava leaf meal in the present study contained 22.5% CP and 18.9% ADF as compared to 14.1% CP and 45.4% ADF in the cassava hay. In the present study both urea treated rice straw and total dry matter intake increased with increasing levels of cassava leaf meal supplementation. The maximum intake of cassava leaf meal was around 18% of the total ration, although the heifers were offered an additional 10% of the total ration of cassava leaf meal.

Effects of cassava leaf meal levels on rumen pH and ruminal NH_3-N

The ruminal pH and NH_3-N concentration were non-significantly increased when the heifers were fed increasing amounts of cassava leaf meal and constant amounts of cassava root meal (table 3). The means of

Table 3. Changes in levels of ruminal pH and NH_3-N, number of protozoa and bacterial population in rumen fluid of local yellow cattle after feeding

Criteria	Hours after feeding	Diets				SE	P
		CLM_0	CLM_{500}	CLM_{1000}	CLM_{1500}		
pH	0	6.74	6.65	6.46	7.43	0.31	0.24
	2	6.22	6.61	6.33	6.49	0.21	0.62
	4	6.04	6.38	6.23	6.39	0.17	0.51
	6	6.00	6.13	6.27	6.35	0.21	0.69
	8	5.72	6.06	6.03	6.07	0.15	0.37
	Mean	6.14	6.36	6.26	6.54		
NH_3-N (mg %)	0	7.93[a]	7.58[a]	8.22[b]	11.45[c]	0.72	0.02
	2	15.54	12.55	17.24	24.69	4.65	0.37
	4	12.05	10.45	11.79	19.87	3.26	0.26
	6	8.38	9.14	8.94	12.71	1.95	0.44
	8	8.16	7.71	7.13	12.51	1.93	0.28
	Mean	10.41	9.48	10.66	16.25		
Protozoa ($\times 10^6$/ml)	0	2.97	3.10	4.72	4.63	0.63	0.18
	2	1.71	2.15	3.82	4.36	1.05	0.31
	4	1.57	2.15	3.33	4.12	1.28	0.53
	6	1.49	2.27	3.28	4.54	1.28	0.43
	8	1.35	2.47	2.74	4.63	1.70	0.61
	Mean	1.82	2.43	3.58	4.46		
Bacteria ($\times 10^9$/ml)	0	1.09	1.38	1.75	1.70	0.36	0.57
	2	0.99	1.00	1.22	1.20	0.25	0.86
	4	1.08	1.14	1.19	1.30	0.17	0.83
	6	1.08	1.14	1.21	1.47	0.21	0.62
	8	1.12	1.21	1.47	1.66	0.23	0.41
	Mean	1.07	1.17	1.37	1.47		

Different letters in the same row are significantly different ($p<0.05$).

rumen pH were 6.14, 6.36, 6.26, and 6.54 for treatments CLM_{1500}, CLM_{1000}, CLM_{500} and CLM_0, respectively. It has long been recognized that animals fed concentrate diets generally have a lower ruminal pH than those fed forages (Lana et al., 1998). When animals are abruptly shifted from forage to concentrate, large amounts of lactic acid can accumulate in the rumen, and lactic acid is a stronger acid than the VFA. Animals that are gradually adapted to concentrate do not usually have significant amounts of rumen lactic acid, but the rumen pH can still be low. Low pH can also be caused by an accumulation of VFA in the rumen (Burrin and Britton, 1986).

Irrespective of treatment, the rumen pH values were highest before feeding (0 h) and decreased up to 8 h after feeding (table 3). This is in agreement with what has been observed in other studies (Hungate, 1966; van Soest, 1982; Kajanapruthipong and Leng, 1998). However, these authors found that the pH values decreased up to 4 h after feeding and then started increasing up to the subsequent feeding.

Urea entering the rumen is hydrolyzed by microbial ureases to CO_2 and ammonia (van Soest, 1982). Later, ammonia is combined with hydrogen ions in the rumen fluid to form ammonia ions. This process depends on ruminal pH (Kajanapruthipong and Leng, 1998). Ammonia moves readily across membranes as compared to ammonium ions, and appears to be more readily absorbed (Mooney and ODonovan, 1970). Chalmers et al. (1971) found that when the pH in ruminal fluid is below 6.9, ammonia concentrations in both peritoneal liquor and jugular blood decrease, whereas the ammonia concentration in ruminal fluid remains constant.

Averages of NH_3-N concentration in the rumen were almost equal on treatments CLM_0, CLM_{500} and CLM_{1000} while it was constantly higher in heifers which consumed 18% of the dietary dry matter as cassava leaf meal (CLM_{1500}), although the difference was statistically significant only at the time of feeding (table 3). The means were 10.41, 9.48, 10.66, and 16.25 mg %, for treatments CLM_0, CLM_{500}, CLM_{1000} and CLM_{1500}, respectively. The high NH_3-N level indicates a surplus of nitrogen from rumen degraded

protein and/or urea in the ration, which is not efficiently used. Microbial growth in the rumen and the use of ammonia as a source for microbial protein formation depend largely on the carbohydrate source and the availability of ATP in its breakdown (van Soest, 1982; Preston and Leng, 1987). Queiroz (1998b) reported 11.72 mg % ruminal ammonia concentration in cattle fed urea treated corn stover, 20% cassava hay and 5% cottonseed meal, all as dry matter. Boniface et al. (1986) and Perdok et al. (1988) showed that the maximum rate of forage digestion for cattle occured at about 4.5 and 6.0 mg % ammonia, respectively. For cattle fed rolled-barley or cracked-maize (Odle and Schaefer, 1987), the minimum concentration of ruminal fluid ammonia required for maximum digestion of grain dry matter was 12.5 and 6.1 mg %, respectively.

