AMMONIUM HYDROXIDE TREATMENT

OF WHEAT STRAW

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Large quantities of cereal grain are produced in many parts of the world. At least 1 kg of residue is left for each kg of grain produced. These residues are high-fiber, lignocellulosic material, and contain vast amounts of energy. Crop residues, when burned are a major source of environmental pollution. Environmental pollution control makes it essential to seek alternatives to customary burning of seed crop residues. To dispose of these wastes by utilizing them to produce valuable products would be doubly advantageous. Crop residues which are low in nutritive quality have potential as a feedstuff for ruminants since ruminants are uniquely adapted to convert large quantities of celluloses into high-quality human food. Unfortunately, voluntary intake of crop residues is low, and digestible energy intake usually is sufficient only for maintenance.

Cellulose and hemicellulose in low-quality roughages are not readily available to digestive enzymes of the rumen microorganism. Therefore, treatment is necessary to expose the fiber structure enough to permit rapid penetration by digestive enzymes.

Physical and chemical treatment of crop residue to improve digestibility and nutritive value have been reviewed recently by many investigators (Jackson, 1977; Klopfenstein, 1976). Many chemicals have been used to enhance digestibility and nutritive value; sodium, ammonium, calcium, and potassium hydroxides have been used most frequently. There is little detailed work with ammonium hydroxide. Ammonium hydroxide (NH_4OH) has the potential to reduce treatment cost as well as increase nitrogen content and digestibility of low-quality roughages.

The objective of this study was to determine (1) the effect of length of time, (days post-treatment) and water content of wheat straw on *in vitro* dry matter digestibility (IVDMD) and nitrogen retention of NH_4OH-treated wheat straw, (2) the relative amount of nitrogen retained as fiber-bound nitrogen (ADF-N) versus free ammonia nitrogen (NH_3-N), and (3) the changes in solubility and digestibility of various fiber fractions of straw due to NH_4OH treatment.

CHAPTER II

REVIEW OF LITERATURE

Wheat Grain

Wheat grain (*Triticum aestivum*, *T. durum*, *T. monocuccum*, *T. dicoccum* and *T. spelta*) is the world's most wide spread grain plant. Since the beginning of the present century, the world wheat production has more than doubled and now exceeds 300 million tons annually. Table 1 shows production of wheat in different areas of the world.

In the United States, wheat is second only to corn (Zea mays) in acreage and production; but it is generally used mainly for human consumption rather than for livestock feed.

Wheat Straw

It is estimated that one ton of wheat straw is available as a potential feedstuff for each acre of wheat which yields 20 to 25 bushels of grain. Straw is essentially the stems, leaves and chaff (non-seed spike parts) plus non-wheat or weed plants.

Agricultural and Industrial Uses

Feed. In the maturation process of wheat grain, most of the energy and protein formed by the wheat plant is transferred to the grain. At maturity, straw is relatively low in protein, starch and fat, but is still rich in energy, mineral and fiber (Table 2). Energy stored as

TABLE I

AREA AND PRODUCTION OF WHEAT IN 1973[a,b]

Country	(1000 HA)	Production (1000 MT)	Productivity MT/HA	Productivity Bu/A	Per Capita Production (kg)
U.S.S.R.	63,012	109,784	1.74	25.8	443.6
U.S.A.	21,800	46,407	2.13	31.6	222.2
China (Red)	25,000	28,000	1.12	16.6	47.4
India	19,463	24,735	1.27	18.8	43.1
France	3,957	17,792	4.50	66.7	347.2
Canada	9,856	16,459	1.67	24.8	754.3
Australia	8,956	12,094	1.35	20.0	933.3
Iran	6,325	4,546	.72	11.0	137.7
World	217,136	366,541	1.69	25.1	96.9

[a]U.S.D.A., 1975. Foreign Agricultural Circular, FG 5-75.

[b]United Nations. 1974. Demographic Yearbook, 1973. United Nations, New York.

TABLE II

COMPOSITION OF WHEAT GRAIN, WHEAT HAY AND WEHAT STRAW (%)[a]

	Wheat grain N.R.C.[b]	Wheat Hay, mature N.R.C.[b]	Wheat straw		
			N.R.C.[b]	VanSoest[c]	Personal observation[d]
Dry matter	88.9	85.2	87.8		90.64
Ash	2.1	6.9	7.2		1.70
Crude fiber	2.8	30.4	43.6		
Sheep dig. coef.	33.0	47.0	51.0		
Ether extract	2.1	1.7	1.5		
Sheep dig. coef.	72.0	43.0	21.0		
N-free extract	79.6	53.7	44.0		
Sheep dig. coef.	92.0	65.0	40.0		
Protein (x 6.25)	13.4	7.5	3.7		4.32
Sheep dig. coef.	78.0	55.0	-16.0		
Sheep dig. protein	10.5	4.1	-0.5		
Cell content				18.2	26.7
Cell wall const. (CWC)				81.8	73.3
Hemicellulose				28.0	24.1
ADF				53.3	49.1
Cellulose			50.1		39.5
Lignin			12.8	14.0	7.7
Starch	77.0				
Energy					
Sheep DE Mcal/kg	3.88	2.51	2.15		
Sheep ME Mcal/kg	3.18	1.98	1.43		
Sheep TDN	88.0	54.8	39.5		
Calcium	.09		.16		
Phosphorus	.39		.08		
Sulfur	.22		.18		
Carotene mg/kg			2.2		

[a]All values are expressed on a dry matter basis except dry matter.

[b]N.R.C. 1972. Atlas of Nutritional Data on U. S. and Canada feeds.

[c]VanSoest, P. J., 1966.

[d]Solaiman, S. G., 1977. Unpublished data.

lignocellulose is not readily available. The fibrous characteristics make straw unsuitable as the main or sole dietary component for beef cattle, dairy cattle or sheep fed for high rates of meat or milk production. However, it can successfully supply part or all of the dietary roughage (Fahmy et al., 1968; O'Donovan and Ghadaki, 1973; Penzhorn, 1956; Richardson et al., 1953).

Bedding or Litter. Wheat straw provides bedding material for livestock to keep animals clean, warm and comfortable. Wheat straw has the potential to absorb moisture in urine and feces and retain nitrogen from excerta to be used as a fertilizer. Chaff is preferred to the stem and leaf for use as litter by poultry enterprises.

Soil Conservation. Wheat straw which is left in the field plays a role in soil conservation, protecting the soil from wind and water erosion. Also, it gives structure and fertility to the soil and aids in evapo-transporation of water.

Industrial Uses. Large amounts of lignocellulose material present in wheat straw make it a favorable source for pulp. Straw pulp is used to manufacture fiber board such as straw board, and paper products (Aronovsky, 1952; Hammond, 1950).

Factors Affecting Utilization of Low-Quality Roughages by Ruminants

The two most important factors which influence utilization of low-quality roughages by ruminant animals are intake and digestibility. These factors and their interrelationships are shown in Figure 1.

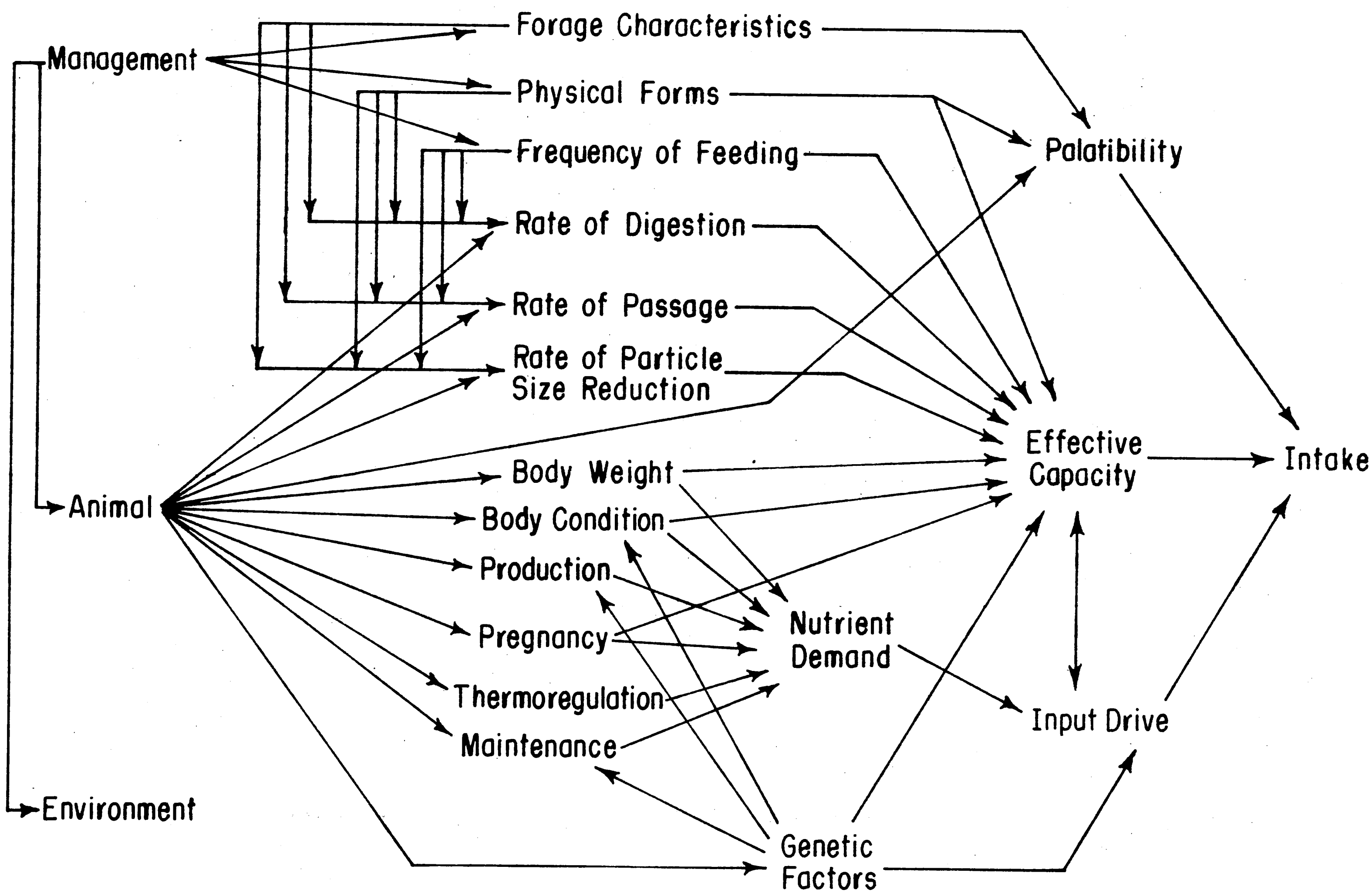


Figure 1. Factors Affecting Voluntary Intake in Ruminant Animals and Their Interrelationship (Mertens, 1973).

Chemical characteristics which reflect these factors include the proportion of cell wall constituents (CWC) which dictate bulk density of forages. Roughages with high cell wall constituents (70-80%) such as straw have higher bulk density values which distends the rumen and limits feed consumption. The proportion of plant CWC tends to have adverse effects on voluntary intake and digestion rate. A lower digestion rate slows rate of passage of digesta through the digestive tract, and decreases rate of passage and further despresses intake (Blaxter, 1950; Blaxter et al., 1961).

Chemical Structure of Cell-Wall Constituents

The chemical structure of CWC, depending on forage species and stage of maturity is made up of variable amounts of cellulose, hemicellulose, lignin, cutin, silica and other minor substances. Cellulose, hemicellulose and lignin exist in close chemical and physical association and account for most of the CWC in plants. Cellulose is a polymer of glucose units, and the degree of polymerization varies within and between sources of cellulose (Timell, 1964). Cellulose is usually the most abudant and insoluble polysaccharide which forms the fibrous structural backbone of CWC. The fibrils of cellulose are located in a matrix of hemicellulose and lignin and are in close physical and chemical association with both (Siegel, 1962). Hemicelluloses are amorphous polysaccharides which include short chain glucans, polymers of xylose, arabinose, manose and galactose plus mixed sugar and uronic acid polymers. The main hemicellulose in forage is xylan which exists in close association with cellulose and lignin. Hemicellulose generally can be separated from the cellulose by extraction with dilute acid or alkali

(Jarrige, 1960; Waite et al., 1964). The amount of hemicellulose in plant cell walls depends on the type of plant, but varies from about 6 to 40% of dry matter (DM). The availability of hemicellulose to rumen microorganisms is generally 45 to 90% (Pigden and Heaney, 1969). Lignin is an aromatic component present from 2% in immature forages up to 15% of dry matter in mature forages. Lignin is closely associated with cell wall polysaccharides and frequently acts as a physical barrier and impedes microbial breakdown of the lignocellulose materials. Thus the lignocellulose complex which comprises most of the organic matter of the CWC consists nutritionally of three fractions: (a) unavailable fraction (i.e. lignin) which is not degraded by rumen microflora; (b) digestible energy (DE) fraction, which represents the available carbohydrates for bacterial degradation; and (c) potentially digestible energy (PDE) fraction, representing the unavailable carbohydrates which have physical or chemical association with the lignocellulose complex, but can be treated effectively by physical or chemical treatments, or supplemented with different nutrients to increase ruminal degradation (Pigden and Heaney, 1969).

Environmental Factors Affecting Cell-Wall Constituents

The nutritive value of forages is usually related to the availability of nutrients in the plant to the animal. Availability of nutrients in forages depends on chemical composition and factors limiting utilization of cellulose and hemicelluloses. Chemical composition of forages is controlled by environmental factors such as light, temperature, and fertilization (Alberda, 1965; Blaser, 1964; Deinum, 1966; Deinum et al, 1968).

Light. Nutritive value of the plant has a positive correlation with light intensity. High light intensity during plant growth increases the water-soluble carbohydrate and decreases crude protein, ash and fiber components (Deinum, 1966).

Temperature. High temperature for growing plants decrease water-soluble-carbohydrate as well as hemicellulose content and increase lignin, CWC, and cellulose which might account for depressed cell-wall nutrients (Deinum, 1966). Generally the negative effect of temperature will out-weigh the favorable effect of light in summer growth (Deinum et al., 1968).

