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STORAGE QUALITY MEASUREMENTS OF TWO LARGE HAY PACKAGES

BY

RONALD EUGENE SCHREMP

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A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Animal Science, South Dakota State
University

1974

STORAGE QUALITY MEASUREMENTS OF TWO LARGE HAY PACKAGES

The subject of this thesis is the storage quality of two large hay packages. The purpose of this investigation was to determine the effect of storage time on the nutritive value of two types of hay packages. The results of this investigation are presented in the following chapters.

Chapter I. Introduction

Chapter II. Literature Review

Chapter III. Materials and Methods

Chapter IV. Results and Discussion

Chapter V. Conclusions

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Date

~~Head, Animal Science Department~~

Date

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RES

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INTRODUCTION

Forage evaluation for utilization by ruminants can be made by several methods. The conventional in vivo digestibility trials, in vitro dry matter digestibility (IVDMD) methods and a number of chemical component analyses have been tested quite extensively in the last 25 years. The in vivo digestibility trial has been the traditional method for feed evaluation, but performance of such a trial is costly and tedious and the resulting total digestible nutrients (TDN) or digestible energy values have been criticized as being an incomplete measure of feed value.

There are many laboratory feed analyses methods available today. However, in vitro digestibility data have been shown to correlate best with in vivo digestibility data with some restrictions, the most important being inconsistent results when attempting to predict intake. Some of the chemical component analyses, on the other hand, may indicate intake to some degree but do not predict digestibility as well as in vitro digestion analyses.

It has been suggested that other laboratory chemical analyses of forages such as acid-detergent fiber (ADF), Crampton and Maynard cellulose (CNC), acid-detergent lignin (ADL), crude protein, neutral detergent fiber (NDF) or ash, alone or in combination with in vitro digestibility data, may indicate forage quality as efficiently as in vivo trials. This presents the possibility of obtaining more intimate relationships between these chemical component analyses and in vivo digestibility than has so far been achieved.

Investigations were conducted with these thoughts in mind to determine the following specific objectives:

1. To determine the effects of field storage on forage quality analyses parameters with two different large hay packaging systems.

2. To determine selected chemical component analyses or combinations of these analyses which may be used with IVDMD in order to obtain a better estimate of in vivo digestibility than in vitro data alone.

3. To incorporate the chemical component analyses which best correlate with in vitro digestibility into a regression equation predicting IVDMD which in turn estimates in vivo digestibility data.

REVIEW OF LITERATURE

Progressive Trends in Forage Evaluation

Proximate Analysis 1864-1967. Extraction of forages with alcohol, dilute acid and alkali, led early chemists to believe a fraction containing fibrous materials represented the indigestible portion of a feed and they used this fraction to predict nutritive value (Hansen, Forbes and Carlson, 1958). Van Soest (1967) found later that in some cases this fiber was more digestible than the nitrogen-free extract (NFE) and that the indigestible portion of the NFE was lignin. Research pertaining to newer and improved systems of forage evaluation has become common because of the shortcomings of the Weende system of proximate analysis. One of the more serious shortcomings being the determination of NFE by difference which included lignin in this fraction instead of in the crude fiber fraction. Low digestibility of fiber resulted partially from extraction of indigestible lignin (Phillips, 1940) and partially from digestible hemicellulose in fiber determination (Ely et al., 1953). Norman (1935) was one of the first to discount the use of crude fiber as an accurate method of predicting forage quality, and Crampton and Maynard (1938) reported that using crude fiber as a basis for estimating nutritive value was inaccurate because of the variable nature of this fraction. Lignin seemed to be the likely replacement for crude fiber because of its indigestibility (Van Soest, 1963a), but its complex chemical makeup presented problems in accurate determination (Ellis, Matrone and Maynard, 1946). It became evident that newer methods must be found in which nutritive value could be estimated.

In Vitro Fermentation 1951-1971. Pigden and Bell (1955) and Baumgardt and Hill (1956) suggested using artificial rumen techniques to evaluate the nutritive value of forages. Baumgardt and Hill (1956) showed that dry matter losses of forages in vitro could be indicated with the artificial rumen and the nylon bag techniques. A technique described by Fina, Teresa and Bartley (1958) and modified by Fina et al. (1962) utilized an in vivo artificial rumen (VIVAR) apparatus made of a stainless steel cylinder or a glass jar suspended in the rumen of a fistulated animal. This procedure equaled the one-stage in vitro fermentation method for digestion of prairie hay or alfalfa and volatile fatty acid (VFA) production methods. This system was still inferior to the two-stage technique of Tilley and Terry (1963) because of the lack of pepsin digestion. Meyer et al. (1971) showed that with a pepsin phase, VIVAR digestion was still inferior to the two-stage technique.

