Effects of Ensiled Cassava Tops on Rumen Environment Parameters, Thyroid Gland Hormones and Liver Enzymes of Cows Fed Urea-treated Fresh Rice Straw

Duong Nguyen Khang and Hans Wiktorsson*
Department of Animal Physiology and Chemistry, Faculty of Animal Husbandry and Veterinary Medicine
Nong Lam University, Vietnam

ABSTRACT : Four rumen-cannulated cows (330 kg average weight at 4 years) were used to evaluate the supplement of ensiled cassava tops (ECT) (variety KM 94, 39% DM) on rumen functions, thyroid hormones and liver enzymes. The treatments, arranged in a 4 ×4 Latin square design, were ECT at 0, 50, 100 and 150 g CP 100 kg^{-1} body weight (BW), and a basal diet of urea-treated fresh rice straw (UFRS) *ad libitum* and 1.1 kg dry matter (DM) cassava root meal (CRM) in each 30 day study period. The results showed a continuous decrease in dry matter intake (DMI) of UFRS with increasing level of ECT supplement ($p<0.001$). The highest total DMI was observed for treatment ECT_{150} (2.68 kg DM 100 kg^{-1} BW day^{-1}) followed by treatments ECT_{100}, ECT_{50} and ECT_0, with 2.47, 2.24 and 2.06 kg DM 100 kg^{-1} BW·day^{-1}, respectively. Increasing levels of ECT supplement increased the concentration of total volatile fatty acids ($p<0.05$) and ammonia nitrogen ($p<0.05$) and resulted in a decrease in pH ($p<0.05$). Overall average plasma triiodothyronine and thyroxine concentrations were 0.80, 0.82, 0.85 and 0.69 ng ml^{-1} ($p>0.05$), and 50.9, 49.5, 50.7 and 42.4 ng ml^{-1} ($p>0.05$) for treatments ECT_0, ECT_{50}, ECT_{100} and ECT_{150}, respectively. There were non-significant differences in alanine aminotransferase and aspartate aminotransferase among treatments. It is concluded that ECT is a valuable protein-rich feed supplement to cattle, and the highest level of on average 2.48 kg DM ECT per cow and day (28% of total DMI) did not significantly affect thyroid gland hormones and liver enzymes in cows. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 936-941)***

Key Words : Cow, Ensiled Cassava Tops, Dry Matter Intake, Rumen, Thyroid Gland Hormones, Liver Enzymes

INTRODUCTION

There are abundant amounts of cassava forage available at root harvesting (Ravindran and Rajaguru, 1988), but they are underutilized and are usually put directly on to the soil as compost (Khang and Wiktorsson, 2000). A factor limiting its utilization as a feedstuff is related to the high cyanogenic glucosides content, which produces the cyanide (HCN) toxin. Cyanide concentrations of nearly 1,000 mg kg^{-1} dry matter (DM) have been reported in cassava forage (Man and Wiktorsson, 2001; 2002). Earlier reports indicated that feeding large amounts of cassava products without treatment could result in the death of animals, particularly non-ruminants (Hill, 1973). In cattle and sheep, HCN can be lethal at 2 to 4 mg HCN kg^{-1} body weight (Kumar, 1992). However, rumen bacteria have the capacity to hydrolyze β-D-glucosides (Majak and Cheng, 1984; 1987) and to utilize the glucose as an energy source. The toxic HCN can be rapidly absorbed, eructated or further metabolized in rumen contents.

Sun-drying alone eliminated almost 90% of the initial HCN content. When combined with chopping and wilting, HCN in the dried meal was reduced to levels which are safe for monogastric animals (Ravindran et al., 1987). However, in the rainy season it is difficult to sun dry, and extending the drying process diminishes the nutritional quality of the product. It is probable that there would also be management advantages if cassava harvesting could be concentrated in a short period. Thus, ensiling has an advantage in this respect (Brown and Chavalimu, 1985). Fermentation contributes to some extent in reducing the deleterious effect of cassava forage. The HCN content was reduced by 68% after 2 months ensiling and the palatability increased (Man and Wiktorsson, 2001; 2002). In general terms, the ensiled feed has better digestibility and contains various microorganisms as well as enzymes (Officer, 2000).

