NUTRITIVE VALUE IMPROVEMENT OF LOW QUALITY FORAGES BY SOME CHEMICAL AND PHYSICAL TREATMENTS

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ABSTRACT

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CHEMICAL AND PHYSICAL
TREATMENTS

By

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The effectiveness of sixteen chemicals, six enzymes, and one physical treatments in improving nutritive value of straws and alfalfa stems was evaluated. The effect of some treatment conditions was also examined. Evaluation techniques were primarily based on resulting chemical composition and *in vitro* fermentations. An animal feeding trial was conducted in order to evaluate the acceptability and digestibility of treated wheat straw.

Sodium chlorite treatment reduced the lignin content in forages and improved both *in vitro* and *in vivo* dry matter digestibility (DMD). Sodium hydroxide treatment only slightly changed chemical composition but markedly increased *in vitro* DMD and digestible cell wall (DCW) of straw. No significant improvement *in vitro* DMD and DCW was noted for sodium hydroxide treated alfalfa stems. Cellulases and pectinases were effective in improving

nutritive value of alfalfa stems but not of oat straw. Chlorine and high energy irradiation treatments solubilized large amounts of cell wall and hemicellulose. These treatments increased forages dry matter solubility but depressed *in vitro* CW digestion. The largest improvement of total *in vitro* cell wall digestion was found for sodium hydroxide or irradiation treatments and the least improvement was noted for organic chlorine, sodium hypochlorite (NaClO) and calcium hypochlorite [$Ca(ClO)_2$] treatments.

Straws treated with chlorine compounds showed a very low acceptability to goats when fed wet and fresh. Washing the treated silages increased intake to a level comparable with that of control straw. However, washing decreased organic matter recovery, dry matter solubility and *in vitro* DMD indicating that soluble nutrients were lost during washing.

Two goats died after being fed sodium chlorite treated straw for about 30 days. No evident abnormality was observed in major organs. The cause of death was possibly due to longterm undernutrition. The *in vitro* DMD of standard alfalfa was decreased to some extent by addition of a silage extract from sodium chlorite treated straw.

The two-fold increase of total *in vitro* cell wall disappearance for sodium hydroxide or high energy

irradiation treated straw indicated that straw contains entrapped nutrients which, if properly liberated, could be solubilized and potentially useful to rumen micro-organisms.

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INTRODUCTION

The world population explosion and possibilities of world-wide famine have recently been subjects of popular concern. Adding to the seriousness of the situation is the fact that domestic animals will eventually compete with humans for cereal grains and other foodstuffs. A higher proportion or all the cereal grains produced may eventually be used directly by man.

Ruminants are adapted, physiologically and anatomically to digest roughages. These animals derive their energy, carbon and nitrogen from grains and forages. With the increasing use of non-protein nitrogen, the importance of plant nitrogen in feeds may diminish. The important function of the plant derived feed component may then be a source of energy and carbon.

Wood and straws contain mainly free cellulose as well as cellulose physically or chemically associated with lignin and pentosans. Reasonable extrapolation of existing information indicates that ruminants and with their rumen microflora could utilize these substances and convert them to a high quality food suitable for human consumption. However, under normal conditions,

only a minor percentage of the carbohydrates in wood, straw and similar roughages can be utilized by the rumen microbial population without some form of pretreatment. The high lignin content and the low cellular contents are the controlling factors in this low degree of digestion of these carbohydrate polymers. The inability of ruminants to utilize fully the carbohydrate portion of wood and straw has led investigators to study the effect of chemical or physical treatments of wood and straw to increase their digestibility.

This study was conducted to evaluate a number of physical and chemical treatments designed to increase the digestibility and intake of straw and alfalfa stems.

LITERATURE REVIEW

I. Classification of Plant Cell Constituents

In general, chemical constituents of plants may be divided into the structural components of the cell wall (lignin, cellulose, hemicellulose and pectins) and more soluble cellular contents (sugars, starch, fructosans, organic acids, lipids, and the nitrogenous fractions consisting of about two-thirds protein and a third non-protein nitrogen compounds). Cellular contents often exceed 50% of the dry matter of forages and represent material having a very high nutritive availability (Van Soest, 1969a).

Lignocellulose is the complex of lignin, cellulose and probably hemicellulose existing in close physical and/or chemical association and accounts for most of the cell wall constituents of plants. As a proportion of the total dry matter it varies widely in forages, depending on species and stage of maturity.

Lignin is an aromatic polymer which varies in content from about 2% in immature forage up to 15% in mature forages; in wood the percentage is somewhat higher. Its main function is to supply strength and rigidity to

plant materials, but its nutritional significance lies in its indigestible nature. It acts not only as an inert diluent in feedstuffs but because of its close physical/chemical association with the cell wall polysaccharides, it frequently acts as a physical barrier and impedes the microbiological breakdown of these compounds (Pigden and Heaney, 1969). The type or structure of lignin rather than quantity of lignin may be of major importance; for example, alfalfa has a higher lignin content than grasses of a similar digestibility (Dehority and Johnson, 1961).

Cellulose is usually the most abundant polysaccharide of the cell wall constituents and the most insoluble. It is a polymer of glucose units and the degree of polymerization varies within and between sources of cellulose (Timell, 1964). Cellulose is degraded by rumen microflora to a variable degree ranging from about 25 to 90%. Purified celluloses from different plant sources differ in _in vitro_ digestion rate (Baker _et al._, 1959; Tomlin and Davis, 1959). Although a relationship between crystallinity and digestion rate was reported (Baker _et al._, 1959), Van Soest (1969b) concluded that crystallinity has not been significantly related to digestibility. The proportion of cellulose to lignin and hemicellulose varies widely. Thus, cellulose may not be quantitatively representative of the fiber constituents of forage (Van Soest, 1969a).

Hemicelluloses are amorphous polysaccharides which include short chain glucose polymers, polymers of xylose, arabinose, mannose and galactose plus mixed sugars and uronic acid polymers (Roelofsen, 1959). Xylan is usually the main hemicellulose in forages. Hemicelluloses exist in close association with the cellulose and lignin and are generally separated from the cellulose by extraction with dilute alkali and acid (Jarrige, 1960; Waite et al., 1964). They vary widely in content from one type of plant material to another with a range of about 6 to 40% (Pidgen and Heaney, 1969). Differences exist in extent and rate of individual sugar digestibility by ruminants. Xylose is less digestible than arabinose (Jarrige, 1960; Gaillard, 1962; Lyford et al., 1963). Uronic acid seemed to have the lowest digestibility of all and should possibly be considered as having a negative relationship to nutritive value (Gaillard, 1966).

With respect of the role of forage carbohydrates in ruminant nutrition, the presence and amount of a particular carbohydrate in the forage is probably of less importance than total digestibility of the carbohydrates. The quantity of the cell wall constituents and degree of lignification are of greater significance than the quantity of more digestible cellular constituents (Sullivan, 1969).

II. Factors Affecting the Nutritive Values of Forages

A general review on factors affecting the nutritive value of forages appears desirable as this study deals with improvement of nutritive value of low-quality forages.

A. Primary Factors

1. Species variation

Although rumen microflora of ruminants can utilize forages as an energy source, a wide variation in the rate or extent of utilization among different plant species has long been apparent. Analysis of chemical composition as well as several *in vitro* evaluation methods sometimes can supply valuable information concerning the forage. Most species of plants used as forages fall into the grass and legume families although under range conditions a wide spectrum is often found. Sullivan (1966) and Van Soest (1966a) have both discussed the digestion of the different proportions of lignin, hemicellulose and cellulose in grass and legume cell walls, and their implications. These differences must have a genetic origin. Grasses possess low ratios of lignin to cellulose that increase markedly as the plant matures (Mowat *et al.*, 1965; Van Soest, 1964). Legumes possess high ratios even at immature stages (Archibald *et al.*, 1962; Maymone, 1962). The ratio of hemicellulose to cellulose is much

higher in the grasses than in the legumes, and is relatively constant for a plant species at any stage of maturity (Burdick and Sullivan, 1963; Sullivan, 1966).

For illustrative and comparative purposes, the chemical composition and *in vitro* dry matter digestibility (IVDMD) of selected plant materials are given in Table 1.

Table 1. The Chemical Composition and *In Vitro* Dry Matter Digestibility of Selected Plant Materials.

Plant Species	CWC[3]	ADF[4]	ADL[5]	Cellulose	Hemicellulose	IVDMD[6]	Ref.
	% DM						
Timothy (inmature)	54	30	2.1	26	24	--	1
Corn cobs	79	42	5.7	36	37	62	2
Orchardgrass	58	37	5.9	31	21	86	2
Alfalfa	52	36	11.1	24	16	74	1
Wheat straw	85	59	11.8	47	26	53	2
Alfalfa stems	68	56	16.8	39	12	58	2
White oak sawdust	91	68	21.7	46	23	17	1

[1]Gard *et al.*, 1968.

[2]Goering and Van Soest, 1968.

[3]Cell wall constituents (Neutral detergent fiber).

[4]Acid detergent fiber (lignocellulose).

[5]Acid detergent lignin.

[6]*In vitro* dry matter digestibility.

Even within a given species considerable variation occurs (Alston, 1963; Wardrop, 1958; Stafford, 1952; Allinson, Elliott and Tesar, 1969). Pigden and Heinrich (1957) analyzed the lignin content of wheat grass and found a significant difference between clones for both leaf and stem. Van Soest and Wine (1967) reported that the lignin fractions of grasses and legumes were different, grass lignin was more soluble and more easily removed than was legume lignin. While McCampbell (1969) concluded that the chemical makeup of the lignin molecules of legumes was different from the lignin of grasses. Allinson (1966) found different ultraviolet spectra for alfalfa clones of high and low nutritive value which indicated the possibility of genetic variation for the cell-wall constituent in the Medicago species. Nevins et al. (1967) also found significant differences in the cell-walls of different species within a given genus.

Dehority and Johnson (1961), Van Soest (1963) and many others have found alfalfa to have a higher lignin content than does a grass of equal digestibility. Van Soest speculated that alfalfa contains a smaller but more highly lignified holocellulose fraction that was considerably less digestible. While Sullivan (1966) concluded that in alfalfa, lignin affected the digestion of hemicellulose more than it influenced the digestion of cellulose.

Tomlin et al. (1965) reported that lignification was linearly but negatively related to cellulose digestibility as a grass matured; however, this relationship did not exist for alfalfa. The same type of phenomena was also reported by Jarrige and Minson (1964), Sullivan (1959), (1964), and Van Soest (1964).

2. The stage of maturity

In general, the forage chemical composition changes as a plant matures. The cellular contents (proteins and soluble carbohydrates) decrease in amount while the cell wall constituents increase, especially in lignin and cellulose (Phillips et al., 1939; Patton and Gieseker, 1942; Patton, 1943; Gaillard, 1962). Qualitative changes in the cell wall complex may also occur (Allinson, 1966). As a result of forage composition change, nutritive value declines with maturity (Baumgardt and Smith, 1962). Most plant physiologists refer to maturation as lignification, which very probably occurs in most forages although wide species difference in responses may be observed.

Lignification is not a sudden process. The large decrease in digestibility following seed formation may be associated with only a small increase in lignin content. This change, however, is very closely related to and may vitally affect energy availability. Stafford (1965) indicated that proteins were degraded with

approaching maturity releasing amino acids of which tyrosine and phenylalanine were lignin precursors. Goodman and Siegel (1959) postulated the lignification was a function of the quantity of cellulose in the cell wall. The hypothetical mechanisms for lignification have been discussed by Freudenberg (1965).

Forage cell wall availability decreases as maturity increases. Dehority and Johnson (1961) and Dehority et al. (1962) indicated that availability of cellulose, hemicellulose and pectins in forages were all negatively affected by lignification. Bolkner (1963) demonstrated the existence of a chemical bond between wood carbohydrates and lignin; xylan may be the component involved. This could explain the depression in digestibility of xylans in grasses as they mature (Pigden and Heaney, 1969). Similar speculation for alfalfa lignification was reported by Sullivan (1966).

The manner in which lignin decreases digestibility is not completely understood (Van Soest, 1963). A number of theoretical possibilities exist such as (a) encrustation of cell components by lignin (Crampton and Maynard, 1938; Dehority et al., 1962); (b) formation of a lignin-carbohydrate compound in plant cell wall material; or (c) formation of a molecular complex due to hydrogen bonding or other attractive forces between lignin-cellulose molecules (Davis et al., 1964).

B. Secondary Factors

1. Environment and cultural practices

Nutritive value of regrowth (following 1st cutting) was generally neither as high at early stages nor as low at later stages compared to the first growth. This was especially true for bromegrass which remained in a vegetative condition (Baumgardt and Smith, 1962).

Ingalls et al. (1965) observed little difference in dry matter intake of first- and second-cut forages but first-cut forages had a higher dry matter digestibility than did second-cut forages.

Baumgardt and Smith (1962) showed that delaying harvests decreased digestibility much less with second and subsequent crops than with the first crop. Days elapsed between first and second cutting was the determining factor.

Norman (1939) and Sullivan et al. (1956) agree that structural constituents are highest in second cuttings. Such growth usually occurs in a period typified by long days. Cellulose and fiber decrease thereafter while lignin decreases only in the fall.

Reid et al. (1959) showed that animals consumed more early first-cut than late-cut forage. Leaf content of first-cut forage was an excellent indicator of forage energy value but leaf content of regrowth was a poor indicator.

Environmental changes are important in producing compositional changes. The principal factors influencing changes in plant composition with time are light, temperature, and fertilization (Alberda, 1965; Blaser, 1964; Deinum, 1966). Changes in nutritive value are generally less marked than compositional changes because of compensatory effects (Van Soest, 1969a). Considerable differences between plant species in the extent and nature of the responses to environmental factors were noted. Meyer et al. (1960) reported the nutritive value of the alfalfa was not greatly influenced by season in that the overall comparison of alfalfa harvested at the same stage of maturity in May and August showed only small differences. Similar results were reported by Meyer et al. (1957) with oat hay. However, Deinum (1966, 1967, 1968) has demonstrated consistent detrimental effects of higher temperatures on nutritive values in both temperate and tropical grass species.

Generally, light intensity had a positive effect on the water-soluble carbohydrates and a negative one on crude protein, ash and fiber components. The net result was a positive relationship between light intensity and nutritive value (Deinum, 1966). Higher temperature decreased water-soluble carbohydrates and increased lignin and cell-wall content resulting in a decline in nutritive quality at increased temperature. The negative

effect of temperature far outweighed the positive effect of light (Deinum, 1968; Brown, 1943), so that a general decline in nutritive value was associated with increasing light and temperature in the spring of the year. A declining temperature might cause compositional change to retrace that of spring since cell-wall components were relatively fixed and a slowed rate of growth in the fall would not rapidly dilute plant tissue already formed. In the spring growth rate increased as the temperature increased (Van Soest, 1969a).

The effects of nitrogen fertilization rate on overall forage nutritive value are controversial. A number of people reported that forage digestibility was unaffected by high-rates of nitrogen fertilization (Reid et al., 1959; Washko and Marriott, 1960; Hart and Burton, 1965; Webster et al., 1965; Allinson, Tesar and Thomas, 1969). Others reported that nitrogen fertilization resulted in increased protein and lignin content of sudan grasses (Reid et al., 1962). Increasing rates of nitrogen fertilization also tended to decrease cell-wall contents during intermediate stages of growth, so that digestibility was not consistently affected. However, at the latest stage of regrowth the increased lignification outweighed other effects and nitrogen fertilization resulted in a depression of digestibility (Van Soest, 1969a).

The general variation of composition effects between lignification, cell-wall, soluble carbohydrates and protein are some reasons for the difficulty of finding consistent effects of environmental changes that allow good prediction of nutritive value (Van Soest, 1969a).

2. Silica in forages

Another important factor influencing forage nutritive value in many species of grasses is silica which is metabolized and largely deposited in the cell wall with cellulose (Jones et al., 1963; Yoshida et al., 1962).

Silica content of grasses is related both to species and to the availability of silica in the soils in which the plants are grown (Jones and Handreck, 1967). Legumes species do not seem to metabolize significant amounts of silica.

Yoshida et al. (1962) speculated that the role of silica in plant physiological functions is mainly to increase the resistance of the plant to pathogens and to reduce water loss in transpiration.

Van Soest (1967) believes that silica depresses the digestibility of the cellulosic carbohydrates in a manner similar to that of lignin. The seriousness of silica in depressing forage digestibility was clearly demonstrated by comparing in vivo digestibility with silica or lignin content of reed canary grass collected

from four states. The correlation coefficient was -0.86 for silica but only -0.58 for lignin (Van Soest, 1969a).

3. Other factors

Water-soluble antimetabolic compounds in alfalfa depressed fermentation through direct inhibition of the cellulose-fermenting bacteria. The amount of these inhibitors varied among clones and had a reasonable heritalibility so that genetic changes could be made (Schillinger, 1964; Elliott, 1963). Forage plants contain tannins and other substances which affect nutritive value (Burns, 1963; Smart et al., 1961). Miller et al. (1967) and Bechtel et al. (1945) reported that the nutritive value of baled hay decreased when stored with a high moisture content. They found that this depression was primarily due to the positive relationship between moisture level and temperature. The higher the temperature the greater the oxidation of nutrients with a proportional increase in the fiber content and a consequent decrease of the nutritive value in the final product. Unexpected rainfall can also solubilize and elute nutrients resulting in a higher percent fiber and a lower digestibility. Van Soest (1964) reported that wet forage samples heated above 50 C during drying increased their apparent lignin content by as much as 300%, and he referred to this increased lignin as

"artifact lignin." He also found that the moisture level was critical in this type of non-enzymatic browning reaction.

III. Composition and Digestibility

Most animal nutritionists believe that there are unseparable and delicate interrelationships among forage chemical composition, voluntary intake and digestibility (Blaxter, 1960, Crampton et al. 1960). However, Van Soest (1965) on the other hand, suggested that voluntary intake and digestibility are not necessarily related, and may be independent. Consequently, measures of digestibility are not necessarily adequate predictors of intake.

The tendency for changes in forage composition to be highly associated with increasing maturity, during which digestibility declines, causes almost all components to be significantly correlated with digestibility (Van Soest, 1964, 1965). However, a primary factor like lignin will not be consistently related to indigestibility of plant fractions because other factors are also operative. The relationship between lignin and digestibility varies with each type of plant (Jarrige, 1964; Sullivan, 1959, 1964; Burdick and Sullivan, 1963). By using Lucas's analysis, Van Soest (1969a) found that there is a variable fraction in forages which is free from lignification and that this fraction forms a

smaller proportion of the dry matter in grasses than it does in legumes. The lignified fraction corresponds to the plant cell wall, which contains quantitatively the indigestible fraction of the forage.

Van Soest (1967) strongly rejected the conclusion of Drapala et al. (1947) that availability of cellular contents is lowered by entrapment in lignified cells, and stressed that the effects of lignin are restricted to the cellulose and hemicellulose carbohydrates of the plant cell wall.

Van Soest (1969a) criticized most digestibility prediction equations proposed by several nutritionists. Many equations that showed promise based on the data from which they were derived performed poorly when applied to a new population of forages. He pointed out that this situation was primarily due to the failure to identify primary factors and further suggested that a complex Lucas test should be used in identification of primary factors. These primary factors should then be more dependable predictors than those secondarily related in the cause-effect sequence.

An instructive comparison is that between lignin (a primary factor) and protein (a secondary factor). Within a plant species an increase in lignin content almost invariably results in a decrease in digestibility (Sullivan, 1959; 1964). In the case of protein, nitrogen

fertilization will increase the plant protein content without inducing an accompanying increase in digestibility. One of the reasons for this is that nitrogen fertilization also promotes formation of lignin in the plant (Deinum, 1968). Lignin is a primary factor limiting availability of certain fractions, but protein is positively associated with digestibility mainly because it decreases as lignin increases in the maturing plant.

IV. Voluntary Intake

"Food intake is both innate and basic to survival. Production and the control mechanisms should therefore be fully understood" (Moir, 1970). However, precise understanding of intake control mechanisms has not been adequately achieved (Baumgardt, 1970). Recently, a number of people agreed with the concept that physical and chemical feedback systems that result in modification of hypothalamic activity appear dominant as physiological mechanisms (Campling, 1970; Baumgardt, 1970; Baile and Mayer, 1970). However, Arnold (1970) reported that the free-living and grazing animal has, in addition, a social and physical environment to cope with and that the directives of the internal physiological mechanisms are modified by interplay with the special senses with individual and group behavior, as well as with weather, climate, and management.

Voluntary intake of food is also regulated by the ingested food itself, by the absorbed nutrients, their level, or their metabolic products (Baumgardt, 1970). With bulky foods intake may be primarily limited by the physical capacity of the reticulorumen and by the degree to which emptying of the rumen is restricted, particularly in young animals (Campling, 1970). The so-called physical regulation or distension factors cease to determine intake when the digestibility of the fodder is equal to or above 65-70 percent (Conrad, 1966). Van Soest (1966) had proposed that cell wall content is correlated to forage intake when the cell wall content is above 55-60 percent. Below that value, intake is limited by other factors. This concept agrees with that of Conrad's since cell wall content is significantly, negatively correlated with digestibility (Van Soest, 1965).

With intensive feeding using highly digestible concentrate diets, chemostatic mechanisms are perhaps dominant in short-term feed regulation (Baumgardt, 1970). Baile and Mayer (1970) considered that neither glucostatic nor thermostatic regulation mechanisms are sufficiently effective, but that volatile fatty acids, which as products of digestion, have characteristics that make them potential factors in control of feeding in ruminants. They found that changes in butyrate concentration in the rumen or in systemic circulation had

little effect, but that increased concentrations of either acetate or propionate resulting from intraruminal injection led to a cessation of eating. They also speculated that sensors sensitive to changes in acetate and propionate concentration may exist in the dorsal rumen. Propionate sensors may also exist within the portal system. In a more practical aspect, Baumgardt (1970) suggested that with more concentrated rations, intake may be appropriately related to the 3/4 exponent of body weight, and that intake is voluntarily regulated according to physiological demand. Based on a range of experimental data, he proposed that intake in growing animals is regulated at a level between 200 and 300 Kcal digestible energy (DE)/body weight $kg^{3/4}$, depending on growth intensity and fattening rate, and between 350 and 500 for lactating cows. These intake levels can be maintained on diets above 2.5 Kcal DE/g.

An overall view of voluntary intake of forages as related to cell wall content, lignin and digestibility was given by Van Soest (1969a). He concluded that forages with very high cell wall volumes (grasses) have low lignin contents, thus promoting relatively higher digestibility with a low intake while legumes present the opposite situation.

V. Cellulose and Cellulase Complex

The ability of cellulolytic microorganisms and of cell free cellulolytic enzymes to degrade cellulose vary greatly with the nature of the substrate. For example, the cellulose in untreated wood is virtually indigestible to ruminants whereas after delignification the cellulose is rapidly and completely digested (Stone et al., 1969). Thus, understanding the structural features of cellulose and its associated substances is important as are features which determine the susceptibility and resistance of cellulose fibers to enzymatic hydrolysis.

A. Structure of Cellulose Fibers

Cellulose exists in various states of purity in plant cell walls. It makes up about 90% of cotton fibers but only about 45% of typical wood cell walls. The cellulose in cotton and wood is very similar in molecular structure. But the two types of cells differ both in gross structure and in the nature and amounts of substances with which the cellulose is associated (Cowling and Brown, 1969).

Both cotton and wood fibers have a thin primary wall that consists of a loose, random fibrillar network and surrounds the relatively thick secondary wall. The secondary wall usually consists of three layers designated S-1, S-2 and S-3. The S-1 and S-3 layers usually are very thin; the S-2 layer is of variable thickness but

usually forms the bulk of the cell wall substance. With each layer of the secondary wall, the cellulose and other cell wall constituents are aggregated into long slender bundles called microfibrils. Within each microfibril, the linear molecules of cellulose are bound laterally by hydrogen bonds and are associate in various degree of parallelism-regions that contain highly oriented moleules. These areas are called crystalline whereas those of lesser order are called paracrystalline or amorphous regions (Cowling and Brown, 1969).

B. Chemical Constituents of Cellulose Fibers

The chemical constituents of wood and cotton fibers include cellulose, several hemicelluloses, lignin, a wide variety of extraneous materials including certain nitrogenous substances, and a small amount of inorganic matter. The chemical properties of cellulose, hemicellulose and lignin have been reviewed in the first part of the text. The extraneous materials are a heterogenous group of non-structural constituents most of which are organic compounds extractable in neutral solvents. The extraneous materials include waxes, fats, essential oils, tannins, resin and fatty acids, terpenes, alkaloids, starch, soluble saccharides, and various cytoplasmic constituents such as amino acids, proteins and nucleic acids (Cowling and Brown, 1969). The non-cellulosic

materials exert a significant influence on the susceptibility of cellulose in natural fibers to enzymatic hydrolysis (Smith et al., 1971).

C. Distribution of Constituents Within Cellulose Fibers

The accessibility of cellulose to the extracellular enzymes of cellulytic microorganisms is determined in part by its distribution within the cell wall and the nature of the structural relationships among the various cell wall constituents. The distribution of constituents is relatively simple in cotton. The secondary walls of cotton fibers consist almost entirely of highly crystalline cellulose. Almost all the hemicelluloses and extraneous materials are contained in the cuticle and primary wall layers. In wood, on the other hand, the non-cellulosic materials are deposited in all regions of the cell walls from the lumen through the compound middle lamella (the primary wall and adjacent intercellular substance). Cellulose is in highest concentration on the secondary wall and diminishes toward the middle lamella. Hemicelluloses predominate among the polysaccharides in the compound middle lamella and decrease toward the lumen. The extraneous materials are deposited in part in the lumen of wood cells and in part within the cell walls. Mineral constituents are distributed in all cell wall layers of both cotton and

wood fibers. Within the various layers of wood cell walls, the hemicelluloses, lignin, extraneous and mineral constituents are concentrated in the spaces between microfibrils or elementary fibrils. The hemicelluloses and lignin form a matrix surrounding the cellulose. Within a given microfibril, lignin and the hemicelluloses may penetrate the spaces between cellulose molecules in the amorphous regions. The lignin apparently prevents the cellulases and hemicellulases of these organisms from contacting a sufficient number of glycosidic bonds to permit significant hydrolysis (Cowling and Brown, 1969).

D. Cellulase Complex

C-1 is an enzyme whose action is unspecified. It is responsible for the hydrolysis of highly oriented solid cellulose by β1-4 glucanases.

β1-4 Glucanases (C_x) are the hydrolytic enzymes. β1-4 glucanase is usually measured by action on soluble cellulose derivatives, usually carboxymethylcellulose. The "x" in C_x emphasizes the multi-component nature of this fraction. The β1-4 glucanases are clearly of two types: (1) exo-β1-4 glucanase, successively removing single glucose unites from the non-reducing end of the cellulose chain; (2) endo-β1-4 glucanases with action in a random nature with the terminal linkages generally being less susceptible to hydrolysis than internal linkages.

β-Glucosidases vary in their specificities. The β-glucosidases that are involved in cellulose breakdown are those highly active on the β-dimers of glucose, including cellubiose. β-Glucosidases and exo-β-1-4 glucanases have substrates in common, cellubiose to celluhexaose. β-Glucosidases hydrolyze the smaller oligomers most rapidly; exo-glucanases, the larger ones. β-glucosidases act by in retention of configuration; exo-glucanases by inversion (King and Vessal, 1969).

E. Organism-Substrate, Enzyme-Substrate Relationship

The cellulytic organisms live either on the exterior surface on the fibers, as is most often the case with cotton, or in the fiber lumina as is the case with forage and wood. Enzymes are then secreted which catalyze the dissolution of the high-polymeric constituents of the fiber to soluble products. These enzymes are usually very highly substrate-specific. The more complex the substrate, the greater will be the number of specific enzymes that will be required to degrade the substrate completely (Cowling and Brown, 1969).

In such general enzymatic reactions a direct physical contact between each particular enzyme and its specific substrate is essential. Thus, cellulytic enzymes secreted from organism must be either bound on the surface of the organisms or secreted into the exterior environment to diffuse some distance away from the

organism and act on the accessible microfibrillar or molecular surfaces within the fine capillary structure of the fiber. However, the cellulose and other major constituents of natural fibers are insoluble molecules and are deposited within the cell walls in an intimate physical mixture of great structural complexity. Formation of a requiste physical association can be achieved only by diffusion of these enzymes to susceptible sites on the gross surfaces of the fiber or the microfibrillar and molecular surfaces within a fiber wall. Thus, any structural feature of the fiber or its constituents that limits its accessibility or the diffusion of cellulolytic enzymes in close proximity to that fiber will exert a profound influence on the susceptibility of the fiber to enzymatic degradation (Cowling and Brown, 1969).

F. Influence of Fiber Structure on Its Susceptibility to Enzymatic Degradation

According to Cowling and Brown (1969), there are five major structural factors that could affect the susceptibility of cellulose to enzymatic degradation. Each are discussed in some detail.

1. Moisture content of the fiber

Moisture plays three major roles in the degradation of cellulose: (a) It swells the fiber by hydrating the cellulose molecules, thus opening up the fine structure in such a way that the substrate is more accessible to

cellulolytic enzymes. (b) It provides, between the organism and the fiber, a medium of diffusion for the extracellular enzymes of the organism and the partial degradation products of the fiber from which the organism derives its nourishment. (c) The elements of water are added to the cellulose during hydrolytic cleavage of each glucosidic link in the molecule.