The amount of gas produced during the fermentation of a feedstuff may also be an indication of the nutritive value of that feed (Barnes, 1965; Gray, Pilgrim and Weller, 1951). Reid et al. (1960a) reported that the use of gas production was beneficial in observing the rate of in vitro fermentation. However, Johnson (1963) indicated that inaccurate conclusions using forages were possible with this method because fermentation gas was not specific and was a product of a variety of substrates. A close correlation was found by Asplund et al. (1958) between total VFA production in vitro and in vivo digestible dry matter. This was unreliable in measuring digestibility because types of VFA's

produced from in vitro digestion instead of total VFA production must be considered. Studies by Packett et al. (1965) demonstrated that VFA production from in vitro fermentation of different forage fractions was similar, but the ratio of these VFA's fluctuated considerably. Further investigation in this area is required before any evaluation of the procedure can be made.

Digestibility Studies by Solvent Solubility 1960-1974. Dehority and Johnson (1961b) initiated the use of solubility methods. They reported that the percent of forage cellulose dissolved was linear and highly correlated to the percent of cellulose digested in the in vitro rumen fermentation. Dehority and Johnson (1963) estimated the digestibility and nutritive value of grasses by solubilizing the hay cellulose in cupriethylene diamine (CED), but inaccurate estimates occurred when using it with alfalfa. Dehority and Johnson (1964) modified this procedure to estimate digestibility as well as relative intake by introducing the dry matter solubility (DMS) method. Using different combinations of CED and DMS methods plus the product of CED \times DMS, they found high correlations with dry matter digestibility (DMD), energy digestibility, nutritive value index (NVI) and intake. Johnson and Dehority (1968) found relative intake and NVI more accurately predicted by in vitro cellulose digestibility (IVCD) \times DMS or CED \times DMS. The two-stage in vitro digestion procedure most accurately predicted dry matter and energy digestibility. Johnson et al. (1964) found IVCD values more highly correlated with in vivo measurement for grasses, while CED, DMS or both were better for

alfalfa and mixed forages. Most accuracy was obtained by combining IVCD or CED values with DMS. Oh, Baumgardt and Scholl (1966) studied solubility methods in which digestible dry matter (DDM) data from conventional digestion trials with cattle and sheep were used as a reference for correlation. They found most satisfactory correlations between DMS and the two-stage in vitro digestion.

Selected Chemical Components 1950-1974. Johnson et al. (1964) reported that forage nutritive value could be predicted by chemical analyses or IVCD and DMD. They indicated good correlation between IVCD and in vivo nutritive value, but in the same study they showed that values of in vitro and in vivo cellulose digestibility were high for grasses but much lower for alfalfa and mixed forages. Quickie et al. (1959) and Reid et al. (1960b) also found similar results. Sullivan (1963) discussed other inaccuracies in using chemical analyses to predict nutritive value.

Van Soest (1963a) introduced sodium hydroxide digestion which was intended to remove nitrogenous fractions from lignin. This failed because lignin was lost due to gelatinization and ultimate loss in the filtrate. Quaternary ammonium compounds were then used to dissolve polysaccharides, proteins and nucleic acids. A number of detergents were then used to dissolve forage nitrogen. Cetyltrimethylammonium bromide in normal sulfuric acid (92%) was found to remove most of the forage nitrogen. Van Soest (1963a) found a high correlation ($r = 0.86$) between nitrogen disappearance and forage dry matter disappearance, but the solubility of protein was found to be

inhibited by detergent under some conditions. Final results included the use of sodium lauryl sulfate in neutral or slightly alkaline solutions (yielding cell-wall constituents) and cetyltrimethylammonium bromide in strongly acid solution. These procedures gave consistent results in obtaining plant fiber of low nitrogen content. This provided a rapid method for the determination of ligno-cellulose in feedstuffs and included silica, which was shown to be closely related to digestibility. Hemicellulose was also determined from the difference between cell walls and ADF. However, some protein attached to the cell walls was included (Van Soest, 1965c). It was obvious that a major problem in chemical fractionation of plant tissues was the separation of protein from lignin (Sullivan, 1959; Thacker, 1954).

It has been known for some time that lignin affects digestibility and pinpointing the reason has initiated some consideration but little experimental evidence. Encrustation of nutritionally significant materials, formation of lignin-carbohydrate compounds of plant cell wall material or the presence of molecular complexes due to hydrogen bonding or other molecular forces have all been studied (Dehority, Johnson and Conrad, 1962).

Scales et al. (1974) and Deinum and Van Soest (1969) agreed that using lignin in summative equations to calculate cell wall digestibility was not adequate because of the nonlinearity of the lignin and cell-wall constituent (CWC) fractions and that a future predictor of in vivo digestibility which looks promising may be the fecal nitrogen method.

