Determination of the Nutritive Value of Tropical Biomass Products for Monogastrics Using Rats: 2. Effects of Drying Temperature, Ensiling and Level of Inclusion of Cassava Leaves and Sweet Potato Vines

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ABSTRACT : In a balance experiment with rats either 0, 25 or 50% of the crude protein (CP) provided as casein in the control diet was replaced with cassava leaves (CL) (*Manihot esculenta Crantz*) or sweet potato vines (SPV) (*Ipomoea batata*). CL were either sun-dried or oven-dried at 60°C or 105°C or ensiled, while the SPV were either sun-dried or ensiled. The experiment included 3 blocks with 30 rats in each and six individuals per treatment group. Drying at 105°C resulted in a reduction of the lysine (Lys) content, suggestive of the occurrence of Maillard reactions. Ensiling CL and SPV slightly decreased the CP content as well as the sum of essential amino acids. The apparent fecal CP digestibility (dCP) and nitrogen retention were negatively affected by increasing the level of replacement ($p<0.01$ and $p<0.001$, respectively). The impaired amino acid profile observed when drying CL at 105°C was found to be related to a slight decrease in dCP ($p<0.001$) as well as N retention ($p<0.005$). The effects of sun-drying and oven-drying in reducing the HCN content in CL were more potent than when ensiling. By increasing the total dietary HCN supply serum thiocyanide level, as well as urinary thiocyanate and linamarin output, were increased, with a weak relationship between them. Sun-drying and ensiling with cane molasses as additive successfully preserved the nitrogenous constituents and could be a means of preserving fresh green feed under tropical conditions. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 7 : 994-1002)*

Key Words : Tropical Biomass, Nutrient Digestibility, Biological Value, Rats, Drying, Ensiling, Protein Quality

INTRODUCTION

Traditionally cassava (*Manihot esculenta Crantz*) and sweet potato (*Ipomoea batatas, L.*) have been cultivated almost exclusively for tuber production for human consumption, while their foliage has mainly been considered as a residue. Cassava is a widely grown crop in most countries in the tropical regions of Africa, Latin America and Asia, and ranks as one of the main crops in the tropical countries (Calpe, 1992). Cassava leaf yields may be as high as 4.6 tonnes dry matter (DM) per ha and are considered as a by-product at root harvest (Ravindran and Rajaguru, 1988). The high content of crude protein (CP) and the nutritive value of cassava leaves are well documented (Ravindran, 1993).

Sweet potato is also one of the five most important food crops in developing countries. Although it is of New World origin, over 90% of developing country output is produced in Asia (Scott, 1992). The productive DM potential per ha of certain varieties of sweet potato vines can be as high as 4.3 to 6.0 tonnes/crop (Dominguez, 1992). Sweet potato vines are mainly used as an animal feed wherever they are produced in developing countries (Scott, 1992). Therefore, information regarding the quality of the protein in cassava leaves and sweet potato vines is important for optimal use of these feedstuffs in monogastric animal production.

Sun-drying is a common way of preserving feedstuffs in tropical countries. However, in the rainy season it is difficult to sun-dry, and extending the drying process diminishes the nutritional quality of the product. Thus, ensiling or high temperature drying has an advantage over sun-drying in this respect (Brown and Chavalimu, 1985). The procedure for drying is an important factor as it has implications for the final product quality (Cooke and Coursey, 1981).

In a previous paper (Phuc et al., 2001) marked differences in the nutritive properties of tropical biomass products potentially useful as animal feed resources have been demonstrated. The aim of the present study was to determine the effect of different techniques and temperatures of drying (such as sun-drying, oven-drying at 60 and 105°C) or ensiling on the protein quality of cassava leaves and sweet potato vines with rats.

MATERIALS AND METHODS

Experimental design

The digestibility and nitrogen utilization of diets with inclusion of cassava leaves and sweet potato vines were studied in balance trials with rats. The experiment included

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3 blocks of 30 rats in each. Two rats were randomly allocated to each of the 15 experimental diets in each block. Thus, there were six rats per treatment group. Each diet was given for a preliminary period of 4 days, followed by a 5 days balance period with quantitative and separate collection of urine and feces. The different biomass products were tested at two levels of inclusion.