Fertilization. Nitrogen fertilization of plants increases total nitrogen (Cowling and Lockyer, 1967; Reid, 1966), alkaloids (Bennet, 1963; Gentry et al., 1969) and carotene (Smith and Wang, 1941), but decreases cellulose (Reid et al., 1967), water-soluble carbohydrate (Blaser, 1964; Bryant and Ulyatt, 1965; Gordon et al., 1962; Jones et al., 1961; Jones et al., 1965), silica (Jones and Handreck, 1967) and acid-insoluble lignin (Reid et al., 1967). Depending on the balance of different factors, digestibility might change in either direction with fertilization (VanSoest, 1969).

Physio-Chemical Factors Affecting Availability of Cell-Wall Constituent

Encrustation. Lignin, cutin and silica, the insoluble chemical components present in CWC, physically encrust cellulose and hemicellulose and protect them from microbial digestion (Ghose and King, 1963; Wardrop and Bland, 1959). The lignin apparently prevents microbial enzymes from contacting a sufficient number of glycosidic bonds to permit rapid hydrolysis (Cowling and Brown, 1969). Consequently, ball-milling of lignified tissue increases the IVDMD of cellulose (Dehority and Johnson, 1961) and hemicellulose (Dehority et al., 1962) due to breakage of lignocellulose fraction and providing more surface area for attachment by microorganisms.

The Covalent Bonds Between Lignin and Carbohydrates (Cellulose and Hemicellulose). Covalent chemical bonds have been suggested to exist between lignin and carbohydrates in CWC (Freudenberg and Harkin, 1960). The combination of lignin with partially crystaline cellulose forms a bond very resistant to enzymatic hydrolysis. By this intimate chemical (and possibly physical) association of cellulsoe with lignin, rumen bacteria are prevented from degrading the cellulose in lignocellulosic material, since rumen bacteria (anaerobic organisms) lack enzymes to cleave this bond. Organisms which possess lignases are aerobic and act by peroxidative cleavage of the aromatic rings in lignin.

Silica (SiO_2). Silica is an important factor in reduction of digestibility of plant CWC (VanSoest and Jones, 1968). The mechanism involved is not like the case of lignin and cutin (encrustation) but rather existance of some as yet unknown chemical linkage(s) between the hemicellulosic carbohydrate and silica (VanSoest and Lovelace, 1969).

Silica is absorbed, metabolized and deposited in large quantities within the cell wall matrix of grasses. In contrast to grass, legumes do not metabolize large amounts of silica. Negative correlations between lignin and silica content in plant cell-wall structures might be due to the function of silica as a physiological replacement for lignin (Van Soest, 1968).

Silica in plants depresses dry matter digestibility by about 3% for each 1% increase in plant silica (VanSoest and Jones, 1968).

Tannin. Tannins have been suggested to depress intake and digestibility of sericea lespedeza (Lespedeza cuneata G. Don) (Donnelly, 1954; Donnelly and Anthony, 1969, 1970; Lyford et al., 1967; Smart et al., 1961). The digestibility of bird resistant sorghum (Sorghum vulgure) may also be decreased by tannins as a result of inhibitation of cellulolytic (Harris et al., 1970; Cummins, 1971) and pectinolytic enzymes (Bell et al., 1965; Hathway and Seakins, 1958).

Begovic and Duzic (1977) indicated that some decomposition of tannins occurs by enzymes present in rumen mucosa of cattle, and showed that a water extraction of rumen mucosa can decompose tannic acid to gallic acid but the rate of process was very slow.

Voluntary Intake and Chemical Composition of Cell-Wall

The cell-wall constituent level of the forages determines the space occupying capacity or bulk density of the forages (Balch and Campling, 1962). Cell wall constituent quantity is negatively correlated to voluntary intake, especially with high fiber diets (VanSoest, 1965).

Even though the chemical composition of forages is more likely related to digestibility of forages, VanSoest (1965) has reported that CWC was the only structural component which was consistently related to voluntary intake. The relationship, however, may be curvilinear. Cell-wall constituents are poorly related to voluntary intake in forages when CWC comprised less than 50 to 60% of the dry matter. This range would include most legumes and immature grasses. But above 50 to 60% CWC, as with straws and low-quality roughages at 70 to 80% CWC, voluntary intake decreases drastically with increasing levels of CWC. In forages with low CWC, dry matter digestibility and voluntary intake do not seem to be related. But as the CWC level in forage increases, dry matter digestibility and voluntary intake show a higher correlation (VanSoest, 1965).

Processing which includes reduction of particle size or pelleting, will destroy the volume-time relationship so that the same digestive tract has the ability to hold more dry matter and voluntary intake will increase. Furthermore, this processing effect has a greater effect on poor quality forages with high CWC (Cate et al., 1955; Moore, 1964; VanSoest, 1966).

Nitrogen, Energy and Mineral Supplementation

Utilization of low quality roughages by ruminant animals is depressed by (1) low feed intake, and (2) low digestibility. Feed intake is affected by a slow rate of passage of ingesta through digestive tract, which may be attributable to unbalanced diet (inadequate nitrogen, energy, mineral) or physical inhibition, both of which limit rate of bacterial digestion (Elliot and Topas, 1963).

The ruminal effects of inadequate nitrogen are probably most important in degressing intake with low quality roughages. When nitrogen becomes limiting, microbial growth diminishes, resulting in depression in fermentation which leads to increased fill due to both lower digestibility of ingesta and lower rate of passage of ingesta. The increased fill resulted in decreased feed intake (Mertens, 1973).

Shin (1976) reported that the increased voluntary intake of low quality roughages, when supplemented with nitrogen is atributable to (1) an increased rate and extent of digestion by cellulotic bacteria, and therefore an increased rate of passage of ingesta through rumen, and (2) a direct effect on the animal's nitrogen status.

Low quality roughages are essentially energy feeds, but the proportion of readily available energy content is too low to optimize utilization of feed by rumen microflora. Supplementation with readily available carbohydrate to the diet enhances microbial growth and microbial protein production due to energy and branched chain volatile fatty acid supplementation (Shin, 1976). Mulholand et al. (1976) have studied the effect of different levels of starch (5, 10, 20, 30, 40%) on utilization of straw diet by wethers. Best results were obtained with 30% starch. Addition of starch increased organic matter digestibility, but at higher levels it depressed cellulose digestion. Intake and live weight gain was depressed with 5% additional starch, but further addition resulted in an increased feed intake, weight gain and wool production. Appreciable energy retention occured only when the diet contained at least 20% starch. Digestibility of nitrogen was similar with all levels of starch supplementation, but nitrogen retention increased at higher starch concentrations due to decreased urinary nitrogen excretion.

Sulfur was one of the first elements after carbon, hydrogen and oxygen which was found to be required for the growth of rumen microflora (Loosli and Harris, 1945). The addition of sulfur to roughage diets generally has increased voluntary intake (Kennedy and Siebert, 1972, 1973; Playne, 1969) digestibility of crude fiber (Bray and Hemsley, 1969; Deif et al.,1970; Hume and Bird, 1970; Kennedy and Siebert, 1972;

Starks et al., 1954) and energy utilization (Bird, 1972). Sulfur content of forages varies between species, stage of maturity, sulfur status of the soil and season of the year. Sulfur and crude protein content is highest in young, actively growing plants, but decreases markedly with the onset of flowering (Begg and Freney, 1960). Also, moisture stress depresses the sulfur and crude protein content of forages.

Phosphorus is another required element for animals. Phosphorus content of forage decreases with maturity (Fraps and Fudge, 1945; Lampkin et al., 1961), and there is a positive correlation between crude protein and phosphorus content in plants (Hemingway, 1967). Shin (1976) summarized that phosphorus supplementation of diets for ruminant animals (a) increases digestibility of dietary components, (b) increases voluntary feed intake, (c) increases metabolic efficiency and (d) increases growth.

Processing Effects on Low-Quality Roughages

Physical Processing of Roughages

Physical processing of low quality roughages improved energy utilization. Most physical treatments alter the association of lignin to structural carbohydrates in plant cell walls and, thereby, free digestible portions for microbial attack in the rumen.

The rate of disappearance from rumen is a function of both rate of passage and particle size (Mertens, 1973). Particle size reduction provides more surface area for microbial attack and allows faster rates of passage and disappearance. Therefore, intake increased. Practically, reduction in particle size has little influence on the rate of digestion of lignocellulose material, but speeds the rate of passage from the rumen, and greatly enhances intake and animal performance (Pigden and Heaney, 1969).

Grinding and Pelleting. Mechanical breakdown of lignocelluloses beyond that achieved by animals tends to (1) reduce the amount of time and effort required by the ruminant and its rumen microbes to breakdown the material into more optimum size to pass through the reticulo-omasal orifice and lower digestive tract, (2) increase surface area for cellulotic bacteria to attack and speed the rate of fermentation and rumen turnover rate, (3) increase the gut capacity of the animal due to increased density of the feed (Pigden, 1971).

An *in vitro* study using two different forages (alfalfa, bromestraw) ground through 2 mm versus .2 mm screen in a micro-Willey mill, indicated that percent carbohydrate fermented was higher with fine particle size. Thus, altering particle size of low quality forages by fine grinding or pelleting, probably breaks down lignocellulose complex, and converts part of the potentially digestible energy into digestible energy (Pigden and Heaney, 1969). It also has produced high *ad libitum* intake (Meyer et al., 1959; Minson, 1962; Moore, 1964).

Reduction of particle size may reduce the time which feed particles are exposed to rumen microbes and accelerate the flow of digesta through the alimentary tract, thereby lowering the efficiency of digestion (Greenhalgh and Wainman, 1972; Hashizume et al., 1975; Laredo and Minson, 1975). The increased fecal energy losses per unit of forage eaten as a result of the rapid rate of passage of ground material from the rumen, is partially compensated by increased voluntary feed intake (Greenhalgh and Reid, 1974; Heaney et al., 1963; Wainman and Blaxter, 1972), and by reduced energy losses as methane and heat increment (Blaxter and Graham, 1956; Paladines et al., 1964).

A study conducted by Piatkowski et al. (1977) to evaluate the effect of pelleted straw on rumen fermentation showed that pelleted straw, included in a ration of concentrated mixture, did not provide optimum conditions for VFA formation and microbial protein synthesis.

In a study conducted by Muller and Bergner (1977), the physiochemical structure of straw was examined by iodine absorption, tritium exchange, bulk density, and digestion with NaOH. Pelleted straw had higher chemical reactivity (higher iodine absorption and tritium exchange). The effect of pelleting was similar to crushing of cellulose. Changes during pelleting are indicative of similar changes in cellulose fraction during crushing in regard to changes of crystalline cellulose regions to an amorphous form. Thus, pelleting probably may have an effect on both cellulose and lignin components.

Grinding and pelleting low-quality roughages increased average daily gain (Lindahl and Terril, 1963; Paladines et al., 1964; Wainman et al., 1972), the molar proportion of propionic acid (Paladines et al., 1964; Wainman and Blaxter, 1972; Wright et al., 1963) and improved nutritive value (Paladines et al., 1964; Wainman and Blaxter, 1972). Therefore, grinding and pelleting are effective ways to improve performance of ruminants fed low-quality roughages by increasing digestible energy intake.

Ball milling of timothy reduced particle size small enough to fracture lignified cellulose in studies of Dehority and Johnson (1961). These processed forages showed marked improvement, in *in vivo* digestibility. However, the practical application of such treatment was not economically feasible.

Steam and Pressure Processing. Another approach to improve the digestibility of lignocellulosic residues is steam and/or pressure treatment. Steam processing produces acetic and formic acid which decompose fiber encrustation (Abele, 1940). Heaney and Bender (1970) reported that ammonia can be introduced at the end of steaming process to neutralize the acids formed. Furthermore, this served as a basis of fixing nitrogen from ammonia addition during the processing which would be available as a source of nitrogen to rumen microbes.

Steam processing has been utilized in the treatment of both straw (Abele, 1940; Waiss et al., 1972) and wood (Heaney and Bender, 1970). Hardwood showed more response in digestibility than soft wood.

An *in vivo* study using steamed aspen (*Populus tremuloides*) with sheep (Heaney and Bender, 1970) and with Holstein steers (Heaney et al., 1973) indicated that processed hardwoods can be utilized practically by ruminants in two ways: (a) as roughage components of finishing rations in feedlots or (b) as part of an all-roughage diet. Up to a 70% level, steamed wood mixed with hay provided energy for maintenance and low levels of production suitable for wintering of beef cows or sheep and breeding flocks.

Steam pressure treatment of lignocellulose material to break the fiber structure has been tested by many investigators (Klopfenstein, et al., 1967; Klopfenstein and Bolsen, 1971; Umunna and Klopfenstein, 1972) and showed improved digestibility. Most studies with steam-pressure treatments have used two chemicals, sodium meta bisulfite ($Na_2S_2O_5$), to reduce protein damage during heating, and sodium hydroxide, to oxidize organic matter. With only water added to the reaction media, optimum increase in IVDMD of wheat straw was obtained at 21 kg/cm^2

(Klopfenstein and Bolsen, 1971).

High pressure-high temperature treatment of rice straw with or without added chemicals and steam has been studied by Garrett et al. (1974). Results of this study indicated that straw treated with steam pressure at 28 kg/cm^2 (231^{o}C) for 90 seconds, did not improve animal performance. Alkali addition improved cellulose digestibility, food intake and daily gain, but depressed nitrogen digestion in lambs fed treated straw. Further growth inhibitors may be produced as a result of high pressure and temperature processing of low quality roughages (Walker et al., 1975).

Irradiation of Roughages. Irradiating low-quality roughages with gamma rays or high velocity electrons physically alters the forage cell wall to make the nutrients readily available to rumen microorganisms.

Gamma irradiation of wheat straw at levels of 1 x 10^8 and 2.5 x 10^8 rads increased the dry matter digestibility *in vitro* from 40% to approximately 70% (Pritchard et al., 1962). Increased VFA production during the *in vitro* fermentation indicated that breakdown products of irradiation were largely available to the rumen microbes. Lawton et al. (1951) concluded that in gamma irradiated wood, cellulose seems to be affected rather than lignin. Mater (1957) reported that one effect of high energy irradiation was the breakage of lignin-cellulose bonds which increased cellulose digestibility.

Electron irradiation was also effective in solubilization of lignocellulose complex. Optimum effectiveness was obtained at about 1 x 10^8 rads. The degree of polymerization of the irradiated cellulose was decreased which indicated extensive bond breakage (Millet et al., 1975).

With equipment presently available for commercial feed processing and current feed prices, the costs of most physical treatment methods are economically infeasible.

