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EXECUTIVE SUMMARY

The project consisted of the following major activities:

1. The Completion of the M.Sc. thesis of the Honduran copartner in Israel and his training in Ruminant Nutrition research in the laboratory of the principal investigator.
2. The experiments of the joint project in which the Honduran copartner was actively involved at various stages.

2.1. The first stage was dedicated to studying the effectiveness of ozone and sodium hydroxide in treating: banana leaves and stems, sugar cane bagasse, cotton stalks, soybean stalks, corn stover, sorghum stover, and screened manure fiber. The response in terms of cell wall solubilization and biodegradation was higher, by the lignin-rich materials to the ozone treatment, and by the low lignin substrates to the alkali treatment. Therefore, we decided to elaborate our nutritional studies on a high lignin, ozone-sensitive substrate.

2.2. In the second phase, a digestion and metabolism study was conducted on sheep fed rations consisting of 70% concentrate + either untreated cotton stalks (UCS) or ozone treated cotton stalks (OCS). OCS is acidic (pH~2) and has a high capacity to absorb ammonia. Accordingly, OCS was titrated to pH 6.5 with ammonia and apart of its improved quality as a roughage, it become also a N-carrier. Thus the N-enriched OCS was the major N contributor (13g N/day) in the OCS ration and soybean meal in the UCS diet.

Digestibility of NDF was almost tripled in OCS as compared to UCS, however, N-enriched OCS was somewhat inferior to the soybean meal as a source of dietary N.

2.3. Third phase was dedicated to examining OCS as a component of a lamb-growth ration. Lambs were grown from 24 Kg to to 40Kg live-weight on a ration consising of 60% concentrate, 20% poultry litter and 20% either UCS or OCS. A third group of lambs was fed a whole concentrate ration (CR) and served as the positive control. Average daily live-weight-gains were: 226 g, 298 g and 395g for the lambs fed the UCS, OCS and CR rations, respectively.

Impact of the project is addressed at the end of the report.

RESEARCH OBJECTIVES

The overall aim of this research was to study the effectiveness of the ozone treatment for converting lignocelluloses into productive ruminant feeds. The specific objectives were:

I. To assess the sensitivity of different lignocelluloses to the ozone treatment regarding cell wall chemistry and biodegradation.

II. To examine the potential of diets containing ozone treated lignocelluloses for providing energy yielding substrates and amino acids to the ruminant.

III. To explore the productive performance of ruminants fed rations containing ozone treated lignocelluloses.

B O D Y

O F

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Effect of Alkali and Ozone Treatments on the Composition and In-vitro Digestion of Various Lignocelluloses

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ABSTRACT

Banana stems, sugar cane bagasse, cotton stalks, soybean straw, corn stalks and sorghum stalks were chemically treated by 4-7% alkali and by ozone, and the effect of treatments on chemical composition and in-vitro digestibility of the substrates was measured.

The cell wall content of the lignocelluloses fluctuated around 80%, except for the bagasse which was higher (89%) and for the sorghum stalks which was lower (71%). Cellulose, the largest CW component in these materials (34-52%) was hardly affected by treatments. The dicotyledonous sources cotton and soybean stalks, were low in nemicellulose (12-13%), and high in lignin (21.4 and 16.2%, respectively), whereas the monocot lignocelluloses were high in hemicellulose (26-35%) and low or medium in lignin (6-13%).

Hemicellulose content was hardly affected by NaOH treatments in the dicot materials, but was decreased to a variable extent in the monocot lignocelluloses. The ozone treatment exerted a remarkable solubilizing effect on the hemicellulose generally, except for the sorghum stalks. Lignin was extensively degraded (oxidized) by the ozone treatment in cotton stalks and in sugar cane bagasse, but was not affected by NaOH treatments.

The in vitro digestibility values of the organic matter (OMD) of the untreated banana, bagasse and soybean lignocelluloses flactuated around 31-36%, cotton stalks was lower (20.7%) whereas corn and sorghum straws were higher in OMD (50-52%).

Banana stems responded positively both to the NaOH and ozone treatments. Sugar cane bagasse responded well to 6-7% NaOH, however its OMD was almost doubled by ozone.

The effect of NaOH treatments on the OMD of the dicotyledonous lignocelluloses was poor, and these materials responded well only to the ozone treatment. For instance, OMD of cotton stalks was tripled and that of soybean straw almost doubled by the ozone treatment.

Both corn and sorghum stalks responded well to the chemical treatments with some advantage to the NaOH over that of the ozone treatment. At the highest application levels of alkali, the OMD of cereal materials was around 70% which is in the level of a good quality forage.

These findings support the differential approach suggesting application of hydrolytic treatments for upgrading the nutritive value of monocotyledonous lignocelluloses and ozone treatment for dicotyledonous materials.

INTRODUCTION

Digestion studies at the monomer level have shown that ozone treatment was more effective than alkali treatments in oxidizing and solubilizing cell-wall (CW) matrix lignin of dicotyledonous lignocelluloses such as: cotton stalks, grape branches, and screened manure fiber. Ozone treatment almost doubled the OM digestibility of those substrates whereas the alkali treatment was less effective (Ben-Ghedalia, 1982; Ben-Ghedalia & Shefet, 1979; Ben-Ghedalia *et al*. 1982; 1989; Shefet & Ben-Ghedalia, 1982; Ben-Ghedalia & Rubinstein, 1986). However, ozone treatment had similar or even smaller effect compared to alkali treatment in increasing the *in vitro* digestibility of monocotyledonous lignocelluloses and cereal straws (Alexander *et al*., 1987; Ben-Ghedalia & Miron, 1981; Bunting *et al*. 1984; Miron & Ben-Ghedalia, 1982; Puri, 1983).

Based on this data, a differential approach for the chemical treatment of lignocelluloses was suggested: Hydrolytic treatments (SO_2 or alkali) were designed for cereal straws since they affect mainly the hemicellulose fraction which to our view is the major obstacle of biodegradation in the CW of monocotyledonos , whereas lignin is the major biodegradation obstacle in CW of dicotyledonous lignocelluloses and therefore, ozone which is an oxidizing agent that degrades the lignin should be used for increasing the digestibility of these materials (Ben-Ghedalia *et al.*, 1989; Miron & Ben-Ghedalia, 1982).

The objective of this study was to assess this differential approach, by treating several monocotyledonous and dicotyledonous lignocelluloses by alkali and ozone and to measure the effect of treatments on chemical composition and in-vitro digestibility of the substrates.

MATERIALS AND METHODS

The monocotyledonous lignocelluloses included: Banana stems, corn stalks and sorghum stalks; and the dicotyledonous lignocelluloses included: soybean straw, and cotton stalks. An industrial byproduct, sugar cane bagasse, was also used in this study. All lignocellulosic substrates were collected in Israel and Honduras, air-dried and ground to pass a 6-mm sieve prior to treatment.

Ozone treatment:

A portion of each of the chopped substrate was moistened to contain 50% DM, divided to three PVC columns (replicates), and treated by a stream of gaseous ozone which was passed through the column until complete bleaching of the materials, as previously described (Ben-Ghedalia et al., 1982).

Sodium hydroxide treatment:

Another part of each substrate was divided to 12 equal portions and placed in hermetically sealed flasks. Solutions containing different concentrations of NaOH were prepared and added to the substrates at levels of 4, 5, 6 and 7 g $100g^{-1}$ DM substrate and the moisture content of substrate in each flask was brought to 50% on a DM basis. Each NaOH application level being tested in three replicates (flasks) for each substrate. The sealed flasks were kept in room temperature for a week.

After ozone or NaOH treatment, material in each flask or column (replicate) was aerated, freeze-dried, ground to pass a 1-mm sieve, and used for chemical analysis and in-vitro digestion measurement.

Analytical methods:

Analysis of cell-wall (CW) content, and determination of CW carbohydrate fractions: hemicellulose, cellulose were according to the detergent fractionation method of (Goering & van-Soest, 1970). Lignin in samples was determined by the potassium permanganate oxidation method (Goering & van-Soest, 1970).

In vitro organic matter digestibility by ruminal microbes was determined by the two stage technique (Tilley & Terry, 1963).

In vitro digestibility results were statistically analyzed using a completely randomized design consisting of six treatments, with three replicates per treatment, each replicate measured in three in vitro tubes. Duncan's multiple range test served to differentiate between means (Little & Hills, 1978).

RESULTS AND DISCUSSION

The chemical composition of the untreated materials is presented in Table I. The lowest content of organic matter was found in banana stems and the highest in the bagasse. The cell wall content of the lignocelluloses fluctuated around 80%, except for the bagasse which was higher (89%) and for the sorghum stalks which was lower (71%). Cellulose, determined as the residue of ADF fraction after the oxidation and removal of lignin, was the largest CW component in these materials (34-52%). Hemicellulose, determined as the difference between concentrations of NDF and ADF fractions, was low in the dicotyledonous sources (12-13%), and high in the monocot lignocelluloses (26-35%). Lignin content was highest in the dicot materials (cotton and soybean straws, 21 and 16%, respectively), lowest in the cereal residues (6-7%), and medium in banana stalks and sugar-cane bagasse, (13 and 12%, respectively). The ration of concentrations between the matrix components hemicellulose/lignin was high in the monocot substrates (banana, 2.03; corn, 4.85; sorghum, 5.35) and lower in the dicotyledonous, cotton stalks and soybean straw (0.61 and 0.77, respectively).

TABLE I NEAR HERE

The effects of sodium hydroxide applied at 4-7g /100g DM of plant residue and of the ozone treatment are demonstrated in **Figures 1,2,** and 3. NaOH even at the highest application level was ineffective in solubilizing the CW. Notwithstanding, ozone exerted a strong solubilizing effect on the CW irrespective of the source of material. Hemicellulose content was not affected by the NaOH treatments in the dicot materials, but was decreased to a variable

FIGURES 1,2,&3 NEAR HERE

extent in the monocot lignocelluloses. The ozone treatment exerted a remarkable solubilizing effect on the hemicellulose generally, except for the sorghum stalks. Lignin was extensively degraded (oxidized) by the ozone treatment in cottons stalks and in sugar cane bagasse.

It is generally accepted that the effect of NaOH treatment is expressed in opening hydrogen bonds and swelling of cellulose microfibrils and in disrupting of alkali labile bonds between the hemicellulose and lignin fractions of the CW with little solubilization of CW components (Alexander et al. 1987; Bacon & Gordon, 1980; Ben-Ghedalia et al., 1982; Ben-Ghedalia & Shefet, 1979; Miron & Ben-Ghedalia, 1982).

