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Effect of Elemental Sulfur Supplementation on Rumen Environment Parameters and Utilization Efficiency of Fresh Cassava Foliage and Cassava Hay in Dairy Cattle

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ABSTRACT : Effect of sulfur (S) on utilization efficiency of fresh cassava foliage and cassava hay in dairy cows was evaluated using thirty-two 1^{st}-2^{nd} lactation Holstein-Friesian crossbred dairy cows. The experimental treatment was a 2×2 factorial arrangement in a randomized complete block design (RCBD) using two roughages (rice straw+fresh cassava foliage (FCF) and rice straw+cassava hay (CH)) and two elemental sulfur (S) levels (0.15 and 0.4% S of dry matter (DM)), respectively. Four dietary treatments (FCF+0.15, FCF+0.4, CH+0.15 and CH+0.4) were offered *ad libitum* in the form of a total mixed ration (TMR) with concentrate to roughage (chopped rice straw+chopped cassava foliage) ratio at 60:40. Fresh cassava foliage or cassava hay resulted in similar dry mater intake, rumen ecology parameters, total tract digestibility, blood chemistry, milk production and composition. However, HCN intake, blood and milk thiocyanate concentration were significantly higher ($p<0.01$) in cows fed fresh cassava foliage with no sign of potential toxicity. Dry matter intake, body weight changes, molar percentage of propionate in rumen, neutral detergent fiber (NDF) digestibility and nitrogen (N) retention of cows tended to be increased while DM digestibility (65.6, 72.7, 68.6 and 72.1% of total DM intake for the respective treatments), rumen bacteria population (1.4, 1.7, 1.6 and 1.7×10^{11} cell/ml for respective treatments), fungal zoospore population (0.4, 0.6, 0.4 and 0.5×10^{6} cell/ml for respective treatments), urinary allantoin (25.3, 28.0, 26.3 and 27.6 g/d for respective treatments), microbial N yield (136.0, 154.6, 142.8 and 151.3 g N/d for respective treatments) and milk protein content (3.4, 3.5, 3.2 and 3.5% for respective treatments) were significantly ($p<0.05$) higher in cows fed on supplemented sulfur at 0.4% of DM in comparison with 0.15% S-supplemented diets. Based on these results, it is concluded that cassava foliage could be used as a portion of roughage for dairy cows and supplementation of S would be nutritionally beneficial. (**Key Words :** Fresh Cassava Foliage, Sulfur, Cassava Hay, Hydrogen Cyanide, Dairy Cow)

INTRODUCTION

Feeding of dairy cattle in the tropics is often difficult because of deficiencies in feed supply, in both quality and quantity (Wanapat and Devendra, 1992). The use of rice straw as a feed in the dry season, in spite of its low nutritive value, has been a common feeding system, generally practiced by dairy farmers in the tropics when green forages are often scarce (Leng and Preston, 1983; Wanapat, 1994). Chemical treatment of rice straw to improve its quality has been reported (Wanapat et al., 1990). The development and utilization of cassava hay (cassava whole crop at a young growth stage, 3-4 months, harvested about 30-45 cm above ground, and sun-dried for 1-2 days until having a final dry matter of at least 85%, Wanapat, 1999; 2003) as an on-farm feed has been recommended as a possible solution to the lack of good-quality roughages during the dry season in the tropics (Wanapat et al., 1997). The cassava hay contains high protein, 20-27% CP, and condensed tannins, 1.5-4%. The use of cassava hay at 0.56 to 1.70 kg/head/d or about 0.1 to 0.5% BW was proved to be an excellent ruminant protein feed (Wanapat, 1999; 2003; Promkot et al., 2007). The use of cassava hay has been successfully implemented in several ways by either direct feeding or as a protein source in concentrate mixtures (Wanapat et al., 2000abc; Hong et al., 2003; Kiyothong and Wanapat, 2004; Wanapat

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et al., 2007; Chantaprasarn and Wanapat, 2008), in combination as a pellet of cassava hay, soybean meal and urea (Wanapat et al., 2006) or inclusion in a high quality feed block (Wanapat and Khampa, 2006).

However, there was a lack of information on feeding fresh cassava foliage as a supplement to ruminants, especially for dairy cows. During the rainy season, it is difficult to make cassava hay, therefore feeding fresh cassava foliage to ruminants could be a possible alternative method. However, cassava foliage, especially fresh cassava, contains cyanogenic glucosides, linamarin and lotaustralin. After tissue damage, these are hydrolyzed by the endogenous enzyme linamarase to cyanohydrins. Further hydrolysis to HCN is responsible for chronic toxicity. In ruminants, HCN can be rapidly detoxified by rhodanase and β-mercaptopyruvate sulfurtransferase (Martensson and Sorbo, 1978; Frankenberg, 1980) by rumen microbes (Majak and Cheng, 1984) and animal tissue (rumen wall, liver, kidney and red blood cell) (Aminlari and Gilapour, 1991; Aminlari et al., 1989). Rhodanase is a sulfur transferase that catalyses the formation of thiocyanate from cyanide and thiosulphate or other suitable sulfur donor, and then the less toxic thiocyanate is excreted in the urine.

