



Influence of Sulfur on Fresh Cassava Foliage and Cassava Hay Incubated in Rumen Fluid of Beef Cattle

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ABSTRACT : Two male, rumen fistulated crossbred Brahman-Thai native beef cattle (body weight = 400±50 kg), fed on rice straw as a source of roughage, were used as rumen fluid sources. The treatments were 2×3 factorial arrangements; two roughages (fresh cassava foliage and cassava hay) and three sulfur levels (elemental sulfur) at 0.2 (control), 0.5 and 1% of DM, respectively. The experiment revealed that the rates (c) of gas production, ammonia-nitrogen concentration, true digestibility, total concentration or molar proportions of VFA and microbial biomass were not significantly different between cassava hay and fresh cassava foliage. However, all parameters for cassava hay were higher than for fresh cassava foliage. The supplementation of 0.5% sulfur to fresh cassava foliage resulted in a significant increase in the rate of gas production, true digestibility, total concentration of VFA, microbial biomass, rate of HCN disappearance, thiocyanate appearance and cyanide percentage conversion into thiocyanate. However, there were no effects of sulfur supplementation at 0.2, 0.5 and 1% to cassava hay. The finding suggests the utilization of cassava foliage for rumen microorganisms in terms of fermentation and HCN detoxification could be improved by sulfur supplementation of 0.5% of DM. (**Key Words :** Cassava Hay, Cattle, Fresh Cassava Foliage, Hydrogen Cyanide, Ruminant, Sulfur)

INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) is a crop of major importance in the tropics. It is grown mainly by smallholder farmers within existing farming systems primarily for the starchy root which is used for human food or as an energy source for non-ruminant or ruminant livestock feed (Khang et al., 2005; Khampa et al., 2006; Wanapat and Khampa, 2007). Cassava can be grown to produce cassava foliage as a protein feed source (Wanapat, 2003; Kiyothong et al., 2004; Khang et al., 2005).

Cassava hay is produced from cassava foliage (green stem, petiole and leaves) at a young growth stage of 3-4 months. It is harvested in a height of 30-45 cm above ground and sun-dried for 1-2 days until it has the dry matter of at least 85%. Cassava hay is high in protein (20-27% CP) and contains condensed tannins (1.5-4%). The use of cassava hay was proved to be an excellent ruminant protein feed (Wanapat, 1999; 2003) and was 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; Granum et al., 2007), as component in soybean meal and urea pellets (Wanapat et al., 2006) and as ingredient in high quality feed blocks. However, there are just few studies about using fresh cassava foliage as a supplement to ruminants. During the rainy season, it is difficult to sun-dry cassava to produce cassava hay. Therefore, feeding fresh cassava foliage to ruminants might be a possible alternative.

However, cassava foliage especially fresh cassava foliage contains cyanogenic glucosides, linamarin and lotaustralin. These are hydrolyzed by the endogenous enzyme linamarase to cyanohydrins after plant tissue damage. Further hydrolysis to HCN is responsible for chronic toxicity. Fresh cassava foliage and cassava hay contains HCN at about 1-100 mg/100 g fresh basis and 3.8 mg/100 g dry basis, respectively. Toxicity of cassava foliage depends on variety, stage of maturity, soil fertility and climate. The sweet varieties are supposed to be much lower in HCN content than the bitter varieties (Sundaresan et al., 1987). The HCN contents of cassava foliage decreases with maturity (Vetter, 2000). De Bruijn (1973) reported that leaf cyanide levels were increased by fertility nitrogen, whereas potassium and farmyard manure had the opposite effect.

