Evaluation of alfalfa hay and factors affecting hay value

by Jack Ira Stivers

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
Four experiments were conducted to evaluate factors affecting nutritional value of alfalfa (Medicago Sativa). Experiment 1 examined digestibility of low moisture (87% dry matter), medium quality alfalfa treated with anhydrous ammonia (NH3) at 3.5% of the dry matter (DM) weight. Treated and untreated alfalfa was fed alternately in two periods to four Holstein bull calves. Ammoniation raised equivalent, crude protein (CP) from 18.9 to 27.3%, a 44.7% increase. Total digestible nutrients and apparent digestion coefficients were unchanged by NH3 treatment (P>.05). In Experiment 2 six sources of first cutting alfalfa, four of second, one brome grass, and wheat straw were treated with NH3 at 3% DM weight. Proximate analyses, neutral detergent fiber (NDF), acid detergent fiber (ADF) and in vitro dry matter digestibility (IVDMD) were conducted before and after treatment. Treatment with NHg did not affect (P>.05) ADF, NDF or IVDMD. Data shows an increase (P<.05) in CP although potential of the rumen to utilize the nonprotein nitrogen with alfalfa does not warrant treatment. Experiment 3 evaluated feeding long-chopped alfalfa with long alfalfa in two lactation trials. Grain was fed to balance the ration using high quality second cutting alfalfa in trial I and medium quality first cutting alfalfa in trial II. Feed intakes, milk production, milk composition and volatile fatty acid composition showed no significant (P>.05) differences which may be attributed to chopping. Experiment 4 consisted of 12 alfalfa harvest schedules based on vegetative maturity to determine DM yields, nutrient yields, nutrient correlations with protein and predicted milk production. Chemical analysis was used to calibrate a near infrared spectrometer (NIR) to determine nutrient correlations between chemical analysis and NIR predictions for Montana conditions and maturity levels. Yields of DM increased until full maturity, then declined. Schedules at 10% bloom furnished higher CP by weight while earlier cuttings resulted in higher CP percentages. Predicted milk yield, DM intake and TVDMD decreased as maturity increased. Protein correlated significantly (r=-.90;P<.05) with NDF, ADF and CF, exclusively. Correlations of r=.90 above were obtained for nutrient variables other than IVDMD where a low correlation of .76 was attributed to the limitations in wavelengths of the NIR used.

EVALUATION OF ALFALFA HAY AND FACTORS AFFECTING HAY VALUE

by

JACK IRA STIVERS

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in
Animal Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 1984

APPROVAL

of a thesis submitted by

Jack Ira Stivers

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

11/28/84 — Date

Mark K Petersen — Chairperson, Graduate Committee

Approved for the Major Department

11-28-84 — Date

[signature] — Head, Major Department

Approved for the College of Graduate Studies

12-17-84 — Date

[signature] Malone — Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his/her absence, by the Director of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Jack Ira Stivers

Date 12/9/84

ACKNOWLEDGEMENTS

I would like to express utmost gratitude to my major professor, Dr. B.R. (Pete) Moss, for initially making a graduate program possible, his patience, direction and implementation of experiments and suggestions in preparation of this thesis. My gratitude also goes out to the members of my graduate committee making completion possible, Dr. Mark Petersen, Dr. Art Linton, Dr. Ray Ditterline, Dr. John Bryant and Dr. Wes Kezar.

The lab analysis portion of this thesis would not have been possible without the extensive assistance from Dr. Nancy Roth and Gayle Watts.

My thanks to John Wenderoth, Kathy Malcolm and the staff of the MSU dairy for their assistance and humbling attitudes. Dr. Leon Welty of the Northwestern Agricultural Research Center Kalispell must be recognized for designing and harvesting the alfalfa schedules.

My thanks also goes out to the fellow graduate students of the Animal Science Department who were very encouraging, informative and helpful, especially Lynn Courtney.

Special thanks to Dr. Rob Keene for initially suggesting I pursue a Master's degree at MSU and to my wife Phyllis for her patience and help in revising this manuscript.

Finally, I owe my special thanks to my parents, Jim Stivers and LaRue Stivers for their encouragement, support and guidance, without which no graduate program would have been attempted.

TABLE OF CONTENTS

LIST OF TABLES

List of Figures

ABSTRACT

Four experiments were conducted to evaluate factors affecting nutritional value of alfalfa (Medicago Sativa). Experiment 1 examined digestibility of low moisture (87% dry matter), medium quality alfalfa treated with anhydrous ammonia (NH_3) at 3.5% of the dry matter (DM) weight. Treated and untreated alfalfa was fed alternately in two periods to four Holstein bull calves. Ammoniation raised equivalent crude protein (CP) from 18.9 to 27.3%, a 44.7% increase. Total digestible nutrients and apparent digestion coefficients were unchanged by NH_3 treatment ($P>.05$). In Experiment 2 six sources of first cutting alfalfa, four of second, one brome grass, and wheat straw were treated with NH_3 at 3% DM weight. Proximate analyses, neutral detergent fiber (NDF), acid detergent fiber (ADF) and in vitro dry matter digestibility (IVDMD) were conducted before and after treatment. Treatment with NH_3 did not affect ($P>.05$) ADF, NDF or IVDMD. Data shows an increase ($P<.05$) in CP although potential of the rumen to utilize the nonprotein nitrogen with alfalfa does not warrant treatment. Experiment 3 evaluated feeding long-chopped alfalfa with long alfalfa in two lactation trials. Grain was fed to balance the ration using high quality second cutting alfalfa in trial I and medium quality first cutting alfalfa in trial II. Feed intakes, milk production, milk composition and volatile fatty acid composition showed no significant ($P>.05$) differences which may be attributed to chopping. Experiment 4 consisted of 12 alfalfa harvest schedules based on vegetative maturity to determine DM yields, nutrient yields, nutrient correlations with protein and predicted milk production. Chemical analysis was used to calibrate a near infrared spectrometer (NIR) to determine nutrient correlations between chemical analysis and NIR predictions for Montana conditions and maturity levels. Yields of DM increased until full maturity, then declined. Schedules at 10% bloom furnished higher CP by weight while earlier cuttings resulted in higher CP percentages. Predicted milk yield, DM intake and IVDMD decreased as maturity increased. Protein correlated significantly ($r=-.90;P<.05$) with NDF, ADF and CF, exclusively. Correlations of $r=.90$ above were obtained for nutrient variables other than IVDMD where a low correlation of .76 was attributed to the limitations in wavelengths of the NIR used.

CHAPTER 1

INTRODUCTION

The economy during the last several decades, has allowed liberal grain feeding to ruminants. The result has been higher energy intakes and greater productivity. This response is due to the lower bulk of concentrate diets and a decrease in the proportion of feed energy used for maintenance. However, animal agriculture has come under attack on the grounds that consumption of cereal grains by animals does not result in efficient energy returns and that an animal's use of grain is in direct competition for potential human food. Concentrate surpluses in the United States will decrease. This will result from world population increasing to 6 billion by the year 2,000, a shift of grain to human diets and continued conversion of grain to sugar and alcohol (Waldo and Jorgensen, 1981). Increased demand for grain will elevate prices and force the animal industry to seek alternative feed energy sources. The ruminant sector of the animal industry should logically lead the way toward maximum forage utilization.

Ruminants are uniquely suited to utilize fibrous feedstuffs due to the interaction of the forage source and rumen microbiota. Feeding forage to ruminants results in production of food for humans from a material that is little digested or utilized by humans.

Ruminant forestomach fermentation may not always be advantageous. Fermentation of diets that contain large proportions of high quality protein and readily available carbohydrate may reduce nutrient utilization due to losses of heat, methane and ammonia.