The main sources of sulfur for HCN detoxification are the sulfur amino acids, cystein and methionine, or elemental sulfur (Oke, 1978). Detoxification of cyanide into SCN causes an increased demand for sulfur-containing amino acids (Maner and Gomez, 1973). NRC (2001) also mentions that the sulfur requirement of dairy cattle consuming cyanogenic plants (such as cassava or sorghum) may be increased because of the need for sulfur in the detoxification of cyanogenic glucosides. Hence, the hypothesis behind this study was that feeding fresh cassava foliage or cassava hay (10% in total ration) combined with adequate sulfur could provide good sources of protein for milk production in dairy cattle with no toxic effects from cyanide. Therefore, the objective of this study was to evaluate the effect of sulfur on utilization efficiency of fresh cassava foliage and cassava hay in milking dairy cows, on i) rumen pH, ammonia nitrogen, total volatile fatty acids, cyanide concentration and microflora population and microbial protein synthesis ii) nutrient digestibility iii) serum concentration of urea nitrogen, thiocyanate, thyroid gland hormones and liver enzymes and iv) milk production and composition.

MATERIALS AND METHODS

Experimental design, animals and treatments:

Thirty-two, 1st-2nd lactation Holstein-Friesian crossbred dairy cows, ranging from 100-150 days-in-milk (DIM) with 12-15 kg/d milk yield, from the dairy farm of the Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus were used in a randomized complete block design (RCBD) to determine the effects of sulfur on utilization efficiency of fresh cassava foliage and cassava hay. The experiment was arranged in two periods (30 days each) using 16 cows for each period. Treatments were total mixed rations with two levels of sulfur and supplemented with a similar amount (10% dry matter in the diet) of two roughages (fresh cassava foliage and cassava hay). Treatments were as follows: T1: 0.15% S, 10% FCF; T2: 0.40% S, 10% FCF; T3: 0.15% S, 10% CH; and T4: 0.40% S, 10% CH. Summary of treatment feeds (total

Table 1 Summary of experimental feeds (total mixed rations, TMR) and animals

Item	Treatments			
	FCF		CH	
	S0.15[a]	S0.4[b]	S0.15	S0.4
Roughages (% DM in TMR)				
Rice straw (R)	30	30	30	30
Fresh cassava foliage (FCF)	10	10	-	-
Cassava hay (CH)	-	-	10	10
Concentrates (% DM in TMR)				
0.2% S[c]	60	-	60	-
0.6% S[d]	-	60	-	60
Roughage:concentrate	40:60	40:60	40:60	40:60
Experimental period (days)	30	30	30	30
Number of animals	8	8	8	8
Lactation	1-2	1-2	1-2	1-2
Range of initial LW (kg)	450-550	450-550	450-550	450-550
Range of initial milk yield	12-16	12-16	12-16	12-16
Range of day-in-milk	100-150	100-150	100-150	100-150

[a] S0.15 = TMR containing sulfur at 0.15% DM. [b] S0.4 = TMR containing sulfur at 0.4% DM.
[c] 0.2% S = the concentrate feed consisting of sulfur 0.2% DM. [d] 0.6% S = the concentrate feed consisting of sulfur 0.6% DM.

Table 2. Ingredients and chemical composition of experimental concentrates, fresh cassava foliage (FCF) and cassava hay (CH)

Ingredient	Sulfur concentration (% dry matter)		FCF	CH
	0.2	0.6		
Cassava chip	52.2	52.0		
Dried tomato pomace	7.2	7.1		
Dried brewer's grain	23.0	23.0		
Palm seed meal	7.6	7.6		
Molasses	5.2	5.1		
Urea	3.4	3.4		
Sulfur	0.2	0.6		
Dicalcium[a]	0.4	0.4		
Salt	0.4	0.4		
Mineral mix[b]	0.4	0.4		
Dry matter (%)	94.5	95.1	26.8	95.8
	---------- % dry matter ----------			
Organic matter (%)	95.7	94.6	92.5	92.1
Crude protein (%)	19.8	19.5	21.3	20.7
Neutral detergent fiber	25.3	25.7	48.8	50.6
Acid detergent fiber	15.0	15.2	32.5	35.3
Ash	4.4	5.3	7.5	8.2

[a] Dicalcium (each kg contains): Calcium 300 g; Phosphorus 140 g.

