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NUTRITIVE VALUE FOR RUMINANTS OF SUGAR CANE BAGASSE ENSILED AFTER SPRAY TREATMENT WITH DIFFERENT LEVELS OF NaOH

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ABSTRACT

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Sugar cane bagasse previously dried to a water content below 25% was sprayed with equal quantities of sodium hydroxide solutions to supply 0, 20, 40 or 60 g alkali/kg of dried product and ensiled for 90 days.

The cell wall content (NDF) decreased with increasing NaOH ($P < 0.001$), whereas the lignocellulose fraction (ADF) remained unaffected. The effect on lignin (ADL) was small. All this suggests a solubilization of the hemicellulose fraction with alkali application. Ensiling reduced the pH in the bagasse owing to partial neutralization of residual NaOH.

From a microbiological point of view, the stability of the NaOH-treated product, whether during the period of silage or when exposed to air, was good. The presence of NaOH in the silo inhibited the development of bacteria and fungi, an effect which was probably enhanced by the reduced soluble carbohydrate content of the substrate.

Sixteen digestibility experiments following a 4 × 2 × 2 factorial design were carried out on wethers of 55.0 kg given, ad libitum, isocaloric diets containing the NaOH-sprayed bagasse supplemented with molasses, a mineral—vitamin mixture and urea—biuret or soya bean meal, to provide a total N content of 1.65 and 2.10%, irrespective of the N source.

Average daily intakes were 29.8, 35.7, 44.7 and 40.6 g/kg $W^{0.75}$ for diets containing bagasse sprayed with 0, 20, 40 and 60 g alkali/kg of dried product, respectively. The effects of NaOH level, N source and N level were highly significant ($P < 0.001$).

The apparent digestibility of dry matter (DM), organic matter (OM) and cell wall components, and the N retention increased linearly ($P < 0.001$) as the level of chemical treatment increased. Except for hemicellulose, the digestibility of the cell wall components was improved when soya bean meal was included in the experimental diets. The effect of N content was not significant. The calculated average organic matter digestibility (OMD) for the sugar cane bagasse ranged from 32.6 for the water-treated product to 56.8% with the highest level of alkali, the effect of the chemical treatment being significant ($P < 0.001$).

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INTRODUCTION

Several authors have recently reviewed the alkali treatment of products rich in lignocellulose (Owen, 1976; Jackson, 1977; Gonzalez Santillana, 1978; Greenhalgh, 1978; Klopfenstein, 1978; Ørskov, 1979).

Sugar cane bagasse (*Saccharum officinarum*) has a nutritive value lower than that of other low-quality roughages such as cereal straws, and the improvement obtained by alkali treatment seems to be inferior (Verma and Jackson, 1975). Ensiling of the treated product probably enhances the NaOH effect on the components of the cell walls, thus providing a more available lignocellulose structure for rumen micro-organisms. The effect of the intensity of alkali spray treatment on the digestibility in vivo of sugar cane bagasse has not been studied, but there are data from experiments in which sugar cane bagasse was treated with alkali according to Beckman's technique (Randel, 1972) and included in complete rations for cattle.

On the other hand, it is now clear that the nitrogen requirements of the rumen micro-organisms depend on the amount of fermented substrate (Ørskov et al., 1972; Miller, 1973), and that the nitrogen uptake is largely a function of both the rate of degradation of the energetic substrate and of the availability of the nitrogen for micro-organisms (Oldham et al., 1977).

The aim of the present work was to determine the effect of NaOH spray treatment on the composition and nutritive value for sheep of sugar cane bagasse. The ability of a biuret–urea mixture, added to the experimental diets as a non-protein source (NPN), to meet the nitrogen requirements of rumen micro-organisms was also studied.

MATERIALS AND METHODS

Experimental procedure

Sugar cane bagasse harvested in 1979 from the coast of Granada was dried at ambient temperature (spread over a concrete floor in the open air) to 75% dry matter. This partially dried bagasse (several lots of 25 kg per treatment) was spread thinly over a polyethylene sheet and sprayed with 25 l of a solution containing 0, 20, 40 or 60 g NaOH l^{-1}, at a rate of 2.5 l min^{-1}. Material from each treatment was then stored in two silos with a capacity of 3 m^3. A representative sample (5 kg) from each silo was kept anaerobically in a sealed polyethylene bag for total viable microflora counts.