Biomass products and their preservation

Leaves of cassava (*Manihot esculenta Crantz*) and vines of sweet potato *(Ipomoea batatas, L)* were studied. Cassava leaves (CL) were harvested and divided into two batches. Batch A was divided into three portions, of which one part was sun-dried (CLM A) and the other two parts were oven-dried at 60°C (CLM60) for 6 h or 105°C (CLM105) for 3 h, respectively. Batch B of cassava leaves was divided into 2 portions, of which one part was sun-dried (CLM B) and the other part was ensiled (CLE) in plastic containers after adding 50 g cane molasses per kg. The harvested sweet potato vines were divided into two portions, of which one part was sun-dried (SPS) and the other part was ensiled (SPE) in plastic containers after adding 50 g cane molasses per kg. After 6 weeks the silages were oven-dried at 60°C. Sun-drying of the biomass products was performed by spreading the material on the ground under the sun until dry, which took 1-2 days. The leaves were ground to pass a 1 mm screen before mixing with the other dietary ingredients. The gross chemical composition and amino acid (AA) content of the products investigated are given in table 1, and the content of different glycosides in the cassava leaf products is shown in table 2. The pH, NH_3 and organic acid contents of ensiled cassava leaves and sweet potato vines are shown in table 3.

Diets

The control diet was a semi-synthetic diet with casein as the sole protein source. The experimental diets were composed of maize starch, sucrose, cellulose, minerals and vitamins and the different protein sources (table 4). The calculated dietary content of metabolisable energy was balanced by admixing soybean oil. In the experimental diets either 0, 25 or 50% of the CP of the diets was replaced by the respective biomass product. Thus, the diets were calculated to be isocaloric and isonitrogenous. In the following the diets will be denoted with capital letter abbreviations and a subscript (25 or 50) for the replacement levels.

Animals, housing, feeding and sampling

A total of 90 male Wistar rats were used in the experiment. The mean initial body weight was 72 g (SD 5). The animals were kept individually in metabolic cages with wire net floors, in a temperature controlled room (23-24°C) with a 12 h light/dark cycle as described previously (Phuc et al., 2001).

Chemical analysis

For descriptions of collection and chemical analyses procedures see Phuc et al. (2001). Ammonia was distilled after increasing the pH to at least 10 with magnesium oxide. Organic acids were determined by HPLC (Andersson and Hedlund, 1983). Blood samples of rats taken at slaughter were drawn into 5 ml tubes containing sodium heparin. Plasma was separated by centrifugation at 2,500 rpm for 10 minutes and stored at -20°C until analysis. The cyanide content of leaves was determined by spectro- photometry (Shimadzu uv-160A) using barbituric acid pyridine reagent at 620 nm wavelength according to Cooke (1979) (in O'Brien et al., 1991). Thiocyanate and linamarin in plasma and urine was determined by thin layer chromatography according to Lundquist et al. (1995)

Statistical analysis

Analyses of variance were performed according to the following model:

$$Y_{ij} = \mu + T_i + P_j + e_{ij}$$

Where Y is the dependent variable, μ is the overall mean, T_i the level effect (i = 1, 2, 3 or 1, 2) or preservation technique (i = 1, 2 or 1, 2 and 3), P_j the block (j = 1, 2, 3) and e_{ij} is the residual error.

The General Linear Model of Minitab Statistical Software Version 12 (1998) was used. Least-squares means (LSM) were compared statistically using the Tukey test ($p<0.05$). Linear regression analyses of the effect of dietary NDF content on the digestibility of organic matter (OM) and CP were performed, as well as of the level of CP replacement on digestibility of CP, N retention and biological value (BV). The effects of HCN intake on the thiocyanide level in plasma and the thiocyanate and linamarin level in urine were determined. Stepwise regression analysis was used to investigate the effect of dietary content of lysine (Lys), methionine (Met) and threonine (Thr) on biological data.

RESULTS

Effect of preservation technique on the chemical composition of the biomass products

The chemical composition values of Batch A and B of CL were quite similar, while the CP content in CL was much higher than that in SPV (table 1). A markedly lower content of ether extract (EE) was observed in SPV compared with CL. Drying at 105°C slightly lowered the CP content and markedly increased the NDF content. Drying CL at 105°C also resulted in a depressed Lys content

Table 1. Analyzed chemical composition (% of DM), and essential amino acid (EAA) and non-essential amino acid (NEAA) content in the biomass products investigated

