OZONE TREATED SORGHUM STOVER FOR RUMINANTS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The feeding of highly cellulosic crop residues to ruminants has potential for red meat production in the United States today. With current harvesting practices, at least one kilogram of residue is produced in the field for each kilogram of grain produced. This means that the amount of crop residues available for ruminant feed is virtually unlimited. Even if one chooses to ignore the probability that one day animals and man may compete for the same grain, the economic advantage of feeding crop residues cannot be disregarded.

The problem with crop residues is that the plants are harvested at a time when cell wall content and lignification are at a maximum in plant tissue. Although these residues are high in the structural carbohydrates, cellulose and hemicellulose, these carbohydrates are bound in an undefined complex with lignin which severely limits their accessibility to rumen bacteria, and thus their value to the ruminant. However, crop residues may be subjected to chemical agents capable of disrupting the lignocellulose complex and be substantially improved in nutritive value.

Ozonation is a chemical treatment which is known to effectively disrupt the lignocellulose complex. Ozone will selectively oxidize lignin, and works most efficiently on those materials which are most highly lignified. The effect of ozone on fiber structure is well documented, however very little is known about the actual feeding value of a crop residue which has been treated with ozone. Therefore, this research was designed to determine the effect of ozone on the nutritive value of a crop residue. Sorghum stover, which is a by-product of grain sorghum production, and very plentiful on the high plains, was used in these studies. The objectives of this research were:

1) to determine the effect of ozonation on the fiber structure of sorghum stover.
2) to determine the effect of ozonation on the overall nutritive value of sorghum stover.

3) to determine the in vivo and in vitro digestibility and utilization of ozone treated sorghum stover.

CHAPTER II

LITERATURE REVIEW

Cellulose is the primary energy constituent of crop residues and low quality roughages. The availability of cellulose will determine both the type of feeding program and chemical treatment, if any, that will be used with a given residue. Crop residues are generally low in protein and usually require protein supplementation, therefore chemical treatment methods for crop residues must be carefully weighed for cost effectiveness, if they are to be used successfully in a feeding program.

Structure of Cellulose and Lignin

Cellulose, a beta 1-4 anhydride polymer of D-glucose, is synthesized by a large number of organisms, ranging from bacteria to trees. It is present in the plant cell wall in the form of highly ordered structures called microfibrils, and comprises from 20 to 50% of the dry matter of most plants (Karr, 1976). Cellulose is an important structural polymer in the plant cell wall and plays a fundamental role in determining cell wall superstructure.

Cellulose is structurally isomeric with starch (amylose) in which the linkages are 1-4 alpha. However, unlike starch, cellulose linkages are not hydrolyzed by either alpha or beta-amylase, and cellulose is nearly completely insoluble (Van Soest, 1973). Since the cellulase enzymes necessary for hydrolyzing beta 1-4 linkages are not secreted by the digestive system of mammals, cellulose is virtually useless to non-ruminants as a carbohydrate source. However, the ruminant animal can rely on rumen bacteria for conversion of cellulose to glucose, since certain strains of bacteria in the rumen produce the cellulase necessary for cellulose hydrolysis (Coen and Dehority, 1970). Kellner concluded in 1947 that one kilogram of digestible cellulose was equal to one kilogram of starch in "fat-producing" value in the ruminant (Wood and Evans, 1947). Cellulose however, never exists independently in plant tissue, and its intimate association with hemicellulose, lignin, cutin and silica, greatly alters

its bacterial availability (Waldo and Smith, 1972).

The association of cellulose with lignin in plant cell structure is the predominant factor which complicates cellulose availability. Lignin represents the "encrusting material" which limits the accessability of cellulose to hydrolytic agents (Allen, et al., 1980). Lignin, which is derived from "lignum", meaning wood (Nikitin, 1966), is a complex polymer which appears to form a barrier to bacterial degradation of plant cellulose and hemicellulose (Dehority and Johnson, 1961). Dry matter digestibility and cellulose availability have been shown to be negatively correlated with the amount of lignin in a given roughage (Crampton and Maynard, 1938; Forbes and Garrigus, 1950; Gaillard, 1962).

Lignin is an amorphous, three-dimensionally cross-linked, high molecular weight polymer, comprised of propyl benzene units with methoxy and hydroxyl groups. Lignin is approximately 61-66% carbon, 5-6% hydrogen and 30% oxygen (Meyer, 1950). Lignin is formed within the cell superstructure by the enzyme-initiated dehydrogenative polymerization of trans-coniferyl, trans-sinapyl and trans-p-coumaryl alcohols (Sarkenen, 1971; Allen, et al., 1980). Increased cross-linking of these chains with advanced plant maturity even further reduces the digestibility of plant material. Cell wall lignins are always associated with hemicellulose through tight covalent bonding of xylans and arabans to the propane side chains of lignin (Allen, et al., 1980; Merewether, et al., 1972). Lignin-hemicellulose bonds have been found to be more resistant to acid hydrolysis than glycosidic bonds between carbohydrate residues (Davydov, et al., 1970). These amorphous lignin-hemicellulose chains pervade throughout the relatively crystalline cellulose lattice, both physically encrusting and covalently binding cellulose in such a manner that penetration by bacteria and hydrolytic agents is limited. Chemical disruption of this lignocellulose "complex" has been demonstrated to greatly improve accessability and digestibility of the carbohydrate fraction of low quality roughages (Pigden, 1969; Klopfenstein, 1978).

Klopfenstein (1978) concluded that the mode of action of effective

chemical treatments on the lignocellulose complex in low quality roughages was three-fold: 1) solubilization of the hemicellulose, 2) increasing the extent of cellulose and hemicellulose digestion, and 3) increasing the rate of cellulose and hemicellulose digestion by swelling. Klopfenstein noted however, that lignin content is usually not reduced, therefore an increase in digestion is usually attributed to breaking of bonds between lignin and carbohydrates, rather than physical lignin removal.

Chemical Treatment of Cereal Grain Crop Residues

The straw remaining after harvest of cereal grains represents both an enduring and substantial source of low quality roughage for the ruminant. However, straw is usually highly lignified and somewhat low in feeding value, necessitating some sort of pretreatment prior to being used in a feeding regimen.

Sodium hydroxide was the first chemical to be used successfully in treating cereal straw. In a series of papers published shortly after World War I, Beckmann outlined an alkali-treating procedure for grain straws (Beckmann, 1921; Beckmann, 1923). The "Beckmann Process" consisted of soaking straw in a 1.5% solution of sodium hydroxide for 24 hours. The soaked straw was then washed free of alkali and air dryed. This process was reported to convert 100 kilograms of grain straw into 75 kilograms of a feedstuff with a nutrient value equal to 56 kilograms of starch. Stone, et al. (1965) successfully used the Beckmann procedure to increase the in vitro dry matter disappearance of rice straw from 20.0 to 49.8%. Saxena, et al. (1971) fed oat straw treated by the Beckmann method to growing lambs. Lambs had significantly better ($P<.05$) gains and feed efficiency when treated straw was included in the ration.

Javed and Donefer (1970) treated oat straw with a 13% solution of sodium hydroxide. The treated straw was washed and neutralized with acetic acid prior to being fed to fattening lambs in a feedlot study. Intake, gains and feed efficiency were all improved by the hydroxide treatment, relative to controls. Ololade, et al. (1970) treated alfalfa stems, barley straw and corn stover with varying concentrations of sodium hydroxide, at differing times and temperatures.

Large increases in in vitro dry matter disappearance were seen when roughages were treated with sodium hydroxide at elevated temperatures. However, Ololade noted a substantial decrease in the cost effectiveness of the process whenever heating was introduced into the process.

Coombe (1979) chopped and ensiled barley straw treated with a 4% solution of sodium hydroxide, or pelleted ground straw mixed with a 4% solution of sodium hydroxide. Coombe found a 10% increase in in vivo digestibility, when either chopped or pelleted treatments were fed ad libitum to fistulated steers.

Other chemical treatments of cereal straws have also been used successfully. Pritchard (1962) subjected wheat straw to gamma radiation at 1 X 10^8 rads. In vitro dry matter disappearance increased dramatically with the treatment, however it was noted that overexposure tended to disintegrate the carbohydrate fraction of the fiber. McManus (1972) fed irradiated wheat straw to adult wethers, both with and without supplementation. Gamma-irradiated diets were always associated with reduced mean retention times, and depressed dry matter digestibilities, although dry matter intake was unaffected. Klopfenstein and Woods (1970) fed wheat straw treated with either 4 or 5 % of a sodium-potassium hydroxide mix to growing lambs. In vivo organic matter digestibilities were increased from 43.4 to 57.6% at the 4% treatment level, and from 43.4 to 60.4% at the 5% treatment level. Sodium hydroxide, potassium hydroxide, ammonium hydroxide, and sodium formate have all been shown to significantly increase the in vitro dry matter disappearance of ryegrass straw (Anderson and Ralston, 1973). Ammonia has also been shown to upgrade straw quality. Garrett, et al. (1974) treated rice straw with 4% ammonia and found significantly improved gains and cellulose digestiblity when treated straw was fed to growing lambs.