Isocaloric diets for sheep were prepared by supplementing the bagasse with beet molasses, a mineral–vitamin mixture and a urea–biuret mixture (60.0% biuret; 36.2% urea) (M) or soya bean meal (S), to provide 1.65 (I) or 2.10 (II) percentage units of N. The composition of the diets is shown in Table I.

The digestibility trials were carried out on 12-month-old Segureña wethers with an average initial weight of 55.0 kg, individually housed in metabolism

TABLE I

Composition of diets containing urea—biuret mixture or soya bean meal as the nitrogen supplement (g kg^{-1})

	Nitrogen source and level (%)			
	Urea—biuret mixture		Soya bean meal	
	1.65	2.10	1.65	2.10
Ensiled treated bagasse (DM)	650	650	650	650
Beet molasses	125	125	125	125
Urea—biuret mixture	25	35	—	—
Soya bean meal (8% N)	—	—	125	175
Starch	150	140	57	7
Calcium triphosphate	12	12	12	12
Calcium diphosphate	15	15	15	15
Calcium monophosphate	3	3	—	—
Magnesium sulphate	14	14	10	10
Magnesium carbonate	4	4	4	4
Mineral—vitamin supplement[1]	2	2	2	2

[1] One kg provides 5 000 000 IU vitamin A; 1 600 000 IU vitamin D_3; 4 g vitamin B_2; 0.2 g Co; 0.75 g I; 1.2 g Cu; 21 g Zn; 30 g Mn; and 42 g Fe.

crates and with free access to water. Sixteen digestibility trials, each on six animals, were performed following a 4 (level of alkali) × 2 (source of supplemental N) × 2 (level of dietary N) factorial design.

The intakes were established on the basis of previous experimental data obtained on individual animals, to be the maximum obtainable without refusals. When the ration was not fully consumed, aliquots were taken for analysis. After a period of adaptation to the diets (25 days when diets were supplemented with the urea- biuret mixture and 10 days for diets including soya bean meal), the digestibility and nitrogen balance were determined individually by collecting the total excreta for a 10-day period, the animals being fed twice daily.

Cumulative samples of feed and excreta were taken daily for each animal and kept at −25°C in airtight reservoirs until analysed.

Chemical analyses

The determinations of dry matter, pH and total N were carried out directly on the samples maintained at −25°C after thawing at room temperature. The dry matter content was determined by freeze-drying. The pH was also measured in samples of ensiled bagasse 2 and 25 days after treatment. The analytical measurements of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ash and silica, and total and residual sodium, were made on samples oven dried at 60°C, equilibrated in air

and milled. The NDF was obtained as described by van Soest and Wine (1967), the ADF and the ADL according to van Soest (1963) and silica as the insoluble mineral fraction remaining after treatment of ADL ash with 48% HBr for 90 min and heating at 550°C. Hemicellulose and cellulose contents were calculated by difference (NDF−ADF and ADF−ADL, respectively). The pH determination was carried out with filtered liquid taken from the sample after maceration for 1 h with an equal volume of distilled water; the total N according to a macro-Kjeldahl procedure utilizing a mixture of K_2SO_4, $CuSO_4$ and Se as catalysts; the mineral content by ashing the sample at 550°C for 3.5 h and the total sodium by flame photometry after mineralization at 450°C and extraction of the residue with HCl. The residual sodium analysis (from NaOH which had not reacted with bagasse) was made by potentiometric evaluation to pH 8 with 0.01 N HCl, after maceration of 10 g of the sample with 50 ml of distilled water.

The organic matter digestibility (OMD) of the bagasse was obtained by assuming that the digestibility coefficient of the rest of the ingredients remained constant at 0.90 in all diets.

Microbiological analysis

The total viable microflora was determined both on treated non-ensiled bagasse and on samples stored anaerobically for 25 days. In the latter case, the product stability was studied by exposing samples to the open air in Petri dishes for 10 days.

In all cases, 10-g samples of bagasse were shaken thoroughly in 100 ml of distilled water, and 10-fold serial dilutions were prepared from the macerate. Aliquots (1 ml) of the solutions were poured in duplicate Petri dishes containing a common agar—yeast extract medium with peptone, glucose and salts, and were incubated at 30°C for 5 days.