In a previous study (Khang and Wiktorsson, 2000), marked differences in DMI of urea-treated rice straw, ruminal fluid parameters and *in sacco* degradability of cassava leaf meal in cattle fed different levels of cassava leaf meal in the diets were demonstrated. The aims of the present study were: to examine the effects of different levels of ensiled cassava tops (ECT) on rumen ammonia, total volatile fatty acids (VFA), HCN concentration, rumen pH and rumen microflora populations in cows fed diets based on urea-treated fresh rice straw (UFRS) and cassava root meal (CRM); and to study the affects of the different rations on the thyroid gland hormones and liver enzymes.

* Corresponding Author: Hans Wiktorsson. Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, P.O. Box 7024, 750 07, Uppsala, Sweden. Tel: +46(0)18-67-10-00, Fax: +46(0)18-67-29-95, E-mail: hans.wiktorsson@huv.slu.se
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MATERIALS AND METHODS

The experiment was conducted at the experimental farm of Nong Lam University, Ho Chi Minh City, Vietnam. The cassava tops, of variety KM 94, were collected at the same time from one field after harvesting the roots, lightly wilted and ensiled in plastic bags, according to the method described by Man and Wiktorsson (2001). The height of the plastic bags was 100 cm and the diameter was 63.7 cm. Four cows of the local yellow breed (four years of age and 330 kg body weight (BW) on average) were fitted with a permanent rumen cannula for the experiment. The cows 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 treatment was arranged in a Latin square with four treatments and four periods. Each treatment period lasted for 30 days. The first two weeks of each period were for adaptation of the cows and of rumen microflora to the new diets. Data on daily feed intake were taken during seven days of the third week. Feed samples for analysis were taken before feeding during the last four days of the same week. Rumen samples to determine ammonia, total VFAs, HCN concentration, pH and microflora population were taken during the following 3 days of the fourth week, and blood samples were taken on the last day of each period.

Diet and treatments

The basal diet consisted of UFRS offered *ad libitum* and supplied once daily at about 07:30 h, together with a supplement (1.1 kg DM animal^{-1} day^{-1}) which consisted of CRM and 20 g of a mixture of salt and minerals. The fresh rice straw was treated with 40 g urea per 1,000 g DM of straw, wrapped in an airtight plastic film and stored for three weeks before feeding. All the CRM was bought on the market at one occasion. In addition 0, 50, 100 and 150 g CP 100 kg^{-1} BW of ECT were offered separately for treatments ECT_0, ECT_{50}, ECT_{100} and ECT_{150}, respectively. The animals had access to the feeds for the whole day (24 h).

Measurements

Dry matter intake : All the feeds were weighed before feeding and supplied separately to the cows. The CRM and the ECT were supplied according to the treatments throughout the experimental period. Refused feeds were weighed each morning during seven days of the third week. The feeds were also sampled at these occasions and analyzed for calculation of daily DM and organic matter (OM) intake, according to the procedure of the AOAC (1990). Ensiled cassava tops were analyzed for toluene DM using the method described by Lingvall and Ericson (1981).

Chemical composition of feed ingredients : Feed samples were taken for analysis of crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents. Ensiled cassava top was also analyzed for HCN and tannin content. The contents of CP and EE in feed samples were determined according to the procedure of the AOAC (1990). The contents of NDF and ADF were determined according to the procedure of Van Soest et al. (1991). Total condensed tannin was determined by the butanol-HCl method (Terrill et al., 1992). The HCN contents of ECT were determined by the alkaline titration method (AOAC, 1990).