Forages comprise about 90 and 84 percent of the diet for sheep and beef cattle respectively. Dairy cattle diets contain about 63 percent forage (Hodgson, 1977). Providing the dairy animal with high quality feed is critical because energy requirements are high and there is a limitation to the physical capacity or intake, controlled by extent and rate of digestion (Moss, 1982). For example, high producing cows need a total ration dry matter (DM) digestibility of 72 percent (Adams, 1977) which is impossible to attain unless excellent hay or corn silage is fed, in addition to grain.

Producing forages capable of meeting the necessary quality has become a major challenge for dairy nutritionists. Many different factors have been shown to influence the feeding value of forages: time of harvest (considered the factor that affects forage quality and yield most), reducing field and storage loss of nutrients through the application of high moisture forage preservatives, physically altering the forage by grinding, pelleting, dehydrating, chopping, etc., and increasing the digestibility and nutritional value of low quality forages by treatment with strong bases or ammoniation.

Accurate and precise assessment of forage quality before a forage is fed to animals will have a marked effect on the economic feasibility of feeding and supplementing such feeds. The most promising new procedure being tested is near infrared reflectance spectroscopy, which can often analyze a sample in less than 1/100 the time of conventional laboratory procedures.

In an effort to evaluate factors that may benefit the forage producer and livestock feeder to optimize the nutritional yield and utilization of forages under Montana conditions, four experiments were conducted with the following objectives:

1. Determine if anhydrous ammonia treatment of medium quality alfalfa (Medicago Sativa) hay will improve nutrient content and digestibility.
2. Determine if treatment with anhydrous ammonia will alter nutritive quality and in vitro digestibility of first and second cutting alfalfa.
3. Determine the effects of chopped and unchopped high and medium quality alfalfa on fat corrected milk yields of lactating dairy cows.
4. Determine nutrient yields for alfalfa harvested from 12 different harvest schedules at Kalispell, Montana.
5. Determine the correlations between crude protein and other nutrients parameters using the data from the 12 harvest schedules.
6. Determine accuracy of near infrared reflectance using the alfalfa analysis obtained from the 12 harvest schedules.
7. Determine if maturity of samples affects the prediction of near infrared reflectance.

CHAPTER 2

REVIEW OF LITERATURE

Variation Effects Within Alfalfa

Genetic variation exists within forage species for nutritive value and/or components contributing to nutritive value (Elliott 1963, Cooper et al. 1962, Chaverra et al. 1967). Allison et al. (1969) determined the variation existing for components of nutritive value within and between populations of Medicago Sativa L., Medicago falcata L., Medicago glutinosa L. and Medicago Coerulea L., using laboratory techniques. The range of values of percent dry matter disappearance (DMD), within populations was from 45.0 to 27.1 percent (Medicago Sativa) to 33.3 to 27.7 percent (Medicago glutinosa). The corresponding range between population means was 35.4 percent (Medicago Sativa) to 26.3 percent (Medicago falcata). Clones of high estimated nutritive value were characterized by being relatively low in fibrous or cell wall constituents.

Hansen and Krueger (1973) evaluated three alfalfa cultivars (T3X-8 hybrid, Saranac, and Vernal). DM yields were higher for Saranac and T3X-8 hybrid alfalfa than for Vernal when moisture was optimum. On dryland, however, there were no yield differences between the cultivars. T3X-8 was generally lowest in crude protein (CP) content and Vernal was highest in CP late in the season. Wilson et al. (1973) compared Anchor, Saranac, Thor, Vernal and Washoe for nutrient availability, digestible energy (DE) and metabolizable energy (ME). Total digestible nutrients (TDN), DE, ME and digestion coefficients of the nutrients, except ether extract (EE), were not influenced by cultivar.

White and Bergman (1980) determined the CP and in vitro dry matter digestibility (IVDMD) of certain varieties grown at Sidney, Montana. Anchor, Thor, Vernal, Ranger, and Olympia produced the most DM yield, while Washoe produced the least in a total of three cuttings for two years. Digestibility varied by cuttings among cultivars. Generally, MSA-75-1, NC-83 and Narragansett were more digestible while Ranger and Washoe were less digestible than the other cultivars with Washoe being considerably lower in digestibility, which does not agree with the findings of Wilson et al. (1973) Crude protein content alone did not indicate digestibility, as MSA-75-1 was one of the most digestible cultivars, but contained the least CP. These findings have been supported by LaMontagne (1980; unpublished data) where 36 alfalfa samples (cultivars unknown) were analyzed for acid detergent fiber (ADF), neutral detergent fiber (NDF), CP and IVDMD. Correlations were not significant among CP and IVDMD, ADF, NDF. Further finding by Wilson et al. (1973) showed Washoe was one of the lowest cultivars for DM production, digestibility, CP and phosphorus levels, and Narragansett was one of the highest in digestibility, CP and phosphorus content.

Ditterline el al. (1979) outlined the recommended cultivars for Montana. Recommended cultivars are based upon yield, disease and insect resistance, and adaptation from extensive testing by state and federal forage research scientists in Montana.

To date, nutrient production and animal response of recommended cultivars is unknown. These data could greatly benefit the forage producers when evaluating varieties.

HARVEST SYSTEMS

When evaluating harvesting systems, the percentage of nutrients and pounds of digestible dry matter (DDM) lost during storage and feeding should be major criteria. Logan and Hillman (1975) stated that field cured hay generally suffers a 2 to 6 percent DM storage loss in addition to a 15 to 20 percent DM harvest loss. Effects of packaging systems on available nutrients and DM losses vary greatly; for example, large package systems (230 - 909 kg) may result in greater storage and feeding losses. Logan and Hillman (1975) compared three large hay package systems weighing 543 kg, 495 kg, 254 kg to a small package system (16 kg). The large packages showed additional weathering losses during storage of 10.1, 10.8 and 19.5 percent DM respectively. Martin (1980) determined that conventional bales lose 3 to 8 percent DM as compared to large round packages that lose 1 to 15 percent DM. Higher harvest losses of large round packages were attributed to: 1) light windows, 2) slow travel speeds, 3) very low moisture concentration and 4) badly weathered hay.

Wells et al. (1977) compared large packages and small bales, stored outside under the same conditions using a ratio of lignin to neutral detergent solubles (NDS) to measure nutrient loss. Large packages were inferior after a 270 day storage (Table 1). Greater losses incurred by large packages are mainly due to increased amounts of exposed surface area. Unweathered portions of large packages do not change appreciably in chemical composition or digestibility during storage. The weathered fractions, (exposed periphery), however, do undergo significant changes.

Handling and feeding losses of large packages vary with type of system used and how the package is fed. Logan and Hillman (1975) predict a 23 to 39 percent DM loss when field feeding hays. Racks can reduce this loss to less than 4 percent. Rides and Bowers (1977) suggest feeding on well drained sites to prevent the cattle and hay from standing in muddy conditions, and not feeding more hay than can be consumed in one week. Hay exposed to the weather for more than one week will become less palatable. Lechtenberg et al. (1974) reported that waste ranged from 35 to 46 percent when large haystacks were fed to cattle and amounts wasted when using a feeding rack dropped to 3.7 percent. Thirty-two percent more hay was needed when hay was fed without racks Lechtenberg et al.(1974).

Large package haymaking systems are becoming very popular with forage producers. These systems greatly decrease labor, reduce the cost of haying and permit rapid, high capacity harvest and storage of hay. Determining if ease and economy of large package systems offset added nutrient loss, is a decision that must be made by the producers.

Table 1 Ratio of Lignin to Neutral Detergent Solubles (NDS)

270 day storage	Lignin: NDS ratio
Initial ratio	1:5.71
<u>Interior portion of bale</u>	
Conventional bales	1:4.76
Big round bales	1:4.16
<u>Surface (15 cm layer)</u>	
Sides	1:1.66
Top	1:2.86
Bottom	1:3.57

Source: Wells et al. 1977.