	Cassava leaves			Cassava leaves		Sweet potato vines	
	Batch A			Batch B			
	Sun Dried	Dried 60°C	Dried 105°C	Sun dried	Ensiled	Sun dried	Ensiled
Chemical composition							
Organic matter	92.8	92.4	92.8	91.4	92.8	89.9	86.6
Crude protein	32.4	32.7	32.2	33.3	31.7	20.6	20.1
Ether extract	6.4	7.5	8.2	7.2	8.1	2.5	3.3
NDF	27.5	25.3	37.6	24.4	24.8	28.4	29.2
Essential AA, g /16 g N							
Arginine	6.3	6.4	5.8	6.5	5.6	6.0	5.9
Histidine	2.2	2.0	2.0	1.8	1.7	2.0	2.2
Isoleucine	4.1	4.4	4.5	4.2	4.2	4.2	4.2
Leucine	8.7	8.9	9.1	8.3	8.3	8.2	8.0
Lysine	5.1	5.1	4.2	5.5	5.4	4.8	4.9
Methionine	1.6	1.4	1.5	1.6	1.4	1.4	1.2
Phenylalanine	6.3	6.2	6.2	6.2	5.6	5.7	5.6
Threonine	4.4	4.2	4.4	4.1	3.9	4.4	4.2
Tyrosine	4.3	4.6	4.6	4.4	4.4	4.1	3.8
Valine	5.9	5.6	5.7	5.6	5.3	5.4	5.5
Σ EAA	48.9	48.8	48.0	48.2	45.8	46.2	45.5
Non-essential amino acids, g /16 g N							
Alanine	6.3	6.5	6.4	6.0	6.4	5.6	6.4
Aspartic acid	9.7	9.4	9.6	10.3	9.3	9.4	9.3
Glutamic acid	11.0	10.4	10.7	9.3	9.6	10.0	9.3
Glycine	4.5	4.4	4.8	4.3	4.1	4.4	4.4
Proline	3.8	3.7	3.6	4.1	4.3	4.3	4.1
Serine	3.4	4.1	4.2	3.3	3.8	4.1	4.0
Σ NEAA	38.7	38.5	39.3	37.2	37.5	37.8	37.5
Σ AA	87.6	87.3	87.3	85.5	83.3	84.0	82.9

Table 2. Contents of total HCN, intermediary products (cyanohydrine), free HCN and glucoside (linamarin) (mg/kg DM) of cassava leaves

	Cassava leaves A			Cassava leaves B	
	Sun dried	Dried 60°C	Dried 105°C	Sun dried	Ensiled dried
Total HCN	59	86	28	255	250
Intermediary (cyanohydrine)	13	47	1	152	215
Free HCN	13	33	9	62	5
Glucoside (Linamarin)	33	6	18	42	30

HCN content of ensiled cassava leaves (not dried), mg/kg DM: total HCN: 762; Cyanohydrine: 673; Free HCN: 17 and Linamarin: 73.

Table 3. pH and content of organic acids and ammonia in ensiled cassava leaves (CLE) and ensiled sweet potato vines (SPV)

	Silage of	
	CLE	SPV
pH	3.8	4.0
Organic acids, % of DM		
Succinic acid	0.4	0.6
Lactic acid	8.5	9.6
Acetic acid	1.0	1.0
Propionic acid	0.2	-
2,3-butane-diol	0.1	0.3
Butyric acid	0.0	0.0
Ethanol	1.7	5.3
Ammonia, g/100 g N	3.5	4.2

of 4.2 g per 100 g CP versus a value of about 5 g in the other CL treatment groups. A comparison of sun-drying and ensiling of CL and SPV, respectively, indicates around 1 percentage unit lower CP content of the silages compared to the sun-dried material. Similarly, negative effects on the essential AA (EAA) contents were noted, particularly in the ensiled CL compared with the sun-dried CL.

The content of total HCN in Batch B of CL was much

Table 4. Ingredients and chemical composition, some limiting amino acids and metabolisable energy content of the experimental diets