However, in ruminants, HCN can be rapidly detoxified

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Table 1. Feed formulation and used in an *in vitro* experiment

Treatment	Cassava chip (mg)	Roughages sources	Amount of roughages (mg)	Elemental S (mg)	Total S % DM
T1	60	Cassava hay	140	0	0.2
T2	60	Cassava hay	134	6	0.5
T3	60	Cassava hay	124	16	1.0
T4	60	Fresh cassava foliage	140	0	0.2
T5	60	Fresh cassava foliage	134	6	0.5
T6	60	Fresh cassava foliage	124	16	1.0

by rhodanese and β -mercaptopyruvate sulfurtransferase (Martensson and Sorbo, 1978; Frankenberg, 1980) by rumen microbes (Majak and Cheng, 1984) 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 excreted in the urine. The main source of sulfur for HCN detoxification is sulfur amino acid, cystein and methionine or elemental sulfur (Oke, 1978). The amount of sulfur required to detoxification of cyanide by rhodanase in the liver is 1.2 g of sulfur per g of ingested HCN (Wheeler et al., 1975). Blakley and Coop (1949) found out that the metabolism of HCN in rumen fluid was enhanced in the presence of sulfur donors they suggested the detoxification of HCN by rumen microbes. This was confirmed by Majak and Cheng (1984) who indicated that thiocyanate formation was also detected in *in vitro* when mixed rumen cultures were incubated with amygdalin or sodium cyanide. Since cassava foliage contains cyanogenic glucoside, linamarin, which on hydrolysis yields HCN is toxic to animals. Cyanide is detoxified to thiocyanate by means of the rhodanase, making use of methionine, cystein or elemental sulfur as the sulfur donor. Thus, feeding cassava foliage to ruminants with sulfur supplementation may be attractive in terms of microbial yield and HCN detoxification. Therefore, the objectives of this study aim to answer the following questions: What is the value of fresh cassava foliage and cassava hay for microbial mass synthesis? Will elemental sulfur supplementation increase microbial mass synthesis? Can rumen microbes detoxify HCN? Will elemental sulfur supplementation increase the rate of HCN detoxification?

MATERIALS AND METHODS

The experiment was carried out at Tropical Feed Resources Research and Development Center (TROFREC), Khon Kaen University, from October to November 2005. During the study the average dairy temperature was 28°C (26-30°C) and the average relative humidity was 75%.

Dietary substrate treatments and design

The dietary treatments were 2 kinds of roughage and 3

levels of sulfur see Table 1.

Sulfur sources were obtained from reducing solution ($\text{Na}_2\text{S}_9\text{-H}_2\text{O}$) and elemental sulfur. Cassava foliage was obtained from the experimental farm of TROFREC, Khon Kaen University, Thailand. Cassava foliage (green stem, petiole and leaves) (variety Ryong 72) was harvested 30-45 cm above ground at a young growth stage of 3-4 months and was separated in two parts: fresh cassava foliage and cassava hay. The fresh cassava foliage was put immediately into ice and transported to the laboratory. The cassava hay was sun-dried for 1-2 days until having a final dry matter of at least 85% according to method of Wanapat et al. (2000a). The experimental design was 2×3 factorial in a Completely randomized design (CRD). The amount of substrates feeds and chemical composition used in this *in vitro* experiment are shown in Table 1 and 2. Cassava chips were used as a source of energy in similar amounts for all treatments.

The Cassava hay was oven-dried at 60°C and milled to a 1-mm screen (Polymix[®] PX-MFC, Kinematica AG, Switzerland) while fresh cassava foliage was frozen in liquid nitrogen for easy milling. Samples were prepared and weighed (total substrate mixture 200 mg dry matter, fresh cassava foliage were weighed in fresh basis to obtain weight in dry basis, based on % DM at 23.3) into 50 ml bottles for incubation. Triplicates set serum bottles of each sampling time within substrate treatments were prepared.

After weighing the bottles were sealed (CO_2 atmosphere) with rubber stoppers and aluminium caps and were placed into the incubator at 39°C for later analyzed *in vitro* gas production.

Chemical analysis

Substrates were analyzed for DM, Ash, CP, and S using the procedure of AOAC (1990), NDF and ADF according to Goering and Van Soest (1970).