The oxidizing effect of ozone on lignin and its solubilizing effect on matrix components found in this study, are supported by previously studies with several ozone treated lignocellulosic substrates (Alexander et al., 1987; Ben-Ghedalia, 1982; Ben-Ghedalia & Rubinstein, 1986; Ben-Ghedalia et al. 1982; Miron & Ben-Ghedalia, 1982; Shefet & Ben-Ghedalia 1982).

The in vitro digestibility values of the organic matter (OMD) of the untreated and chemically treated lignocelluloses are presented in **Table II**. Banana stems responded positively both to the NaOH and ozone treatments. However, the major problems of this material are: its high content of ash (16%), consisting mainly of Si compounds, and lignin (13%), and its low content of dry matter in the fresh material, which renders the nutritional upgrading of banana stems by chemical treatments in-feasible.

TABLE II NEAR HERE

TABLE I. Chemical Composition of the Untreated Lignocelluloses (g/100g DM)

Constituents	Lignocelluloses					
	Banana stems	Sugar cane bagass	Cotton stalks	Soybean straw	Corn stalks	Sorghum stalks
Organic matter	84.5	96.1	95.1	92.7	92.9	90.7
Cell wall	78.3	89.1	79.7	80.9	83.0	71.4
Cellulose	41.6	46.0	45.6	51.7	41.1	33.7
Hemicellulose	26.4	30.3	13.1	12.4	34.7	31.7
Lignin	13.0	12.4	21.4	16.2	7.15	5.94

TABLE II. In Vitro Digestion of Organic Matter (g/100g OM) of the Treated and the Untreated Lignocelluloses

Lignocellulose material	Untreated	NaOH Treatment				Ozone Treatment	SEM
		4%	5%	6%	7%		
Banana stems	35.9^{f}	50.0^{e}	55.7^{c}	61.7^{b}	66.1^{a}	51.7^{d}	0.05
Sugar cane bagass	31.4^{f}	36.9^{e}	42.8^{d}	52.5^{c}	57.0^{b}	65.6^{a}	0.05
Cotton stalks	20.7^{e}	25.7^{d}	29.2^{cd}	32.5^{c}	37.1^{b}	61.8^{a}	1.19
Soybean straw	32.0^{b}	35.4^{c}	37.0^{b}	38.0^{b}	37.3^{b}	56.7^{a}	0.05
Corn stalks	50.3^{e}	62.0^{d}	63.8^{c}	66.5^{b}	71.9^{a}	64.0^{c}	0.05
Sorghum stalks	52.0^{d}	61.7^{c}	66.6^{b}	67.4^{b}	72.2^{a}	60.5^{c}	0.06

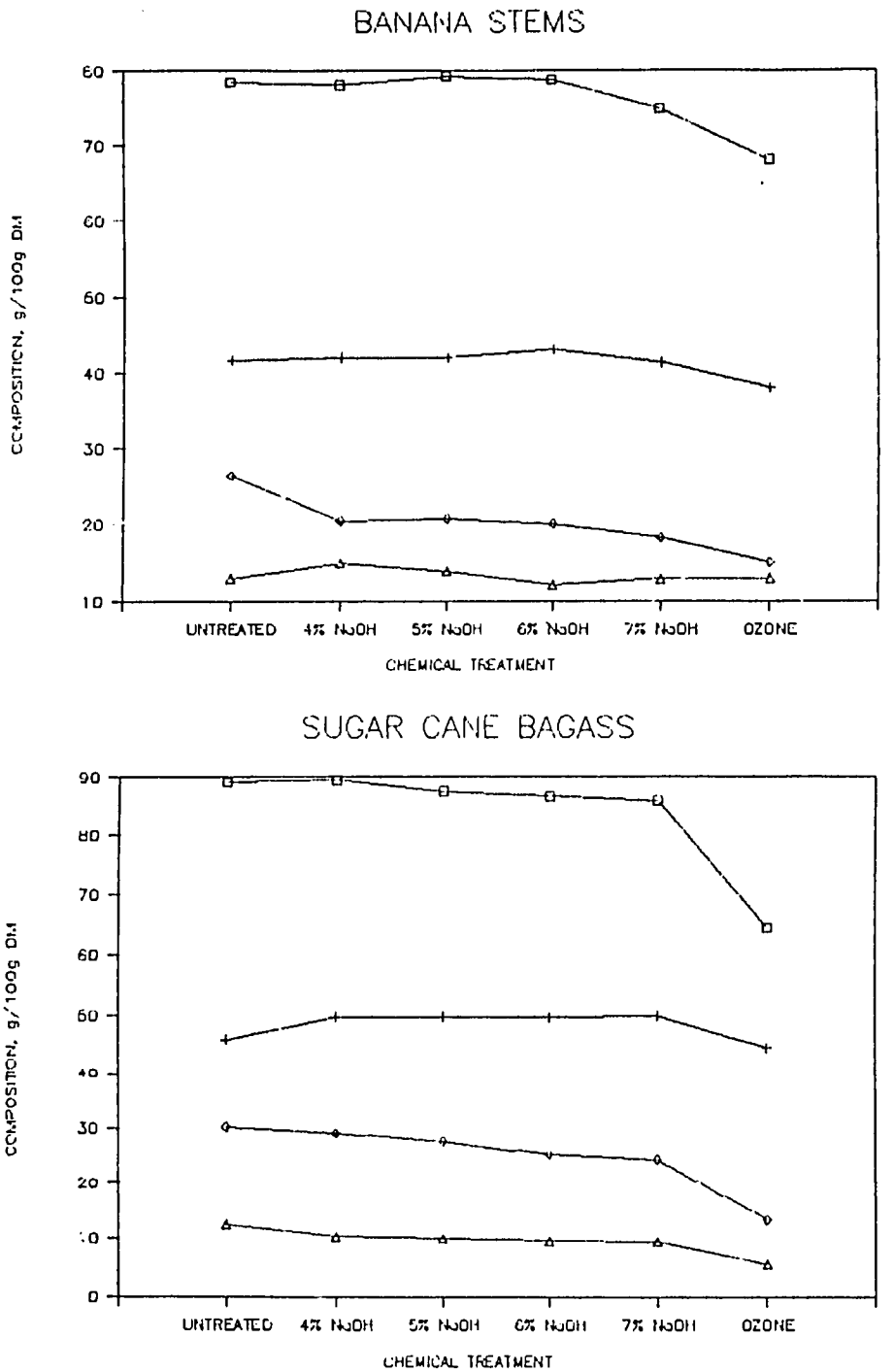
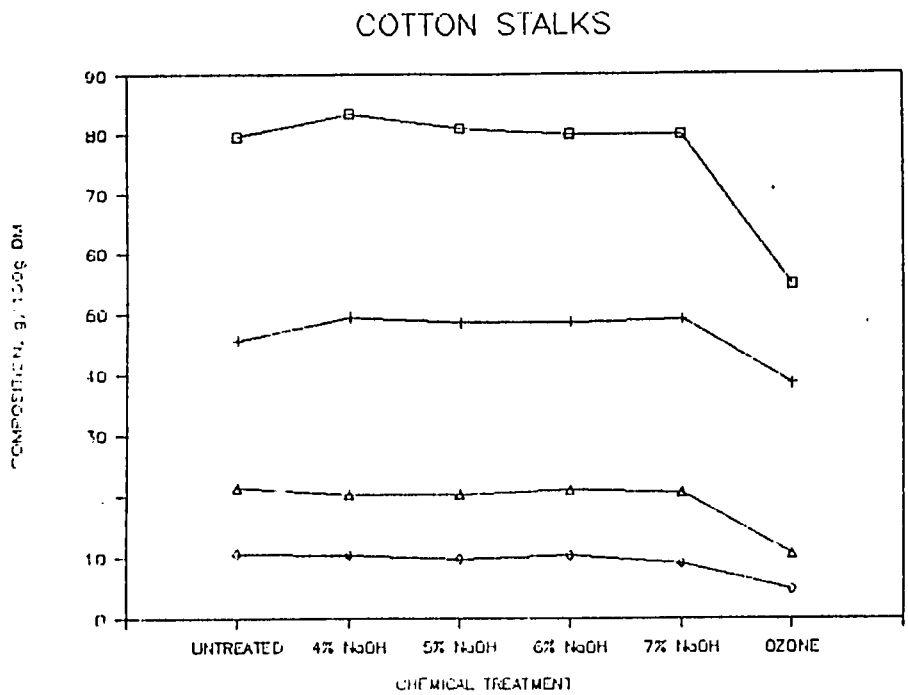


Fig. 1. The effect of sodium hydroxide (g/100g DM lignocellulose) and ozone treatments on the concentration of CW and CW constituents of banana stems and sugar cane bagass. □ =CW; + =cellulose ◇ =hemicellulose; △ =lignin.



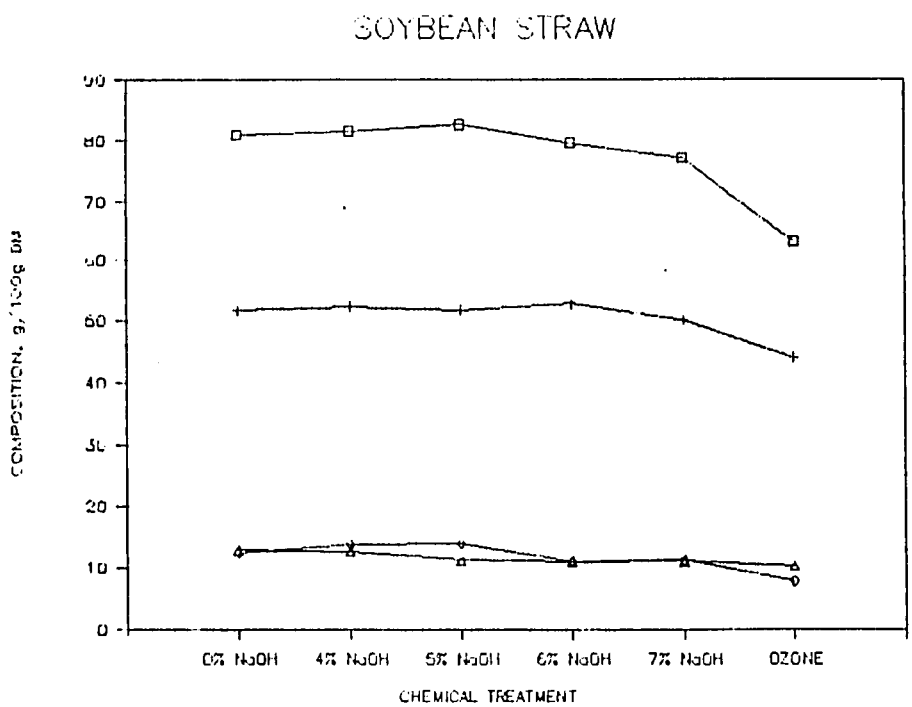


Fig. 2. The effect of sodium hydroxide (g/100g DM lignocellulose) and ozone treatments on the concentration of CW and CW constituents of cotton stalks and soybean straw. □ =CW; + =cellulose; ◇ =hemicellulose; △ =lignin.