Feeding and Chemical Treatment of Grain Sorghum Stover

Almost fifty million metric tons of residue from grain sorghum production are produced annually in the United States (Ward, 1978). The majority of this residue is classified as grain sorghum stover or stubble, and is the part of the sorghum plant remaining after combing. Sorghum stover is relatively low in nutritive value, and is often

grazed or returned to the soil. However, stover may be harvested and effectively used as an alternative roughage source.

Sherrod, et al. (1974) conducted extensive investigations into the feeding value of sorghum stover. In vitro studies were conducted to measure the digestibility of sorghum leaves, stalks and complete stover. Stalks were found to be slightly more digestible than leaves in total dry matter, but leaves were higher in digestibility of cell wall constituents, acid detergent fiber, cellulose and hemicellulose. In vitro dry matter digestibilities were found to closely parallel in vivo digestibilities by sheep. Stover was found to contain adequate digestible energy to be used as a maintenance feed for ruminants, although the low protein content of stover would necessitate protein supplementation for adequate performance.

Sherrod, et al. (1975) fed grain sorghum stover to gestating beef cows at 83% of maintenance with either liquid or dry supplements, and concluded that the nutritive value of sorghum stover was high enough that it could be fed as the only roughage source for wintering beef cows. Ward, et al. (1979) grazed gestating beef cows on sorghum residue pasture, and also found that satisfactory performance and weight gain could be realized, if stover is properly supplemented with protein. In further studies with protein supplemented stover (Summers and Sherrod, 1975), sorghum stover digestibility was measured when stover was supplemented with either cottonseed meal, urea or biuret, and fed to mature sheep. The results indicated that the digestibility of stover supplemented with cottonseed meal was the same as unsupplemented stover, but that the digestibility tended to decrease with urea and biuret supplementation.

Once sorghum stover has been harvested and handling becomes more easily facilitated, chemical treatment offers a viable method for upgrading its feeding value. Chandra and Jackson (1970) treated ground sorghum stover with varying concentrations of sodium hydroxide, hydrogen peroxide and chlorine dioxide. The in vitro dry matter digestibility of stover increased almost linearly with sodium hydroxide treatment. However, dry matter digestibility tended to level off above a 15% solution. Hydrogen peroxide and chlorine dioxide treatments also improved stover digestibility. Koers (1972) fed ensiled

corn stalklage and milo tailings treated with 4% sodium hydroxide to growing lambs in a 69-day trial. Satisfactory growth and performance were demonstrated when treatments were compared to a corn silage control ration.

Summers and Sherrod (1974) conducted studies on the effect of sodium hydroxide treatment on the in vitro digestibility of sorghum stover and other coarse roughages. It was found that the in vitro dry matter digestibility of sorghum stover could be increased from 57 to 65% with hydroxide treatment, and that sodium hydroxide treatment had the greatest effect on hemicellulose digestibility, increasing in vitro digestibility from 61 to 82%. Sherrod, et al. (1978b) treated ground sorghum stover with 4% sodium hydroxide, 4% calcium hydroxide and a 2%-2% mixture. Sodium hydroxide alone demonstrated the greatest ($P<.05$) improvement in in vitro dry matter disappearance of the three treatments. Measurements were also made on the rate of dry matter disappearance with hydroxide treatment. All treatments were found to have a higher ($P<.05$) rate of digestion than controls. It was suggested that the increased digestibility and feed intake, and the improved feed efficiency observed with hydroxide treatment of sorghum stover may be a function of rate of digestion.

Improving Low Quality Crop Residues Using Oxidative Reagents

Phenolic in nature, the aromatic nuclei of the lignin molecule are quite susceptible to oxidative attack by a wide variety of reagents (Sarkenen, 1971). As noted by Sarkenen (1971), chemicals such as permanganate and dichromate very efficiently degrade the lignin macromolecule, but the potent oxidative capacity of these two reagents tends to be very destructive to surrounding carbohydrates as well, making these chemicals unsuitable for treating roughages. However, weaker oxidants such as chlorite, chlorine dioxide, sodium hypochlorite, peracetic acid, hydrogen peroxide, sulfur dioxide and ozone may selectively degrade lignin, with only minor nutritive damage to the carbohydrate fractions. Use of oxidative reagents in chemical treatment of roughages facilitates the physical removal of lignin as a barrier to microbial action, rather than simple disruption of the lignocellulose complex.

Chlorine dioxide gas is a very effective oxidant and is used extensively in sewage treatment processes (Rice and Cortuvo, 1978). Sullivan and Hershberger (1959) treated orchard grass, canary grass, and wheat straw with gaseous chlorine dioxide. The lignin content of wheat straw was reduced from 6.7 to 1.85%, with a subsequent increase in in vitro cellulose digestibility of 29.4%. Chandra and Jackson (1970) reported the in vitro dry matter disappearance of sorghum stover could also be improved with chlorine dioxide treatment.

Jones (1967) conducted extensive work with the chemical oxidation of corn cobs. Ground cobs were treated with sodium hydroxide, sodium peroxide and hydrogen peroxide, at levels varying from 1.5 to 12 g / 100 g of cob dry matter. Cobs were also treated with a combination of 4% sodium hydroxide and varying levels of hydrogen peroxide. The greatest increases in in vitro dry matter digestibility occurred with 9% sodium hydroxide, 9% sodium peroxide, and 4% sodium peroxide + 3% hydrogen peroxide. Hydrogen peroxide alone was found to depress digestibility at all treatment levels.

Koers (1970) conducted further investigations into treatment of corn cobs with sodium hydroxide and sodium peroxide. Ground corn cobs were treated with either 4% sodium hydroxide, 4% sodium peroxide, or 4% sodium hydroxide + 4% sodium peroxide, and fed to steer calves. All treatments were higher in in vivo digestibility than an untreated control, with sodium peroxide alone demonstrating the greatest improvement in digestibility. Similar trends were noted for the in vitro digestibility of treated cobs.

Klopfenstein (1972) treated alfalfa stems, corn cobs, whole corn plants and corn stalks with either 4% sodium hydroxide, 4% sodium peroxide or 4% sodium peroxide + 3% hydrogen peroxide. Peroxide treated cobs were higher in in vivo dry matter digestibility than sodium hydroxide treated cobs, when fed to growing lambs. Greater delignification was also noted with peroxide treatments, although this difference was not found to be significant.

The use of sulfur dioxide in combination with a salt of sulfurous acid to solubilize lignin in wood, forms the basis of the

sulfite pulping process. Sulfur dioxide has also been used to disrupt the lignocellulose complex in wood to produce a product with highly digestible carbohydrates, without actual removal of lignin (Allen, et al., 1980).

Sherrod, et al. (1978c) treated sunflower stalks, cotton burrs and ground sorghum stover with 6% sulfur dioxide for 2 hours at 150^{o} C. The in vitro dry matter digestiblity was improved 93% for the sunflower stalks, 113% for cotton burrs and 40% for sorghum stover. Sherrod concluded that sulfur dioxide could be used effectively to improve the digestibility of low quality roughages. Sherrod, et al. (1978a) treated ground mesquite wood with sulfur dioxide at different reaction temperatures, times and concentrations. The best treatment of mesquite was found to increase the in vitro dry matter digestibility over 100%. McCarthy, et al. (1980) fed sulfur dioxide treated mesquite to growing lambs in a series of feeding trials. It was determined from the studies that sulfur dioxide treated mesquite could support animal performance equal to controls if fed at less than 20% of a ration. However, at intake levels of 20% or greater of treated mesquite, growth rate was depressed, probably due to high sulfur intakes.

The ability of ozone to degrade plant lignin and structural carbohydrates has been well established (Mester, 1959; Kiryushina and Tishchenko, 1968; Katuscak, et al., 1972). In recent years, the reaction of ozone with lignin has attracted considerable attention, particularly with its potential use in the industrial bleaching of chemical and mechanical pulps (Mbachu and Manley, 1981a). However, the potent delignifying capability of ozone also makes it an effective agent for upgrading the nutritive value of low quality roughages.

Ozone, which can function as a 1, 3 dipole, a nucleophile or an electrophile (Bailey, 1978), will selectively oxidize carbon-carbon double bonds and unsaturated ring structures. The mechanisms of ozone reactions with lignin and carbohydrate fractions of wood have been studied in great detail (Katai and Schuerch, 1966; Mbachu and Manley, 1981b). The attack of ozone on wood components has been shown to be limited by diffusive resistances which block or inhibit ozone penetration into wood structure. However, these diffusive

resistances can be decreased by presoaking wood with an equal quantity of water (Schuerch). Mbachu and Manley (1981c) conducted studies with ozone treatments of spruce wood and the subsequent effect on lignin, hemicellulose and cellulose. Ozone was found to react with lignin four times faster than with carbohydrates, and to have a relative rate of degradation of wood components in the order of lignin > hemicellulose > cellulose. The rapid rate of lignin degradation relative to carbohydrates was attributed largely to the chemical mechanism of degradation, while the rate difference between hemicellulose and cellulose was determined to be a function of physical structure. The crystallinity of cellulose was found to limit its accessability to ozone molecules, whereas hemicellulose was relatively amorphous, and therefore more readily attacked.