[b] Mineral mix (Dailymin®) (each kg contains): Iron 2.14 g; Iodin 0.15 g; sulfur 11.82 g; Copper 0.23 g; Magnesium 0.96 g; Sodium 2.68 g; Manganese 7.21 g; Cobalt 0.03 g; Phosphorus 19.60 g; Selenium 0.003 g; Zing 0.16; Calcium 204.03 g.

mixed rations, TMR) and animals in the experiment are shown in Table 1.

Feeds and cows management

Feed ingredient compositions of concentrate and cassava foliage are shown in Table 2. Whole fresh cassava foliage crop (variety Rayong 72, bitter variety) was harvested about 30-45 cm above ground (at the point where the woody stem changes to the green stem) at a young growth stage, 3-4 months, at the university farm once a day in the morning (07:00 am) throughout the experimental period and kept in the cool room (4°C) for fresh cassava foliage until total mixed rations (TMR) were provided. The cassava foliage crop (variety Ryong 72) was also harvested for making cassava hay according to the method of Wanapat et al. (1997, 2000ab). In brief, cassava foliage was chopped and sun-dried for 1-2 days until having final dry matter of at least 85%. Concentrate and roughage (30% chopped rice straw and 10% chopped FCF or CH) feeds were provided every day (09:00 am and 15:00 pm) in the form of a total mixed ration (TMR) with concentrate:roughage at 60:40 by using a mixing machine, and water was added to obtain TMR moisture at an average 45-50%.

The TMR diets were formulated for two levels of S and two supplemental roughages (FCF and CH), and made iso-caloric (TDN) and iso-N for crude protein (CP). The composition of the TMR diets is shown in Table 3. Cows were housed individually for each treatment and had *ad libitum* access to a TMR (TMR was offered three times a day), fresh water and mineral block. Cows were milked

Table 3. Chemical composition (% of dry matter) of four total mixed rations (TMR) fed to dairy cows

Cassava foliage / Ingredient (% S)	FCF[a]		CH[b]	
	S0.15[c]	S0.4[d]	S0.15	S0.4
DM	50.2	50.7	56.1	57.6
OM	92.8	92.1	92.8	92.1
CP	15.2	15.0	15.2	15.0
NDF	46.9	47.1	47.1	47.3
ADF	28.8	28.9	29.1	29.2
Ash	7.3	7.8	7.3	7.9
S	0.15	0.40	0.15	0.40

[a] FCF = Fresh cassava foliage. [b] CH = Cassava hay.

[c] S0.15 = Sulfur in ration at 0.15% dry matter. [d] S0.4 = Sulfur in ration at 0.4% dry matter.

twice daily by milking machine. The cows were adjusted for the first two weeks, and actual intake and measurements were taken subsequently during the experimental 30 days of milk collection.

Feed, feces, rumen fluid and blood samplings

Feed intakes were recorded daily. Feed, feces and urine were collected during the last 5 days of each period. Feed (fresh feed offered and feed residues) was randomly collected (250 g) once a day in the morning before new fresh feed was offered and was kept in a refrigerator until analysis.

Four cows from each treatment in each period were randomly selected for metabolism crates for total collection of feces to measure nutrient digestibility and volume of urine excretion. Feces and urine were collected daily before the morning feeding and were weighed to determine output; a 1% subsample of urine and a 3% subsample of feces were collected, composited by period and stored at -20°C until later analysis. Urine was collected into a plastic container with a suitable amount of 50% H_2SO_4 to reduce pH to <2.5. An additional urine subsample was collected daily, immediately diluted, and stored at -20°C for analysis of purine derivatives (PD), a marker of ruminal bacteria production.

Rumen fluid and blood samples were collected at 0 and 4 h-post feeding on the last day of each period. About 100 ml of whole rumen fluid was collected by stomach tube from individual cows and pH was determined immediately after collection using a glass electrode pH meter. Ruminal fluid samples were then filtered through four layers of cheesecloth and were centrifuged (3,000×g, 4°C for 15 min) immediately and the supernatant was divided into two parts. The first 50 ml rumen fluid portion was kept in a plastic bottle to which 5 ml of 1 M H_2SO_4 was added and stored at -20°C to be used later for NH_3-N and VFA analysis. The second portion of 1 ml rumen fluid was kept in a plastic bottle to which 9 ml of 10% formalin solution was added and stored at 4°C to be used later for total direct count of bacteria, protozoa and fungal zoospores. Blood samples were taken from a coccygeal vessel into heparinized Vacutainer tubes and centrifuged immediately to separate plasma that was stored at -20°C before analysis. Milk yields of each cow were recorded daily. Milk samples of 60 ml (in a ratio of morning milk samples:afternoon milk samples of 60:40) were collected twice daily during milking (05.00 and 17.00 h) on the last five days of each period for later chemical analysis.