HARVESTING ALFALFA

The primary factor which influences forage quality is the maturity of plants at the time of harvest (Hibbs and Conrad 1975). Yield of DM and contribution of leaves and stems to DM yield at different stages of maturity were investigated by Kilcher and Heinrichs (1979) by taking first cuttings of Roamer alfalfa at a wide range of maturities (very immature to late bloom). Dry matter yield increased at a constant rate from the very immature stage to the half-bloom stage of maturity. Thereafter, yield increased but at a declining rate. Stem DM yield increased linearly throughout the entire period. Leaf DM yield, increased linearly until flowering commenced, then did not increase after that. Thus, at the early flowering stage, the yield of stems and leaves was equal; whereas at the late bloom stage, stems made up 60 percent and leaves only 40 percent of the total yield. This change in leaf-to-stem ratio affects energy and CP content of the total harvested plant.

Kilcher and Heinrichs (1974) determined the DE of leaves to be nearly a constant value throughout all stages of development. The DE of the leaves declined in their study only from 73 to 70 percent. However, DE in stems declined rapidly from 70 percent at the early leaf stage to 47 percent at the early bloom stage. Yield of both CP and DE of leaves leveled out when flowering commenced; whereas in stems, yield continued to increase. Crude protein and DE yields for whole plant material declined at nearly a constant rate from very immature stage to late bloom. Porter and Reynolds (1975) showed similar results when testing 11 alfalfa cultivars for specific leaf weight, plant density, and concentrations of CP, phosphorus, potassium, calcium, and magnesium. Dry matter yield of cultivars was positively correlated with plant density but not correlated with specific leaf weight or with concentration in the forage of any of the elements stated.

First cutting date not only affects forage yield and quality but also yields of succeeding cuts (McGuffey and Hillman 1976). Singh and Winch (1974) compared three harvest schedules of early bud (4 cuttings), first flower (3 cuttings), and full flower (2 cuttings). During regrowth, following harvest, many early developing buds failed to produce mature stems. After each harvest, regrowth originated mainly from stubble of the most recently harvested stems. Yield reductions occurring in successive regrowths of each harvest schedule were mainly due to the production of smaller stems. Increased growth rates following more mature cutting stages resulted mainly from faster elongation of stems developing from larger buds.

Peterson and Hagan (1953) demonstrated that the largest total DM yield for a season was obtained when alfalfa and grass were cut at five-week intervals in comparison with either two, three or four week cutting intervals. Jones et al. (1953) showed that DM yield per hectare increased per cutting until full bloom, which may be as long as 50 to 60 days after the previous cutting; but the protein and carotene per hectare declined and crude fiber (CF) and lignin increased as the alfalfa becomes more mature and as cutting interval is lengthened. Weir et al. (1960) conducted a trial for 4 years in which alfalfa was cut at the pre-bud, bud, 1/10 bloom and 1/2 bloom stage for 3 years and then all plots cut at the same stage in the fourth year. During the 3 years of differential treatment, the greatest DM yield was produced by alfalfa cut at the bloom stages. The greatest protein yield alfalfa was cut at the 1/10 bloom stage. In the fourth season, when all alfalfa was cut at the same stage, there was no significant difference in yield despite the difference in treatment during the previous three seasons. However, total yield may not be decreased in all circumstances and nutrient yield per hectare may be enhanced as shown by McGuffy and Hillman (1976) when they employed three harvest schedules ([A] 3 early cuttings, [B] 3 late cuttings, [C] 2 late cuttings) to determine yearly yield and quality. For the year schedule, A produced 563.63 and 800 kg more DM, 217.2 and 41.81 kg more CP, 512.72 and 700 kg more TDN than schedules B and C, respectively.

Stand Survival

The goal of the alfalfa producer is to obtain the largest yield of high quality forage consistent with reasonable stand survival. Recovery rate after harvesting, total vegetative growth and winter survival are all closely associated with the carbohydrate root reserves of alfalfa (Ditterline et al. 1979). Root carbohydrates are used to produce new top growth, and continue to be used until there is sufficient top growth to manufacture enough carbohydrates to meet plant growth requirements (Grandfield 1935). In general, research has shown that the concentration of nonstructional carbohydrates in alfalfa roots decreases for a time after forage is harvested and then increases as photosynthate is translocated to the roots. Pearce et al. (1969) found that during a 18-day regrowth period, 45 percent of the carbon in the defoliated plant was lost to respiration, leaching, and sloughing while 19 percent appeared in the new top growth. Smith and Silva (1969) accounted for 15 percent of root nonstructional carbohydrates in respiration, while 66 percent was used in production of new roots and tops. Frequent defoliation of alfalfa decreased the carbohydrates; low carbohydrate was associated with stand loss and reduced yields (Cooper and Watson 1968). Reynolds (1971) compared nonstructional carbohydrate trends in Buffalo alfalfa at six harvest schedules (8, 6, 5, 4, 3, and 2 cuts per year) for 2 years. Root carbohydrate levels in the first year were generally lowest when cut eight times, and the stand was very sparse at the end of the first year. The greatest drop in carbohydrate concentration usually occurred after the first harvest of the year. Under a uniform harvest schedule in the third year, the two cut, three cut, and four cut treatments had the most vigorous and productive stands.

Alfalfa was harvested at various stages of development of first cut to determine the effects of both summer harvests and different fall rest periods on the productivity, quality of crops and persistence of Saranac and Narragansett alfalfas (Macleod et al. 1972). Satisfactory DM yields with high CP and IVDMD of Saranac, and Narragansett were obtained in the first year under early maturity cutting managements. However, early maturity cutting regimes resulted in a rapid decline of vigor, severe stand deterioration and weed invasion in the second year. First harvest of alfalfa at prebud stage, with two subsequent cuts at early bloom stage before fall rest period and a late fall harvest, did not markedly improve alfalfa persistence over systems in which all cuts during the season were taken at vegetative stages. Ditterline et al. (1979) recommends cutting alfalfa under Montana conditions at 10 percent bloom, stating this is the best time to obtain high concentrations of feed nutrients in the forage, high yields and to allow for high root carbohydrate replenishment.

Animal Response to Hay Quality and Maximum Yearly Nutrient Yield

The best indicator of forage quality is the amount of digestible forage DM or DE a cow can eat in 24 hours per unit of metabolic size (Hibbs and Conrad 1975). As forage crops advance in maturity prior to harvesting, forage digestibility decreases and forage intake is reduced (Moss 1982). Meyer et al. (1960) conducted a four year study on the influence of stage of maturity on the value of alfalfa as an energy source for sheep. Changes in lignin content and gains of lambs fed hay harvested at different stages of maturity indicated the critical turning point in feeding value appears to be when 10 percent of the stems have one or more blossoms. After 10 percent bloom, feeding value did not change as markedly as between earlier maturity stages. Horton and Holmes (1977) compared the feeding value between alfalfa harvested at first, second and third cutting. Alfalfa intake decreased as the digestibility of organic matter and cellulose increased, to result in similar intakes of digestible organic matter for all treatments. Reid et al. (1959) noted a linear decline in maximum intake from 2.5 - 3.0 kg of hay equivalent per 45.45 kg of body weight for forage harvested early June to 0.5 - 0.77 kg for forage harvested in mid-July when dairy cows were used as test animals and concentrates were fed at the rate of 0.45 kg for each 1.36 or 1.81 kg of milk produced. Conrad et al. (1962) showed the effects of advancing maturity on digestibility, DM intake and milk production. Dry matter intake decreased from 15.45 kg per 454.54 kg live weight to about 11.81 kg which is a result of the slower rate of passage of the ingested material. Decreased intake along with the decreased digestibility caused milk production per 454.5 kg live weight to decrease from 19.31 kg to less than 9.09 kg. Hibbs and Conrad (1975) showed that as daily green chopped forage matured, percent digestibility, voluntary DM intake and milk production decreased, requiring increased grain supplementation to keep production at 19.31 kg a day.