Chemical Treatment

Chemical treatment of crop residues has increased digestibility and improved nutritive value due to cell-wall disruption and by solubilization of hemicellulose (Chandra and Jackson, 1971; Fernandez and Greenhalgh, 1972; Waller, 1976), lignin (Chandra and Jackson, 1971; Fernandez and Greenhalgh, 1972; Jones and Klopfenstein, 1967) and silica (Chandra and Jackson, 1971; Fernandez and Greenhalgh, 1972). Improved cellulose digestibility (Garrett et al., 1974; Waller, 1976) as a result of swelling has also been reported. Chemicals are needed to disrupt the close physical and chemcial association between lignin-cellulose and lignin-hemicellulose complex, which would contribute to the higher digestibility of alkali treated roughages.

A number of chemicals have been tested for their ability to increase the digestibility of crop residues, but none has proven as effective and as easy to apply as alkali (NaOH). Alkalis which have been widely used to increase digestibility of crop residues include NaOH, NH_4OH, $Ca(OH)_2$ and KOH.

Sodium Hydroxide (NaOH). Sodium hydroxide is the most widely studied alkali. For increasing the digestibility of crop residues, *in vitro* studies and digestion trials with ruminants have indicated that NaOH has high potential for treating crop residues due to its effectiveness and relative safety. Sodium hydroxide has been applied to a variety of residues.

Wheat straw was one of the first residues to be effectively treated. Beckmann (1921), one of the early workers in this area, had soaked wheat straw in 1.5% NaOH solution for 24 hours. The treated and washed product had a digestibility of about 70%.

To evaluate nutritive value of wheat straw treated with different levels of NaOH (0, 3, 6 and 9 gram/100 g DM), lambs were fed ad libitum a ration that consisted of 60% wheat straw (Shin, 1976). Average daily gain of the lambs fed the 0, 3, 6 and 9% un-neutralized NaOH-treated straw was 43.8, 153.4, 66.6 and -4.0 grams, respectively. Straw dry matter and organic matter digestibility tended to increase linearly with the level of NaOH which was consistent with the results of Wilson and Pigden (1964). However, animal performance was more closely related to feed intake rather than observed digestibilities.

In a study conducted by Wilson and Pigden (1964), various amounts of NaOH was sprayed onto straw, while water was added at a constant rate of 30 ml/100 g of straw DM. The treated wheat straw was then allowed to react for different periods of time prior to analysis. Digestibility of un-neutralized NaOH-treated wheat straw was 70% as measured in vitro, and increased linearly with increasing levels of NaOH up to 8 to 9%. In the same study, NaOH additions to the in vitro fermentation media caused no improvement in IVDMD for either treated or untreated wheat straw. Only 70% of 6% NaOH added was utilized during the 21 day reaction period.

In a study of Braman and Abe (1977), wheat straw was treated with NaOH, KOH and NH_4OH at either 2 or 4% (w/w) levles. Sodium hydroxide was the most effective alkali. In vitro organic matter digestibility of NaOH-treated straw was increased (144 and 227% over control untreated straw for 2 and 4% levels of NaOH, respectively), while acid detergent fiber (ADF), acid detergent lignin (ADL), cell-wall constituents (CWC) and hemicellulose were decreased (from 50.6 to 38.3; 20.4 to 15.0; 80.4 to 47.4; 30 to 9.1) with increasing concentrations of NaOH (0-16%) respectively. Crude protein (CP) (5.2 to 6.1), ash (9 to 11.9) and ADF (46.9 to 52.7) increased, while hemicellulose decreased (24.6 to 17.5) as duration of chemical treatment increased from 0 to 56 days. In a steer feeding trial, apparent dry matter and crude fiber digestibilities were improved by NaOH treatment of wheat straw.

A number of studies relative to the chemical treatment of rice straw have been conducted by different investigators (Garrett et al. 1974; Rexen and Moller, 1974). Garrett et al. (1974) pressure treated rice straw with and without added NaOH. Pressure treating at 28 kg/cm^2 and $231^{o}C$ with 4% added NaOH improved performance, feed intake, and daily gain of lambs fed 65-72% treated rice straw ration. Sodium hydroxide treatemnt increased cellulose digestion. However, nitrogen digestibility was depressed. In another study ground rice straw was treated with 4% NaOH as a concentrated solution. The treated material was pelleted to compact the product which requried no dehydration for safe storage. Daily gains of lambs fed treated straw were increased approximately 50% (Rexen and Moller, 1974).

In a paper reported by Saxena et al. (1977), oat straw was treated with 1.5% NaOH solution for 22 hours. The treated material was washed with water and neutralized with 1% acetic acid solution. Lambs were fed treated and untreated oat straw with 3 different sources of supplemented nitrogen, soybean oil meal, urea, or diammoniumphosphate. Dry matter intake, crude protein, daily gain and feed efficiency were improved by straw treatment. Lambs fed treated straw had lower fecal out put, due to higher dry matter digestibility of treated straw. This increased the protein percentage in feces; however, ADL and cellulose fractions were higher and neutral detergent fiber (NDF) was lower. Differences between composition of feces regarding to supplemental N sources were small.

Chemical treatment of barley straw with various levels of NaOH (0-16%) demonstrated that the optimum level of NaOH to improve digestibility was 8% NaOH of DM (Ololade et al., 1970). In vitro dry matter digestibility of treated barley straw reacting at 23^{o} and $121^{o}C$ were 59.3 and 84.8%, respectively. These results indicated that temperature is an important factor in rate and extent of response produced (Ololade et al., 1970).

Maeng and Mowat (1971) reported that digestible energy was increased by feeding 6% NaOH treated barley straw which had been exposed to steam for 30 minutes at atmospheric pressure.

Koers et al. (1972) conducted an in vivo study to evaluate chemically treated combine tailings. Moisture content was adjusted to 50% by adding water to ground tailings, and NaOH was added at 4% of dry matter. Lambs were fed 80% treated-tailings plus 20% supplement. A corn silage based ration was used as the control. Average daily gains (kg) and feed efficiencies were .13, 7.5 and .17, 8.3 with treated tailings and corn silage rations, respectively. Digestibility was improved 16.7% over untreated tailings. In the same study IVDMD of stalkage increased 55% when treated with 4% NaOH. In a lamb digestion trial, however, the improvement noted for the treated stalkage was not as large as that demonstrated for corn cobs, grass hay and milo tailings.

Calcium Hydroxide /$Ca(OH)_2$/. Calcium hydroxide was selected as a chemical reagent for treating crop residues because of its low cost and ease of handling compared with NaOH. Since the cost of $Ca(OH)_2$ is only about one-third that of NaOH, its use could markedly reduce chemical costs of treatment. Since $Ca(OH)_2$ is less caustic and less hygroscopic than NaOH, it does not requrie as much safety equipment and is therefore easier to apply. It also provides supplemental calcium for the total ration.

Rounds et al. (1976) reported that $Ca(OH)_2$ in combination with NaOH in a total of 4 or 5% alkali treatment improved IVDMD. However, $Ca(OH)_2$ treatment alone was ineffective for IVDMD improvement. This may be due to its lower dissociation constant than NaOH or KOH. Calcium hydroxide may also require a longer reaction period for complete effectiveness. Lambs consumed more feed, gained faster and more efficiently when fed cobs treated with 3% NaOH plus 1% $Ca(OH)_2$ than cobs treated with 4% NaOH alone.

Verma and Jackson (1975) and Gharib et al. (1975) found $Ca(OH)_2$ to be much less effective than NaOH, probably because of low solubility. However, when $Ca(OH)_2$ treated-material was allowed to react for 150 days, digestibility of treated material was increased as much as the digestibility of NaOH treated material.

In a study conducted by Waller and Klopfenstein (1975), 4% NaOH treated cobs produced significantly ($P < .05$) greater lamb gains than that of 4% $Ca(OH)_2$ treated cobs. Combination of $Ca(OH)_2$ and NaOH produced somewhat better gain than either alkali alone. It is not clear at this point whether the synergism is due to chemical or nutrition effects (Klopfenstein, 1976). Cornstalks treated with NaOH and $Ca(OH)_2$

in different combinations have been fed to lambs. When at least 3% NaOH was presented in a total of 4 to 5% treatment combination, rate and efficiency of gain was increased.

Potassium Hydroxide (KOH). Potassium hydroxide has been used in treating crop residue effectively. Braman and Abe (1977) observed an improvement in IVDMD of wheat straw treated with KOH at either 2 or 4% level of DM. Anderson and Ralston (1973) reported significant increase in IVDMD of ryegrass straw when a combination of NaOH and KOH were used for treatment.

In a study conducted by Rounds et al. (1976), ground corn cobs were adjusted to 50% moisture level and were treated with combinations of NaOH plus KOH or NaOH plus $Ca(OH)_2$. Total amount of added chemicals were 4 or 5 g/100 g DM. Digestibility was increased over untreated samples in both cases, but as the replacement of NaOH by either KOH or $Ca(OH)_2$ increased, the IVDMD was depressed. Potassium hydroxide in combination with NaOH also was used to treat corn cobs (Klopfenstein and Woods, 1970). Potassium hydroxide and NaOH were applied in a ratio of 1:1 or 1:2 at 4 and 5% total treatment combinations. Organic matter digestibility was improved in treated samples and NaOH or KOH were of equal value for enhancing energy utilization from treated corn cobs. There was little effect of Na:K ratio. Since KOH is more expensive than NaOH, replacement is not economically desirable for treating crop residues.

Ammonium Hydroxide (NH_4OH). Another long-standing approach to improve the nutritive value of lignocellulosic material involves treatment with aqueous or gaseous ammonia. Lehmann (1905) is probably one of the earliest researchers that treated straw under pressure with aqueous ammonia. Later on, Oehme and Koln-Rath (1943) described a two stage process of chemical treatment. In the first stage, moist wood was reacted with ammonia (NH_3) at 130°C and, in the second stage, it was oxidized with air under 10 atm pressure. The product contained 5 to 8% nitrogen.

In recent studies NH_4OH attracted attention for treatment of low quality roughages because of low treatment cost. In addition to low cost, NH_4OH adds no Na or Ca ions. Sodium hydroxide adds excess Na to the soil to which the urine and feces are applied and these excess ions may cause a depression of mineral colloid, and change soil structure (Buckman and Brady, 1972). The nitrogen supplied by NH_4OH treatment also could offset part of the ration cost. Ammonium hydroxide also is effective as a preservative to reduce microbial activity and dry matter losses in hay stored above 20% moisture (Knapp et al., 1974). Other workers (Waiss et al., 1972) reported that as long as ammonia was present in treated rice straw, there was no apparent spoilage or decomposition from microbial action. Ammonia is a slow reacting alkali requiring a processing time of several days (Waiss et al., 1972). It has been studied by the ensiling method or similar methods and has been shown to be effective for treating crop residues (Garrett et al., 1974; Waller, 1976). There was an intake problem when 4% NH_4OH treated corn cobs were fed alone. The intake problem was overcome by combining 4% NH_4OH treated cobs with corn silage in a 1:1 ratio at feeding time (Waller, 1976). The ammonium hydroxide treated-cobs and corn silage combination produced animal performance equal to corn silage alone (Rounds et al., 1976).

German researchers, Bergner et al. (1977) fed ammoniated wheat straw pellets as a sole feed for ruminants. Ammoniated wheat straw pellets produced performance equivalent to conventional green feeds. ruminal fermentation was similar to that for silage. In comparison with grazed pasture, chemically treated straw produced higher levels of total fatty acids and increased the proportions of butyrate and propionate in the rumen.

Naik and Shah (1975) conducted a study to evaluate the nutritive value of NH_4OH-treated wheat straw. Wheat straw was treated with 40 and 80 ml of 30% ammonia solution (6 and 12% ammonia/100 g straw) in plastic bags. Nylon bag dry matter digestibility was increased from 44 to 54 and 55% and crude protein was increased from 2.7 in untreated straw to 7 and 7.5% in the treated samples, respectively. In the same study dry wheat straw was treated with ammonia vapor by placing 100 g of ground straw above a liquid ammonia solution containing 17 g ammonia in a desicator with 30 mm of Hg pressure at room temperature for 40 minutes. Digestibility was not improved. In contrast, Millett et al. (1970) observed an average increase of 19 units in dry matter digestibility by treating aspen saw dust with ammonia vapor under pressure.

Garrett et al. (1974) conducted a study to evaluate NaOH and NH_4OH treated rice straw. Digestion and comparative slaughter feeding trials were conducted with lambs. Sodium hydroxide was sprayed at 4% of DM basis before pelleting. Ammonia was sprayed at 4 to 7% of DM followed by 30 day storage period post-treatment. Nutritive value of rice straw was improved by both chemicals. Rates of gain of lambs fed 65 to 72% rice straw (treated with NaOH and NH_4OH) rations were increased by 50%. This was mostly due to improved cellulose digestibility and greater feed consumption. Ammonia treated straw contained twice as much nitrogen as untreated straw. Digestible energy content of straw with either chemical treatment was improved. A depression in nitrogen digestibility (65% average of untreated control and 54% for treated samples) was consistent but unexplained.

Waiss et al. (1972) reported that digestibility and nutritive value of rice straw could be improved by aqueous ammonia treatment. Rice straw was treated with various amounts of NH_3, water and was ensiled for different periods of time post-treatment. Optimum conditions to convert rice straw to a more valuable and nutritive feed was to treat it with 5% NH_3 and 30% added water and ensiled for 30 days post-treatment as well as air dried prior to feeding. NH_4OH treatment increased _in vitro_ digestibility almost 100% (from 29% in untreated samples to 61% in treated rice straw) and apparent crude protein almost 200% (from .56% in original straw to 1.4% in treated sample). Results of a digestibility trial with this material using sheep fed treated straw at 65% of the ration showed that dry matter digestibility was 56.3% compared with 50.5% for the control ration containing 65% untreated straw.