Weakley and Owens (1975) experimented with the application of ozone as a pretreatment method for low quality roughages. Wood hemicellulose, alfalfa hay, and a low quality range forage were treated with 4.5% ozone for 2-4 hours. The in vitro dry matter digestibility of the low quality range forage was significantly improved with the ozone treatment, however the alfalfa hay was only slightly improved, and the digestibility of the wood hemicellulose was depressed with ozone treatment. It was particularly noted that no toxic compounds were produced by ozonation of roughages.

Ben-Ghedalia, et al. (1980) treated cotton stalks with ozone, ammonium hydroxide and a combination of the two treatments. Cotton stalks, which were classified as "woody" in structure, responded well to ozonation with a 50% reduction in lignin content noted. In vitro dry matter disappearance was increased 100% with ozone treatment, and 120% with the hydroxide-ozone combination. Cotton stalks were only improved 20% with ammonium hydroxide treatment alone. Ben-Ghedalia and Miron (1981b) treated wheat straw with 5% sodium hydroxide, ozone and 5% sulfur dioxide (70^{o} C for 72 hours). The straw was ozonated until it had a bleached appearance. In vitro organic matter disappearance was increased from 44 to 80% with sulfur dioxide treatment, and from 44 to 66% for both ozone and sodium hydroxide treatments. Large increases in the percent of

reducing sugars were also noted with sulfur dioxide and ozone treatments. Reducing sugars were increased from 2.2 to 15.6 and 24.3% by ozone and sulfur dioxide, respectively.

The efficiency of sodium hydroxide, sulfur dioxide, and ozone treatment of wheat straw as a preparatory step for the saccharification of wheat straw with cellulases was examined by Ben-Ghedalia and Miron (1981a). The effect of chemical and cellulase treatment of wheat straw on in vitro dry matter disappearance was measured, with sulfur dioxide having the most potent pretreatment effect, followed by ozone in a medium position, and sodium hydroxide last.

Tock, et al. (1982) found that ozone could be successfully used to improve the nutritive value of ground mesquite, increasing the in vitro dry matter disappearance of ground mesquite nearly 100% with ozone treatment. It was also noted that ozone treatment produced no toxic chemical residues, giving it a feeding advantage over other chemical treatment methods. Runte, et al. (1981) included ozone treated mesquite as 10% of a lamb ration, with no significant depression in performance relative to controls.

CHAPTER III

EFFECT OF OZONE TREATMENT ON COMPOSITION, IN VITRO DIGESTIBILITY, AND NYLON BAG DISAPPEARANCE OF SORGHUM STOVER

Summary

Sorghum stover was contacted with ozone for either 24 or 72 hours (h) in a continuously stirred pilot plant reactor. The effect of ozone on sorghum stover composition, in vitro digestibility and nylon bag disappearance was studied. Lignin content was significantly reduced by all treatments ($P < .05$), with 12 and 23% delignification occurring with the 24 h and 72 h treatments, respectively. Cellulose content was not affected ($P > .05$). The amount of total reducing sugars measured in a 10% methanol extract of stover was increased by 35% by the 24 h treatment, and 75% by the 72 h treatment, demonstrating that considerable carbohydrate hydrolysis had occurred. In vitro organic matter digestibility was increased ($P < .05$) from 48.06 to 55.16 by the 24 h treatment. However, digestibility was slightly depressed by the 72 h treatment. Rate of digestion of untreated and 24 h treated stover both in vitro and in nylon bags was determined. Differences ($P < .05$) were noted at all incubation times in nylon bags, and the magnitude of these differences were much larger than those noted with in vitro incubations.

Introduction

Current crop harvesting practices generate a significant amount of low quality crop residues. These residues, which are high in structural carbohydrates, represent a very important alternative energy source for the ruminant animal. However, the nutritive value of these residues is often diminished by the association of the structural carbohydrates, cellulose and hemicellulose, with lignin in the plant cell wall. Chemical treatment may be used effectively to disrupt the lignocellulose complex in crop residues, and free carbohydrates for microbial attack. A number of chemical agents have been effective in improving low quality crop residues (Stone, et al., 1965; Klopfenstein and Woods, 1970; Chandra and Jackson, 1970; Arndt and Richardson, 1982).

Ozonation is a chemical treatment method which is known to

selectively oxidize lignin and disrupt the lignocellulose complex. The potent delignifying capability of ozone, which has been used effectively by the paper pulping industry, has only recently been applied to improvement of roughages for animal feeds (Runte, et al., 1981; Ben-Ghedalia and Miron, 1981; Tock, et al., 1982). Although crop residues have been ozonated in small laboratory reactors (Weakley and Owens, 1975; Ben-Ghedalia, et al., 1980), very little information is available on ozonation of crop residues in larger pilot plant reactors.

Sorghum stover is the stalky portion of the sorghum plant, remaining in the field after grain harvest. The nutritive value of stover is relatively low, and its use in animal feeding programs is limited without some sort of chemical pretreatment. These studies were conducted to determine the effect of ozone on sorghum stover in a large pilot plant reactor.

Experimental Procedure

Treatment of sorghum stover

For large scale treatment, sorghum stover was ground in a Gehl grinder-mixer with a 9.5 mm screen. Ground stover was treated with ozone in a continuously stirred batch reactor (0.05 m^3), with an oxygen-ozone mixture (50 ppm ozone) introduced at 8 standard liters per minute. The stover was treated at ambient temperature, and no provision was made to remove the heat of reaction. After desired contacting time was achieved, the material was dried to 6% moisture in a forced air dryer.

Chemical analysis

Acid detergent fiber (ADF), cellulose and permanganate lignin were determined by methods described by Goering and Van Soest (1970) on triplicate samples. Gross energy was determined in a Parr bomb calorimeter. For determination of reducing sugars and glucose, approximately 300 mg samples of stover were extracted with 10 ml a 10% methanol solution at 60^{o} C for 1 h. After extraction, samples were centrifuged and the supernatant analyzed for concentrations of reducing sugars and glucose. Glucose concentration was spectrophotometrically determined by the glucose oxidase procedure using

the "Flozyme" reagent produced by Worthington Diagnostics, Freehold, New Jersey. Total reducing sugars were determined spectrophotometrically by the dinitrosalicyclic acid method. Chemical analysis data was analyzed using a completely randomized design. Duncan's multiple range test was used to determine differences between means (Steel and Torrie, 1980).

Estimation of digestibility

In vitro digestibility was determined by the Moore modification of the two-stage Tilley-Terry procedure (Harris, 1970), which was further modified by using 2.0 g samples and 100 ml rumen fluid: buffer solution inoculum in 250 ml centrifuge bottles (Sherrod and Summers, 1974). Rumen fluid was obtained from a mature steer fitted with a permanent ruminal cannula and fed alfalfa hay. Duplicate bottles for the 24 h ozonation treatment were removed after 3, 6, 12, 24, and 48 h of fermentation, and placed in the 48 h pepsin digestion stage. Forty-eight h fermentation only was determined for the 72 h ozonation treatment. Organic matter digestibilities were determined, with organic matter residues corrected for residue blanks.

Nylon bag disappearance was determined by placing 2 g "as is" samples in nylon bags (6 X 15 cm) and incubating for 3, 6, 12, 24 and 48 h in a ruminally fistulated steer fed alfalfa hay. The bags were randomly attached to a leader line weighted at one end. After incubation, the bags were removed, rinsed with cold water to remove any debris, and dried overnight at 100^{o} C. The bags were then reweighed and dry matter disappearance calculated. Nylon bag data were analyzed as a randomized complete block design using the general linear model procedures (Barr, et al., 1979). Duncan's multiple range test was used to determine differences between means.

Results and Discussion

Compositional changes in ground sorghum stover as a result of ozone treatment are shown in table 1. Both ozone treatments were lower ($P < .05$) than untreated stover in ADF content. Cellulose content was slightly reduced by both treatments, however this reduction was not significant. Permanganate lignin content of

TABLE 1. COMPOSITION, GROSS ENERGY CONTENT AND IN VITRO DIGESTIBILITY OF SORGHUM STOVER TREATED WITH OZONE

Item	Treatment		
	Control (untreated)	Ozonation (24 h)	Ozonation (72 h)
Composition, %[a]			
ADF	49.2 ± 1.00[b]	46.7 ± .53[c]	46.5 ± .01[c]
Cellulose	35.1 ± 1.10[b]	33.4 ± .62[b]	31.6 ± .01[b]
Lignin	8.0 ± .29[b]	7.1 ± .44[c]	6.2 ± .84[c]
Gross Energy, kcal/g[a]	3884 ± 27[b]	3762 ± 35[c]	3634 ± 26[d]
In Vitro Digestibility, %			
Dry matter	44.6 ± .75[b]	50.3 ± .59[c]	43.6 ± .26[b]
Organic matter	48.1 ± .67[b]	55.2 ± .54[c]	47.4 ± .46[b]

[a]Dry matter basis.