The optimum time to harvest depends on the producer's goals; higher returns from his forage due to increased animal performance are more important than the price received for higher yields of lower digestible forages. If the producer's goal is maximum DM production, he must sacrifice maximum digestibility of energy and protein, (Stallcup 1979).

HEAT DAMAGE TO FORAGE

Chemical Examples of Browning Reaction

Rohweder and Collins (1980) explain that heat damage is caused by chemical oxidation (burning of sugars in forage material), and that such oxidation produces a compound known as artifact lignin formed by combining nitrogen with lignin compounds in the plant. This process is known as the Maillard Reaction of organic chemistry, in which amino groups of proteins react with carbonyl groups of carbohydrates to form an indigestible compound (Waldo, 1979).

The reaction of carbonylic compounds with amino compounds can initiate a sequence of reactions frequently referred to as non-enzymatic browning which lead to the formation of brown pigments and off flavors. The most extensively studied form of non-enzymatic browning is the Maillard Reaction (Hodge, 1953). In general, amino compounds, amines, amino acids, peptides, and proteins are active in reactions, usually with carbohydrates, which produce highly reactive carbonylic intermediates and involve condensation with these intermediates to produce highly colored pigments (McWeeny et al., 1974). Almost all feeds contain these reactants and the extent to which the browning reaction occurs will depend on the moisture content, pH, and temperature a feed is exposed to during processing and/or storage. Frequently it is the reducing sugar content of a feed and the type of sugars present such as pentoses, hexoses, or disaccharides, which determine the rate at which nonenzymatic browning occurs and the reaction of these sugars with amino acids (McWeeny et al., 1974).

McWeeny et al. (1974) summarized the known chemistry of non-enzymatic browning and indicated the major features of the reaction. Figure 1 of the appendix shows the initial condensation of glucose with glycine, a reversible reaction, the equilibrium lying to the right in low-moisture systems, therefore favoring formation of the resultant glycosylamine to a ketoseamine. This arrangement requires acid catalysis, and the amino acid function acts as its own catalyst, the ketoseamine being formed immediately. These are stable compounds but more reactive than ketoses.

Analogous products from a ketose amino acid reaction are the corresponding aldoseamine which can add a second mole of amino acid or amine to give the diamino sugar and is illustrated in Figure 2 of the appendix. The fourth step is the degradation of the amino sugars to amino; and non-amino containing compounds which are believed to be the reactive intermediates lead to the production of brown colors and/or aromas.

Decomposition of difructoseglycine is a complex stage and involves a series of degradations probably occurring concurrently, the relative importance of the various routes depending on the particular reaction system. The decomposition of difructoseglycine (Figure 3, appendix) has a maximum rate at pH 5.5 and yields a quantative amount of fructoseglycine together with other carbonylic compounds such as 3-deoxy-hexosuloses and the cis- and trans- forms of unsaturated hexosuloses (Anet, 1962).

In Maillard reactions involving glucose and glycine, (at pH 5.5), the concentration of defructose-glycine is comparatively low, but its large turnover insures the formation of a large amount of the carbonyl decomposition products (Anet 1959). Since these decomposition products browned rapidly they, and in turn defructose-glycine, may be main precursors of the brown pigments. This mechanism should also apply in the case of other aldoses and other primary aliphatic amines but may not be the most important under more acid or alkaline conditions (Anet 1959).

Carbonylic compounds of all these types can be formed directly from sugars by thermal decomposition, though the temperature must in this case be much higher (McWeeny et al., 1974). Brown pigments or melanoidins are produced by a fifth stage involving the carbonylic intermediates, especially the unsaturated carbonylis, condensing either with each other or with amino moities, possibly in a random manner but leading eventually to highly colored, fluorescent macromolecular pigments (McWeeny et al., 1974).

Goering and Van Soest (1967) determined the effect of moisture, temperature, and pH on the relative susceptibility of forages to non-enzymatic browning. This experiment was conducted by heating orchardgrass and alfalfa in flasks with varying amounts of water (8-82%) and buffer in an oven (40 to 100 C) for various lengths of time (4-72 hrs.). Extent of browning was assayed by acid detergent fiber insoluble nitrogen or pepsin digestion. Buffering orchardgrass at pH 4.5 and 6.5 with acetic acid and phosphate, respectively, caused an increase in acid-detergent fiber nitrogen with the lower pH. In alfalfa, no differences with pH could be found. Susceptibility appeared greatest over 20-40% moisture range with orchardgrass. Alfalfa susceptibility was high and relatively constant over the 20-80% moisture range. Hemicellulose content decreased in severely browned samples and it appeared to be one of the carbohydrate sources for browning.

STORAGE AT PROPER MOISTURE

Biological processes that cause nutrient losses and lower digestibility are closely linked to moisture content and temperature of hay during storage. As moisture content increases, DM is lost and nutrients become less digestible (Von Bergen 1978). Table 2, adapted from Miller et al. (1967), demonstrates this concept. The chemical analyses, air dry basis, of the hays in Table 3 showed that the percentage CP was relatively constant regardless of moisture content at time of baling. There was more ash, cell wall constituents, cellulose and ADF and lignin in the hays baled at higher moisture content than in those baled at lower moisture content (Miller et al. 1967). From this, Miller et al. (1967) determined that remaining portions of the forage, primarily readily fermentable carbohydrates, decreased as the moisture content at time of baling increased. This confirms Barnes and Gordon's (1972) observations that progressively higher moisture contents at baling were subsequently shown to be related to increased levels of ADF and lignin after storage, along with depressed digestibility of both gross energy and CP.

Performance of beef animals decreases, in general, as moisture content at baling of alfalfa and native hay increases (Miller et al. 1967). Miller et al. (1967) showed average weight gains of calves fed hay baled at lower moisture contents were higher than of those fed hays baled at higher moisture content (Table 2). Daily feed consumption was similar, although there was a trend toward lowered consumption of the hay baled at higher moisture content. However, Von Bergen (1978) noted milk production in dairy cows was maintained when fed hay at moisture contents below 40 percent level.

If hay is put into stacks or packaged at low moisture and protected from weathering, fewer nutrient losses occur during storage (Moser 1980). However, Moser (1980) states that, due to oxidation, vitamin A loss is significant, and loss is greater on the outside of stacks or packages than toward the center. Carotene losses of 50 to 75 percent after one year of storage are common regardless of the hay storage conditions. Absolute losses are greatest in hay with high initial content of carotene, such as in high quality alfalfa.

Moisture concentrations for safe storage of all hay types are not well defined. Lechtenberg (1978) suggests a moisture concentration of less than 20 percent regardless of package type, whereas Simms (1977) suggests less than 25 percent for large hay packages stored outside. Overall recommendations (Martin 1980, Von Borgen 1978, Hibbs and Conrad 1975) generally agreed that alfalfa should be baled at less than 20 percent moisture, native hay at 15 percent and large packages at less than 25 percent.

Perennial forages generally contain between 70 and 80 percent moisture at the suggested stages of harvest for silage (Rohweder and Collins 1980). Rohweder and Collins (1980), recommend that moisture contents above 70 percent in direct cut silage may result in undesirable

Table 2. Average Performance of Calves Fed Hays Baled at Various Moisture Contents during 112-day Growth Study[a].

	Percent moisture at time of baling							
	Alfalfa hay				Native hay			
Item	26.2	35.2	53.4	58.5	19.2	34.1	43.5	50.8
Weight, kg.								
Initial	169	166	173	169	175	181	181	184
Daily gain	0.42[b]	0.41[b]	0.27[c]	0.19[d]	0.25[b]	0.17[c]	0.16[c]	.08[d]
Feed consumed, kg.								
Daily[e]	5.4[b]	5.2[b]	4.8[b]	4.4[b]	4.0[b]	4.0[b]	3.8[b]	3.8[b]
Per kg. gain	13.1[b]	13.2[b]	18.3[c]	24.6[d]	15.7[b]	23.2[c]	23.7[c]	47.2[d]

[a] Each value is the average of 5 steers. The alfalfa hay data and the native hay data were subjected to separate analysis of variance with Duncan's Multiple Range Test used to indicate which treatments differ from each other within each type of hay.