Animals rumen inoculum

Two male, rumen fistulated crossbred Brahman-Thai native beef cattle (body weight = 400±50 kg) were used as rumen fluid source. The cows were individually penned, clean fresh water and mineral blocks were offered free choice. Rice straw as a roughage was fed on *ad libitum* basis and concentrate (12% crude protein, 75.4% TDN, consisting of: 72.7% cassava chip, 22% rice bran meal,

Table 2. Chemical composition (% dry matter basis) of feed stuffs used in an *in vitro* experiment

Item	Cassava chip	Cassava hay	Cassava foliage
Dry matter	92.7	98.2	23.3
Organic matter	97.9	92.6	93.4
Crud protein	2.0	22.5	22.3
Neutral detergent fiber	6.4	50.6	51.0
Acid detergent fiber	3.1	34.3	35.1
Ash	2.1	7.4	7.2
S	0.08	0.13	0.14
HCN mg/100 g	3.7	4.9	117.9

HCN = Hydrogen cyanide.

2.5% urea, 1% minerals and vitamins, and 1% salt) was fed at 0.5% body weight in two equal portions, at 08.30 am and at 16.30 pm. The animals were given the diets for 20 days before the rumen fluid was collected. 1,000 ml rumen liquor was obtained from each of cattle before morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks. Strict anaerobic techniques were used and during of the rumen fluid collection according to the method of Menke et al. (1979). It was then transported to the laboratory.

Preparation rumen inoculums mixed

Preparation of Artificial Saliva was done according to Menke and Steingass (1988). In order to reduce sulfur for this experiment, magnesium sulphate was replaced by an equal weight of magnesium chloride and sodium sulfide was replaced by cysteine hydrochloride (Mould et al., 2005). The artificial saliva and rumen fluid was mixed in a 2:1 ratio to a rumen inoculums mixed. Three bottles of each sampling time contained only rumen inoculums mixed solution were included with each run and the mean gas production value of these bottles was termed the blank value. The blank value was subtracted from each measurement to give the net gas production.

Substrate incubation

The serum bottles with the mixture of substrate treatments were prewarmed in a water bath at 39°C for 1 hour before filling with 30 ml of rumen inoculums mixed. Thirty minutes after the start of incubation the bottles were gently mixed and then mixed three times in every three hour.

Sampling and analysis

During the incubation the gas production was recorded after 0, 0.5, 1, 1.5, 2, 4, 6, 8, 16, 24, 36 and 48 h. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follow:

$$y = a + b(1 - e^{-ct})$$

where a = the gas production from the immediately

soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production, y = gas produced at time 't'.

The pH values of each incubation were immediately measured after each sampling time. Twenty milliliters of rumen inoculums mixed was pipetted each sampling time and divided into three parts (10, 5 and 5 ml). These were put into plastic bottles and stored at -20°C until analysis. The 10 ml samples were used for NH₃-N analysis using the procedure of AOAC (1990) and VFA analysis using High performance liquid chromatography (HPLC: Model Water 600; UV detector, Millipore Corp) according to the method of Samuel et al. (1997). One of the 5 ml samples were used for cyanide concentration measurement by spectrophotometrical (SpectroSC, LaboMed, inc. USA) with the 2,4-quinolinediol-pyridine reagent (Lambert et al., 1975). The second 5 ml samples were used to determine thiocyanate concentration according to method of Lambert et al. (1975).

In vitro true digestibility was determined according to Van Soest and Robertson (1985). The true digestibility was used to calculate microbial mass according to the method of Blümmel et al. (1997).

Statistical analysis

The collected data were further used to analysis of variance (ANOVA) according the General Linear Model of SAS Version 6.12 (1996). On this analysis significant differences between individual means were identified by using orthogonal contrasts. The following model was used:

$$Y_{ij} = \mu + A_j + B_k + AB_{jk} + E_{ijk}$$

Y = observation, μ = overall mean, A_i = factor A effect (A = type of roughage, i = 1-3) B_j = factor B effect (B = level of sulfur supplementation, j = 1-3), AB = interaction effect, and E_{ij} = error.