	Cassava leaves A							Cassava leaves B				Sweet potato vines			
	Control	CLM		CLM 60°C		CLM 105°C		CLM		CLE		SPS		SPE	
	0	25	50	25	50	25	50	25	50	25	50	25	50	25	50
Ingredients, % of DM															
Maize	62.6	70.2	64.6	70.4	65.1	69.4	63.2	70.5	65.5	70.5	64.0	65.0	54.6	63.8	51.8
Soya oil	7.0	2.8	4.1	2.6	3.6	3.6	5.5	2.7	3.7	2.7	3.9	4.0	6.2	4.5	7.2
Sucrose	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Cellulose	7.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Casein	10.0	7.5	5.0	7.5	5.0	7.5	5.0	7.5	5.0	7.5	5.0	7.5	5.0	7.5	5.0
Biomass Products	00	6.5	13.3	6.5	13.3	6.5	13.3	6.3	12.8	6.5	14.1	10.5	21.2	11.2	23.0
Premix ‡	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
Chemical composition, % of DM															
Organic matter	95.1	95.7	95.4	95.6	95.7	95.8	95.3	96.0	96.3	96.2	95.6	95.5	94.8	94.9	93.5
Crude protein	9.3	9.3	9.4	9.4	9.4	9.3	9.3	9.3	9.3	9.4	9.3	9.4	9.4	9.3	9.3
NDF	7.4	1.8	3.7	1.6	3.4	2.4	5.0	1.5	3.1	1.6	3.5	3.0	6.0	3.2	6.7
Essential amino acids, g/16 g N															
Lysine	7.9	7.3	6.6	7.3	6.6	7.1	6.2	7.4	6.8	7.4	6.7	7.2	6.4	7.2	6.4
Methionine	3.2	2.8	2.5	2.8	2.3	2.8	2.4	2.8	2.5	2.8	2.4	2.8	2.4	2.7	2.2
Threonine	4.6	4.6	4.5	4.5	4.4	4.6	4.5	4.5	4.4	4.5	4.3	4.6	4.5	4.5	4.4
ME, MJ/kg	15.1	15.1	15.0	15.1	15.0	15.1	15.0	15.1	15.0	15.1	15.0	15.1	15.0	15.1	15.0

† Experimental diets: CLM A: cassava leaves sun-dried Batch A; CLM.60: cassava leaves dried at 60°C; CLM 105: cassava leaves dried at 105 °C; CLMB: cassava leaves sun-dried Batch B; CLE: cassava leaves ensiled; SPS: sweet potato vines sundried; SPE: sweet potato vines ensiled.

‡ The supplement provided/ kg diet: Calcium: 3.5 g/kg; Phosphorus: 0.93 g/kg; Magnesium: 0.08 g/kg, Sodium chloride: 4.6 g/kg; Iodine: 1.04 mg/kg; Selenium: 0.16 mg/kg; Iron: 32 mg/kg; Cobalt: 0.32 mg/kg; Copper: 9.6 mg/kg; Manganese: 40 mg/kg; Zinc: 48 mg/kg, vit. A: 31 500 IU; D_3: 3,152 IU; E: 210 mg; K_3: 40 mg; B_1: 30 mg; B_2: 24 mg; B_6: 30 mg; B_{12}: 0.08 mg; Choline chloride: 200 mg; Folic acid: 4 mg; Calcium pantothenate: 30 mg; Niacin: 60 mg; Biotin: 0,40 mg.

higher than in Batch A (table 2). The total HCN and cyanohydrides decreased after oven drying at 105°C. The undried silage of CL had the highest HCN content, but this was reduced to around 30% by drying at 60°C.

The pH of the CL and SPV silages were acceptably low and differed only slightly (3.8 versus 4.0, respectively) (table 3). A marked difference between the silages was found in the content of ethanol (1.7 versus 5.3% of DM for the CL and SPV silages, respectively).

Effect of drying technique on rat biological data

Daily feed consumption of the dried biomass product diets was acceptable (table 5). The feed intakes of the 25% of replacement groups were similar to the control group and slightly higher than of the 50 % groups ($p<0.05$) and resulted in corresponding differences in weight gains ($p<0.05$). The LSM given in Table 5 for the five day weight gains of the rats were on average 10.4 g versus 15.0 g for the control group animals ($p<0.001$). However, the weight gains of the 25% replacement group were only slightly higher than of the 50% group (11.0 versus 9.8 g/5days, $p>0.05$). Digestibility of CP and N utilization were reduced with inclusion of the biomass products ($p<0.001$). However, there were no significant differences in BV between replacement levels ($p>0.05$).

The $CLM105_{50}$ animals gained only 8.0 g, which was lower than the average weight gain of the experiment. Digestibility of OM was not affected by drying temperature, whereas dCP was significantly lower for CLM105 compared with CLMA and CLM60 (73 versus 81 and 80%, respectively, $p<0.01$) (table 5). Nitrogen utilization, i.e. N retention as a percentage of N ingested, showed differences between treatment groups ($p<0.05$) and was 52% for the CLM105 diet, versus 58% for the CLMA diet.