Chemical analyses

Feed (both fresh feed offered and feed residues) and fecal samples were analyzed for DM, ash, CP (AOAC, 1990), NDF and ADF (Goering and Van Soest, 1970). Hydrogen cyanide content of feed and cassava foliage was determined spectrophotometrically (SpectroSC, LaboMed, inc. USA) with 2, 4-quinolinediol-pyridine reagent (Lambert et al., 1975). In brief, feed (TMR, CH, FCF) was extracted with 0.1 M phosphoric acid and the acid extract (feed material was removed by filtration) hydrolyzed in 2 M H_2SO4 at 100°C for 50 minutes (Bradbury et al., 1991). To 4.0 ml of the acid solution was added 5 ml of 3.6 M NaOH, after which the solution was filtered. After five minutes, a 1 ml aliquot was taken for colorimetric determination of cyanide by modified Konig reactions (Lambert et al., 1975).

Rumen fluid samples were analyzed for NH_3-N using the procedure of AOAC (1990), and for VFA using high performance liquid chromatography (HPLC; Model Water 600; UV detector, Millipore Crop) according to the method of Samuel et al. (1997). Total direct counts of bacteria, protozoa and fungal zoospores content of rumen fluid were done according to the method of Galyean (1989).

Plasma samples were analyzed for urea-nitrogen composition (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triiodothyronine (T_3), and thyroxine (T_4) using automated clinical chemistry analyzers (Vitallab Flexor E). Thiocyanate was analysed using the method of Lambert et al. (1975). Plasma samples (1 ml) were added to 3 ml 15% w/v trichloroacetic acid and centrifuged for 15 min at 1,000 g. One milliliter of supernatant was used for the colorimetric procedure with 2, 4-quinolinediol-pyridine as the coupling reagent (Lambert et al., 1975). Milk samples were analyzed for fat, protein, lactose and solids-not-fat using infared apparatus (Milko-scan 104, Foss Electric, Denmark). A sub-sample of the composite was analyzed for milk urea nitrogen according to the method of Roseler et al. (1993), using a Sigma diagnostics kit #535 reading at 540 nm using a spectrophotometer (Spectonic 20, Milton Roy company, USA), and milk thiocyanate was analysed using the method of Lambert et al. (1975). One milliliter of milk sample was added to 3 ml 15% w/v trichloroacetic acid and centrifuged for 15 min at 1,000 g. One milliliter of supernatant was used for the colorimetric procedure with 2, 4-quinolinediol-pyridine as the coupling reagent (Lambert et al., 1975).

Urinary ammonia N was measured using the procedure of AOAC (1990). Purine derivatives were measured in urine by HPLC (Waters Model 600; UV detector, Millipore Corp) according to the method of Chen et al. (1993).

Statistical analyses

Data were analyzed using the GLM Procedure (SAS, 1996). The following models were used to determine treatment mean differences using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Chemical composition of feed

The values for composition of feed ingredients are shown in Table 2 and 3. The chemical compositions of cassava hay and fresh cassava foliage were in the range reported in the literature (Wanapat et al., 2000a; Man and Wiktorsson, 2001; Wiktorsson and Man, 2002; Wanapat, 2003; Kiyothong and Wanapat, 2004; Wiktorsson and Khang, 2004; Wanapat et al., 2006). These values would be expected to support normal performance of these lactating cows.

Dry matter intake, digestibility and body weight change

Daily DM intake did not differ significantly among treatments but there was an increasing trend when sulfur was supplemented in the diet ($p = 0.065$) (Table 4). These results could be due to significant increase ($p<0.05$) in dry matter digestibility, especially in the fresh cassava foliage fed group when sulfur was supplemented. Hydrocyanic acid (HCN) intake was highly significant ($p<0.01$) in cows fed fresh cassava foliage (but no toxic signs were evident) than in cassava hay due to a higher level of HCN in the former, similar to the findings of Ravindran et al. (1987) and Promkot et al. (2007).

Cassava foliage (fresh or hay) and interaction of cassava foliage and sulfur did not affect nutrient digestibility (Table 4) with the exception of DM digestibility which was higher ($p<0.05$) in animals fed 0.4% S than in those fed 0.15% S. Moreover, ADF digestibility tended to be higher in cows fed fresh cassava foliage with 0.4% S as compared to other treatments. This result could be attributed to increased numbers of rumen bacteria and fungal zoospores in animals fed with supplemental sulfur at 0.4% DM as compared with 0.15% S diets, leading to increased fiber digestibility. The cows fed cassava foliage with high content of HCN might require increased sulfur supplement, in order to facilitate S for detoxification into thiocyanate (NRC, 2001). Therefore it is possible that the ration of 0.15% S might be deficient in sulfur content especially for the diet with fresh cassava foliage. Therefore, sulfur supplementation to a ruminant ration deficient in sulfur could increase numbers of cellulolytic bacteria (Slyter et al., 1986) which could increase fiber digestibility (Bull, 1984), digestibility of cellulose and lignocellulose (Barton et al., 1971), feed intake (Bouchard and Conrad, 1973) or feed utilization (Garrigus et al., cited by Goodrich et al., 1978).