[bcd] Coefficients with different subscripts are different at the 0.01 level of probability.

[e] Includes 0.45 kg. of supplement each calf received daily with the native hay.

Source: Miller et al. 1967.

Table 3. The Chemical Composition of the Hays Baled at Various Moisture Contents[a].

	Percent moisture at time of baling							
	Alfalfa hay				Native hay			
Composition, %	26.2	35.2	53.4	58.5	19.2	34.1	43.5	50.8
Dry Matter	91.5	90.2	90.6	58.5	90.9	90.4	88.6	87.6
Ash	9.6	9.8	11.0	11.4	8.8	8.8	8.9	9.2
Crude protein	18.3	19.4	20.4	18.1	8.2	8.2	8.0	8.6
Water-soluble carbohydrates	4.9	4.8	4.5	4.9	4.9	3.2	3.2	3.2
Cell wall constituent	44.9	41.8	46.7	46.8	63.8	67.2	69.4	67.3
Acid detergent fiber	30.4	31.6	39.9	40.0	42.7	49.6	48.8	50.3
Cellulose	22.9	23.5	27.1	28.0	30.6	31.7	33.2	31.6
N-free extract	38.4	37.2	33.8	29.5	42.5	41.3	38.2	37.8
Acid detergent lignin	7.2	7.5	10.7	10.3	7.0	10.3	10.5	11.0

[a]Chemical analyses were made after samples were standardized at atmospheric conditions.

Source: Miller et al. 1967.

fermentation, seepage due to squeezing of water out of plant material and movement of soluble nutrients out of the silo with the water. Logan and Hillman (1975) found alfalfa will retain 70 percent moisture without seepage at normal silo pressures, suggesting filling silos when the forage has wilted to 70 percent moisture. Later loads will be dryer, but most of the loads will be 50 percent moisture or more. A desirable moisture for silages is 65 percent (Rohweder and Collins 1980).

Moisture content during storage is related to the degree of heating. Heating will occur to some extent in all forage material unless it contains less than 15 percent moisture (Martin 1980). Waldo (1979) explains that allowing forages to wilt before ensiling to the suggested 65 percent will reduce field losses, but raise storage losses. Wilting can produce silages that have excessive heating. Corn silage that is too wet has excessive protein degradation; corn silage that is too dry has excessive heating and energy loss during feeding. Rohweder and Collins (1980) state that ensiling material at 50 to 65 percent moisture, excluding air by packing and storing in a tight container are the best ways of preventing heat damage.

DETRIMENTAL EFFECTS OF MOLDING

Molding due to Moisture

Forage, to be stored satisfactorily as hay (long or baled), should be about 80 percent DM, depending on environmental conditions. At this and greater DM content, field loss of leaves is considerable during harvesting. Rapid drying of leaves as compared to stems reduces nutrient and DM yields due to leaf shatter (Thomas, 1978).

Hay storage at a moisture content higher than the critical level results in continued plant respiration, mold growth, and the development of excess heat. Detrimental effects of high moisture content at the time of storage have been measured as is the degree of continued respiration, the extent of heat development, the amount of mold developed, reduction in digestibility, chemical composition of hay, and animal production responses when fed this material (Barnes and Gordon, 1972). Using a controlled environment chamber, Wilkinson and Hall (1965) determined heat production, moisture, and weight losses of wilted and fresh alfalfa at different storage temperatures. They showed fresh material stored at 15.5 C would exceed DM losses of field-cured alfalfa after approximately 13 days of storage. Fresh material stored at 7.2 C would exceed field curing losses after three to four weeks in storage. Fresh material stored at -1.1 C would not exceed field-cured losses until it had been in storage several months. After freezing, alfalfa may be stored at 3.8 C indefinitely without loss. Respiration losses of stored alfalfa increased at storage temperature above 25 C and moisture contents greater than 10 percent.

Miller et al. (1967) investigated the effect of moisture content at time of baling, as reflected through changes in temperature following baling, upon the nutritive value of alfalfa and native hay. Alfalfa hay was baled at moisture contents of 26.2, 35.2, 53.4, and 58.5 percent, and native hay was baled at moisture contents of 19.2, 34.1, 43.5, and 50.8 percent. Maximum temperatures were reached at four to eight days following storage and peaked at 45 to 60 C. Ash, ADF and lignin increased as moisture content at time of baling increased.

Apparent digestibility of CP decreased 11.5, 11.5, 8.0, and 5.1 percentage units for the four moisture levels of alfalfa and 3.3, 1.7, 0.9, and 0.6 percentage units for the four moisture levels of native hay. Digestible energy also declined as moisture levels increased: 2.2, 2.0, 1.8, 1.3; and 2.7, 2.5, 1.9, and 1.9 percentage units for alfalfa and native hay, respectively. When beef calves were fed the forage in digestibility trials, calves that received hay baled at lower moisture contents gained faster and more efficiently than those that received hay baled at higher moisture contents. There was no significant difference in feed intake between the two groups.

Influence of Mold on Animal Performance

Poorer animal performance from feeding moldy hay has been related to lower nutrient digestibility. Mohanty et al. (1967) reported that weight gains of dairy steers fed badly molded alfalfa were only 75 percent of those fed good quality hay. When steers were supplemented with 1.8 kg grain daily as compared to 0.9 kg of grain daily, gains on the moldy hay decreased to 85 percent of those on good-quality hay. Dry matter needed per kg body weight gain was 14.54 and 17.44 for the two-grain feeding levels with well-cured hay; 16.29 and 22.71 kg respectively for moldy hay. Average digestible DM, energy, protein, CF, and nutritive value index for good and moldy hay were 64.5, 60.3; 63.7, 63.9; 76.0, 65.3; 56.2, 71.2 and 61.2, 50. for well-cured and moldy hay fed steers, respectively.

In two feeding experiments by Burt et al. (1976), two hays of similar chemical composition produced rates of decline in milk yield of 2 percent and 6 percent of initial yield per week when used as part of the maintenance ration. It was found that when the hay associated with the 6 percent decline was included in the maintenance ration, together with molassed sugar beet pulp and 1.8 kg concentrate per 3.785 liter of milk, milk yield was depressed and the digestibility of dry and organic matter, energy, nitrogen, and nitrogen-free extract (NFE) were reduced. Addition of 900 grams per day of concentrates to the diet containing the poor hay did not alleviate these effects. Examination of this hay for fungal contamination showed total spore counts of more than 2,000,000 per gram including large contaminations of Aspergillus and Penicillium species. These results indicate that hay which is not apparently moldy may give poor productive results owing to fungal contamination, and may be an important cause of field cases of unexplained poor productivity in dairy herds.

CHEMICAL CURING

Hay Preservatives

Knapp et al. (1976) stated that DM losses, compositional changes and digestibility decreases due to heating, weathering, and packaging at greater than 25 percent moisture are serious problems in forage production. Mold growth not only decreases hay quality but it is primarily responsible for the heating of hay which causes further deterioration. It is desirable to devise treatments so that mold development in inadequately cured hay can be prevented due to the lack of optimal conditions for packaging.

Early investigators treated wet hay with over 100 chemicals and found trichlorophenol to be one of the most mycostic chemicals investigated. With 40 percent DM hay in jars, a relation between days for mold to appear and application rate of trichlorophenol was established. Later, trichlorophenol was sprayed on hay at the baler pick-up apron at the rate of 4.09 to 15.9 kg/1.016 metric tons of 70 percent DM hay but did not prevent mold sufficiently to be termed a success (Hopkins and Wiant, 1956).