[b,c,d]Means in the same row with different superscripts differ ($P < .05$).

sorghum stover was reduced by both ozone treatments, with a 23% reduction in lignin content with the 72 h treatment. This is consistent with the data of Ben-Ghedalia and Miron (1981), which showed a 22% reduction in permanganate lignin content with ozone treatment. Ozonation was also found to diminish the gross energy content of sorghum stover (table 1). All ozone treatments were significantly lower ($P < .05$) in gross energy content than untreated stover, probably due to oxidation of stover by ozone. The 72 h treatment was lower than the 24 h treatment.

Figure 1 shows changes in reducing sugars and free glucose in sorghum stover resulting from treatment with ozone. The amount of total reducing sugars was increased by ozone treatment, whereas free glucose concentration was proportionally diminished by treatment. Although a limited amount of cellulose degradation does appear to occur with treatment (table 1), essentially any glucose which might be produced is subsequently destroyed by ozone. The very large non-glucose fraction of the total reducing sugars is probably composed primarily of 5-carbon sugars resulting from the degradation of hemicellulose.

Table 1 shows the effects of ozone treatment on in vitro dry and organic matter digestibility of sorghum stover. The 24 h treatment was higher ($P < .05$) in digestibility than untreated stover. Digestibility of stover was slightly depressed by the 72 h ozonation, although this depression was not significant ($P < .05$). The depressed in vitro digestibility and the 6.4% loss of gross energy noted with the 72 h treatment may indicate that a detrimental amount of carbohydrate destruction may occur with longer treatment times. Based on in vitro digestibilities, it would appear that stover treated for 24 h in the large reactor would be a practical method for ozonating sorghum stover.

The rate of in vitro organic matter digestion of 24 h treated and untreated sorghum stover is shown in figure 2. Although differences ($P < .05$) were noted at all incubation times except 24 h, the increase in digestion rate for ozone treatment was not as great as that noted by other workers (Ben-Ghedalia and Miron, 1981; Ben-Ghedalia, et al.,

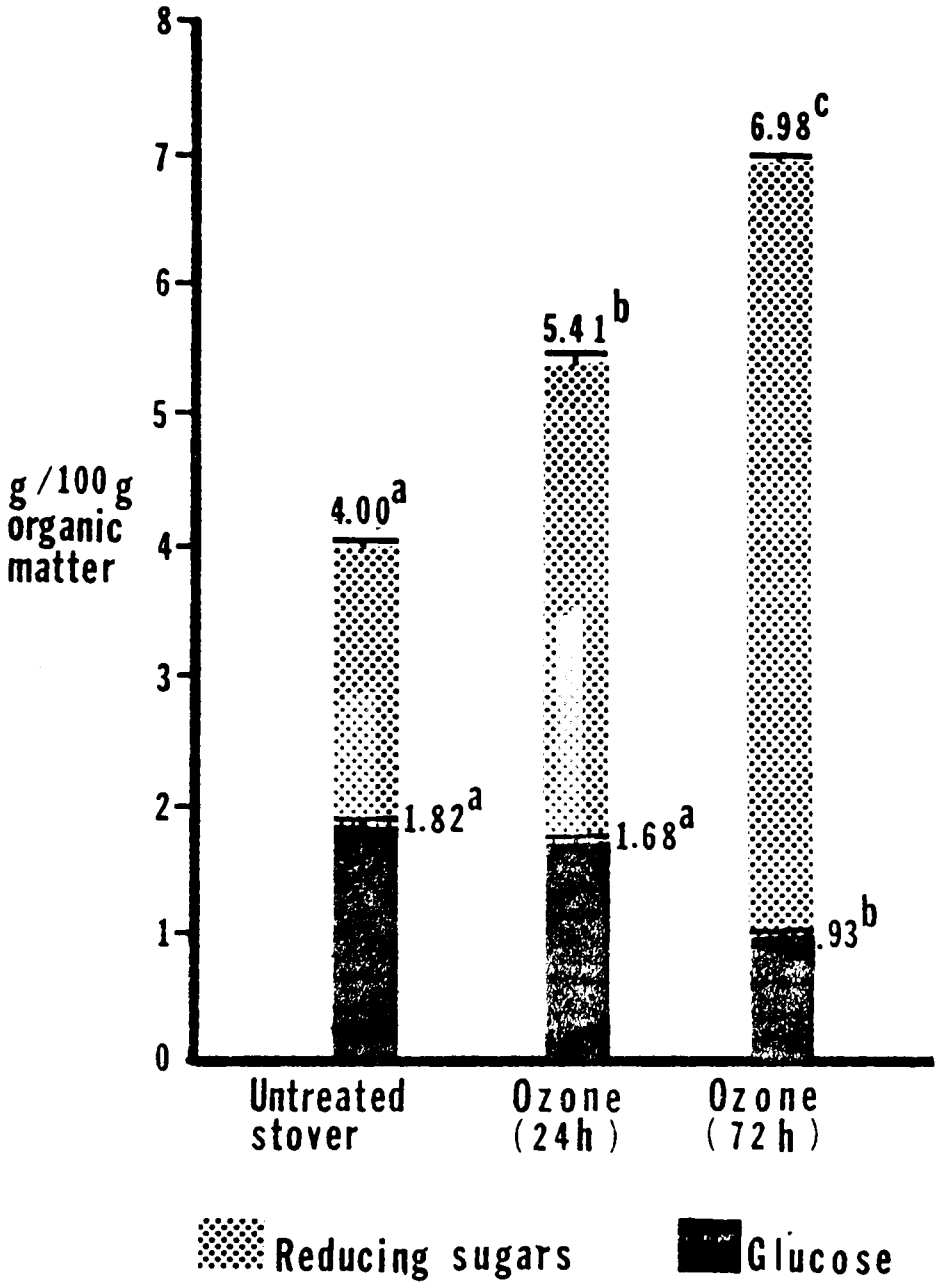

Figure 1. Total reducing sugars and glucose in a 10% methanol extract of treated and untreated sorghum stover.

^{a,b,c}Means in the same row with different superscripts differ ($P < .05$).

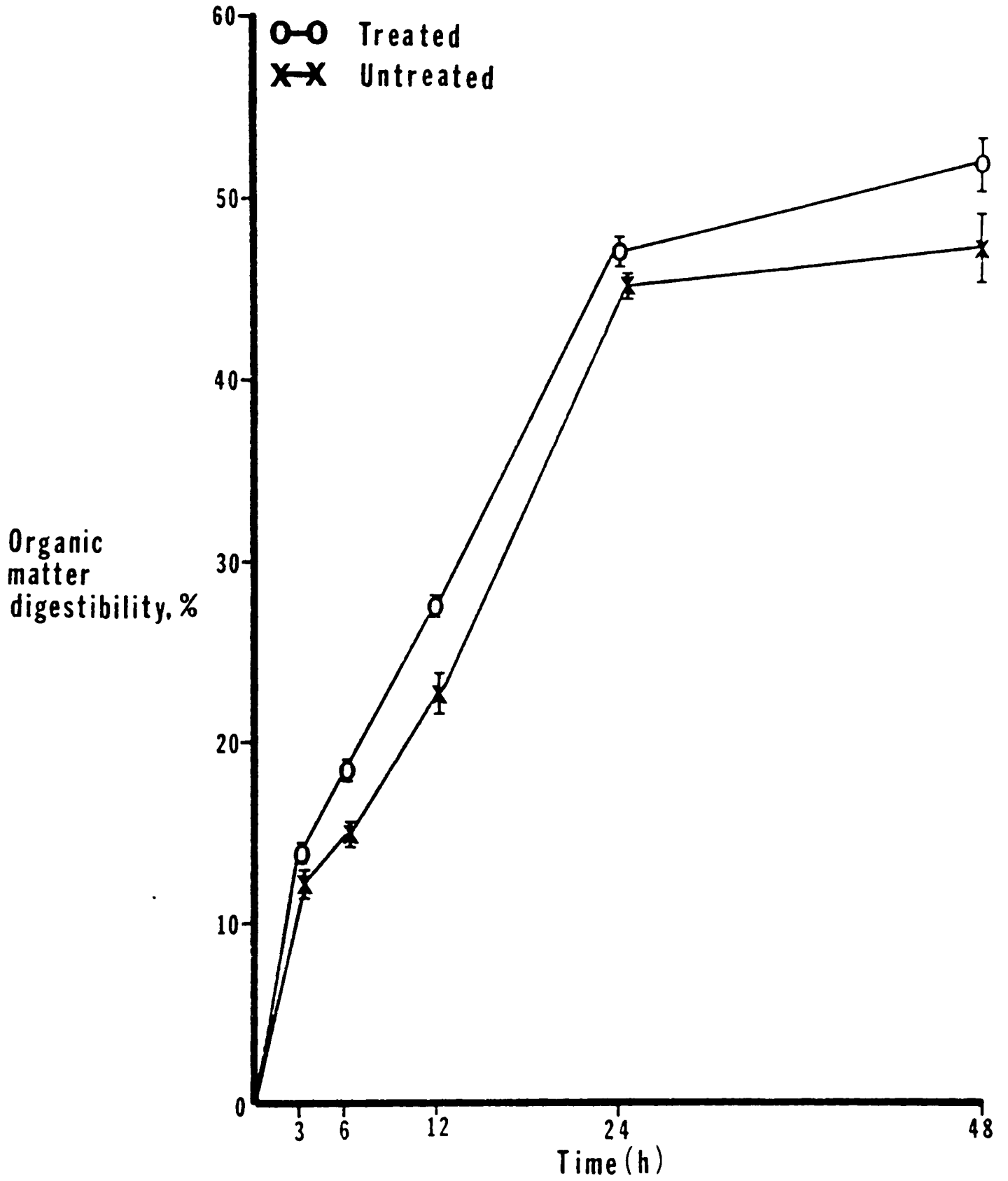

Figure 2. Rate of in vitro organic matter disappearance of untreated and 24 h treated sorghum stover.

