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Short communication

Misleading relationships between in situ rumen dry matter disappearance, chemical analyses and in vitro gas production and digestibility, of sugarcane bagasse treated with varying levels of electron irradiation and ammonia

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Abstract

Twelve samples of sugarcane bagasse, representing untreated material or bagasse irradiated with doses of 200, 400, 600, 800 and 1000 KGy in an electron accelerator and afterwards with or without 20 g/kg dry matter (DM) of NH₃. These were analysed for changes in neutral detergent fibre (NDF), phenolic compound and reducing sugars content. DM disappearance was evaluated using both 48 h in situ and in vitro incubations, 0 h DM disappearance (washing losses) and in vitro volatile fatty acid (VFA) production. In situ disappearance was highly correlated with NDF (negatively), phenolic compound (positively) and reducing sugar (positively) content. However, washing losses were similarly correlated to NDF, phenolic compounds and reducing sugars, and little variation in either in vitro gas or VFA production was recorded. It is therefore concluded that the variations in situ disappearance observed were an artefact of washing losses and did not accurately reflect differences in rumen fermentability. These results may help to explain why the performance of animals, reported in the literature, offered treated bagasse is not as good as that anticipated from in situ analyses. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electron irradiation; Bagasse; In situ dry matter disappearance; In vitro gas production

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1. Introduction

Each tonne of sugarcane produces around 300 kg of bagasse by-product; a large quantity is therefore generated in sugar producing countries such as Brazil. Sugar-factory bagasse is a high moisture (ca. 500 g dry matter (DM) kg^{-1}), high crude fibre (500 g kg DM^{-1}), high lignin (200 g kg DM^{-1}) and low nitrogen (6.3 g kg DM^{-1}) feed stuff (Gohl, 1981) of low digestibility (e.g. 48 h in situ rumen DM disappearance, 360 g kg^{-1} ; Basile and Machado, 1990). Therefore, efforts have been made to improve the digestibility of bagasse using treatment with chemicals (sodium hydroxide and/or ammonia, Abdalla et al., 1990; Martin et al., 1976; phosphoric acid, Deschamps et al., 1996) or steam and pressure (Basile and Machado, 1990; Vitti, 1984). Although treatments have improved in situ rumen DM disappearance, intake and digestibility in cattle (Mello et al., 1989) and in sheep (Abdalla et al., 1990) have been disappointing. Recently, bagasse and other fibrous crop residues have been treated with electron irradiation and ammonia and effects on structural (electron microscopy) and chemical properties reported (Vitti et al., 1998). The study by Vitti et al. (1998) also showed that in situ rumen DM disappearance increased with electron irradiation dose over the range 200–1000 KGy. However, a parallel study (Abdalla and Vitti, 1996) to compare hay and bagasse treated with a 600 KGy of electron irradiation and 20 g $\text{NH}_3 \text{ kg DM}^{-1}$ showed treated bagasse to be no better than hay as a maintenance feed. In addition, radiophosphorous uptake by rumen micro-organisms tended to be higher in sheep fed hay, indicating a lower microbial growth in animals fed treated bagasse.

In the present study, samples of treated bagasse generated by Vitti et al. (1998) were reassessed and also subjected to in vitro fermentation with rumen liquor to measure gas production, volatile fatty acid production and digestibility. The objective was to establish whether in vitro fermentation data, together with chemical analyses, would help explain why growth performance of animals fed treated bagasse was not as good as that anticipated from in situ rumen DM disappearance.

2. Materials and methods

Samples of fresh sugarcane bagasse (two replicates of 5 kg, with DM content of 500 g kg^{-1}) were irradiated with doses of 200, 400, 600, 800 or 1000 KGy using an electron accelerator (Dynamitron, Radiation Dynamics). Each sample was placed in a metal tray at a density of 0.45 g cm^{-2} , covered with aluminium foil and passed through the accelerator at a rate of 0.84 cm min^{-1} . With setting of 1.5 MeV and a current flux of 7.0 mA, one passage result in an exposure of 50 KGy. Multiple passages were therefore used to obtain the required dose rate. Following treatment, half of each sample was placed in a closed cylinder (0.42 m^{-3}) and treated with 20 g kg DM^{-1} of ammonia gas for 5 min at ambient temperature (25°C). A non-irradiated sample was also treated with ammonia. Untreated and treated samples were dried at 60°C, milled to pass a 2 mm screen and assessed for in situ DM disappearance (48 h, Ørskov and McDonald, 1979). This was assessed by placing 3 g samples in artificial fibre bags (inner size 7.5 × 10.5 cm; pore size = 50 μ) with three bags per sample inserted before the