Rounds et al. (1976) compared different chemicals to treat corn cobs. Chemicals included NaOH, KOH, $Ca(OH)_2$ and NH_4OH. Chemical addition was on a NaOH percentage basis and KOH, $Ca(OH)_2$ and NH_4OH were added on an equimolar basis. Dry matter was treated with an equal amount of water plus appropriate amount of base in combinations of 4 or 5 g alkali/100 g DM. Treated materials were ensiled for 28 days post-treatment. Combinations of 3% NaOH and 1% $Ca(OH)_2$ gave the highest digestibility when fed to the lambs in this experiment. Ammonium hydroxide treated cobs did not ferment and a strong odor of NH_3 may have contributed to the reduced intake of treated cobs. Gains were less ($P < .01$) than for those lambs fed NaOH treated cobs. Lambs fed rations consisting of equal amounts of 4% NH_4OH treated cobs and corn silage performed as well as lambs fed 4% NaOH or corn silage rations. Also, cobs treated with 4% NH_4OH when fed 50:50 with cobs treated with a combination of 3% NaOH plus 1% $Ca(OH)_2$ resulted in slightly better response than lambs fed 4% NaOH treated cobs. In conclusion, NH_4OH treatment reduced feed comsumption. This may be overcome by aerating the samples after treatment. Since after 28 days reaction period NH_4OH treated samples did not ferment, acid or molasses was added to initiate fermentation and lower pH. Best results were obtained by combining treated corn cobs with fermented feed such as corn silage or chemically treated cobs which had been fermented. Ammonium hydroxide treatment proved to be most economical (cheaper than NaOH) and provided supplemental nitrogen.

Oji et al. (1977) reported that wethers were fed corn stover treated with different levels of NaOH, $Ca(OH)_2$ and NH_3. Ammonia treated material were aerated before feeding. Organic matter intakes were increased by 45 to 51%. Gross energy digestibility was improved from 12 to 14%. Treatment with 3% NH_3 increased total nitrogen content by 94% over the control silage. Most of the added nitrogen was recovered as NH_3, which agrees with Waiss et al. (1972) who recovered 50% NH_3-N in NH_4OH treated rice straw. However, true protein increased markedly ($P < .05$) over control silage. Negligible amounts of lactic acid were present in NH_3-treated material and NH_3 treatment increased acetic acid content ($P < .05$).

Waller (1976) evaluated NaOH, $Ca(OH)_2$ and NH_4OH for treating corn cobs. NaOH was most effective when combined with $Ca(OH)_2$ which is in agreement with Rounds et al. (1976). In this experiement, NH_4OH treated samples were aerated before feeding to lambs. Ammonium hydroxide treated cobs produced gain equivalent to 4% NaOH when fed in a 1:1 ratio with NaOH and/or $Ca(OH)_2$-treated cobs at feeding time which is in agreement with results of Garrett et al. (1974), and Rounds et al. (1976).

In conclusion, chemical treatment other than NaOH has attracted the attention of many researchers, due to the reduced Na load for animals, reduced Na buildup in the soil, and reduced cost. Potassium hydroxide showed results equivalent to NaOH treatment, but since it is expensive, it is not economically feasible to use. Calcium hydroxide alone was a very slow reactant (Waller, 1976) but 3% NaOH plus 1% $Ca(OH)_2$ gave the best response in treating corn cobs. It also could supply some Ca for the diet. Ammonium hydroxide treated material depressed feed intake (Rounds et al., 1976) but when aerated before feeding, gave results equivalent to NaOH treatment (Garrett et al., 1974). Feed intake and animal performance were improved when animals were fed NH_4OH treated material in combination with a fermented feed (corn silage) or chemically treated material [NaOH or $Ca(OH)_2$] at feeding time (Rounds et al., 1976; Waller, 1976). In addition to improved digestibility and nitrogen content, NH_4OH produces no ions which remain in animal or soil. Yet it supplies NPN to the ration, and thereby offsets rations costs as well as treatment cost.

<u>Mechanism of Action of Alkali on Lignocelluloses</u>. Physically, alkali or ammonia markedly increase water holding capacity (fiber saturation point) or internal surface area, extending enzyme (cellulase) accessibility to its substrace (Stone et al., 1969; Tarkow and Feist, 1969). The chemical effects of alkali includes saponification of ester linkages (uronic and acetic esters) and ammonolysis of 1) esters of 4-O-methyl glucuronic acid and 2) acetyl group of the xylan chain

(hemicellulose portion). Linkages in the encrusting lignin may also be saponified. Thereby, both lignin and silica are partially dissolved and hemicellulose is solubilized. This increases digestibility of lignocellulosic material (Walker et al., 1975; Jackson, 1977).

Cellulose swells under the influence of alkali (Whistler and Teng, 1970) and looses crystalinity which promotes digestibility. Alkalis further reduce the strength of inter-molecular hydrogen bonds which bind cellulose molecules together, and thus cause swelling. Swollen cellulose is more easily penetrated by rumen microflora and thus it would account for greater digestibility of cellulose.

Morris and Bacon (1976) indicated that hemicellulose of grain is esterified with acetic acid. These acetyl groups impede digestion of hemicellulose. Therefore, through hydrolysis of such ester lingages, alkali also increases digestibility of hemicellulose.

Waller (1976) has described the mode of action of alkali treatment into three components. First, solubilization of hemicellulose, accompanied by a greater rate and extent of digestion. Second, changes in lignin-cellulose and lignin-hemicellulose complex, producing a more digestible residue. Cellulose was not solubilized by alkali, but rate and extent of digestion was improved. Thirdly, swelling of cellulose could increase rate of digestion, due to exposure of more area for microbial attack.

Methods of Application of Chemicals. The first method of treatment application was introduced by Kellner and Kohler (1900). In this method straw was pressure cooked in diluted NaOH solution followed by washing with clean water to remove alkali.

In later versions another method was developed by a German researcher, Beckmann (1921). In this process, wheat straw was soaked in 1.5% NaOH solution for a 24-hour period, washed with water until free from alkali, and air dried before feeding to animals. The product had a digestibility of about 70%.

Godden (1920) presented another procedure which yielded two different products, the crude and the washed concentrate. Crude concentrate was acquired by soaking the straw in 1.5% NaOH solution overnight, followed by one hour of steaming. Excess water and alkali was removed by a press and the samples were air dried before being fed to the animals. Washed concentrate also was obtained by soaking and steaming as mentioned for crude concentrate. The processed material was neutralized by soaking in water. Excess water was removed and treated straw was air dried before being fed.

In the Beckmann method, some 25% of the original dry matter was lost, probably as a result of the washing phase (Sen, 1942; Saxena et al., 1971). Sen (1942) observed that most of the dry matter lost from treated oat straw was nitrogen free extract (NFE) and ash. In a study conducted by Saxena et al. (1971), 25% DM loss from washing of treated oat straw was attributable to decrease in crude protein, while NDF, ADF, and IVDMD were increased. In the same study, lambs responded to chemical treatment by enhanced weight gain, feed efficiency and carcass quality. Residue treated by the Beckmann method showed appreciable loss of nutrients (Sen, 1942; Saxena et al., 1971) and were more expensive than conventional feeds.

A still simpler and cheaper method to reduce the DM loss and water required for washing in the Beckmann procedure was introduced by Wilson and Pigden (1964). Sodium hydroxide solution was sprayed at various levels while water was added at a rate of 30 ml/100 g of straw dry matter. After the treatment, wheat straw was allowed to react for different periods of time prior to analysis and the NaOH was not neutralized. In vitro dry matter digestibility was improved linearly with increasing levels of NaOH up to 8 to 9%. In vitro digestibility of treated straw was about 70%.

Hasimoglu et al. (1969) conducted a lamb performance study to evaluate the spray method of Wilson and Pigden (1964). Wheat straw mositure level was raised to 50% and NaOH was sprayed at a level of 4% of dry matter. In vitro DMD for control and treated straw were 46 and 61%, respectively, and in vivo organic matter digestibility was 49 and 59%.

Several workers (Donefer et al., 1969; Maeng and Mowat, 1971; Singh and Jackson, 1971) concluded that using the dry process developed by Wilson and Pigden (1964) increased water intake and urine output of alkali by animals. Ololade et al. (1973) reported three physiological abnormalities of sheep fed NaOH treated diets: alkaline urine, osmotic diuresis and hemoglobinuria.

Neutralizing the treated material was an appropriate approach to reduce the DM losses and eliminate the detrimental effects of NaOH on animals and the environment.

Stone et al. (1965) introduced a mixture of VFA's which contained 3 parts acetate, 6 parts propionate and 1 part butyrate to neutralize the alkali treated oat straw. Neutralized alkali-treated oat straw had a higher IVDMD than a washed alkali-treated straw.

In another study, growing lambs were fed a ration of 77.5 to 85%

NaOH treated oat straw neutralized with acetic acid. Molasses was added at 7 to 10% of the ration. Feed intake was lower and feed per pound of gain was higher than with dehydrated alfalfa meal, although, digestibility and intake were improved by treatment and gain approached the control alfalfa ration (Jared and Donefer, 1970).

The recently developed large scale process (Rexen et al., 1975; Rexen and Thomsen, 1976) involves an application of NaOH solution prior to pelleting. The added heat and pressure of pelleting enhances chemical reaction. Also excess moisture is removed in the pellet cooling process. The final product is easily transported and stored.

The other process for large scale of production is an on-the-farm ensiling process which has been developed at the Univeristy of Nebraska (Klopfenstein, 1975; Klopfenstein and Koers, 1973). By this method, concentrated chemcial is added to ground residue with 50 to 60% moisture. The chemicals and residue are mixed and stored. This method provides a new feedstuff for cattle feeders without the added investment needed for washing or pelleting of treated materials.

CHAPTER III

AMMONIUM HYDROXIDE TREATMENT OF WHEAT STRAW

Summary

Two laboratory studies were conducted to determine the effect of level of added water and duration of ammonium hydroxide (NH_4OH) treatment upon the nutritive value of wheat straw. Water was added to chopped wheat straw to result in final levels of 10, 20, 30, 40 and 50% DM. Ammonia (3.3% of DM as NH_4OH) was sprayed onto the straw after adding water. Straw samples were then sealed in double plastic bags, and stored at room temperature (21 to 23^{o}C) for 10, 20, 30, 40 and 50 days (Experiment I) or 1, 5, 10, 20, 40 and 60 days (Experiment II) before being frozen and ground with dry ice to pass a 1 mm screen. Dry matter (DM), total nitrogen, in vitro dry matter digestibility (IVDMD), fiber-bound nitrogen (ADF-N) versus free ammonia nitrogen (NH_3-N), solubilization (direct chemical effects) and changes in digestibility (IVDMD) of cell-wall constituents (NDF) acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicellulose fractions were measured on treated and untreated straw. Days post-treatment ($P < .05$ in Experiment I, $P < .0001$ in Experiment II) and water level ($P < .0001$) had significant linear relationship with both IVDMD and total nitrogen content; however, further improvement in IVDMD after 10 days was very small and most of the improvement in digestibility had occured by 5 days post-treatment. Total nitrogen content of treated straw continued to

increase as days post-treatment increased. Both IVDMD and total nitrogen content of treated straw increased as water content increased. The overall improvement (e.g., mean of all day x water treatment combinatons) in IVDMD of treated straw over untreated straw was 33%. Crude protein content was increased from 4.6 percent to 11.0 percent of dry matter. Fiber bound nitrogen and NH_3-N accounted for 12.6 and 43.4%, respectively, of the total nitrogen retained when averaged across all day x water treatment combinations. Ammonium hydroxide treatment increased straw digestibility due to reduction in the amount of hemicellulose and NDF present by 42.7 and 8.6%, respectively. Additionally, NH_4OH increased the digestibility of remaining hemicellulose, cellulose, ADF and NDF fiber fractions in straw by 10.4, 28.2, 29.6 and 39.9 g/100g DM, respectively.

Introduction

Interest in the use of crop residues for livestock feeds is increasing as the prices of higher quality feeds increase. Only a small portion of the millions of tons of wheat straw available annually is utilized as feed. The potential use of straw is worthy of consideration in view of the fact that ruminatns are uniquely adapted to utilize the cellulose from high fiber material. Straws are essentially energy feeds, low in protein and mineral. Even their energy yield as livestock feed is only 40 to 50% readily digestible. Voluntary intake is also low; therefore, digestible energy intake of livestock on straw diets is usually sufficient only for maintenance conditions. Any treatment, physical or chemical, which increases energy availability of straw could tremendously increase the world's food resources.

Processing wheat straw to increase energy availability could enable use of this crop residue for growing and lactating beef cattle in addition to maintaining dry beef cows. Chemical treatment of low-quality roughages has been studied for nearly a century. Sodium hydroxide (NaOH) has proven to be an effective chemical treatment for increasing the digestibility of crop residues (Ololade et al., 1970; Singh and Jackson, 1971; Klopfenstein et al., 1972; Klopfenstein and Woods, 1970). Prolonged intake of crop residues treated with NaOH increases the Na content of the urine (Quintero, 1972) and eventually may increase Na content of the soil to which the wastes are applied. Other hydroxides may be as effective as NaOH in enhancing digestibility.

Very little detailed work has been reported on the use of aqueous ammonia (NH_3) to increase digestibility of low quality forages. Ammonium hydroxide (NH_4OH) treatment has been used to increase energy availability and crude protein content of cereal straws (Martynov, 1972; Waiss et al., 1972). By using NH_4OH treatment, no mineral residue remains which might be detrimental to animal and soil. Use of ammonium hydroxide also may reduce the costs of chemicals for treatment.

The objective of these studies was to determine (1) the effect of length of time (days post-treatment) and water content of wheat straw on *in vitro* dry matter digestibility (IVDMD) and nitrogen retention of NH_4OH treated straw, (2) the relative amounts of nitrogen retained as fiber-bound nitrogen versus free ammonia nitrogen (NH_3-N) and (3) the changes in digestibility of various fiber fractions of straw due to NH_4OH treatment.

Experimental Procedure

Experiment I

Wheat straw containing approximately 92% dry matter and 4.7% crude protein was chopped by a hammer mill with screens removed and mixed well. Five different levels of water and five different ensiling periods (days) were chosed to provide a 5 x 5 factorial arrangment of treatments. Samples of straw (approximately 100 g DM) were placed in double plastic bags. Treatment combinations were randomly assigned to duplicate samples to give a completely randomized design with two replications. Water was sprayed on the straw samples to result in final levels of approximately 10, 20, 30, 40 and 50 percent of dry matter. Ammonium hydroxide (28-30% NH_3) was then sprayed on the straw in the bags at a level to provide 3.3 percent ammonia (NH_3), being added on a dry matter basis. Level of NH_3 was the same for all treatment combinations. Treated straw was sealed in double plastic bags, and stores at room temperature (21 to 23^{o}C) for 10, 20, 30, 40 and 50 days post-treatment. After the end of the reaction period, treated samples were frozed and ground with dry ice through a 1 mm screen of a laboratory Wiley mill. Ground samples were then tightly sealed in double plastic bags and frozen for later analysis. Dry matter (DM), total nitrogen (Tot-N) and _in vitro_ dry matter digestibility (IVDMD) were measured before aerating the samples. For DM analyses, straw samples (approximately 2 grams) were placed in aluminum pans and dried for 24 hours at 100^{o}C. Total nitrogen was analyzed by the Kjeldahl procedure. _In vitro_ dry matter digestibility was measured by a modification of the two stage procedure of Tilly and Terry (1963). Urea was added to the buffered-rumen fluid at a level of .5 g/liter and incubation with acid pepsin was for only 24 hours.