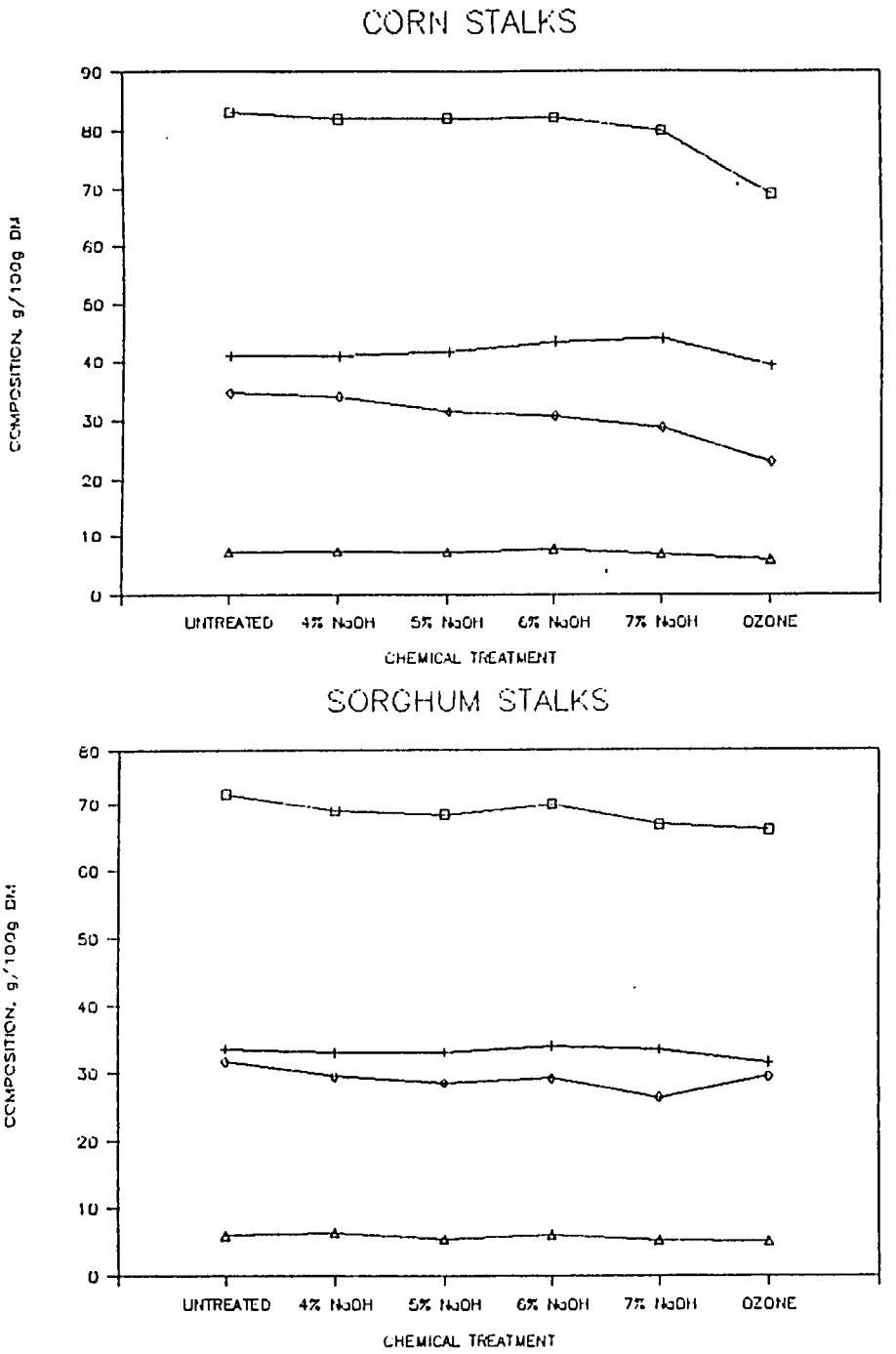


Fig. 3. The effect of sodium hydroxide (g/100g DM lignocellulose) and ozone treatments on the concentration of CW and CW constituents of corn stalks and sorghum stalks residues. □ =CW; + =cellulose; ◇ =hemicellulose; △ =lignin.

Sugar cane bagasse responded well to 6-7% NaOH and its OMD was almost doubled by ozone. Thus, it seems that in this monocot origin industrial by-product core lignin and related phenolics are the major biodegradation obstacles, and their removal by ozone enable the better degradation of its CW carbohydrates by ruminal bacteria as previously reported also by Puri (1983).

The effect of NaOH treatments on the OMD of the dicotyledonous lignocelluloses was poor, and these materials responded well to the ozone treatment. For instance, OMD of cotton stalks was tripled and that of soybean straw almost doubled by the ozone treatment. These findings are supported by previous studies on ozone treated dicot substrates (Ben-Ghedalia, 1982; Ben-Ghedalia & Rubinstein, 1986; Ben-Ghedalia & Shefet, 1979; Ben-Ghedalia et al., 1982; Shefet & Ben-Ghedalia 1982), and support the differential approach suggesting to use ozone for upgrading the nutritive value of dicot lignocelluloses.

The initial OMD values of the cereal lignocelluloses was pretty high. Both corn and sorghum stalks responded well to the chemical treatments with some advantage to the NaOH over that of the ozone treatment. At the highest application levels of alkali, the OMD of cereal materials is araised to the level of a medium quality forage. These findings are supported by previous studies on ozone and NaOH treated wheat straw (Ben-Ghedalia & Miron, 1981; Miron & Ben-Ghedalia, 1982), and support the differential approach suggesting the application of hydrolytic treatments for upgrading the nutritive value of monocot lignocelluloses.

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Effect of Ozone and Sodium Hydroxide Treatments on the Composition and in Vitro Digestion of Screened Manure Fiber from Different Sources

Daniel Ben-Ghedalia,* Aharon Rubinstein, and Yosef Dror

Screened manure fiber (SMF) was separated from manure of heifers fed complete diets consisting of a concentrate + either vetch hay (VH) or oat hay (OH) at 30 and 70% of the ration's dry matter (DM). The four SMF sources were subjected to sodium hydroxide and ozone treatments. Chemical treatment was the major factor affecting the neutral detergent fiber (NDF) content of SMF. Accordingly, three distinct groups of NDF values with no overlap were obtained: untreated > NaOH-treated > ozone-treated SMF. Sodium hydroxide had a slight solubilizing effect on the NDF of SMF (≥10%), whereas ozone was far more effective (~38%). Lignin content in SMF was in the range 17–22%. The effect of 6% NaOH on the content of lignin was in the direction of a slight increase; ozone, however, degraded up to two-thirds of the lignin of SMF. As with the NDF fraction, three distinct groups of hemicellulose values, distributed according to chemical treatments, were clearly noticeable: untreated > NaOH-treated > ozone-treated SMF. Ozone exerted the most effective solubilizing action upon the matrix polysaccharides. In vitro organic matter digestibility of untreated SMF was very low (12–20%). Ozone increased the IVOMD to the range 70–75%; the effect of 6% NaOH was more modest, upgrading the SMF to the range 36–53%.

Digestion studies at the monomer level have shown that cellulose and xylans are not equally digested by ruminants. More extensive digestion of the cellulose component was demonstrated in a series of experiments with different forages both in vitro and in vivo (Ben-Ghedalia and Miron, 1984; Ben-Ghedalia and Rubinstein, 1984, 1985; Morris and Bacon, 1977). Consequently, the proportion of hemicellulose in the residual CW is increased. The cell walls (CW) of cereal and leguminous forages, representing mono- and dicotyledonous sources, differ in terms of chemical, structural and biodegradational properties. For example, the ratio of lignin to matrix polysacchrides in the CW is approximately 1:5 in monocots as compared with 1:1 in dicots (Ben-Ghedalia and Rubinstein, 1984, 1985). The digestibility of the matrix polysaccharides is determined by the degree of branching of the xylan chains and by the extent to which they are interlinked with lignin (Brice and Morrison, 1982; Tanner and Morrison, 1983). Although it is understood that the digestive tract of the ruminant exerts a leveling effect with respect to the composition of the ingested CW, there is a question whether the original "fingerprint" of the forage plant CW could still be recognized in the fecal material. This is of particular interest when considering the response of screened manure fiber (SMF) of various sources to chemical treatments, since it was found that hemicellulose-rich lignocelluloses respond better to hydrolyzing or saponifying treatments whereas lignin-rich materials are more effectively treated by oxidative agents (Miron and Ben-Ghedalia, 1982; Shefet and Ben-Ghedalia, 1982).

Another question of interest is the effect of the level of forage intake (within a fixed total dry matter (DM) intake) on neutral detergent fiber (NDF) composition of SMF, the biodegradation of SMF by rumen microbes, and its response to chemical treatments. This is of particular interest, as it is well-known that increasing forage intake speeds the rate of passage of fiber from the rumen and increases the amount of undigested, but potentially digestible, fiber in the feces (Evans, 1981).

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Table I. Composition (g/100 g of DM) of Hays Fed to the Heifers

component	VH	OH
NDF	59.4	71.7
cellulose	37.4	37.6
hemicellulose	9.45	28.2
lignin (permanganate)	12.5	5.73
NDF non-glucose polysacch	15.6	21.9
ND-sol carbohydr	27.1	13.1
in vitro OMD	53.0	55.5

The use of vibrating screeners for the separation of cattle manure into fiber and liquid presents a well-balanced solution to the ecological problem of manure disposal in the dairy herd, since the fiber can be refed and the liquid and fine particles recycled to the soil (Wilkinson, 1980). The expanding use of this technology motivated this research.