morning feed into the rumen of each of two rumen fistulated steers (ca. 250 kg LW and six months of age) offered *Brachiaria decumbens* hay ad libitum and 1.0 kg commercial concentrate (180 g kg⁻¹ crude protein) daily. Following incubation the bags were removed from the rumen, rinsed in cold water and washed by hand under running water until the water became clear. Zero hour DM disappearance was determined with six bags per sample using the same rinsing and washing procedure as that for bags incubated in the rumen. Samples were analysed in triplicate for neutral detergent fibre (NDF) (Van Soest et al., 1991), phenolic compounds (Swain and Hills, 1959) and reducing sugars (Somogyi, 1952). Triplicate samples were also assessed in Reading (UK) for in vitro gas production (48 h incubation) (Theodorou et al., 1994) using rumen liquor obtained from a rumen-fistulated, mid-lactation cow fed a grass silage base diet. In addition, in vitro organic matter (OM) digestibility and volatile fatty acid (VFA) production was determined following 48 h incubation. Due to an analytical capacity restriction only nine bagasse samples were selected, on the basis of in situ DM disappearance, for the in vitro investigation. Three treatments (untreated NH₃, 400 and 800 KGy NH₃) were, therefore, not examined. Pearson correlation coefficients were computed (SAS, 1985) using mean values of parameters, to investigate the relationships between each of in situ 48 h DM disappearance and 0 h DM disappearance with NDF, phenolic compounds, reducing sugars, in vitro 48 h OMD, in vitro 48 h gas production and in vitro 48 h VFA. Correlations between in vitro 48 h gas production and NDF, phenolic compounds, reducing sugars, in vitro 48 h OMD and in vitro 48 h VFA were also computed.

3. Results

Compared to untreated bagasse, irradiation treatment increased the content of both phenolic compounds and reducing sugars while decreasing NDF content (Table 1), with the magnitude of this response increasing with the level of radiation up to 1000 KGy, with the exception of sugars where increasing the dose rate above 800 gave no further improvement. Irradiation also appeared to affect particle size as losses at zero hour increased with treatment (Table 2). In situ disappearance values followed those of washing losses increasing with the level of radiation. In vitro 48 h gas production values were variable and no consistent treatment effect could be detected. In vitro degradability were also inconsistent but all treated substrates showed an increase compared with the controls. Treating with 20 g NH₃ kg DM⁻¹ after irradiation appeared to have little effect on DM degradation in situ. Total in vitro VFA production increased as a result of irradiation, although this effect was lowest at the highest rate (1000 KGy). Similar proportions of VFA were produced (Table 2) although there was a tendency for acetate and butyrate to be increased relative to propionate ($[C_2 + C_4]/C_3$) following exposure to medium levels of irradiation. An increase, due to treatment, was also observed with in vitro OMD but this improvement was much lower than that found with in situ DM degradation.

Table 3 shows that in situ DM disappearance was highly correlated ($p < 0.001$), with NDF (negatively) and positively with both phenolic compounds and reducing sugars.