2. Size and diffusibility of cellulolytic enzymes in relation to the capillary structure of cellulose

The accessible surface of cellulose to a cellulolytic enzyme complex is regulated by the size, shape, and surface properties of the microscopic and submicroscopic capillaries within the fiber in relation to the size, shape, and diffusibility of the enzyme molecules themselves. The influence of these relationships on the susceptibility of cellulose to enzymatic hydrolysis has not been verified experimentally in natural fibers but the concepts that follow are demonstrated by the work of Stones et al. (1969).

The total surface area exposed in the gross capillaries is large (approximately one square meter per gram of wood or cotton) but is several orders of magnitude smaller than the total surface area potentially available to a small molecule within the water-swollen cell wall (approximately 300 square meters per gram). Thus, if cellulolytic enzymes can penetrate the cell wall

capillaries, substantially greater rates of dissolution of cell wall constituents can be expected than if the enzymes are so large that they are confined to the surfaces of the gross capillaries. The area exposed on the gross capillary surfaces of one gram of wood or cotton is sufficient to accommodate about $3x10^{15}$ randomly oriented enzyme molecules 200x35 Å. in size. That is equivalent to approximately 3 mg. of enzyme protein per gram of wood or cotton (Cowling and Brown, 1969).

The dimensions of cell-wall capillaries have been determined recently by using a series of dextran or polyethylene glycol polymers of known molecular size to measure the dimensions of cell wall capillaries (Aggebrandt and Samuelson, 1964; Stone and Scallan, 1967; 1968; Tarkow et al., 1966; Cowling and Brown, 1969). The data of Cowling and Brown showed that the approximate median and maximum dimensions of cell wall capillaries in water-swollen wood (Black spruce), Cotton (Scoured) and wood pulp (Black spruce kraft, 44.6% yield) were, respectively, about 10 and 35, 5 and 75, and 25 and 150 Å. The substantial increase in median and maximum pore size during pulping results in part by removal of lignin and hemicelluloses from the cell wall capillaries.

Estimates of the dimensions of the cellulolytic enzymes of Myrothecium verrucaria and other microorganisms had been studied by Whitaker et al. (1954), Tanford (1961),

Cowling and Brown (1969). These workers showed that if the enzymes are spherical, they range from about 25 to 80 Å. in diameter with an average of 60 Å. If the enzymes are ellipsoids with an axial ratio of about six, they range from about 15x80 Å. to 40x250 Å. in width and length, respectively, with an average of 35x200 Å.

The mechanism of penetration of capillaries by cellulolytic enzyme molecules in natural fibers has been examined and reported by Cowling and Brown (1969). They indicated that the maximum dimensions of cellulolytic enzyme molecules known to date are all smaller than the gross capillaries of both wood and cotton fibers. Thus, from consideration of size and shape alone, these molecules would be expected to diffuse readily within the gross capillaries and act on cellulose molecules exposed on the surface of these capillaries. However, only a small fraction of the cell wall voids in water swollen wood and cotton fibers are sufficiently large to permit penetration of most of the cellulolytic enzyme molecules. The pulp prepared from spruce wood contains cell wall capillaries that are adequate to admit many of the enzyme molecules as spheres or by endwise penetration if they are ellipsoids. But since both rotational and translational modes of motion are generally assumed during diffusion of dissolved solute molecules, it is

realistic to assume that pores as large or larger than the largest (rather than the smallest) dimension of a given enzyme molecule would be required for unimpeded accessibility. For this reason, cellulolytic enzyme molecules are physically excluded from all but the largest capillaries. Consequently, they must gain access to the interior regions of the cell by enlarging the existing capillaries (Cowling and Brown, 1969).

3. Degree of crystallinity

The influence of degree of crystallinity on the susceptibility of cellulose to enzymatic hydrolysis has been studied by Norkrans (1950), Walseth (1952), Reese et al. (1957), Cowling and Brown (1969) and Stone et al. (1969). Using several cellulose samples precipitated after swelling in phosphoric acid, Walseth showed that the preparations with higher crystallinity as indicated by low moisture regain values were more resistant to hydrolysis by enzymes Aspergillus niger than those with lower crystallinity. Using X-ray differaction data, Norkrans (1950) concluded that the cellulolytic enzymes were degrading the more readily accessible amorphous portions of her regenerated cellulose and were unable to attack the less accessible crystalline material. Preferential attack of amorphous cellulose over native cellulosic material was indicated by some organisms but not by all (Cowling and Brown, 1969). For example,

Poria monticola preferentially attack the amorphous portion of holocellulose obtained from sweetgum wood while *Polyporus versicolor* degraded the crystalline and amorphous cellulose of holocellulose simultaneously. Crystallinity of cellulose has not been successfully related to digestibility in forages (Van Soest, 1969b).

The amorphous fraction may determine the accessibility of the hydroxyl groups to water or the glucosidic bonds to dilute mineral acid but is not necessarily related to the accessibility of the cellulose to a large molecule such as an enzyme. For example, the amorphous material in wood is accessible to water and hydrogen ions but is completely inaccessible to an enzyme. If, therefore, a correlation is found between the amount of amorphous cellulose in a sample and its susceptibility to attack by cellulase, this is coincidental, and the true reason for the increase in reactivity must be caused by some other change which has occurred simultaneously with the change in crystallinity (Stone *et al.*, 1969). A further demonstration that crystallinity of the cellulose is not of itself a controlling factor in reactivity is the common observation that a cellulosic material which had never been dried is much more reactive than one which has been dried and reswollen in water, even though the crystallinity is increased only slightly if at all by such a drying treatment (Stone *et al.*, 1969).

Although variations exist among microorganisms in attacking amorphous cellulose; any treatment which will alter the proportion of crystalline material or the degree of perfection (parallelism) of the crystallites present may modify the susceptibility of the material to enzymatic attack. Treatments that would increase susceptibility include: reprecipitaion from solution, mechanical disruption such as vibratory ball mill, or ionizing radiation (Cowling and Brown, 1969).

4. Degree of polymerization of the cellulose

The length of cellulose molecules in a fiber varies over a wide range from less than 15 glucose units to as many as 14,000. This variation would be expected to considerably affect the rate of hydrolysis, particularly by enzymes that cleave the cellulose molecules by an endwise mechanism. But most isolated cellulases studied to date apparently hydrolyze cellulose at random along the length of the molecules. Only slight differences have been noted in the resistance of cellulose preparations of different average DP (degree of polymerization) to a wide variety of fabric-destroying fungi (Greathouse, 1950; Siu, 1951).

When the DP of cellulose is reduced during acid hydrolysis, the disaggregated cellulose chain ends in the amorphous regions have a tendency to recrystallize and make the residue more resistant to enzymatic

hydrolysis. Thus, DP is of limited significance in determining the susceptibility of cellulose to enzymatic hydrolysis except in the relatively rare case of enzymes that act by an endwise mechanism.

5. The nature of the substances with which the cellulose is associated and the nature of that association

(a) Mineral constituents: Cellulose fibers usually contain about 1% ash. The mineral elements contained in the ash includes all those essential for the growth and development of cellulolytic microorganisms. Mandels and Reese (1957) have studied the induction of cellulase by various metal ions and have shown that cobalt is particularly stimulatory to cellulase production. Gascoigne and Gascoigne (1960) concluded that mercury, silver, copper, chromium, and zinc salts are generally inhibitory whereas manganese, cobalt, magnesium and calcium in the presence of phosphate have been reported as stimulatory, though exceptions to these generalizations also have been reported.

(b) Extraneous materials: Cowling and Brown (1969) have summarized the influence of extraneous materials on the susceptibility of cellulose fibers to enzymatic degradation. These influences are: (i) Growth promoting substances such as vitamins and certain soluble carbohydrates provide substrates for rapid growth and development of cellulolytic microorganisms within cellulose fibers;

(ii) Poisonous substances, particularly toxic phenolic materials, inhibit the normal growth and development of cellulolytic organisms; (iii) Certain specific enzyme inhibitors act directly to reduce the rate or extent of enzymatic hydrolysis of the cellulose; (iv) Various substances (e.g., lignin, silica) are deposited within the fine capillary structure of the cell wall and thus reduce the accessibility of the cellulose to extracellular enzymes; (v) The very small amounts of nitrogen and phosphorous present make both wood and cotton comparatively deficient substrates for microorganisms that are not specifically adapted to cope with these deficiencies.

The combination of lignin with the partially crystalline cellulose that occurs in wood are the natural materials most resistant to enzymatic hydrolysis. Many cotton-fabric-destroying fungi and rumen bacteria, are prevented from degrading the cellulose in wood by this association with lignin. To degrade the cellulose in wood, organisms must possess not only the ability to degrade cellulose but also to degrade the lignin or at least break down its association with cellulose.

A chemical bond between lignin and cellulose has been postulated by many investigators (Freudengberg, 1965). Present evidence suggests, however, that the association is largely physical in nature with the lignin and amorphous cellulose forming a mutually interpenetrating

system with a high degree of polymerization. Lignin apparently decreases the accessibility of wood cellulose to enzyme molecules that diffuse within the fine structure of wood fibers (Cowling and Brown, 1969).

VI. Forage Nutritive Value Evaluation

The Weende system of feed analysis has been a widely accepted procedure for measuring chemical composition and predicting digestibility of forages. The crude fiber content is generally used as a reference in this system. The Weende procedure has been very persistant and useful despite the questionable theory and frequently faulty results obtained (Deinum and Van Soest, 1969).

In recent years, some procedures have been developed which give a more accurate prediction of forage digestibility. In 1965, Van Soest and Wine developed procedures, using detergents, to separate forage constituents into cell walls and cell contents. The cell contents, being independent of lignin, are almost completely utilizable while the digestibility of the holocellulose in the cell walls is influenced by the degree of lignification. These authors suggested that the percent of lignin in the cell walls or in the acid detergent fiber is a good chemical predictor of forage digestibility. Other workers (Lucas, 1961; Mott and Moore, 1969) had discussed the validity and precision of forage dry matter

digestibility prediction by chemical constituents. The concept of "Nutritive entity" proposed by Lucas (1961) have received particularly heavy emphasis by Van Soest (1969a, b). Mott and Moore (1969) have summarized the recent literature on the forage dry matter digestibility prediction by chemical components. They concluded that the measurement of a single forage constituent satisfactorily predicted digestibility of dry matter if the investigator restricted the kinds of forages to a few closely related species; to a fairly narrow spectrum of management systems and to similar environmental conditions. As one broadens the kinds of forages and growing circumstances the complexity of the problem is magnified by inclusion of additional nutritive entities.

Dehority and Johnson (1964) have suggested that the solubility of cellulose in cupriethylene diamine (CED) is an indicator of cellulose digestibility and therefore, could be used to predict dry matter digestibility *in vivo*. They also suggested that dry matter solubility (DMS) of a forage in 1.0 *N* H_2S04 is highly correlated to relative intake and that a combination of CED and DMS is a good predictor of the nutritive value index (Crampton *et al.*, 1960). On the other hand, Donefer *et al.* (1963) found that the simplest measurement, that obtained with dry matter dissolved with distilled water resulted in correlation coefficients of 0.86 and 0.90 when compared to

relative intake and nutritive value index (NVI), respectively; though the highest correlation obtained with dry matter dissolved by a 0.075 N HCl solution of pepsin. These high correlations were confirmed in a later experiment (Donefer et al., 1966).

Thomas and McCampbell (1969) discussed the usefulness and precision of forage nutritive evaluation by ultra-violet spectra of extracted lignin. Their results indicated that a good estimation of forage intake by sheep can be obtained by the use of these lignin parameters.

Brudick and Sullivan (1963) reported that a significant correlation coefficient of 0.96 was found between the degree of solubilization of the xylose and in vivo digestion coefficient of dry matter in eleven forages including grasses and legumes. In addition, they found that correlation coefficient between the rate of hydrolysis and the digestion coefficient of dry matter was 0.63 ($p<0.01$).

Recently, several in vitro systems have been developed. These have been used not only for the evaluation of forages but also for the elucidation of rumen reactions. Excellent reviews of these techniques are given by Barnett and Reid (1961), Annison and Lewis (1962), El-Shazly et al. (1960), Barnes et al. (1964), Johnson (1963), (1966), (1969), Barnes (1965).

The *in vitro* systems are superficially ideal in that they offer a rapid, inexpensive evaluation while require minimum quantities of forage. Most *in vitro* systems were developed and used to study extent of digestive or metabolic activities rather than rate phenomena. This was especially true with studies of fiber or cellulose digestion. However, mere extent of digestion did not adequately relate to all the factors of forage quality observed in other studies, not the least of which were animal growth or production studies. Passage of forages through the rumen was obviously related not only to the total digestibility, but also to the rate at which digestion proceeded. Thus, it became the double task to develop a technique or techniques to relate mathematically, if not biologically, to both digestibility and intake (Johnson, 1969).

The older *in vitro* digestion methods based on cellulose digestion often showed fairly precise predictions of apparent *in vivo* digestibility with the small number of forages used (Johnson *et al.*, 1962; Kamstra *et al.*, 1958; Donefer *et al.*, 1960; Baumgardt *et al.*, 1962).

The Tilley and Terry (1963) method of *in vitro* evaluation of forage digestibility has lately received wide acceptance. It is a relatively simple and precise technique. A strong support for the usefulness of this technique was given by Oh *et al.* (1966). However,

Van Soest et al. (1966) indicated that the Tilley method did not actually simulate in vivo digestion. They suggested that the determination of undigested cell wall constituents gave a more meaningful measurement of forage quality. They suggested a summative equation using both in vitro digestion and chemical analysis as the best predictor of forage digestibility. The high precision in prediction of forage dry matter digestibility by using this combined technique has been reported by Deinum and Van Soest (1969).

Oh et al. (1966) pointed out that in vitro fermentation techniques often yield more accurate estimates of forage digestibility than chemical methods. This superiority was not surprising because energy releasing bacteria were very likely influenced by unassayed limiting factors (e.g., silica) active on the substrate (Van Soest, 1969b).

In vitro dry matter disappearance (IVDMD) is the material most often measured. It is an analytically simple determination and subject to relatively low variability (Baumgardt and Oh, 1964; Bowden and Church, 1962). IVDMD is especially well correlated with in vivo dry matter digestibility (DMD) or digestibility of energy (DE). It is very poorly related to intake parameters (Johnson and Dehority, 1968). Long term in vitro cellulose digestibility values (30 to 48 hr.) were generally

found to be well related to *in vivo* cellulose digestibility and dry matter digestibility but not to the intake parameters. While shorter incubation period (12 hr.) was more highly related to both digestibility and intake (Donefer *et al.*, 1960; Johnson *et al.*, 1962). Ingalls (1964) found a 6-hour fermentation measuring dry matter disappearance was significantly correlated with NVI; a 36-hour fermentation period was more highly correlated to animal digestibility.

For several years, Klopfenstein and Woods (1968, 1969, 1970) have examined the validity and usefulness of *in vitro* techniques, which have been the acceptable methods for *in vitro* evaluation of conventional forage, in evaluating the nutritive value of chemically or physically treated forages. Their results indicated that *in vitro* techniques are important tools in evaluating the effects of treatments though there are some discrepancies between *in vitro* values and *in vivo* values.

Modified forages may be unique in that their constituent content could remain the same as in the original material, even with increased digestibility. Also, lignin content could be reduced without a change in the ratio of solubles to fiber (Klopfenstein and Woods, 1967). These facts may indicate that chemical components are not good nutritive value predictors of modified forages.

Smith *et al.* (1969) introduced the term "total *in vitro* digestible cell wall units" for evaluating nutritive value of chemically or physically treated forages. They proposed that total *in vitro* digestible cell wall units could be partitioned into those units which resulted directly from chemical solubilization and those resulting from bacterial cell wall digestion. This method may be useful for evaluating nutritive value of treated forages or for assistance in understanding treatment effects.

VII. Improvement of the Nutritive Value of Low-Quality Forages by Chemical and Physical Methods

It has been recognized for many years that changes in the structure and composition of a variety of cellulosic substrates could influence the susceptibility of these materials to enzymatic attack. Such changes are: (1) particle size reduction, (2) delignification, (3) change in crystallinity of the cellulose, (4) hydrolysis and reduction of cellulose chain length, (5) swelling, and (6) disruption the lignicellulose complex by radiation or ball-milling (Cowling, 1963). Some chemical and physical treatments which have been evaluated for their effectiveness in changing structure composition and degradation rate of cellulosic materials will now be discussed.

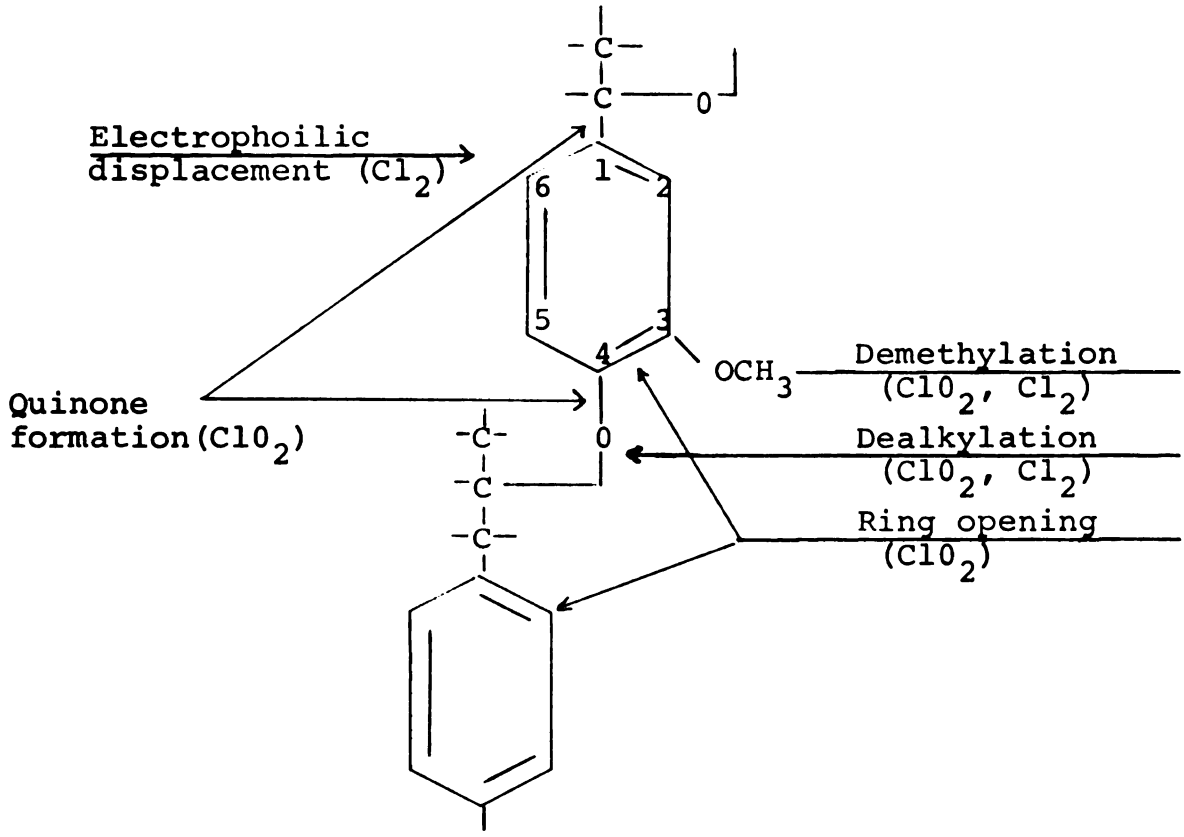
A. Chemical Methods

1. Chlorine and its oxides

Lignin polymers are susceptible to decomposition by chlorine and its oxides (Sullivan et al., 1959). In practice, the removal of lignin from plant material by sodium chlorite in aqueous acid solutions is part of the procedure for the preparation of holocellulose. Sodium chlorite also has been used as a pulp bleaching chemical in the wood industry (Sarkanen et al., 1962). The oxidative delignification mechanisms of chlorine dioxide and sodium chlorite were extensively studied by Sarkanen et al. (1962) and Dence et al. (1962) using lignin models (vanillyl, α-methylvanillyl, and syringyl alcohols), lignin preparations plus hard and softwood meals. Although the oxidative reactions are not completely understood, the following three major reactions occur: (a) Oxidative ring opening--between carbon atoms 4 and 5 of vanillin--resulting in the formation of derivatives of muconic acid and monomethylesters; (b) Quaiacyl-type compounds are oxidized to substituted p-benzoquinones and (c) Demethylation. They also found that the isolation of chlorine-substituted intermediates suggests that during the course of the oxidation chlorine dioxide or sodium chlorite, or both, are reduced to elemental chlorine. The possible reaction routes through which lignin residues

are ultimately degraded to colorless, water-soluble fragments were discussed by these workers.

The following sketch indicates possible sites of degradative attack by chlorine dioxide and chlorine on lignin as proposed by Dence et al. (1962).



Ely and Moore (1954) reported that recoveries of cellulose and hemicellulose were essentially 100% when boiled with acid chlorite for 1 to 2 hours but that boiling for longer periods decreased recovery of these carbohydrates. The residue contained lignin and nitrogen and minimum levels were not attained until the fifth or sixth

hour of treatment. Sullivan and Hershberger (1959) treated orchardgrass, red canary, and wheat straw with dry chlorine dioxide in order to prevent loss of the soluble constituents. The amount of sodium chlorite ranged from 0.2 to 3.2 grams per 100 grams substrate. The results showed an average of forty percent reduction of lignin and thirty percent increase in the *in vitro* cellulose digestibility of all three substrates. The rate of reduction or increase was proportional to the amount of sodium chlorite used.

Considerable preliminary information about the level of sodium chlorite, optimal pH, and effect of length of reaction was given by Goering and Van Soest (1968). Five different lignocellulosic materials were used. The chemical composition of treated roughages indicated that sodium chlorite was specifically effective in reducing the amount of lignin. About 70% reduction in lignin was noted for corn cobs, wheat straw, orchardgrass, and alfalfa stems when 15% (air-dry basis) of sodium chlorite was used. However, sodium chlorite treatment did not change the lignin value of peanut hulls even at the 25% level. Sodium chlorite treatment also lowered other fibrous constituents about 30%. Generally, *in vitro* studies showed that sodium chlorite can be used to increase the *in vitro* digestibility of low-quality forages. It improved the *in vitro* digestibility of

434, 75, 55, 53 and 9% for peanut hulls, wheat straw, alfalfa, corn cobs and orchardgrass, respectively. Apparently, some of the increase in digestibility was largely due to cell wall carbohydrate solubilization and quantitative reduction of lignin by sodium chlorite. However, the large increase for peanut hulls could not be accounted for simply by solubilization of cell walls. Perhaps sodium chlorite broke bonds between carbohydrates and lignin to achieve this increased digestibility. In another experiment, they determined the effect of conditions (temperature, pH, treatment DM) on $NaClO_2$ treatment of wheat straw for 8 days on composition and *in vitro* digestibility. The lowest lignin value (4.0%) and highest *in vitro* digestibility (89%) was obtained from a treatment combination of 15% $NaClO_2$, pH 4 and 18% DM. They also evaluated the effect of ensiling time and levels of $NaClO_2$, using cotton stalks, peanut hulls and alfalfa stems as substrates. The results indicated that length of treatment needed to achieve a highly digestible material varied with the type of material. For example, peanut hulls were not as digestible (51%) with 35% $NaClO_2$ for 3 days as with 25% $NaClO_2$ for 15 days (91%).

Barley straw was treated with sodium chlorite at a 15% level, and ensiled for 60 days (Goering *et al.*, 1969). The resulting silage was then dried, mixed with isolated soybean protein, starch, etc. and pelleted. Straw was

the only source of plant cell walls in the ration. Treated straw had a lower content of CWC, ADF and lignin than did the control straw. However, the magnitude of the decrease was less than that observed in laboratory values indicating that the efficiency of the chemical treatment was greatly reduced by treatment of large amounts of material. In vivo digestibility of dry matter, organic matter, CWC, ADF, cellulose and hemicellulose were all significantly higher for the chlorite silage ration than the control ration. Total concentration of ruminal volatile fatty acids showed no marked difference between sodium chlorite and control ration. However, the former one had a slightly higher proportion of propionic acid.

In studying the influence of chemical treatments upon digestibility of ruminant feces, Smith et al. (1969) selected eleven chemicals to treat feces from cattle fed orchardgrass or alfalfa hay. Potassium chlorate ($KClO_3$) and NaClO treatments at 3% (wet feces basis) level showed no improvement in in vitro digestibility of fecal cell walls, even though the treatment lasted 3 weeks. When wet feces were treated with eight levels of $NaClO_2$ (0 to 7 g/100 g wet feces), lignin reduction was linearly related to increasing levels of $NaClO_2$. The maximum reduction was about 50% at level of 7 g/100 g wet feces. No significant reduction in cellulose or hemicellulose

was noted with increasing levels of $NaClO_2$. The *in vitro* digestibility of fecal cell walls was markedly improved by $NaClO_2$ treatment. A seven-fold increase was noted for orchardgrass feces and a 15-fold increase for alfalfa feces. However, these increases were related almost linearly with the level of $NaClO_2$. Thus, they suggested that up to 10g $NaClO_2$/100 g wet feces might be required for maximum digestion.

2. Dilute alkali and liquid ammonia

The effect of NaOH in improving the digestibility of straw has long been known (Beckmann, 1919). Several early workers (Ferguson, 1942; Godden, 1920; Sen *et al.*, 1942) had promising results from sodium hydroxide treatment.

Mechanism: The chemical and physical action of NaOH on cellulose was not clear until very recently. Tarkow and Feist (1968, 1969) described and verified two major modes of action: (a) The important chemical reaction is saponification of esters of uronic acid associated with the xylan chains. Since these esters bridge polymeric units, the net effect of saponification is a breaking of "cross-link"; (b) The physical effect is a marked increase in fiber saturation point or moisture content of the substance at 100% relative humidity. This can be quantitated. The increase in fiber saturation

point not only provides for improved diffusion conditions of water soluble material, but also provides for improved enzyme-substrate interactions.

Concentration and amount of sodium hydroxide: Beckmann (1919) proposed 12 grams NaOH per 100 grams straw as a 1.5% solution for treatment. He obtained a two-fold increase in the amount of crude fiber utilized by ruminants. While Lampila (1963) used a more dilute solution and about half the amount of NaOH used in the Beckmann procedure and obtained a similar result. Wilson and Pigden (1964) reported that the improvement of *in vitro* DMD of wheat straw and popular wood was approximately linear with NaOH levels up to about 7 gram per 100 gram substrate. Further improvement of *in vitro* DMD was small as greater amounts of NaOH was used. Similarly Ololade *et al.* (1970) evaluated the effect of different amounts of NaOH on *in vitro* DMD of barley straw. They found that alkali treatments up to the level of 8g/100 g DM increased IVDMD with no further increase to 12g/100 g DM. Nebraska workers (Kloplenstein and Woods, 1968, 1969, 1970) consistently use 3 to 5 grams NaOH per 100 grams of dry matter in treating corn cobs or straw. Both levels significantly improved *in vitro* DMD values, although the 5g/100 g treatment showed a greater response. Donefer *et al.* (1969) reported that greatest *in vitro* cellulose digestibility of oat straw was obtained by the treatment

of NaOH at a level of 16g/100 g straw in a 13.3% solution. Smith et al. (1969) suggested that, for treating ruminant feces, the optimal NaOH level was in the range of 15g NaOH per 100 g dry feces. The most comprehensive concentration-level-substrate relationships were reported by Tarkow and Feist (1969). They proposed that the amount of NaOH required for treatment can be calculated when substrate parameters concerning amount of esterfied and non-esterfied uronic acid groups and acetyl groups are known. They also indicated that treatment time was inversely proportional to NaOH concentration. This idea was supported by Feist et al. (1970) and Millett et al. (1970).

Ideally, both the amount of NaOH and water should be minimized in large-scale treatment procedures. Excess alkali either should be removed by washing or neutralized by acid or balanced by potassium, so that the resultant sodium levels do not overload the animal's ability to maintain acid-base equilibrium (Donefer et al., 1969; Kloplenstein and Woods, 1970).

Effect of sodium hydroxide on chemical composition of treated forages: Dilute alkali removes a portion of the lignin from wood or forages (Harris, 1933). However, a number of researchers also reported opposite results (Feist et al., 1970; Donefer et al., 1969). Ololade et al. (1970) reported that NaOH treatment at a level of

8g/100 g DM solubilized the hemicellulose of alfalfa stem and barley straw by 22 and 16%, respectively. However, no reduction in lignin and ADF was noted in either materials. Smith et al. (1969) found that the amount of CW, hemicellulose, cellulose and lignin of both orchardgrass and alfalfa feces decreased when treated with increasingly larger amounts of NaOH. Hemicellulose was almost completely solubilized by the higher levels of alkali. In addition, they found that NaOH treatment also solubilized a major portion of the acid insoluble ash (primarily silica) associated with the undigested CW in orchardgrass feces.

Effect of sodium hydroxide on in vitro fermentation: Stranks (1959) reported that rate of in vitro digestion of hardwoods measured by succinic acid production was markedly increased by pretreatment with a NaOH solution (1 to 5 g/100 g wood). Pew and Weyna (1962) and Stone et al. (1965) both have shown the digestibility of hardwoods by cellulase to be markedly improved by pretreatment with aqueous NaOH. Ololade et al. (1970) reported that NaOH treatment was much more effective in improving IVDMD of barley straw as compared to alfalfa stems. Feist et al. (1970) evaluated the effectiveness of NaOH in improving in vitro digestibility of hardwoods. Twelve species of hardwoods were treated with 1% NaOH solution (NaOH:wood ratio = 20:100). The in vitro DMD

of treated woods showed a marked increase. Treatment response was related to lignin content of the wood. Yet, little lignin was removed by treatment. A very similar type of study with comparable results was reported by Millett et al. (1970). Both Feist et al. and Millett et al. concluded that dilute NaOH treatment of certain hardwoods could be a potential method to provide energy feeds for ruminants.