Rumen inoculum was obtained from a steer fed the diet shown in Table III. Rumen fluid was collected at 0730 before feeding. It was immediately strained through 4 layers of cheese cloth, and transported to the laboratory in a tightly sealed, insulated container. Equal volumes of prewarmed, CO_2-saturated rumen fluid was transferred to a prewarmed (39°C) CO_2-saturated buffer solution (McDougall, 1948).

Straw samples (approximately .5 grams of dry matter) were placed in 100 ml polypropylene centrifuge tubes and prewarmed (39°C) before inoculation. Tubes were inoculated with 25 ml of buffer rumen fluid (1:1 ratio of filtered rumen fluid and buffered solution) and immediately stoppered and placed in a water bath (39°C). Rubber stoppers with a 3/16 inch diameter hole were utilized to provide adequate gas release. Temperature (39°C), CO_2-saturation and agitation were maintained during inoculation. After pepsin digestion for 24 hours, residual DM was collected in gooch crucibles fitted with oven dried Whatman number 4 filter paper. Blanks that consisted of an equal volume of buffered rumen fluid were included in each assay to correct for residual DM from ruminal fluid.

Bermudagrass and untreated wheat straw reference standards were included with each assay. Treated straw samples and reference standards were analyzed in triplicate, and replicates were assayed at an interval of one week.

Dry matter, total nitrogen, fiber-bound nitrogen (ADF-N) and free ammonia nitrogen (NH_3-N) were also measured on the NH_4OH treated straw samples after they had been spread in a pan and aerated for 24 hours at

TABLE III

COMPOSITION OF DIET FED TO RUMEN INOCULUM DONOR STEER

Item	% of Ration[a]
Alfalfa hay	50.00
Dry rolled corn grain	31.71
Cottonseed hulls	7.00
Soybean meal	5.00
Dehydrated alfalfa	3.00
Liquid molasses	2.50
Trace mineralized salt	0.25
Calcium carbonate	0.25
Dicalcium phosphate	0.25
Urea	0.05
Aureo 50	0.015

[a]As-fed basis.

room temperature. Fiber-bound nitrogen (ADF-N) was determined by analyzing ADF residue (Goering and VanSoest, 1970) for total nitrogen by the Kjeldahl procedure. Free ammonia nitrogen (NH_3-N) was analyzed by the magnesium oxide distillation step of the Kjeldahl procedure. Use of the magnesium oxide distillation step for NH_3-N quantification was checked for accuracy before use. One ml of NH_4OH solution (28-30% NH_3) was diluted to 10 ml with glass distilled water. The amount of NH_3 of this diluted NH_4OH solution was determined by magnesium oxide distillation. One, two or three mls of the diluted NH_4OH solution were added to treated straw samples and recovery of NH_3 was determined by magnesium oxide distillation procudure. Results indicated that ammonia recovery was above 98% (Table IV).

Changes in the concentrations and digestibility of various fiber fractions as a result of NH_4OH treatment were determined by analyzing the untreated straw and the three treated straw samples which had the highest IVDMD for neutral-detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicellulose both before and after assaying the samples for IVDMD. Residual DM that remained after IVDMD on treated and untreated samples was transfered to a 600 ml Berzelius beaker with neutral-detergent solution as well as with acid-detergent solution, to analyze for NDF, ADF, and ADL. Analyses of NDF and ADF were conducted using the procedure described by Goering and VanSoest (1970). Cellulose and hemicellulose concentrations were calculated by difference from analyses of NDF, ADF and ADL.

Experiment II

Chopped wheat straw was treated in a fashion similar to that

TABLE IV

RECOVERY OF ADDED AMMONIA TO TREATED STRAW SAMPLES

Treated Straw Samples	Added NH_3-N, mg[a]	Recovery, mg	Recovery (%)
b c d			
10% - 10 - A	16.985	17.58[e]	>100
20% - 10 - A	16.985	16.49	97
50% - 10 - B	16.985	16.97	99
50% - 10 - B	33.970	33.44	98
50% - 10 - B	50.955	50.46	99

[a]1, 2, or 3 ml of 1:10 diluted NH_4OH solution was added to the samples.
[b]Water (%) added to the straw.
[c]Days post-treatment.
[d]Replications.
[e]Mean of duplicate samples.

described for Experiment I except that a straw sample with addition of neither water nor ammonia was carried through each of the storage periods. Storage times were 1, 5, 10, 20, 40 and 60 days. Treated samples were analyzed for IVDMD and total nitrogen after aeration as described for Experiment I. The experiment had a 6 x 6 factorial arrangement of treatments in a completly randomized design with two replications.

Statistical Analysis

All data were analyzed by analysis of variance, orthogonal polynomials, using General Linear Model (GLM) subroutine of Statistical Analysis System (SAS). Significant differences between means of treatment combinations within days and moisture levels (e.g. Appendix Tables VIII and XIII) and overall means of treated and untreated straw samples were tested for significant differences by using least significant difference (LSD) procedure (Steel and Torrie, 1960).

Results and Discussion

Experiment I

After 10 days reaction period, further days post-treatment did not show a significant effect on IVDMD ($P > .10$) (Figure 2). Digestibility at 10 days post-treatment (across water levels) was not significantly different ($P > .05$) from digestibility after 50 days post-treatment. Waiss et al. (1972) reported that optimum *in vitro* digestibility was obtained from NH_4OH-treated rice straw when it was stored for 30 days post-treatment. Oji et al. (1977) also suggested that ammonia was a slow reacting alkali requiring storage time of several days. This delay may have misled previous investigators to believe that NH_3 was not

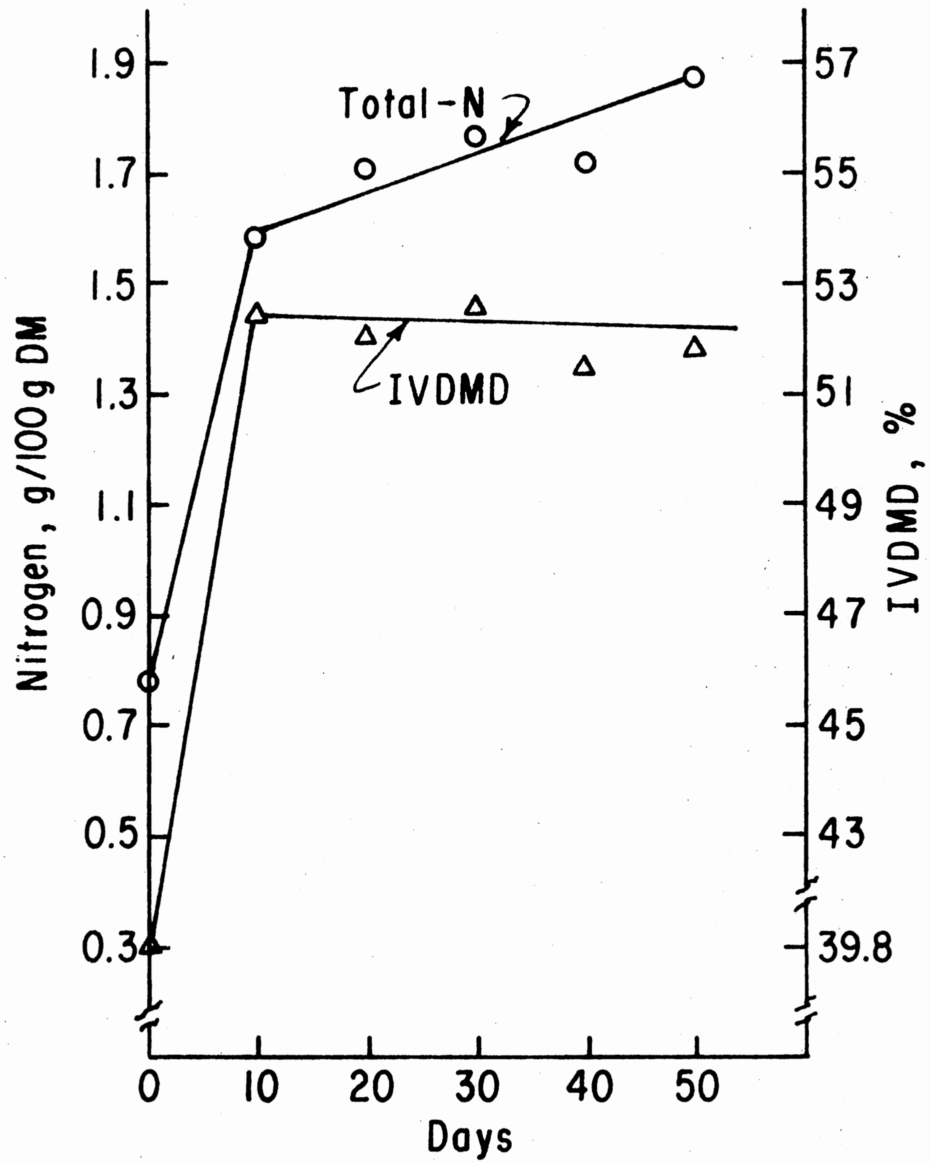


Figure 2. Total Nitrogen Content and IVDMD Response to Days Post-Treatment (Experiment I)

effective. Water level (measured on treated samples before aeration) had a significant linear relationship with IVDMD ($P < .0001$) (Figure 3). Digestibility continued to increase as water content of treated wheat straw increased. This is not in agreement with Waiss et al. (1972) who reported that there was no further increase in <u>in vitro</u> digestibility of NH_3 treated rice straw with moisture levels higher than 30%.

The overall mean of <u>in vitro</u> dry matter digestibility (IVDMD) of wheat straw was significantly ($P < .01$) increased by NH_4OH treatment (Table V). Digestibility was improved 12.2 percentage units and this is greater than that reported by Waiss et al. (1972) for rice straw treated with 5% NH_4OH which showed a 5 percentage unit increase in digestibility. Braman and Abe (1977) reported 14.1 and 22.5 percentage unit increases in IVDMD of wheat straw treated with either 2 or 4% (W/W) levels of NH_4OH respectively. The overall increase in IVDMD (e.g., mean of all days x water treatment combinations) due to NH_4OH treatment was 30.6%, whereas maximum or near maximum improvement was 34.4% over untreated straw (Table V).

Total nitrogen was measured on treated samples before aeration. As shown in Figure 4, only 76.5% of added nitrogen (2.72 g N added 100 g straw DM or 3.3% NH_3) was recovered before aeration. The overall mean quantity of nitrogen added that was retained in treated straw after aeration was .95 g per 100 g dry matter (e.g., 1.73 g minus .78 g) or approximately 34.9% of added nitrogen was retained after aeration (Figure 4, Table VI). This means that more than one third of the nitrogen applied was lost during aeration of treated straw.

Number of days post-treatment ($P < .0001$) and water level ($P < .0001$) had significant linear relationship with total nitrogen content of

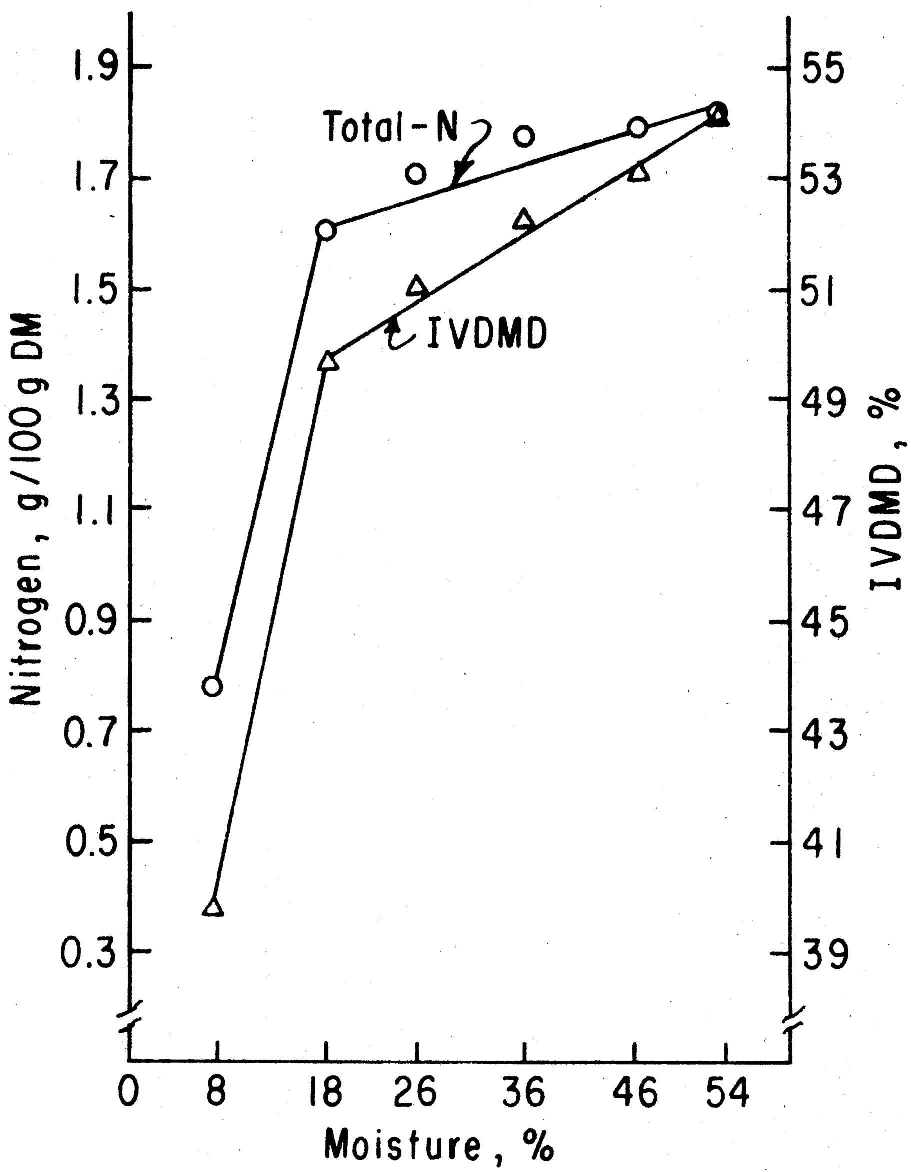


Figure 3. Total Nitrogen Content and IVDMD Response to Straw Moisture Level (Experiment I)

TABLE V

IVDMD AND TOTAL N CONTENT IN WHEAT STRAW

	IVDMD (%)		Total N content (g/100 g DM)	
Experiment No.:	I	II	I	II
Untreated straw	39.8	36.8	.78	.69
Treated straw[a]	52	49.64	1.73	1.79
% increase over untreated straw	30.6	34.9	122	159
Treated straw[b]	53.5[c]	52.4[d]	1.90[e]	2.13[f]
% increase over untreated straw	34.4	42.4	144	209

[a]Overall means of all day x moisture treatment combinations.