RESULTS AND DISCUSSION

Chemical composition of feeds

The chemical compositions of the feed are shown in Table 2. The chemical compositions of cassava hay analyzed in this study are in a range of reports in the literatures (Wanapat et al., 2000; Wanapat, 2003; Kiyothong and Wanapat, 2004; Wanapat et al., 2006). In this experiment the CP and HCN in fresh cassava foliage was slightly higher as compared to previous researches (Man and Wiktorsson, 2001; Wiktorsson and Man 2002; Wiktorsson and Khang, 2004). This result could be explained by the fact that the protein and HCN (cyanogenic

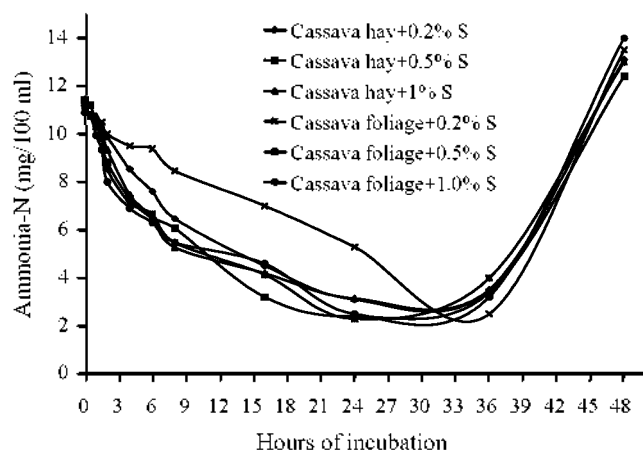


Figure 1. Effect of sulfur supplementation on ammonia-N concentration during *in vitro* gas fermentation.

glycosides) content of cassava foliage depends on variety, stage of maturity, soil fertility and climate. The crude protein (Ravindran and Ravindran, 1988) and HCN (cyanogenic glycosides) (Vetter, 2000) contents of cassava foliage decreased with maturity.

In this study the cassava foliage was from younger plants compared to previous reported (Man and Wiktorsson, 2001; Wiktorsson and Man 2002; Wiktorsson and Khang, 2004) in which the cassava foliage was used after root harvesting which happen with 1 year of age of the plant.

Ruminal pH and ammonia-nitrogen

The pH value of the inoculum was very similar in all treatments (6.5-6.75). However, these values do not necessarily reflect the values which could be generated in an *in vivo* system, due to the buffering activity of the inoculum. The pH values in this experiment represent a monitor of the fermentation and did not fall below the normal range (pH range 6.5-7.0) for rumen microbe growth especially for cellulolytic bacteria.

From the data in Figure 2, it is apparent that the decrease in ammonia nitrogen concentration during the fermentation which started after 0.5 h for all substrate treatments. After this lag period, the pronounced decline was observed for all substrate treatments except for fresh cassava foliage with a sulfur level at 0.2% (control). That indicates ammonia incorporation (Bryant and Robinson, 1961) or utilization of the protein by rumen microorganisms was lower (Stern et al., 1978; Hening et al., 1991) than in the other treatments. The results of this study confirm those of Khang and Wiktorsson (2004) who found out that rumen ammonia nitrogen concentration increased with the level of supplementation of fresh cassava foliage. High rumen ammonia nitrogen concentration in those researches could be due to high CP intake. However another possible explanation for this is that high HCN intake can result in sulfur deficiency. Bacteria can use carbohydrates