Intake of sodium hydroxide treated forages: Donefer et al. (1969) treated oat straw with 8g NaOH and 600 ml of solution per 100 g straw, then fed this to lambs as the sole feed. They found that NaOH treatment had no effect on relative intake. However, when treated straw supplemented by urea was fed the relative intake was significantly improved. The value observed was similar to that found for an excellent quality forage. They concluded that nitrogen became a limiting nutrient when the energy digestibility was improved by NaOH treatment. Klopfenstein and Woods (1969) treated wheat straw with NaOH solution at the level of 4g/100 g straw and ensiled the product at a 50% moisture content. When fed to lambs as 70% of a mixed ration 1% potassium chloride was added. Sodium hydroxide treatment of the straw significantly increased average daily gain and feed consumption. In addition, they found that supplemental soybean meal supported better daily gain than did urea. Donefer et al.

(1969) reported that the combination of sucrose and urea did not result in any greater increase in intake or digestibility than urea alone indicating that a source of readily available energy was not a factor limiting utilization of the treated straw. Klopfenstein and Woods (1969) treated corn cobs with various amounts of NaOH and after ensiling 50% of the added sodium in the ration was neutralized with hydrochloric acid. A ration was fed to sheep consisting of 60% of the resulting silage, 30% alfalfa pellets and 10% cereal supplement. Daily feed intake was highest for treated silages with maximum intake for 5g NaOH/100 g corn cobs. Average daily lamb gains were also appreciably increased by the sodium hydroxide treatment (5g/100 g corn cobs).

In vivo digestibility of sodium hydroxide treated forages: Beckmann (1919) reported a two-fold increase in crude fiber digestibility. Lampila (1963) determined the digestion coefficient of the crude fiber fraction of straw treated by Beckmann's procedure using sheep. The value was 88%. Sarrinen et al. (1959) reported that wood pulps obtained by alkali treatment were more digestible by rams than those resulting from treatment with acid or chlorite. Donefer et al. (1969) reported that the dry matter digestibility of NaOH treated oat straw was significantly increased as compared with control (54 vs. 36%). The digestibility of treated straw

could be further increased by either urea or sucrose supplementation. Klopfenstein and Woods (1969) found a similar type of response for wheat straw. However, when corn plant, corn stalk or corn cobs were treated with various amounts of NaOH and fed to lambs as 60% of the ration, the dry matter digestibility of treated materials were not increased compared to that of controls. In fact, they found that as NaOH increased dry matter digestibility decreased. Since the *in vitro* DMD was increased and did not follow the *in vivo* pattern they conducted a series of experiments in order to obtain more knowledge about the effect of feeding NaOH treated roughages on mineral metabolism in the animal.

Na^+, Cl^- and K^+ relationships: Several hypotheses about mineral imbalance *in vivo* caused by feeding large amounts of Na^+ were proposed and evaluated by Klopfenstein and Woods (1968, 1969, 1970). They believed that there is probably a delicate balance between sodium, potassium and chloride that must be maintained in order to obtain optimum digestibilities. Although the absolute relationships have not been established, the result of several *in vivo* trials indicated that mineral balance seems to be related to a small increase in *in vivo* dry matter digestibility.

Although NaOH treatment increased digestibility and perhaps rate of digestion the bulk characteristics

of the resulting material was not decreased. Therefore, intake as affected by fill may still limit performance (Klopfenstein and Woods, 1970).

3. Cellulase

Theoretically, at optimum pH and temperature, cellulase should be able to hydrolyze and reduce the chain length of any cellulose polymer. Thus, the availability of forage cellulose to ruminants might be improved by predigestion before consumption. However, the results of few *in vivo* experiments have not supported this idea.

McCullough *et al.* (1966) evaluated the effectiveness of cellulase for improving cellulose utilization of wheat straw and corn silages by measuring milk production, efficiency of production and *in vitro* cellulose digestibility as guides. Animals fed the cellulase (0.5%) treated corn silage produced significant higher amounts of milk than did controls. The efficiency of production was higher for the former group. The extent and rate of *in vitro* cellulose digestibility of treated corn silage was slightly but significantly higher than that of the control silage. However, the chemical composition of both silages showed that there was essentially no difference in cellulose content. The authors concluded that in practice silage made with cellulase should reduce the problems of lowered feed utilization with high levels of grain feeding. However, neither the milk production,

efficiency of production nor *in vitro* cellulose digestibility was improved by cellulase treatment in wheat straw silage.

Further studies of cellulase on corn silage were conducted by Thomas and Benne (1968) who also referred to unpublished work by investigators at Wisconsin. Both groups of researchers found that cellulase did not decrease the cellulose or crude fiber content of the resulting silage. No significant difference in *in vivo* cellulose or dry matter digestibility was noted between cellulase treated silage and control corn silage.

Pew and Weyna (1962) reported that a commercial cellulase (RH-19) digested fairly large amounts of cellulose of alfalfa meal (nitrogen-free extract 35%, and crude fiber 27%). Total sugars analyzed 15% after alfalfa meal was digested twice by the enzyme while the original value was 41%. Percentage of lignin in this alfalfa meal was comparable to that in aspenwood but the latter was completely resistant to enzyme digestion. The authors concluded that cellulytic enzymes do not digest coarsely ground wood but may digest lignified annual plants. The lignification in alfalfa does not protect the carbohydrate to the extent it does in wood. Furthermore, the quantity, chemical nature and physical organization of cellulose in forage fibers are variable (Waite and Gorrod, 1959; Lagowski *et al.*, 1958) so that a great difference in

action of cellulases might be expected. These facts may partially explain the variable and contradictory results about the effectiveness of cellulases (Cowling and Brown, 1969).

B. Physical Methods

1. High temperature-pressure

When lignocellulosic materials are exposed to high pressure steam or sulfuric acid and nitric acid under both atmospheric and increased pressure, the cellulose chain length or degree of polymerization is reduced and hydrolysis occurs. The ligno-carbohydrate bond may also be altered sufficiently to allow a microbial population to more extensively utilize the carbohydrate portion.

Stamm (1964) reported that at high temperatures lignin behaved much like a plastic, i.e., flowing at a temperature of 350 F, and pressure of 1500-2500 p.s.i. Krupnova and Sharkov (1963) reported that milling of wood cellulose at high temperatures produced a substrate that was readily hydrolyzable in 10% H_2SO_4. Using the same technique, Katz and Reese (1968) showed that the enzymatic hydrolysis of wood cellulose thus treated gave concentrations of glucose (30%) comparable with the enzymatic hydrolysis of starch. Stranks (1959) reported that hot water cooking (150 C) increased *in vitro* succinic acid production in elm, basswood and birch, while only a

limited effect was observed in Douglas-fir and pine. Acid cooking had a detrimental effect on aspen, which fermented without pretreatment but showed no reaction after acid cooking. Hajny et al. (1951) reported that pretreatment of delignified cellulose with dilute acid (0.4 or 0.8% H_2SO_4) at high temperature (180 C) for 15 minutes decreased the rate of fermentation of the residual cellulose by thermophilic cellulose bacteria. They speculated that this unexpected result was due to: (a) the treatment solubilized most of the amorphous cellulose so that the residual cellulose contained a high level of crystallinic cellulose and had low susceptibility to enzyme degradation; (b) toxic decomposition products formed from cellulose during the acid hydrolysis.

Klopfenstein et al. (1967) subjected roughages to a pressure of 28 Kg/cm^2 in the presence of water, 0.5% HCl or 4% H_2O_2 and increased the in vitro dry matter disappearance from 9 to 22%. This increase was mainly due to the hydrolysis of hemicellulose to soluble constituents. Lignin content was not reduced and in some cases may have been increased by the acid hydrolysis.

Alfalfa stems, corn cobs and corn stalks were exposed to steam under 400 p.s.i. pressure for 45 seconds (Klopfenstein and Woods, 1968). The in vivo dry matter digestibility did not show significant improvement over that of control values. Yet in vitro studies showed a significantly higher value for DMD than did controls.

They considered that some kind of toxic material was produced in high temperature-pressure treated forages and cause this discrepancy.

High temperature-pressure treatment increased *in vitro* DMD of several forages. Limited animal feeding trials indicated that treated forages depressed *in vivo* digestibility. Formation of a toxic-like compound was proposed, but clearly, more research work is needed to extend and verify existing contradictory findings.

2. Ball-milling

Ball-milling is a pure physical treatment that can cause a four-dimensional change in forage fiber. These changes are: (a) particle size reduction, (b) disruption of the lignocellulose complex, (c) change in crystallinity of the cellulose, (d) reduction of cellulose chain length (Dehority, 1961; Dehority and Johnson, 1961; Dehority *et al.*, 1962; Ott *et al.*, 1954).

The preliminary effects of the ball-milling on particle size reduction and *in vitro* rumen fermentation of purified cellulose were reported by Dehority (1961). The particle size of wet ball milled cellulose appears to be proportional to the length of ball-milling. However, additional ball-milling (more than 24 hours) only slightly reduced particle size. The *in vitro* fermentation results indicated that reduced particle size resulted in only a shorter lag-phase for digestion, with no marked

difference in the rate or extent of digestion. The result seems to indicate that ball-milling can be used to improve intake but not digestibility.

When various stages of maturity of timothy, alfalfa, orchardgrass and red clover were ball milled for 72 hours, the *in vitro* cellulose, hemicellulose, pectin digestion showed not only a marked shorter lag-phase but also a significant net increase in total carbohydrate digestion. There was a basic difference in grasses and legumes in regard to the amount of cellulose, hemicellulose and pectin that could be digested per given amount of lignin in the plant. Carbohydrate digestion increased as forage maturity increased (Dehority and Johnson, 1961; Dehority *et al.*, 1962). These workers concluded that ball-milling was very effective in disrupting the lignocellulose complex and that ball-milling did not change the chemical structure of any of the constituents in the plants. In other words, they proposed that the function of disruption of the lignocellulose complex was equal to delignification. However, Stranks (1959) has shown that partially pulped (lignin removed by $NaClO_2$) Douglas-fir was much more fermentable than ball-mill treated Douglas-fir. Pew (1957), Pew and Weyna (1962) reported that the properties of lignin in wood is changed after ball-milling. Cowling and Brown (1969) and Ott *et al.* (1954) believed that ball-milling decreased cellulose crystallinity and chain length of plant cellulose.

With wood residues, the benefits derived from ball milling are varied from species to species. This inconsistency is very likely due to species variation in quantity, chemical nature, and distribution of lignin within cell walls (Millett et al., 1970). The experiment conducted by Millett et al. (1970) is exemplary. Aspen and red oak meals were ball-milled for various times ranging from 5 min. to 240 min. In vitro dry matter digestibility (5-day fermentation period), sugar production and water solubility were used as complementary evaluators. In vitro DMD of both samples increased rapidly with milling time to about 30 min., further milling increased digestibility slowly and at 140 min. approached a plateau. The maximum improvement was a one-fold increase for aspen and a six-fold increase for red oak milled 240 min. Enzymatic hydrolysis (Cellulase-Onozuka SS, 1,500u/g) ndicated that this significant improvement of in vitro D was not merely a solubilization effect. The 240 min. lled aspen and oak produced 63 and 57% of their weight glucose, respectively, while the sugar production from untreated aspen and oak was 10 and 0.0%, respectively. ulations indicated that of the total carbohydrates ese two wood residues 70 to 80% were made accessible llulase digestion by vibratory ball milling. Water- ility measurement indicate a similar pattern in that alf the water-extracted solids was carbohydrate and

of probable nutritive values to the ruminants. Positive responses to ball milling have been reported for birch (Stranks, 1959), aspen, sweetgum, red oak (Virtanan, 1946; Millett et al., 1970; Pigden and Heaney, 1969). On the other hand, hickory, red alder, basswood, black spruce and cotton failed to show a favorable response to ball-milling (Millett et al., 1970; Stranks, 1959; Lawton et al., 1951).

Pew and Weyna (1962) concluded from their research that when wood was ground in the vibratory mill, the resulting fragments were in the size range of several microns. However, these pieces must have been agglomerates since the material was readily put into solution. The dissolved particles were submicroscopic but may still have been large enough to constitute a "cage" of lignin that enmeshed the carbohydrate chain. If the ultimate particles produced by grinding were small enough then an enzyme can remove most of or all of the cellulose even when the particles were agglomerated.

Although ball-milling has increased the in vitro digestibility of cellulose in most forages and some of woods, it did not produce similar responses when fed to ruminants. Digestibility was then decreased (Rodrique and Allen, 1960; Moore, 1964; Meyer et al., 1965). This was primarily due to the short time that finely ground

materials would remain and be fermented in the rumen. Balch (1960) suggested that a critical size of a 2 mm existed.

3. High energy irradiation

This type of treatment is a product of modern science. It is also the least time consuming and least in labor required. Dosage and type of substrate are the only variables involved in treatment and as a result, the quality of the end product can be well controlled and uniform. Presently the cost of irradiation is high, but has practical potential in the future. Consequently, future irradiation treatment may be a promising way for improving nutritive value of low-quality forage.

Although a number of reports about the effects of irradiation have appeared, the one by Lawton et al. (1951) is the most outstanding. By using in vitro fermentation techniques and chemical methods, Lawton et al. reported that, in the case of basswood, a dosage of less than 6.7×10^6 rads had little effect on any properties of the original material. In vitro DMD increased markedly when exposed to more than 6.7×10^6 rads with a maximum value at the highest dose level of 3.3×10^8 rads. The in vitro production of volatile fatty acids (VFA) showed a sharp increase to a maximum at an irradiation dose of 1×10^8 rads then decreased at 3.3×10^8 rads. Irradiated products produced at this high dosage may not have been readily

fermented, since the minimum chain length was estimated to be approximately six glucose units. Dry matter solubility, amount of reducing surgars, pentoses and phenolic groups all showed a positive relationship with dosage. The analytical data indicated that irradiation had a greater measurable action on the cellulose than on the lignin component of the wood. Possible reasons for the results observed are: (a) although the lignin is affected less than the cellulose, it is nevertheless disrupted enough to expose the cellulose; (b) the irradiation breaks a particular bond between lignin and cellulose which rumen bacteria are unable to hydrolyze; (c) natural lignin contains bacteriostatic compounds which are destroyed by irradiation; (d) the irradiation renders the cellulose in wood digestible not by destroying the lignin but by breaking the cellulose micellae themselves, thus exposing to enzyme attack the open ends of the glucose chains.

The effects of cathode rays on sprucewood, isolated cell substance and cotton linters was made by Saeman et al. (1952). They found a decrease in the degree of polymerization in all three substances with an increase in the radiation dosage from $1x10^6$ rads to $1x10^8$ rads. At a dosage of 10^8 rads, 14% of the cotton linters, 17% of the wood pulp, and 9% of the wood were depolymerized. Up to 70% of the carbohydrate portion in Douglas-fir sawdust

could be made soluble by rumen microorganisms depending upon the treatment compared with 1-3% solubilized in untreated wood (Mater, 1957).

The decomposition of wood constituents by different levels of gamma irradiation was studied by Seifert (1964). Based on chromotagraphic and radiographic observations, he concluded that gamma irradiation first led to the decomposition of micelles that were still in their lattice-like order. This was found to be different from enzymatic wood decay where the intermolecular bonds between the chains are attacked initially. Using *in vitro* fermentation techniques, Pritchard *et al.* (1962) investigated the effects of gamma radiation upon the feeding value of wheat straw. Optimum dosage for the release of nutrients from wheat straw was 2.5×10^8 rads.

Kitts *et al.* (1969) evaluated the effect of gamma irradiation on wood cell wall components and *in vitro* DMD and cellulose digestibility using Hemlock sawdust. They found that ADF and lignin fractions of sawdust decreased as the irradiation dosage increased from 0 to 1.46×10^8 rads. The *in vitro* DMD and cellulose digestion showed a steady increase with increasing irradiation which supported the earlier report of Lawton *et al.* (1951).

By measuring *in vitro* DMD, Millett *et al.* (1970) evaluated the effect of various dosages of electron irradiation on aspen and spruce. They reported that

aspen digestion was essentially quantitative assuming that only carbohydrate was solubilized by an electron dosage of 10^8 rads. However, the lignin content of this aspen was 19.5%. They speculated that some lignin-degradation products would be formed at this dosage level. In the case of spruce, the maximum digestibility was only 14% at the highest dosage level (10^8 rads). They concluded that electron irradiation was an ineffective means for enhancing the digestibility of spruce. The low *in vitro* DMD of irradiated spruce could be due to its high lignin content or due to an inhibitory effect of lignin degradation products.

Pigden *et al.* (1969) examined the effect of gamma irradiation on fecal material from sheep fed low quality forages. The *in vitro* volatile fatty acid (VFA) production from these irradiated feces was measured. The results showed that at the highest dosage (2.7×10^8 rads), the VFA concentration of feces from sheep fed timothy was about equal to the concentration of the original feed.

Although the physical and chemical effects of gamma irradiation on plant cell wall constituents are not fully known, most reports reviewed amply supported the concept that low quality forages or woods contain substantial quantities of potential digestible energy which can be irradiated and made available to rumen microorganisms.

MATERIALS AND METHODS

Material Description

Oat and wheat straw were coarsely chopped (about 4 cm) and used in all laboratory silo studies. Wheat straw for the irradiation experiments was reground through a 1 mm screen using a Wiley mill.

Alfalfa stems and leaves remaining from expressing the soluble material in a high pressure Carver Laboratory Press (Model B) formed the starting material. It contained about 30% dry matter (DM) and was stored frozen until needed. The material was oven dried and ground through a 1 mm screen of Wiley mill for the irradiation experiment.

Experimental Procedures and Evaluatory Techniques

I. Laboratory Silo Studies

Relatively small quantities of samples are needed to evaluate the effects of chemical or enzyme treatments of ensiled forages using composition and *in vitro* values as evaluating parameters. For preliminary study, quart or pint Mason jars with a bunsen valve installed in the metal lid may simulate a large silo. The effect of 16

chemical treatments, 6 enzymatic treatments and 3 ensiling conditions were evaluated by using the Mason glass jars as ensiling vessels. The capacity of a 1 quart jar for air dry (93% DM) and coarsely ground (4.0 cm) straw was about 160 grams, for wet alfalfa stems (30% DM) 500 grams.

A. Forages Treated and Ensiled with Chlorine Compounds

Six chlorine compounds were selected and tested for their influence on composition and in vitro values of straws and alfalfa stems. The chemicals and levels of these chemicals used are presented in Table 2. The level of $NaClO_2$ used was primarily based on results reported

Table 2. Levels of Chlorine Compounds Used in Laboratory Silo Studies.

Chemical	$NaClO_2$	NaClO[1]	$Ca(ClO)_2$	$KClO_3$	organic Cl[2]	Cl_2
	---------- g/100 g DM----------					
	2.5, 5	81	6.3	7.1	12.5	6, 12

[1]NaClO (Bleach)- ml/100 g DM

[2]Disodiumdichloro-(S)-triazinetrione

by Goering and Van Soest (1968). Levels for NaClO, $Ca(ClO)_2$, $KClO_3$ and organic Cl were derived from calculations so that these treatments had comparable amounts of available Cl with that of $NaClO_2$ treatment. The amount

of Cl_2 used was selected arbitrarily. Distilled water was used as solvent for chemicals (except for Cl_2) and used to adjust the DM content in resulting products to about 20%. The chemical solution was gradually spread over the straw or stems. After mixing by hand the treated material was transferred to a quart jar and immediately sealed under a CO_2 atmosphere. In the case of Cl_2 treatment DM content of forage was adjusted to 20% by adding water. After thorough mixing, this material was transferred to a plastic beaker placed in a hood into which four sections of glass tubing (0.5 cm diameter) were inserted down the side and to the bottom. These glass tubing sections were attached to the Cl_2 gas cylinder by Y connectors. A sheet of plastic was placed over the container to help contain any escaping Cl_2. The gas cylinder was placed on a nearby balance and the amount of Cl_2 used obtained by weight difference. The treated material was allowed to stand for about five minutes then transferred to a quart jar and sealed. The ensiling processes were at room temperature for 14 days. At the end of the ensiling period one aliquot was taken for pH and VFA analysis and the remainder dried for 72 hours at 85 C. Dried silage was ground in a Wiley mill (1 mm screen) and stored in a sealed glass jar for analysis.

Silage extracts were prepared by homogenizing a 25 gram aliquot of the sample in an Lourdes homogenizer

with 100 ml distilled water for one minute and straining through two layers of cheesecloth and pH determined on the extract. The extract was deproteinized using 3 ml of 50% sulfosalicylic acid (SSA) and 27 ml of extract. The sample was then centrifuged at 12,000 x g for 10 minutes and stored in refrigerator for later analysis. Volatile fatty acid content of the silage was determined by injecting samples of the deproteinized silage fluid into an aerograph model A 600 D, "Hi Fi" gas chromatograph with a flame-ionization detector. VFA concentrations were expressed in micromoles per gram of wet silage.

Neutral detergent fiber (NDF or CWC), acid detergent fiber (ADF), and acid detergent lignin (ADL) values were determined according to the procedures of Goering and Van Soest (USDA Agriculture Handbook No. 379, 1970). Hemicellulose was obtained by substracting the value for ADF from that for CWC. Cellulose was determined by the procedure of Crampton and Maynard (1938). Ash content was determined according to the Association of Official Agricultural Chemists (1960).

Three different in vitro fermentation techniques were used for each sample.

(1) Six and 36 hours dry matter disappearance (DMD). These procedures were well outlined by Allinson (1966) and were based on the work of Bowden and Church (1962),

Baumgardt et al. (1962), and modifications by Ingalls (1964). A 1.0 gram sample was weighed into a 100 ml polyethyene tube. Approximately one hour before the rumen inoculum was to be added, the tube was charged with 20 ml of phosphate-urea-carbonate buffer. Tubes were caped with a stopper and bunsen valve and placed in a water bath at 39 C. Twenty-four ml of settled rumen fluid was added to initiate fermentation. At the end of the fermentation period, a few drops of a 20% thymol solution were added to stop microbial activity. The fermentation mixture was then filtered through a tared filtering crucible and dried at 80 C for 36 hours. At the end of this period, the crucibles were weighed, and the loss of dry matter obtained. The dry matter content of all samples was determined separately. All DMD values were expressed as the percentage of dry matter lost in the fermentation period.

(2) Two-stage 48 hours DMD. This procedure was adapted from that of Tilley and Terry (1963) with modifications were made by various personnel in this laboratory. Quantities of sample, buffer and inoculum were exactly one-half of those used in the former techniques. The fermentation period was 48 hours at 39° C. A 0.9 ml of 6 N HCl and 0.5 ml of 20% pepsin solution were added to stop fermentation and digest microbial protein. Fermentation tubes (50 ml) were then placed back in the water

bath for another 48 hours. Determination of dry matter loss and calculation of DMD values were the same as those for the 6 and 36 hour fermentations.

(3) In vitro digestibility of cell wall (IVDCW). The procedure was based on the report of Van Soest et al. (1966) combining in vitro fermentation and chemical analysis. The forage sample was incubated with rumen fluid and buffer for 48 hours then digested by HCl-pepsin solution for another 48 hours as described by Tilley and Terry (1963) procedure. However, the quantity of forage sample, buffer and inoculum was double that used by Tilley and Terry (1963) procedure. When the total incubation period (96 hours) was completed , fermentation tubes were centrifuged at 12,000 x g for 30 minutes and the supernant decanted. The residue was transferred to a 600 ml beaker using of 100 ml neutral detergent solution. The quantity of CW in the residue was determined by the procedure of Van Soest (1962). The DM and CW content of respective samples was determined separately. The difference in quantity of CW between fermented and unfermented sample was attributed to microbial digestion during the in vitro fermentation. The digestibility of cell wall was expressed as percent of CW in the unfermented sample.

B. Forages Treated and Ensiled with NaOH and NH_4OH Solutions

Wheat straw and alfalfa stems were treated with two levels of NaOH but only wheat straw was treated with two levels of NH_4OH. Treatment conditions are presented in Table 3. Concentrations and ratios of NaOH to DM used were based on values reported by Wilson and Pigden (1964). The DM content of treated and untreated forages, treatment procedures and ensiling conditions, were comparable to those for the chlorine compounds studies. Silage pH, organic acid concentration, composition and *in vitro* values were determined according to the procedures described previously.

Table 3. Quantity, Concentration and Amount of Solution of NaOH and NH_4OH used in Laboratory Silo Studies.

	NaOH and NH_4OH		
	Quantity	Concentration	Solution
	g/100 g DM	%	ml/100 g DM
Wheat straw	6	1.33	400
	2	0.50	400
Alfalfa stems	6	4.00	133
	2	1.54	133

C. Forages Treated and Ensiled with NaOH Solution and Cl_2

Data reviewed indicated that NaOH and Cl_2 gas had different modes of action (Tarkow and Feist, 1969; Dence et al., 1962). Thus a combination of the two was used. Wheat straw and alfalfa stems were soaked in NaOH solution for 24 hours at room temperature then Cl_2 was introduced as described previously. The ensiling processes were carried out at room temperature for 14 days. Resulting silage pH, organic acid concentration, composition and in vitro fermentation values were determined as described previously.

D. Forages Treated and Ensiled with Cellulases and Pectinases

Oat straw and alfalfa stems were used to evaluate the effectiveness of 4 cellulases and 2 pectinases in improving nutritive value. The enzymes and the level of these enzymes used in this study are presented in Table 4. Each enzyme was weighed and then dissolved in about 30 ml distilled water and the solution gradually mixed with the forage. The DM content of oat straw and alfalfa stem mixture was reduced to the level of about 25% by adding 440 and 100 ml distilled water respectively before they were mixed with enzyme solutions. The mixed forage was then transferred to glass quart jar and ensiled at 34 C for 14 days.

Table 4. Levels of Cellulases and Pectinases Used in Laboratory Silo Studies.

		Cellulase				Pectinase	
		HP-150[1]	36[1]	MD-164[2]	H-39[1]	41-p[1]	10-M[1]
		g/100 g DM					
Oat straw		0.6 +	0.6 +	0.6 +	0.6		
Alfalfa stems	1.	0.6 +	0.6 +	0.6 +	0.6		
	2.					1.3 +	1.3
	3.					0.6 +	0.6
	4.	0.6 +	0.6 +	0.6 +	0.6 +	0.6 +	0.6

[1]Obtained from Rohm and Haas Co., Philadelphia, Pa.

[2]Obtained from Wallerstein Co., Marton Grove, Ill.

E. Ensiling Condition Evaluation

Several environmental factors can affect the characteristics of treated and ensiled forages. Some important ones are length of ensiling, ensiling temperature and moisture content. Three trials with two chemical treatments were conducted to evaluate the influence of ensiling time, ensiling temperature and moisture content of silage on composition and *in vitro* values of wheat straw.

1. Ensiling time

The design was a 2x7 factorial with 2 chemicals (NaClO and Cl_2 gas) and 7 times (0, 0.5, 1, 4, 12, 30 hours and 14 days). Sample preparation, chemical levels and treatment procedures were as previously described.

The ensiling temperature was 25 C. In the case of zero time treatment, forage was put into the drying oven immediately after treatment procedures were completed. The oven dried silages were finely ground (1 mm) and composition and in vitro fermentation values determined.

2. Ensiling temperature study

The temperature study was a 3x4 factorial design with 3 treatments (NaClO, Cl_2 and control) and 4 temperatures (-4, 25, 34 and 80 C). Experimental procedures were the same as previously described.

3. Treatment and ensiling moisture content study

The experiment was designed especially for the Cl_2 gas treatment. Eight moisture contents (80, 70, 60, 50, 40, 30, 20, and 10%) were tested. The moisture content of wheat straw was adjusted to the desired level by adding water. The well-mixed material was then treated with Cl_2 at the level of 12 gram per 100 gram of straw DM. A thermometer was inserted at the center of ensiling jar and maximum temperature was recorded. Ensiling temperature and time were 25 C and 14 days respectively.

II. Washing Treated Silages

Animal feeding trials showed that intake of the treated wet silages was low. The simplest means of removing an undesirable taste influencing palatability would be washing with water. Palatability, composition

and nutritive value of treated and washed straws were studied. Two sets of washed samples were used. Those silages made in glass laboratory vessels and those made in barrel silos at the barn. About 6 parts water to 1 part straw w/w was used. A metal pail with a 0.5 cm screen on the bottom and holding approximately 4 kg of silage was used. The water was then poured over the silage and excessive moisture was removed by drainage and pressing the wet silage by hand. For comparison unwashed samples were also collected and analyzed. Variables measured were pH, organic acid concentration, ash content, dry matter recovery, organic matter recovery, chemical composition and in vitro fermentation values. Analysis procedures are given in section IA.

III. In Vitro Fermentations with Silage Extract

Laboratory fermentations used dried and ground silages as substrate. Biologically active compounds could be inactivated during the drying and grinding processes and to test this possibility water extracts from aliquot of 25 grams of unwashed and washed silages were prepared with three different silage to water ratios (1:1, 1:2, 1:4) using a Lourdes homogenizer. Forty-eight hour in vitro fermentation was conducted using standard alfalfa as substrate with 3.0 ml of silage extract added to each fermentation tube along

with normal amounts of buffer and rumen fluid. Three ml of distilled water was added to control tubes. Disappearance of dry matter was quantitated as for the usual Tilley-Terry procedure (1963).