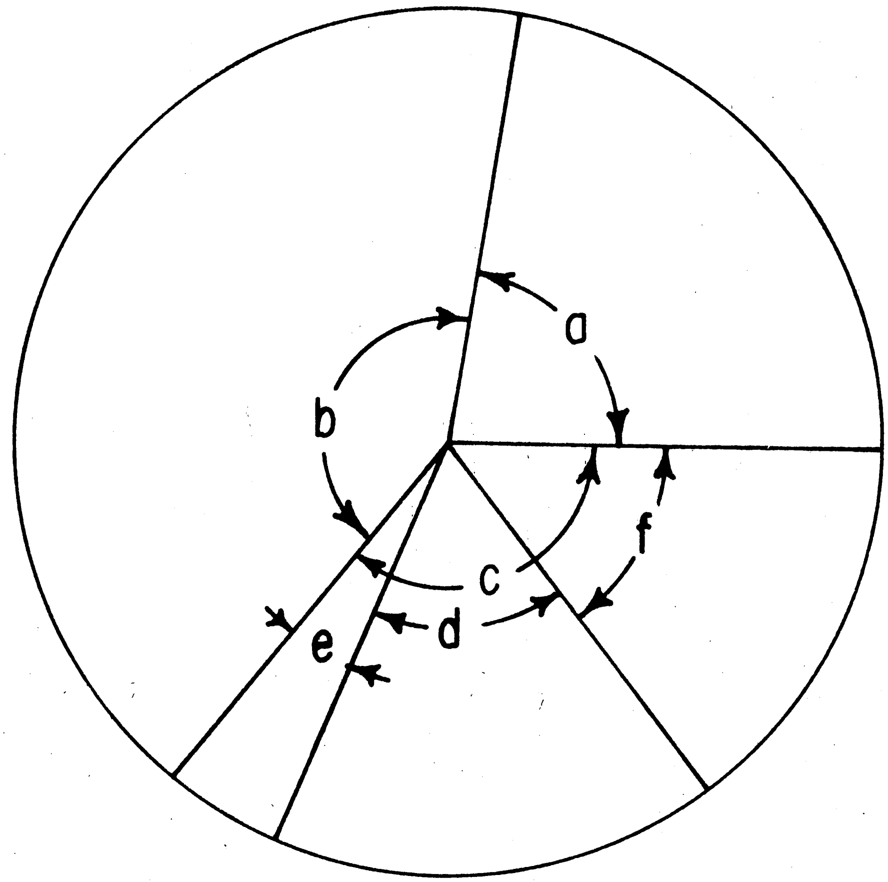
[b]Treated straw samples which showed maximum or near maximum increases in IVDMD or total nitrogen content.

[c]10 days at 54 percent moisture.

[d]10 days at 50 percent moisture.

[e]50 days at 54 percent moisture.

[f]60 days at 50 percent moisture.



a. Not recovered before aeration, 23.5% of total added nitrogen, .64 g/100 g DM

b. Lost during aeration, 41.6% of total added N, 1.13 g/100 g DM

c. Retained after aeration, 34.9% of total added N, .95 g/100 g DM

d. NH_3-N, 15.1% of total added N, 412 g/100 g DM or 43.4% of nitrogen retained

e. ADF-N, 4.4% of total added N, .12 g/100 g DM or 12.6% of total N retained

f. Unidentified nitrogen, 15.4% of total added N, .418 g/100 g DM or 44% of nitrogen retained

Total N added = 2.72 g/100 g DM

Figure 4. Distribution of NH_3 as N in treated samples before and after aeration

TABLE VI

TOTAL NITROGEN, ADF-N AND NH_3-N IN TREATED WHEAT STRAW

	Untreated straw	Treated[a] straw	Treated[b] straw
Total N added			
g/100 g DM straw	----	2.72	2.72
Total N content			
g/100 g DM	.78	1.73	1.90
Total N retention,			
% of added	----	34.9	41.2
ADF-N			
g/100 g DM	.34	.46	.49
% of total N added	----	4.4	5.5
% of total N retained	----	12.6	13.4
NH_3-N			
g/100 g DM	.008	.42	.56
% of total N added	----	15.1	20.3
% of total N retained	----	43.4	49.3

[a]Overall means of all day and moisture treatment combinations.

[b]Treated straw sample (50 days at 54 percent moisture which showed maximum or near maximum increase in total nitrogen content.

treated wheat straw measured after aeration. Total nitrogen content continued to increase as days post-treatment (10 to 50 days) and water level (18% to 54%) increased (Figures 2 and 3).

Total nitrogen content (e.g., mean of all day x water treatment combinations) was increased 122% over control, untreated straw (Table V). This agrees with results obtained by Waiss et al. (1972) who reported a 133% increase in total nitrogen content of NH_4OH-treated rice straw. Oji et al. (1977) reported an increase of 94% in total nitrogen content for 3% NH_3 treated corn stover. Maximum or near maximum increase in total nitrogen content was 144% over control untreated straw (Table V).

Number of days ($P < .0001$) post-treatment and water level ($P < .0001$) had a significant linear relationship with free ammonia nitrogen (NH_3-N). Free NH_3-N continued to increase as the reaction period (10 to 50 days) and water level (18 to 54%) increased (Figures 5 and 6).

Free NH_3-N accounted for 15.1% of the added nitrogen (Figure 4), 43.4% of the retained nitrogen (Table VI) or 52.8% of the increase in total nitrogen content of treated wheat straw. However, Oji et al (1977) reported that 53% of the added nitrogen was retained for feeding by stover treated with 3% NH_3. Most of the retained nitrogen was presented as free NH_3-N. Waiss et al. (1972) reported that about 50% of the increased nitrogen was NH_3-N and the remaining 50% was more tightly bound.

Number of days post-treatment ($P < .002$) and water level ($P < .002$) had a significant linear relationship with fiber-bound nitrogen (ADF-N), and ADF-N increased further as days post-treatment and water level increased in treated samples (Figures 5 and 6). Fiber bound nitrogen accounted for 4.4% of added nitrogen or 12.6% of nitrogen retained

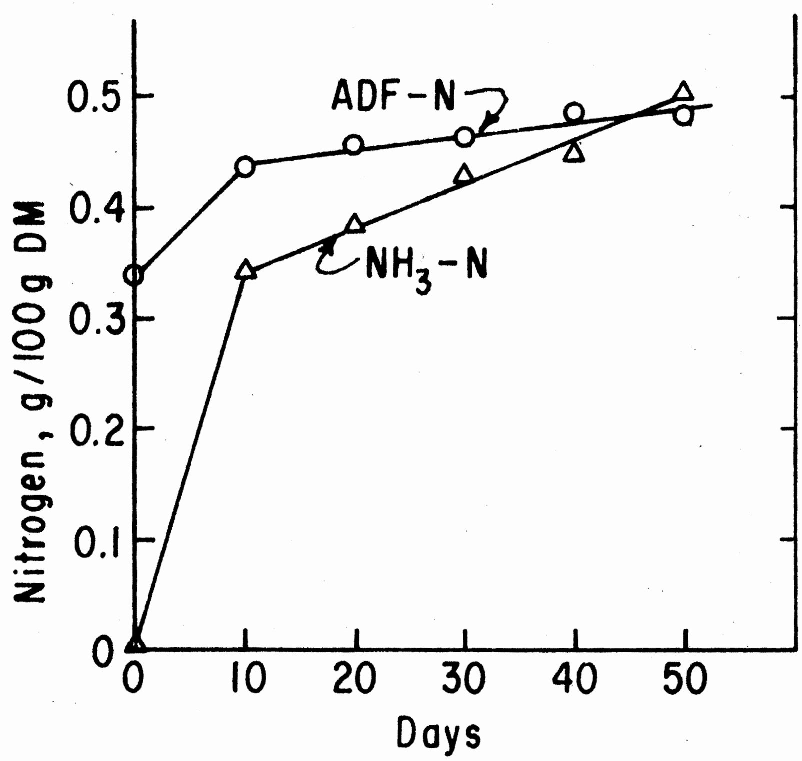


Figure 5. Free NH_3-N and ADF-N Response to Days Post-Treatment (Experiment I)

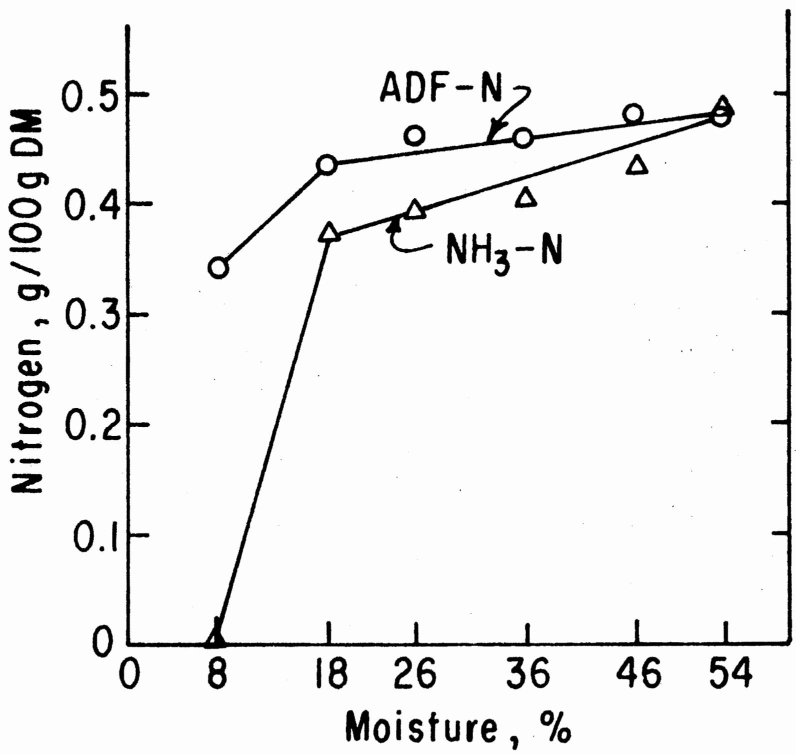


Figure 6. Free NH_3-N and ADF-N Response to Straw Moisture Level (Experiment I)

(Figure 4, Table VI).

Oji et al. (1977) indicated that NH_3 treatment markedly increased true protein content of corn stover compared to control stover silage. Although non-protein nitrogen content of material increased with ensiling due to microbial proteolysis of plant protein (Bergen et al., 1974) some silage studies have shown that alkali additives such as ammonia (Henderson et al., 1971; Mowat et al., 1976) may increase true protein content presumably because of decreased microbial proteolysis of plant proteins. However, true protein content was not measured in Experiment I.

The fiber fractions of treated straw were altered as a result of NH_4OH treatment. Cell-wall constituents of treated wheat straw were 8.6% lower than untreated straw as shown in Table VII. The decrease in total cell-wall may be due to solubilization of hemicellulose, which has been suggested by Waller (1976). Hemicellulose is the fibrous portion of cell-wall solubilized by dilute alkali (Donnelly et al., 1974). Therefore, solubilization of hemicellulose was expected in alkali treated residues. The reduction in cell-wall constituents (CWC) shown in Experiment I agrees with results reported by Jones and Klopfenstein (1967) in which CWC of NaOH-treated corn cobs were significantly lower ($P < .01$) than untreated control corn cobs. Cell-wall constituents of corn cobs treated with 4% NaOH were 4.8 percentage units lower than untreated cobs in another study reported by Klopfenstein et al. (1972). Ololade et al. (1970) reported CWC solubilization for alfalfa stems, barley straw, and corn stover treated with 8% NaOH to be 1.6, 11.5, and 7.4 percentage units, respectively. Summers and Sherrod (1975) concluded that improvement in IVDMD, using different residues treated with 5%

TABLE VII

SOLUBILIZATION AND CHANGES IN DIGESTIBILITY OF WHEAT STRAW FIBER FRACTION[a] DUE TO NH_4OH TREATMENT

	Initial	Solubilization		In Vitro Digestion		Sum[b]	
	g/100 g DM	g/100g DM	% untreated straw	g/100g DM	% untreated straw	g/100g DM	% untreated straw
NDF							
Untreated straw	73.3	0	0	27.1	36.97	27.1	36.91
Treated straw	67.0	6.3	8.6	39.91	54.44	46.21	63.04
NDS							
Untreated straw	26.7	0	0	12.7	47.5	12.7	47.5
Treated straw	33.0	-6.3	-23.6	15.14	56.7	8.84	33.1
ADF							
Untreated straw	49.1	0	0	15.8	32.18	15.8	32.18
Treated straw	53.2	-4.1	-8.3	29.6	60.28	25.5	51.93
ADL							
Untreated straw	7.7	0	0	0	0	0	0
Treated straw	8.5	-.8	-10.4	1.03	13.37	.23	2.98
Hemicellulose							
Untreated straw	24.1	0	0	11.2	46.5	11.2	46.5
Treated straw	13.8	10.3	42.7	10.38	43.1	20.68	85.8
Cellulose							
Untreated straw	39.5	0	0	15.3	38.7	15.3	38.7
Treated straw	42.3	-2.8	-7.00	28.21	71.4	25.41	64.33

[a]Neutral-detergent fiber (NDF), Neutral-detergent solubles (NDS), Acid-detergent fiber (ADF), Acid-detergent lignin (ADL).

[b]Sum = NH_4OH solubilization + *In vitro* digestion

[c]IVDMD of untreated and treated straw samples were 39.8 and 55.1 ± .37 percent, respectively.

NaOH, closely corresponded to the decrease in hemicellulose content.