The negative effect of the cassava foliage with 0.15% S diet on body weight condition in the present study may have been due to reduction in muscular development as a result of depletion of the sulfur-containing amino acids necessary for formation of S amino acids as well as for cyanide detoxification (Onwuka et al., 1992). Similar results were reported by Onwuka et al. (1992) who indicated that goats on a low sulfur cassava-based diet had the greatest weight losses as compared to S-supplemented groups. They proposed that a dietary level of 0.5% elemental S ensured adequate cassava cyanide detoxification in sheep and goats.

Rumen ecology

Cassava foliage, supplementation of sulfur and their interactions did not affect rumen ecology, namely rumen pH, ammonia nitrogen, and total volatile fatty acids. Similar findings were reported by deOliveira et al. (1996) that moderately high percentages of sulfur (0.4 to 0.6%) in the

Table 4. Effect of fresh cassava foliage (FCF), cassava hay (CH) and different concentrations of S in total mixed rations on daily intakes, HCN intake, total-tract digestibility, live weight and weight change of dairy cows

Cassava foliage / Item (% S)	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Daily intake								
kg	11.6	12.4	11.0	11.8	0.40	NS	0.065	NS
% BW	2.5	2.6	2.3	2.6	0.10	NS	0.057	NS
HCN intake (mg/hd/d)	1180.4	1256.4	99.4	107.1	41.2	***	NS	NS
Apparent total-tract digestibility								
DM	65.6	72.7	68.6	72.1	2.14	NS	*	NS
OM	73.3	77.0	73.5	75.3	3.60	NS	NS	NS
CP	72.5	76.8	73.8	75.3	3.50	NS	NS	NS
NDF	59.3	71.3	64.1	65.8	3.15	NS	0.052	NS
ADF	53.19	60.2	56.3	56.4	2.09	NS	0.114	0.131
BW (kg)								
Initial	472.8	472.4	472.8	450.9	18.29	NS	NS	NS
Final	463.5	474.4	471.8	457.2	16.62	NS	NS	NS
ADG (kg/hd/d)	-0.48	0.10	-0.06	0.33	0.24	NS	0.058	NS

NS = Non-significant, * $p<0.05$, ** $p<0.01$.

Table 5. Effect of fresh cassava foliage (FCF), cassava hay (CH) and different concentrations of S in total mixed rations on rumen ecology in dairy cows

Item (S %) \ Cassava foliage	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Rumen ecology								
pH								
0 h, post-feeding	7.0	7.1	7.1	7.1	0.10	NS	NS	NS
4 h	6.8	6.8	6.7	6.7	0.08	NS	NS	NS
Mean	7.0	7.0	6.9	6.9	0.07	NS	NS	NS
NH_3-N (mg %)								
0 h, post-feeding	11.8	13.8	12.4	10. 9	2.11	NS	NS	NS
4 h	15.3	13.9	11.2	13.7	1.97	NS	NS	NS
Mean	14.2	13.61	11.9	12.3	2.24	NS	NS	NS
Total VFA (mM/ml)						NS	NS	NS
VFA (mol/100 mol)	106.3	109.9	104.4	107.7	6.57	NS	NS	NS
Acetate (C2)	67.5	67.5	68.6	67.2	6.82	NS	NS	NS
Propionate (C3)	17.2	19.5	18.0	20.2	1.16	NS	0.081	NS
Butyrate (C4)	14.0	13.9	13.1	13.7	0.72	NS	NS	NS
C2: C3	3.9	3.5	3.9	3.4	0.26	NS	NS	NS

VFA = Volatile fatty acid. NS = Non-significant. * $p<0.05$, ** $p<0.01$.

diet generally had no effects on ruminal VFA and ammonia-nitrogen concentrations. Qi et al. (1993) indicated that ruminal fluid ammonia nitrogen was not affected by added sulfur. Similar findings were also reported by Wora-anu et al. (2004; 2007) that cattle fed cassava hay or fresh cassava foliage had no effects on ruminal pH, VFA and ammonia-nitrogen concentrations.