Effects of cassava leaf meal levels on microbial populations in the rumen

The means of rumen protozoa populations were 1.82, 2.43, 3.58, and 4.46×10^6/ml, respectively for 0, 10, 17, and 18% of total DMI as cassava leaf meal. The corresponding means of rumen bacteria populations were 1.07, 1.17, 1.37, and 1.47×10^9/ml, respectively.

The results presented in table 3 show that protozoal and bacterial populations in the rumen were influenced by the levels of cassava leaf meal, especially the protozoa population. With the cassava leaf meal supplementation at 18% of total dry matter intake, the protozoal and bacterial populations were highest, and also influenced by the highest level of ruminal fluid ammonia. This is similar to the results of Kanjanapruthipong and Leng (1998), who found that when the concentration of ruminal fluid ammonia was 6.0 to 18 mg %, the protozoal population increased. At a concentration of about 20 mg % of ruminal fluid ammonia the protozoal growth decreased, while the bacterial growth increased. This is the requirement for ammonia for optimum bacterial growth on roughage based diets.

Effects of cassava leaf meal levels on *in sacco* degradability of feeds in the rumen

Cassava leaf meal and cassava root meal were more highly degraded *in sacco* than urea treated rice straw (figure 1). The degradation of cassava root meal and cassava leaf meal was high; 75 and 85% of the dry matter having disappeared after 24 h of incubation. Degradability of the urea treated rice straw was maximal at about 65% of the dry matter after 72 h of incubation. Increasing levels of cassava leaf meal also affected the degradability of urea treated rice straw, cassava leaf meal, and cassava root meal at 6, 12, 24, 48, and 72 h of incubation. The high ruminal degradabilities supported high intakes of urea treated rice straw, as a result of good ruminal fermentation by microorganisms. These results also suggest that cassava leaf meal and cassava root meal are a potential source of nutrients for cattle, with supplementation of cassava leaf meal up to 18% of total dry matter intake.

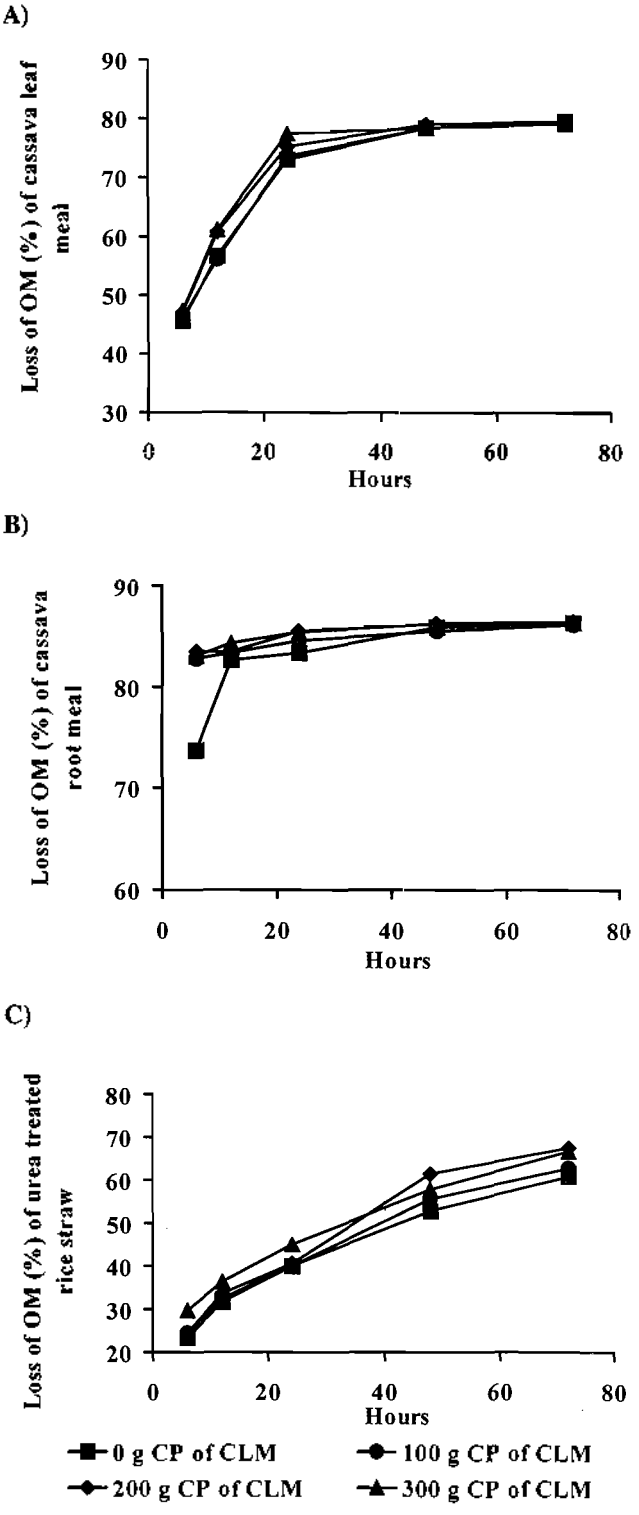

Figure 1. Effect of diet on the relative degradability of cassava leaf (A), cassava root meal (B) and urea treated rice straw (C)

These results are similar to the results of Wanapat et al. (1997), who found that degradability of cassava leaf meal after 72 h of incubation was high (78.7%) and it is concluded that it is a good potential source of nutrients for dairy cows due to its high crude protein content and high protein degradation.

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