Results of Experiment I indicated that during the IVDMD procedure, 39.9 and 10.4 g/100 g DM of the neutral-detergent-fiber (NDF) and hemicellulose fractions, respectively of that which remained after chemical treatment, were digested (Table VII). Therefore, chemical treatment of wheat straw not only resulted in solubilization but also improved digestion of CWC and hemicellulose.

Cellulose and lignin content of treated wheat straw were not solubilized due to chemical treatment. This agrees with results reported by Ololade et al. (1970) for alfalfa stems, barley straw, and corn stover treated with 8% NaOH, and results of Waller (1976) for corn cobs treated with different combinations of NaOH, $Ca(OH)_2$ and NH_4OH. However, 28.2g/100 g DM of cellulose was digested in this study (Table VII). In total, 46.2, 25.5, 20.68 and 25.4 g/100 g DM of NDF, ADF, hemicellulose and cellulose fiber fractions, respectively that were present in the initial untreated straw were lost due to the combination of (1) chemical effects on the fiber fraction (probably due to solubilization), and (2) digestion during the IVDMD procedure (increased digestibility of fiber fraction).

In Experiment I there was not a significant interaction between days post-treatment and water level in regard to IVDMD ($P > .05$) or total nitrogen content ($P > .10$). Means and analysis of variance tables for Experiment I are presented in Appendix Tables VIII, and IX, X, XI, XII.

Experiment II

Number of days post-treatment ($P < .0001$) and water level ($P < .0001$) had a significant linear relationship with both digestibility and total nitrogen content. Further improvements in digestibility after day 10 was very small, and a large percentage of the improvement in digestibility had occurred by 5 days post-treatment (Figure 7). Total nitrogen content of aerated treated straw, however, continued to increase as days post-treatment increased from 1 to 60 days (Figure 7). Both digestibility and total nitrogen content of treated straw continued to increase as water content increased (Figure 8).

Overall means (e.g., mean of all days and water treatment combinations) of IVDMD ($P < .01$) and total nitrogen ($P < .01$) were increased significantly with NH_4OH-treatment. Overall improvements in IVDMD and total nitrogen content were 34.9 and 159%, respectively (Table V). Maximum or near maximum improvements in IVDMD and total nitrogen content were 42.4 and 209%, respectively (Table V).

In Experiment II there was a significant interaction between days post-treatment and water level in regard to IVDMD ($P < .03$) and this significant level of interaction seems to be due to days quintic x moisture interaction (Figure 9 and Appendix Table XIV). Since the magnitude of mean square main effects of days post-treatment and water level was greater than the magnitude of mean square interection between these two factors, it was concluded that main effect of days post-treatment on IVDMD ($P < .005$) was much more significant than the interaction between these two factors. Also, main effect of water level on IVDMD ($P < .025$) was more significant than the interaction between these two factors. There was not significant interaction ($P > .25$) between days post-treatment and water level in regard to nitrogen content of treated wheat straw (Figure 10 and Appendix Table XV). Means and anlaysis of variance tables for Experiment II are presented in Appendix Tables XIII, XIV and XV.

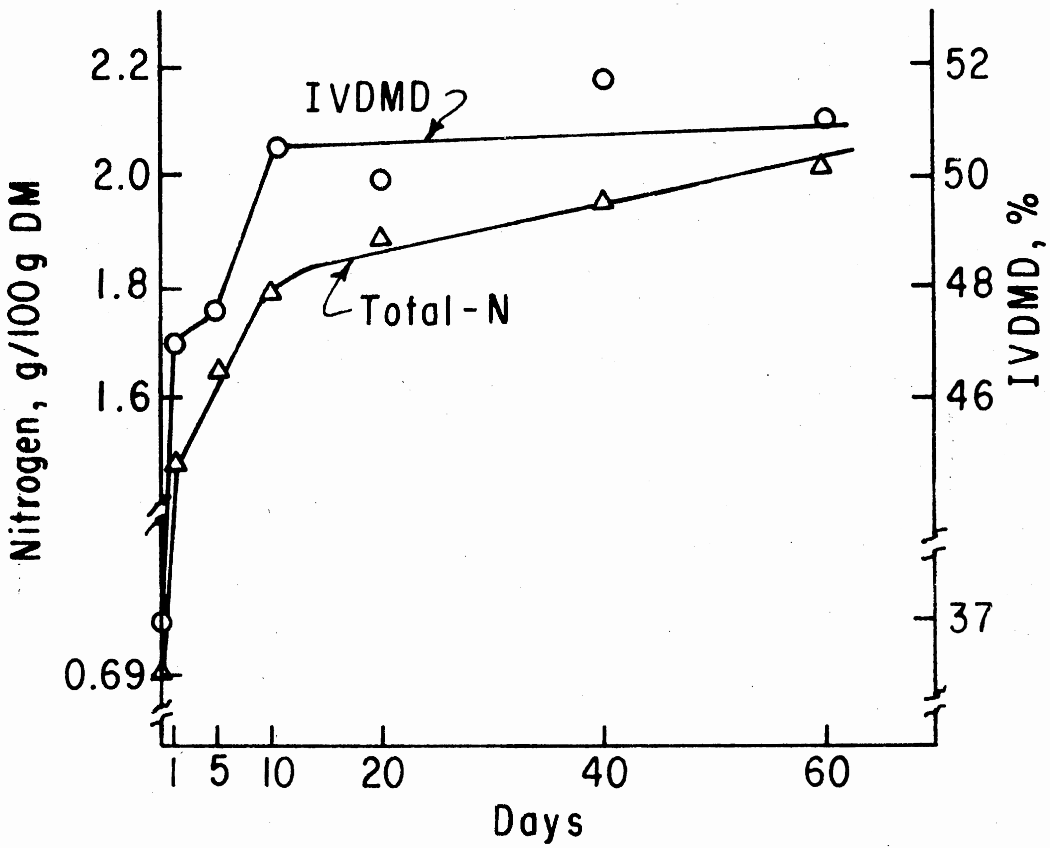


Figure 7. Nitrogen and IVDMD Response to Days Post-Treatment (Experiment II)

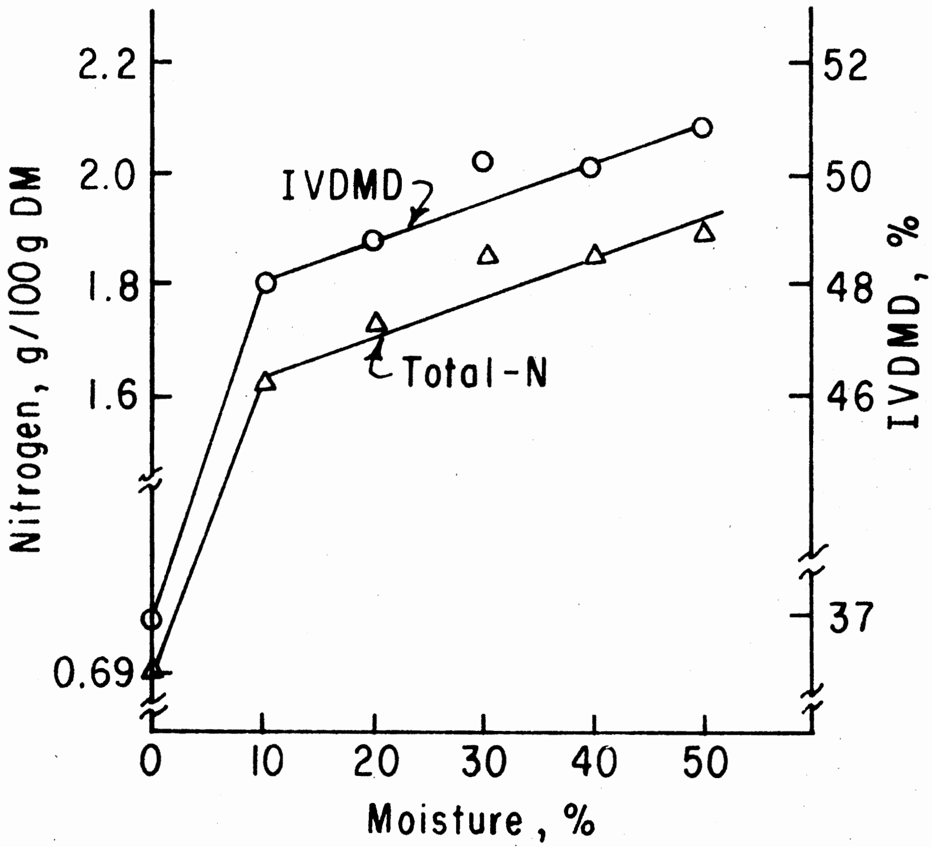


Figure 8. Nitrogen and IVDMD Response to Straw Moisture Level (Experiment II)

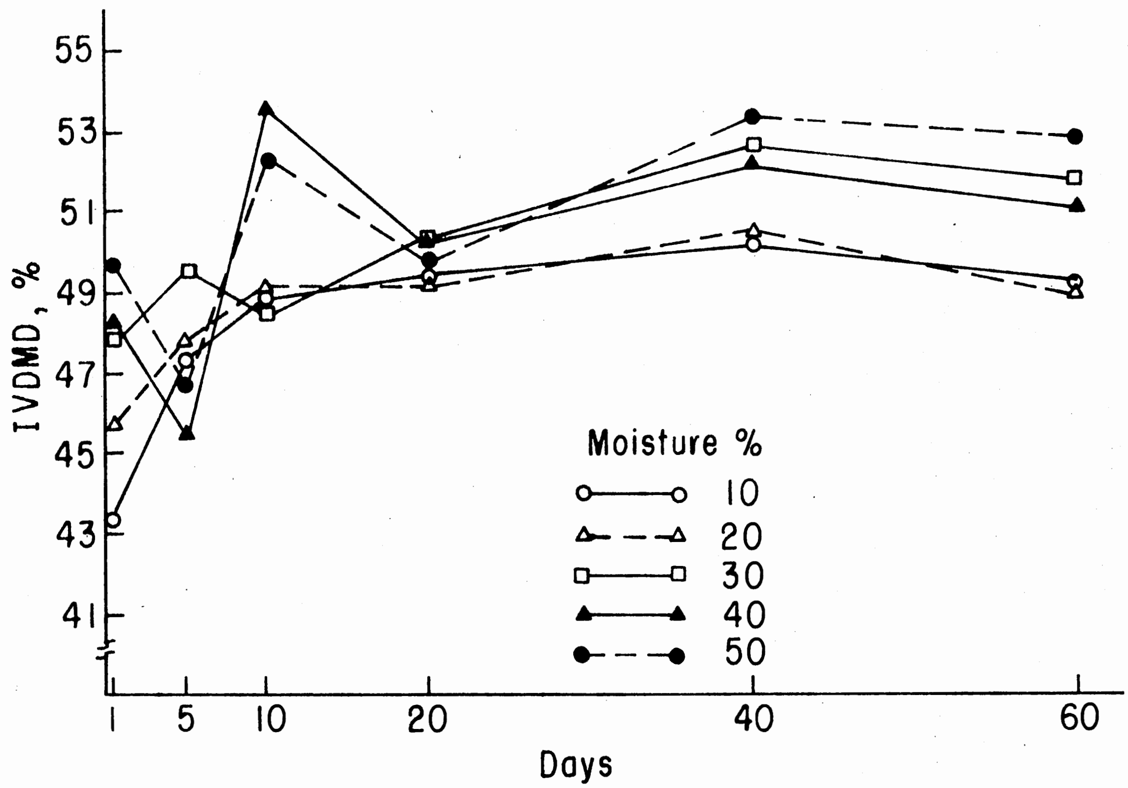


Figure 9. IVDMD Response to Days X Moisture Interaction (Experiment II)

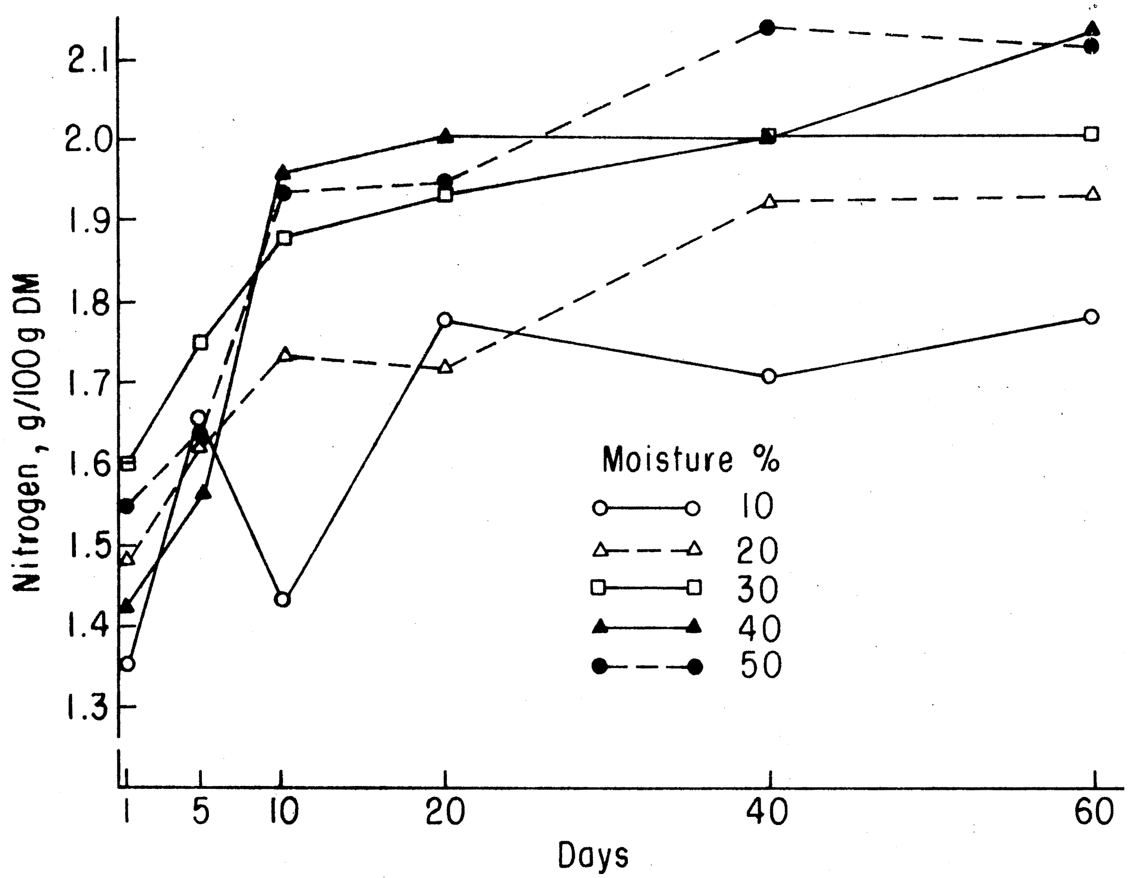


Figure 10. Total Nitrogen Content Response to Days X Moisture Interaction (Experiment II)

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APPENDIX A

THE PRINCIPAL CHEMICAL CONSTITUENTS OF THE COMPONENTS OF PLANT CELL-WALLS (CLOWES AND JUNIPER, 1968; PRESTON, 1974)

1. Lignin

P-Hydroxycinnamyl alcohol, coniferyl alcohol, sinapyl alcohol, p-Coumaryl alcohol.

