



**Full Length Article**

# Fermenting Cell Walls of Processed Sugarcane Pith by Ruminal Bacteria, Protozoa and Fungi

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## ABSTRACT

The objectives of this study were to determine contribution of ruminal bacteria, protozoa and fungi to the fermentation of cell walls of sugarcane pith treated with low temperature steam (121°C, 120 min.) and or sulfuric acid (0.0, 6, 12 & 18 g/kg DM), and effectiveness of this method for increasing the feed value of sugarcane pith and negative effects of this method on rumen microorganisms. Gas production parameters were determined and cumulative gas production data were fitted to the exponential equation. The results showed that processing with low temperature steam resulted in significant increase in rate (C) and potential gas production (B) by all microbial fractions, as the highest (B) (112.9 mL) was for condition 18 g/kg of dry matter (DM) acid. The highest gas produced by total microorganism and rumen isolated bacteria was followed by fungi and protozoa fractions. *In vitro* cell wall degradation, organic matter digestibility (OMD) and metabolisable energy (ME) of sugarcane pith treated with steam and 18 g/kg acid was the highest and microbial biomass production (MB) was the lowest for all microbial groups. Therefore, it appears that the gas production parameters and degradability of sugarcane pith by rumen microbial fractions are influenced by low temperature steam and sulfuric acid treatment, bacterial group generates a larger volume of gas production than fungal or protozoan group and this method had no negative effect on rumen microorganisms. © 2011 Friends Science Publishers

**Key Words:** Low temperature steam; Rumen microbial groups; Sugarcane pith; Sulfuric acid

## INTRODUCTION

Rumen microorganisms; bacteria, protozoa and fungi have a key role in the degradation of polysaccharides of plant cell walls (Lee *et al.*, 2000), but cellulolytic bacteria are a major microorganisms responsible for ruminal digestion of plant cell walls due to their metabolic diversity and numerical predominance (Cheng *et al.*, 1991; Krause *et al.*, 2003). Williams and Withers (1991) found that although the addition of protozoa to previously defaunated sheep reduced the bacterial population, it appears that protozoa may stimulate fiber digestion in the rumen either directly or by stimulation of the cellulolytic bacteria and digest fiber in the rumen by 5–21% (Dijkstra & Tamminga, 1995; Lee *et al.*, 2000). Lee *et al.* (2000) concluded that fungal activity was mostly responsible for cell wall degradation. The production of gas during the microbial fermentation of plant material by rumen contents has been used as an indirect measure of substrate digestibility (Menke & Steingass, 1988).

Much amount of sugarcane pith is produced annually in the world including Iran. However, the low digestibility, high lignin and very low nitrogen content, are considered as the main reasons for unsatisfactory performance of animals fed these roughages (Osorio & Cruz, 1990). Many methods

have proven successful in disrupting cell wall material e.g., using acids (Shambe & Kennedy, 1984) and high-pressure steam (Castro & Machado, 1990). Under steam conditions, acetyl groups are released from the hemicellulose matrix and suitable levels of cell wall disruption are achieved (Muzzy *et al.*, 1983). Steam and pressure treatments alone or allied with chemical treatments are known to disrupt lignocellulosics in a way, which allows improved utilization of cell wall polysaccharides by cell-free enzymes (Grohmann *et al.*, 1985) and rumen microbes (Castro & Machado, 1990).

The objective of these experiments was to estimate the relative roles of bacteria, protozoa and fungi in plant cell wall digestion of sugarcane pith processed with low temperature steam and sulphuric acid by *in vitro* gas production and investigation effectiveness of this method for increasing the feed value of sugarcane pith, and probably negative effects of this processing method on rumen microorganisms.

## MATERIALS AND METHODS

**Preparation of samples and inoculums:** Sulphuric acid solution was added to sugarcane pith (USP) (100 g, about 92% DM) to obtain samples of approximately 30% dry

mater (DM) content of 0.0, 6, 12 and 18 g/kg H<sub>2</sub>SO<sub>4</sub> on a DM basis. Then, a part of samples were autoclaved (at 121°C for 120 min, 2.2 bar) and then oven-dried at 55°C. Rumen fluid was collected from two fistulated sheep prior to the morning feeding. Animals fed 250 g concentrate and 750 g forage once at day. Collected rumen contents were strained through four layers of cheesecloth; different microbial fractions for evaluating ruminal fermentation were prepared according to the method described by Zhang *et al.* (2006) as: the strained rumen fluid (total microorganism, TM), protozoa was isolated using centrifuged procedure (1000 RPM, 5 min), fungi (F) was prepared from non protozoa strained rumen fluid using antibacterial agent (streptomycin sulphate, penicillin G, potassium & chloramphenicol [0.1 mg/mL each]) and bacteria was isolated from non protozoa strained rumen fluid using antifungal agent [benomyle (500 mg/L) and metalaxyle (10 mg/L)]. The antibiotics and other chemicals were used 100 µL/mL of mixed culture solution was injected into the syringes to give desired concentrations before the mixed culture solution (30 mL) was added to the syringes.