Ruminal fluid parameters : During the last three days of each 30 day period, ruminal fluids were collected before feeding in the morning and then at intervals of two h during an 8 h period through a probe placed in a caudal position in the ventral part of the rumen. The ruminal pH was determined immediately after collection by pH meter. Protozoan and bacterial populations in ruminal fluids were estimated by counting under light-microscopy, as described by Khang and Wiktorsson (2000). Protozoan population in ruminal fluids 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. For bacteria counting, 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 μm-pore-size nuclepore 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. Samples were later filtered through cheesecloth and poured into centrifuge tubes containing 1 ml of 0.1 N HCl for determination of the concentration of ammonia nitrogen (NH_3-N) in ruminal fluids by the standard Kjeldahl procedure (AOAC, 1990), or 1 ml of 25% metaphosphoric acid for total VFA determination by gas liquid chromatography.

Thyroid gland hormones and liver enzymes in the blood : Blood samples were taken from the jugular vein before feeding in the morning at the end of each experimental period. Samples were centrifuged at 1,500× g at 4°C for 20 min, and the serum was analyzed for triiodothyronine (T_3), thyroxin (T_4), free thyroxin (FT_4) and thyrotropin-stimulating hormone (TSH) by ELISA procedures using Diagnostic Automation kits Cat. No. 3,144, 3,149, 3,146 and 3,122, respectively (Diagnostic Automation Inc., Calabasas, California, USA); for alanine aminotransferase (ALT) and aspartate aminotransferase

Table 1. Chemical composition of experimental feeds (mean and SE on DM basis)

	ECT	UFRS	CRM
DM (g kg^{-1})	390.1±1.96	643.4±4.83	914.4±4.99
pH	4.38±0.02	-	-
HCN (mg kg^{-1})	399.1±28.20	-	-
Composition of DM (g kg^{-1})			
CP	200.4±2.44	90.3±2.47	18.5±1.48
EE	65.2±1.46	11.5±0.52	23.7±1.14
ADF	361.2±4.48	420.7±4.26	24.1±1.14
NDF	476.9±6.25	666.2±5.20	35.4±1.22
Ash	69.3±2.22	197.8±3.32	72.6±3.16
Tannin	26.9±0.54	-	-
WSC	17.2±1.40	-	-

ECT: ensiled cassava top, UFRS: urea treated fresh rice straw, CRM: cassava root meal, HCN: cyanide, WSC: water soluble carbohydrates.
All the numbers are averages of sixteen observations ±SE.

(AST) by Sigma diagnostic kits, Cat. No. ALSL-0500 and ASSL-0500 (SIGMA, St. Louis, Missouri, USA).

Statistical analysis

Data were analyzed by ANOVA using General Linear Model and pair-wise comparison in Minitab Statistical Software version 12.21.

RESULTS

Chemical composition of diet ingredients

Values for HCN and tannin content in the samples of ECT, and DM content and chemical composition of all feeds are presented in Table 1. The wilting of the fresh cassava tops before ensiling resulted in a DM content of 39% in ECT. ECT had a pH 4.38 indicating that the ensiling process was successful.

Feed intake

The effects of ECT on total DM intake are presented in Table 2. Increasing the level of ECT supplementation slightly decreased UFRS intake ($p<0.001$), but to less extent than the added amount of ECT. Therefore, the highest total DMI of 2.68 kg DM 100 kg^{-1} BW day^{-1} was on the highest level of ECT supplement (ECT_{150}). The daily average DMI per cow of ECT was 2.48 kg, 1.65 kg and 0.82 kg, equivalent to 28, 20 and 11% of the total dietary DMI for treatments ECT_{150}, ECT_{100} and ECT_{50}, respectively. Similarly, the daily intake per cow of HCN was 2.51, 1.68 and 0.83 g for treatments ECT_{150}, ECT_{100} and ECT_{50}, respectively.