IV. In Vivo Study

In vivo digestibility and animal performance would be the most valid criteria with which treated forages could be evaluated. Wheat straw was treated with $NaClO_2$, NaClO, $Ca(ClO)_2$, Cl_2 gas and NaOH plus Cl_2 gas in sufficient quantities for a limited in vivo feeding trial. A beneficial effect of $NaClO_2$, NaClO, Cl_2 gas and NaOH treatments had been shown in several laboratory silo trials. The reason for evaluating the effectiveness of $Ca(ClO)_2$ treatment in this trial was that a large quantity of $Ca(ClO)_2$ had been purchased before this study began although the result of in vitro fermentation values did not indicate that $Ca(ClO)_2$ would be an effective chemical in improving the nutritive value of straw. For each treatment, a 55 gallon barrel was used as treatment and ensiling vessel. The barrel was lined with a polyethylene bag having the same volume. Wheat straw with similar physical and chemical properties to that used in laboratory silo studies was used. The proportion of straw, chemical level and water used were the same as used in laboratory silos (Table 2). One-third straw by weight was placed in the barrel followed by the addition

of a third of the volume of treatment solution and mixed. This procedure was repeated twice until 55 pounds straw and 207 pounds of solution were used allowing a more uniform treatment of the forage than one mixing. Chlorine was distributed into the bottom of the water or NaOH solution treated straw mixture. As the treatment procedures were completed the bag was sealed, lids placed on the barrels and the barrels occasionally inverted to help distribute the water which tended to accumulate at the bottom. The ensiling processes were at room temperature for 14 days.

Six young goats with an average initial body weight of 30 pounds were fed the six silages. The first trial was a 6x6 Latin square design in which voluntary intake was measured on each silage and weight changes monitored as diet changed every three days. Forty grams of SBOM (50% CP), 100 ml liquid molasses, 1 gram of vitamin mixture (A.D.E.) and 1 gram of trace mineralized salt were well mixed with the fresh silage before feeding. The crude protein level for the complete ration was about 10%. Daily dry matter fed and refused were obtained.

The second trial was subdivided into two periods of about 14 days each. In the first period, the intake and dry matter digestibility of $NaClO_2$, NaClO and $Ca(ClO)_2$ treated wheat straw were determined. Ration preparation

was similar to trial 1 except that all silages were washed before mixing. Dry matter content of washed silage was determined daily. In the second period control and Cl_2 gas treated straws were evaluated. Chlorine gas treated silage was neutralized by NaOH and washed. The neutralization process was done by soaking 15 pounds fresh silage in 3 to 4 gallons of water to which NaOH was added to obtain a neutral solution. This neutralized silage-water mixture was allowed to stand for 24 hours then transferred to the screen pail and washed as described in section II. Because of the poor animal performance observed in the first period, the crude protein level of rations used in period 2 was increased to about 12% by increasing the amount of SBOM to 80 grams and energy was added as 40 grams of a grain mixture (14% CP). In the first and second period feces were collected for 6 days preceded by a 7-day preliminary period. At the end of each period, rumen samples were taken and analyzed for pH and volatile fatty acids (VFA). Animals were weighed at the beginning and at the end of each period.

Rumen samples were strained through 4 layers of cheesecloth and an aliquot used for pH determination using a Beckman pH meter Model G. Ten ml of strained rumen fluid was mixed with an equal volume of 1 $\underline{N}$ H_2SO_4 solution the mixture allowed to stand for 24 hours and

then centrifuged at 12,000 x g for 10 minutes. Rumen VFA concentrations were determined by injecting the clear-supernatant described above into an aerograph model A 600 D, "Hi Fi" gas chromatograph with a flame ionization detector. The peak heights were converted to micromoles per ml and molar percentages by comparison to standard solutions analyzed at the same time.

V. Irradiation Study

Irradiation was chosen as a physical treatment to compare its effectiveness in improving nutritive value of low quality forages with chemical treatments. Two trials were conducted but since the type of radiation and the sample preparations were different the experimental procedures are described separately.

A. Cobalt-Gamma Ray Irradiation

The design was 2x5 factorial with wheat straw using 2 different particle sizes (4 cm and 1 mm) and 5 dosages. Dose, log dose and radiation time are presented in Table 5A. Dosages were somewhat arbitrarily selected but were based on results reported by Lawton et al. (1951). A 50 gram sample was vacuum sealed in a 18x18 cm polyethylene bag. Thickness of the filled bag was about 2 to 3 cm. Samples were placed at the central position of radiation chamber. The radiator delivered a dose of $2x10^6$ rad per hour. The radiation was done in the Food

Table 5A. Dose, Log Dose and Radiation Time Used in the Gamma Ray Radiation Experiment.

Dose Level	Log Dose Level	Radiation Time
------ rad ----------		hr./min.
2.0×10^6	6.30	1/ 0
20.0×10^6	7.30	10/ 0
43.4×10^6	7.64	21/42
96.6×10^6	7.99	48/18
143.0×10^6	8.16	71/30

Science Department at Michigan State University under direction of Mr. George Giddings. After radiation, finely ground samples were placed in labeled glass jars while coarsely ground samples were further ground through a 1 mm screen of Wiley mill then stored in glass jars for later composition and in vitro fermentation analysis.

B. High-Energy Electron Irradiation

Dried (93% DM) and finely ground (1 mm) wheat straw and alfalfa stems were exposed to ten different dosages. Dose, log dose and radiation times are presented in Table 5B. Sample quantity and preparation was the same as those for the gamma ray radiation trial. Samples were placed at a distance of 47 cm from the tube window of the electron beam generator. At this distance the generator, of a resonant transformer type, delivered a dose of 2.97×10^6 rad per minute. The operation was done through the cooperation of Mr. Hock Patrick of the Agricultural

Engineering Department at Michigan State University. Radiated samples were analyzed for composition and *in vitro* DMD and DCW according the procedures described in the section IA.

Table 5B. Dose, Log Dose and Radiation Time Used in the High-Energy Electron Radiation Experiment.

Dose Level	Log Dose Level	Radiation Time
------- rad ----------		hr./min./sec.
1.00×10^6	6.00	0/ 0/20
2.14×10^6	6.33	0/ 0/43
4.57×10^6	6.66	0/ 1/20
10.00×10^6	7.00	0/ 3/20
21.40×10^6	7.33	0/ 7/20
45.70×10^6	7.66	0/15/20
100.00×10^6	8.00	0/33/20
214.00×10^6	8.33	1/53/20
457.00×10^6	8.66	2/53/20
1000.00×10^6	9.00	5/33/20

RESULTS AND DISCUSSION

I. Characterization of Original Materials

Alfalfa stems contained less CWC, ADF, cellulose and more lignin than straws. In vitro rumen fermentation DMD values for three different periods (6, 36 and 48 hr. Tilley-Terry, 1963) were two-fold greater for alfalfa stems than for straws. The in vitro cell wall digestibility was two-fold higher for alfalfa stems than for straws.

Within the species of straws, wheat straw contained lesser amounts of lignin and more CWC and hemicellulose than did oat straw. The ADF and cellulose content of both straws were similar. Wheat straw was more fermentable in vitro than oat straw. The in vitro cell wall digestibility for wheat straw was about two-fold higher than the value for oat straw.

II. Characterization of Silage

pH, organic acid concentration, ash content, recovery of dry matter (DM) and organic matter in the treated and ensiled wheat straw and alfalfa stems are presented in Tables 6 and 7. Chlorine treatment produced a very low pH, even when combined with NaOH treatment.

Table 6. pH, Organic Acid Concentration, Ash Content, Recovery of Dry Matter (DM) in Treated and Ensiled Wheat Straw.

	$NaClO_2$	NaClO (Bleach)	$Ca(ClO)_2$	Organic Cl	Cl (12%)	Cl (6%)	NaOH (6%)	NaOH (2%)
pH	5.7	5.5	7.0	5.4	1.4	1.6	7.0	5.1
Acetate (umoles/g)	0.36	0.90	3.02	0.25	7.38	6.51	118.37	92.81
Butyrate (umoles/g)							24.42	13.92
DM recovery (%)	109	114	111	117	103	102	97	99
Ash (% of DM)	8.3	12.4	9.4		4.2			
Organic matter recovery (%)	103	104	104		102			

	NaOH (6%)+ Cl_2 (12%)	NaOH (6%)+ Cl_2 (6%)	Control
pH	1.5	2.5	4.2
Acetate (umoles/g)	9.02	80.23	0.13
Butyrate (umoles/g)			
DM recovery (%)	114	109	96
Ash (% of DM)	11.9		3.3
Organic matter recovery (%)	104		

Table 7. pH, Organic Acid Concentration, Ash Content, Recovery of Dry Matter (DM) in Treated and Ensiled Alfalfa Stems.

	$NaClO_2$	NaClO (Bleach)	$Ca(ClO)_2$	Organic Cl	Cl_2 (12%)	Cl_2 (6%)	NaOH (6%)	NaOH (2%)	NaOH (6%)+ Cl_2 (12%)
pH	4.9	5.2	5.0	4.4	1.2	1.8	5.7	5.2	4.1
Acetate (umoles/g)	0.68	0.60	1.97	0.55	6.68	5.33	112.56	33.38	5.92
Butyrate (umoles/g)							88.42	73.30	
DM recovery (%)	107	116	122	118	107	106	99	102	87

	NaOH (2%) + Cl_2 (12%)	Cellulase (2.6%)	Pectinase (2.6%)	Pectinase (1.3%)	Cellulase (2.6%) + Pectinase (1.3%)	Control
pH	1.8	3.7	3.5	3.5	3.6	3.6
Acetate (umoles/g)	5.80	3.39	4.42	4.33	4.03	4.03
Butyrate (umoles/g)						
DM recovery (%)	84	97	97	95	94	94

Sodium hydroxide treatment alone, surprisingly, gave a neutral pH, indicating that considerable amounts of organic acids were produced during the ensiling period. Acid production was positively related to the level of NaOH used. Sodium chlorite ($NaClO_2$), bleach (NaClO), $Ca(ClO)_2$ and organic Cl treatments produced a similar pH of about 5 for both forages. Alfalfa stems when treated with enzymes had a slightly lower pH than control silage (3.5 vs. 4.4). Organic acid concentration indicated that a very limited fermentation occurred in forages treated with chlorine compounds.

Dry matter recovery of straws and alfalfa stems treated with NaOH, and enzymes were similar to that for control silages. All chlorine treated silages showed a higher DM recovery than did controls. Calculations indicated that the higher dry matter recovery was due to the added inorganic chemicals. Organic matter recovery was high and similar among the chlorine compound treated straws.

In summarizing these observations, the following points can be made: (1) The extremely low pH produced by Cl_2 treatment may affect *in vivo* silage intake and microbial growth during ensiling; (2) For most treatments, prolonged ensiling time did not result in increased amounts of acids produced. However, in the case of NaOH treatments, large amounts of organic acids were produced.

Such acid production might indicate that the insoluble complex carbohydrate fractions of original materials became available to microorganisms after NaOH treatment; (3) Organic matter recovery was satisfactory for most treatments.

III. Laboratory Silo Studies

A. Wheat Straw

The composition of chemically treated wheat straw is presented in Table 8. The apparent and quantitative changes of each constituent are present in Table 9A. The difference between the value of apparent and quantitative changes is due to the percent of dry matter recovery for any particular treatment.

Sodium chlorite treatment reduced apparent lignin by about 50 percent but only slightly reduced other fibrous constituents. Chlorine treatment very effectively reduced CWC (28%) and hemicellulose (78%). Surprisingly, Cl_2 treatment produced little reduction in apparent or quantitative change in lignin. Alkaline treatments apparently did not cause marked changes in any of the fibrous constituents except perhaps solubilization of hemicellulose when 6 g NaOH/100 g straw was used. In fact, these treatments considerably increased the lignin content of the resulting product. Similar results were reported by Ololade *et al.* (1970). Changes

Table 8. Composition of Wheat Straw Treated and Ensiled With Given Materials.

Treatment	CWC[1]	ADF[2]	Lignin	Cellulose	Hemi-cellulose	N
	----------% DM----------					
$NaClO_2$	78.1±0.3[3]	50.8±0.2	3.6±0.2	45.4±0.1	27.7	2
NaClO(bleach)	76.4±0.5	51.4±0.5	6.8±0.2	43.4±0.4	25.0	4
$Ca(ClO)_2$	80.9±0.1	53.8±0.2	6.7±0.3	44.8±0.1	27.1	2
$KClO_3$	79.8±0.4	50.4±0.4	5.4±0.4	43.3±0.1	26.0	2
Organic Cl	72.7±0.2	47.5±0.1	5.5±0.3	42.6±0.1	28.2	2
Cl_2(12%)	59.8±0.6	53.7±0.3	6.2±0.2	48.5±0.1	6.1	4
Cl_2(6%)	61.6	55.7	7.9	49.8	5.9	1
NaOH(6g/100 g)	75.3±1.4	59.1±1.3	9.9±0.5	49.1±0.2	16.2	2
NaOH(2g/100 g)	84.5±0.5	58.8±0.8	9.6±0.3	49.1±0.2	25.6	2
NH_4OH(6%)	85.3	58.1	9.1	47.4	27.2	1
NH_4OH(2%)	86.7	57.9	8.2	47.3	28.9	1
NaOH(6%) + Cl_2(12%)	54.0±0.4	49.6±0.1	6.0±0.3	46.7±0.3	4.9	2
NaOH(6%) + Cl_2(6%)	53.4	42.0	7.7	44.2	9.4	1
NaOH(2%) + Cl_2(12%)	57.4	51.6	5.8	48.0	5.7	1
NH_4OH(6%) + Cl_2(12%)	60.2	53.7	6.6	48.2	6.6	1
NH_4OH(2%) + Cl_2(12%)	58.9	52.6	5.9	46.7	6.3	1
Control	84.2±0.4	56.8±0.4	7.9±0.3	47.3±0.3	27.9	4

[1]CWC = cell wall constituents

[2]ADF = acid detergent filer

[3]= ± standard error mean

Table 9A. The Apparent and Quantitative Changes of Fibrous Constituents in Treated and Ensiled Wheat Straw.

Treatment	CWC		ADF		Lignin		Cellulose		Hemi-Cellulose	
	A	Q	A	Q	A	Q	A	Q	A	Q
$NaClO_2$	-7.2	+5.4	-10.6	+1.5	-55.0	-49.0	-4.0	+9.0	-0.8	+12.6
NaClO(Bleach)	-9.3	+7.7	-9.5	+7.4	-15.0	+1.0	-8.1	+9.1	-10.4	+6.4
$Ca(ClO)_2$	-3.9	+11.1	-5.3	+9.5	-16.0	-2.9	-5.2	+9.5	-2.9	+12.2
$KClO_3$	-8.8	-3.1	-11.2	-5.7	-31.6	-27.3	-8.3	-2.5	-7.1	-1.3
Organic Cl	-13.6	+5.3	-16.3	+2.0	-30.2	-15.0	-9.9	+9.8	+1.0	+23.1
Cl_2(12%)	-28.9	-23.8	-5.5	+1.4	-21.5	-15.7	+2.6	+10.1	-78.1	-76.5
Cl_2(6%)	-26.9	-22.2	-2.0	+4.2	-0.3	+6.0	+5.4	+11.9	-79.0	-77.6
NaOH(6%)	-10.6	-9.6	+4.0	+5.0	+25.1	+26.4	+3.9	+4.9	-41.9	-41.3
NaOH(2%)	+0.4	+3.5	+3.5	+6.7	+20.4	+24.1	+3.9	+7.1	-8.4	-5.6
NH_4OH(6%)	+1.3	+0.2	+2.2	+1.1	+14.0	+12.9	+0.03	+0.7	-2.6	-3.7
NH_4OH(2%)	+3.0	+3.0	+1.8	+1.8	+3.4	+3.4	0.0	0.0	+3.3	+3.3
NaOH(6%) + Cl_2(12%)	-35.9	-23.9	-12.7	+3.7	-23.9	-9.6	-1.2	+17.3	-82.6	-79.3
NaOH(6%) + Cl_2(6%)	-36.6	-28.0	-8.6	+3.8	-2.6	+10.6	-6.4	+6.3	-66.2	-61.7
NaOH(2%) + Cl_2(12%)	-31.9	-31.2	-9.1	-8.2	-26.7	-25.9	+1.7	+2.7	-79.5	-79.2
NH_4OH(6%) + Cl_2(12%)	-28.4	-25.5	-5.5	-1.6	-17.0	-13.5	+2.1	+6.3	-76.5	-75.5
NH_4OH(2%) + Cl_2(12%)	-30.0	-30.8	-7.4	-8.4	-26.2	-26.9	-1.2	-2.2	-77.4	-77.6

A = Apparent change = % of constituent in original forage minus percent in ensiled material x 100 ÷ by original percentage.

Q = Quantitative change = Total quantity of that constituent in original forage minus quantity after ensiling x 100 ÷ initial total quantity.

in composition by combined treatment of dilute alkali and chlorine were similar to those of chlorine gas treatment alone. Organic chlorine treatment was fairly effective in reducing all fibrous components except hemicellulose. However, in quantitative changes, potassium chlorate was more effective in reducing lignin and ADF than was organic chloride. Bleach (NaClO), $Ca(ClO)_2$, and NH_4OH treatments showed the least effectiveness in reducing fibrous constituents among these sixteen treatments.

Quantitative effects of these chemical treatments are presented in Table 9A. Some chemical constituents showed a net increase after treatment. This increase might be due to improper handling or analysis.

Nutritive value estimations based on chemical composition and *in vitro* values of treated wheat straw is presented in Table 10. The ratio of lignin to acid detergent fiber (ADF) is the best predictor of CW digestibility of normal forages (Van Soest and Wine, 1965). This value is negatively related to CW digestibility. In this study, only $NaClO_2$ treatments produced a marked decrease in L/A ratio (50%). About a 26% reduction was noted for $KClO_3$, Cl_2 and organic Cl treatments. This ratio was not reduced by the alkali treatments. The consequences of these changes were clearly reflected in the calculated values for CW digestibility. *In vitro* cell wall digestibilities were

Table 9B. The Apparent and Quantitative Changes of *In Vitro* Dry Matter Disappearance (IVDMD) at Three Fermentation Periods of Treated and Ensiled Wheat Straw.

Treatment	6 hr. DMD		36 hr. DMD		T.T. 48 hr. DMD	
	A	Q	A	Q	A	Q
$NaClO_2$	+46.6	+66.4	+66.4	+89.0	+46.4	+66.2
NaClO(Bleach)	+124.9	+167.0	+54.2	-7.8	+41.4	+67.9
$Ca(ClO)_2$	-18.9	-6.3	-26.6	-15.2	+5.3	+21.7
$KClO_3$	+88.4	+100.1	+37.6	+46.2	+15.0	+22.1
Organic Cl	+128.5	+178.3	+43.3	+74.7	+28.5	+56.6
Cl_2(12%)	+190.9	+211.9			-4.0	+2.9
Cl_2(6%)	+109.9	+122.9			-6.0	-0.2
NaOH(6%)	+86.4	+88.3			+72.4	+74.1
NaOH(2%)	-8.7	-5.9			+13.3	+16.8
NH_4OH(6%)	-4.6	-5.6			+2.4	+1.3
NH_4OH(2%)	-33.8	-33.8			-6.8	-6.8
NaOH(6%) + Cl_2(12%)	+232.3	+294.4			+18.2	+40.3
NaOH(6%) + Cl_2(6%)	+152.2	+186.3			+10.6	+25.5
NaOH(2%) + Cl_2(12%)	+174.3	+177.0			+4.0	+5.1
NH_4OH(6%) + Cl_2(12%)	+149.2	+159.5			-11.4	-7.8
NH_4OH(2%) + Cl_2(12%)	+176.0	+173.0			-5.6	-6.6

A = Apparent change = % IVDMD of original forage minus percent IVDMD of ensiled material x 100 ÷ original percentage.

Q = Quantitative change = Total quantity of dry matter disappearance *in vitro* fermentation of original forage minus quantity of ensiled material x 100 ÷ initial total quantity.

Table 10. Several Nutritive Value Estimations of Chemically Treated Wheat Straw.

Treatment	CW Digestibility Predictors			DM Digestibility Predictors				Intake Predictors	Total CW Digestion		
	L/A[1]	D_{CWC}[2]	IVD_{CWC}[3]	ETD[4]	IV ETD[5]	36 hr. DMD	T.T. 48 hr. DMD	6 hr. DMD	Chem Soly[6]	Bact Dig.[7]	Sum Dig.[8]
	--------%----------			----------%----------				%	-g/100 g original CW-		
$NaClO_2$	7.3	80	33	84	47	38.2	37.9	13.7	-5.4	34.7	29.3
Bleach	13.1	59	22	68	40	35.4	36.6	21.0	-7.7	23.3	15.6
$Ca(ClO)_2$	12.4	61	25	68	39	16.8	27.2	7.6	-11.1	27.5	16.4
$KClO_3$	10.7	66	24	73	39	31.6	29.7	17.6	3.1	23.2	26.3
Organic Cl	11.7	63	19	73	41	32.9	33.2	21.3	-5.3	20.0	14.7
Cl_2(12%)	11.6	64	2	77	40	----	24.8	27.1	23.8	1.1	24.9
Cl_2(6%)	14.2	57	7	73	42	----	24.3	19.6	22.2	5.4	27.6
NaOH(6%)	16.8	50	45	62	58	----	44.6	17.4	9.6	40.6	50.2
NaOH(2%)	16.3	52	32	59	42	----	29.3	8.5	-3.5	32.6	29.1
NH_4OH(6%)	15.6	54	--	60	--	----	26.5	8.9	----	----	----
NH_4OH(2%)	14.2	57	--	62	--	----	24.1	6.2	----	----	----
NaOH(6%) + Cl_2(12%)	12.2	62	5	79	48	----	30.6	31.0	23.9	3.7	27.6
NaOH(6%) + Cl_2(6%)	14.9	55	11	75	52	----	28.6	23.5	28.0	7.9	35.9
NH_4OH(6%) + Cl_2(12%)	12.3	61	--	76	--	----	22.9	23.3	----	----	----
NH_4OH(2%) + Cl_2(12%)	11.1	65	--	79	--	----	24.4	25.8	----	----	----
Control	15.3	55	24	62	36	22.9	25.9	9.3	0.0	24.0	24.0

[1] L/A = 100 x Lignin (%)/Acid detergent fiber (%) - (Van Soest and Wine, 1965, J. An. Sci 74:814).

[2] D_{CWC} = 181-96.6 log (Lx100/ADF) (Deinum and Van Soest, 1969, J. Agric. Sci. 17:119).

[3] IVD_{CWC} = In vitro cell wall digestibility = 100x ($\frac{\text{1-undigested CWC}}{\text{CWC in sample}}$) (Deinum and Van Soest, 1969, J. An. Sci. 17:119).

[4] ETD = estimated true digestibility = 0.98 CC+CWC (1.81 = 0.966 log (Lx100/ADF). (Deinum and Van Soest, 1969).

[5] IVETD = in vitro estimated true digestibility = 0.98 CC+CWC (IVD_{CWC}) (Deinum and Van Soest, 1969).

[6] Chem Soly = (Chemical Solubilized) = 100x (Amount CW in original material - amount CW in product)/(Amount CW in original material) (Smith et al., 1969).

[7] Bact. Dig. = (Bacteria Digested) = 100x (amount CW in product x IVD_{CWC})/(Amount CW in original material. (Cornell University Conference on Agriculture, New York).

[8] Sum of 6 and 7 (Cornell University Conference on Agriculture, New York).

obtained by measuring the amount of undigested CW in the sample after a 24 hr. incubation with buffered rumen fluid. Data in Table 10 indicates that these values were generally much lower than those for calculated CW digestibilities (Van Soest, 1965). This difference for forages was 55 vs. 24%. For example, Van Soest's formula indicated an 18% increase in CW digestibility of Cl_2 gas treated straw but the *in vitro* fermentation method gave a value twelve-fold lower than that for the control. On the other hand, NaOH treatment increased the *in vitro* CW digestibility about two-fold but the calculated value showed no improvement.

Estimated true digestibility (ETD) was calculated from forage composition by using CWC, ADF and lignin values in equations proposed by Van Soest and Wine (1965). These values indicate that all treatments, except alkali treatments, improved straw digestibility with the greatest improvement of 35% (-control minus treatment X 100 ÷ control) which was noted for $NaClO_2$ treatment. This value was followed by those for NaOH (6%) plus Cl_2 (12%), NaOH (2%) plus Cl_2 (12%), NH_4OH (2%) plus Cl_2 (12%), Cl_2 gas (12%), Organic Cl and $KClO_3$ treatments which gave improvements of 27, 27, 27, 24, 17, and 17% respectively.

In vitro estimated true digestibility (IVETD) calculated from values of CW and *in vitro* CW digestibility also showed that all treatments improved estimated true

digestibility. However, in contrast to values of ETD, IVETD showed that the most effective treatment for improving estimated true digestibility was (6%) NaOH (+ 61%).

Similar to the results for *in vitro* CW digestibility, the *in vitro* 48 hr DMD method also predicted that the most effective treatment for improving dry matter digestibility of wheat straw was NaOH (6%) (Tables 9B and 10). A 72% increase (Table 9B) in percent DMD was found with this treatment. This value was followed by those for $NaClO_2$ (46%), NaClO (41%) and organic Cl (29%) treatments. The large improvement in *in vitro* 48 hr. DMD and *in vitro* CW digestibility observed for NaOH treatment can probably be explained, in part, by increased water solubility; solubilization of hemicellulose; swelling of the cell; or possibly by alternating certain lignin-carbohydrate bonds (Ololade *et al.*, 1970; Sullivan *et al.*, 1960; Tarkow and Feist, 1969). Another possible explanation is that NaOH treatment solubilized the forage silica more than did other treatments. Van Soest (1967) has shown that silica can be an important factor in depressing forage digestibility.

Long-term *in vitro* fermentation (48 hr.) showed very low values of DMD or IVDCW for Cl_2 treated straw though rather high values were calculated from chemical constituents for Cl_2 treated straws. The reason for this discrepancy is not clear. However, some possible explanations could be: (1) there is no more available cell

wall constituents for bacterial degradation after straw was treated with Cl_2, or (2) a toxic or microbial growth inhibiting compound was formed when straw was treated with Cl_2 gas.

There was no significant correlation between any two dry matter digestibility prediction methods. This fact indicates that unusual forages were probably produced by some treatments; since in normal forages, these evaluation methods are correlated to each other. Reasons for discrepancies among different prediction methods may be clarified when sufficient *in vivo* data become available on these modified forages. However, $NaClO_2$ treatment improved digestibility of wheat straw by both chemical and *in vitro* evaluation methods.

Chlorine treatment and treatments involving Cl_2 markedly increased (three-fold) *in vitro* 6 hr. DMD (Table 10). A two-fold increase was found for organic Cl and NaClO treatments. In normal forages, 6 hr. DMD has been shown to be highly correlated with animal intake (Ingalls, 1964). If this relationship is also valid for chemically treated forages, then Cl_2 treatment should markedly improve the intake of straw.

The equations developed by Smith *et al.* (1969) for total CW digestion calculation are primarily for modified forages. NaOH (6%) treatment increased the total CW digestion by two-fold. This increase was mainly due to

improved bacterial CW digestion. Some improvement was noted for NaOH (6%) plus Cl_2 (12%), $NaClO_2$ and $KClO_3$ treatments, while NaClO, $Ca(ClO)_2$, Organic Cl and Cl_2 treatments showed no improvement.

Figure 1A shows DMD values of wheat straw treated with six chlorine compounds at three in vitro fermentation periods (6, 36 and 48 hr.). The following conclusions can be made: (1) The rates of DMD for organic Cl, NaClO, $KClO_3$ treated straws were above and parallel with controls; (2) Cl_2 gas treated straw showed a negative slope; and (3) $Ca(ClO)_2$ treatment was generally ineffective in improving the value of wheat straw.

To specifically determine treatment effect on in vitro dry matter disappearance degraded by bacteria, it may be necessary to adjust the values by eliminating the solubility component. When this was done for each treatment (Figure 2B), a comparison with control values indicated actual enhancement of rumen microbial digestibility which occurred when treated with $NaClO_2$ and $Ca(ClO)_2$. However, using this method of analysis NaClO, $KClO_3$ and organic Cl exhibited no improvement of microbial digestibility above the control. In addition chlorine gave a decrease in digestibility when compared to the control values.