Effect of ensiling versus sun-drying on rat biological data

Table 6 shows that the same patterns were found for the effect of level of protein replacement, and drying versus ensiling on the feed intake, weigh gain, dOM, dCP and Nu ($p<0.001$) (table 5).

For the CLE_{50} and the SPE_{50} treatment groups the feed intake was reduced by at least 75%. In analyzing the effects of ensiling versus sun-drying the CL diets and SPV diets were combined, since no interactions could be found. As shown in table 6 feed intake of the rats was slightly lower

Table 5. Effect of drying technique and temperature on feed intake and weight gain of rats, digestibility of organic matter (dOM), and crude protein (dCP), nitrogen utilization (N_u; N retention,% of N ingested), BV and intake of HCN, free N and intermediary products of experimental cassava diets, Bach A

	Level			SEM	Drying			SEM	P	
	0	25	50		Sun	60 °C	105 °C		Level	Drying
Intake, g/5days	49.8[a]	49.8[a]	49.3[b]	0.16	49.6	49.5	49.6	0.21	0.050	0.814
Weight gain, g/5days	15.0[a]	10.9[b]	9.9[b]	0.37	10.4[a]	11.6[a]	9.2[b]	0.45	0.001	0.006
DOM, %	92[a]	96[b]	93[c]	0.1	95	95	94	0.01	0.001	0.564
DCP, %	94[a]	83[b]	73[c]	0.3	81[a]	80[a]	73[b]	0.01	0.001	0.002
N_u	69[a]	60[b]	51[b]	0.4	58[a]	57[a]	52[b]	0.02	0.001	0.031
BV	88	87	87	0.34	87	86	87	0.48	0.702	0.078
HCN intake, mg/5 days										
Total HCN	-	0.21	0.43	0.03	0.31	0.48	0.15	0.03	0.001	0.001
Intermediary (Cyanohydrin)	-	0.07	0.16	0.03	0.07	0.26	0.01	0.01	0.033	0.001
Free	-	0.07	0.14	0.02	0.07	0.18	0.04	0.01	0.002	0.001
Glucoside (Linamarin)	-	0.07	0.13	0.01	0.17	0.03	0.09	0.01	0.007	0.001

[a, b] Means with different superscripts within rows are significantly different ($p<0.05$)

for the diets with the ensiled biomass products, but dOM and dCP of the diets were not significantly affected. N retained as a proportion of N ingested and BV were significantly lower for the diets with the ensiled products compared with the sun-dried diets (50 vs 57%, $p<0.01$ and 83 vs 88 %, $p<0.001$, respectively).

LSM for the CLM and SPV diets have been included in table 6, and show slightly higher values for the digestibility of OM and CP in the CLM diets compared with the SPV diet ($p<0.001$ and $p<0.01$, respectively).

Regression analyses

The dOM of all dietary treatment groups (Y_{dOM}) was significantly related to the dietary NDF content (X in g per kg DM) and is expressed by the regression equation $Y_{dOM}=97-0.90X$ ($R^2=0.79$)

There were close negative relationships between dCP (Y_{dCP}) and the protein replacement level for all diets with biomass products, implying a decrease in dCP with an increasing level of replacement. The same pattern was found for N retained of the N ingested (g/5 days) (Y_{Nu}). Expressing these respective relationships as overall on experimental effects gave the the following regression equations: $Y_{dCP}=93-0.39X$ ($R^2=0.81$), and $Y_{Nu}=69-0.36X$ ($R^2=0.82$) , where X is the replacement level (either 0, 25 or 50%).

The relationship between weight gain, nitrogen utilization (as proportion of N ingested) and BV, with dietary AA content (g/16 gN) are shown in Figures 1A, B and C, respectively.

HCN intake and content in serum and urine

Intakes varied considerably with the varying contents in the cassava leaf products of HCN-containing constituents and were highest in the treatment groups CLMB as compared with those in Batch A (with an average of 1.22 vs 0.31 mg/5days, respectively, $p<0.001$) (tables 5 and 6). As the HCN intake increased, thiocyanate content in serum and urine and the content of linamarin in urine increased. The relationships between total HCN intake and these constituents are shown in figures 2A, B and C.