Ruminal fluid parameters

The mean ruminal fluid parameters from feeding until eight h after feeding are presented in Table 3 and Figure 1. While the means are statistically analyzed and shown in Table 3, Figure 1 only illustrates the mean levels and trends during the eight h of rumen fluid sampling. All rumen fermentation variables determined in the present study were significantly altered by the treatments. However, only the mean HCN concentrations increased gradually with increasing levels of ECT, while pH, VFA concentration and number of protozoa in the ruminal fluid were not

Table 2. Daily intakes of dietary ingredients per 100 kg body weight by the rumen-cannulated cows (DM)

Item	Treatment				SE	p
	ECT_0	ECT_{50}	ECT_{100}	ECT_{150}		
	----------Intake 100 kg^{-1} BW day^{-1} ----------					
ECT DMI, kg	0	0.25	0.50	0.75	-	-
HCN intake, g*	0	0.25	0.51	0.76	-	-
Tannin intake, g*	0	6.71	13.50	20.20	-	-
UFRS DMI, kg	1.74^{a}	1.66^{b}	1.64^{c}	1.60^{c}	0.01	0.001
CRM DMI, kg	0.32	0.33	0.33	0.33	0.004	0.21
Total DMI, kg	2.06^{a}	2.24^{b}	2.47^{c}	2.68^{d}	0.01	0.001
Total CP DMI, g	162^{a}	206^{b}	254^{c}	300^{d}	0.46	0.001
ECT as % of total DMI	0	11.16	20.24	27.98	-	-

ECT: ensiled cassava top, DMI: dry matter intake, HCN: cyanide, UFRS: urea treated fresh rice straw, CRM: cassava root meal.
$^{a, b, c, d}$ Means within rows with differing superscript letters are significantly different ($p<0.05$). * Based on ensiled cassava top intakes.

Table 3. Effects of increased supplemental levels of ECT on ruminal fluid parameters measured every 2 h until 8 h post feeding

Item	Treatment				SE	p
	ECT_0	ECT_{50}	ECT_{100}	ECT_{150}		
pH	7.03^{a}	6.77^{b}	6.77^{b}	6.72^{b}	0.05	0.05
HCN (mg 100 g^{-1})	0^{a}	0.52^{b}	1.03^{c}	1.40^{d}	0.01	0.001
VFA (mmol L^{-1})	80.87^{a}	90.42^{b}	91.16^{b}	93.36^{b}	1.85	0.05
NH_3-N (mg 100 g^{-1})	11.23^{a}	12.18^{ab}	12.08^{ab}	12.70^{b}	0.21	0.05
Protozoa (×10^{6} ml^{-1})	1.14^{a}	0.97^{b}	0.95^{b}	0.91^{b}	0.35	0.05
Bacteria (×10^{9} ml^{-1})	1.16^{a}	1.39^{ab}	1.41^{ab}	1.57^{b}	0.07	0.05

ECT: ensiled cassava top, HCN: cyanide, VFA: volatile fatty acid, NH_3-N: ammonia nitrogen.
$^{a, b}$ Means within rows with differing superscript letters are significantly different ($p<0.05$).

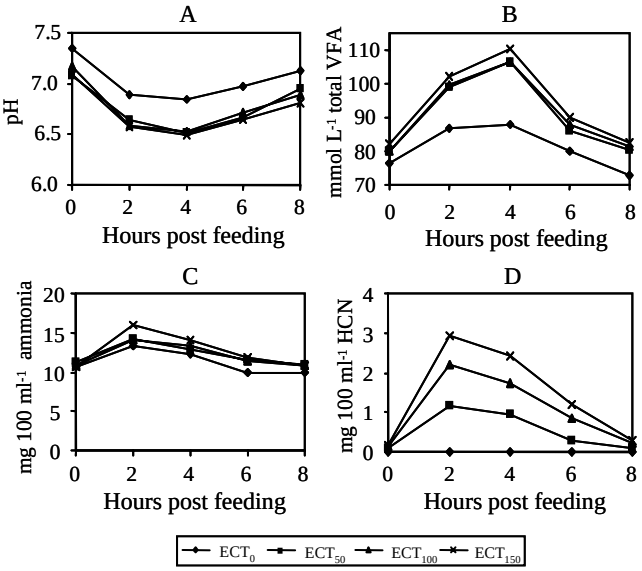

Figure 1. Effects of increased supplemental levels of ECT on the rumen pH (A), total VFA concentration (B), ammonia concentration (C) and rumen HCN (D). ECT: ensiled cassava tops, HCN: cyanide, VFA: volatile fatty acid.

significantly different between the three treatments containing ECT, but were significantly different from treatment ECT_0 (Table 3). When feeding ECT the pH was lowered, VFA concentration was higher and the number of protozoa was reduced. Averages of NH_3-N concentration and number of bacteria in the rumen only differed between treatments ECT_0 and ECT_{150}. In both cases the NH_3-N level and the number of bacteria increased with high amounts of ECT in the ration.