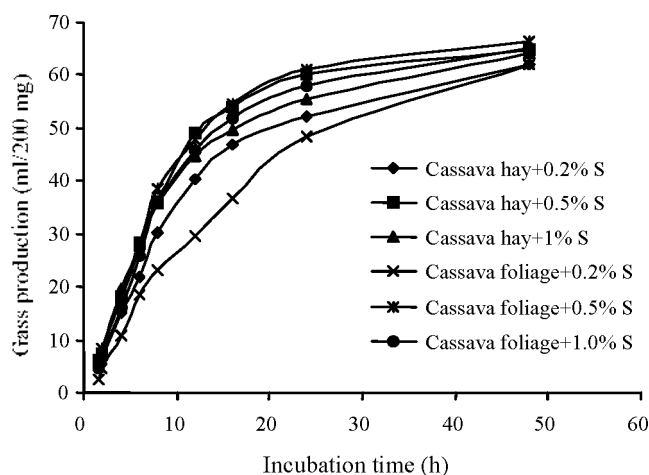


Figure 2. Effect of sulfur supplementation on cumulative gas production of cassava foliage at different time of incubation. 0.2%, 0.5% and 1% S = concentration of sulfur in substrates at 0.2, 0.5 and 1% of DM.

as carbon skeletons for protein synthesis in combination with ammonia (Bach et al., 2005) and also utilize sulfur (organic or inorganic sulfur) to synthesize sulfur-containing amino acids (Kandyliis, 1984) to produce microbial protein. Therefore a possible explanation for this might be that fresh cassava foliage contains a high level of HCN (Table 2) which requires more sulfur (HCN reacts with sulfur to form thiocyanate) (Blakley and Coop 1949; Wheeler et al., 1975; Onwuika et al., 1992) for the rumen microorganisms to cyanides detoxification. It can, therefore be assumed that substrate of fresh cassava foliage with sulfur level at 0.2% was deficient in sulfur. A number of studies have found that added sulfur has improved ruminal fermentations, but only when the diet was deficient in sulfur. Hegarty et al. (1994) reported a greater number of bacteria in the rumen of sheep which were fed a high sulfur diet compared to a low sulfur diet (<0.25% dry matter basis). Slyter and Chalupa (1986) found that the number of cellulolytic bacteria were reduced with sulfur deficiency (0.04% the dry matter) (when continuously cultured ruminal microbes were studied). This view is supported by Patterson and Kung (1988) who reported that added sulfur (0.3% of the dry matter) from methionine, methionine hydroxy analog, or sodium sulfate improved cellulose digestion threefold in *in vitro* fermentations that were void of sulfur. Cellulolytic ruminal bacteria use NH_3 as their sole main source of N (NRC, 2001). In the current study lower microbial biomass and truly digestibility in fresh cassava foliage which deficiency of S (0.2% S, Table 4) seem to be consistent with those of previous reports (Slyter and Chalupa, 1986; Patterson and Kung, 1988; Hegarty et al., 1994). After 24 h of fermentation, the ammonia nitrogen concentration for the bottles containing sulfur supplementation in cassava hay (all level of sulfur) and fresh cassava foliage containing

Table 3. The effect of level of sulfur (S) (0, 0.5 and 1% dry basis) supplementation and roughage source on rate of gas production (c, %/h), extent of gas production (a+b) from *in vitro* incubation with rumen fluid

Treatment	Gas kinetics				Gas (48 h) ml/200 mg DM
	a	b	c	Lag h	
CH+S0.2	-0.8	64.9	0.070	0.3	63.1
CH+S0.5	-0.2	64.7	0.083	0.1	64.7
CH+S1.0	-0.1	63.6	0.078	0.1	64.0
FCF+S0.2	-0.7	64.5	0.058	0.3	61.1
FCF+S0.5	-0.4	66.5	0.078	0.1	66.3
FCF+S1.0	-0.6	66.0	0.070	0.1	65.2
SEM	0.35	0.47	0.001	0.04	0.32
Comparison					
Roughage	ns	*	*	ns	ns
Sulfur level	0.052	*	**	*	**
Interaction	ns	0.06	**	ns	**
Orthogonal comparison					
CH+S0.2 VS CH+S0.5 and CH+S1.0	*	ns	ns	*	ns
CH+S0.5 VS CH+S1.0	ns	ns	ns	ns	ns
FCF+S0.2 VS FCF+S0.5 and FCF+S1.0	*	***	***	*	***
FCF+S0.5 VS FCF+S1.0	ns	ns	*	ns	ns