DISCUSSION

Effect of preservation on chemical composition of biomass products

The CL used in this study are characterised by rather high contents of OM and CP, averaging 92.4 and 32.4% of DM, respectively, which is in agreement with previous reports (Ravindran, 1993; Phuc and Lindberg 2000; Phuc et al., 2000). The CP content of 20.7% of DM in SPV was lower than for CL and may partly be explained by a higher stem proportion, but is in agreement with the value reported by Göhl (1998) and slightly higher than the 18.2 to 18.5% reported by Dominguez (1992).

The contents of AA in CL and SPV were similar to values reported for other green biomass products, e.g. dehydrated lucerne (Ravindran, 1993; Degussa, 1996). Our observation of a rather low level of Met in CL and SPV also seems to be in agreement with earlier studies (Dominguez, 1992; Ravindran, 1993; Phuc et al., 2000).

The reduced Lys content of CLM dried at 105°C was probably the result of a Maillard reaction between reactive sugars in CL and the α-amino group of Lys, which occurs at high temperatures (van Soest and Mason, 1991). The main products are lignin-like polymers of a distinctively high nitrogen content (van Soest and Mason, 1991), which also could explain the higher NDF content in CLM105.

Table 6. Effect of sun-drying and ensiling on feed intake and weight gain of rats, digestibility of organic matter (dOM), and crude protein (dCP), nitrogen utilization (N_u; N retention, % of N ingested), BV and intake of HCN, free HCN and intermediary products of experimental diets of CL B and SPV

	Level			SEM	Preservation		SEM	Leaf		SEM	P		
	0	25	50		Sun drying	Ensi ling		CLM	SPV		Level	Preser vation	Leaf
Intake, g/ 5 days	49.8[a]	48.2[a]	39.1[b]	0.5	45.5	41.9	0.5	43.6	43.8	1.1	0.001	0.020	0.903
Weight gain, g/ 5 days	15.0[a]	11.1[b]	8.5[c]	0.6	10.3	9.3	0.6	9.9	9.8	0.6	0.001	0.256	0.912
DOM, %	92[a]	96[b]	92[a]	0.3	94	94	0.2	95	93	0.2	0.001	0.946	0.001
DCP, %	94[a]	82[b]	74[c]	0.3	79	78	0.3	81	77	0.3	0.001	0.216	0.007
N_u	69[a]	60[b]	47[c]	0.5	57	50	0.5	55	52	0.5	0.001	0.002	0.110
BV	88	87	84	0.4	88	83	0.4	87	88	0.4	0.001	0.001	0.789
HCN intake, mg/5 days													-
Total HCN	0	0.93	1.49	0.02	1.17	1.26	0.020		-		0.001	0.014	-
Intermediary (Cyanohydrin)	0	0.69	1.09	0.02	0.70	1.08	0.02		-		0.001	0.001	-
Free	0	0.12	0.19	0.003	0.28	0.03	0.003		-		0.001	0.001	-
Glucoside (Linamarin)	0	0.13	0.21	0.003	0.19	0.15	0.003		-		0.001	0.001	-

[a, b] Means with different superscripts within rows are significantly different ($p<0.05$).

The limited effect of ensiling on the content of essential AA (EAA) as compared with drying is in agreement with an earlier study on SPV (Brown and Chavalimu, 1985). This is also confirmed by the high content of lactic acid and low content of ammonia as a percentage of total N, which can be explained by the ideal ensiling conditions caused by cane molasses addition and which resulted in a rapid pH drop.

In the present investigation a large variation in HCN content in CL (28 to 255 mg/kg DM) was observed, which agrees with other reports (Chew, 1972; Yeoh and Chew, 1979; Ravindran et al., 1988; Ravindran, 1993). It seems that a slow drying rate achieved e.g. by sun-drying, resulted in an increase of cyanogen activity and an elimination of cyanides as compared with a fast artificial drying at 60°C (O'Brien and Jones, 1994) and is explained by the HCN produced being volatilised during sun-drying. Thus, the drying temperature has a marked effect on the cyanide content of CL and their consequent toxicity (Ravindran, 1993; Nambisan, 1994; Cooke and Coursey, 1981; Oke, 1994). The higher total HCN content in CL silage as compared to drying is confirmed by previous studies (Gomez and Valdivieso, 1988; Oke, 1994; Westby, 1994; Phuc et al., 2000) and can be explained by the fact that linamarase, which hydrolyses cyanogenic glucosides, is inactivated at pH 2 to 4 (Oke, 1994; Phuc et al., 2000).