The objective of this study was to examine the response of SMF of different sources, originating from cereal or legume hay fed at different levels, to ozone and NaOH treatments in terms of chemical composition and biodegradation by rumen microbes.

MATERIALS AND METHODS

Animals, Feeds, and Separation of SMF. Sixteen Holstein heifers with an average weight of 360 kg each were allocated to four dietary treatments with four heifers per treatment. The rations (7.89 kg of DM/day) consisted on a DM basis of (I) 70% vetch hay (VH) + 30% concentrate, (II) 30% VH + 70% concentrate, (III) 70% oat hay (OH) + 30% concentrate, and (IV) 30% OH + 70% concentrate. The chemical composition and in vitro digestibility of the forages are given in Table I. The concentrate mixture was a 15% crude protein commercial concentrate, consisting per 100 kg of 37 kg of corn, 25 kg of wheat bran, 14 kg of soybean meal, 10 kg of barley, 10 kg of sorghum, and 4 kg of a mineral mixture. The heifers were fed the complete feed mixture (hay + concentrate) twice daily at 0800 and 1400 h. Following 14 days of adaptation, fecal grab samples were taken twice per day, 2 h postfeeding for 4 days. Fecal samples were pooled on a DM basis into one large sample for each treatment and kept at -20 °C until separation of the fiber fraction.

SMF was separated on a laboratory scale using a 2-mm net screen and repeated washings with tap water until complete clearing of nonfibrous material. The four SMF materials were

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Table II. Effect of Chemical Treatments on the Content (g/100 g of DM) of NDF Fractions in SMF Originating from Cattle Fed either VH or OH (Mean Values of Three Replicates per Treatment ± SE)

source of fecal fiber in diet	chemical treatment		
	control	NaOH	O_3
	NDF		
30% VH	87.4 ± 1.79	79.0 ± 1.66	57.3 ± 0.29
70% VH	86.0 ± 0.24	77.9 ± 0.18	54.3 ± 0.31
30% OH	88.7 ± 0.98	81.2 ± 0.49	55.1 ± 0.78
70% OH	84.9 ± 0.68	76.9 ± 0.56	53.5 ± 1.18
	Lignin		
30% VH	22.3 ± 0.27	24.6 ± 0.90	9.93 ± 0.32
70% VH	19.6 ± 0.17	22.4 ± 0.58	7.17 ± 0.45
30% OH	16.6 ± 0.83	17.5 ± 0.40	5.53 ± 0.09
70% OH	17.2 ± 0.34	18.2 ± 0.26	7.50 ± 0.25
	Hemicellulose		
30% VH	28.4 ± 0.61	17.4 ± 0.42	12.6 ± 0.84
70% VH	32.3 ± 0.93	17.9 ± 0.82	14.6 ± 1.25
30% OH	33.2 ± 0.75	19.7 ± 0.89	13.4 ± 1.11
70% OH	32.5 ± 1.07	21.9 ± 0.78	12.3 ± 1.67
	Cellulose		
30% VH	35.4 ± 0.13	[illegible] ± 0.25	32.6 ± 0.56
70% VH	32.7 ± 1.13	35.4 ± 0.30	33.2 ± 0.49
30% OH	36.7 ± 0.03	39.8 ± 0.64	35.4 ± 0.24
70% OH	33.3 ± 1.00	34.5 ± 1.02	32.0 ± 0.36

dried in an oven at 50 °C and kept in the laboratory until subjected to the chemical treatments.

Chemical Treatments. Each of the four SMF materials was divided into three subsamples to be used for control (untreated SMF), NaOH treatment, and ozone treatment. Chemical treatments were performed in triplicate. Each of the control SMF materials was subdivided into three replicates, as done with the SMF subjected to the chemical treatments. The NaOH treatment was done by adding 100 mL of 6% NaOH to 100 g of SMF-DM. The NaOH-treated SMF samples were kept at room temperature for 1 week, after which the materials were freeze-dried and used for analyses. For the ozone treatment, batches of 50 g of SMF-DM were moistened to 40% and ozonized in glass columns (80 × 5 cm) until complete decolorization. For this purpose a Fischer ozone generator, Model 501, was used, supplying 2 g of ozone/h. Under these conditions, ozone consumption was 1 g/5 g of DM of treated SMF.

Analytical Procedures. The detergent fractionation system of Goering and Van Soest (1970) was used to determine and prepare the neutral detergent fiber, acid detergent fiber (ADF), and permanganate lignin. Hemicellulose was calculated by difference (NDF − ADF).

In vitro digestibility (IVD) of the organic matter and monosaccharide residues was measured in triplicate by using the rumen liquor-acid pepsin method of Tilley and Terry (1963). Monosaccharides in whole SMF material and in NDF preparations were determined in ball-milled material after hydrolysis in 12 M H_2SO_4 for 1 h at room temperature and continued hydrolysis in 0.5 M H_2SO_4 at 100 °C for 5 h. Inositol was added as an internal standard, and the released monosaccharides were determined as their alditol acetate derivatives after reduction and acetylation according to Blakeney et al. (1983). The conditions for separation by gas-liquid chromatography were those of Bacon and Gordon (1980). Uronic acids in hydrolysates were determined colorimetrically according to Blumenkrantz and Asboe-Hansen (1973).

Calculations. Monosaccharides were determined in whole SMF and in NDF preparations, and the fraction of soluble monosaccharides was derived by difference. For any of the parameters examined, the effect of treatments was the predominant factor affecting the variation within SMF samples. Therefore, standard error (SE) was calculated to show the variation within the replicates of the chemically treated SMF samples.

RESULTS

The effect of treatments on the content of NDF and NDF fractions in SMF is shown in Table II. There was only a slight variation in NDF content within the untreated SMF sources. Three distinct groups of NDF values, distributed according to chemical treatments with no overlap, are clearly noticeable, implying that the major factor affecting the variation of NDF content in the SMF sources of this study was the effect of chemical treatments. The NaOH treatment had a slight solubilizing effect (10% or less), whereas ozone was far more effective in this regard (~38%). Lignin content of untreated SMF was somewhat higher in materi[illegible] originating from vetch than in those originating fron[illegible] hay; however, the between-feeds difference in lignin content (see Table I) has been leveled off. The effect, if any, of NaOH on the content of lignin was in the direction of a slight increase. Notwithstanding, ozone degraded up to two-thirds of the lignin of SMF, almost equaling its content to that of the forages ingested. (For comparison, see Table I.)

As with the NDF fraction, three distinct groups of hemicellulose values, distributed according to chemical treatments, are clearly noticeable. Considering the hemicellulose content, it seems that the limited efect of the NaOH treatment on NDF content of SMF was mediated via its solubilizing action on the hemicellulose fraction. Ozone was more effective than NaOH in solubilizing hemicellulose. Cellulose content of SMF sources was not affected by chemical treatments. Moreover, the variation in cellulose content within and between chemical treatments was small.

The effect of treatments on the composition of monosaccharide residues in whole SMF materials is shown in Table III, and their distribution between NDF and cell solubles is presented in Table IV. Neither ozone nor NaOH had any appreciable effect on monosaccharide composition. Moreover, between-forages differences in carbohydrate composition (Table I) were almost completely eliminated in SMF as a result of the digestive processes in the gut. Whereas chemical treatments did not affect overall carbohydrate composition of SMF, they did affect the distribution of monosaccharides in NDF and cell solubles fractions. Generally, glucose was NDF-bound and hardly affected by treatments. The effect of chemical treatments was expressed mainly on the xylan and the related matrix (hemicellulose) monosaccharides. Ozone exerted the most effective solubilizing action on those

Table III. Effect of Chemical Treatments on the Composition (g/100 g of DM) of Monosaccharide Residues[a] of Screened Manure Fiber Originating from Cattle Fed either VH or OH

monosacch residue	30% VH in the diet			70% VH in the diet			30% OH in the diet			70% OH in the diet		
	control	NaOH	O_3	control	NaOH	O_3	control	NaOH	O_3	control	NaOH	O_3
glucose	30.9	31.4	30.2	33.4	29.7	32.7	32.1	32.7	34.9	33.9	31.0	31.8
xylose	16.8	16.0	15.8	15.4	14.0	16.0	18.4	17.8	17.5	20.0	16.8	17.3
arabinose	5.58	5.47	4.91	2.61	2.70	3.30	5.23	5.94	5.29	3.13	3.70	3.52
galactose	1.92	1.58	1.86	1.15	1.45	2.05	1.60	1.60	1.06	1.35	1.32	1.40
mannose	1.02	0.98	0.99	0.85	1.03	1.32	0.80	0.93	0.50	0.79	0.70	1.03
rhamnose	0.26	0.24	0.27	0.40	0.27	0.33	0.19	0.13	0.21	0.16	0.11	0.31
uronic acids	5.56	5.25	5.27	6.31	5.93	5.24	5.02	4.98	4.34	4.44	4.47	5.53

[a] Released by hydrolysis; see Materials and Methods.

Table IV. Effect of Chemical Treatments on the Distribution (%) of Monosaccharide Residues[a] in NDF and in Cell Solubles (CS) of Screened Manure Fiber

		30% VH in the diet			70% VH in the diet			30% OH in the diet			70% OH in the diet		
		control	NaOH	O_3	control	NaOH	O_3	control	NaOH	O_3	control	NaOH	O_3
glucose	NDF	100	100	97.6	96.7	94.2	100	100	93.2	96.2	100	100	100
	CS			2.36	3.30	5.80			6.80	3.80			
xylose	NDF	96.2	71.9	49.5	98.1	78.0	58.2	100	89.6	51.7	98.0	100	52.8
	CS	3.80	28.1	50.5	1.90	22.0	41.8		10.4	48.3	2.00		47.2
arabinose	NDF	94.0	74.8	21.0	94.6	60.0	27.0	100	100	26.6	100	83.4	25.3
	CS	6.00	25.2	79.0	5.40	40.0	73.0			73.4		16.6	74.7
galactose	NDF	57.2	48.5	19.6	64.8	31.2	18.0	53.0	51.5	50.2	39.4	33.0	13.0
	CS	42.8	51.5	80.4	35.2	68.8	82.0	48.0	48.5	49.5	60.6	67.0	87.0
uronic acids	NDF	74.7	83.4	43.5	78.4	74.0	40.6	82.2	99.6	43.3	100	71.2	45.2
	CS	25.3	16.6	56.5	21.6	26.0	59.4	17.8	0.40	56.7		29.8	54.8

[a] Released by hydrolysis; see Materials and Methods.