**Gas production method:** *In vitro* fermentation by various microbial groups of rumen was estimated by gas production (GP) method as described by Menke and Steingass (1988). Rumen fluid (total microorganism) and the isolated microbial groups (P, B & F) were added to the anaerobic buffer before transfer to the syringes (three parallel syringes of each treatment). The glass syringes were prewarmed to 39°C, then 30 mL of mixed culture medium (consisting of ruminal microbial groups & buffer; ratio 1:2) were pipetted into each glass syringe followed by incubation in a water bath at 39°C for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h.

Syringes were used to measure the gas production and DM degradation of experimental samples within 96 h. The syringes were taken out of the incubator at 96 h. culture fluid of each sample carefully removed and residues in the syringes washed into a tube, carefully with distilled water separately. Then the residues were dried at 105°C for 12 h and used to calculate the degradation of samples. Cumulative gas production data were fitted to the exponential equation  $Y = B(1 - e^{-Ct})$ , where B is the gas production from the fermentable fraction (mL), C is the gas production rate constant C (mL/h), t is the incubation time (h) and Y is the gas produced at time t. The values of organic matter digestibility (OMD) and metabolisable energy (ME) of samples were calculated by the equation of (Menke & Steingass, 1988),  $OMD (g/kg OM) = 148.8 + 8.89 GP + 4.5 CP + 0.651A$  and  $ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CP^2$ . Microbial biomass production (MB) was estimated by method of Blümmel *et al.* (1997).

Data of gas production, cell wall degradability, OMD, ME and MB were subjected to analysis as a completely randomized design using the General Linear

Model procedures of SAS institute (1996). Duncan's multiple range test was used to compare the means at  $P < 0.05$ .

## RESULTS AND DISCUSSION

*In vitro* gas production parameters (B & C) of experimental samples by total rumen microorganism (TM) and other microbial groups over 96 h incubation are shown in Table I. Processing with steam associated with acid caused to increase gas production by microbial fractions, and increased in amount of acid, resulted in a significant increase of gas production parameters ( $P < 0.05$ ). The most amount of gas production by all microbial groups was for 18 g acid/kg DM ( $P < 0.05$ ). The highest gas production for all the samples was observed in TM group, followed by the group of fungi and protozoa. Steam treatment may be caused to partial or complete hydrolysis of hemicellulose fraction of raw pith and decrease of NDF, changing it into more soluble components (Toussaint *et al.*, 1991), partial depolymerization of lignin (Morjanoff & Gray, 1987) and following increasing digestibility (Chaji *et al.*, 2010). Using lower temperatures with an acid can achieve comparable cell wall disruption to steam treatment at high temperatures (Castro *et al.*, 1994). Steam and pressure treatments alone or allied with chemical treatments are known to disrupt lignocellulosics in a way, which allows improved utilization of cell wall polysaccharides by cell-free enzymes (Grohmann *et al.*, 1985) and rumen microbes (Castro & Machado, 1990).

The result of this study showed that treating acidified sugarcane pith with low temperature (121°C for 120 min. & 18 g/kg DM acid) had the highest cell wall degradability, OMD and ME and the lowest MB of both samples by microbial groups (Table II). The researchers reported under these steam conditions hemicellulose fraction hydrolysed and acetyl groups are released from the hemicellulose matrix and suitable levels of cell wall disruption are achieved (Grohmann *et al.*, 1985). By applying the steam explosion process to sugarcane bagasse, the susceptibility of cellulose to enzymatic hydrolysis was increased (Kling *et al.*, 1987), also cellulose will be more accessible for rumen microbial enzymes (Castro & Machado, 1990) and cell-free enzymes (Liu & Orskov, 2000). Chaji and Naserian (2006) reported increased *in vitro* dry matter digestibility for sugarcane pith treated with steam by about 14%. Liu *et al.* (1999) reported any improvement in digestibility of bagasse resulting from steam treatment was due to the formation of water soluble substances.