1980). However, much larger differences in digestion rate were noted in nylon bags as shown in figure 3. Digestibilities for the 24 h treated stover were higher ($P < .05$) than the untreated control at all incubation times. Ozone appeared to give much greater improvement in stover digestibility in nylon bags than with the in vitro determination. The increased dry matter disappearance noted in nylon bag studies may be a function of ozone's ability to "open up" fiber structure, since particle size was not reduced as it was for the in vitro determination.

Data indicate that sorghum stover can be successfully contacted with ozone in a large pilot plant reactor, and that ozonation can be used effectively as a chemical treatment method for stover. Results from these studies and other work (Weakley and Owens, 1975; Ben-Ghedalia and Miron, 1981; Tock, et al., 1982), indicate that a considerable amount of variation in the extent that ozone improves roughage quality is to be expected. Both the degree of lignification and the overall fiber structure of a given roughage appear to be important factors in improving the nutritive value of a roughage using ozone.

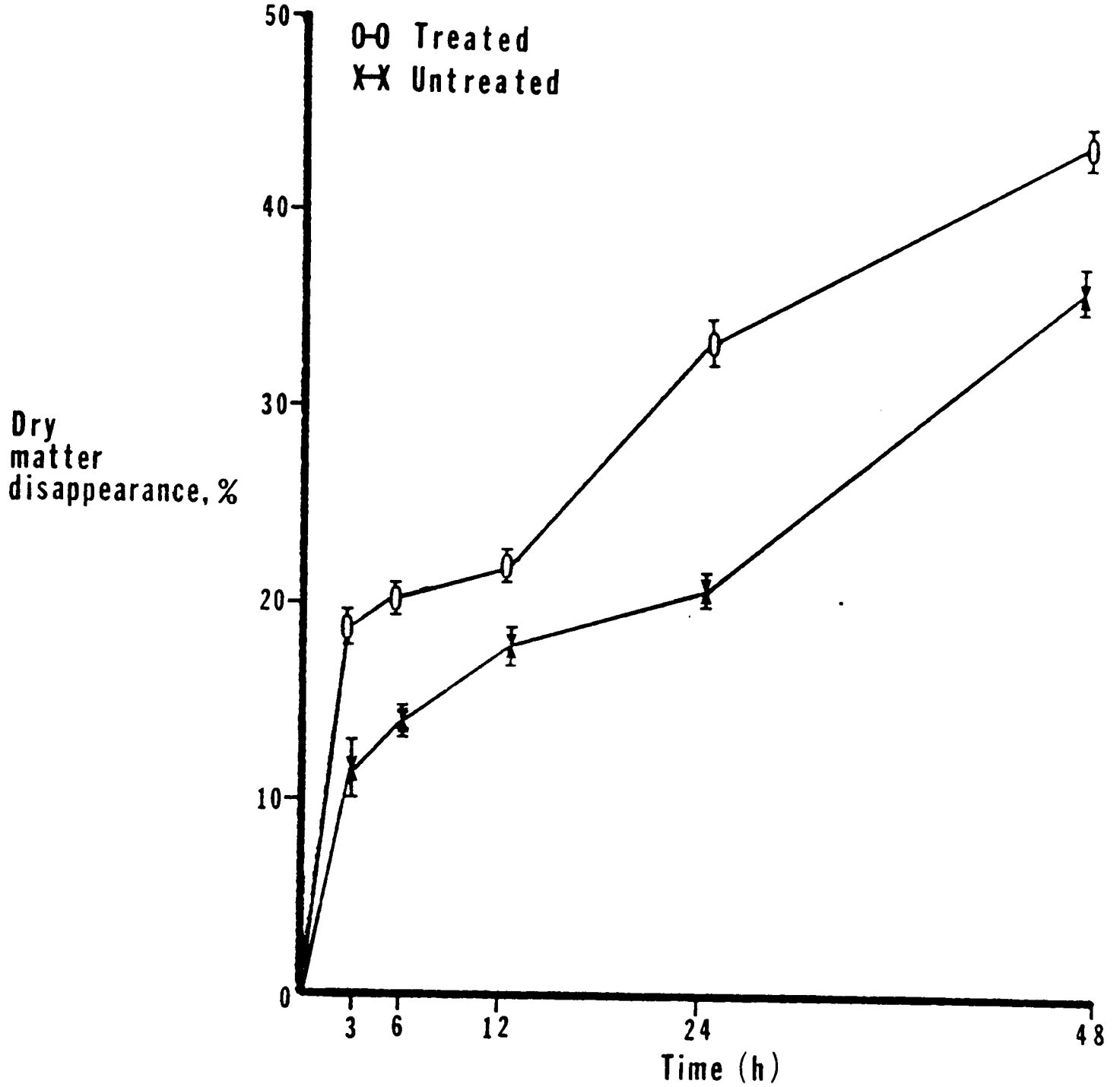

Figure 3. Rate of nylon bag dry matter disappearance of untreated and 24 h treated sorghum stover.

CHAPTER IV

DIGESTIBILITY OF OZONE TREATED SORGHUM STOVER BY LAMBS

Summary

The effects of ozone treatment of grain sorghum stover on apparent digestibilities of dry matter, protein, energy, ADF, cellulose and lignin were studied in an experiment with growing lambs. In vitro dry matter, cell walls, hemicellulose, ADF, cellulose and lignin digestibilities were determined, as well as in vitro hydrogen ion concentrations with both untreated and ozonated sorghum stover. In experiment 1, in vivo dry matter digestibilities were different only between the control and the 50/50 treatment (50% untreated stover + 50% ozone treated stover), with the 50/50 treatment exhibiting greater ($P < .05$) digestibility. Protein, energy, ADF and lignin digestibilities were not different ($P > .05$) among stover treatments, however, all stover treatments had greater ($P < .05$) protein and energy digestibilities than the control. Cellulose digestibility was greater ($P < .05$) for the ozone treated stover than for untreated stover. In vitro digestibilities for dry matter and fiber components were generally higher ($P < .05$) for ozonated stover than for untreated stover, and a 50/50 mixture of treated and untreated stover was found to have a very favorable effect on in vitro digestibility. In vitro pH was monitored to determine the effect of ozone treatment on in vitro hydrogen ion concentration. No detrimental decreases in in vitro hydrogen ion concentration were found to occur during incubation. Untreated sorghum stover and prairie hay were innoculated in vitro with a water extract of ozonated sorghum stover. Dry matter digestibility was depressed ($P < .05$) at 24 h of incubation for both prairie hay and untreated stover, and continued to be depressed for prairie hay at 48 h of incubation. High pressure liquid chromatography of the water extract revealed no compounds known to be anti-bacterial.

Introduction

A variety of chemical reagents and treatment methods have been tested for their potential to enhance the digestibility of crop

residues. The most universally used chemicals for animal experimentation in residue treatment are sodium or ammonium hydroxide (Chandra and Jackson, 1970; Koers, et al., 1972; Klopfenstein, 1978; Arndt and Richardson, 1982), which are representative of most conventional chemical treatments used for crop residues. Chemical treatments such as sodium and ammonium hydroxide usually effect improvement in digestibility of roughages by solubilizing hemicellulose, and increasing the extent and rate of cellulose and hemicellulose digestion (Klopfenstein, 1978). Delignification is not usually considered to be an important aspect of chemical treatment (Klopfenstein, et al., 1972), and increases in digestion are usually attributed primarily to breaking of bonds between lignin and carbohydrates rather than lignin removal. However, the aromatic nuclei of the lignin molecule are quite susceptible to oxidative attack (Sarkenen, 1971), and crop residues may be significantly delignified by oxidative chemical reagents (Sullivan and Hershberger, 1959; Sherrod, et al., 1978; Ben-Ghedalia, et al., 1980). Oxidative treatments facilitate the actual physical removal of lignin as a barrier to microbial action, rather than simple disruption of the lignocellulose complex.