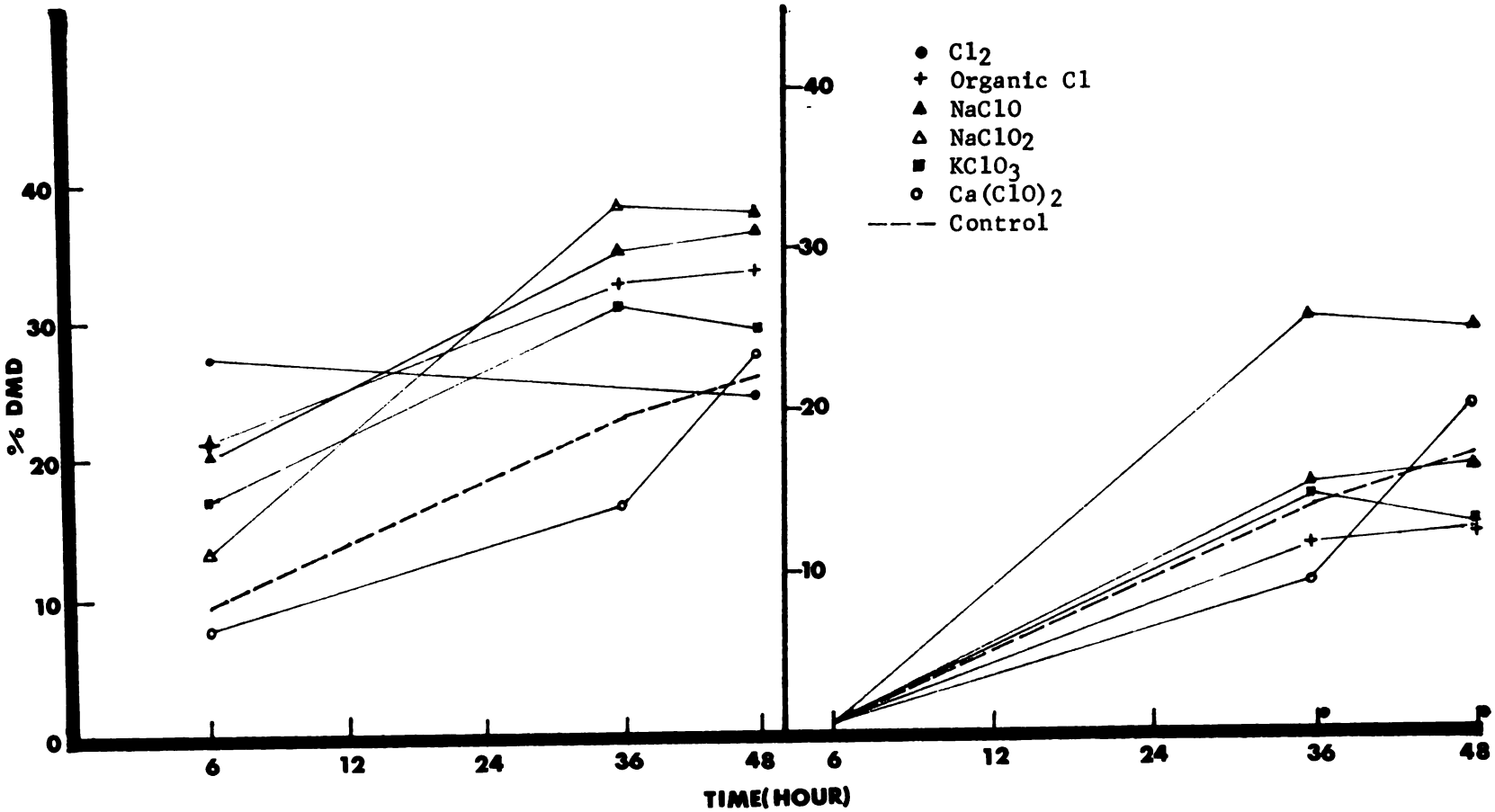


Figure 1A. *In Vitro* DMD of Wheat Straw Treated with Various Chemicals.

Figure 1B. *In Vitro* Insoluble DMD of Wheat Straw Treated with Various Chemicals. Values were Adjusted to Zero at 6 Hrs.

B. Oat Straw

Six chlorine compounds and cellulase were used with oat straw. In general, oat straw showed responses to chlorine compounds similar to those shown by wheat straw. The chemical composition of treated oat straw is presented in Table 11. The apparent and quantitative changes of chemical constituents and *in vitro* DMD are presented in Table 12A and 12B. Sodium chlorite treatment reduced lignin content more than did other treatments (36% vs 15 to 27%). It also reduced ADF and cellulose about 11%. Chlorine treatment reduced CWC, lignin and hemicellulose by 27, 23 and 72% respectively. $KClO_3$, $Ca(ClO)_2$ and NaClO treatments reduced all fibrous constituents to some extent. $Ca(ClO)_2$ was the least effective chlorine treatment in reducing fibrous constituents in oat straw. Cellulases treatment did not affect the chemical composition of oat straw.

Estimations of nutritive value for treated oat straw are presented in Table 13 along with quantitation of cell walls digested *in vitro*. Treatments with chlorine compounds improved the estimated true digestibility (ETD) of oat straw. Cl_2 treatment improved digestibility by 26%. IVETD values indicated that all treatments improved true digestibility. The magnitude of improvement was 51, 41, 41, 39, 34, 31 and 10% for $NaClO_2$, NaClO, organic Cl, Cl_2, $Ca(ClO)_2$, $KClO_3$ and cellulase treatments,

Table 11. Composition of Treated and Ensiled Oat Straw with Several Materials.

Treatment	CWC	ADF	Lignin	Cellulose	Hemi-cellulose	N
	----------%DM----------					
$NaClO_2$	74.2±0.2[1]	51.8±0.6	6.9±0.4	41.9±0.5	21.5	3
NaClO(Bleach)	70.1±0.8	51.4±0.7	8.7±0.4	41.0±0.6	17.2	3
$Ca(ClO)_2$	74.0±0.9	53.1±0.9	9.2±0.4	42.3±0.6	21.1	2
$KClO_3$	72.0	49.9	7.7	41.3	15.4	1
Organic Cl	69.2±0.3	49.1±0.9	8.6±0.3	38.8±0.4	20.1	3
Cl_2(12%)	58.5±1.8	54.0±0.3	8.2±0.2	44.9±0.7	6.2	3
Cellulase	81.0±0.2	57.9±0.7	10.9±0.9	47.2±0.9	23.0	2
Control	80.4±0.3	58.1±0.3	10.8±0.4	47.9±1.7	22.3	4

[1] ± Standard error mean.

Table 12A. The Apparent and Quantitative Changes of Fibrous Constituents in Treated and Ensiled Oat Straw.

Treatment	CWC		ADF		Lignin		Cellulose		Hemi-Cellulose	
	A	Q	A	Q	A	Q	A	Q	A	Q
$NaClO_2$	-7.9	-5.9	-10.7	-8.9	-35.6	-34.3	-12.4	-10.6	-3.8	-1.8
NaClO(Bleach)	-12.9	-4.7	-11.5	-3.2	-19.2	-11.6	-14.5	-6.4	-22.9	-15.7
$Ca(ClO)_2$	-8.0	-2.3	-8.6	-2.9	-14.6	-9.3	-11.8	-6.2	-5.6	+0.3
$KClO_3$	-10.5	-4.9	-14.2	-8.8	-28.6	-24.2	-13.8	-8.4	-31.0	-26.7
Organic Cl	-13.9	-3.1	-15.4	-4.8	-20.5	-10.6	-19.0	-8.8	-10.0	+1.3
Cl_2(12%)	-27.2	-27.2	-7.0	-7.0	-23.4	-23.4	-6.3	-6.3	-72.2	-72.2
Cellulase	+0.7	+0.7	-0.2	-0.2	+1.2	+1.2	-1.5	-1.5	+3.1	+3.1

A = Apparent change (Calculations are described in 9A).

Q = Quantitative change (Calculations are described in 9A).

Table 12B. The Apparent and Quantitative Changes of In Vitro Dry Matter Disappearance at Three Fermentation Periods of Treated and Ensiled Oat Straw.

Treatment	6 hr. DMD		36 hr. DMD		T.T. 48 hr. DMD	
	A	Q	A	Q	A	Q
$NaClO_2$	+138.1	+143.0	+99.6	+103.7	+76.2	+79.9
NaClO(Bleach)	+175.4	+201.0	+75.0	+91.4	+63.4	+78.7
$Ca(ClO)_2$	+3.6	+10.0	+8.2	+15.0	+45.0	+54.1
$KClO_3$	+134.7	+149.2	+51.0	+60.5	+51.0	+60.5
Organic Cl	+204.6	+242.3	+74.0	+95.7	+55.5	+75.0
Cl_2(12%)	+220.4	+220.4	+37.1	+37.1	+10.3	+10.3
Cellulase	+8.9	+8.9	-2.3	-2.3	-7.2	-7.2

A = Apparent change (Calculations are described in Table 9B).

Q = Quantitative change (Calculations are described in Table 9B).

Table 13. Several Nutritive Value Estimations of Chemically Treated Oat Straw.

Treatment	CW Digestibility Predictors			DM Digestibility Predictors				Intake Predictor 6 hr. DMD	Total Cell Wall Digestion		
	L/A[1]	D_{CWC}[2]	IVD_{CWC}[3]	ETD[4]	IVETD[5]	36 hr DMD	T.T. 48 hr DMD		Chem[6] Soly.	Bact[7] Dig.	Sum[8] Dig.
	--------%----------			----------%----------				%	--g/100 g original CW------		
$NaClO_2$	14.3	57	26	68	44	35.6	38.4	15.2	5.9	24.1	30.0
Bleach	17.8	48	16	63	41	31.2	35.6	17.5	4.7	15.3	20.0
$Ca(ClO)_2$	17.6	49	18	62	39	19.3	31.6	6.6	2.3	18.0	20.3
$KClO_3$	15.4	54	15	66	38	26.9	32.9	15.0	4.9	14.5	19.4
Organic Cl	17.4	49	15	64	41	31.0	33.9	19.4	3.1	14.7	17.8
Cl_2(12%)	15.3	54	- 2	72	40	24.4	24.0	20.4	27.2	- 1.5	25.8
Cellulase	18.8	46	16	56	32	17.4	20.2	6.9	- 0.7	16.5	15.8
Control	18.5	47	13	57	29	17.8	21.8	6.4	0.0	12.7	12.7

1, 2, 3, 4, 5, 6, 7, 8 See Table 10.

respectively. Values for *in vitro* 48 hr. DMD of treated oat straw were similar to those of treated wheat straws. However, since the control value of oat straw was 4% units lower than the control value of wheat straw, greater improvement was noted for treated oat straw. *In vitro* 48 hr. DMD ranked the effectiveness of treatments in a similar pattern to that found for IVETD for improving nutritive value.

Values of *in vitro* 6 hr. DMD of treated oat straw were comparable with those for wheat straw. Chlorine and organic Cl treatments gave a three-fold increase. A two-fold increase was noted for $NaClO_2$, NaClO and $KClO_3$ treatments, while $Ca(ClO)_2$ and cellulase treatments improved *in vitro* 6 hr. DMD only slightly.

Sodium chlorite and Cl_2 treatments increased total CW digestion of oat straw two-fold. A 1.67-fold improvement followed treatment with NaClO and $Ca(ClO)_2$ treatments. The increase in total digestion was due mainly to improved *in vitro* bacterial CW digestion with the exception of Cl_2 gas treatment.

Figure 2A shows the trends of *in vitro* DMD of oat straw treated with six chlorine compounds at three fermentation periods (6, 36 and 48 hr.). Generally, the *in vitro* response of oat straw to treatments was comparable with that of wheat straw. At the 6 hr. fermentation period, Cl_2 and the other four treatments

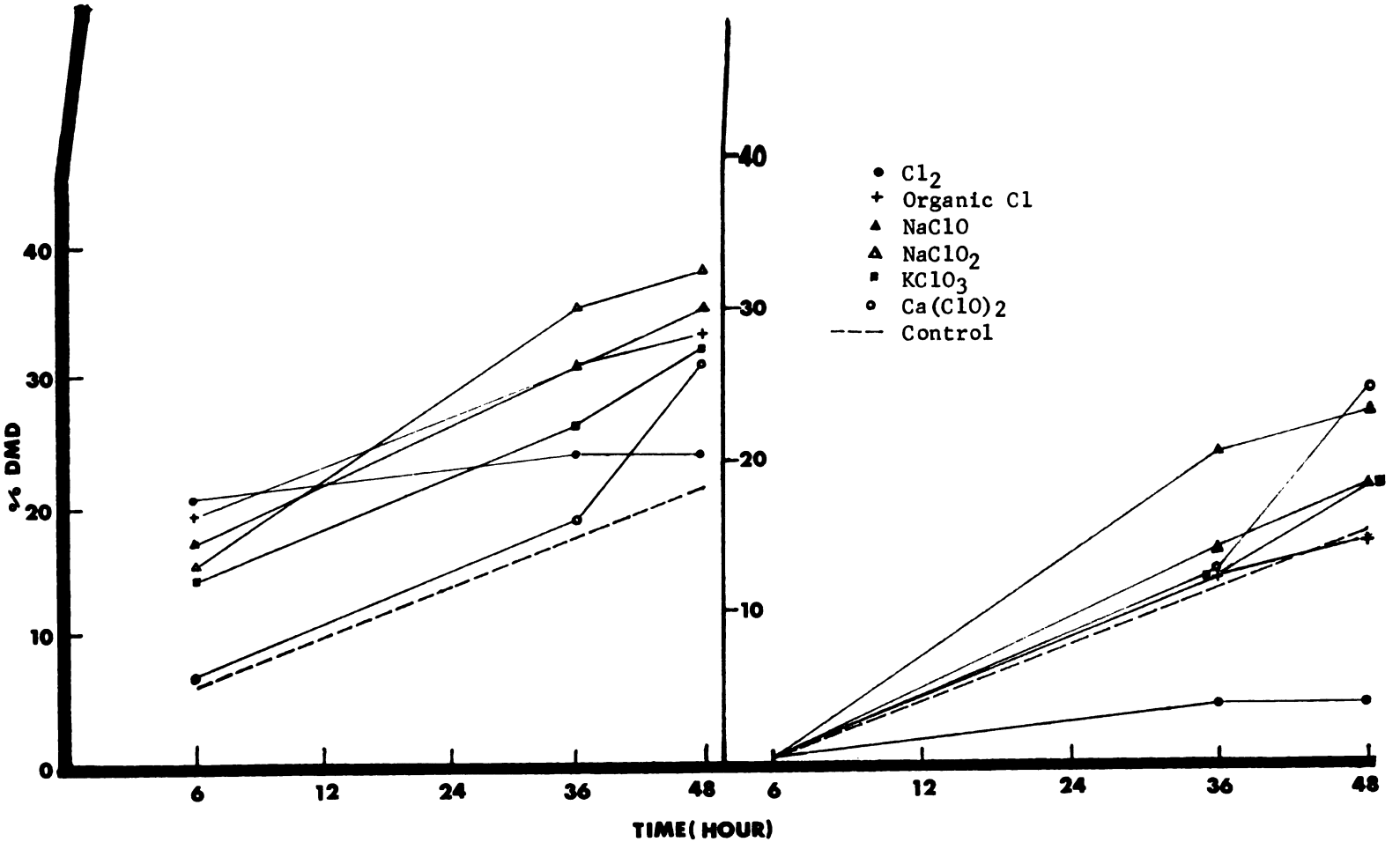


Figure 2A. In Vitro DMD of Oat Straw Treated with Various Chemicals.

Figure 2B. In Vitro Insoluble DMD of Oat Straw Treated with Various Chemicals. Values were adjusted to zero at 6 hrs.

increased the percentage of dry matter disappearance of oat straw markedly while $Ca(ClO)_2$ treatment showed no improvement. This result was not surprising since composition analysis indicated that Cl_2 treatment solubilized a large amount of CW and hemicellulose while $Ca(ClO)_2$ treatment did not reduce any fibrous constituents to a marked extent. Figure 2B shows the trend of *in vitro* insoluble dry matter digestibility of oat straw with and without chemical treatments at 3 different times. The influence of chemical treatments on availability of insoluble component of oat straw to rumen microorganism and/or their activity was similar to that of wheat straw. $Ca(ClO)_2$ and $NaClO_2$ treatments increased the insoluble dry matter disappearance. Organic Cl, NaClO and $KClO_3$ treatments had slight effect on *in vitro* insoluble dry matter disappearance of oat straw. However, Cl_2 treatment had a significant negative effect on the *in vitro* insoluble dry matter disappearance of oat straw.

C. Alfalfa Stems

Two trials were conducted utilizing alfalfa stems. Sources of alfalfa stem residues were different, hence the results are presented separately. Table 14 and 15 show the composition of treated alfalfa stems for trial 1 and 2, respectively. The control alfalfa stems used in trial 2 were greater in fibrous constituents than were stems for trial 1. Apparent and quantitative changes of

Table 14. Composition of Treated Alfalfa Stems (Trial I).

Treatment	CWC	ADF	Lignin	Cellulose	Hemi-cellulose
	----------%DM----------				
$NaClO_2$(5%)	47.2	35.5	6.1	31.0	11.7
NaClO(Bleach)	52.0	36.5	7.8	28.3	15.5
$Ca(ClO)_2$	54.6	39.5	8.8	31.2	15.2
Organic Cl	46.1	31.5	5.8	27.4	14.7
$KClO_3$	49.8	35.7	6.2	29.7	14.0
Cellulase(2.6%)	41.5	36.1	8.4	25.3	5.4
Cellulase(2.6%) + $NaClO_2$(2.5%)	45.1	35.0	6.0	28.8	10.2
$NaClO_2$(2.5%)	50.1	38.2	7.4	27.5	11.9
Control	56.4	44.3	9.5	33.6	12.1

Table 15. Composition of Treated Alfalfa Stems (Trial II).

Treatment	CWC	ADF	Lignin	Hemi-cellulose
	%DM			
$NaClO_2$	54.8	39.5	9.7	15.3
NaClO(Bleach)	56.1	40.1	11.1	16.0
$Ca(ClO)_2$	60.2	42.3	11.3	17.9
Organic Cl	55.3	38.8	10.1	16.5
Cl_2(12%)	39.7	39.1	7.1	0.6
Cl_2(6%)	43.2	40.4	9.2	2.8
NaOH(6%)	62.7	49.2	13.4	13.5
NaOH(2%)	65.4	50.5	12.9	14.9
NaOH(6%) + Cl_2(12%)	65.7	41.7	9.2	24.0
NaOH(2%) + Cl_2(12%)	45.0	40.5	7.4	4.5
Cellulase(2.6%)	55.7	47.3	13.6	8.4
Pectinase(2.6%)	51.2	41.0	13.9	10.2
Pectinase(1.3%)	54.3	43.5	14.2	10.8
Cellulase(1.3%) + Pectinase(1.3%)	50.6	42.7	13.8	7.9
Control	68.5	48.2	15.5	20.2

fibrous constituents and *in vitro* DMD are presented in Tables 16A, 16B, 17A and 17B for trials 1 and 2, respectively. Generally, all treatments caused some reduction in fibrous constituents. Similar to the response observed in straws, $NaClO_2$ treatment caused a large reduction in lignin (36%). $NaClO_2$ treatment reduced ADF and CWC by 20 and 16%, respectively. The greatest reduction in all fibrous constituents among the 8 treatments evaluated in the trial was due to organic Cl. It reduced lignin, ADF, CWC and cellulose by 39, 29, 18 and 19%, respectively. Some reduction in lignin and ADF was noted for NaClO, but only slight changes in fibrous constituents were observed for $Ca(ClO)_2$ treated alfalfa stems. Cellulase treatment reduced large amounts of CWC (26%), cellulose (25%), but only slightly reduced lignin in alfalfa stems (Table 16A). Changes in composition by the combined treatment of cellulase and $NaClO_2$ appeared to be primarily due to the cellulase treatment. In trial 2, $NaClO_2$ and organic Cl treatment reduced fibrous constituents to an extent similar as noted in trial 1. Among 14 treatments used in trial 2, Cl_2 (12%) was the most effective treatment in reducing CWC, lignin and hemicellulose with reduction of 42, 54 and 97%, respectively. $Ca(ClO)_2$ and NaClO treatments showed a larger reduction in fibrous constituents of alfalfa stems in trial 2 than those treatments did in trial 1 but these

Table 16A. The Apparent and Quantitative Changes of Fibrous Constituents in Treated and Ensiled Alfalfa Stems (Trial I).

Treatment	CWC		ADF		Lignin		Cellulose		Hemi-cellulose	
	A	Q	A	Q	A	Q	A	Q	A	Q
$NaClO_2$(5%)	-16.3	-6.7	-19.9	-10.7	-35.8	-28.4	-7.7	+2.8	-3.3	+7.8
NaClO(Bleach)	-7.8	+11.4	-17.6	-0.5	-17.9	-0.8	-15.8	+1.8	+24.8	+54.7
$Ca(ClO)_2$	-3.2	+23.0	-10.8	+13.3	-7.4	+17.8	-7.1	+18.0	+25.6	+60.0
$KClO_3$	-11.7	-1.6	-19.4	-10.2	-34.8	-27.3	-11.6	-1.5	+15.7	+28.9
Organic Cl	-18.3	+0.5	-28.9	-12.6	-39.0	-25.0	-18.5	+0.2	+21.5	+49.3
Cellulase(2.6%)	-26.4	-16.4	-17.6	-6.4	-11.6	+0.4	-24.7	-14.5	-55.4	-49.3
Cellulase(2.6%) + $NaClO_2$(2.5%)	-20.0	-5.0	-21.0	-6.2	-36.8	-25.0	-14.3	+1.8	-15.7	0.0
$NaClO_2$(2.5%)	-11.2	-12.1	-13.8	-14.7	-22.1	-22.9	-18.2	-19.0	-1.7	-2.7

A = Apparent change (Calculations are described in Table 9A).

Q = Quantitative change (Calculations are described in Table 9A).

Table 16B. The Apparent and Quantitative Changes of In Vitro Dry Matter Disappearance (IVDMD) at Three Fermentation Periods of Treated and Ensiled Alfalfa Stems (Trial I).

Treatment	6 hr. DMD		36 hr. DMD		T.T. 48 hr. DMD	
	A	Q	A	Q	A	Q
$NaClO_2$(5%)	+9.6	+22.1	-9.9	-0.4	+7.9	+20.3
NaClO(Bleach)	+34.1	+62.0	+3.9	+25.5	+12.3	+35.7
$Ca(ClO)_2$	-31.8	-13.4	-16.4	+6.2	+5.1	+33.5
$KClO_3$	+40.0	+56.1	-4.7	+6.3	+6.6	+18.8
Organic Cl	+53.6	+88.8	+12.6	+38.4	+13.3	+39.3
Cellulase(2.6%)	+73.6	+97.2	+7.3	+21.8	+3.5	+17.6
Cellulase(2.6%) + $NaClO_2$(2.5%)	+46.6	+73.8	+1.2	+20.2	+8.1	+28.4
$NaClO_2$(2.5%)	+1.8	+0.8	-11.4	-12.3	-0.5	-1.6

A = Apparent change (Calculations are described in Table 9B).

Q = Quantitative change (Calculations are described in Table 9B).

Table 17A. The Apparent and Quantitative Changes of Fibrous Constituents in Treated and Ensiled Alfalfa Stems (Trial II).

Treatment	CWC		ADF		Lignin		Hemi-cellulose	
	A	Q	A	Q	A	Q	A	Q
$NaClO_2$	-20.0	-12.7	-18.1	-10.5	-37.4	-31.7	-24.3	-17.3
NaClO(Bleach)	-18.1	-3.1	-16.8	-1.5	-28.4	-15.2	-20.8	-6.3
$Ca(ClO)_2$	-12.1	+9.4	-12.2	+9.3	-27.1	-9.2	-11.4	+10.3
Organic Cl	-19.3	-2.8	-19.5	-3.1	-34.8	-21.5	-18.3	-1.7
Cl_2(12%)	-42.0	-36.7	-18.9	-11.4	-54.2	-50.0	-97.0	-96.8
Cl_2(6%)	-36.9	-31.8	-16.2	-9.4	-40.7	-35.8	-86.1	-85.0
NaOH(6%)	-8.5	-7.5	+2.1	+3.1	-13.6	-12.6	-33.2	-32.5
NaOH(2%)	-4.5	-0.6	+4.8	+9.0	-16.8	-13.4	-26.2	-23.2
NaOH(6%) + Cl_2(12%)	-4.1	-14.9	-13.5	-23.2	-40.7	-47.3	+18.8	+5.5
NaOH(2%) + Cl_2(12%)	-34.3	-43.7	-16.0	-28.0	-52.3	-59.1	-77.1	-80.9
Cellulase(2.6%)	-18.7	-19.5	-1.9	-2.9	-12.3	-13.2	-58.4	-58.8
Pectinase(2.6%)	-25.3	-26.0	-14.9	-15.8	-10.3	-11.3	-49.5	-50.1
Pectinase(1.3%)	-20.7	-23.2	-9.8	-12.5	-8.4	-11.2	-46.5	-48.2
Cellulase(2.6%) + Pectinase(1.3%)	-26.1	-29.2	-11.4	-15.0	-11.0	-14.6	-60.9	-62.5

A = Apparent change (Calculations are described in Table 9A).

Q = Quantitative change (Calculations are described in Table 9A).

Table 17B. The Apparent and Quantitative Changes of *In Vitro* Dry Matter Disappearance (IVDMD) at Two Fermentation Periods of Treated and Ensiled Alfalfa Stems (Trial II).

Treatment	6 hr. DMD		T.T. 48 hr. DMD	
	A	Q	A	Q
$NaClO_2$	+99.1	+117.4	+25.1	+36.6
NaClO(Bleach)	+135.7	+179.0	+16.9	+38.4
$Ca(ClO)_2$	+16.5	+45.1	+20.1	+49.5
Organic Cl	+171.3	+226.7	+29.6	+56.0
Cl_2(12%)	+193.9	+220.9	+1.4	+10.7
Cl_2(6%)	+187.9	+210.4	+12.2	+21.4
NaOH(6%)	+131.3	+133.6	+2.3	+3.3
NaOH(2%)	+87.8	+95.5	+2.9	+7.1
NaOH(6%) + Cl_2(12%)	+132.2	+106.1	+28.7	+14.2
NaOH(2%) + Cl_2(12%)	+136.5	+102.8	+20.5	+3.3
Cellulase(2.6%)	+133.0	+130.7	+9.5	+8.4
Pectinase(2.6%)	+185.2	+182.3	+4.3	+3.2
Pectinase(1.3%)	+111.3	+104.9	+5.6	+2.4
Cellulase(2.6%) + Pectinase(1.3%)	+196.5	+184.4	+19.0	+14.1

A = Apparent change (Calculations are described in Table 9B).

Q = Quantitative change (Calculations are described in Table 9B).

two treatments were still less effective in changing chemical composition of alfalfa stems than $NaClO_2$, organic Cl and Cl_2 treatments. Sodium hydroxide (6%) treatment solubilized 33% of hemicellulose but it reduced other fibrous constituents only slightly. Similar results were reported by Ololade et al. (1970). The effect of pectinase treatment on chemical composition of alfalfa stems was similar to cellulase treatment. Pectinase treatment (2.6%) reduced CWC and hemicellulose by 25 and 50%, respectively. In trial 2, cellulase treatment did not reduce fibrous constituents to the same extent as the treatment did in trial 1. A slight additive effect on reducing fibrous constituents was observed for the combined treatment of cellulase and pectinase.

Data in Tables 18 and 19 show nutritive value estimates of treated alfalfa stems for trial 1 and 2. Much closer agreement between chemical and in vitro nutritive value estimates were found with alfalfa stems than with straws. However, for Cl_2 treatment, a very poor relationship between these two evaluation methods was evident for both forage materials. The data for estimated true digestibility (ETD) indicated that all treatments improved the digestibility of alfalfa stems with Cl_2 treatment showing the greatest improvement (56%). Sodium chlorite, calcium hypochlorite, organic Cl and enzymes treatments gave similar improvement of about 20%.

Table 18. Nutritive Value Estimation of Treated Alfalfa Stems (Trial I).

Treatment	CW Digestibility Predictors			DM Digestibility Predictors				Intake Predictor	Total Cell Wall Digestion		
	L/A[1]	D_{CWC}[2]	IVD_{CWC}[3]	ETD[4]	IVETD[5]	36 hr. DMD	T.T. DMD	6 hr. DMD	Chem[6] Soly	Bact[7] Dig	Sum[8] Dig
	----------%----------								g/100 g original CW		
$NaClO_2$(5%)	17.2	50	29	75	65	44.4	64.0	24.1	6.7	27.1	33.8
NaClO(Bleach)	21.3	43	43	69	69	51.2	66.6	29.5	- 11.4	47.6	36.2
$Ca(ClO)_2$	22.4	41	48	67	70	41.2	62.3	15.0	- 23.0	58.6	35.5
$KClO_3$	17.3	50	36	74	67	47.0	63.2	30.8	1.6	35.1	36.7
Organic Cl	18.6	47	40	75	71	55.5	67.2	33.8	- 0.5	40.1	39.6
Cellulase(2.6%)	23.2	40	29	74	69	52.9	61.4	38.2	16.4	23.9	40.3
Cellulase(2.6%) + $NaClO_2$(2.5%)	17.3	50	38	76	71	49.9	64.1	32.2	5.0	35.7	40.7
$NaClO_2$(2.5%)	19.3	46	36	72	67	43.7	59.0	22.4	12.1	31.3	43.4
Control	21.4	43	47	67	69	49.3	59.3	22.0	0.0	47.4	47.4

[1], [2], [3], [4], [5], [6], [7], [8] See Table 10.

Table 19. Nutritive Value Estimation of Treated Alfalfa Stems (Trial II).

Treatment	CW Digestibility Predictors			DM Digestibility Predictors			Intake Predictor	Total CW Digestion		
	L/A[1]	D_{CWC}[2]	IVD_{CWC}[3]	ETD[4]	IVETD[5]	T.T. 48 hr. DMD	6 hr. DMD	Chem[6] Soly	Bac[7] Dig	Sum[8] Dig
	----------%----------			---------%----------			%	g/100 g original CW		
$NaClO_2$	24.6	38	39	65	66	55.4	22.9	16.2	31.6	47.8
Bleach	27.7	33	36	62	63	51.8	27.1	8.2	32.6	40.8
$Ca(ClO)_2$	26.7	35	44	60	65	53.2	13.4	0.0	45.1	45.1
Organic Cl	26.0	35	43	63	68	57.4	31.2	9.0	39.4	48.4
Cl_2(12%)	18.2	48	7	78	62	44.9	33.8	40.9	4.2	45.1
Cl_2(6%)	22.8	40	6	73	58	49.7	33.0	36.1	3.9	40.0
NaOH(6%)	27.2	34	44	58	64	45.3	26.6	17.6	36.1	53.7
NaOH(2%)	25.5	36	41	57	60	45.6	21.6	11.5	35.8	47.3
NaOH(6%) + Cl_2(12%)	22.1	42	44	61	62	57.0	26.7	20.4	34.7	55.1
NaOH(2%) + Cl_2(12%)	18.3	48	14	75	60	53.4	27.2	47.0	7.3	54.3
Cellulases(2.6%)	28.8	31	33	61	62	48.5	26.8	28.5	23.6	52.1
Pectinase(2.6%)	33.9	26	25	61	61	46.2	32.8	33.7	16.8	50.5
Pectinase(1.3%)	32.6	27	29	60	61	46.8	24.3	31.3	19.9	51.2
Cellulases + Pectinase	32.3	28	24	63	60	52.7	34.1	36.4	15.0	51.4
Control	32.2	28	40	50	58	44.3	11.5	0.0	40.1	40.1

[1], [2], [3], [4], [5], [6], [7], [8] See Table 10.