Two types of hay preservatives have been widely investigated. They are organic acids or their salts, such as propionic acid, ammonium isobutyrate, and anhydrous ammonia. Organic acids, especially propionic acid, have strong fungicidal properties, and have been promoted as effective preservatives of high-moisture hay. Huber et al. (1972) applied a 3:2 mixture of acetic and propionic acid at 0.5 percent and 1.5 percent of the DM weight to 40 percent moisture hay in large three-ton stacks, compared to stacks of 23 percent and 40 percent moisture and conventional bales at 23 percent moisture without acid treatment. Acid decreased DM losses (28 percent vs 17 percent) and visible mold of high-moisture hay, but heifers readily ate the moldy feed. Dry matter intakes (percent of body weight) were lower and daily gains were higher for acid-treated hays with 0.5 percent treatment being higher than all others. Although organic matter digestibility (OMD) was higher for 23 percent moisture stacks and bales and 1.5 percent acid treatment than 0.5 percent acid treatment, it appears acid treatment of 40 percent moisture had decreased DM losses and temperature increases which appeared to be the cause of decreased OMD.

McGuffey et al. (1973) reported that alfalfa at 17 to 28 percent moisture had less temperature rise when a higher concentration of acid treatments were used. For example, 0.70 percent propionic and 0.42 percent ammonium propionate had lower temperatures than 0.35 percent propionic and 0.21 percent ammonium propionate, but untreated hay exceeded all temperatures. Acid detergent fiber and ADF-nitrogen (ADF-N) were highest for untreated hay at 28 percent moisture and lowest for untreated at 17 percent moisture, and 13.4 and 29.6 percent greater respectively than the average ADF and ADF-N for the other treatments combined (McGuffey et al., 1973). McGuffey et al. (1974) treated 45 percent moisture alfalfa with 0.35 to 1.6 percent ammonium isobutrate or 0.5 to 3.9 propionic, as hay entered the baler. They found reduced temperatures (2 to 15 degrees C lower) during the first one to three weeks of storage, after which temperature was similar for treated and untreated lots. Treatment with 0.65 percent ammonium isobutrate or 0.51 to 3.9 percent propionic had lower ADF-N (0.4 to 0.17 percent compared to .59% for untreated).

Two experiments were conducted by Sheaffer and Clark (1975) to evaluate the effectiveness of propionic acid and ammonium isobutrate in preventing mold growth. Application was done manually on alfalfa-timothy hay with 31 or 40 percent moisture. Preserving effects of the two compounds were not significant; however, there were significant differences in rates necessary to preserve the hay at a given moisture level. Hay baled at 31 percent moisture content and treated with preservatives at rates of 1.5 to 2.0 percent by weight had significantly lower storage temperatures and significantly higher IVDMD than untreated hay and hay sprayed at the 1.0 percent rate. Application rates of 3.0 and 5.0 percent were effective in significantly reducing storage temperatures and maintaining forage quaility of hay baled at 40 percent moisture.

Similarly, Knapp et al. (1976) reported that 32 percent moisture hay was effectively preserved from mold and fungi by propionic acid when the amount applied equalled one percent of the hay weight. Application rates of less than one percent did not effectively prevent heating or dry weight loss during storage. Even when applied at an effective rate, acid treatment did not increase protein percentage, IVDMD, or the fiber digestibility of the hay. Results found by Kjelgaard et al. (1977) after applying organic acids to high moisture hay as compared to field- cured were: 1) high-moisture alfalfa hay, 25 to 35 percent moisture, treated with organic acid at 1 to 2 percent of wet forage weight showed increased DM yield per hectar; 2) chemically treated hay had less temperature rise in storage when compared with untreated hay baled at the same moisture content; 3) no change in CF content was observed, but there was higher available protein and ADF; 4) animal intake and acceptability were higher for acid treated than field-cured; 5) addition of water to the acid reduced intensity of irritating vapors, improved safety, and did not reduce effectiveness of the chemical.

Lord and Lacey (1978) recognized the problem of nonuniform distribution of the chemical in the bale permitting localized growth of some fungi. The fungi metabolize the chemical, spread through the rest of the hay, and permit colonization by other, often more harmful, fungi. They found that addition of 8-hydroxyquinoline to propionic acid diminished the amount of the latter required to prevent mold growth, possibly by inhibiting organisms tolerant of fatty acids and able to metabolize them. Thus, 8-hydroxyquinline enables conservation to be made in the amount of propionic acid used and alleviates some of the problems of obtaining a uniform distribution within the stored crop.

Animal Responses Due to Acid Treatment

Nehrir et al. (1978) investigated acid application methods to 30 percent moisture hay at the mower conditioner, at the rake, and at the baler. Heat-dried and high-moisture untreated hays were used as controls. Results from ewes fed ad libitum alfalfa with treatment applied at either the baler or mower gained significantly more weight than ewes fed heat-dried hay. Weight gains for animals fed alfalfa treated with 2 percent acid at the baler did not differ significantly from those fed heat-dried hay. Animals refused more hay treated with 1 percent acid at the mower than with any other treatment.

A commercial hay preservative of propionic acid was used to treat bermudagrass at moisture contents of 24.2 and 16.0 percent; counterpart controls were baled at 21.5 and 14.9 percent moisture. After 245 days of storage, each hay was fed to four Jersey heifers and digestibilities were estimated by lignin ratio technique. Apparent digestibilities of EE, CF, and DM of the treated 24.2 percent moisture hay were higher but the DM loss of 3.8 percent during storage was 310 percent of the average of the other three hays. Treatment of either high or low moisture hay failed to reduce DM losses during storage, raise voluntary intake, increase efficiency of utilization, or improve weight gains of heifers. Intakes of the untreated high and low moisture hays were 6.2 and 5.0 percent more than that of treated counterparts. Gains of heifers receiving untreated high and low moisture hays were 200 and 153 percent of those by heifers fed treated counterpart hays (Johnson and McCormick, 1976).

Jafri et al. (1979) investigated the effects of applying one percent of the hay weight with 70 percent propionic acid and 30 percent formalin diluted 50:50 with water to 28 percent moisture alfalfa. They compared the chemically treated hay with dry (19 percent moisture) control hay using lactating Holstein cows. Both were accepted readily by the cows, resulting in no difference in average intake. While insignificant, average milk yield of cows fed chemically treated hay was slightly higher than for those fed dry baled hay. Milk fat and nonfat solids percentages were similar for cows fed the two kinds of hay. Overall, feed value of chemically treated hay was at least equal to that of dry baled hay.

Anhydrous Ammonia as a High Moisture Forage Preservative

Anhydrous ammonia (NH_3) is a good fungicide for controlling a number of fungal organisms (Bothast et al., 1973). It controls fungi and molds on fruit and high moisture corn (Bothast et al., 1973, Hawkes et al., 1966). Knapp et al. (1975) investigated the effectiveness of NH_3 as a preservative to prevent microbial activity and consequent DM and digestibility losses in high-moisture hay, using one percent NH_3 of the hay DM weight applied immediately after baling. Results indicated that NH_3 treatment reduced molding, heating, and DM loss in stored hay, with ADF-N increasing significantly in both treated and untreated hay during storage. However, when ADF-N was expressed as percent of total nitrogen, the original samples were 7.0 percent

ADF-N compared to 6.7 percent ADF-N in the treated hay. Acid detergent fiber-nitrogen was 9.1 percent of total N in untreated hay, this suggests that only a small amount, if any, of the added NH_3-N became part of the indigestible ADF-N fraction. In vitro dry matter disappearance was significantly greater in treated than in untreated hay after storage (66.1 and 60.5 respectively; with 70.5 before storage). Ammonia treatment did cause an increase in in vitro cell wall disappearance (IVCWD) of 11 percent in alfalfa. Presumably, the increase is due to NH_4OH formed in the hay hydrolyzing some lignin-cellulose bonds.