RESULTS

Effect of alkali treatment on the composition of ensiled sugar cane bagasse

The pretreatment mean composition of the sugar cane bagasse, as well as the average analytical values of this product treated with NaOH solutions and ensiled for 90 days, appear in Table II. Over 90% of the dry matter was accounted for by NDF, which consisted mainly of cellulose (56.0%) and hemicellulose (31.5%), whereas lignin formed only 12.5% of this fraction. As expected, the N content was very low.

The pH increased considerably with the incorporation of 2% NaOH solution and levelled off thereafter. The pH values in the treated product decreased by about 2.5 units during the first 25 days in the silos. An extension of the period of storage did not induce further changes (Fig. 1).

The content of NDF in the ensiled sugar cane bagasse decreased ($P < 0.001$)

TABLE II

Effect of alkali treatment and ensiling on the composition of sugar cane bagasse (% dry matter)

	Pre-treated bagasse	Level of NaOH (g/100 g dried bagasse)				SEM[1]	Level of significance		
		0	2	4	6		Treatment	Linear effect	Quadratic effect
NDF	93.6	94.5^{c}	89.5^{b}	86.0^{b}	72.7^{a}	1.02	***	***	**
ADF	64.1	65.1	64.6	65.2	62.2	1.36	NS	—	—
ADL	11.7	12.0^{b}	11.5^{bc}	9.93^{ac}	9.00^{a}	0.55	**	**	NS
NDS	6.4	5.47^{a}	10.5^{b}	14.9^{c}	27.3^{d}	1.00	***	***	**
Hemicellulose	29.5	29.4^{c}	24.9^{bc}	20.8^{b}	10.5^{a}	1.31	***	***	NS
Cellulose	52.5	53.1	53.2	55.3	53.2	1.36	NS	—	—
Total N	0.54	0.49	0.52	0.49	0.57	0.03	NS	—	—
Ash	3.99	3.28^{a}	7.85^{b}	9.93^{b}	16.3^{c}	0.63	***	***	NS
Silica	1.50	1.50	1.34	1.34	1.54	0.23	NS	—	—
Total Na	—	Traces	1.31	2.32	3.70		—		
Residual Na	—	Traces	0.32	0.43	1.17		—		
Dry matter	41.5	43.9	45.1	45.6	44.2	1.64	NS	—	—
pH	—	6.95	9.70	10.0	10.2				

[1] Standard error of mean.

^{a,b,c}Means within a row with different superscripts differ significantly; $P < 0.05$.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS = $P > 0.05$.

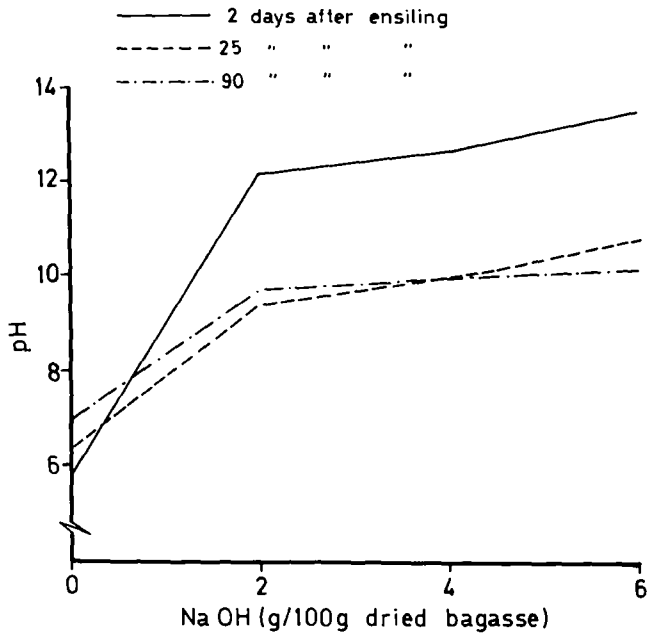

Fig. 1. Effect of alkali treatment and period of ensiling on pH of sugar cane bagasse.

as the level of alkali increased, whereas no significant differences were found in ADF. The fall in NDF content was basically due to solubilization of hemicellulose ($P < 0.001$) and, to a lesser extent, to the decrease in the ADL fraction ($P < 0.001$) caused by the alkali treatment.

Significant differences in the total mineral content of the product ($P < 0.001$) were found with the addition of NaOH. Conversely, both the silica and the N contents of bagasse remained unaffected by the treatments.