Ozonation is a chemical treatment method which is known to selectively oxidize lignin and disrupt the lignocellulose complex. Ozone has been used successfully by the paper pulping industry, both to delignify and improve the brightness of wood pulps. Several researchers have successfully applied the delignifying capability of ozone to the treatment of roughages for animal feeds (Weakley and Owens, 1975; Ben-Ghedalia and Miron, 1981; Tock, et al., 1982). Very little however, is known of the actual in vivo feeding value of crop residues treated with ozone.

Sorghum stover, a by-product of sorghum grain production, is produced each year in large quantities in the Southwestern United States. Sorghum stover, which is relatively low in nutritive value, is often grazed or used as a wintering roughage for beef cows. Once sorghum stover has been harvested, chemical treatment may be more easily facilitated. These studies were conducted to determine the effect of ozone on the feeding value of sorghum stover.

Experimental Procedure

Sorghum stover was ground in a Gehl grinder-mixer with a 9.5 mm screen. Ground stover was treated with ozone in a continuously stirred batch reactor (.05 m^3), with an ozone-oxygen mixture introduced at 8 standard liters per minute with an ozone concentration of 50 ppm. Only ambient temperatures were used and no provision was made to remove the heat of reaction. After desired contacting time was achieved, the material was dried to 6% moisture in a forced air dryer.

Experiment 1

Four crossbred wether lambs and four crossbred ewe lambs were used in a twice replicated 4 X 4 Latin square design, with lambs allotted to squares by sex. The lambs (40 kg) were housed in individual crates equipped for total collection of feces. The lambs were kept in a thermostatically controlled building, with the temperature remaining relatively constant throughout the experimental period. Clean water was furnished ad libitum. The diets were composed of 60% concentrate and 40% of either cottonseed hulls, untreated sorghum stover, ozone treated sorghum stover, or a 50/50 mixture of treated and untreated sorghum stover (table 2). The chemical analysis of the experimental diets is given in table 3.

Each period consisted of a 14-day adjustment to the diet and a 7-day collection period. At the end of each collection period, the feces were homogenized with water and a composite sample taken and frozen for subsequent analysis. Samples were analyzed for dry matter, nitrogen, gross energy, ADF, cellulose and lignin. Dry matter, protein, energy, ADF, cellulose and lignin digestibilities were determined.

The data were analyzed using the general linear model procedures (Barr, et al., 1979). Duncan's multiple range test was used to determine differences between means (Steel and Torrie, 1980).

Experiment 2

Single stage in vitro runs were conducted for 3, 6, 12, 24 and 48 h of incubation on untreated sorghum stover, ozone treated sorghum stover, and a 50/50 mixture (dry matter basis) of untreated and ozonated stover.

TABLE 2. COMPOSITION OF DIETS[a]-EXP. 1

Ingredient	Diet A	Diet B	Diet C	Diet D
Sorghum (IFN 4-04-444)	54.00	54.00	54.00	54.00
Cottonseed hulls (IFN 1-01-599)	40.00	...	...	...
Sorghum stover (IFN 1-04-302) (untreated)	...	40.00	...	20.00
Sorghum stover (IFN 1-04-302) (treated)	...	...	40.00	20.00
Soybean meal (IFN 5-04-604)	4.50	4.50	4.50	4.50
Calcium carbonate (IFN 6-01-069)	.60	.60	.60	.60
Plain salt (IFN 6-04-152)	.50	.50	.50	.50
Ammonium sulfate	.35	.35	.35	.35
Vitamins A and D[b]	+	+	+	+

[a]Dry matter basis.

[b]Vitamin A-636 iu/kg, Vitamin D-139 iu/kg.

TABLE 3. CHEMICAL ANALYSIS OF DIETS-EXP. 1

Item	Percentage[a]						
	Dry matter	Crude protein	Ash	ADF	Cellulose	Lignin	G. Energy kcal/g
Diet A	90.8	10.7	2.6	30.0	19.2	5.4	4.37
Diet B	91.2	10.3	4.5	25.0	17.2	5.0	4.17
Diet C	91.6	10.4	4.5	24.0	16.5	4.6	4.16
Diet D	91.4	10.4	4.5	24.5	16.8	4.8	4.17
Cottonseed hulls	92.0	5.0	3.0	61.7	40.2	9.0	4.30
Sorghum stover (untreated)	93.0	4.0	8.0	49.2	35.0	8.0	3.80
Sorghum stover (treated)	94.0	4.1	8.2	46.7	33.4	7.0	3.78

[a]Dry matter basis.

Disappearance of dry matter, cell walls, ADF, hemicellulose, cellulose and lignin was determined at each incubation time. All samples were ground through a 1 mm screen, and triplicate samples of 2 grams each were weighed into 250 ml centrifuge bottles and innoculated with 100 ml of a solution of 30% rumen fluid and 70% McDougal's buffer. Rumen fluid was obtained from a mature steer fitted with a permanent ruminal cannula and fed alfalfa hay. Residue blanks were run at all incubation times. After the desired incubation time, fermentation was stopped by the addition of .5 ml of a saturated mercuric chloride solution. The samples were filtered through a fritted glass crucible, dried at 100^{o} C for 24 h, and weighed.

Cell walls, hemicellulose, ADF, cellulose and permanganate lignin were determined by methods described by Goering and Van Soest (1970), on samples, sample residues, and residue blanks. In vitro digestibilities were calculated for each component.

A completely randomized design was used, with one-way analysis of variance and Duncan's multiple range test used to determine differences between means (Steel and Torrie, 1980).

Experiment 3

The in vitro hydrogen ion concentration (pH) in samples of ozone treated and untreated stover was determined after 0, 1, 2, 3, 4, 5, 6, 12, and 24 h of incubation in buffered and unbuffered in vitro incubations. Buffered in vitro incubations were prepared using triplicate samples of 2 grams each ground through a 1 mm screen. Samples were weighed into 250 ml centrifuge bottles and innoculated with 100 ml of a solution of 30% rumen fluid and 70% McDougal's buffer. Unbuffered in vitro incubations were prepared similarly, with distilled water substituted for McDougal's buffer solution. The initial (h=0) hydrogen ion concentration was determined 5 minutes after innoculation. Incubation flasks were agitated and pH determined with a glass electrode after each period of incubation. Anaerobic conditions were then regenerated by flushing flasks with carbon dioxide.

Experiment 4

Ozone treated sorghum stover was extracted with distilled water (50 ml of distilled water per gram of stover) for 1 h at 39^{o} C. The suspension was then filtered through a buchner funnel with about 80% of the water extract recovered. This extract was then used to prepare McDougal's buffer solution, with the extract substituting for distilled water on an equal basis.

Untreated sorghum stover and prairie hay were ground through a 1 mm screen, and samples of 2 grams each were weighed into 250 ml glass centrifuge bottles. Duplicate sets of 10 bottles each were prepared for both untreated stover and prairie hay samples. One set of bottles for each roughage was innoculated with 100 ml of a solution of 30% rumen fluid and 70% McDougal's buffer. The other set was innoculated with 100 ml of a solution of 30% rumen fluid and 70% McDougal's buffer prepared from the extract of ozonated stover. Sample blanks were run for both types of innocula at each incubation time. The water extract was analyzed on a high pressure liquid chromatograph to determine if compounds were present which might be toxic or inhibitory to bacterial growth.

Results and Discussion

Average daily intakes and apparent digestibilities of treatments for experiment 1 are given in table 4. Average dry matter intakes in g per day for lambs on diets B, C, and D were not different ($P > .05$). Dry matter intake of lambs on treatment A was greater ($P < .05$) than intake on treatment B, but was not different ($P > .05$) than treatments C or D. Nitrogen intakes in g per day were not different ($P > .05$) for lambs on diets B, C, and D, but nitrogen intake for lambs on the control diet was significantly higher ($P < .05$) than nitrogen intake for lambs on all other diets. Gross energy intake in kcal per day was not different ($P > .05$) for lambs on diets B, C, and D. Gross energy consumption for lambs on diet A was higher ($P < .05$) than all other treatments.

The dry matter digestibility of diet D was greater ($P < .05$) than the control diet with no other differences ($P > .05$) in dry matter

TABLE 4. INTAKE AND APPARENT DIGESTIBILITIES-EXP. 1

Item	Treatment A Control	B Untreated stover	C Treated stover	D 50% treated + 50% untreated	SEM[a]
Intake					
Dry matter, g/d	913[b]	890[c]	904[b,c]	907[b,c]	3.63
Nitrogen	15.9[b]	15.3[c]	15.0[c]	15.3[c]	.09
Gross energy, kcal/d	3914[b]	3649[c]	3697[c]	3705[c]	15.72
Digestibility, %					
Dry matter	62.2[b]	63.9[b,c]	63.9[b,c]	64.6[c]	.43
Crude protein	41.4[b]	59.3[c]	57.3[c]	57.7[c]	1.08
Energy	60.3[b]	63.3[c]	64.0[c]	64.7[c]	.52
ADF	30.8[b]	32.0[b]	31.6[b]	33.8[b]	.92
Cellulose	46.4[b]	41.0[c]	45.3[b]	44.4[b,c]	.77
Lignin	-18.5[b]	17.3[c]	16.6[c]	14.2[c]	2.39

[a]Standard error of the mean

[b,c]Means in the same row with different superscripts differ ($P < .05$).

digestibility noted among treatments. Digestible protein and energy were not different (P >.05) among the stover diets (B, C and D), but all stover diets exhibited greater (P <.05) protein and energy digestibilities than the control diet. The lower dry matter, protein and energy digestibilities of the control diet appear to be in part the result of the greater (P <.05) intakes noted for lambs on the control diet.