Knapp et al. (1975) reported IVCWD was 51 percent in untreated alfalfa hay and 57 percent in NH_3 treated alfalfa. A more recent study by Weiss et al. (1982) showed NH_3 treatment of alfalfa increased IVCWD from 53.2 to 57.6 percent. Alfalfa harvested at the proper stage of maturity has relatively high fiber digestibility, and therefore, NH_3 treatment should not improve it significantly, whereas low quality roughages, such as straws, undergo large increases in fiber digestibility after NH_3 treatment (Horton and Stacey, 1979).

These results compare closely to the findings of Lechtenberg et al. (1977) where a similar experiment was conducted applying one percent NH_3 of the hay DM weight to 32 percent moisture in alfalfa. Dry matter losses during storage were reduced from 15.1 to 9.9 percent by NH_3 treatment. In vitro cell wall disappearance was increased from 51.1 percent initially to 56.9 percent following NH_3 treatment, with the control dropping to 47 percent. This suggested NH_3 has an effect on fiber digestibility similar to that of strong bases (Guggolz et al., 1971).

Lechtenberg et al. (1977) results also showed the alfalfa hay baled at 32 percent moisture and treated with NH_3 did not heat during storage. They stated that the hay was bright green and free of mold after two months of storage. Untreated high moisture hay, in comparision, heated to more than 50 C and was extremely moldy at the end of the storage period.

Neiss et al. (1982) conducted an experiement where alfalfa hay was baled either at 32 percent moisture and treated with 1.87 percent NH_3 of the DM weight, or at 19.5 percent moisture and left untreated. Moistures for the treated and untreated hays after 6 months storage were 12.4 percent and 11.2 percent respectively. At harvest, 9.56 percent CP equivalent from NH_3 was added to the treated hay with 52.3 percent of this nitrogen retained after storage. This raised the CP content of the treated hay from 18.8 to 23.8 percent which was a 27 percent increase in CP. However, when treated and control hays were analyzed for acid detergent insoluble nitrogen (ADIN), expressed as a percent of total nitrogen, ADIN's were similar for both hays. Forages with 20 percent or more total nitrogen as ADIN are considered heat damaged (Van Soest, 1965); hence, Weiss et al. (1982) states that the relatively low ADIN obtained for NH_3 treated hay indicates that ammonia successfully prevented heating in high moisture hay.

Intake data indicate that high moisture grass and alfalfa treated with NH_3 caused increased consumption for both cattle and sheep (Knapp et al., 1975). Lechtenberg et al. (1977) showed significant intake differences with sheep consuming NH_3 treated alfalfa (1.94 percent of body weight) as compared to untreated alfalfa (1.71 percent of body weight). Weiss et al. (1982) showed no significant differences in intake between lactating Holsteins consuming NH_3 treated alfalfa or untreated alfalfa as the diet roughage source. Actual and fat-corrected milk yields and percent milk protein in this study showed no statistical difference for cows receiving NH_3 treated or conventionally harvested alfalfa. Percent milk fat, however, did show a significant increase (P=0.10) for those cows receiving NH_3 treated alfalfa, 3.83 percent milk fat as compared to the cows receiving untreated alfalfa, 3.70 percent.

Application of NH_3 treatment to cereal straws to improve its nutritive value has been practiced in Europe for several decades, and commercial processing plants have been developed in a number of countries (Knipfel, 1982). Many investigators have shown NH_3 treatment to increase the CP and the digestibility of DM and organic matter of wheat straw (Horton, 1978; Sundstl et al., 1978; Horton, 1979; Horton and Stacy, 1979; Kernan et al., 1979). However, Herrara-Saldana et al. (1981) stated that the CP content of the straw can decrease up to 20 percent after the stack is opened due to ambient factors such as wind, temperature and humidity.

Morrison (1974) postulated that at least 3 types of bonding may be present between lignin and carbohydrates: namely, one cleaved on borohydride reduction, another cleaved by alkali and a third type of linkage resistant to alkali. The effect of NH_3 treatment of straw may break down the first 2 types of bonds, producing an increase in lignin digestibility, but no clear explanation was found in the literature for lignin digestibility in untreated straw.

Application of Anhydrous Ammonia

Applying NH_3 to forages is relatively simple, requiring an effective cover to create an air-tight atmosphere, a perforated steel pipe and a source of NH_3. The procedure generally adopted by researchers is well-documented by Sundstol et. al. (1978) and is referred to as the Norwegian Method. There are no specific requirements regarding the shape and size of the container in which the reaction between the material and the NH_3 takes place. The number of bales varies according to their size, it being easier to get a good stack if the bales are 1-1/2 or 2 times as long as they are wide. A lath should be placed between the third and fourth layers to provide an entrance for the injection pipe.

The stack is placed on an undersheet of 0.20 mm polyethylene leaving a 0.7 m margin of plastic on each side for closure of the stack. The stack is covered with a top sheet of black polyethylene leaving a free margin of 0.7 m polyethylene on each side corresponding to that on the undersheet. When the stack is completed, three sides are sealed by rolling the two edges of the under and top sheets around a wooden lath at the base of the stack. It is then pinned down by sand bags placed at the top of the roll. The fourth side remains unsealed until the NH_3 has been injected. The NH_3 is transported in pressure tanks on trucks and is injected through a perforated metal pipe which is put three quarters of the way into the stack.

Syndstøl et al. (1978) recommends length of treatment for low quality roughages treated with 3-4 percent NH_3 at ambient temperature to be:

Temperature	Length of Treatment
Below 5 degrees C	More than 8 weeks
5-15 degrees C	4-8 weeks
15-30 degrees C	1-4 weeks
Above 30 degrees C	Less than 1 week

Extending the treatment beyond the time indicated above does not harm the forage.

FACILITATING NUTRIENT ANALYSES WITH NEAR-INFRARED REFLECTANCE

Development of Near-Infrared for Biological Samples

The region of electromagnetic spectrum known as the infrared is next to the visible region of the spectrum. Infrared waves, although not visible, are often known as heat waves because they cause a warm sensation to the skin and because the most important sources of these waves are usually heated solids. In this spectral region lie the rotational and vibrational spectra of molecules, the manifestations of the changes in the molecular rotational and vibrational energy that can occur under certain conditions by the interaction of infrared radiation with matter (Brugel, (1962). Notable advances in experimental infrared spectroscopy, that part of the science of optics concerned with the infrared region, have contributed to the understanding of the structure of molecules. Determination of precise values for internuclear distances and bond angles and evaluation of potential functions have indeed broadened the application of infrared spectroscopy to evaluate molecular parameters.

Infrared radiation is characterized by its wavelength using microns (M) on the y axis and apparent absorbance peaks shown on the x axis.

Instruments used for research are equipped to illuminate samples with monochromatic light making it possible to scan multiple wavelength spectra, the range depending on the monochromatic used. This enables the investigator to determine exactly which wavelength is being absorbed when scanning a sample for nutrient parameters. Reflectance emission, from a sample after illumination with infrared wave lengths from a monochromatic source, is detected by a photocell or a lead-sulfide detector. The signal can be amplified in different ways, the two most popular being, (1) amplification with a logrithmic response amplifier, digitized and fed to a digital computer, or (2) channel signal output through a filter synchronizer, and then processed by an analog computer. Once wavelengths are determined, a narrower region from the emission of a radiation source can be employed. Such a rough spectral resolution is generally carried out by means of suitable filters of the required transmittance characteristics. Filters can be equipped with the wavelengths necessary to predict the desired constituents of a sample without scanning a wide spectrum then amplified and digitized in logarithmic response. Fixed wavelength instruments are amenable for marketing in that they provide excellent precision if the proper wavelengths for estimating a specific property are known. However, such instruments do not provide the flexibility of those that are capable of scanning the wavelengths in the infrared region.

The latter type is certainly best for research uses (Barnes and Marten, 1979).