The results of the microbial study are shown in Table III. Higher counts

TABLE III

Total viable count of micro-organisms (N × 10^7/g fresh matter) in sugar cane bagasse treated with NaOH. Product stability after exposure to air

NaOH (g/100 g dried bagasse)	Non-ensiled bagasse		Ensiled bagasse			
			0 days[1]		10 days[1]	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
0	155	14	—	—	62	2.0
2	270	30	25	3.0	7.4	0.8
4	311	17	38	0.5	1.3	0.1
6	23	20	18	0.8 .	1.7	0.1

[1] Time of exposure to air.

were found in the non-ensiled product. Ensiling of bagasse for 25 days decreased 10-fold the content of viable micro-organisms, irrespective of the level of alkali treatment. The greater levels of alkali also reduced the number of micro-organisms in non-ensiled bagasse. After its exposure to air for 10 days the numbers of both total viable bacteria and fungi were substantially reduced. It thus seemed that ensiled alkali-treated sugar cane bagasse was sufficiently well preserved.

Effect of the alkali treatment on intake and digestibility

In general, the daily intakes of the rations containing 0 or 20 g NaOH/kg treated bagasse (29.8 and 35.7 g/kg $W^{0.75}$, respectively) were too low and losses of liveweight were observed. Higher alkali levels gave higher intakes (44.7 and 40.6 g/kg $W^{0.75}$, respectively, for 40 and 60 g NaOH/kg treated bagasse), adequate to meet maintenance requirements or to promote slight gains. The addition of alkali, as well as the level and source of nitrogen in the diet, affected intake significantly ($P < 0.001$). The intake of the diets containing soya bean meal was superior to that of rations containing a biuret—urea mixture.

As the level of NaOH in the diet increased, the water intake, volume of urine and the water content of faeces increased markedly.

The mean digestibility coefficients and the nitrogen retention results appear in Table IV. Figures 2 and 3 show the main effects of treatments. The mean digestibility of diets which included untreated bagasse was 47.4 and 50.2, respectively, for DM and OM, and rose to 66.5 and 68.0% with the highest concentration of alkali. Significant linear and quadratic components of variance showed the improvement in digestibility of the diet as the level of alkali increased and also the tendency for the magnitude of the effect to decline with the highest level of NaOH addition. The effects of level and source of dietary nitrogen were non-significant.

Table V gives the calculated digestibility of organic matter (OMD) of the sugar cane bagasse alone. The average OMD for bagasse ranged from 32.6 for untreated material to 56.8% with the highest level of NaOH addition.

Table IV shows the digestibility coefficients for NDF. The effect of alkali treatment was highly significant; the linear and quadratic components indicated an increase in the digestibility of this fraction that was less pronounced as the amount of NaOH in the bagasse increased. The digestibility of NDF was also significantly affected by the N source but not by the N level. A quite similar pattern was found for ADF digestibility.

With the highest level of NaOH in the diet faecal ADF was higher than NDF so the digestibility of hemicellulose was over 100%. Its digestibility was also affected by both NaOH concentration and N level. The digestibility of the cellulose was similar to that of hemicellulose, as far as the NaOH level was concerned. Nevertheless, two important differences should be outlined; the absence of statistical significance for the effect of dietary N level and the high significance for the N source ($P < 0.01$).

TABLE IV

Mean digestibility coefficients and nitrogen balance in wethers fed on diets based on ensiled NaOH-treated sugar cane bagasse (%)