There were no differences (P >.05) among treatments in the digestiblity of ADF. Cellulose digestibility of treatment B was not different (P >.05) than treatment D, but was lower (P <.05) than treatments A and C. Although ozone treatment results in a slight decrease in the amount of cellulose in stover (table 3), treatment appears to greatly increase cellulose availability. There were no differences (P >.05) among treatments B, C and D in lignin digestibility, however treatment A was lower (P <.05) than all other treatments. The large negative lignin digestibility of the control diet (cottonseed hulls) is consistent with previous reports on the lignin digestibility of hulls, where the permanganate lignin procedure is used to determine lignin content (Muntifering, et al., 1981).

The tendency of the 50/50 treatment (diet D) to be slightly higher in digestible ADF, energy, protein and dry matter than ozonated stover (diet C), could indicate that mixing treated and untreated stover influenced rate of passage of the diet. Mixing treated and untreated stover may also dilute any factor or compound which may have depressed digestiblity.

Experiment 2

The composition of untreated and ozonated stover is given in table 5. An increase (P <.05) in cell solubles was noted as a result of ozone treatment, which is consistent with the findings of other workers (Ben-Ghedalia and Miron, 1981). Cell walls, ADF, and lignin content were all significantly reduced (P <.05) by ozone treatment. Cellulose content of sorghum stover was only slightly reduced by ozonation, which coincides with results obtained with ozonated wheat straw (Ben-Ghedalia and Miron, 1981). Hemicellulose content of sorghum stover was not significantly reduced (P >.05) by ozone treatment.

TABLE 5. COMPOSITION OF UNTREATED AND OZONE TREATED SORGHUM STOVER-EXP. 2[a,b]

Constituent	Ozone treated sorghum stover	Untreated sorghum stover
Cell solubles	32.3 ± .5[c]	27.8 ± .3[d]
Cell walls	67.7 ± .5[c]	72.2 ± .3[d]
Hemicellulose	27.0 ± .5[c]	28.9 ± .5[c]
ADF	40.4 ± .2[c]	43.5 ± .5[d]
Cellulose	32.7 ± .3[c]	35.0 ± .3[c]
Lignin	6.2 ± .3[c]	7.4 ± .5[d]

[a]g/100 g dry matter

[b]Treatment means ± SE.

[c,d]Means in each row with different superscripts different ($P < .05$).

The in vitro dry matter digestibilities of untreated stover, ozonated stover, and a 50/50 mixture of treated and untreated stover are given in table 6. Digestibilities of treated stover and the 50/50 treatment were not different ($P > .05$) at 3 and 6 h of incubation, however, both treatments were greater ($P < .05$) than untreated stover. Ozonated stover had a higher ($P < .05$) digestibility than untreated stover at 12 h of incubation. The 50/50 treatment was not different from the other treatments at 12 h. No differences ($P > .05$) in digestibility were noted between treatments at 24 h of incubation. After 48 h of incubation, treated stover and the 50/50 treatment were not different ($P > .05$), but both treatments exhibited higher ($P < .05$) digestibilities than untreated stover.

In vitro cell wall disappearance for the three treatments is given in table 7. No differences ($P > .05$) among treatments in cell wall disappearance were noted at 3 and 6 h of incubation. The treated and 50/50 treatment were not different ($P > .05$) at 12 h of incubation, but both treatments were greater than untreated stover at 12 h. Cell wall disappearance was greater ($P < .05$) for ozone treated stover than for all other treatments at 24 and 48 h of incubation.

In vitro hemicellulose digestibilities for treatments are given in table 8. No differences ($P > .05$) were noted among treatments at any incubation time, largely due to the large degree of error encountered with hemicellulose determination. It is difficult to determine from these data if ozone had any effect on the hemicellulose digestion rate.

In vitro ADF disappearance was not different ($P > .05$) among treatments for 3, 6, and 12 h of incubation (table 9). At 24 h of incubation, ozone treated stover was higher ($P < .05$) in ADF digestibility than untreated stover, but was not different ($P > .05$) from the 50/50 treatment. Ozonated stover and the 50/50 treatment were not different ($P > .05$) at 48 h of incubation, but both treatments were greater ($P < .05$) than untreated stover.

In vitro cellulose digestion exhibited a slightly different pattern of disappearances than did other fiber components for the three treatments (table 10). The 50/50 treatment had the greatest ($P < .05$) cellulose disappearance after 3 h of incubation. Treated

TABLE 6. TOTAL IN VITRO DRY MATTER DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	11.03 ± .49[b]	14.62 ± 1.21[c]	13.69 ± .90[c]
6	15.24 ± .66[b]	18.69 ± .69[c]	17.69 ± .72[c]
12	20.99 ± .82[b]	25.57 ± .48[c]	23.37 ± 1.40[b,c]
24	37.08 ± 1.26[b]	39.63 ± .71[b]	38.24 ± .54[b]
48	44.62 ± .75[b]	50.33 ± .59[c]	49.48 ± .38[c]

[a]g/100 g original dry matter.

[b,c]Means in each row with different superscripts differ ($P < .05$)

TABLE 7. TOTAL IN VITRO CELL WALL DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	9.19 ± .45[b]	11.91 ± .09[b]	10.59 ± .35[b]
6	12.84 ± .64[b]	12.87 ± 1.40[b]	14.19 ± .96[b]
12	17.84 ± .07[b]	19.53 ± .02[c]	19.74 ± .08[c]
24	37.67 ± .06[b]	45.23 ± .30[c]	42.75 ± .25[d]
48	39.36 ± .29[b]	47.30 ± .35[c]	44.71 ± .20[d]

[a]g/100 g original cell walls.

[b,c,d]Means in each row with different superscripts differ ($P < .05$).

TABLE 8. TOTAL IN VITRO HEMICELLULOSE DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a,b]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	9.29 ± 1.30	15.02 ± 1.40	14.99 ± .90
6	12.28 ± 1.50	14.87 ± 1.40	16.00 ± 2.00
12	15.60 ± 1.27	14.52 ± 1.00	15.00 ± 1.20
24	38.26 ± 1.10	41.36 ± .75	40.94 ± .98
48	45.18 ± 2.33	51.97 ± 1.98	44.45 ± 1.52

[a]g/100 g original hemicellulose.

[b]There were no differences between treatment means ($P < .05$).

TABLE 9. TOTAL IN VITRO ACID DETERGENT FIBER DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	11.82 ± 2.00[b]	13.62 ± 1.20[b]	13.03 ± 1.30[b]
6	13.89 ± 2.10[b]	17.01 ± 1.70[b]	16.79 ± 1.40[b]
12	17.84 ± .95[b]	20.06 ± .83[b]	19.89 ± 1.20[b]
24	36.18 ± 1.20[b]	42.02 ± .45[c]	40.37 ± .85[b,c]
48	36.76 ± 1.60[b]	43.81 ± 1.74[c]	44.85 ± .81[c]

[a]g/100 g original ADF

[b,c]Means in each row with different superscripts differ ($P < .05$).

TABLE 10. TOTAL IN VITRO CELLULOSE DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	13.76 ± 1.00[b]	7.44 ± .94[b]	16.99 ± .19[c]
6	11.36 ± .18[b]	18.04 ± .91[c]	18.87 ± .11[c]
12	16.84 ± 1.50[b]	29.13 ± .94[c]	20.89 ± .73[b]
24	38.74 ± 1.26[b]	50.78 ± .33[c]	44.96 ± .72[d]
48	45.09 ± .59[b]	56.66 ± .55[c]	50.69 ± .47[d]

[a]g/100 g original cellulose.

[b,c,d]Means in each row with different superscripts differ ($P < .05$).

stover and the 50/50 treatment were not different ($P < .05$) after 6 h of incubation, although cellulose disappearance was greater ($P < .05$) for both treatments than for untreated stover after 6 h. Ozone treated stover had the greatest ($P < .05$) cellulose disappearance after 12, 24, and 48 h of incubation. The 50/50 treatment was not different ($P < .05$) than untreated stover at 12 h of incubation, but was greater ($P < .05$) at 24 and 48 h of incubation. It appears from the data that ozone treatment causes a marked increase in the rate of cellulose disappearance, which indicates that ozone could be significantly opening up the fiber structure. In vitro data for cellulose digestibility closely parallel in vivo findings. The effect of ozone on stover cellulose is consistent with the results of Ben-Ghedalia, et al.(1980) with ozonated cotton straw.