Table V. Effect of Chemical Treatments on the in Vitro Organic Matter Digestibility (%) of SMF Originating from Cattle Fed either VH or OH (Mean Values ± SE)

source of fecal fiber in diet	control	NaOH	O_3
30% VH	11.5 ± 0.29	36.3 ± 0.61	70.0 ± 1.16
70% VH	17.7 ± 0.97	46.5 ± 0.42	74.9 ± 0.45
30% OH	11.8 ± 1.01	51.5 ± 0.06	70.7 ± 0.62
70% OH	20.5 ± 0.87	53.2 ± 0.26	75.1 ± 1.07

polysaccharides. It is interesting to note that, in the untreated SMF samples (controls, Table IV), glucose, xylose, and arabinose—the typical NDF components—were virtually NDF-bound, whereas galactose and uronic acids, which may be found in microorganisms and in pectin, were present also in the cell solubles fraction. In vitro digestibility values of the organic matter (IVOMD) are presented in Table V and those of the monosaccharide components of whole SMF in Figure 1. In vitro organic matter digestibility (OMD) values of untreated SMF were very low, but the effect of treatments was remarkable and the difference between treatments was clearly distinguishable. The ozone treatment increased the IVOMD up to the range 70–75%. The effect of 6% NaOH was more modest, upgrading the SMF to the level of a low-quality forage (36–53%). It appears that, among the untreated SMF materials, those originating from the high-forage treatments (70% hay) were more digestible than those from the low-forage treatments (30% hay). It seems also that the oat hay originating SMF samples responded better to the NaOH treatment than those originating from vetch hay.

The increase in digestibility of the monosaccharide components of whole SMF as affected by treatments was higher than that found with the OM. The IVD of the individual monosaccharides was raised to the level of 80–90% in the ozone-treated materials. The effect of sodium hydroxide on the digestibility of glucose and xylose, the major monosaccharides, was weaker, but the effect of ozone and of NaOH on the IVD of arabinose was similar.

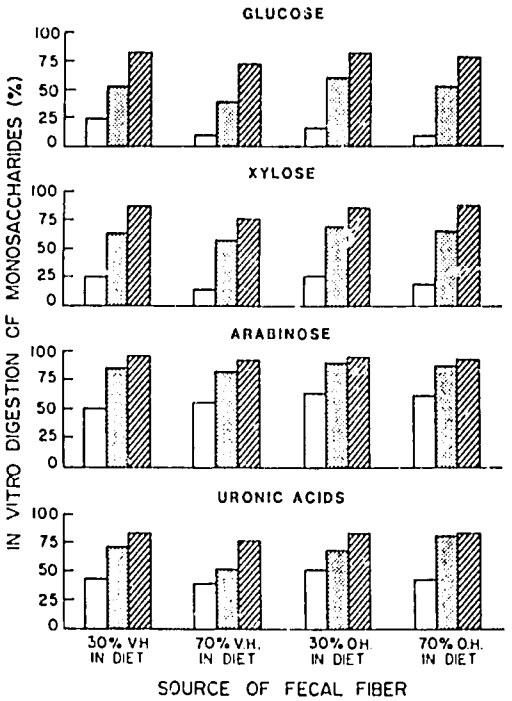

Figure 1. In vitro digestibility of monosaccharides released by hydrolysis in four sources of chemically treated SMF: □, control; ▨, 6% NaOH-treated; ▨, ozone-treated.

DISCUSSION

The question whether SMF could be characterized chemically according to its origin, i.e., the type of forage ingested, was raised in this study. Tables II and III provide

Table VI. In Vitro Digestibility (%)[a] of Monosaccharides in OH and VH[b]

monosacch residue[c]	in whole material		in cell walls	
	OH	VH	OH	VH
glucose	65.7 ± 1.01	58.7 ± 0.88	61.0 ± 1.15	55.3 ± 0.95
xylose	52.2 ± 1.90	42.4 ± 2.19	51.5 ± 1.95	34.3 ± 2.52
arabinose	61.1 ± 0.21	74.8 ± 1.30	58.6 ± 0.28	61.8 ± 1.97
galactose	75.3 ± 0.14	84.0 ± 0.18	49.7 ± 0.25	58.8 ± 0.45
mannose	89.8 ± 0.19	76.7 ± 0.33	57.7 ± 0.09	63.5 ± 0.52
rhamnose	61.3 ± 3.26	70.0 ± 0.83		46.7 ± 1.46
uronic acids	75.0 ± 0.25	83.2 ± 1.10	62.7 ± 0.50	42.6 ± 3.70
total monosacch	62.8 ± 1.06	64.4 ± 0.78	57.3 ± 1.22	51.2 ± 1.07
IVOMD	57.4 ± 0.16	57.6 ± 0.03		

[a] Mean values ± SE based on three in vitro replicates. [b] From Ben-Ghedalia and Rubinstein (1984). [c] Released by hydrolysis; see Materials and Methods.

an answer to this question: The digestive tract exerts a remarkable leveling effect on the composition of the ingested forages. Differences in composition between oat and vetch hay (Table I) are almost completely eliminated and cannot be recognized in the corresponding SMF samples. However, it appears that the original nature of the chemical bonds within the CW macromolecule is still preserved to some extent in the SMF materials. This is evident from the differential response of oat-SMF and vetch-SMF to NaOH treatment with respect to IVOMD (Table V).

It is clear from this study that the predominant effect on SMF composition and digestibility was that of the chemical treatments. Irrespective of SMF source, ozone was the most effective treatment in terms of both NDF solubilization and of increasing the IVOMD (Table V; Figure 1). These phenomena correspond well with previous data on SMF (Ben-Ghedalia and Rubinstein, 1986) and with other lignocelluloses showing that high-lignin (dicot) plant residues are sensitive to oxidizing (ozone) treatments (Shefet and Ben-Ghedalia, 1982). SMF, irrespective of source, can be considered as high-lignin materials. To illustrate the magnitude of the treatments effect, some data on the IVD of monosaccharide residues of oat and vetch hays are presented in Table VI. Ozone increased the in vitro digestibility of total glucose and total xylose of SMF to a level (80–90%) much higher than found in the hays (Table VI; Figure 1). The effect of NaOH in this respect was within the range of, or somewhat higher than, the respective values of the forages. The significance of this finding is that the chemical treatments applied to SMF were able to convert the later into a foragelike material.

In summary, untreated SMF is of little value as a feed alternative irrespective of origin. The variation in its composition is not large as compared with conventional forages (Ben-Ghedalia and Rubinstein, 1986). Treated SMF from cattle seems to be suitable for recyling through ruminants since it contains a substantial fraction of potentially digestible carbohydrates. Sodium hydroxide treatment, which proved to be particularly effective with SMF of monocot origin, is suggested as a starting point, since the technology is simple and available. NaOH-treated SMF is suggested as a feed alternative for growing heifers and other low-producing ruminants. Ozone-treated SMF can be considered as a potential component in the rations of high-producing ruminants. This is, however, closely associated with developing the ozonation technology. Further experiments, in vivo, are necessary to substantiate the results of this study prior to application in the field.

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Registry No. NaOH, 1310-73-2; O_3, 10028-15-6; glucose, 50-99-7; xylose, 58-86-6; arabinose, 147-81-9; galactose, 59-23-4; mannose, 3458-28-4; rhamnose, 3615-41-6; lignin, 9005-53-2; hemicellulose, 9034-32-6.

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Digestibility of Ozone Treated Cotton Stalks by Sheep

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ABSTRACT

A metabolism experiment was conducted with GIT-cannulated sheep fed two isonitrogenous rations consisting on a DM basis of 30% cotton stalks (CS) either untreated or ozone treated (OCS) plus 70% concentrate mixture. Ozonation increased the proportion of soluble carbohydrates in cotton stalks, however in both diets the cotton stalks contributed the major part of rations' NDF. The effect of ozone treatment was expressed in increasing the digestibility of NDF from 23.6% to 63.8%. In the CS ration most of NDF digestibility occurred in the lower gut whereas in OCS fed sheep 69% of NDF digestion occurred in the reticulorumen. Ozonation nearly doubled the digestibility of both total and CW-glucose. The calculated digestibility values of total xylose originating from cotton stalks were by 150% higher in the OCS than in CS.

OCS had a low pH (~2) and therefore it was used as a N-(NH_3) carrier and accordingly ammonia was the major N contributor in the OCS diet whereas soybean-meal was the major N-contributor in the CS diet. The ratios of duodenal NAN/dietary N were 0.79 and 0.96 for the OCS and CS rations respectively, suggesting an incomplete capture of the NH_3 in the OCS. Nevertheless the percentage of intestinal absorption of N flowing to the duodenum was similar in in both rations (61%).

INTRODUCTION

Much attention has been paid in our laboratory to the developing of the ozone treatment for increasing the nutritive value of dicotyledonous lignocelluloses (Ben-Ghedalia & Shefet, 1979; Ben-Ghedalia, 1982; Ben-Ghedalia et al., 1982). It was shown in the initial studies that by treating cotton stalks (CS) with ozone, the cell-walls content was reduced from 74% to 52%, and about half of the lignin fraction of the CS was solubilized or oxidised to organic acids (Ben-Ghedalia & Shefet, 1979; Ben-Ghedalia et al., 1982). These compositional changes in ozone treated CS were expressed in increasing significantly the in vitro biodegradation of organic matter and total monosaccharides (Ben-Ghedalia & Shefet, 1979; Shefet & Ben-Ghedalia, 1982).

Results of the early in vitro studies should be confirmed in vivo. Accordingly, the objectives of the present experiment were: I. To explore the effect of ozonation in vivo, focusing on the digestibility of cotton stalks carbohydrates, and II. to examine the potential of OCS as a nitrogen carrier in a concentrate-rich diet.

MATERIALS AND METHODS

Feeds and Animals:

Cotton stalks harvested at a commercial farm were chopped to an average size of 10 mm pieces and treated with ozone as described in Ben-Ghedalia et al., (1982); final pH of the ozonated material was 2. Subsequently, the OCS was ammoniated with a concentrated solution

of NH_4OH to reach 38.4% crude protein on a DM basis and pH 6.5. This pH level was set to avoid losses of ammonia from the ammoniated OCS and to prevent any chemical effect of the alkali upon the lignocellulose. Two isonitrogenous rations were formulated consisting on a DM basis of 30% cotton stalks (CS) either untreated or treated as above (ammoniated OCS) + 70% of a concentrate mixture. Full details of rations composition are in Tables 1&2.