A 16% improvement was noted for NaOH (6%) treatment. On the other hand, values for IVETD showed that all treatments tested in trial 1 and 2 only slightly improved the dry matter digestibility of alfalfa stems. Values of *in vitro* 48 hr. DMD also indicated chemical and enzyme treatments did not markedly improve DMD of alfalfa stems. A 28% increase in DMD resulted from organic Cl and NaOH plus Cl_2 treatments. However, all treatments except $Ca(ClO)_2$ increased *in vitro* 6 hr. DMD of alfalfa stems by approximately 2-fold. Cl_2 and enzyme treatments were most effective in increasing DMD at 6 hr. *in vitro* indicating that these treatments solubilized a large amount of cell wall constituents in alfalfa stems. Sodium hydroxide treatment was ineffective in improving *in vitro* DMD of alfalfa stems although it gave promising results with straws. This species difference response to NaOH treatment was also found by Ololade, *et al.* (1970). One explanation may be related to the different type and extent of lignification in legumes and grasses (Korers, *et al.*, 1969; Sullivan, 1962, 1969). Lignin in grasses is more alkali-soluble than that in legumes (Van Soest, 1969). Also, the silica content of these two species are different; grasses usually contain much higher amounts of silica than legumes (Jones and Handreck, 1967).

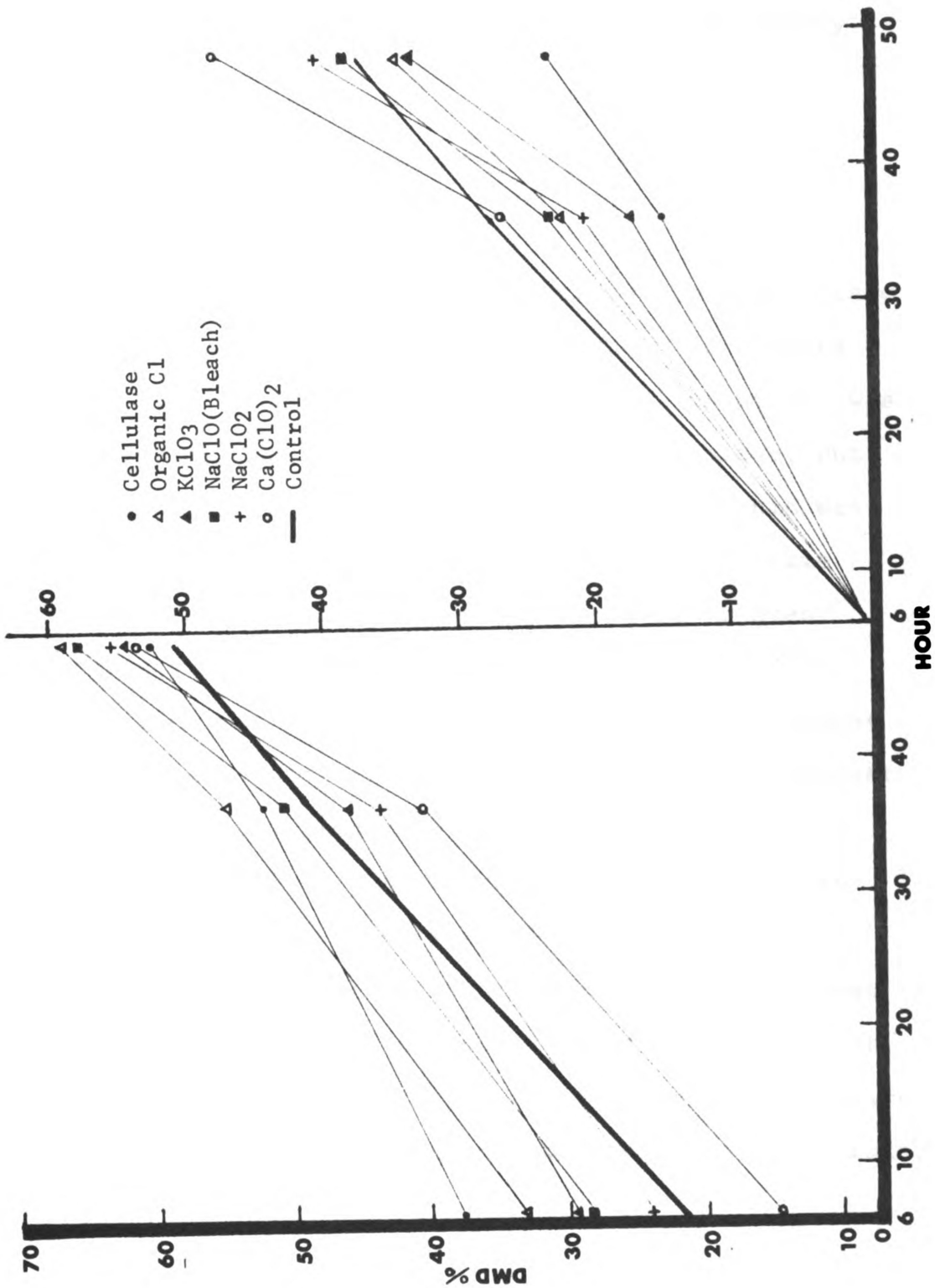
In trial 1, none of the treatments produced an amount of digestible CW comparable to that of control

alfalfa stems. This negative response was mainly due to the ineffectiveness of chemical or enzyme treatment to solubilize any CW in the original material. However, in trial 2 most treatments improved the total CW digestibility of alfalfa stems. The combined treatment of NaOH (6%) and Cl_2 (12%) gave maximum improvement of 1.35-fold over the control value. In other words, 55% of the original CW can be solubilized and/or digested after alfalfa stems were treated with NaOH and Cl_2. The next best improvement (25%) was noted after enzyme treatments. Sodium chlorite, organic Cl, calcium hypochlorite and Cl_2 treatments improved total CW digestion to a similar extent of about 17%.

Figure 3A shows the trends of *in vitro* dry matter disappearance (DMD) of alfalfa stems treated with five chemicals and one cellulase at three fermentation times (6, 36 and 48 hr.). In general, the rate of increase in percent of DMD was much greater than for straws. A wide difference in 6 hr. DMD was evident among treated alfalfa stems, but only a slight difference was found in 48 hr. DMD. This figure indicated that dry matter solubility of alfalfa stems could be improved by cellulase, organic Cl, $KClO_3$ and NaClO treatments. Since *in vitro* 6 hr. DMD is highly correlated with intake (Ingalls, 1964), the treatments that increased 6 hr. DMD would be expected to improve intake of alfalfa stems.

Figure 3A. *In Vitro* DMD of Alfalfa Stems Treated with Various Chemicals.

Figure 3B. *In Vitro* Insoluble DMD of Alfalfa Stems Treated with Various Chemicals. Values were adjusted to zero at 6 hrs.


Cellulase
Organic Cl
KClO3
NaClO(Bleach)
NaClO2
Ca(ClO)2
Control
DMD %
HOUR
0
10
20
30
40
50
60
70
6
10
20
30
40
50

However, Figure 3B indicates that none of the treatments improved *in vitro* insoluble dry matter digestibility significantly.

IV. Supplementary Studies

A. Time Variations

The effects of different ensiling times on forage composition and *in vitro* values are shown in Figures 4 and 5. At all ensiling times tested, Cl_2 treatment was more effective in reducing CWC and hemicellulose, but less effective in reducing cellulose and ADF than was NaClO treatment. Chemical composition of zero time treated straws (straws were put into an 80° C oven immediately after treatment) was only slightly different from that at 14 days (Figure 4). The treatment effect of both chemicals on wheat straw occurred almost immediately. Ensiling time longer than 4-10 hours caused only small further changes in fibrous constituents. In fact, for any particular constituent, no significant ($P < 0.05$) difference was observed between the value at 10 hr. and the value at 14 days. However, variations in values at less than 10 hours ensiling time suggest that this may be the minimum period for accurate estimations.

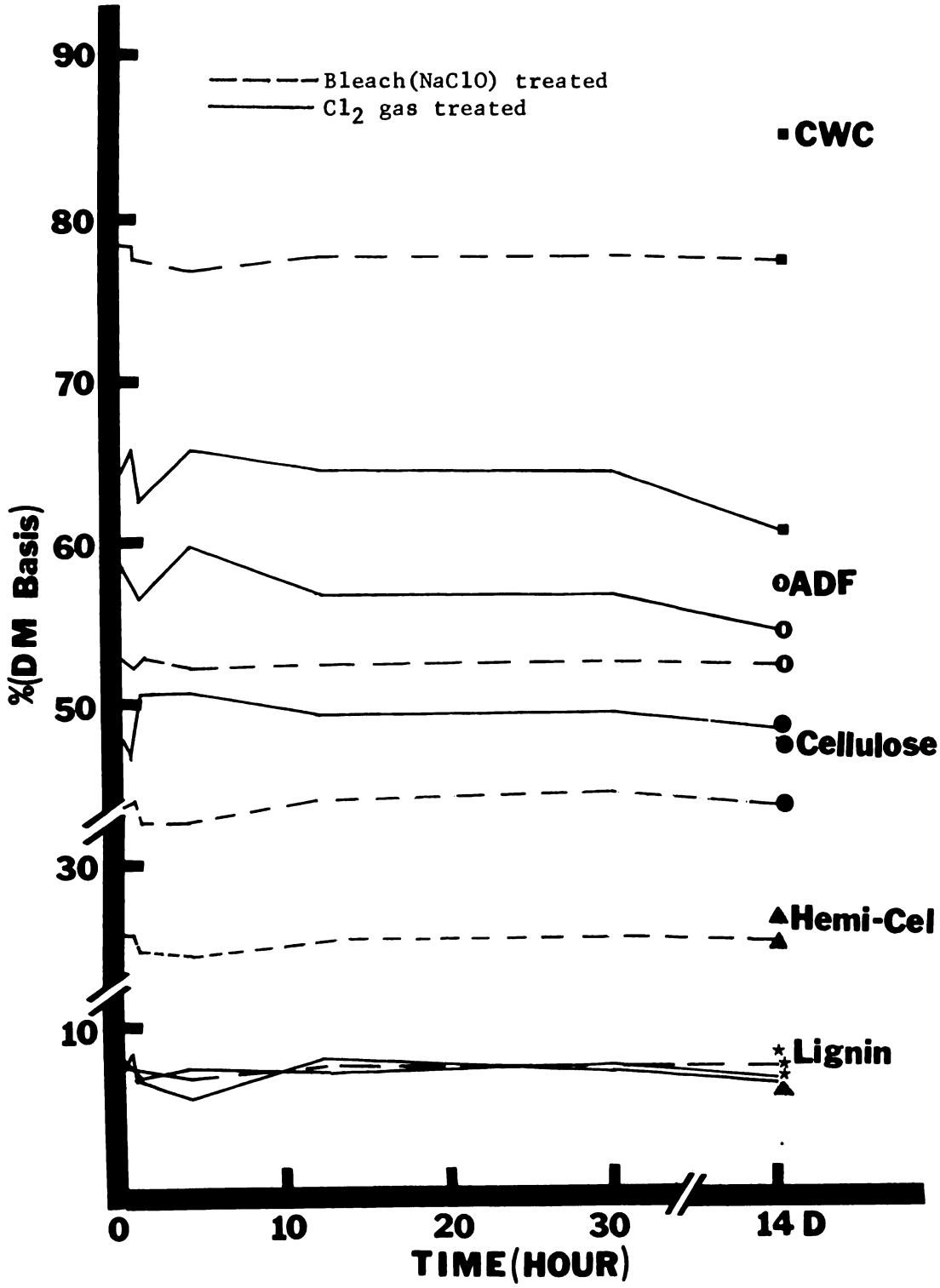


Figure 4. Changes in NaClO and Cl_2 Gas Treated Wheat Straw Composition with Ensiling Time. Control values are given at day 14.

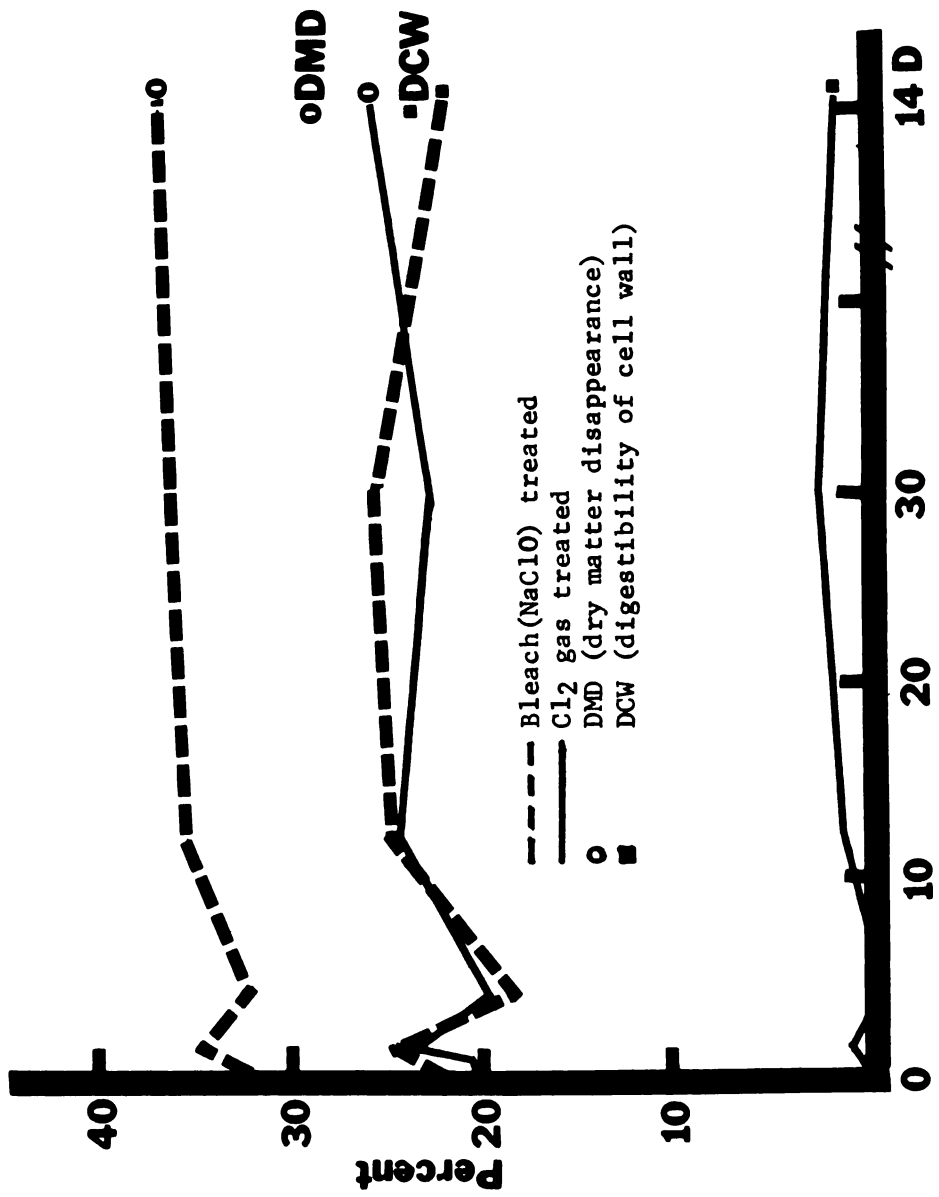


Figure 5. Relationship Between Ensiling Time and In Vitro 48 Hr. DMD and DCW of Wheat Straw. Control values are given at day 14.

In vitro studies also showed that for both treatments no marked changes in 48 hr. DMD or DCW were found between values at 10 hours and those at 14 days (Figure 5).

These findings suggested that a very short time (perhaps less than 10 hr.) is required for the reaction to become complete when treating wheat straw with either Cl_2 gas or NaClO.

B. Temperature Variations

The effect of ensiling temperature on composition of Cl_2 and NaClO treated wheat straws is presented in Figure 6. The chemical composition of NaClO treated straw showed very slight changes at different ensiling temperatures. For Cl_2 treated straw, a marked increase in cell wall constituents (CWC), acid detergent fiber (ADF) and lignin content was found at the higher temperature (80° C). The large increase in lignin content could be due to non-enzymatic browning reaction (Van Soest, 1964). Thus, high temperature with Cl_2 treatment is probably contraindicated.

The effect of ensiling temperature on in vitro 48 hr. DMD and DCW of Cl_2 and NaClO treated wheat straw is presented in Figure 7. Both in vitro DMD and DCW values of NaClO treated and control straw showed slight and parallel increases with increasing temperature, while the values of DMD and DCW of Cl_2 treated straw were not influenced by temperature. Although this experiment

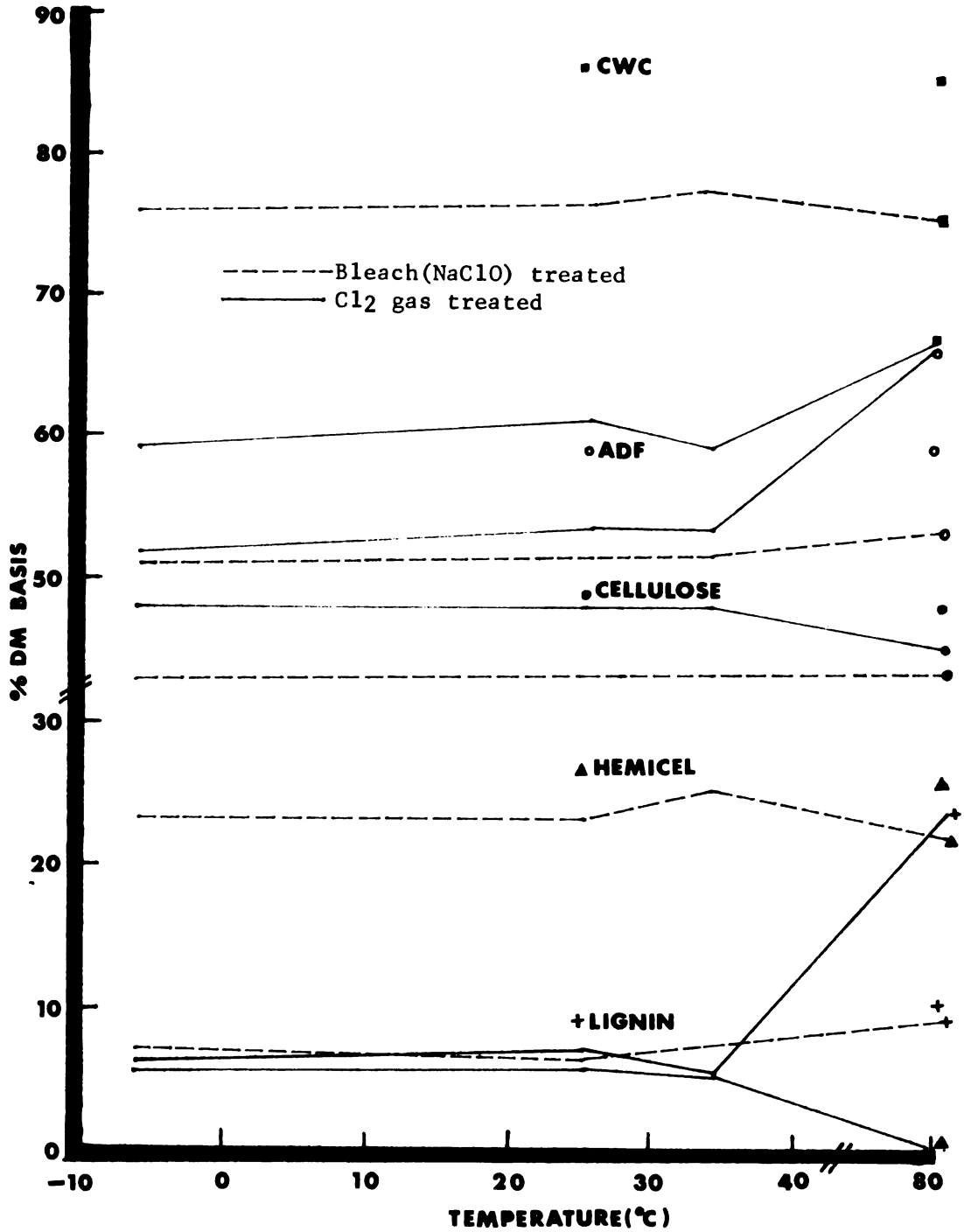


Figure 6. Relationship Between Ensiling Temperature and Wheat Straw Composition. Control values are given at 26 and 80 C.

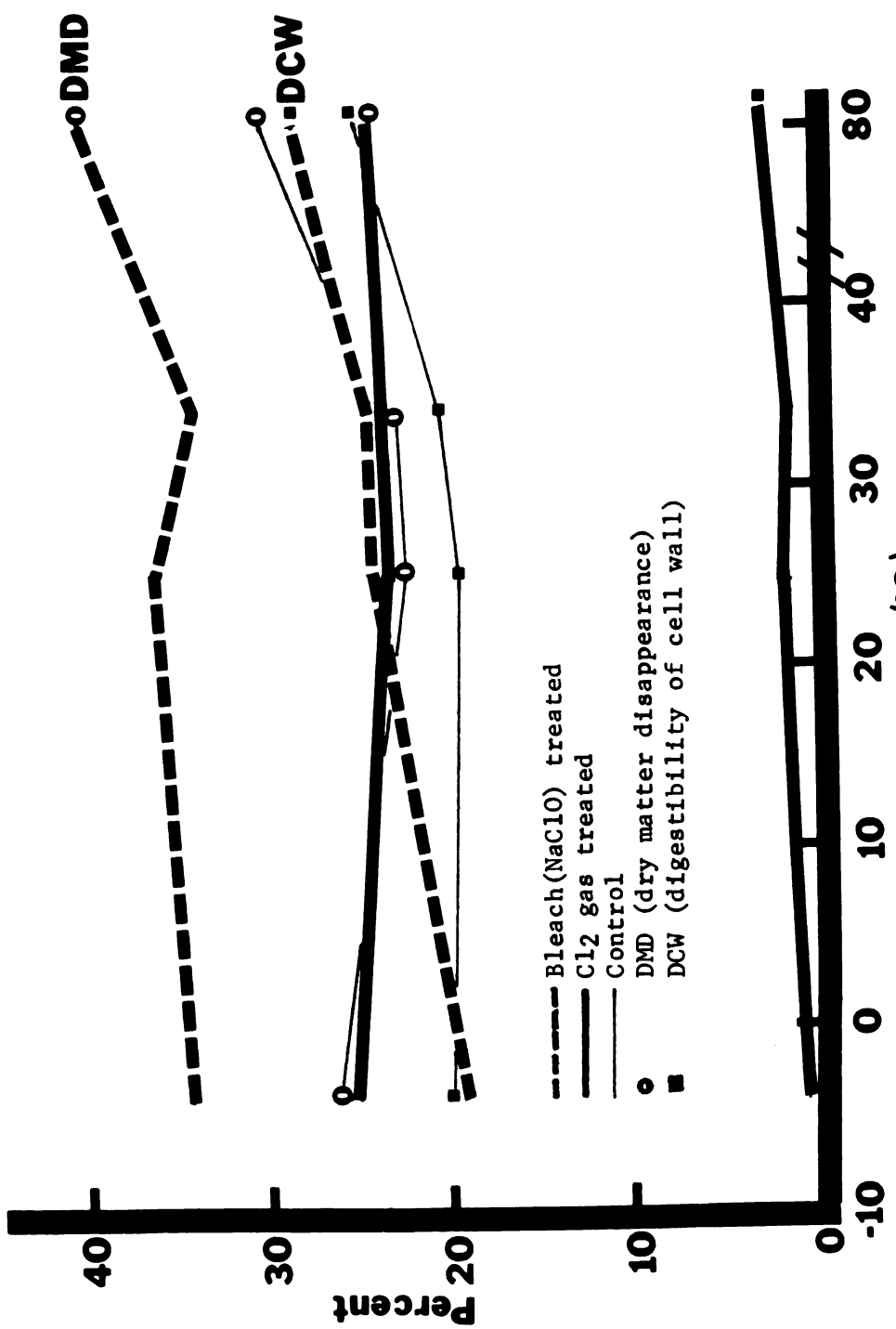


Figure 7. Ensiling Temperature Influence on In Vitro 48 Hr. DMD and DCW of Wheat Straw.

did not clearly indicate any optimum ensiling temperature for treating wheat straw with Cl_2 or NaClO, room temperature could be adapted for the treatment.

C. Ensiling-Treated Forage at Variable Moisture Contents

Difficulties were encountered in uniformly wetting straw when the desired moisture levels were below 40%. The temperature increase during treatment was negatively related to moisture content. Localized burning was unavoidable as moisture levels were below 30%.

The effects of treatment moisture levels on chemical composition, *in vitro* 6 hr. DMD and 48 hr. DMD, of Cl_2 gas treated wheat straw are presented in Figure 8. There were small changes in chemical composition at dry matter (DM) levels at 40% or lower. However, at high dry matter levels (90%), a marked increase in ADF and lignin was found. Since a high temperature (90° C) was observed at this low moisture level, the increases in lignin and ADF were possibly due to this factor.

Values for *in vitro* 6 hr. DMD and 48 hr. DMD of straw treated with Cl_2 gas showed no definite trend when DM content of straw was less than 70%. At DM levels greater than 70%, there was a trend for increased 6 hr. DMD values but decreased 48 hr. DMD values. These changes were presumably due to the effect of increased temperature during treatment.

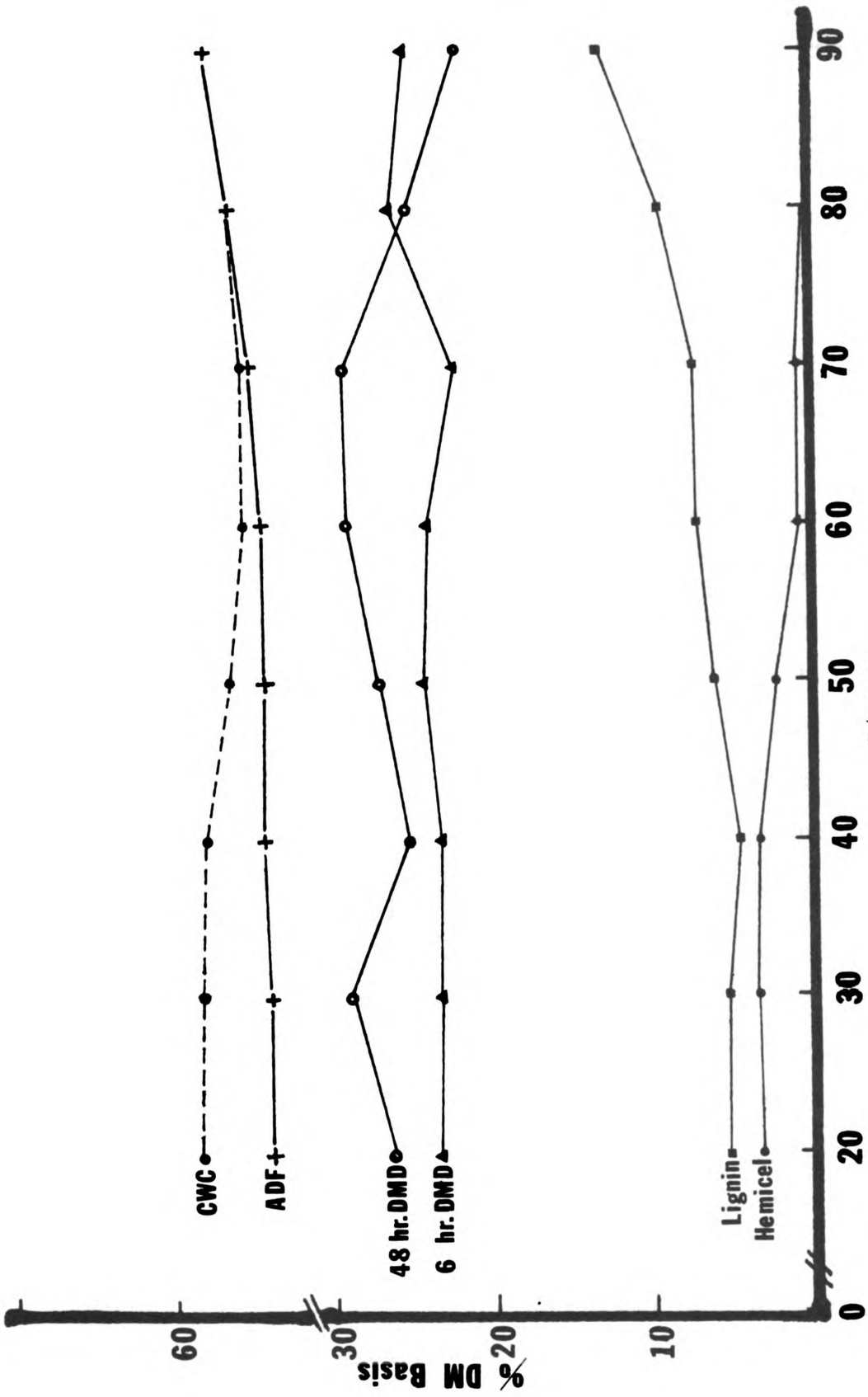


Figure 8. Relationship of DM Content to Composition and In Vitro DMD of Cl_2 Gas Treated Wheat Straw.