The level of inclusion of the biomass products, as well as the method of their preservation, significantly affected feed intake. Bitterness may be associated with high cyanogenic glycoside contents in CL (Lee and Hutagalung, 1972; Mahendranathan, 1971; Sundaresan et al., 1987), which could have reduced the feed intake of treatment group CLMB. Also, the poor feed intake that was observed when including SPV and CLM silages, which reduced the weight gain of the rats, could be the result of an unpleasant taste related to the low pH and possibly the occurrence of organic acids. In accordance with the results of a previous study (Phuc et al., 2001), rats fed diets with biomass products had low weight gains compared with a control diet, and there were negative influences on dOM, dCP and N utilisation. Explanations for these responses include the negative effect of fibre (Eggum, 1992; Beames and Eggum, 1981; Champ et al., 1989), the imbalance in EAA supply and their lowered availability in the biomass diets (Bergner et al., 1975; Eggum, 1992; McDonald et al., 1995) as well as the presence of antinutritional factors (Kumar and Singh, 1984; Makkar, 1993).

SPV diets had lower dOM and dCP than the CLM diets, which may have been caused by a higher degree of lignification in the stems of SPV compared with the entire leaves, decreasing the availability of the cell walls and non-cell wall components for digestion (van Soest, 1994).

Drying CL at 105°C clearly reduced dCP and N utilisation as well as weight gain of the rats, which can be explained by the limited dCP, and also by the lowered Lys content, obviously as a result of Maillard reactions reducing the supply of EAA and particularly of Lys (van Soest and Mason, 1991). Furthermore, the rats fed CLM diets might have suffered from a reduced supply of the S-containing AA, as these may be used by the rats in metabolic processes to detoxify cyanide (Tewe and Maner, 1981; Ravindran, 1993). However, stepwise regression analysis indicated no differences between dietary contents of Met (R^2=0.76) and Lys (R^2=0.81) as explanatory factors

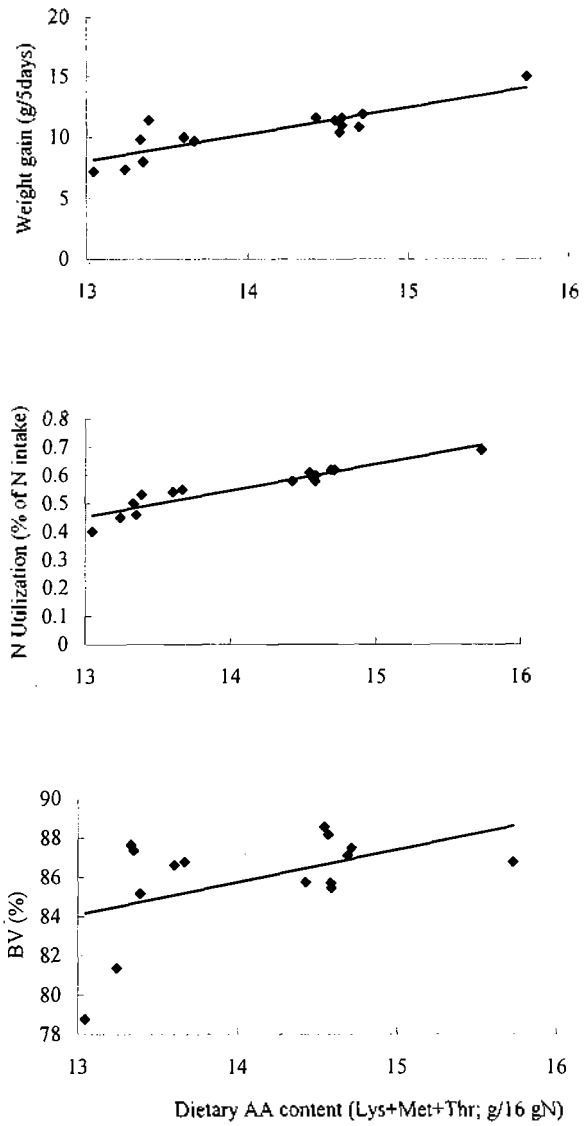

Figure 1. A/. Relationship between weight gain (g/ 5days) and dietary amino acid content (Lys+ Met+Thr; g/ 16 gN); Y=2.21X-20.7, R^2= 0.74

B/ Relationship between N utilization (% of N intake) and dietary amino acid content (Lys+Met+Thr; g/ 16 gN); Y=0.093X-0.76, R^2=0.88.