The mean rumen pH decreased in all treatments from the time of feeding until four h later and then increased gradually to near the original values (Figure 1A). None of the pH means fell below 6.5. The highest total VFA concentrations were observed in the rumen fluid collected four h after feeding. While the mean figures from the three treatments including ECT showed great similarities during the 8 h post-feeding, the peak was much less pronounced in treatment ECT_0 (Figure 1B). After a slight peak two h post-feeding, ruminal NH_3-N concentrations declined gradually during the remaining observation time until eight h post-feeding. None of the observed means was below 10 mg NH_3-N 100 ml^{-1} (Figure 1C). Similarly, HCN concentration rapidly increased within two h after feeding in the ECT supplemented diets. The gradual mean increase in HCN with increasing amounts of ECT in the rations is well illustrated in Figure 1D.

Thyroid hormones and liver enzymes

The affects of ECT levels on thyroid hormones and liver enzymes are presented in Table 4. Triiodothyronine, T_4, FT_4 and TSH concentrations were not significantly affected by ECT ($p>0.05$). Free T_4 was the only parameter that showed any tendency towards varying between treatments. Plasma ALT and AST did not differ among treatments ($p>0.05$).

DISCUSSION

The current experiment was undertaken in order to study the effects of different levels of ECT on the rumen environmental parameters, thyroid hormones and liver enzymes of cows fed UFRS and CRM. The ECT was well preserved, as indicated by its pH (4.38). The ECT was a good protein supplement, although the CP content of 20% was slightly lower than the values reported by Man and Wiktorsson (2002) and Hong et al. (2003). The lower value may have been the result of a higher ratio of stem to leaf in the ECT. The ECT used in this study was also slightly lower in tannin content, but higher in DM, due to wilting.

The cows readily consumed all the ECT given, while the daily intake of UFRS was significantly reduced with increasing levels of ECT. However the substitution rate was small. During treatment ECT_{150}, the mean level of 2.48 kg DM day^{-1} of ECT reduced the UFRS intake by 0.46 kg DM day^{-1}, thus resulting in a net increase in daily DM intake of 2.0 kg DM day^{-1}, as compared to no ECT in the ration.

Ensiling and storage of silage reduce HCN content. Man and Wiktorsson (2001) showed that by ensiling, HCN content was reduced from 840 mg kg^{-1} DM fresh cassava tops to 369 mg and 251 mg kg^{-1} DM silage after two and three months storage, respectively. In the present study, HCN content was not determined in the fresh cassava tops, but the HCN content was only slightly higher than

Table 4. Effects of increased supplemental levels of ECT on thyroid hormones and liver enzymes

	Treatment				SE	p
	ECT_0	ECT_{50}	ECT_{100}	ECT_{150}		
T_3 (ng ml^{-1})	0.80	0.82	0.85	0.69	0.10	0.68
T_4 (ng ml^{-1})	50.90	49.53	50.70	42.43	4.26	0.49
FT_4 (ng ml^{-1})	9.99	11.77	12.58	9.78	0.79	0.12
TSH (μUI ml^{-1})	0.23	0.21	0.17	0.16	0.06	0.84
ALT (units L^{-1})	19.43	20.35	21.50	21.85	1.04	0.40
AST (units L^{-1})	35.08	35.40	36.20	37.28	0.90	0.39

ECT: ensiled cassava top, T_3: triiodothyronine, T_4: thyroxin, F T_4: free thyroxin, TSH: thyrotropin, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

mentioned above, or 399 mg kg^{-1} DM after ensiling and storage.