Treatment diet[1]	DM	OM	NDF	ADF	ADL	NDS	Hemi-cellulose	Cellulose	N retention
0MI	45.3	47.4	37.0	39.6	25.1	58.6	31.4	42.6	−19.9
II	51.7	53.7	42.5	42.5	24.3	66.0	42.3	47.1	−4.2
SI	52.7	56.4	49.4	50.6	30.0	58.8	46.8	54.2	−21.4
II	41.3	44.8	35.1	31.2	22.6	52.8	43.4	33.0	−14.9
2MI	56.4	59.3	51.8	46.3	24.8	66.9	47.2	54.6	4.3
II	57.3	61.0	54.7	47.2	4.4	60.6	79.3	56.8	5.0
SI	56.8	60.1	53.4	50.5	33.9	62.5	60.0	54.2	0.1
II	59.4	62.5	58.7	58.0	40.9	60.7	60.0	61.6	9.5
4MI	62.9	63.7	61.7	57.5	9.5	65.8	74.1	65.5	28.8
II	62.5	65.2	60.6	58.2	31.6	65.8	67.7	63.3	10.3
SI	62.2	64.6	61.9	62.4	35.7	62.8	59.9	67.4	2.9
II	63.1	65.9	65.0	65.0	35.2	60.2	65.0	69.8	2.1
6MI	64.8	65.6	61.6	45.9	−38.6	67.4	130.0	62.5	14.4
II	65.8	67.4	66.9	59.1	−5.9	64.6	115.5	69.7	9.1
SI	71.8	74.5	74.5	69.3	2.1	69.1	104.7	78.3	15.8
II	63.6	64.5	65.9	52.5	−33.1	61.5	187.3	67.2	10.8
SE of means	1.61	1.60	2.21	2.59	5.26	2.12	7.48	2.05	5.41
Statistical significance of treatments									
NaOH (1)	***	***	***	***	***	***	***	***	***
Linear component	***	***	***	***	***	***	***	***	***
Quadratic component	***	***	***	***	***	NS	***	***	***
Cubic component	NS	NS	NS	*	***	NS	***	NS	NS
N source (2)	NS	NS	**	***	***	**	NS	**	NS
N level (3)	NS	NS	NS	NS	NS	*	***	NS	NS
1 × 2	NS	NS	NS	*	*	NS	*	NS	*
1 × 3	NS	*	*	**	NS	NS	*	***	***
2 × 3	***	***	**	***	**	NS	*	***	NS
1 × 2 × 3	***	***	***	***	***	*	***	***	**

[1] 0, 2, 4, 6 = NaOH level; M = urea—biuret mixture, S = soya bean meal; I and II = levels of supplementation.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS = $P > 0.05$.

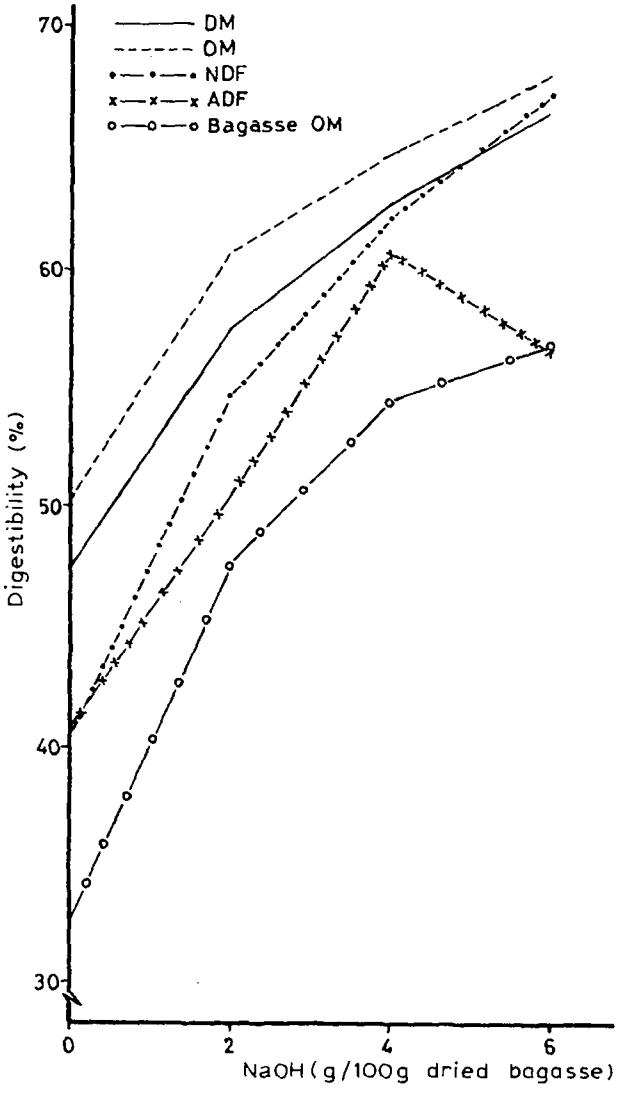

Fig. 2. Effect of alkali treatment of sugar cane bagasse on digestibility in vivo.

The digestibility of the neutral detergent solubles (SDN) was significantly affected by NaOH concentration, N level and N source. The average values of ADL digestibility for the diets with 40 g NaOH/kg bagasse were ca. 26%. A negative digestibility coefficient was found for the highest level of alkali. Both NaOH treatment and N source were significant.