Adaptation of Near-Infrared to Grain Analysis

Although the near-infrared (NIR) reflectance technique is capable of rapid evaluation of various components within a biological sample, the instrument does not directly measure chemical components. It must be calibrated against standards determined through wet chemistry procedures. A stepwise multiple linear regression program is used to analyze the data and determine optimum wavelengths for predicting the chemical components and animal responses, conducted between the known (y) and the infrared predicted values (x) within each of the parameters desired. Researchers have applied this technique of transmission spectroscopy to study NIR absorption of various proteins (Ellis and Bath, 1938; Bath and Ellis, 1941; Sutherland et al., 1954; Fraser, 1956; Hermans and Scheraga, 1960), carbohydrates (Ellis and Bath, 1938; Mitchell et al., 1957), lipids (Holman and Edmoson, 1956), and bound and free water (Hart et al., 1962; Ben-Gera and Norris, 1968; Bayly et al., 1963), of cereals and oilseeds.

Commercial instruments using the NIR reflectance principle were introduced to the grain trade in 1971 (Ben-Gera and Norris, 1968; Williams, 1975). Research subsequently demonstrated that the percent protein, moisture, and oil content of various cereals and oil seed could be estimated with a precision comparable to standard laboratory analysis. Several papers have been presented on the advantages and accuracy of the instruments (Murakami, 1973; Trevis, 1974; Williams, 1975), particularly Hymowitz et al. (1974). In an intensive study, estimates of protein in corn, soybean, and oat seed meals, made by an NIR light reflectance instrument coupled to an analog computer, were compared to protein determinations by chemical laboratory Kjeldahl methods. Multiple correlations between Kjeldahl proteins and NIR analysis were .994, .996, and .982 for corn, soybeans, and oats, respectively. A study conducted by Law and Tkachuk (1977) gives the fundamental background of the technique in assigning absorption waves for wheat and its components. Near-infrared diffuse reflectance spectra between 1.0 and 2.5 were recorded for wheat, protein, starch, pentosans, lipids, and water. Spectral absorption waves were assigned to various overtone and combination vibrations of C-H, N-H, O-H, and C-O bonds. The spectrum for wheat was determined largely by the carbohydrate components. Major peaks in the gluten spectrum occurred at 1.19, 1.50, 1.73, 1.98, 2.18, 2.29, and 2.47. Absorption peaks at 1.98 are characteristic of gluten and are due to high concentration of primary amide groups in gluten. Spectra for starch and pentosans were similar, with major peaks at 1.20, 1.45, 1.54, 1.93, 2.09, 2.32, and 2.49. The position of peaks associated with hydroxyl groups was determined, in part, by the degree of hydrogen bonding. Lipid spectrum was characterized by intense absorption due to CH_2 groups. Waves at 1.17, 2.14, and 2.17 were due to the C-H vibrations associated with C's double bonds, while absorption at 1.41 and 2.07 was due to OH groups. Liquid water had absorption peaks at 1.445 and 1.928, while water in undried wheat, gluten, starch, and pentosan samples exhibited absorption at slightly longer wavelengths, probably due to hydrogen bonding.

Speed of analysis is the primary advantage of NIR. A finely ground sample of grain or forage can be analyzed for multiple nutrients in less than two minutes. No special handling of the sample other than grinding is required. The sample does not need to be weighed or corrected for dry matter. One of the major uses of NIR is in wheat marketing. As of the 1978 wheat harvest, the Federal Grain Inspection Service of the U.S. Department of Agriculture has accepted the infrared method of measurement of protein and the method is being employed at all wheat export stations around the U.S.

Adaptation of NIR to Forage Analyses

Less progress has been made, however, in the application of this NIR principle to estimating quality parameters of forage crops and mixed feeds. The potential application of the technology to forages was first reported by Norris et al. (1976). In this study, the NIR reflectance spectra (1.4 to 2.4 mm) was recorded for 87 samples of ground dry forages. Temperate forage species analyzed were alfalfa, tall fescue, and alfalfa bromegrass mixtures preserved as hay, silage, and fresh frozen forages. Eleven samples were prepared by mixing various amounts of alfalfa and smooth bromegrass forage to represent a range of legume: grass ratios and chemical constituents. Laboratory analysis of CP, ADF, NDF, L, IVDMD, as well as in vivo digestibility (IVVD), dry matter digestibility (DMD), dry matter intake (DMI), and digestible energy intake (DEI) were determined for the samples. Reflectance (R) spectra were recorded with a multipurpose computerized spectrophotometer with a monochromator operated in a single-beam mode. Samples were packed into a sample holder which holds the sample between a clear glass window and a pressure pad to maintain good contact between the granular sample and the window. Samples were illuminated with monochromatic light through the window and the radiation was collected by four lead-sulfide detectors equally spaced around the incident beam. Signals from the detectors were amplified by a logarithmic-response amplifier, digitized and fed to a digital computer.

Powdered teflon was used as a reference standard. Reflected signals from the teflon were stored in the computer and used to correct the curves recorded with the forage samples. This gives a resultant curve of true reflectance relative to the teflon standard. Spectral reflectance curves were recorded using the second derivative of the log (1/R) reflectance curve rather than log (1/R), because preliminary calculations indicated that performance could be improved by using the second derivative. This showed a much greater difference than log (1/R) between samples.

Data processing for the 2,000-point spectral curves was smoothed by a computer program which averaged adjacent points and compressed the curves to 500 points. A stepwise multiple-linear regression program was used to analyze the data and determine the optimum wavelengths for predicting the chemical components and animal responses. Multiple linear regression analysis of second derivative reflectance data to predict crude protein resulted in a correlation coefficient of .98, indicating that the second derivative values are related to Kjeldahl protein with a high degree of linearlity. Results for NDF, ADF, lignin, IVDMD, IVVD, DMI, and DEI are summarized in Table 4. Correlations were highly linear for each chemical component even though the samples

Table 4. Summary of Results with Log 1/R Reflectance Data to Predict Forage Components.

Components	CP	NDF	ADF	L	IVDMD	DMD	INTAKE
N	87	87	87	87	75	75	75
R	.98	.92	.90	.72	.90	.81	.79
SE	1.07	5.3	2.5	2.1	3.5	4.4	7.8
a	5.98	12.80	5.60	2.88	7.75	6.90	13.26

CP - Crude Protein

NDF - Neutral detergent fiber

ADF - Acid detergent fiber

L - Lignin

IVDMD - InVitro dry matter digestibility

DMD - Dry matter digestibility

N - Number of samples

R - Correlation

SE - Standard error

a - Standard deviation

Source: Norris et al. 1967.

included the variables of hay, silage, and fresh-cut grass combined with alfalfa and four different grasses. Errors in predicting animal responses were greater than those in predicting chemical composition. The authors attribute this to be the result of errors in the animal response data, and much of the error appeared to be in only a few samples. An elimination of these few samples improved the correlation from .78 to .87. By use of the six wavelengths normally used in the instrument manufactured for grain analysis (1.680, 1.940, 2.100, 2.180, 2.230, and 2.130 m), an accurate prediction of CP and NDF of forages could be determined but not for other components. Analyses were then made to choose six new wavelengths which would give the best prediction of all components (Table 5).

Researchers in the area of forage spectroscopy agree that the chemical composition of forages which determines its nutritional values may be less uniform over geographic areas, years, and species than cereal grains (Shenk et al., 1979; Shenk et al., 1981; Templeton et al., 1981; Fales and Cummins, 1982). Therefore, recalibration of the instrument or new equations are expected to be required for each situation. Shenk et al. (1979) states that the reason for this is that forage is a complex material both chemically and physically. A forage sample usually will contain a mixture of plant parts (leaves, stems, sheaths) and differenct species. Each of these plant parts and plant species will have different chemical, physical properties and thereby different IR spectra. Proteins in a sample of alfalfa are not all alike. The qualitative differences are due to differences in amino acid composition. Each amino acid has a specific NIR spectrum