The molar percentage of propionate exhibited an increasing trend when sulfur was supplemented in the diet ($p = 0.081$) (Table 5). This finding is supported by Thompson et al. (1972) and Zinn et al. (1997) who reported increased ruminal propionate in feedlot cattle or Holstein steers when dietary sulfur was increased from 0.12 to 0.37 and 0.15 to 0.25%, respectively. Low rumen propionate concentration can occur when ruminants are fed sulfur-deficient diets (Goodrich et al., 1978), because little lactate is converted to propionate via the acrylate pathway (Goodrich et al., 1978). It is hypothesized that in cattle fed with cassava foliage, rumen microbes might need more sulfur for hydrogen cyanide detoxification (Onwuka et al., 1992; Promkot et al., 2007) leading to sulfur-deficiency in cattle fed a low sulfur diet that might result in low rumen propionate concentration.

Rumen microbes

Rumen bacteria were significantly ($p<0.05$) higher in animals fed with supplemental sulfur at 0.4% of DM in comparison with 0.15% S diets. Similar results were reported by by dePaiva (2007) that a dose of 0.31% sulfur could increase the rumen microorganisms (including protozoa) in heifers. Slyter et al. (1986) reported that there were reduced numbers of cellulolytic bacteria in sulfur-deficient sheep (0.04% S) in comparison to sulfur-supplemented sheep (0.34% S). Sulfur is one of many important factors for microbial growth (Leng and Preston, 1983). Low levels of sulfur supplementation in the diet containing cassava foliage could reduce microbial biomass in the rumen (Promkot et al., 2007).

Fungal zoospore population was significantly ($p<0.05$) higher in animals fed with supplemental sulfur at 0.4% of DM in comparison with 0.15% S diets. Cassava foliage (hay or fresh) and interaction of cassava foliage and sulfur did not affect the population of rumen microbes; however, there was a trend ($p = 0.085$) for higher fungal zooospores in animals fed fresh cassava foliage with 0.4% S in the diet. Ruminal fungi concentrations and activity may be increased by supplementation with a variety of sulfur sources (Morrison et al., 1990; Gutierrez et al., 1996). In Australia, sulfur-fertilized grass (Akin et al., 1983) and a methionine-supplemented diet (Gordon et al., cited by Akin and Borneman, 1990) resulted in increased fungal populations. Under this study it was also found that there were higher fungal zoospore populations in animals fed a 0.4% S diet as compared with 0.15% S. In addition, similar work conducted by Orpin and Greenwood (1986) reported that *N. patriciarum* required a reduced form of sulfur.

Blood metabolites and hormones

Cassava foliage (hay or fresh) and sulfur supplementation had no effect on blood chemistry, namely BUN, T3, T4, ALT, and AST (Table 7). BUN concentration is positively correlated with ruminal ammonia nitrogen

Table 6. Effect of fresh cassava foliage (FCF), cassava hay (CH) and different concentrations of S in total mixed rations on rumen microorganisms in dairy cows

Item (S %) \ Cassava foliage	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Total direct counts								
Bacteria ($\times10^{11}$ cell/ml)	1.4	1.7	1.6	1.7	0.09	NS	*	0.162
Protozoa ($\times10^{6}$ cell/ml)	0.6	0.7	0.8	0.7	0.07	NS	NS	NS
Fungal zoospores ($\times10^{6}$ cell/ml)	0.4	0.6	0.4	0.5	0.05	NS	**	0.085

NS = Non-significant, * p<0.05, ** p<0.01.

(Hammond, 1983; Promkot and Wanapat, 2005). This study also found that treatment with higher ruminal ammonia nitrogen resulted in higher BUN. Thyroid hormones and liver enzymes were in normal physiological ranges (Table 6) which indicated that feeding fresh cassava foliage or cassava hay at 10% of the diet did not damage the liver and thyroid gland. Similar findings have been reported by Khang and Wiktorsson (2004) that 11% of total DMI as fresh cassava foliage did not affect liver enzymes and triiodothyronine and thyroxine concentrations of the thyroid gland in local yellow cattle. Soto-Blanco et al. (2001) also found that the level of thyroid hormones was unaffected when lactating goats were orally dosed with 3 mg/kg/d KCN for 90 days.

The concentrations of SCN in plasma were significantly greater (p<0.01) in cows given fresh cassava foliage diets than in cows on the cassava hay diet (p<0.01) (Table 7). The increased levels of thiocyanate in plasma of cows fed fresh cassava foliage in this study were due to a relative increase of HCN consumption which was furthered detoxified to thiocyanate (Martensson and Sorbo, 1978; Frankenberg, 1980). Within the fresh cassava foliage and cassava hay diets, the overall concentrations of SCN in plasma were much higher (p<0.01) when cows were fed with 0.4% S than when fed with 0.15% S, especially in cows fed with fresh cassava foliage. However, there was an interaction (p<0.05) effect between cassava foliage and S treatments on plasma SCN concentrations and the highest plasma SCN concentrations were found in animals fed 0.4% S and fresh cassava foliage. This finding indicated that sulfur supplementation could increase HCN detoxification (thiocyanate is a metabolic product of cyanide detoxification) in dairy cows fed with cassava foliage. Similar findings were reported by Promkot et al. (2007) who reported that sulfur can stimulate the rate of HCN detoxification by rumen microbes. Onwuka et al. (1992) also similarly found that percentage of S in the diet was correlated with serum SCN when sheep were fed a dried cassava-based diet.