Nitrogen retention improved with the level of alkali, but was not affected by either the N source or level.

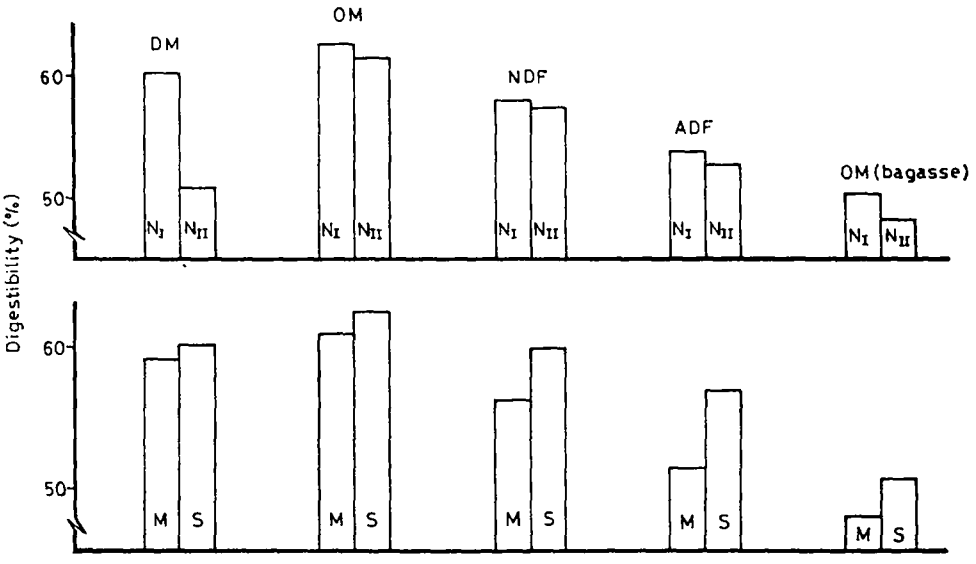

Fig. 3. Effect of level and source of dietary nitrogen on digestibility in vivo.

TABLE V

Calculated digestibility and digestible organic matter content of sugar cane bagasse sprayed with NaOH solutions and ensiled (%)

Treatment[1]	OMD	DOM in DM		Level of significance[1]	
0MI	27.4	26.5			
II	37.7	36.5		(1) NaOH	***
SI	42.6	41.2		Linear component	***
II	25.1	24.3		Quadratic component	***
2MI	46.8	43.1			
II	46.1	42.5	Main effects	Cubic component	NS
SI	46.3	42.7		(2) N source	NS
II	50.6	46.6		(3) N level	NS
4MI	52.1	46.9			
II	54.3	48.9		(1) × (2)	**
SI	53.8	48.5	First order	(1) × (3)	NS
II	57.5	51.8	interactions	(2) × (3)	***
6MI	54.6	45.7			
II	55.3	46.3			
SI	67.2	56.3	Second order interactions	(1) × (2) × (3)	***
II	49.7	41.6			
SE of means	2.31	2.09			

[1] See footnotes to Table IV.

DISCUSSION

Our average chemical values for sugar cane bagasse are very close to those of Randel (1972), Sharma (1974) and Marshall and van Horn (1975), and clearly show that its nutritive value is lower than that of cereal straws. The decrease observed in NDF with increasing NaOH has been reported for various roughages, mainly cereal straws, by several workers (Klopfenstein et al., 1972; Gharib et al., 1975; Jackson, 1977; Wilkinson and Gonzalez Santillana, 1978) and must be attributable to solubilization of hemicellulose as the ADF fraction is not significantly affected by the alkali treatment (Thomsen et al., 1973; Wilkinson and Gonzalez Santillana, 1978).

Following the technique of immersion of the material in alkali solution and its subsequent washing, Sharma (1974) obtained orderly increases in the degree of solubilization of sugar cane bagasse upon increasing the alkali concentration up to 15 g/100 g of bagasse, and no response to higher levels of alkali. A slight reduction in ADL content was found by Ololade et al. (1970), Yu et al. (1975) and Wilkinson and Gonzalez Santillana (1978).