The results of this experiment indicated that as the DM content of straw increased above 70%, the chemical composition and the values for *in vitro* DMD of Cl_2 treated wheat straw could be affected.

V. Rumen In Vitro Fermentation with Silage Extract

Sodium chlorite treatment showed a 46% increase in *in vitro* 48 hr. DMD which could indicate that rumen bacteria were not inhibited by the treated straw. The sample used for the *in vitro* evaluation was dried and ground, while in the *in vivo* study straw was fed wet and unwashed. Two goats died in a feeding trial when fed treated straw. This mortality might indicate that certain toxic compounds were formed during $NaClO_2$ treatment. If a toxic compound existed it may have been destroyed or removed by the drying and/or grinding process and to examine this possibility extracts of wet treated silage were added to a standard substrate for *in vitro* fermentation.

Values for *in vitro* 48 hr. DMD of standard alfalfa with and without silage extracts added are presented in Table 20. The *in vitro* 48 hr. DMD of alfalfa was not affected by adding silage extracts from washed silages. However, when the silage extract was prepared from unwashed $NaClO_2$ treated straw and added to fermentation tubes, a 12% decrease in DMD of alfalfa was observed.

Table 20. In Vitro 48 Hr. Dry Matter Disappearance (DMD) of Standard Alfalfa When Incubated with Buffer, Rumen Innoculum and Chemically Treated Washed or Unwashed Silage Extract.

Treatment[1]		Tilley-Terry 48 hr. DMD
		----------%----------
I.	$NaClO_2$	
	(1) Unwashed	
	1. 1:1[2]	51.9
	2. 1:2	51.7
	3. 1:4	
	(2) Washed	
	1. 1:1	56.1
	2. 1:2	56.3
	3. 1:4	56.8
II.	NaClO(Bleach)	
	(1) Unwashed	
	1. 1:1	56.8
	2. 1:2	56.7
	3. 1:4	56.8
	(2) Washed	
	1. 1:1	57.3
	2. 1:2	57.5
	3. 1:4	57.9
III.	$Ca(ClO)_2$	
	(1) Unwashed	
	1. 1:1	55.8
	2. 1:2	57.3
	3. 1:4	55.8
	(2) Washed	
	1. 1:1	56.0
	2. 1:2	55.8
	3. 1:4	58.8
IV.	Control[3]	57.0

[1]0.5 g of standard alfalfa was incubated with 10 ml phosphate buffer, 12 ml rumen innoculum and 3 ml silage extract.

[2]Ratio of weight of wet silage to distilled water used in extraction.

[3]Standard alfalfa was incubated with 3 ml distilled water substituted for silage extract.

This decreased DMD indicates that certain compounds in fresh $NaClO_2$ treated silage can decrease the activity of rumen microorganisms. Such a decrease is perhaps not sufficient to explain the mortality observed in goats. More information about the possible toxicity of feeding unwashed $NaClO_2$ treated straw is needed.

Extracts from unwashed NaClO and $Ca(ClO)_2$ treated straws had no detrimental effect on *in vitro* DMD values of a standard alfalfa.

VI. Washing Treated Silages

The effects of washing the ensiled-treated silage on the resulting pH, organic acids, chemical composition, *in vitro* DMD and total CW digestion of the residue are summarized in Table 21. The washing process only slightly reduced the pH value. In general, most organic acids were lost during the washing process. For example, 98% of the acetate and 85% of the butyrate in NaOH (6%) treated silage was lost during washing. The range in dry matter loss was 7 to 22% during washing. Organic matter lost due to washing of $NaClO_2$, NaClO and $Ca(ClO)_2$ treated silages was 9, 2 and 7%, respectively. However, dry matter lost due to washing of these three respective treated silages was 19, 9 and 12%. The difference between these two sets of values indicated that relatively large amounts of ash were solubilized and eluted during the washing process. Calculations supported this

Table 21. Influence of Washing on Characteristics, Composition and In Vitro Fermentation Values of Treated Wheat Straw.

	NaClO		Bleach		$Ca(ClO)_2$		Organic Cl	
	U	W[1]	U	W	U	W	U	W
pH	5.7	5.8	5.5	5.8	7.0	6.9	5.4	5.2
Acetate(umoles/g)	0.36	0.12	0.90	0.29	3.02	0.90	0.25	0.11
Butyrate(umoles/g)					16.20	5.06		
D.M. Recovery %	109	88	114	103	111	98	117	104
D.M. lost due to treatment %		-12	14	3	11	-2	17	4
D.M. lost due to washing %	9	18.8		9.3		11.8		10.3
Ash(% D.M.)	8.3	3.9	12.4	5.2	9.4	5.2		
Ash lost due to washing %[3]		42.9		53.6		45.6		
Org. matter Recovery(%)[4]	103	94	104	101	104	96		
Org. matter lost due to treatment %[5]		5.9		0		3.7		
Org. matter Recovery %[6]		91.2		97.8		92.7		
Org. matter lost due to washing %[7]		8.8		2.3		7.3		
CWC(% D.M.)	80.8	86.0	77.1	84.2	79.9	86.5	74.2	82.4
CWC lost due to washing %[8]		35.4		1.3		3.9		1.0
ADF(% D.M.)	53.5	57.8	53.2	58.3	56.1	59.5	50.9	56.5
ADF lost due to washing %[8]		5.9		0.9		6.0		1.1
Lignin(% D.M.)	6.1	7.2	8.1	8.1	8.7	9.2	6.7	8.3
Lignin lost due to washing %[8]		2.2		9.7		6.0		11.0
Hemicellulose(% D.M.)	27.3	28.2	23.9	25.9	23.8	27.1	23.3	25.9
Hemicellulose lost due to washing %[8]		-10.2		2.0		0.9		0.9
In vitro 6 hr. DMD(% D.M.)	10.6	6.1	15.8	7.8	1.9	2.2	18.4	9.8
In vitro T.T. 48 hr. DMD(% D.M.)	33.6	33.7	32.2	25.0	30.7	26.4	35.3	25.3
In vitro DCW(% CWC)[9]	32.9	35.2	27.2	30.3	28.2	30.5	23.0	22.6
Solubilized CWC[10](g/100 g original CW)	3.4	37.5	3.1	4.4	2.7	6.6	4.6	5.6
Digested CWC(g/100 g original CW)[11]	31.8	22.0	26.3	29.0	27.4	28.5	22.0	21.4
Total Digested CWC(g/100 g original CW)[12]	35.2	59.5	29.4	33.4	30.1	35.1	26.6	27.0

Table 21. (Continued)

	Cl_2 (12%)		Cl_2 (6%)		NaOH (6%)		NaOH (2%)	
	U	W[1]	U	W	U	W	U	W
pH	1.4	2.1	1.6	2.1	7.0	7.3	5.1	5.5
Acetate(umoles/g)	7.38	1.81	6.51	2.13	118.37	2.44	92.81	1.85
Butyrate(umoles/g)					24.42	3.63	13.92	3.28
D.M. Recovery %	103	89	102	90	97	85	99	92
D.M. lost due to treatment %	3	-11	2	-10	-3	-15	-1	-8
D.M. lost due to washing %[2]		14.2		11.8		12.5		7.0
Ash(% D.M.)	4.2	4.0						
Ash lost due to washing %[3]		0						
Org. matter Recovery(%)[4]	102	88						
Org. matter lost due to treatment %[5]		12.0						
Org. matter Recovery %[6]		86.4						
Org. matter lost due to washing %[7]		13.6						
CWC(% D.M.)	56.7	63.1	61.6	65.5	72.8	84.4	83.5	88.5
CWC lost due to washing %[8]		4.2		6.0		2.0		1.8
ADF(% D.M.)	53.0	57.7	55.7	57.9	61.4	67.5	60.1	62.8
ADF lost due to washing %[8]		6.2		8.2		1.8		3.2
Lignin(% D.M.)	6.4	5.9	7.7	6.5	10.7	12.4	10.1	10.1
Lignin lost due to washing %[8]		20.8		25.4		2.5		7.1
Hemicellulose(% D.M.)	3.7	5.3	5.9	7.6	11.5	16.9	23.3	25.7
Hemicellulose lost due to washing %[8]		25.1		14.6		29.7		1.9
In vitro 6 hr. DMD(% D.M.)	26.5	20.5	19.7	15.8	19.7	6.2	8.8	3.1
In vitro T.T. 48 hr. DMD(% D.M.)	28.9	20.8	34.3	20.4	45.1	37.2	29.0	26.2
In vitro DCW(% CWC)[9]	6.2	6.3	6.9	5.8	44.9	42.7	31.5	30.2
Solubilized CWC[10](g/100 g original CW)	35.9	38.6	30.9	35.1	22.7	21.1	9.0	10.6
Digested CWC(g/100 g original CW)[11]	4.0	4.0	4.8	3.8	34.7	33.7	28.7	27.0
Total Digested CWC(g/100 g original CW)[12]	39.9	42.6	35.7	38.9	57.4	54.8	37.7	37.6

Table 21. (Continued)

	NaOH(6%) + Cl_2(12%)		NaOH(6%) + Cl_2(6%)		Control
	U	W[1]	U	W	
pH	1.5	2.4	2.5	3.7	4.2
Acetate(umoles/g)	9.02	1.34	80.23	1.17	0.13
Butyrate(umoles/g)					
D.M. Recovery %	114	89	109	92	96
D.M. lost due to treatment %	14	-11	9	-8	
D.M. lost due to washing %[2]		22.3		16.0	
Ash(% D.M.)	11.9	6.9			3.3
Ash lost due to washing %[3]		35.7			
Org. matter Recovery(%)[4]	104	86			
Org. matter lost due to treatment %[5]		14.0			
Org. matter Recovery %[6]		82.5			
Org. matter lost due to washing %[7]		17.5			
CWC(% D.M.)	53.4	64.9	61.4	80.1	91.0
CWC lost due to washing %[8]		5.1		9.7	
ADF(% D.M.)	49.7	58.6	52.0	61.1	61.3
ADF lost due to washing %[8]		8.0		1.2	
Lignin(% D.M.)	6.5	6.8	7.7	7.8	11.4
Lignin lost due to washing %[8]		17.6		15.3	
Hemicellulose(% D.M.)	3.7	6.3	9.4	19.0	29.7
Hemicellulose lost due to washing %[8]		33.4		69.8	
In vitro 6 hr. DMD(% D.M.)	30.0	21.5	23.5	7.2	7.2
In vitro T.T. 48 hr. DMD(% D.M.)	30.4	19.4	28.6	15.3	30.9
In vitro DCW(% CWC)[9]	4.8	2.5	10.9	19.0	27.1
Solubilized CWC[10](g/100 g original CW)	32.9	36.3	26.2	19.0	0
Digested CWC(g/100 g original CW)[11]	3.2	1.6	8.1	15.4	27.1
Total Digested CWC(g/100 g original CW)[12]	36.1	37.9	34.3	34.4	27.1

Table 21. (Continued)

[1]U = Unwashed straw, W = washed straw.

[2]100 x (Total DM in unwashed straw - total DM in washed straw) / (Total DM in unwashed straw).

[3]100 x (Total ash in unwashed straw - total ash in washed water) / (Total ash in unwashed straw).

[4]100 x (Total organic matter in either unwashed or washed treated straw) / (Total organic matter in original central material).

[5]100 x (Total organic matter in original straw - total organic matter in either unwashed or washed treated straw) / (Total organic matter in original straw).

[6]100 x (Total organic matter in washed treated straw) / (Total organic matter in unwashed treated straw).

[7]100 x (Total organic matter in unwashed straw - Total organic matter in washed straw) / (Total organic matter in unwashed straw).

[8]100 x (Total fibrous constituent in unwashed straw - total fibrous constituent in washed straw) / (Total fibrous constituent in unwashed straw).

[9]*In vitro* digestibility of cell wall (IVDCW) = $100 \times \frac{\text{(1-undigested CW)}}{\text{CW in sample}}$

[10]100 x (Amount CW in original material - amount CW in product) / Amount CW in original material.

[11]100 x (Amount CW in product x IVDCW) / (Amount CW in original material).

[12]Sum of 10 and 11.

observation. Percent of the total ash lost due to washing for $NaClO_2$, NaClO and $Ca(ClO)_2$ treated silages was 43, 54 and 46%, respectively. On the other hand, no detectable amount of ash was lost during washing of Cl_2 treated silage. As a result, the percent of organic matter lost due to washing of Cl_2 treated silage was identical with the value of dry matter lost due to washing (14%). Percent of organic matter lost due to treatment of washed silage was low (0 to 6%) for NaClO, $Ca(ClO)_2$ and $NaClO_2$ treatments. While a comparatively higher value of a 12% and 14% of organic matter loss due to washing was observed for Cl_2 and NaOH (6%) plus Cl_2 (12%) silages. The chemical analysis showed that for all eleven treatments used in this study, washed silages contained a higher apparent content of fibrous constituents than did the unwashed silages. However, quantitative calculations indicated that fibrous constituents were reduced to some extent by the washing process.

In general, washed silages had much lower values for in vitro 6 hr. DMD and 48 hr. DMD than did unwashed silages but both had comparable values of digestible cell wall (DCW). For example, values for 6 hr. DMD, 48 hr. DMD and in vitro DCW of organic Cl treated unwashed silage were 18, 35 and 23%, respectively, while the respective corresponding values for washed silage were 10, 25 and 23%. For most treatments, washed silages had

a higher total amount of CW digestion than did unwashed silages. Increased cell wall digestion was due to the additional solubilization of CW during the in vitro fermentation process as a result of the washing process. However, since the soluble constituents in the treated silages were eluted out, the overall nutritive value might actually have been decreased although the total CW digestion was increased.

In summary, washing chemically treated silages eluted out organic acids, reduced the content of soluble nutrients and in vitro fermentability, slightly increased cell wall digestibility but most likely decreased the overall nutritive value of the silages.

VII. In Vivo Study

The chemical composition and in vitro fermentation values for treated straws prepared in 55 gallon barrel silos is presented in Table 22. The corresponding values obtained for straws prepared in laboratory glass silos are also given in this table. In general, good agreement was noted between chemical composition of treated silages made from silos of different sizes. This was true for washed and unwashed silages. However, the values of in vitro DMD and IVDCW of unwashed silages indicated that barrel silages were more fermentable than were laboratory silages. For example, the values of in vitro 6 hr. DMD, 48 hr. DMD and IVDCW of $Ca(ClO)_2$ treated,

Table 22. Chemical and *In Vitro* Fermentation Values for Pilot Type Barrel Silos Compared to Values for Laboratory Glass Silos When Wheat Straw was Treated with Five Chemicals. Values are given for treated silages and treated-washed silages.

	$NaClO_2$		NaClO		$Ca(ClO)_2$		Cl_2(12%)		NaOH(6%) + Cl_2(12%)		Control
	L	B[1]	L	B	L	B	L	B	L	B	
Unwashed											
CWC	78.1	77.9	76.4	76.0	80.9	75.4	59.8	59.7	54.6	51.6	84.2
ADF	50.8	49.6	51.4	49.2	53.8	51.0	53.7	53.5	49.5	49.9	56.8
Lignin	3.6	4.3	6.8	4.4	6.7	6.8	6.2	5.7	5.6	7.0	7.9
Hemicellulose	27.7	28.3	25.0	26.8	27.1	24.4	6.1	6.2	5.0	1.6	27.9
6 hr. DMD	13.7	18.9	21.0	21.4	7.6	8.1	27.1	28.5	32.0	36.7	9.3
48 hr. DMD (T.T.)	37.9	37.6	36.6	43.3	27.2	38.6	24.8	26.5	30.7	33.6	25.9
IVDCW[2]	32.9	30.1	21.6	35.4	24.7	31.2	1.5	2.6	4.0	2.1	24.0
Washed											
CWC	86.0	87.8	84.2	86.1	86.5	84.7	63.1	72.5	64.8	70.8	
ADF	57.8	57.8	58.3	58.9	59.5	59.1	57.3	61.2	58.6	65.7	
Lignin	7.2	6.3	8.1	8.7	9.2	8.4	5.9	9.7	6.8	10.3	
Hemicellulose	28.2	30.1	25.9	27.2	27.1	25.6	5.3	10.9	6.3	4.6	
6 hr. DMD	6.1	7.2	7.8	7.5	2.2	5.9	20.5	14.0	21.5	19.0	
48 hr. DMD (T.T.)	33.7	34.1	25.0	32.4	26.4	33.4	20.7	16.5	19.4	12.1	
IVDCW	35.2	41.5	30.3	36.6	30.5	39.2	6.3	26.0	2.5	27.4	

[1]L = Laboratory glass silo; B = Barrel silo.

[2]IVDCW = *In vitro* digestibility of the cell wall.

unwashed laboratory silage were 8, 27 and 25%; while the respective values for these *in vitro* values of barrel silages were 8, 39 and 31%. The values of *in vitro* 6 hr. DMD, 48 hr. DMD and IVDCW of washed $NaClO_2$, NaClO and $Ca(ClO)_2$ treated barrel silages were higher than comparable values for washed treated laboratory silages. However, for Cl_2 and NaOH (6%) plus Cl_2 gas (12%) treatments, washed barrel silages showed lower values for *in vitro* 6 hr. DMD and 48 hr. DMD than washed laboratory silages. On the other hand, values for IVDCW were much higher for washed barrel silages than for the laboratory silages of these two treatments. In summary, the data presented in Table 21 indicates that the efficiency of chemicals in reducing fibrous constituents and in improving *in vitro* DMD, IVDCW of large pilot silos was comparable with that of small glass silos.

Responses when goats were fed treated straw with and without subsequent washing are presented in Table 23. In trial 1, treated straws were fed to animals fresh and unwashed. Extremely low ration dry matter intake was observed for Cl_2, NaOH (6%) plus Cl_2 (12%) and $NaClO_2$ treated silages. Ration intake of NaClO and $Ca(ClO)_2$ treated silages was comparable with or slightly lower than the control straw. These low intakes of treated silages were presumably due to low palatibility, high salt concentration or low pH. The negative weight

Table 23. Responses When Goats Were Fed Treated Straw With and Without Subsequent Washing.

	$NaClO_2$	NaClO	$Ca(ClO)_2$	Cl_2(12%)	NaOH(6%) + Cl_2(12%)	Control
Trial I - treated straw direct from Silo						
Ration DM intake(lb/cwt)	0.5	1.4	1.1	0.2	0.2	1.4
Weight gain(lb/day)	-0.4	-0.03	-0.1	-1.1	-0.8	+0.6
Trial II - washed treated straw						
Period[1]	1	1	1	2		2
Intake(lb.DM/cwt)						
Total ration	2.3	2.3	2.2	1.8		2.7
Straw	1.9	1.9	1.7	1.0		1.9
Weight gain(lb/day)	-0.2	-0.2	-0.3	+0.7		+0.4
DM digestibility(%)						
Total ration	62.8	57.7	56.1	67.9		60.4
Straw	56.4	51.2	46.2	49.3		47.1
In vitro Tilley-Terry 48 hr. DMD(%)	34.1	32.4	33.4	16.5	12.1	25.9
Relative position to control						
In vivo(straw digestibility)	120	109	98	105		100
In vitro(straw digestibility)	132	125	129	64		100
Number of goats started	2	2	2	2	2	4
Mortality	2	1	0	0	0	0

[1]Treated straws fed mixed with 40g SBM, 3% molasses, TM salt, vitamins for period I; treated straw plus 80g SBM, 40g grain mix., 5% molasses, TM salt and vitamins for period II.

gain for goats fed treated silages was possible a direct consequence of low feed intake. In period 1 of trial 2, treated silages were washed and fed to goats as 80% of the total ration dry matter. The crude protein content in the rations was the same as that in trial 1. The intake of total ration and straw dry matter of all three treated silages increased considerably as compared to the data of trial 1. Increased intake presumably was due to improved acceptability of silages by the washing process. However, during the trial, animals showed a weak, unvigorous appearance though the feed intake was improved. In fact, all goats lost body weight to some extent. In period 2 of trial 2, neutralized, washed Cl_2 gas-treated silage was fed to animals as 56% of the total ration dry matter. The control ration was fed as 70% of the total ration dry matter. The intake of neutralized, washed Cl_2 treated straw was lower than that of control and other washed, treated silages. This result was not expected based on laboratory evaluations of these silages. Six hour DMD, was highly correlated with intake, values indicated that Cl_2 treated straws would be consumed in greater quantity than control straw. Animals in this period showed a positive weight gain which could be attributed to relatively higher level of concentrate in the rations.

The dry matter digestibilities of straws were calculated by difference with the assumption that the supplemental DM was 90% digestible. These values indicated that washed $NaClO_2$ treated straw was 20% more digestible than control straw. Little improvement was noted for NaClO and Cl_2 treated straws, while $Ca(ClO)_2$ treatment did not improve straw digestibility. The effectiveness of $NaClO_2$ treatment in improving *in vivo* dry matter digestibility of straw was reported by Goering *et al.* (1969). They obtained a 15% increase in dry matter digestibility of $NaClO_2$ (15%) treated barley straw using sheep. For comparative purposes, the *in vitro* 48 hr. DMD (Tilley and Terry, 1963) of washed-treated straws is also given in Table 23. This *in vitro* evaluation method gave greater magnitude of improvement for $NaClO_2$, NaClO and $Ca(ClO)_2$ treatments than was found *in vivo.* Smith *et al.* (1971) reported that *in vivo* digestibility of $NaClO_2$ treated feces did not increase to the extent observed *in vitro*. In addition, Table 23 shows that these two evaluation methods ranked the effectiveness of these chemicals in improving straw digestibility differently. However, these *in vivo* data should not be considered as absolute since only 2 goats were used per treatment but they do strongly indicate that *in vitro* procedures developed for usual forages may not be appropriate indication of *in vivo* results with modified forages. Klopfenstein and

Woods (1967, 1968) have also made this suggestion. Nevertheless, among the four chemicals evaluated in this *in vivo* study, $NaClO_2$ improved straw digestibility both *in vitro* and *in vivo*.

During the course of the digestion trial, two goats died while consuming $NaClO_2$ treated straw and one goat died while consuming NaClO treated straw. The autopsy report did not indicate any malfunction of body organs. Malnutrition was suggested as a major cause, yet these goats had been on the straws only 10 to 30 days when they died.

Table 24 shows the pH and volatile fatty acids (VFA) concentrations of ruminal fluid taken from goats fed treated silages and control straw. The rumen samples were contaminated with excessive saliva, thus the pH values given in Table 24 are perhaps higher than actual values.

Silages treated with $NaClO_2$, NaClO and $Ca(ClO)_2$ produced higher concentrations of rumen VFA than did control straw by ratios of 1.23, 1.09 and 1.16, respectively. Rumen VFA concentration for the Cl_2 gas treated silage was only 0.64% that of the control ration. The relatively low rumen VFA concentrations of control and Cl_2 treated straw rations were unexpected, since 10 and 25% more concentrate was added to these two rations as compared to the other three treated silage rations. Molar

Table 24. Ruminal Fluid pH and VFA Analyses of Goats Fed Four Treated and One Control Silages.

	$NaClO_2$	Bleach	$Ca(ClO)_2$	Cl_2	Control
pH	6.9	7.4	6.7	8.5	9.0
VFA(ug moles/ml)					
Acetate	22.14	19.03	20.65	9.78	23.76
Propionate	15.44	13.77	14.50	8.79	6.35
Butyrate	2.65	2.82	2.72	2.42	2.65
Total VFA(ug moles/ml)	40.23	35.62	37.86	20.99	32.76
Molar percent					
Acetate	55.1	53.6	54.9	46.6	72.4
Propionate	38.5	38.4	38.1	41.7	19.4
Butyrate	6.4	7.9	7.0	11.7	8.2
Acetate = Propionate	1.4	1.4	1.5	1.1	3.8

ratios of VFA indicated much higher proportions of propionate was found in rumen fluid from goats fed treated straws than for goats fed control ration.

Cursary microscopic examination of these rumen samples showed that feeding treated silages possibly changed rumen microbial population qualitatively and quantitatively. In general, protozoa were seldom seen in any sample. In rumen fluid samples of $NaClO_2$ treated silage, there was a large number of rod-shaped, large motile bacteria. The significance of these findings are not known.

Five conclusions can be made: (1) Fresh, wet, chlorine compound treated straws possessed extremely low acceptability to goats; (2) Washing $NaClO_2$, NaClO and $Ca(ClO)_2$ treated straws markedly improved the intake to a level comparable with control straw; (3) The intake of Cl_2 gas treated straw could be improved to some extent by washing and neutralization. However, the intake of washed and neutralized straw was still lower than for control straw. Methods for further improvement in intake should be investigated; (4) Among four chemicals evaluated, $NaClO_2$ (5%) treatment showed a 20% increase *in vivo* dry matter digestibility of wheat straw. $Ca(ClO)_2$ and Cl_2 treatments showed no improvement in dry matter digestibility of straw; (5) Rumen acetate:propionate ratio was markedly reduced in goats fed these treated silages.

VIII. High-Energy Radiation

A. Wheat Straw

1. Trial 1

Composition and *in vitro* values of straws exposed to variable amounts of Cobalt radiation are presented in Table 25. Wheat straw was ground before radiation at low dosage levels ($2x10^6$ and $20x10^6$ rads) and there was no marked difference in composition and in *in vitro* DMD between finely ground (1 mm.) and coarsely ground (4.0 cm.) wheat straw. However, at high dosage levels ($96.6x10^6$ and $143x10^6$ rads), the amount of CWC and hemicellulose in coarse samples were degraded to a greater extent than were finely ground samples. At the highest dose ($143x10^6$ rads), the value of *in vitro* DCW and total cell wall digested were 85 and 13% higher for coarse samples than for fine samples.

Generally, the reduction in fibrous constituents with increasing radiation dose levels followed a negative cubic type response. The resulting CWC, ADF, lignin and hemicellulose after the highest dosage ($143x10^6$ rads) were 67, 75, 74 and 46% of the original values, respectively. When these values are compared with values of chemically treated wheat straws, the effectiveness of irradiation in reducing CWC and lignin are comparable with the combined treatments of NaOH (6%) and Cl_2 gas (12%).

Table 25. Influence of Gamma Radiation on Straw Composition and *In Vitro* Values of Two Particle Sizes.

Treatment			CWC	ADF	Lignin	Hemi-cellulose	L/A[2]	6 hr. DMD	T.T. 48 hr. DMD	IVDCW[3]	Total CW Digested		
Dose Level	Log Dose	Particle Size[1]									Soly[4]	Dig[5]	Total[6]
-------rads-----			----------% DM basis----------								g/100 g original CW		
143.0x10^6	8.16	F	57.2	43.0	6.6	14.0	15.4	20.9	29.4	6.9	32.9	4.7	37.6
		C	55.5	43.2	6.7	12.3	15.6	21.8	29.9	12.7	34.9	8.3	43.2
96.6x10^6	7.99	F	66.1	46.9	7.0	19.2	15.0	15.2	29.4	18.3	22.4	14.2	36.6
		C	64.4	47.0	6.9	17.4	14.7	16.0	28.7	18.9	24.4	14.3	38.7
43.4x10^6	7.64	F	74.9	51.2	6.5	23.7	12.7	10.3	32.4	26.3	12.1	23.1	35.2
		C	71.8	51.7	7.8	20.1	15.1	11.1	31.9	21.6	15.7	18.2	33.9
20.0x10^6	7.30	F	79.9	54.8	8.2	25.1	15.0	7.5	30.6	22.5	6.3	21.1	27.4
		C	77.8	54.8	8.3	23.0	15.1	8.9	31.3	19.3	8.7	17.6	26.3
2.0x10^6	6.30	F	83.0	57.1	8.8	25.9	15.5	6.1	26.9	21.7	2.6	21.1	23.7
		C	83.2	57.5	8.9	25.7	15.4	5.8	26.7	20.2	2.4	19.8	22.2
Control			85.2	57.5	8.8	27.7	15.2	7.3	27.3	27.4	0	27.4	27.4

[1]F = Fine (1 mm.); C = Coarse (Approx. 2.3 cm.).

[2]L/A = 100 x Lignin (%) / Acid detergent fiber (ADF) (%).

[3]IVDCW = *In vitro* digestibility of the cell wall = 100 x (1-undigested CWC / CWC in sample)

[4]Soly (Amount CW solubilized by radiation) = 100 x (Amount CW in original material - amount CW in product) / (Amount CW in original material).

[5]Dig (Amount CW digested by *in vitro* bacteria) = 100 x (Amount CW in product x IVDCW) / (Amount CW in original material.

[6]Total amount CW digested = Sum of 4 and 5.

None of the chemical treatments reduced ADF in wheat straw to the extent that gamma irradiation did. Table 25 also indicates that irradiation did not change the ratio of acid detergent lignin (ADL) to acid detergent fiber (ADF) which might imply that irradiation would not increase the CW digestibility of straw.

The *in vitro* 6 hr. DMD of irradiated straws showed a positive cubic response with the increase of 2.93-fold over control at the dose level of 143×10^6 rads. This increase was comparable with the value of Cl_2 treated straw. The responses for *in vitro* 48 hr. DMD did not follow the pattern for 6 hr. DMD. At the highest dosage, irradiation increased 48 hr. DMD of wheat straw by only 1.09 above the control value. This value was just slightly higher than the value given by $Ca(ClO)_2$ treatment which was one of the least effective chemical treatments for improving *in vitro* DMD in the laboratory silo studies. Pritchard *et al.* (1962) irradiated the same type of material at comparable dosage levels as used in this study and reported a two-fold increase for *in vitro* 48 hr. DMD values. This conflicting result might be partially explained by the compositional variation of substrates. In that study the original wheat straw contained 41% crude fiber while the straw used in this study contained 58% ADF.