Values for in vitro lignin digestibility are given in table 11. There were no differences in lignin disappearance among treatments at any incubation time. In vitro lignin digestibilities were highly variable, consequently no conclusions can be drawn from the data.

Experiment 3

Ozonation of roughages is known to generate a large number of organic acids which lower the pH of suspensions containing ozonated roughages (Ben-Ghedalia and Miron, 1981). The hydrogen ion concentration (pH) of in vitro incubations of both treated and untreated stover were monitored to determine if ozone treatment altered the pH of the in vitro environment (table 12). Both buffered and unbuffered incubations were used. In a standard, buffered in vitro, pH was slightly depressed ($P < .05$) after 1 h of incubation with ozone treated stover. There were no detectable differences ($P > .05$) at other incubation times. In an unbuffered in vitro (rumen fluid and water), the pH was lower ($P < .05$) for ozonated stover incubations at the initial incubation time ($t=0$), and continued to be lower for 3 h. Treated stover was not significantly different ($P > .05$) from untreated stover from 4 to 24 h of incubation. The slight drop in pH in both buffered and unbuffered systems may be the result of rapid fermentation of readily available carbohydrates which are known to be a result of ozonation of roughages (Ben-Ghedalia and Miron, 1981),

TABLE 11. TOTAL IN VITRO LIGNIN DIGESTION OF TREATED AND UNTREATED SORGHUM STOVER-EXP. 2[a,b]

Incubation time, h	Treatment		
	Untreated	Ozone treated	50% treated + 50% untreated
3	9.29 ± 1.30	15.02 ± 1.40	14.99 ± .90
6	12.28 ± 1.50	14.87 ± 1.40	16.00 ± 2.00
12	15.60 + 1.27	14.52 + 1.00	15.00 + 1.20
24	38.26 ± 1.10	41.36 ± .75	40.94 ± .98
48	45.18 ± 2.33	51.97 ± 1.98	44.45 ± 1.52

[a]g/100 g original lignin.

[b]There were no differences between treatment means ($P < .05$).

TABLE 12. EFFECT OF OZONE TREATMENT OF SORGHUM STOVER ON THE pH OF BUFFERED[a] AND UNBUFFERED[b] IN VITRO SYSTEMS-EXP. 3

Incubation time, h	Treatment			
	Buffered		Unbuffered	
	Untreated stover	Ozonated stover	Untreated stover	Ozonated stover
	pH[c]			
0	6.96[d]	6.97[d]	6.32[d]	6.24[e]
1	7.00[d]	6.91[e]	6.25[d]	6.05[e]
2	6.82[d]	6.80[d]	6.24[d]	6.09[e]
3	6.90[d]	6.85[d]	6.27[d]	6.15[e]
4	6.94[d]	6.92[d]	6.38[d]	6.34[d]
5	6.99[d]	6.98[d]	6.55[d]	6.51[d]
6	6.92[d]	6.93[d]	6.53[d]	6.52[d]
12	6.90[d]	6.88[d]	6.44[d]	6.42[d]
24	6.81[d]	6.82[d]	6.38[d]	6.36[d]

[a]In vitro buffered using McDougal's buffer solution.

[b]Distilled water substituted for buffer solution.

[c]Treatment means, standard errors and statistical differences determined from hydrogen ion concentrations.

[d]Means within same row and treatment with different superscripts differ ($P < .05$).

and does not appear to be great enough to significantly alter fermentation.

Experiment 4

Samples of prairie hay and untreated sorghum stover were innoculated with water extracts of ozonated stover and incubated with rumen microbes. In vitro dry matter digestibilities of controls and controls innoculated with extracts are given in table 13. The in vitro dry matter disappearance of untreated sorghum stover was depressed 14% after 24 h of incubation by the addition of the extract. However, at 48 h of incubation, stover controls were not different ($P > .05$) from extract innoculated controls. The in vitro dry matter disappearance of prairie hay was depressed 4% after 24 h of incubation by extract addition. This was reduced to 2% at 48 h of incubation. The cause of the depression in digestibility after 24 h of incubation is unknown, however this observation parallels the apparent depression in dry matter digestibility of ozone treated stover after 24 h of incubation (table 6). Ozonation of roughages may produce small quantities of organic products which are slightly inhibitory to bacterial growth. However, high pressure liquid chromatography of the water extract did not reveal detectable concentrations of products known to be antibacterial.

Based on in vitro digestibilities, it would appear that some mixture of treated and untreated stover might be more economical than ozonated stover alone. However, it is unclear from in vivo data what practical implications ozonation will have for roughage treatment. These data also indicate that ozonated stover could contain products which might inhibit bacterial growth, and more research is needed to determine what products are created, and the levels at which they become inhibitory.

TABLE 13. EFFECT OF OZONATED STOVER EXTRACT ON THE IN VITRO DIGESTIBILITY OF SORGHUM STOVER AND PRAIRIE HAY-EXP. 4

Roughage	Incubation time, h	Treatment	
		Control	Control + extract
Sorghum stover	24	37.1 ± 1.3[a]	32.0 ± .06[b]
Sorghum stover	48	43.2 ± .1[a]	43.6 ± 1.04[a]
Prairie hay	24	54.6 ± .2[a]	52.7 ± .34[b]
Prairie hay	48	63.7 ± .1[a]	62.5 ± .26[b]

[a,b]Means in the same row with different superscripts differ ($P < .05$).

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LIST OF APPENDICES

APPENDIX A. SPECTROPHOTOMETRIC DETERMINATION OF REDUCING SUGARS IN ROUGHAGES

Procedure

1. Weigh 350 to 500 mg. of each sample and place in 20 ml centrifuge tubes with screw caps. Add 10 ml of a 10% methanol solution to each tube, and extract samples in a shaker bath at 60^{o} C for 1 h. After extraction, centrifuge tubes at 400 X G, and filter supernatant to remove impurities. Save extract for determination of reducing sugars.
2. Preparation of D. N. S. reagent. Dissolve 5 g of 3-5 dinitrosalicyclic acid in 100 mls of water (heating may be necessary), and add 100 mls of 2N NaOH. Dissolve 150 g of potassium sodium tartrate in this solution, and make up to a final volume of 500 ml.
3. Preparation of standard curve. From a 1% dextrose solution, make the following subdilutions:

   10 mls to 100 (c = 100 mg/100 ml)
   5 mls to 100 (c = 100 mg/100 ml)
   2.5 mls to 100 (c = 100 mg/100 ml)

   Use 2 ml of each dilution for standard curve preparation.
4. Place 2.0 ml of extract in a 30 ml test tube (with screw cap) and add 2.0 ml of D. N. S. reagent. Additionally, add 2.0 ml of D. N. S. reagent to standard tubes and 2.0 ml to a water blank. Place all tubes in a boiling water bath and hold for five minutes.
5. Cool tubes to room temperature under running water. Add 15 mls of distilled water to each tube and mix well. Read absorbance on a spectrophotometer adjusted to a wavelength of 540 mu, using the water blank to zero the spectrophotometer.

APPENDIX B. EFFECT OF OZONE TREATMENT OF SORGHUM STOVER ON THE HYDROGEN ION CONCENTRATION IN A BUFFERED[a] IN VITRO SYSTEM-EXP. 3

Incubation time, h	Treatment	
	Untreated stover	Ozone treated stover
0	1.10×10^{-7}	1.07×10^{-7}
1	9.92×10^{-8}	1.24×10^{-7}
2	1.52×10^{-7}	1.58×10^{-7}
3	1.27×10^{-7}	1.41×10^{-7}
4	1.16×10^{-7}	1.20×10^{-7}
5	1.03×10^{-7}	1.06×10^{-7}
6	1.19×10^{-7}	1.17×10^{-7}
12	1.23×10^{-7}	1.31×10^{-7}
24	1.57×10^{-7}	1.51×10^{-7}

[a]In vitro buffered using McDougal's buffer solution.

APPENDIX C. EFFECT OF OZONE TREATMENT OF SORGHUM STOVER ON THE HYDROGEN ION CONCENTRATION IN AN UNBUFFERED[a] IN VITRO SYSTEM-EXP. 3

Incubation time, h	Treatment	
	Untreated stover	Ozone treated stover
0	4.76×10^{-7}	5.76×10^{-7}
1	5.63×10^{-7}	8.96×10^{-7}
2	5.79×10^{-7}	8.09×10^{-7}
3	5.42×10^{-7}	7.09×10^{-7}
4	4.15×10^{-7}	4.60×10^{-7}
5	2.80×10^{-7}	3.00×10^{-7}
6	2.94×10^{-7}	3.13×10^{-7}
12	3.64×10^{-7}	3.80×10^{-7}
24	4.17×10^{-7}	4.23×10^{-7}

[a]Distilled water substituted for McDougal's buffer solution.