In ruminants, HCN can be rapidly detoxified by rhodanase and β-mercaptopyruvate sulfurtransferase (Martensson and Sorbo, 1978; Frankenberg, 1980) by rumen microbes (Majak and Cheng, 1984; Promkot et al., 2007) and animal tissues (rumen wall, liver, kidney and red blood cell) (Aminlari and Gilapour, 1991; Aminlari et al., 1989). Rhodanase is a sulfur transferase that catalyses the deformation of cyanide and thiosulphate, or other suitable sulfur donor, to less toxic thiocyanate which is secreted into the blood steam and futher excreted in the milk and urine. The main source of sulfur for HCN detoxification is sulfur amino acid, cysteine and methionine, or elemental sulfur (Oke, 1978). Therefore, adding sulfur to feeds containing

Table 7. Effect of fresh cassava foliage (FCF), cassava hay (CH) and different concentrations of S in total mixed rations on rumen, serum and milk thiocyanate (SCN), blood urea nitrogen (BUN), thyroid hormones and liver enzymes

Item (S %) \ Cassava foliage	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Serum SCN (ppm)	18.5	21.4	15.7	17.0	0.37	**	**	*
Milk SCN (ppm)	15.4	16.0	14.5	14.7	0.43	*	NS	NS
BUN (mg %)	15.5	14.8	16.3	17.5	1.21	NS	NS	NS
MUN (mg %)	16.7	15.56	17.03	16.26	0.50	NS	0.069	NS
T3 (nmol/L)	1.8	1.7	1.7	1.6	0.21	NS	NS	NS
T4 (nmol/ml)	7.3	6.9	7.3	4.9	1.14	NS	NS	NS
ALT (units/L)	58	62.3	55.3	50.7	5.09	0.150	NS	NS
AST (units/L)	24.8	27.8	27.5	25.9	3.87	NS	NS	NS

T3 = Triiodothyronine, T4 = Thyroxine, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase.
NS = Non-significant, * p<0.05, ** p<0.01.

Table 8. Nitrogen balance (g/d), excretion of allantoin and microbial nitrogen supply in dairy cows fed cassava foliage supplemented with sulfur

Item (S %) \ Cassava foliage	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Nitrogen balance (g/d)	280.9	286.3	280.3	279.3	18.1	NS	NS	NS
Fecal N (%of intake)	30.2	26.2	28.7	28.3	2.2	NS	NS	NS
Urinary N (% of intake)	39.9	36.4	39.3	33.6	2.3	NS	0.068	NS
Milk N (% of intake)	25.1	24.8	21.6	25.5	3.1	NS	NS	NS
N Retention (% of intake)	4.7	12.6	10.4	12.6	2.6	NS	0.080	NS
Urinary Allantoin (g/d)	25.3	28.0	26.3	27.6	0.8	NS	*	NS
Microbial N yield (g N/d)[1]	136.0	154.6	142.8	151.3	5.4	NS	*	NS

[1] Microbial N yield (g N/d) = (urinary allantoin (mmol/d)×1.043)- (0.329×$BW^{0.75}$) (Chen and Gomes, 1992), based on 82% of purine derivatives as allantoin and 18% as uric acid in this study.
NS = Non-significant, * p<0.05, ** p<0.01.

HCN is essential for HCN detoxification.

Nitrogen balance

Nitrogen metabolism data are summarized in Table 8. Nitrogen intake and fecal N output were not different across the treatment diets and urinary N output tended to be increased (p = 0.068) as a result of supplemental S. Nitrogen retention (g/d and as a percentage of intake) was numerically higher (p = 0.080) for S-supplemented cows. Nitrogen retained as a percentage of N intake suggests that N utilization was lower for animals fed 0.15% S, whereas those supplemented with S tended to utilize their N more efficiently. This finding may be due to cows fed cassava foliage with low sulfur supplement (0.15% S) providing low sulfur for microbial growth leading to a high ratio of N: S in the rumen. Qi et al. (1992) suggested that if a diet contains an unsuitable ratio of N to S, especially high N and low S, the animals will adjust to this ratio by wasting N by increasing N excretion. Therefore, a decrease in efficiency of feed protein utilization is the principal effect of a S deficiency. Moreover, Fron et al. (1990) suggested that all three forms of supplemental S (elemental S, sodium sulfate and DL-methionine) increase the efficiency of N utilization (retention) for cattle consuming a fescue hay-based diet. Johnson et al. (1971) also mention that elemental sulfur is less available than methionine and other sulfur sources. However, when elemental S was added to a cassava diet it improved protein utilization efficiency by pigs. Presumably, the added S improved N utilization.