These results indicate that the cellulolytic activity of whole ruminal groups was highest, and there was a complementary synergism on the degradation of plant cell walls among ruminal bacteria, protozoa and fungi. The highest gas production for all the samples was observed in TM group, whereas a less gas volume was produced by the group of fungi and protozoa. The gas production and

**Table I: Gas production coefficients (mean ± SE, mL/200 mg DM) of sugarcane pith treated with low temperature and acid by rumen microbial fractions**

Temp (°C)	Time (min.)	Acid (g/kg DM)		Microbial fractions			
				TM	B	F	P
0.0	0.0	0.0	B	64.2±2.8e	54.2±1.3d	28.5±0.3e	10.1±0.1d
121	120	0.0	B	76.4±2.3d	67.5±1.2c	37.7±0.8d	15.5±0.4c
121	120	6	B	80.4±1.6c	71.1±1.1b	43.3±0.7c	19.5±0.6b
121	120	12	B	105.3±2.6b	89.6±2.7a	52.3±1.0b	23.3±0.6a
121	120	18	B	112.9±3.5a	92.6±1.1a	54.7±0.9a	23.5±0.5a
		SEM		2.06	0.98	0.34	0.08
0.0	0.0	0.0	C	0.05±0.003a	0.03±0.002a	0.01±0.002b	0.02±0.004b
121	120	0.0	C	0.06±0.001b	0.01±0.002b	0.01±0.004b	0.02±0.001b
121	120	6	C	0.03±0.001c	0.01±0.003b	0.02±0.003a	0.03±0.005a
121	120	12	C	0.04±0.002d	0.03±0.004a	0.02±0.004a	0.03±0.005a
121	120	18	C	0.04±0.008d	0.01±0.005b	0.01±0.004b	0.02±0.002b
		SEM		0.002	0.0022	0.0032	0.0024

B: Gas production from the fermentable fraction (mL for 96h) ; C: Gas production rate constant (mL/h)

**Table II: Estimated parameters of sugarcane pith treated with low temperature and acid by rumen microbial fractions**

Temp (°C)	Time (min.)	Acid (g/kg DM)		Microbial fractions			
				TM	B	F	P
0.0	0.0	0.0	CEL	42.1e	30.3e	11.7e	4.6e
121	120	0.0		56.1d	40.2d	15.7d	6.5d
121	120	6		63.4c	45.2c	17.8c	7.3c
121	120	12		72.5b	51.6b	20.3b	8.3b
121	120	18		78.5a	56.2a	22.1a	10.4a
		SEM		0.2	0.1	0.2	0.3
		P		<0.05	<0.05	<0.05	<0.05
0.0	0.0	0.0	OMD	425.20e	347.32e	196.10e	79.81e
121	120	0.0		535.62d	433.21d	243.70d	95.51d
121	120	6		550.46c	454.12c	261.20c	110.83c
121	120	12		733.26b	600.61b	341.91b	135.80b
121	120	18		822.16a	676.90a	382.83a	155.31a
		SEM		1.5	2.2	3.2	2.4
		P		<0.05	<0.05	<0.05	<0.05
0.0	0.0	0.0	ME	6.60e	5.45e	3.50e	1.20e
121	120	0.0		7.50d	6.59d	3.76d	1.44d
121	120	6		8.23c	6.83c	3.90c	1.56c
121	120	12		11.30b	9.18b	5.19b	2.14b
121	120	18		12.36a	10.75a	5.82a	2.22a
		SEM		0.02	0.01	0.02	0.01
		P		<0.05	<0.05	<0.05	<0.05
0.0	0.0	0.0	MB	24.08a	110.40a	122.30a	134.21a
121	120	0.0		22.80b	101.29b	109.40b	120.10b
121	120	6		20.70c	96.35c	104.91c	115.01c
121	120	12		19.12d	84.80d	92.30d	104.02d
121	120	18		16.70e	77.80e	85.20e	96.10e
		SEM		0.4	1.1	1.2	0.4
		P		<0.05	<0.05	<0.05	<0.05

CEL: cell wall degradability; OMD: organic matter digestibility; ME: metabolisable energy; MB: microbial biomass production

TM: total rumen microorganism; P: protozoa; B: bacterial; F: fungal

If means within each column have different letters are significantly different ( $P < 0.05$ )

degradability by B group was higher than fungi and protozoa. Although, the rumen cellulolytic bacteria are believed to be responsible for most of the feed digestion in the rumen (Cheng *et al.*, 1991; Flint & Bayer, 2008), the

anaerobic fungi to penetrate deeply into plant tissues that are not normally accessible to bacteria suggests that they have a special role in fiber digestion (Thareja *et al.*, 2006). The quantitative estimation revealed that rumen protozoa cause

25-30% of total rumen microbial fiber digestion in the rumen (Lee *et al.*, 2000).

## CONCLUSION

Ruminal bacteria, protozoa and fungi together contribute to ruminal microbial fermentation of plant cell walls. Within the protozoal, bacterial or fungal group, bacterial group generates a larger amount of gas than fungal or protozoal group and this method had no negative effect on rumen microorganisms. Low temperature steam (121°C, 120 min, 2.2 bar) associated H<sub>2</sub>SO<sub>4</sub> (18 g acid/kg DM) increased gas production parameters, cell wall degradability, OMD and ME, and decreased MB by all rumen microbial fractions and therefore, improved nutritional values of sugarcane pith.

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