The total CW digestibility was increased a factor 1.40 for the finely ground straw and by 1.66 for the coursely ground sample. These improvements were similar to those for NaOH (6%) treatment. Table 25 also indicates that with increased dosage levels, there were opposite trends for changes in the amount of CW solubilized by irradiation and that solubilized by bacterial digestion. In other words, the amount of CW solubilized by irradiation was positively related to the dosage, while the amount of CW digested by bacteria was negatively related to dosage. The increased amount of CW solubilized as irradiation dosage increased was very possibly due to a progressive decrease in the degree of polymerization of cellulose and hemicellulose complexes in the CW of wheat straw (Stanks, 1959 and Stone et al., 1969). Reasons for the progressive decrease in amount of residual CW digested by bacteria are only speculative. Possible explanations are: (1) Compounds inhibitory to bacterial activity are formed by high levels of irradiation as degradation products of CW constituents (possibly lignin); (2) The availability of the residual CW to bacteria was gradually decreased as increasing amounts of CW were solubilized by irradiation. The structure of the residual CW might be more lignified and less susceptible to bacterial enzymatic degradation than the original CW; (3) There was a maximum limit to the amount

of straw CW that could be degraded by irradiation and/or bacterial digestion. This maximum limit might be defined by the structure of the fibrous constituents and substances associated with them. Furthermore, this maximum limit could be species dependent. This appears to be in contradiction with the findings of Lawton (1951). He measured only total dry matter disappearance crediting all disappearance to bacterial digestion. The present study divides dry matter disappearance into two fractions: that solubilized by irradiation, and that due to bacterial action. When divided in this manner, it is apparent that the solubilization by bacterial action actually decreases with increasing levels of irradiation. Total dry matter disappearance continues to increase because solubilization by irradiation increases faster than bacterial solubilization decreases. Total dry matter disappearance is the sum of the two solubilization forces.

2. Trial II

Wheat straw was finely ground (1 mm., Wiley mill) and exposed to high-energy electron irradiation (1,000,000 Volt electron beam generator). The effect of irradiation on composition of wheat straw is presented in Figure 9. For comparative purposes, the composition of finely ground irradiated straw from trial 1 is also plotted. Reduction in all fibrous constituents was greatest with the highest levels of irradiation ($1x10^9$ rads). The

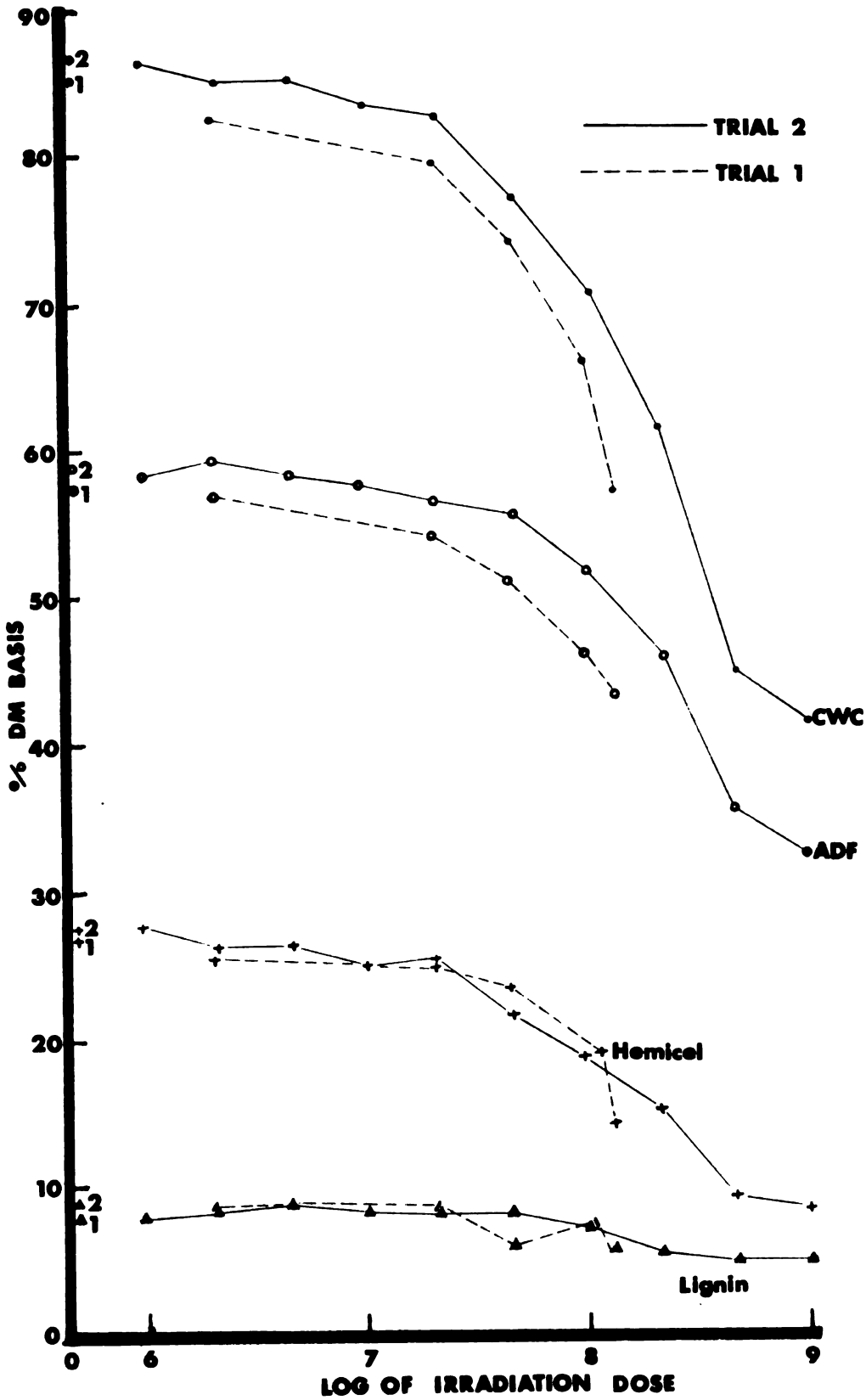
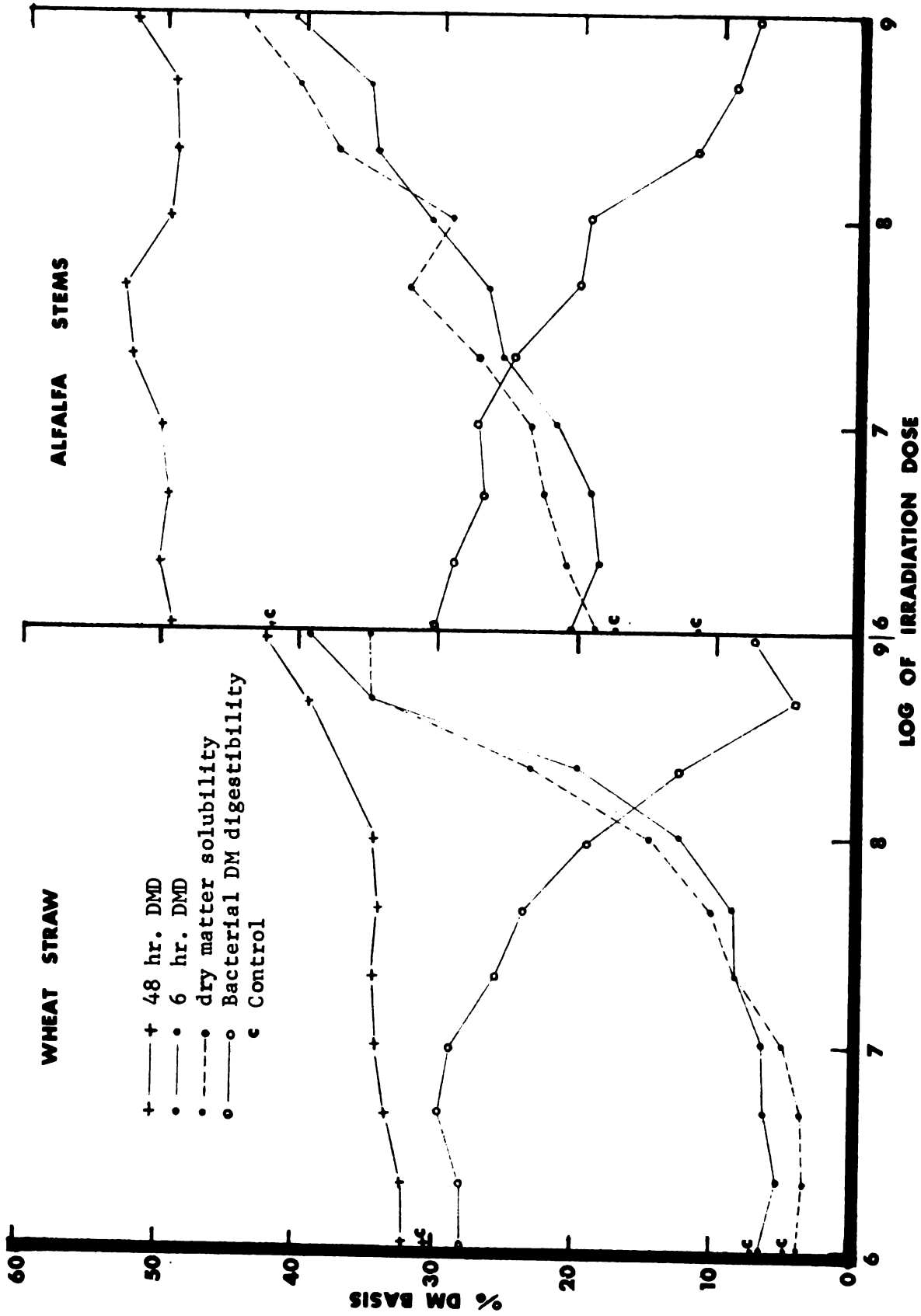


Figure 9. Influence of High-Energy Irradiation on Composition of Wheat Straw.

greatest percentage reduction with increasing dosage was found between $1x10^8$ and $4.57x10^8$ rads. The maximum reduction of CWC, ADF, hemicellulose and lignin at the dosage level of $1x10^9$ rads were 52, 45, 70 and 44%. These figures indicate that at high irradiation levels, the amount of all fibrous constituents was solubilized about one-half that in the original straw. The effect of irradiation on *in vitro* 6 hr. DMD, 48 hr. DMD and dry matter solubility (DMS) of wheat straw is presented in Figure 10. The greatest values for 6 hr. DMD, 48 hr. DMD and dry matter solubility were obtained with the straws that received the highest level of radiation. Extent and rate of increase for *in vitro* 6 hr. DMD was almost identical to dry matter solubility. At the highest level of radiation ($1x10^9$ rads), a three-fold increase in *in vitro* 6 hr. DMD and DMS was found. These large increases might have been predicted since chemical analysis indicated that 52% of the original CW was solubilized at this dose level. *In vitro* 48 hr. DMD was increased by approximately 33% at a dose of $1x10^9$ rads due primarily to the increase of dry matter solubility. The true portion of dry matter disappearance caused by rumen bacterial digestion was the difference between *in vitro* 48 hr. DMD and dry matter solubility values. As shown in Figure 10, dry matter disappearance due to bacterial digestion decreased progressively with increasing levels

Figure 10. Influence of High-Energy Electron Radiation of Wheat Straw and Alfalfa Stems on:

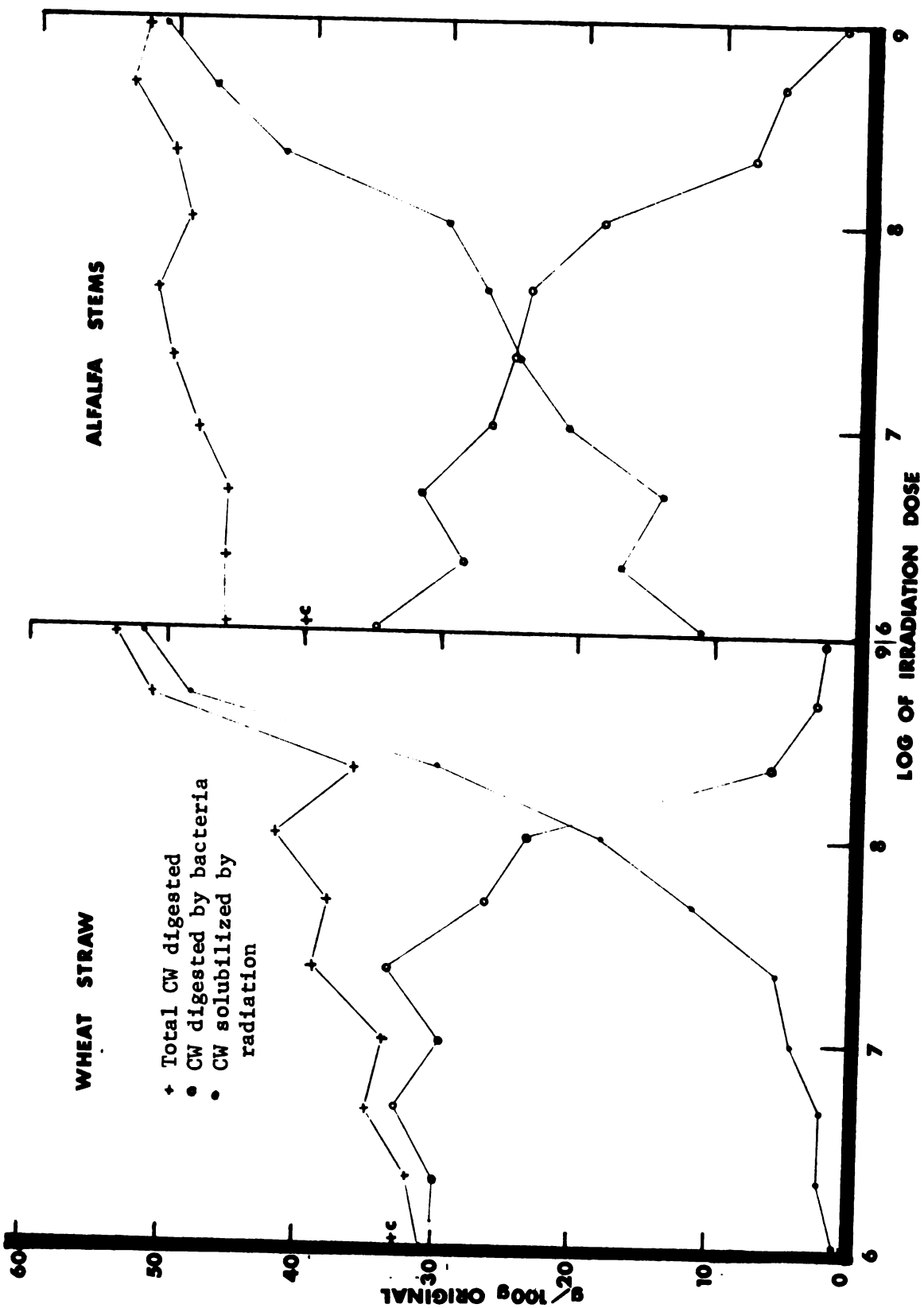
(1) In vitro 6 hr. dry matter disappearance (DMD)
(2) In vitro 48 hr. dry matter disappearance (DMD)
(3) In vitro bacterial dry matter digestibility
(4) Dry matter solubility (DMS)


WHEAT STRAW
ALFALFA STEMS
+——+ 48 hr. DMD
•——• 6 hr. DMD
•- - -• dry matter solubility
o——o Bacterial DM digestibility
c Control
% DM BASIS
LOG OF IRRADIATION DOSE
0
10
20
30
40
50
60
6
7
8
9/6
7
8
9

of radiation. The effect of radiation on total CW digestion of wheat straw is presented in Figure 11. The trends for changes in total CW digestibility, CW solubilized by irradiation and residual CW digestion by bacteria were similar to trends for in vitro total DMD (48 hr.), dry matter solubility and dry matter disappearance due to bacterial digestion, respectively. The difference between these two evaluation systems is that in the CW digestion system (Figure 11), the in vitro bacterial digestion was actually quantitatively determined, while in the total dry matter disappearance system (Figure 10), bacterial digestion was calculated by difference. On the other hand, the amount of total dry matter disappearance in vitro was actually measured for the latter system (Figure 10) but for the former system (Figure 11) the total CW digestion was calculated by adding the CW of solubilized and the CW digested by bacteria. Both results strongly indicated that high levels of irradiation on wheat straw depressed the extent of bacterial degradation of fibrous constituents in in vitro systems. These evaluation systems, however, did not clearly indicate the optimum dose for the release of nutrients from wheat straw. The maximum total CW digestion was obtained with the straw which had received the highest of radiation. About 52% of the original CW was solubilized. If the solubilized products can be utilized and

Figure 11. CW Digestibility of Wheat Straw and Alfalfa Stems as Influenced by Extent of Radiation.

Total CW digested = CW solubilized by radiation and CW digested by bacteria.
CW digested by bacteria = 100 x (Amount CW in product x IVDCWC) / C Amount CW in original material.
CW solubilized by radiation = 100 x (Amount CW in original material - amount CW in product) / (Amount CW in original material).
C = Total CW digested for original forage.


WHEAT STRAW
ALFALFA STEMS
+ Total CW digested
● CW digested by bacteria
• CW solubilized by radiation
g/100g ORIGINAL
LOG OF IRRADIATION DOSE
9/6
0
10
20
30
40
50
60
6
7
8
9

fermented to useful energy sources by the bacterial host, then the highest dose of $1x10^9$ rads should be recommended. However, Pritchard et al. (1962) found that the maximum in vitro VFA production was noted at a dose of $2.5x10^8$ rads; dosage levels higher than this depressed VFA production. Furthermore, other workers (Saeman et al., 1952; Millett et al., 1970) reported that high level irradiation (upward of $1x10^8$ rads) on cotton fiber or wood increased the level of carbohydrate depolymerization. For example, carbohydrate depolymerization of irradiated wood was about 15% at $1x10^8$ rads and increased to about 45% at $5x10^8$ rads. No study to date has adequately related solubilization and biological value of the solubilized constituents. In the present study, the greatest depression in bacterial digestion occurred between dosage levels of $1x10^8$ rads and $2.14x10^8$ rads. This finding and results from other workers might lead one to consider that the optimum dose for the release of nutrients from wheat straw would be close to $1x10^8$ rads.

B. Alfalfa Stems

The effect of irradiation on composition of alfalfa stems is presented in Figure 12. Similar to responses for irradiated wheat straw, the greatest reduction of fibrous constituents was obtained when alfalfa stems received the highest levels of radiation ($1x10^9$ rads). High levels of radiation only slightly degraded the

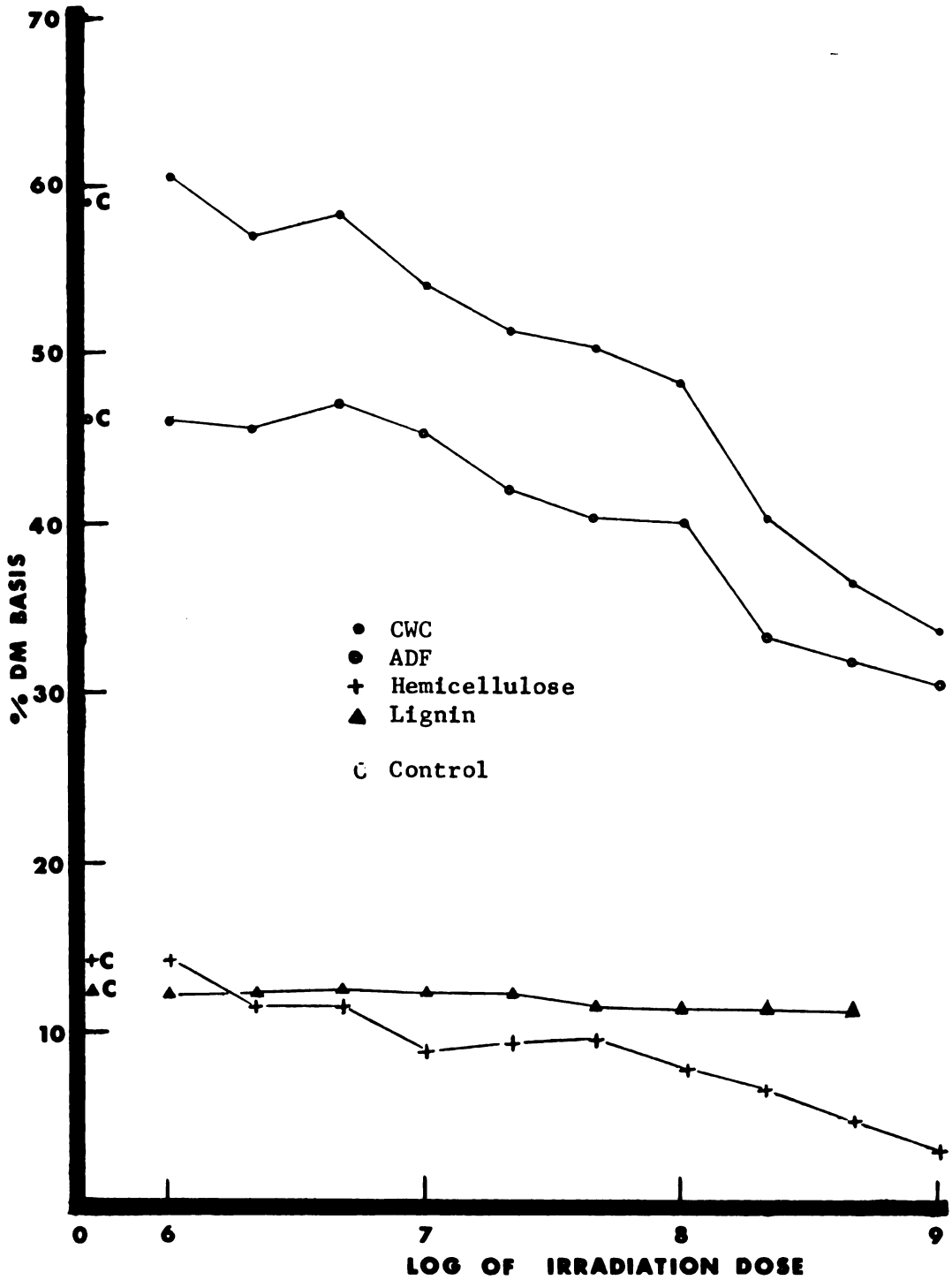


Figure 12. Influence of High-Energy Irradiation on Composition of Alfalfa Stems.

lignin of alfalfa stems. The maximum reduction in CWC, ADF and hemicellulose was 45, 34 and 80%, respectively, compared to values of 52, 45 and 70%, respectively, for wheat straw. The effect of irradiation on in vitro 6 hr. DMD, 48 hr. DMD and dry matter solubility of alfalfa stems is presented in Figure 10. The trends for in vitro 6 hr. DMD, dry matter solubility and in vitro bacterial DM digestion with dosage levels were linear type responses. A three-fold increase in 6 hr. DMD and a two-fold increase in dry matter solubility (DMS) were noted at the highest dose ($1x10^9$ rads). The percentage improvement in 6 hr. DMD was comparable to that for treatment with Cl_2 gas, pectinase (2.6%) and cellulase (2.6%) plus pectinase (1.3%). At the lowest dosage level ($1x10^6$ rads), in vitro 48 hr. DMD was increased by 16%. However, only a further increase of about 8% was found for in vitro DMD as dosage levels reached $1x10^9$ rads. Alfalfa stems showed a limited response to high levels of radiation in this respect. Furthermore, several chemical and enzymatic treatments such as $NaClO_2$, $Ca(ClO)_2$, organic Cl and cellulase (2.6%) plus pectinase (1.3%) were more effective in improving this value than was radiation.

As for wheat straw, values for bacterial DM digestion of alfalfa stems were calculated by the differences of in vitro 48 hr. DMD and dry matter solubility. The trend of changes with dosage levels was negative and comparable

to that for wheat straw indicating that high levels radiation also depressed bacterial degradation on fibrous constituents in alfalfa stems (Figure 10).

The effect of irradiation on total CW digestion of alfalfa stems is presented in Figure 11. The maximum improvement (32%) of total CW digestion was given at a dosage level of 4.57×10^8 rads (8.66 log irradiation dose). The change in amount of CW solubilized by irradiation and by bacterial digestion was comparable to that shown for wheat straw. As irradiation level increased there was a similar increase in CW solubilization but a decrease in CW microbial digestion. Similar improvements in total CW digestion were obtained from the highest irradiation level as from the chemical treatments of NaOH plus Cl_2 gas, cellulase plus pectinase, cellulase pectinase or NaOH.

With respect to optimum dose for the release of nutrients from alfalfa stems, similar conclusions can be made to those for wheat straw.

In summary the following conclusions appear warranted: (1) High-energy irradiation solubilized a larger amount of fibrous constituents in wheat straw than in alfalfa stems; (2) There was a decrease in cell wall digestibility by bacteria as doses increased for alfalfa and for straw; (3) High-energy irradiation increased total CW disappearance of wheat straw and alfalfa from values of 32 and

45 up to a value of approximately 53g/100 g of original CW. Similar increases were obtained by NaOH (6%) treatment; (4) Estimated treatment cost for irradiation was $150 per ton of air dry forages with irradiation at a level of 10^8 rads (Anonymous, 1959) compared to $60 per ton for NaOH (technical flake) treatment at a level of 6 grams per 100 grams forage (E. H. Sargent Co., Illinois, 1970) and $66 per ton for $NaClO_2$ industrial grade $NaClO_2$ treatment administered at a level of 5 grams per 100 grams dry forage (Goering and Van Soest, 1968). On the basis of these estimates, chemical treatment appears to be more economical than irradiation.

CONCLUSIONS

This study yielded the following conclusions:

1. Most treatments used in this study improved the nutritive value of wheat, oat straw and alfalfa stems based on *in vitro* and chemical evaluation methods.
2. The degree of response by forages to treatments were in the following order: oat straw>wheat straw>alfalfa stems. The nutritive value of the original materials were ranked in the opposite direction.
3. Chemical evaluation method predicted a greater nutritive value of treated straws than did *in vitro* evaluation method, but both methods predicted similar nutritive values for treated alfalfa stems. These facts indicated that some forage evaluation methods which are useful and valid for normal forages may need to be adjusted or modified in order to predict nutritive value of chemically or physically modified forages.

4. Sodium chlorite treatment specifically greatly reduced lignin content of both straws and alfalfa stems. It improved *in vitro* 6 hr. DMD slightly but markedly improved *in vitro* 48 hr. DMD indicating that $NaClO_2$ treatment increased the availability of the cellulose fraction to microorganisms.
5. Chlorine gas treated forages showed a very low pH and this treatment was very effective in reducing CW and hemicellulose. *In vitro* 6 hr. DMD was increased two to three fold by the Cl_2 treatment. However, *in vitro* 48 hr. DMD and DCW values showed that Cl_2 gas treatment depressed the digestibility of dry matter by bacteria.
6. When forages were treated with NaOH (6g/100 g DM) and ensiled for two weeks at room temperature, large amounts of acetate and butyrate were produced and the pH of silages was about neutral. This treatment solubilized hemicellulose to some extent but only slightly changed other fibrous constituents. *In vitro* 48 hr. DMD of NaOH treated wheat straw was greatly improved (72%) but only a slight improvement (2%) was noted for treated alfalfa stems.
7. The effect of the combined treatment of NaOH and Cl_2 gas on forage composition and *in vitro* DMD was similar to Cl_2 gas treatment alone.

8. Cellulase treatment was ineffective in improving nutritive value of oat straw but it markedly improved *in vitro* DMD of alfalfa. Pectinase treatment improved the nutritive value of alfalfa stems comparable to that with cellulase treatment.
9. Among sixteen chemical treatments, $Ca(ClO)_2$ treatment was the least effective in reducing forages fibrous constituents or in improving *in vitro* DMD.
10. Different treatment-ensiling times and temperatures did not cause marked changes in the resulting composition or *in vitro* DMD of NaClO treated wheat straw. High treatment temperature (80 C) considerably increased the lignin and ADF of Cl_2 treated wheat straw.
11. Wheat straws treated with chlorine compounds had very low acceptability to goats when fed wet and fresh.
12. Washing the treated straws with water markedly improved their intake. However, treated washed straws had a decreased organic matter recovery, dry matter solubility (*in vitro* 6 hr. DMD) and higher proportions of fibrous constituents than did unwashed straws. But both washed and unwashed straws had comparable values for *in vitro* CW digestibility.

13. Sodium chlorite and NaClO treatments increased *in vivo* dry matter digestibility of wheat straw. However, these increases were lower than those found in the *in vitro* studies.
14. Sodium chlorite, $Ca(ClO)_2$, NaClO and Cl_2 treated wheat straw produced a much higher proportion of propionate in the rumen than did the control straw.
15. Two goats died while consuming $NaClO_2$ treated straw. Silage extracts of $NaClO_2$ treated straw decreased *in vitro* 48 hr. DMD of standard alfalfa by 12%. This might indicate that there were inhibitory compounds to cellulytic organisms in treated-fresh silage.
16. High levels of irradiation caused a large amount of CW solubilized, but caused only a slight reduction in lignin of alfalfa stems.
17. High levels of radiation depressed *in vitro* bacterial CW digestibility of alfalfa stems and wheat straw.
18. The largest improvements in total CW digestion of straw and alfalfa stems were obtained either chemically from NaOH treatment or physically from high-energy electron irradiation. However, the treatment cost of irradiation is much higher

than NaOH treatment. Thus, in a practical situation, NaOH treatment is more feasible than irradiation.

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