The excretion of allantoin in urine was greater (p<0.05) for cows fed the 0.4% S diet than for those fed 0.15% S. Allantoin excretion in urine reflected significant differences (p<0.05) in microbial N for the 0.4% S diet as compared with 0.15% S. Purine derivatives in the urine (uric acid, allantoin, xanthine, and hypoxanthine) have been related quantitatively to the post-ruminal microbial protein supply in cattle (Chen and Gomes, 1992). It is therefore obvious that 0.4% S-supplemented cassava foliage supported higher rumen microbial protein synthesis. Recently, an *in vitro* study by Promkot et al. (2007) found that sulfur supplementation (0.5% substrate) increased microbial biomass especially with a substrate of fresh cassava foliage. Stimulation of microbial protein synthesis by S addition has been observed *in vivo* with semi-purified diets containing a high proportion of urea (Elliott and Armstrong, 1982) and with natural diets in 23 reports as summarized by Durand and Komisarczuk (1988). Increased microbial protein synthesis in cows fed a 0.4% S diet in this study might be due to decreased rumen retention time, due to significantly increased DM digestibility, and an increasing trend of DM intake that may result in higher rate of passage than in cows fed with 0.15% S diet. As reported by Faichney (1993), it has often been observed that mean retention time of consumed feeds decreased with increased intake.

Milk yield and milk production

Milk production and composition did not differ significantly among treatments, except for protein content which was higher in the sulfur-supplemented group (Table 9). The higher milk protein production for the 0.4% S diet in our study may be related to improved nutrient digestibility and nitrogen utilization since there was an increased digestibility of DM, increased ADF and NDF digestibility and N retention for cows fed the 0.4% S diet, as compared with those fed 0.15% S. Similar results have been reported by Stobbs and Wheeler (1977) that milk yield and protein content of cows were increased following S supplementation.

The concentrations of SCN in milk were significantly greater (p<0.01) in cows given fresh cassava foliage diets than in those on cassava hay (p<0.05) (Table 9). This result could be due to higher HCN intake leading to higher levels of HCN metabolite (thiocyanate) in blood serum and milk. Sulfur treatments had no influence on the concentration of

Table 9. Effect of fresh cassava foliage (FCF), cassava hay (CH) and different concentrations of S in total mixed rations on milk yield and composition in dairy cows

Cassava foliage / Item (S %)	FCF		CH		SEM	Contrast		
	0.15	0.40	0.15	0.40		Cassava	S	Interaction
Milk yield (kg/d)	12.2	12.8	12.1	12.0	0.54	NS	NS	NS
3.5% FCM (kg)	12.2	13.0	12.7	12.5	0.56	NS	NS	NS
Milk composition (%)								
Fat	4.0	4.1	4.5	4.2	0.33	NS	NS	NS
Protein	3.4	3.5	3.2	3.5	0.08	NS	*	NS
Lactose	4.4	4.1	4.4	4.3	0.17	NS	NS	NS
Solid-not-fat	8.6	8.4	8.3	8.6	0.16	NS	NS	NS
Total solids	12.6	12.5	12.8	12.9	0.37	NS	NS	NS

NS = Non-significant, * $p<0.05$, ** $p<0.01$.

SCN in milk, while SCN in plasma was much higher ($p<0.01$) when cows fed with 0.4% S than in those fed 0.15% S. This finding might be due to passage of SCN from the blood stream into milk being apparently slow. Grun et al. (1995) reported that in lactating cows with healthy udders the SCN content of blood plasma was higher than in milk (on average twice). Jose (2004) stressed that the mammary gland barrier reduces the passage of SCN from maternal serum to milk.

CONCLUSIONS AND RECOMMENDATIONS

Based on these results, the conclusion can be made that sulfur supplementation at 0.4% (DM) in the ration was beneficial to cows consuming fresh cassava foliage or cassava hay (10% diet) and improved utilization of fresh cassava foliage or cassava hay in terms of dry matter intake, concentration of propionate in the rumen, dry matter and NDF digestibility, nitrogen retention, rumen microbial protein synthesis and high milk protein content. Supplementation of 0.4% elemental S to cassava foliage-based diets also ensured adequate detoxification of cassava cyanide in dairy cattle in this trial.

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