The increases in pH and sodium content found with the alkali spray treatment are in agreement with those obtained by Fernandez Carmona and Greenhalgh (1972) and Wilkinson and Gonzalez Santillana (1978). The fall of 2.5—3 pH units in the bagasse silages could be due to partial neutralization of the residual NaOH, both by organic acids formed in the process of ensiling (mainly by residues of uronic acids), and also from hydrolyses of ester linkages of phenyl and acetyl groups bound to xylan chains in the cell wall, which are especially frequent in sugar cane bagasse (Tarkow and Feist, 1969). These reactions seem to be practically completed 25 days after ensiling. Our results agree with those of Agrawal (1975), Flipot et al. (1976), Wilkinson and Gonzalez Santillana (1978) and Fernandez and Gonzalez (1979). Nevertheless, Greenhalgh et al. (1978a) indicate only small reductions in the pH of barley straw treated with 6.6 g of NaOH/100 g of product after being ensiled for 1 year.

The low population of micro-organisms in the ensiled NaOH-treated bagasse can be explained by the high pH which affects mainly the fungi, whose optimal pH is below that of bacteria, as well as the inadequacy of the substrate for the development of extensive microbial activity. This deleterious effect of NaOH addition on the microflora has been observed by Greenhalgh et al. (1978a, b) and Wilkinson and Gonzalez Santillana (1978) in ensiled cereal straws.

The stability of ensiled bagasse after 10 days of exposure to air is rather good, as demonstrated by the decline in total counts of micro-organisms and, particularly, fungi, which are below those found by Wilkinson and Gonzalez Santillana (1978) and Greenhalgh et al. (1978b) in barley straw.

The dry matter digestibility (DMD) and OMD of diets with untreated bagasse were slightly lower than those obtained by Stone et al. (1966) and Randel (1972) in rations containing smaller amounts of bagasse.

The OMD of bagasse increased by 24.2 percentage units after spraying with NaOH, which implies an average improvement of about 4 units per g of alkali/100 g dried bagasse. This is more then the improvements described by Fernandez Carmona and Greenhalgh (1972), Greenhalgh et al. (1976), Rexen and Thomsen (1976) and Pirie and Greenhalgh (1978), for diets based on treated cereal straws; by Kategile and Frederiksen (1979) for diets based on alkali-treated maize cobs and close to those obtained by Klopfenstein et al. (1972). Nevertheless, the nutritive value of treated sugar cane bagasse was lower than that of alkali-treated cereal straws, in close agreement with a previous work of Verma and Jackson (1975), and similar to that of more lignified roughages after alkali treatment (Feist et al., 1970; Guggolz et al., 1971; Choung and McManus, 1976).

The extent of the NaOH effect in improving the digestibility of the sugar cane bagasse declined as the NaOH concentration increased; this was possibly due to a rise in the ruminal osmotic pressure (Ololade et al., 1972), which could reduce the microbial activity in the rumen (Bergen, 1970), and to a faster rate of passage through the digestive tract caused by the increase in water intake with the addition of NaOH (Bolduan et al., 1974; McManus et al., 1976; Berger et al., 1980). Moreover, greater intakes were found for diets with the highest levels of NaOH, which would reduce the retention of digesta in the rumen and, therefore, the length of time for microbial activity.

The improvement in OMD of treated bagasse is due to an increase in the digestibility of the cell wall components (except lignin), derived from both their partial solubilization and the greater availability of the non-solubilized material for microbial breakdown. The increase in the digestibility of hemicellulose is probably due to the release of phenyl and acetyl groups, especially abundant in the Graminae (Morris and Bacon, 1976). Lignin artifacts soluble in neutral detergent but insoluble in acid detergent appear in faeces with the higher levels of alkali and imply that the faecal ADF is higher than the NDF fraction; thus, digestibility coefficients for hemicellulose above 100% are obtained. Rexen and Thomsen (1976) found similar results with barley straw. These compounds similar to lignin, collected in the faecal ADF (Hartley and Jones, 1978), could explain the negative digestibility coefficients of ADL found with the highest levels of alkali. Allison and Osbourn (1970) and Fahey et al. (1979) obtained negative digestibility coefficients even lower than those reported in this paper.

The increase in the digestibility of cellulose after alkali treatment is attributable to both the breakage of hydrogen bonds (Whistler and Teng, 1970; Bacon, 1979) and the saponification of ester linkages between cellulose and hemicellulose molecules (Feist et al., 1970).