a = the gas production from the immediately soluble fraction. b = the gas production from the insoluble fraction. c = the gas production rate constant for the insoluble fraction (b). (a+b) = the potential extent of gas production.

CH = Cassava hay, FCF = Fresh cassava foliage, S0.2 = 0.2% S, S0.5 = 0.5% S, S1.0 = 1.0% S, *** p<0.0001; ** p<0.01; * p<0.05; ns = non-significant.

Table 4. Effect of level of sulfur supplementation and roughage sources on *in vitro* ruminal fermentation, true digestibility and microbial mass from *in vitro* incubation 48 h with rumen fluid

Treatment	Total VFA mM/L	C2 %	C3 %	C4 %	C2:C3	True digestibility %	Microbial mass (mg)
CH+S0.2	50.6	66.5	23.1	10.4	2.9	82.9	22.5
CH+S0.5	50.9	66.8	22.9	10.3	2.9	83.3	24.2
CH+S1.0	50.1	66.8	22.9	10.3	2.9	82.4	23.9
FCF+S0.2	45.5	67.5	22.3	10.1	3.0	79.7	20.2
FCF+S0.5	48.2	67.0	22.7	10.2	2.9	84.7	23.6
FCF+S1.0	48.7	66.9	22.7	10.2	2.9	82.5	21.5
SEM	0.25	0.09	0.06	0.02	0.01	0.44	0.34
Comparison							
Roughage	***	***	***	**	**	ns	***
Sulfur level	***	ns	ns	ns	ns	**	**
Interaction	***	*	*	*	**	**	*
Orthogonal comparison							
CH+S0.2 VS CH+S0.5 and CH+S1.0	ns	0.069	ns	*	0.078	ns	ns
CH+S0.5 VS CH+S1.0	ns	ns	ns	ns	ns	ns	ns
FCF+S0.2 VS FCF+S0.5 and FCF+S1.0	***	**	**	*	**	***	***
FCF+S0.5 VS FCF+S1.0	ns	ns	ns	ns	ns	*	*

C2 = acetate, C3 = Propionate, C4 = butyrate. CH = cassava hay, FCF = fresh cassava foliage.

S0.2 = 0.2% S, S0.5 = 0.5% S, S1.0 = 1.0% S, *** p<0.0001; ** p<0.01; * p<0.05; ns = non-significant.

sulfur level at 0.5 and 1% dry matter basis reached a value of 2.3-3.1 mg% and then increased. While the bottles with fresh cassava foliage which contained sulfur at 0.2%, decrease for 36 h, and increased after the ammonia nitrogen concentration reached the values of 2.5 mg%. It has been reported that concentration of ammonia nitrogen lower than 2.0 mg% of rumen fluid can be limiting for microbial growth (Satter and Slyter, 1974). In this present experiment, the critical concentration was observed after 36 h of fermentation for the treatment of fresh cassava foliage containing sulfur at 0.2% dry matter basis while in other

treatments reached the critical concentration after 33 h of fermentation.

The ammonia nitrogen concentration increased after reaching the critical concentration which was found in all treatments. This condition it can be assumed that recycling of bacterial nitrogen (bacteria were lysed and their soluble nitrogen recycle) could occur (Russell and Hespell, 1981).