Table 1
NDF, phenolic compounds and sugar

Bagasse samples	NDF (g kg DM ⁻¹)	Phenolic compounds (g kg DM ⁻¹)	Sugar (g kg DM ⁻¹)
0 KGy	889	7.4	5
0 KGy, NH ₃	870	5.6	10
200 KGy	767	11.6	35
200 KGy, NH ₃	767	10.2	28
400 KGy	672	14.4	38
400 KGy, NH ₃	671	15.4	28
600 KGy	598	21.1	40
600 KGy, NH ₃	599	17.9	35
800 KGy	547	20.6	40
800 KGy, NH ₃	535	22.7	45
1000 KGy	493	24.6	50
1000 KGy, NH ₃	478	28.7	45

Compared with the latter, correlations between in situ DM disappearance and in vitro 48 h OMD and in vitro 48 h gas production were lower and less significant ($p < 0.05$). Correlations between 0 h DM disappearance and the various parameters were similar to those between in situ 48 h DM disappearance and the various parameters. There was no correlation between in vitro 48 h gas production and in vitro 48 h VFA production. There was also no correlation between in situ 48 h DM disappearance and in vitro 48 h VFA production.

4. Discussion

A significant linear reduction ($r^2 = 0.98$, $p < 0.0001$) in NDF content occurred when sugarcane bagasse was treated with increasing levels of radiation, a similar finding to Flachowsky et al. (1990). They reported decreasing NDF and ADF contents of wood by-products irradiated with increasing doses of gamma rays over the range 100–2000 KGy. In the same study, in situ DM disappearance also increased with increasing irradiation dose, but the authors questioned whether the DM solubilised would be fermentable in the rumen. They cited the early work of McManus et al. (1972) showing inhibition of microbial activity and depression of digestibility after feeding irradiated straw and that of Sorokin et al. (1989) showing a decrease in digestibility when feeding irradiated sawdust. On the other hand, Flachowsky et al. (1985) found a higher in vivo digestibility of irradiated wheat straw compared to untreated straw. Both Flachowsky et al. (1985) and Vitti et al. (1998) commented on the apparent decrease in particle size and increase dustiness of irradiated material. In the present study and that of Flachowsky et al. (1985) it is likely that part of the increased DM disappearance during in situ incubation was due to loss of fine particles from the artificial fibre bags rather than solubilisation per se. Reduction in particle size of bagasse following steam treatment and the implications for in situ rumen DM disappearance measurement was also commented on by Basile and Machado (1990). In the present study, it is unlikely that the increase in the quantity of

Table 2
In situ 48 h DM disappearance and other fermentation parameters of irradiated and ammonia treated samples of sugarcane bagasse

Bagasse samples	In situ 48 h DM disappearance (g kg DM ⁻¹)	0 h DM disappearance (g kg DM ⁻¹)	In vitro 48 h gas (ml)	In vitro 48 h VFA					In vitro 48 h OMD (g kg ⁻¹)
				total (mg ml ⁻¹)	proportion			ratio (C ₂ + C ₄)/C ₃	
					acetic	propionic	butyric		
0 KGy	300	135	108	1.6	0.53	0.34	0.13	1.9	332
0 KGy, NH ₃	304	162	—	—	—	—	—	—	—
200 KGy	353	208	128	2.6	0.54	0.33	0.13	2.0	385
200 KGy, NH ₃	320	198	114	2.4	0.55	0.33	0.12	2.1	374
400 KGy	371	211	—	—	—	—	—	—	—
400 KGy, NH ₃	423	270	106	2.5	0.63	0.26	0.11	2.9	377
600 KGy	455	418	109	2.7	0.61	0.28	0.12	2.6	396
600 KGy, NH ₃	516	424	85	2.3	0.63	0.26	0.11	2.9	338
800 KGy	574	522	101	2.3	0.55	0.33	0.12	2.0	433
800 KGy, NH ₃	560	538	—	—	—	—	—	—	—
1000 KGy	541	517	103	2.3	0.56	0.32	0.12	2.1	442
1000 KGy, NH ₃	557	532	101	1.7	0.53	0.34	0.13	2.0	415

Table 3
Pearson correlation coefficients between in situ 48 h DM disappearance and other parameters