TABLES 1 & 2 NEAR HERE

Four Merino rams cannulated in the rumen (simple cannula) and in the proximal duodenum and terminal ileum (T shaped cannulas) were allocated per treatment in a change-over design. The animals were kept in metabolism cages equipped with automatic feeders and free water supply. The cages were located in an air conditioned animal house. Each experimental period consisted of the following activities: an adaptation period of 17 days followed by 10 days dedicated to total collection of feces and urine and sampling of rumen duodenal and ileal digesta. From the first day of this period, Cr_2O_3 impregnated paper was given twice daily via the rumen cannula at 0800 and 2000 hours. During the last 4 days, rumen liquor, duodenal and ileal digesta were sampled 4 times per day at different intervals. Ten ml of rumen liquor plus 10 ml HCl 1N, from each rumen sampling were pooled in one composite sample per animal and stored at -18°C until analysed for NH_3 concentration. Duodenal and ileal digesta (25ml) samples were pooled to provide one composite sample per animal and stored at -18°C until analysed for DM, OM and total N. About 150 ml of duodenal and ileal digesta was freeze dried until analysed for Cr_2O_3, NDF, and monosaccharide residues.

Table 1. Daily DM intake of dietary components by sheep (g).

Ingredients	Dietary treatments	
	Cotton Stalks (CS)	Ozonated Cotton Stalks (OCS)
Cotton stalks	203	-
Ozonated cotton stalks*	-	228
Corn grain	230	402
Soybean meal	186	-
$NaHCO_3$	13.7	13.7
NaCl	2.83	2.83
KCl	5.66	5.66
MgO	2.27	2.27
Na_2SO_4	2.83	2.83
Dicalcium phosphate	11.3	11.3
Vitamins+microelements mix	1.13	1.13
Total	659	670

*Ozonated to pH 2 and subsequently ammoniated to pH 6.5.

Table 2. Chemical composition (g/100 g DM) of the feeds

Components	CS	OCS	Corn	Soybean meal
Organic matter	94.6	92.3	98.4	93.1
NDF (ash free)	77.4	45.9	11.0	13.6
Total N	0.95	5.69	1.53	7.57

*Cotton stalks ozonated to pH 2 and subsequently ammoniated to pH 6.5.

Analytical procedures

Samples of food, faeces and digesta were analyzed for organic matter by ashing in a muffle furnace at 600°C for 2 h. Neutral detergent fibre in feeds, in freeze-dried duodenal digesta and in faeces was determined and prepared as described by Goering & van Soest (1970). Amyloglucosidase was used to eliminate starch completely from NDF preparation of the concentrate mixtures (Marlett & Lee 1980).

Monosaccharides in the whole material and NDF preparations of feeds, duodenal digesta and faeces were determined in ball-milled material, after hydrolysis in 24N H_2SO_4 for 1 h at room temperature and continuing the hydrolysis in 1 N H_2SO_4 at 100°C for 5 h. Inositol was added as an internal standard, and the released monosaccharides were determined as their alditol-acetate derivatives after reduction and acetylation according to Blakeney et al. (1983). The conditions for separation by gas-liquid chromatography were those of Bacon & Gordon (1980).

Uronic acids in hydrolysates were determined colorimetrically according to Blumenkrantz and Asboe-Hansen (1973).

Total N in feeds, wet faeces and intestinal digesta was determined by the Kjeldahl method. Ammonia N concentration in rumen liquor and in duodenal and ileal digesta was determined by the microdiffusion method of Conway (1947).

Measurement of Cr_2O_3 in duodenal digesta was done according to Stevenson & de Langen (1960). The amounts of digesta constituents passing to the duodenum were calculated from Cr_2O_3 concentrations in digesta. Disappearance of NDF and monosaccharides before the

duodenal cannula is referred to herein as digestion in the reticulorumen and used to calculate the proportion reticulorumen/ total gastrointestinal-tract digestion. The results were analysed by the Latin square analysis of variance (Little & Hills 1978).
Uronic acids in hydrolysates were determined colorimetrically according to Blumenkrantz and Asboe-Hansen (1973).

RESULTS AND DISCUSSION

Monosaccharides composition in feeds is given in Table 3. Ozonation increased the proportion of soluble carbohydrates in cotton stalks, but unfortunately the subsequent ammoniation increased pH and enabled aerobic bacterial fermentation which resulted in some decrease in the concentration of soluble sugar components. Glucose comprises more than 90% of the total monosaccharide components of corn; whereas in soybean meal glucose and galactose are the major ones.

TABLES 3 & 4 NEAR HERE

NDF digestibility is presented in Table 4. The rations consisted of 30% cotton stalks + 70% concentrate mixture, however the cotton stalks contributed the major part of rations' NDF. NDF digestibility of the whole ration was higher by 86% in OCS than in CS. The calculated digestibility for the NDF of cotton stalks, showed a 170% increase in OCS as compared to CS. These results confirmed the previous in-vitro findings (Shefet & Ben-Ghedalia, 1982). The NDF of the CS ration was hardly digested in the rumen, probably as a result of the depressive effect of the concentrate. The major part

Table 3. Monosaccharides composition of the feeds

Component	Cotton stalks			Ozonated cotton stalks			Corn			Soybean meal		
	Composition g/100 g DM	Distribution In CW %	Distribution In CS %	Composition g/100 g DM	Distribution In CW %	Distribution In CS %	Composition g/100 g DM	Distribution In CW %	Distribution In CS %	Composition g/100 g DM	Distribution In CW %	Distribution In CS %
Glucose	31.19	91.75	8.25	26.27	86.25	13.75	76.25	3.25	96.75	14.10	42.93	57.07
Xylose	10.99	99.15	0.85	8.33	60.88	39.12	2.06	100	0	1.53	84.68	15.32
Arabinose	1.15	80.08	19.92	0.60	31.33	68.66	1.54	100	0	2.80	37.71	62.29
Galactose	1.11	68.32	31.68	0.61	25.56	74.44	0.96	37.45	62.55	8.21	12.36	87.64
Mannose	0.61	78.65	21.35	0.43	44.79	55.21	0.78	26.18	73.82	1.89	26.42	73.56
Uronic acids	5.67	53.23	46.77	4.55	29.23	70.77	1.04	26.3	73.7	2.67	19.24	80.76

Table 4. Amounts (g/day) of NDF present in the feed, entering the small intestine and excreted in the feces of sheep fed rations consisting of cotton stalks (CS) or ozonated cotton stalks (OCS), plus concentrate.

Criteria	CS	OCS	SEM
Intake			
In cotton stalks	157	104	
In corn	25.4	44.3	
In soybean meal	25.3	-	
Total	208	148	
At proximal duodenum	199	80.2	
In feces	133	49.3	
Digestibility (%)			
In reticulo-rumen	4.32[b]	45.8[a]	4.20
In entire GIT	36.1[b]	66.7[a]	1.37
Cotton stalk component	23.6[**]	63.8[**]	

a,b Means in the same raw with different superscripts are significantly different ($P<0.05$).

[**]Calculated, based on CW digestibility (74%) of a reference concentrate diet.

of its digestion occurred in the lower gut. The digestibility values of total and cell wall (CW) glucose are in Table 5. Since both CS and OCS treatments were basically concentrated diets, the difference between rations regarding total glucose digestibility was small. However the difference was more strongly expressed in the CW glucose parameter. The effect of chemical treatment is clearly demonstrated in the calculated values of CW-glucose digestibility of the cotton stalks component only. Ozonation nearly doubled the digestibility of both total and CW glucose, and these high digestibility values obtained in-vivo are similar to the previous in-vitro results (Shefet & Ben-Ghedalia, 1982). Values of xylose digestibility are shown in Table 6. Dicot lignocelluloses are lower in xylans than cereal straws (Ben-Ghedalia et al., 1982; Ben-Ghedalia, 1982; Ben-Ghedalia & Miron, 1984; Miron & Ben-Ghedalia, 1982), therefore the absolute quantities of xylose component in the rations are small. The digestibility patterns of xylose which is a genuine CW component resemble very closely those of the NDF fraction; including the partition of digestion between the stomachs and the lower gut, and the upgrading effect of ozone treatment. Since almost the entire xylose component originates from the CW (including the solubilized fraction in OCS), it would be more meaningful to refer to the digestibility values of total xylose. The calculated digestibility values of total xylose originating from cotton stalks are by 150% higher in OCS than in CS. The extraordinary effect of ozonation in increasing the in-vivo digestibility of CW-glucose and CW-xylose is explained by the partial solubilization and modification of CS matrix components: lignin and hemicellulose by ozone (Ben-Ghedalia

Table 5. Digestibility (%) of total and CW glucose in the retiçulo-rumen and in the entire gastro-intestinal tract (GIT) of sheep fed rations consisting of cotton stalks (CS) or ozonated cotton stalks (OCS), plus concentrate.

	Total glucose			CW glucose		
Criteria	CS	OCS	SEM	CS	OCS	SEM
Intake	g/day			g/day		
In CS	63.4	-		58.2	-	
In OCS	-	59.8		-	51.6	
In corn	176	306		5.01	8.8	
In soybean meal	26.2	-		13.3	-	
Total	266	366		76.5	60.4	
At proximal duodenum	76.8	55.9		58.4	24.0	
In feces	40.3	15.5		35.0	12.3	
Digestibility	%			%		
In reticulo-rumen	71.1[b]	84.7[a]	1.79	23.7[b]	60.3[a]	5.77
In entire GIT	84.8[b]	95.8[a]	0.33	54.2[b]	79.6[a]	1.93
Cotton stalk component	43.1[*]	84.5[*]		48.1[*]	80.7[*]	

a,b Means in the same raw and parameter with different superscripts are significantly different ($P<0.05$).

[*]Calculated, based on digestibility of monosaccharides in a reference concentrate diet (total glucose, 97.9%; CW glucose, 73.6%).

Table 6. Digestibility (%) of total and CW xylose in the reticulo-rumen and in the entire gastro-intestinal tract (GIT) of sheep fed rations consisting of cotton stalks (CS) or ozonated cotton stalks (OCS), plus concentrate.