The ADL digestibility coefficients ranged between 25 and 28% for the bagasse treated with 0—40 g NaOH/kg; values greater than those for gross sugar cane bagasse found by Fahey et al. (1979). It seems that in the presence of molasses and starch, soluble lignin–carbohydrate complexes are formed by microbial action in the rumen, which are not recovered in the acid deter-

gent residue (Gaillard and Richards, 1975). This would explain the partial digestibility of lignin, an effect found also by Allison and Osbourn (1970), Minson (1971) and Grant et al. (1974), which raises doubts on the suitability of lignin as an indicator for digestibility trials.

In our experiments the microbial growth was probably more limited by the rate of degradation of the energetic substrate than by the availability of N, and thus the N level did not affect either the DMD, OMD, cell wall digestibility (with the exception of hemicellulose) or the nitrogen retention. Our results are in disagreement with those of Donefer et al. (1969), Ørskov and Grubb (1978) and Kategile (1979), who found a higher digestibility of the diet after the addition of NPN.

The source of supplemental nitrogen affected the digestibility of cell wall components (except hemicellulose), which was higher with soya bean meal, suggesting more efficient utilization of soya protein than of NPN by the ruminal microflora. This low efficiency of utilization of NPN can also be the

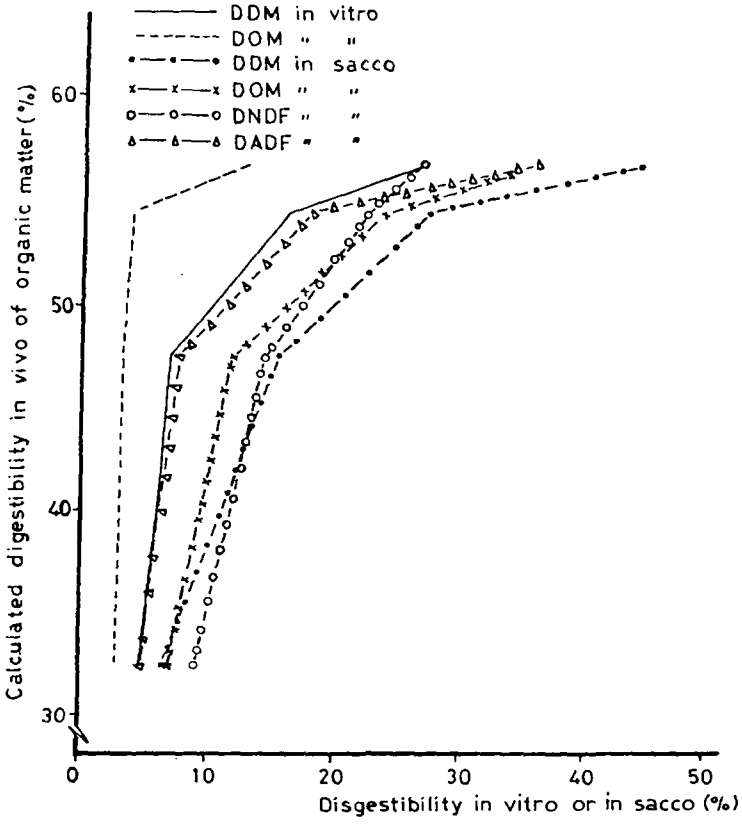

Fig. 4. Digestibility in vitro or in sacco of NaOH-treated bagasse as predictors of digestibility in vivo.

result of an incomplete adaptation of the ruminal microflora to biuret. Schroder (1970) found that up to 70 days were necessary in order to attain the maximum biuretolytic activity in the rumen, depending on the protein content in the supplemented ration.

With regard to the effect of alkali treatment, the N balance showed the same pattern as the DMD and OMD. We have obtained similar results to those found by Klopfenstein et al. (1972) and Donaldson et al. (1976), who reported better N retention with NaOH treatment. It follows that the structure of the bagasse prevents adequate microbial growth, which is limited by the availability of energy. This fact would explain the negative nitrogen balances reported (Ørskov and Grubb, 1978; Ørskov, 1979).

The appearance of significant second- and third-order interactions is difficult to explain and restrains the magnitude of the effect to our experimental conditions. Figure 4 shows the relationship between digestibility of organic matter in vivo and in vitro or in sacco, previously determined for different fractions in samples of the alkali-treated sugar cane bagasse. With the exception of OMD in vitro, all these parameters can provide a reasonable estimate of the digestibility of the bagasse sprayed with levels of alkali ranging from 20 to 60 g NaOH/kg dried product.

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