All ECT supplemental levels increased HCN concentration in the rumen, particularly a few hours after the morning feeding. Following the peak observed in the ruminal fluid sampled two h after feeding, ruminal HCN concentrations declined rapidly. There is no data available on how the HCN released from ECT during the first two h was related to the rate of dissociation of cyanohydrins (from a glycosidic bond) released as HCN in ruminal fluid. However, at eight h after feeding the mean HCN concentrations in the rumen fluid were close to zero, which also was observed immediately before feeding on all diets (Figure 1D). This finding corroborates previous reports in the literature that HCN can be rapidly absorbed, eructated or further metabolized in rumen contents (Majak, 1992). It has also been observed that the rate of dissociation of cyanohydrins in ruminal fluid depends on the pH, with high rates of dissociation occurring at pH>6 (Majak et al., 1990). In the present study, mean pH levels below 6.5 were never observed (Figure 1A).

Inclusion of ECT and thus increased CP content in the ration (Table 2) had an obvious positive effect on ruminal microbial growth. The bacteria number and VFA and NH_3-N concentrations increased (Table 3), and were in all cases significantly higher at the highest inclusion level of ECT compared to the basic ration. Although the NH_3-N concentrations in the rumen fluid even on the basic ration (ECT_0) were above 10 mg 100 ml^{-1} NH_3-N, which is about double what is considered as minimum required NH_3-N level in the rumen to support microbial synthesis (Satter and Slyter, 1974), ECT contributed with carbohydrates as energy source for the microbes and low degradable protein due to a tannin-protein complex formed in the rumen (Wanapat et al., 2000). It was expected that the basic ration based on UFRS and CRM should imply sufficient degradable N, because the CP in UFRS was to a great extent present as NH_3-N. Furthermore, the comparatively small but gradual increase of NH_3-N concentrations in the rumen on the ECT-supplemented rations was a result of the low degradation of the protein of ECT. These results agree with an earlier study that also found that NH_3-N concentration was gradually increased with increasing levels of cassava leaf supplementation (Khang and Wiktorsson, 2000).

The other objective of ECT supplementation undertaken in the experiment was to determine the levels of thyroid hormones and liver enzymes of cows after being fed ECT, which may depress thyroid gland and liver functions as a result of HCN toxin. Our results show that thyroid hormones and liver enzymes (Table 4) were within the normal physiological range. There was a slight reduction in the plasma T_3, T_4 and TSH concentrations at the highest supplementation level of ECT. Different authors have found values of thyroid hormone concentrations for bulls ranging between 0.77 and 1.90 ng ml^{-1} for T_3, and 37.5 and 94.1 ng ml^{-1} for T_4 (Singh and Goel, 1986; O'Kelly and Spiers, 1994). The different values of the thyroid hormones were found to be due to differences in breeds (Marai et al., 1999), age (Mitin et al., 1983), climatic conditions (Khurana and Madan, 1986) and energy level (Barash et al., 1998), or iodine deficiency in diets (Han et al., 1998). Khurana and Madan (1986) found that T_3 and T_4 concentrations in cattle were significantly lower in hot humid conditions (0.19 ng ml^{-1} for T_3 and 28.2 ng ml^{-1} for T_4) compared with cold conditions (1.09 ng ml^{-1} for T_3 and 50.5 ng ml^{-1} for T_4). However, there is no information available on how the thyroidal response to cassava products is affected by season in cattle under tropical climatic conditions. Results concerning the concentrations in circulating thyroid hormones indicate that ECT did not affect thyroid function during the feeding trial except for a slight decrease in plasma T_3, T_4 and TSH concentrations with the highest supplement of ECT. This finding corroborates previous reports in the literature that in cattle the ingestion of cassava forage with low HCN content has no adverse effects on animal performance (Ffoulkes and Preston, 1979; Garcia and Herrera, 1998, Wanapat et al., 1999).

In conclusion, large amounts of ECT could be used as a protein supplement in cow diets with positive effects on rumen function and without negative effects on thyroid gland and liver functions, but more investigations are still needed to determine the long-term effects of ECT and cassava products on reproductive processes.

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