Gas production, kinetic analysis of gas production, true digestibility, microbial biomass and volatile fatty acid

Cumulative gas production for each of the substrate

Table 5. Effect of level of sulfur supplementation on hydrogen cyanide (HCN) disappearance, thiocyanide (SCN) appearance during *in vitro* gas fermentation of fresh cassava foliage

Treatment	SCN appearance rate (ppm HCN/h)	HCN disappearance (ppm HCN/h)	% HCN incorporation into SCN
FCF+S0.2	0.18 ^a	0.40 ^a	46.2 ^a
FCF+S0.5	0.47 ^b	0.60 ^b	77.9 ^b
FCF+S1.0	0.41 ^c	0.56 ^b	77.4 ^b
SEM	0.01	0.02	2.28
p value	0.0001	0.0013	0.0003

^{a-c} Means within columns with differing superscript letters are significantly different ($p < 0.05$).

FCF = Fresh cassava foliage, S0.2 = 0.2% S, S0.5 = 0.5% S, S1.0 = 1.0% S.

treatments was presented as gas production curves (Figure 1) and values for the estimated parameters obtained from the kinetics of gas production models for substrates studied are given in Table 3. Cumulative gas production (48 h) was not influenced by different type of roughage while there were effects of sulfur supplementation in which higher cumulative gases were found in sulfur supplementation (0.5 and 1% dry basis) than those in control (sulfur at 0.2% dry matter basis). The rate of gas production (c) increased ($p < 0.001$) with the level of sulfur inclusion in the substrate of fresh cassava foliage. The *in vitro* gas production study by using fresh cassava foliage is lack in literatures, however, since the *in vitro* gas production technique has been used as a measure of ruminal degradation of feed (Menke and Steigass, 1988) or hay (Karabulut et al., 2007) high gas production indicate that high digestibility. Under the current study, it was found that the treatments with higher gas production (with sulfur level at 0.5 and 1% dry matter basis especially for fresh cassava foliage) were also higher in *in vitro* true digestibility (Tables 3 and 4). Higher *in vitro* true digestibility reflects higher microbial biomass (Blümmel et al., 1997), the result was also found in the current study see Table 4. The effect of sulfur supplementation on total volatile fatty acid production and individual VFAs is given in Table 4. The total volatile fatty and each VFAs concentrations in substrate of cassava hay were higher than those in fresh cassava foliage. Increasing of volatile fatty acid production were only found in sulfur supplemented fresh cassava foliage and not in cassava hay.

Hydrogen cyanide and thiocyanate

It seems possible that these results (gas production, true digestibility, microbial biomass and volatile fatty acid) are due to sulfur deficiency in fresh cassava foliage with sulfur level at 0.2% dry matter basis. From the data in Table 5, it is apparent that higher rates of HCN concentration disappearance, thiocyanide appearance and percentage of thiocyanide incorporation into thiocyanide during *in vitro* fermentation were observed in sulfur supplemented fresh cassava foliage. Majak and Cheng (1984, 1987) demonstrated that many ruminal bacteria have the ability to release free cyanide from the glycosides amygdalin (laetrile), prunasin, and linamarin (cyanogenic glucosides in

cassava). The conversion of cyanide to thiocyanate occurs by means of the specific enzyme rhodanase (which catalyses the formation of thiocyanate from cyanide in the presence of sodium thiosulfate or colloidal sulfur) (Martensson and Sorbo, 1978; Frankenberg, 1980). The detoxication mechanism of the organism, an important role is played by the availability of sulfur. The metabolism of HCN in rumen fluid was enhanced in the presence of sulfur donors (Blakley and Coop, 1949). The required amount of sulfur for detoxification of cyanide by rhodanase of rumen microbes is not know yet, however detoxification of 1 g HCN theoretically requires 1.2 g S for the conversion to thiocyanate (Wheeler et al., 1975). This study confirms that HCN could be detoxified in the rumen (Majak and Cheng, 1984) and sulfur is associated with HCN detoxification by rumen microbe (Blakley and Coop, 1949).