Reid et al. (1950) introduced a plant chromogen pigment of chlorophyll and its degradation products to study digestibility and consumption of pasture forage. This material, which is easily extracted in an aqueous acetone mixture, can be used to measure the indigestibility of pasture forage in cattle and sheep. Lignin could be used as an indicator if in fact, it is indigestible. Lignin is digestible to some extent, depending upon the forage, so a correction factor for digested lignin may be valuable. Cook and Harris (1951) used lignin as an indigestible indicator for range studies in Utah and showed the lignin technique to give less variability than the chromogen method. Wallace and Van Dyne (1970) found, however, that inconsistencies existed with adjusted lignin ratios and suggested the fecal nitrogen method might give a better estimate of digestibility values in conventional digestibility trials. McCullough (1959) discussed other factors which cause variable results when using lignin as an indicator.

Generally it has been shown that many techniques, with certain restrictions, are highly correlated to DMD (Baumgardt, Cason and Taylor, 1962a; Baumgardt and Oh, 1964; Hershberger et al., 1959; Reid, Jung and Murray, 1964). Meyer et al. (1971) compared three systems of forage evaluation which seemed important in future forage quality studies. These methods were (1) the two-stage dry matter disappearance method of Tilley and Terry (1963), (2) the digested NDF method of Van Soest and Wine (1967) and (3) the one-stage in vitro fermentation method similar to that described by Baumgardt, Taylor and

Cason (1962b). The weight assigned to each of these methods to accurately solve the many problems which still exist in forage evaluation needs to be reviewed and analyzed.

In Vitro Cellulose Digestibility. Cellulose is the primary source of energy for ruminants (Reid et al., 1959a). It is utilized by the ruminant through symbiotic microbial fermentation (Barnes, 1965). In 1938, Crampton and Maynard modified the old methods of cellulose digestion by Cross and Bevan (1911) and Norman and Jenkins (1933) by using nitric and acetic acids to dissolve non-cellulose components of forage samples. This method favored the chlorite-sulfite method of Cross and Bevan because it contained less of the celluloses. It seemed logical to include an in vitro cellulose technique as a criterion for forage evaluation studies; therefore, many have been established (Bryant and Burkey, 1953; Hungate, 1947). Most inoculum or buffer-medium solutions for in vitro fermentation were made according to McDougall (1949) based on the analysis of sheep saliva. Burroughs et al. (1950b,c, 1951), Bentley et al. (1951) and Hall, Baxter and Hobbs (1961) used this solution with the modification of adding trace minerals to increase digestion. Donefer, Crampton and Lloyd (1960) eliminated the need of adjusting for pH by increasing the buffering capacity of the solution. Bentley et al. (1954) used centrifugation and unwashed bacterial cells, and Wasserman et al. (1952) and Huthanen and Elliot (1956) used whole rumen fluid.

IVCD showed promise as a means of evaluating nutritive quality of forages and as a result, comparisons between in vitro and in vivo

cellulose digestion also became numerous. A survey of the various reports generally demonstrated high correlations between in vitro and in vivo cellulose digestibility (Barnett, 1957; Karn, Johnson and Dehority, 1964; LeFevre and Kamstra, 1960; Bowden and Church, 1962; Clark and Mott, 1960; Quickie and Bentley, 1959).. Positive correlations were also found between IVCD and in vivo digestible dry matter (Reid et al., 1960a).

Many factors have been shown to influence IVCD. Therefore, its use as a single factor in forage evaluation has been misleading. Barnes et al. (1964) compared two modifications of the Crampton and Maynard (1938) procedure, two adaptations of the Tilley, Deriaz and Terry (1960) procedure and combinations of these methods. Results indicated increases in standard deviation as the time of fermentation increased, but because digestibility increased with increased time, the coefficient of variation decreased. Data also indicated that differences in methods were apparent only in the first part of fermentation. LeFevre and Kamstra (1960) found a significant difference between the 24-hour IVCD and in vivo coefficients but found little difference between the 48-hour in vitro and in vivo cellulose digestion. Differences were found between bromegrass hay and alfalfa but not between alfalfa samples. Quickie et al. (1959) found that the digestibility of forage cellulose measured in vitro was related to the nutritive value of the forage. When IVCD was compared with lignin (Tomlin, Johnson and Dehority, 1965), it was found that lignin was

linearly related to IVCD in grasses as they matured but did not relate highly to IVCD in alfalfa.

Barnes (1967) reported considerable variability in IVCD between laboratories due to variability in technique, and Johnson (1966) especially warned that a great source of error existed in inoculum sources and preparation. Van Dyne and Weir (1964) and Scales et al. (1974) noted higher variability of in vitro digestion in grazing donor animals than stall-fed donor animals. LeFevre and Kamstra (1958) and Van Dyne (1962) observed that feeding the inocula donor animal similar feed as that being evaluated maintained a higher correlation. Scales et al. (1974) reported that sheep inocula did not give as consistent results as inocula from cattle.