C/ Relationship between BV (%) and dietary amino acid content (Lys+Met+Thr; g/ 16 gN); Y=1.66X+62.5; R^2=0.23.

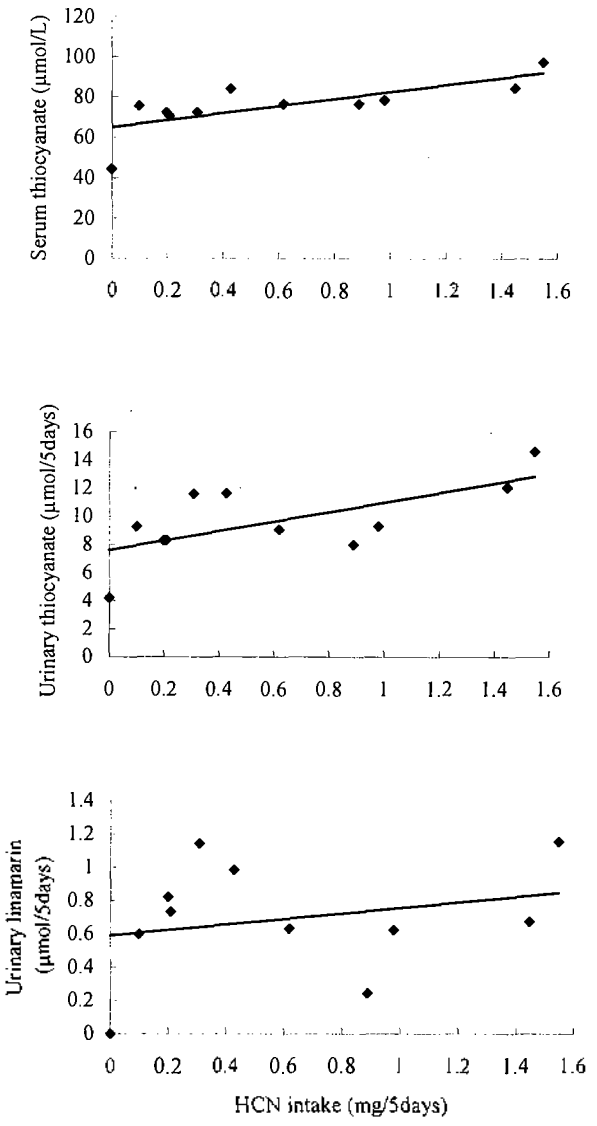

Figure 2 A/. Relationship between HCN intake (mg/5 days) and serum thiocyanate (µ mole/5 days); Y=17.6X+64.9; R^2=0.54.

B/ Relationship between HCN intake (mg/5 days) and urine thiocyanate (µ mole/5 days); Y=3.40X+7.60; R^2=0.44.

C/ Relationship between HCN intake (mg/5 days) and urinary linamarin (µ mole/5 days); Y=0.17X+0.59; R^2=0.07.

on biological traits (data not shown). The biomass products also reduced N retention but had very little effect on BV (figures 1 B and C), in agreement with part 1 of this study.

The albino rat has been reported to tolerate a dietary cyanide level of up to 1 000 ppm without serious effects on its performance and metabolism (Kumta and Harper, 1961; Tewe, 1982). This could explain the acceptable performance of the rats as the dietary HCN content varied from only 2 to 31 ppm (for $CLM105_{25}$ and CLE_{50}, respectively). However, the serum levels of the different cyanide products were positively correlated with the daily HCN intake, which is consistent with other reports on pigs and rats (Kumta and Harper, 1961; Tewe and Maner, 1981) and humans (Osuntokun, 1981). However, there was a low correlation of serum thiocyanide, and thiocyanate and excreted linamarin in urine.

CONCLUSION

There were no differences found between SPV and CL in rat weight gains, intake and protein quality, although nutrient digestibility was lower for the SPV. Ensiling with molasses was less effective than sun-drying in reducing total HCN, and at high levels of inclusion intake, digestibility of nutrients and weight gains were also lower for the ensiled products.

High temperature oven-drying reduced protein digestibility and quality, and therefore weight gains. Sun-drying and low temperature oven-drying were the most effective of the methods evaluated in that biomass products were well preserved, but there was some reduction in nutrient digestibility and performance.

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