	Total xylose			CW xylose		
Criteria	CS	OCS	SEM	CS	OCS	SEM
Intake	g/day			g/day		
In CS	22.3	-		22.2	-	
In OCS	-	19.0		-	11.5	
In corn	4.75	8.27		4.21	7.38	
In soybean meal	2.84	-		2.84	-	
Total	29.9	27.3		29.2	18.9	
At proximal duodenum	26.8	11.2		27.3	11.0	
In feces	17.6	6.96		17.0	5.99	
Digestibility	%			%		
In reticulo-rumen	10.4[b]	59.0[a]	4.08	6.68[b]	41.8[a]	5.77
In entire GIT	41.1[b]	74.5[a]	1.63	41.8[b]	68.3[a]	3.28
Cotton stalk component	31.8[*]	77.1[*]		33.3[*]	68.0[*]	

a,b Means in the same raw and parameter with different superscripts are significantly different ($P<0.05$).

[*]Calculated, based on digestibility of monosaccharides in a reference concentrate diet (total xylose, 68.4%; CW xylose, 68.9%).

et al., 1982; Shefet & Ben-Ghedalia, 1982) in which the cellulose microfibrils are enbedded. This creates large spaces within the matrix of CS and enable better accessibility of ruminal bacteria to the cellulose and xylan and better degradability of glycosidic bonds within these structural polysaccharides (Miron & Ben-Ghedalia, 1982; Ben-Ghedalia & Miron, 1983; Ben-Ghedalia et al., 1982; Shefet & Ben-Ghedalia, 1982).

TABLE 7 NEAR HERE

N metabolism at the GIT level is shown in Table 7. OCS has a low pH (~2) and therefore it could be considered as a $N(NH_3)$-carrier for supplementing low N diets. In the present study the two rations were isonitrogenous, however soybean meal was the major N contributor in CS and ammonia in the OCS ration. The ratios of duodenal NAN/dietary N were 0.79 and 0.96 for the OCS and CS rations respectively suggesting an incomplete capture of the NH_3 in the OCS. This interpretation is supported by the relative high value of rumen ammonia concentration in OCS (43.1 mg/100 ml). Nevertheless the percentage of intestinal absorption of N flowing to the duodenum were similar in both rations (61%). The quantity of N absorbed in the small intestine was somewhat higher, though not significantly, in the CS than in the OCS (11.9 vs 10.1).

<u>In Summary</u>: Ozonation is effective in increasing the digestibility of the carbohydrate fraction of dicot lignocelluloses. The effect is maintained even in a 70% concentrate ration. Ammonia enriched OCS can be considered for supplementing low N diets. Matching the NH_3-N in OCS with a source of starch more fermentable than corn and setting the ration N content to a level lower than 2.85% would be desirable.

Table 7. Mean quantities of N (g/day) present in the feed, entering the small intestine and terminal ileum and excreted in the feces of sheep fed rations consisting of cotton stalks (CS) or ozonated cotton stalks (OCS), plus concentrate.

Criteria	CS	OCS	SEM
Intake (g/day)			
In CS	1.94	-	
In OCS	-	13.0	
In corn	3.52	6.13	
In soybean meal	13.3	-	
Total	18.8	19.1	
At proximal duodenum	19.7	16.6	
NAN at the duodenum (g/day)	18.1[a]	15.0[b]	0.70
Duodenal NAN/dietary N	0.96[a]	0.79[b]	0.03
At terminal ileum	7.75	6.54	
Intestinal absorption of duodenal N			
g/day	11.9	10.1	0.67
%	60.5	60.8	1.90
Fecal N	3.99	3.99	
Apparent absorption of N (%)	79.7	79.2	0.72
Rumen NH_3 (mg/100 ml)	22.9	43.1	1.45

a,b Means in the same raw with different superscripts are significantly different ($P<0.05$).

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Ozone-treated cotton stalks as a component of a ration for growing lambs

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ABSTRACT

Solomon, R., J. Miron, A. Rubinstein and D. Ben-Ghedalia, 1991. Ozone-treated cotton stalks as a component of a ration for growing lambs. Anim. Feed Sci. Technol.,

Lambs of the Assaf dairy breed were assigned at 23-24 kg liveweight, to one of three nutritional treatments: I, a high energy concentrate mixture (CD); II, a diet in which 40% of the concentrate was replaced by a 1:1 mixture of untreated cotton stalks and poultry litter (UT-CS); and III, as II, but the cotton stalks were treated with ozone gas (O_3-TCS). The diets were fed ad libitum for 42-65 days until the lambs reached approximately 40 kg liveweight. The average daily liveweight gains were 226, 298 and 395 g for the UT-CS, O_3-TCS and CD rations, respectively. The better performance of the O_3-TCS group of lambs as compared with the UT-CS group was the result of a 9.6% higher feed intake and 16.2% more efficient utilization of the diet.

It was calculated that 1:1 mixture of O_3 treated CS and poultry litter could save 18.2% of the concentrates needed to grow lambs to reach 41.2 Kg liveweight.

INTRODUCTION

In this laboratory it have been shown that when treating cotton stalks (CS) with ozone gas, the cell-wall (CW) content was reduced from 74% to 52%, and about half the lignin fraction was solubilized or oxidized to organic acids (Ben-Ghedalia & Shefet, 1979; Ben-Ghedalia et al., 1982). Ozone treatment increased the biodegradation both in-vitro and in vivo of CS organic matter from 30.0% up to 60.8%. This treatment effect was a result of both carbohydrate solubilization and an increase in biodegradation of residual CW carbohydrates of CS (Ben-Ghedalia & Shefet, 1979; Shefet & Ben-Ghedalia, 1982; Ben-Ghedalia et al., 1983; Ben-Ghedalia & Shefet, 1983). However, to the best of our knowledge, the nutritive value of ozone treated CS have not been examined in performance experiments.

In this study, the nutritive value of untreated and ozone-treated CS was evaluated in a lamb growth experiment in which 1:1 mixture of CS and poultry litter replaced 40% of the concentrate given to lambs in an ad-libitum feeding regime. The CS experimental diets were compared with the conventional system of ad libitum concentrate feeding used in Israel.

MATERIALS AND METHODS

Animals, feeds, and feeding regime

Fifteen Assaf lambs were weaned at an average age of 38 days and were given concentrates ad libitum for an additional 36 days until reaching an average liveweight of 23.8 kg. The lambs were then divided into three experimental groups of five lambs each and were housed in three separate pens, with free access to fresh water. Lambs were group fed to maximize voluntary feed intake by: I, an untreated cotton stalks based diet (UT-CS); II, an ozone-treated cotton stalks based diet (O_3-TCS); or III, a concentrated diet (CD). The composition of the CS-based diets (TABLE I) was identical except for the CS component, which was untreated in the UT-CS group and ozone- treated in the O_3-TCS group. The concentrate mixture of the CD diet was similar to that used for intensive lamb growth in Israel (Ben-Ghedalia et al., 1988).

TABLE I NEAR HERE

The animals were fed ad libitum with fresh feed supplied each morning, after collection of feed residues of previous day to be kept in freezer until analyzed.

The CS were chopped to pass through a 6-mm screen and were mixed with the other components of the UT-CS diet plus dry urea (TABLE I). Another portion of the chopped CS was placed in a PVC column and treated by a stream of gaseous ozone which was passed through the column until complete bleaching of the CS at pH 2 (Ben-Ghedalia et al., 1983), and subsequently ammoniated to pH 6.5 in the ozone treated CS diet. Thus, the three diets were iso-nitrogenous, containing 28.8 g N \kg dry matter (DM) feed.

TABLE I.
Composition of the experimental diets (kg $100kg^{-1}$ DM)

Ingredients	Cotton stalks diets (UT-CS and O_3-TCS)	Concentrate diet (CD)
Cotton stalks (CS)	20.0	-
Poultry litter	20.0	-
Corn	58.9	36.1
Barley	-	34.2
Soya-bean meal	-	25.6
Wheat bran	-	0.92
Corn-gluten meal	0.96	-
Calcium carbonate	-	2.15
Dicalcium phosphate	-	0.19
Sodium chloride	-	0.70
Microelements + vitamins mix**	0.14	0.14

** Composition per kg mix: retinol equivalent, 2.4g; cholecalciferol equivalent, 0.04g; α-tocopherol, 20g; Zn, 100g; Mn, 30g; Fe, 20g; Co, 1g; I, 0.5g; Se, 0.1g.

The poultry litter (PL), obtained from a commercial broiler plant, was sun-dried, and as frequently found in Israel it contained 40 ppm Cu on a DM basis. The chemical composition of the poultry litter and of the O_3 treated and untreated cotton stalks is given in **TABLE II**.

TABLE II NEAR HERE

Weighing and slaughter procedure

The growth experiment lasted for 42-65 days until the lambs of each group reached an average liveweight of approximately 40 kg. During this period the lambs were weighed at 14-day intervals, prior to morning feeding. Last weighing was at morning of slaughter day. The lambs were slaughtered at noon (12.00-13.00 h) and the entire gut + contents, liver, kidney, gut fat, kidney fat and tail fat were weighed separately. The carcass was weighed after removal of the internal organs, including the kidney fat and tail fat.

Chemical analyses

Total N in ingredients and diets was analyzed by the Kjeldahl procedure, and organic matter by ashing the samples in a muffle furnace for 4 h at 550°C. The neutral detergent fiber (NDF) fraction (cell walls) and permanganate lignin were measured according to Goering & van Soest, (1970). Monosaccharides in feeds were analyzed by gas-liquid chromatography as their alditol-acetate derivatives after hydrolysis, reduction and acetylation as described in a previous study (Miron & Ben-Ghedalia, 1987). Uronic acids in feeds were analyzed according to Blumenkrantz & Asboe-Hansen, (1973).

TABLE II.
Chemical composition of the cotton stalks (CS) and poultry litter used in the diets (g 100g-1 DM)

Constituent	Untreated cotton stalks	Ozone treated cotton stalks	Poultry litter
Organic matter	94.6	92.3	82.9
Cell walls	74.0	51.0	36.0
Lignin	20.3	10.6	-
Total nitrogen	3.91	3.91	5.47
Glucose	33.2	32.4	25.6
Xylose	10.4	8.2	5.78
Arabinose	1.17	0.72	6.12
Galactose	1.01	0.53	4.97
Mannose	0.62	0.57	1.58
Uronic acids	9.78	9.41	1.20
Total monosaccharides	56.2	51.8	45.3

Statistical analysis

The live weight and slaughter data were analysed statistically using a completely randomized design. Since the feed intake was not measured individually, there is no statistical analysis for that parameter or for the feed conversion. Duncan's multiple range test served to differentiate between means (Little & Hills, 1978).