	In situ 48 h DM disappearance	0 h DM disappearance	In vitro 48 h gas	NDF	Phenolic compounds	Sugar	In vitro 48 h VFA	In vitro 48 h OMD
In situ 48 h DM disappearance	—	0.98 ^a	−0.65 ^c	−0.94 ^c	0.91 ^c	0.76 ^b	0.02 ^d	0.65 ^a
0 h DM disappearance	0.98 ^c	—	−0.58 ^d	−0.95 ^c	0.94 ^c	0.78 ^b	0.006 ^d	0.71 ^a
In vitro 48 h gas	−0.65 ^d	−0.58 ^d	—	0.53 ^d	−0.46 ^d	−0.18 ^d	0.29 ^d	0.07 ^d
NDF	−0.94 ^c	−0.95 ^c	0.53 ^d	—	−0.97 ^c	−0.89 ^c	−0.08 ^d	−0.73 ^a
Phenolic composition	0.91 ^c	0.94 ^c	−0.46 ^d	−0.97 ^c	—	0.83 ^b	−0.05 ^d	0.72 ^a
Sugar	0.76 ^b	0.78 ^b	−0.18 ^d	−0.89 ^c	0.83 ^b	—	0.37 ^d	0.80 ^b
In vitro 48 h VFA	−0.02 ^b	−0.006 ^d	0.29 ^d	−0.083 ^d	−0.05 ^d	0.37 ^d	—	0.19 ^d
In vitro 48 h OMD	0.65 ^a	0.71 ^a	0.07 ^d	−0.73 ^a	0.72 ^a	0.80 ^b	0.19 ^d	—

^a $p < 0.05$.

^b $p < 0.001$.

^c $p < 0.0001$.

^d Non-significant.

phenolic compounds released due to irradiation inhibited microbial *in vitro* fermentation. This is because even the highest level of phenolic compounds detected (28.7 g kg DM⁻¹ bagasse) would have only resulted in a concentration of 3.2×10^{-4} g m⁻¹ fermentation inoculum. This level corresponds to ca. 0.01 of the minimum inhibitory concentration (0.025 g ml⁻¹ inoculum) reported when phenolic compounds (as tannic acid) were added to rumen fluid inoculum (Vitti et al., 1986). The decreases in NDF observed are typical of those following treatment of straw with NaOH and which are normally associated with increased *in vitro* and *in vivo* digestibility (Wilkinson, 1984). However, early work on treatment of barley straw with NaOH (Jayasuriya and Owen, 1975) showed that responses measured using the *in vitro* digestibility technique (Tilley and Terry, 1963) overestimated those measured *in vivo*. This was considered to be due to phenolic compounds released during treatment, being deemed to be digestible in the *in vitro* technique on account of their being soluble and the fact that the technique measures DM and/or OM disappearance. *In vivo*, however, phenolic compounds are known to be indigestible (Khazaal et al., 1994).

The improvement in *in vitro* 48 h OMD with irradiation observed in the present study was likely to be due to a combination of the decreased particle size increasing the surface area exposed for microbial attachment, a possible increase in solubility and the alteration in the chemical composition (especially the reduction in NDF content and corresponding increase in sugar content). Gas released during *in vitro* fermentation originates either directly from fermentation or from the neutralisation of the organic acids produced by the buffered medium. It is, therefore, interesting that *in vitro* gas production remained largely unaltered in this study, and found to be non-correlated with any of the other fermentation parameters examined. Although increased fermentation (OMD) does not necessarily have to result in increased gas production, fermentation stoichiometry suggests that both the increased VFA production and slight shift towards a C₂/C₄ fermentation, observed in this study, should have resulted in increased gas production. It is proposed that the apparently lower *in vitro* estimate for degradation provides a better assessment of the effect of treatment than the *in situ* technique as the latter values are greatly influenced by the substantial washing losses (soluble and particle) which have been reported to occur as a result of particle size reduction following irradiation (Flachowsky et al., 1985).

5. Conclusions

The present study provides evidence that irradiation improves the degradation of sugarcane bagasse. *In vitro* techniques indicate that this results in an increase in VFA production, with possibly a slight shift towards a C₂/C₄ fermentation. However, the greater washing losses associated with the *in situ* technique, a direct result of irradiation on particle size, tend to overestimate this improvement and may be the cause of the poor correlation often observed between *in situ* and *in vivo* studies when this material has been examined. The study helps to explain literature studies where the performance of animals fed steam treated and irradiated treated bagasse were not as good as anticipated from *in situ* rumen DM disappearance.

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