2. Pectic substances

D-Galacturonic acid, L-Arabinose, D-Galactose, L-Rhamnose

3. Hemicelluloses

D-Xylose, L-Arabinose, D- Mannose, D-Glucose, D-Galactose, D-Glucuronic acid, D-Galacturonic acid, Glucomannan, Galactoglucomannan.

4. Cellulose

D-Glucose (β-1,4 linked chains)

5. Fatty constituents

a. Cutin: Mono-, di-, and tri-hydroxyoctadecanoic acid; Di-hydroxyhexadecanoic acid.

b. Suberin: Hydroxy-docosanoic acid, Di- and Tri-hydroxydecanoic acid, Friedeline, Pentacyclic triterpene.

c. Waxes: Esters of higher aliphatic acids and higher aliphatic or cyclic alcohols, Ursolic acid, paraffin hydrocarbons, Triterpeneketone (Cerin, friedelin).

6. Tannins

a. Hydrolysable Tannin: Glucose, Phenolic acid (e.g., gallic acid).

b. Non-hydrolysable Tannin: Flavan-3-01, Flavan-3,4 diol.

7. Proteins

Hydroxyproline, Serine, Glycine, Aspartic acid.

8. Gums

Xylose, Arabinose, Glucose, Galactose, Uronides.

9. Mucilages

Xylose, Arabinose, Glucose, Galactose, Uronides.

10. Callose

D-Glucose (β-1,3 linked chains).

11. Sporopollenin

An intimate mixture of cellulose and xylan.

12. Enzymes

Ascorbic acid oxidase, Hydrolase, Invertase, Pectin methyles terase.

Phosphatases, Glucanase, Peroxidases, ATP-ase, DNA-ase, RNA-ase.

APPENDIX B

TABLES

TABLE VIII

MEANS OF IVDMD, TOTAL N, ADF-N, NH_3-N, IN EXPERIMENT I[a]

	Days				
	10	20	30	40	50
Water, %					
10					
IVDMD, %	51.2^{cf}	49.4^{bcf}	51.0^{cf}	48.5^{bf}	48.7^{bf}
Total N, %	1.54^{bf}	1.60^{bf}	1.62^{bf}	1.55^{bf}	1.68^{bf}
ADF-N, %	$.451^{bfg}$	$.430^{bf}$	$.462^{bf}$	$.449^{bfg}$	$.476^{bf}$
NH_3-N, %	$.304^{bf}$	$.316^{bf}$	$.383^{cf}$	$.410^{cfg}$	$.477^{df}$
20					
IVDMD, %	50.8^{bf}	49.8^{bf}	50.9^{bf}	51.5^{bg}	51.7^{bg}
Total N, %	1.54^{bf}	1.66^{bcf}	1.77^{bcf}	1.65^{bcfg}	1.91^{cf}
ADF-N, %	$.431^{bfg}$	$.512^{cg}$	$.456^{bcf}$	$.439^{bf}$	$.488^{bcfg}$
NH_3-N, %	$.314^{bf}$	$.381^{cg}$	$.407^{cfg}$	$.398^{cf}$	$.493^{df}$
30					
IVDMD, %	53.6^{bg}	52.1^{bg}	51.4^{bf}	52.8^{bg}	51.9^{bg}
Total N, %	1.62^{bf}	1.74^{bcf}	1.76^{bcf}	1.80^{bcfg}	1.93^{cf}
ADF-N, %	$.396^{bf}$	$.421^{bf}$	$.436^{bf}$	$.504^{cgh}$	$.541^{cg}$
NH_3-N, %	$.338^{bfg}$	$.370^{bcg}$	$.405^{cdfg}$	$.422^{dfg}$	$.474^{ef}$
40					
IVDMD, %	53.7^{bg}	53.7^{bgh}	53.9^{bg}	52.1^{bg}	51.9^{bg}
Total N, %	1.61^{bf}	1.82^{bcf}	1.80^{bcg}	1.76^{bceg}	1.97^{cf}
ADF-N, %	$.450^{bfg}$	$.457^{bfg}$	$.462^{bf}$	$.532^{cg}$	$.504^{bcfg}$
NH_3-N, %	$.363^{bgh}$	$.409^{bcgh}$	$.443^{cdg}$	$.459^{cdg}$	$.501^{df}$
50					
IVDMD, %	53.5^{bcg}	55.3^{ch}	55.6^{cg}	52.2^{bg}	54.4^{bch}
Total N, %	1.58^{bf}	1.74^{bcf}	1.91^{cf}	1.86^{cg}	1.89^{cf}
ADF-N, %	$.466^{bg}$	$.455^{bf}$	$.490^{bf}$	$.500^{bg}$	$.488^{bfg}$
NH_3-N, %	$.389^{bh}$	$.433^{bh}$	$.501^{ch}$	$.541^{cdh}$	$.560^{dg}$

[a] All values are expressed on 100% dry matter basis.
LSD = 2.23 for IVDMD ($P < .05$)   LSD = .298 for Total Nitrogen ($P < .05$).
LSD = .050 for NH_3-N ($P < .05$)   LSD = .064 for ADF-N ($P < .05$)

[bcde] Means in the same row followed by the same superscript are not significantly different ($P < .05$).

[fgh] Means in the same column followed by the same superscript are not significantly different ($P < .05$).

TABLE IX

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR IVDMD, EXPERIMENT I

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	149	1462.3			
Treatment	24	503.43	20.976	5.96	P < .005
Days	4	29.34	7.336	2.08	P > .100
D Linear	1	15.41		4.38	.046
D Quadratic	1	.004		0.00	.97
D Cubic	1	.609		0.17	.68
D Quartic	1	13.321		3.79	.062
Moisture (%)	4	367.908	91.97	26.16	P < .005
M Linear	1	364.662		103.74	.0001
M Quadratic	1	1.504		.43	.519
M Cubic	1	.101		.03	.866
M Quartic	1	1.643		.47	.500
Days x Moisture	16	106.174	6.636	1.89	P > .05
DLxM	4	33.299		2.37	.0799
DQxM	4	12.004		.85	.505
DCxM	4	39.306		2.80	.048
DQuarxM	4	21.566		1.53	.2229
Experimental Error	25	87.880	3.515		
Sampling Error	100	870.988	8.709		

TABLE X

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR TOTAL N, EXPERIMENT I

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	99	2.2301			
Treatment	24	1.71	.0712	4.813	P < .005
Days	4	.9366	.2341	15.820	P < .005
D Linear	1	.7514		50.61	.0001
D Quadratic	1	.0066		.45	.5106
D Cubic	1	.1475		9.93	.0042
D Quartic	1	.03151		2.12	.1576
Moisture	4	.5652	.1413	9.54	P < .005
M Linear	1	.4693		31.61	.0001
M Quadratic	1	.0905		6.09	.0207
M Cubic	1	.0010		.07	.7927
M Quartic	1	.00025		.02	.8976
Days x Moisture	16	.21181	.01324	.894	P > .10
DLxM	4	.0774		1.3	.2956
DQxM	4	.05727		.96	.4443
DCxM	4	.0445		.75	.5677
DQuarxM	4	.0326		.55	.7017
Experimental Error	25	.3711	.0418		
Sampling Error	50	.1489	.00297		

TABLE XI

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR NH_3-N, EXPERIMENT I

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	49	.2434			
Treatment	24	.2281	.0095	15.65	P < .005
Days	4	.1491	.0373	61.4	.0001
D Linear	1	.14678		241.69	.0001
D Quadratic	1	.0000013		0.00	.9632
D Cubic	1	.000999		1.65	.2114
D Quartic	1	.001389		2.29	.1430
Moisture	4	.0698	.0174	28.74	.0001
M Linear	1	.06251		102.94	.0001
M Quadratic	1	.005654		9.31	.0053
M Cubic	1	.001158		1.91	.1794
M Quartic	1	.000496		.82	.3746
Days x Moisture	16	.0092	.00057	.9487	.5323
DLxM	4	.00299		1.23	.3227
DQxM	4	.00179		.74	.5753
DCxM	4	.00410		1.69	.1842
DQuarxM	4	.00033		.14	.9668
Error	25	.0152	.000607		

TABLE XII

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR ADF-N, EXPERIMENT I

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	49	.10025			
Treatment	24	.07595	.00316	3.264	P < .005
Days	4	.01381	.00345	3.55	.0197
D Linear	1	.01223		12.59	.0016
D Quadratic	1	.00043		.45	.5090
D Cubic	1	.000367		.38	.5439
D Quartic	1	.000777		.80	.3795
Moisture (%)	4	.01494	.00373	3.845	.0143
M Linear	1	.01180		12.15	.0018
M Quadratic	1	.00109		1.12	.2993
M Cubic	1	.00022		.23	.6325
M Quartic	1	.00182		1.88	.1827
Days x Moisture	16	.0472	.00295	3.0365	.0065
DLxM	4	.0275		7.08	.0006
DQxM	4	.00376		.97	.4420
DCxM	4	.01356		3.49	.0214
DQuarxM	4	.0024		.51	.659
Error	25	.0243	.00097		

TABLE XIII

MEANS OF IVDMD AND TOTAL NITROGEN FOR EXPERIMENT II[a]

	Days					
	1	5	10	20	40	60
Water, %						
0[b]						
IVDMD, %	35.6	36.7	38.7	36.4	38.1	35.7
Total N, %	.70	.65	.71	.64	.76	.68
10						
IVDMD, %	43.3^{cg}	47.4^{dgh}	48.9^{deg}	49.3^{deg}	50.2^{eg}	49.1^{deg}
Total N, %	1.36^{cg}	1.65^{deg}	1.43^{cdg}	1.78^{eg}	1.71^{eg}	1.78^{eg}
20						
IVDMD, %	45.8^{cgh}	47.9^{cdgh}	49.0^{dg}	49.4^{dg}	50.5^{dg}	50.0^{dgh}
Total N, %	1.48^{cg}	1.63^{cdg}	1.75^{deh}	1.73^{deg}	1.93^{egh}	1.93^{egh}
30						
IVDMD, %	47.9^{chi}	49.7^{ch}	48.7^{cg}	50.4^{cdg}	52.9^{dgh}	52.0^{dhi}
Total N, %	1.60^{cg}	1.76^{cdg}	1.88^{deh}	1.94^{degh}	2.02^{eh}	2.02^{egh}
40						
IVDMD, %	42.8^{cdhi}	45.4^{cg}	53.5^{fh}	50.3^{deg}	52.3^{efgh}	51.0^{efghi}
Total N, %	1.43^{cg}	1.57^{cg}	2.05^{dh}	2.02^{dh}	2.02^{dh}	2.14^{dh}
50						
IVDMD, %	49.8^{di}	46.9^{cg}	52.4^{deh}	49.8^{dg}	53.4^{eh}	52.9^{ei}
Total N, %	1.56^{cg}	1.63^{cg}	1.95^{dh}	1.95^{dgh}	2.14^{dh}	2.13^{dh}

[a]All values are expressed on 100% dry matter basis.

[b]Represent samples of straw without NH_3 and water added, which was not included in treatments mean comparison.

LSD = 2.78 for IVDMD ($P < .05$)

LSD = .246 for Total nitrogen ($P < .05$)

[cdef]Means in the same row followed by the same superscript are not significantly different ($P < .05$).

[ghi]Means in the same column followed by the same superscript are not significantly different ($P < .05$).

TABLE XIV

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR IVDMD, EXPERIMENT II[a]

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	179	1951.89			
Treatment	29	1007.66	34.747	6.23	P < .005
Days	5	569.10	113.82	20.42	.0001
D Linear	1	335.396		60.18	.0001
D Quadratic	1	142.780		25.62	.0001
D Cubic	1	4.358		.78	.3835
D Quartic	1	27.544		4.94	.0339
D Quintic	1	59.010		10.59	.0028
Moisture	4	194.64	48.661	8.73	.0002
M Linear	1	176.512		31.67	.0001
M Quadratic	1	6.442		1.67	.2909
M Cubic	1	0.020		0.00	.9516
M Quartic	1	11.671		2.09	.1582
Day x Moisture	20	243.92	12.196	2.18	.0253
DLxM	4	4.493		0.20	.9355
DQxM	4	23.086		1.04	.4052
DCxM	4	31.616		1.42	.2519
DQuarxM	4	6.498		0.29	.8812
DQuinxM	4	178.227		8.00	.0002
Experimental Error	30	167.18	5.573		
Sampling Error	120	777.04	6.475		

[a]Untreated wheat straw is not included in analysis.

TABLE XV

ANALYSIS OF VARIANCE BY USE OF ORTHOGONAL POLYNOMIALS FOR TOTAL N, EXPERIMENT II[a]

Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Total	119				
Treatment	29	5.99331	.20666	7.0802	P < .005
Days	5	3.95249	.790498	27.08	.0001
D Linear	1	2.9363		100.6	.0001
D Quadratic	1	0.74563		25.55	.0001
D Cubic	1	0.23238		7.96	.0084
D Quartic	1	0.03506		1.20	.2818
D Quintic	1	0.003106		.11	.7465
Moisture	4	1.28435	.321088	11.00	.0001
M Linear	1	1.07878		36.96	.0001
M Quadratic	1	0.16954		5.81	.0223
M Cubic	1	0.00199		.071	.7954
M Quartic	1	0.03403		1.17	.2888
Days x Moisture	20	0.75647	.037823	1.29582	.2542
DLxM	4	0.15426		1.32	.2847
DQxM	4	0.075154		.64	.6356
DCxM	4	0.118538		1.02	.4152
DQuarxM	4	0.057449		.49	.7416
DQuinxM	4	0.351063		3.01	.0337
Experimental Error	30	0.87567	.029189		
Sampling Error	60	0.11436	.001906		

[a]Untreated wheat straw is not included in analysis.

VITA

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