RESULTS

TABLE III NEAR HERE

The average daily feed intake and liveweight gain data are presented in **TABLE III**. The average initial liveweight of the groups ranged from 23.6 to 23.9 kg. The experiment lasted for 42, 58 and 65 days for the CD, O_3-TCS and UT-CS groups, respectively, until each dietary group reached the average final liveweight of ~40 kg.

The daily liveweight gain of the O_3-TCS lambs was 32% higher than that of the UT-CS lambs, but 25% less than the rate of growth of the CD lambs. Feed intake tended to be higher and feed utilization to be better in the O_3-TCS group than in the UT-CS lambs. However, feed utilization was probably better in the CD group than in the UT-CS and O_3-TCS lambs.

TABLE III.

Average performance values of male Assaf lambs on diets containing untreated (UT) or O_3-treated (O_3-T) cotton stalks (CS) plus poultry litter, as compared to a concentrate diet (CD).

Criterion	Dietary treatment			
	UT-CS	O_3-TCS	CD	S.E.
Initial age (days)	78	74	74	-
Growth period (days)	65	58	42	
Initial live weight (kg)	23.9	23.9	23.6	-
Final live weight (kg)	38.7	41.2	40.2	1.03
Daily gain (g)	226[a]	298[b]	395[c]	19.7
Feed intake (g DM/day)	1226	1344	1272	-
Feed conversion (feed/gain)	5.38	4.51	3.22	-

[a,b,c] Means with different superscripts in the same row are statistically different, $P<0.05$

TABLE IV
NEAR HERE

Since this study is the first to demonstrate performance data on lambs fed ration containing ozone treated cotton stalks, slaughter data are of particular interest and shown in **TABLE IV**. The weight of the gastrointestinal tract plus contents was somewhat lower in the O_3-TCS group than in the UT-CS lambs, but 28% higher than that of the CD group. Therefore, although the O_3-TCS lambs were 1.0 kg liveweight heavier than the CD ones, differences in average weight of the carcasses were small. The average carcass weight was 1.8 kg higher in the O_3-TCS group than in the UT-CS lambs, and since gut+contents weights were not statistically different this reflected the difference in the final liveweight between the CS groups (Table 3). Differences in liver weights corresponds with data of carcass weights without any abnormal findings in the CS groups. The largest kidneys both in weight and as percentage of carcass weight, were found in the O_3-TCS lambs. There was no significant difference between the O_3-TCS group and the CD lambs in total fat (gut + kidney + tail) weight or percentage. Generally, the UT-CS lambs were leaner than those of the O_3-TCS and CD groups.

Finally, the lambs underwent a general veterinary examination, including the internal organs of the thorax and abdominal cavities, at slaughter; there were no abnormal or pathological findings.

TABLE IV.

Slaughter data of Assaf lambs grown on the experimental diets.

Criterion	Dietary treatments			
	UT-CS	O_3-TCS	CD	SEM
Hot carcass weight (HCW) (kg)	17.5[a]	19.3[b]	19.7[b]	0.48
% of live weight	45.4	47.1	49.0	1.09
Gut + contents (g)	7972[a]	7582[a]	5939[b]	376
% of live weight	20.6[a]	18.5[a]	14.7[b]	0.83
Liver weight (g)	664[a]	774[b]	803[b]	25.3
% of HCW	3.79	4.01	4.08	0.12
Kidney weight (g)	107[a]	147[b]	127[c]	2.35
% of HCW	0.61[a]	0.77[b]	0.65[a]	0.02
Total fat (gut + kidney + tail) (g)	700[a]	1161[b]	978[b]	88.2
% of HCW	3.99[a]	6.03[b]	4.99[ab]	0.50

[a,b,c] Means with different superscripts in the same row are statistically different, $P<0.05$

DISCUSSION

Daily liveweight gain, voluntary feed intake and feed utilisation of the lambs group given the O_3-TCS diet were increased by 31.9%, 9.6% and 16.2%, respectively, in comparison with the untreated CS group (**Table III**). Thus, the ozone treatment of CS which decrease cell-walls and lignin contents with little effect on the concentration of total carbohydrates (**Table II**), caused not only an increase in the digestibility of CS carbohydrates as shown by Ben-Ghedalia & Shefet (1983), but also an increase in overall ration ingestibility (Table III). The better rate of growth of the O_3-TCS lambs in comparison with the UT-CS group is attributed to this combined effect.

TABLE V NEAR HERE

The amounts of concentrate saved by including the CS plus PL in the rations are presented in **TABLE V**.

Calculations in TABLE V are based on the performance data of Table III, assuming that within the range of 38.7-41.2 kg final liveweight, daily feed intake and daily gain of the UT-CS and CD lambs were constant, and accordingly the growth periods needed to obtain a final liveweight of 41.2 kg in the UT-CS, O_3-TCS and CD groups of lambs are 76, 58 and 45 days, respectively.

TABLE V shows that the UT-CS lambs would utilise mostly the concentrate component of their diet to attain that weight. Only 1.3 kg (2.27%) of the concentrate could be saved by consuming 37.3 kg of untreated CS plus PL. On the other hand, in the O_3-TCS group of lambs, 10.4 kg (18.2%) of the concentrate was replaced by 31.2 Kg of ozone-treated CS plus PL components to reach this final liveweight.

TABLE V.

Calculated values of the dry matter (DM) intake of ration components required to obtain 17.4 kg liveweight gain in lambs up to a final liveweight of 41.2 kg.

Calculated Criterion	Dietary treatment		
	UT-CS	O_3-TCS	CD
Growth period (days)	76	58	45
Total ration intake (kg DM)	93.2	78.0	57.2
Total concentrate intake (kg DM)	55.9	46.8	57.2
Total CS + PL intake (kg DM)	37.3	31.2	0
Concentrate saved by inclusion of CS + PL in diets (kg DM)	1.3	10.4	0

Discussing the issue of feed intake by ruminants, Waldo (1986) concluded that "intake is generally more important than digestibility in causing differences in the digestible DM intake". The findings of the present study (Tables III and V) and previous digestibility data (Ben-Ghedalia & Shefet, 1983; Ben-Ghedalia et al., 1983) suggest that this view can be applied not only to conventional forages, but also to straws and treated lignocelluloses.

The replacement of 40% of a whole concentrate ration by a 1:1 mixture of ozone treated CS plus PL resulted in a 25% lower daily liveweight gain. Previous studies showed that lambs can perform intensively on SO_2-treated wheat straw diets. For this reason the term "concentrate-like feed" has been applied to that product (Ben-Ghedalia & Miron, 1987; Ben-Ghedalia et al., 1988).

In view of the above mentioned, ozone-treated CS can be regarded as a "hay-like feed".

Recently, the issue of ozone treatment of lignocelluloses has gained renewed interest (Akin and Morrison, 1988; Alexander et al., 1987; Vidal & Molinier, 1988). These efforts are essential for establishing the potential of the ozonation treatment and its application in the field of ruminant nutrition.

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I M P A C T S

Firstly some basic facts: An Agricultural Research Institution does not exist in Honduras. Students interested in further studies (M.Sc., Ph.D.), are leaving for other countries and usually forget to return. Although there is the basis in Honduras for a sound ruminant industry, the lack of knowledge, research facilities and laboratories are serious obstacles in the way of realizing their potential. Therefore, although having vast resources of high quality feed alternatives, Honduras is still importing most of its grains for feeding the dairy cattle, and is not self sufficient in milk production. The country is spending some $50 million annually for importing milk powder and products.

The Honduran copartner completed his M.Sc. studies in ruminant nutrition in Israel during the project and returned to Honduras, motivated by the goal of maximizing the use of local feed alternatives in ruminant rations. At the end of the project we are leaving in Honduras a highly trained ruminant nutritionist who returned to his country after his training period.

During the visit of the P.I. in Honduras, a comprehensive program for field observations was jointly planned, the objectives being: to explore the production of high quality silages made of local waste materials for serving as a complete-feed alternative in dairy rations. Consequently, a series of preliminary field observations were started by the Honduran copartner with a remarkable initial

success and stimulating a tremendous interest among Honduran dairymen. In the last meeting organized by the Honduran copartner on this issue, he succeeded to bring together 200 dairymen from all over the country. The efforts included operative plans for knowledge dissemination. These efforts were cut by the negative response of AID to our research preproposal C11-010, 1991. Thus, a most important and most promising project was hampered and the goal of establishing a ruminant research laboratory, so badly needed in Honduras, was not realized.

PROJECT ACTIVITIES

List of publication

BEN-GHEDALIA, D. & RUBINSTEIN, A. (1984)
The digestion of monosaccharide residues of the cell walls of oat and vetch hays by rumen contents in vitro.
J. Sci. Fd Agric. 35:1159.

BEN-GHEDALIA, D. & RUBINSTEIN, A. (1985)
The effect of dietary starch on the digestion by sheep of cell wall monosaccharide residues in maize silage.
J. Sci. Fd Agric. 36:129.

BEN-GHEDALIA, D. & RUBINSTEIN, A. (1986)
The response of screened manure fiber to ozone and sodium hydroxide treatments.
Anim. Fd Sci. Technol. 15:47.

BEN-GHEDALIA, D., RUBINSTEIN, A. & DROR, Y. (1989)
The effect of ozone and sodium hydroxide treatments on the composition and in vitro digestion of screened manure fiber from different sources.
J. Agric. Food Chem. 37:1275.

SOLOMON, R., MIRON, J., RUBINSTEIN, A. & BEN-GHEDALIA, D. (1991)
Ozone-treated cotton stalks as a component in rations for growing lambs.
Anim. Fd Sci. Technol., Accepted

SOLOMON, R., RUBINSTEIN, A. & BEN-GHEDALIA, D. (1991)
Effect of alkali and ozone treatments on the composition and in-vitro digestion of various lignocelluloses
Submitted

SOLOMON, R., RUBINSTEIN, A. & BEN-GHEDALIA, D. (1991)
Digestibility of ozone treated cotton stalks by sheep.
Submitted

PROJECT PRODUCTIVITY

The objectives of the research proposal were met.

FUTURE WORK

Not approved by AID.