Sulfur requirements of the rumen microbes

Requirements of beef cattle for sulfur are not well defined. The recommended concentration in beef cattle diets is 0.15 percent (NRC, 1996). Supplementation of such diets with some form of sulfur may be necessary to provide optimum dietary nitrogen to sulfur (N:S) ratios. The desirable dietary N:S ratio required for the most efficient utilization by rumen microorganisms is about 10-13.5:1 for sheep and about 13.5-15:1 for cattle (Kandyliis, 1984). Gutierrez et al. (1996) found out that the required N:S ratio of rumen microbes ranges from 8:1 to 31:1 (mean of 21.6:1) and concludes that a 20:1 ratio of available nitrogen and sulphur should be optimum ration and adequate for rumen microbes. However, sulfur requirements for rumen microorganisms may be higher when fed fresh cassava foliage, because the sulfur is required for the detoxification of the cyanogenic glucosides which are found in fresh cassava foliage. In the present study, of cassava hay and fresh cassava foliage the N:S ratio was 18.8, 18.9, 8.3, 8.3; and 3.9, 3.9 for substrate diets with 0.2, 0.50 and 1.0% S, respectively. Theoretically, after detoxification of HCN (1 g HCN theoretically requires 1.2 g S) N:S ratio of the above in the *in vitro* becomes effective 19.4, 32.9, 8.3, 10.2; and 4.0, 4.4, respectively.

It is very obvious that the N:S ratio (32.9:1) after detoxification of HCN in substrate of fresh cassava foliage

with 0.2% sulfur which was higher than optimum ratio (20:1) as suggested by Kandyliis (1984) or Gutierrez et al. (1996). However, increasing sulfur supplementation above 0.5% (DM) of total substrate did not improve the gas production, total VFA production, microbial biomass yield, true digestibility and rate of HCN disappearance. Moderate high percentages of sulfur (0.4 to 0.6%) in the diets have generally had no effects on ruminal volatile fatty acids and ammonia-nitrogen concentrations. However, the effect of extremely high percentages of sulfur (>1% of the DM) in the diet of ruminants is equivocal. Kahlon et al. (1975) reported that 1.3% sulfur in the diet inhibits microbial protein synthesis in the rumen, but Kennedy et al. (1986) reported that a similar percentage of sulfur was not toxic to ruminal microorganisms. In calves, dietary sulfur as high as 1.72% had no effect on ruminal VFA or ammonia-nitrogen relative to calves consuming a diet with 0.34% sulfur (Slyter et al., 1988). The reason is that the production of VFA from carbohydrates in the rumen is coupled with microbial growth (Bergen and Yokoyama, 1977). The maximal microbial yield can only be attained only if precursors for protein synthesis are made simultaneously available and in adequate quantities to the microbe. This study suggests that diet which are high HCN (fresh cassava foliage) and low in sulfur are utilized better when sulfur is adequate and the N:S is at the optimum ratio.

CONCLUSIONS AND RECOMMENDATIONS

Sulfur supplementation at 0.5% DM (of substrate) especially in fresh cassava foliage significantly increased the rate of gas production, total VFA production, microbial biomass yield, true digestibility and the rate of HCN disappearance. However, increasing sulfur supplementation above 0.5% (DM) of total substrate did not additionally improve rumen fermentation. That leads to the conclusion that sulfur supplementation at 0.5% (DM) of total substrate diet of fresh cassava foliage was beneficial for rumen microorganism in terms of fermentation and HCN detoxification. Based on these results, it indicates (i) that the value of fresh cassava foliage for microbial mass yield is slightly lower than for cassava hay, (ii) that sulfur supplementation increases microbial biomass especially in fresh cassava foliage, (iii) that rumen microbes can detoxify HCN, and (iv) that sulfur can stimulate the rate of HCN detoxification by rumen microbes. However further studies with focus on the role of sulfur on HCN detoxification by rumen microbes are therefore suggested and investigates the use of fresh cassava foliages in productive ruminants especially in lactating cows and fattening cattle.

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