El-Shazly, Dehority and Johnson (1961) showed inhibition of cellulose digestion via the nylon bag technique in vivo by feeding higher quantities of concentrates, but nitrogen supplementation in the form of urea caused cellulose digestion to appear similar to the all-forage ration. Very fine grinding increased IVCD but decreased in vivo cellulose digestibility (Minson, 1963). Since grinding is a physical treatment, it would appear that a physical barrier between cellulose, hemicellulose, pectin and rumen bacteria is present. Grinding may serve to degrade the macromolecular structure or remove encrusting lignin. This "barrier" becomes more obvious as the plant matures.

Rumen microorganisms produce enzymes which are responsible for cellulose digestion in forages. These microorganisms are dependent

on certain nutrients to fulfill this function. Some nutrients which proved effective in increasing IVCD were a complex salt solution, the ash of alfalfa extract, autoclaved rumen liquid and an autoclaved water extract of manure (Burroughs et al., 1950a). Roughage digestion in cattle (Burroughs et al., 1950b) and in sheep (Swift, Cowan and Barron, 1950) can be increased by mineral additions to the feed. These researchers named available energy, available nitrogen and minerals as essentials in cellulose digestion. MacLead and Murray (1956) showed that certain amino acids, short chain fatty acids and certain vitamins stimulated cellulose digestion. McNaught, Owen and Smith (1950) have shown that some metals stimulate utilization of nonprotein nitrogen in vitro, and Arias et al. (1951) increased urea utilization in vitro by supplementation of energy and protein sources.

Packett et al. (1965) reported that hemicellulose, soluble proteins, soluble carbohydrates and other soluble forage components were used first by the rumen population and that a high ratio of these more soluble materials reduced cellulose digestion. In this case, if IVCD is the sole predictor of nutritive value, a higher quality forage which is more highly solubilized may indicate a lower nutritive value. These limits of accuracy must be taken into consideration along with the fact that in vitro digestion trials can evaluate only the potential, and not the realizable value of a feed. Specific indicators such as in vitro fermentation may result in inaccurate results. Consequently, several chemical analysis measures may be functional in forage evaluation.

In Vitro Dry Matter Digestibility. IVCD appears to measure forage quality quite well, but information on the digestibility of total plant constituents is not available with this method. Good correlations between IVDMD and in vivo dry matter digestibility were found by Tilley et al. (1960) in forages low in protein content and digestibility. Forages high in protein content and digestibility had IVDMD values 10% lower than in vivo values. Tilley and Terry (1963) improved on this shortcoming by using a proteolytic enzyme called pepsin to simulate protein digestion further down the digestive tract. This increased the digestibility of feeds high in protein and digestibility. Higher correlations with pepsin were found than with rumen fluid inoculum alone. The pepsin stage reduced the standard error and also altered the slope of the regression line. The increased digestibility figures obtained by using pepsin resembled those attained in vivo, and as a result, continuation of cellulose analysis of the sample and residue was no longer needed. More recent methods for evaluating forage digestibility now available include in vitro systems (Van Soest, Wine and Moore, 1966; Mellenberger et al., 1970), nylon bag techniques (Tomlin, Anderson and Harris, 1967), summation equations (Van Soest, 1967), lignin ratio systems (Wallace and Van Dyne, 1970) and fecal nitrogen indices (Jeffery, 1971).

Barnes (1965) reported that in vitro digestibilities may be highly correlated to in vivo digestibility; but because there was more deviation in in vivo results, the possibility of more variability existed between these two methods. Since differences in

digestibilities of different forages is considerable, the in vitro rumen fermentation procedure seems primarily important in determining digestibility or even intake. Baumgardt and Oh (1965) showed that IVDMD of all forage species best indicated nutritive value.

In vitro fermentation as a lab technique depends upon the accuracy of in vivo comparisons. Therefore, Barnes (1968) made a collaborative study to test and develop a reliable in vivo method to estimate digestibility and voluntary intake, to determine variability within and among stations associated with these studies and to obtain samples with precise in vivo measurements for use in the development of laboratory methods to be used in estimating forage quality. The study indicated that variabilities in sampling procedures, in analyses for chemical constituents and in preparation of forages for chemical analysis were responsible for inconsistent results in sheep experiments. Variability in in vivo measurements among stations resulted because of differences in species used in the trial, the age and health of the animals, the level of feed intake and the manner in which feed was prepared.

Kamstra, Ross and Ronning (1973) found a high correlation ($r = 0.95$) between in vivo and IVDMD of bromegrass. Asplund et al. (1958) found a pooled correlation of 0.71 between in vivo and IVDMD with hays of quality and origin variations, and Reid et al. (1959b) found in vivo DMD most accurately predicted from IVDMD of oven dried samples ($r = 0.98$), although the oven and freeze dried forages had

high in vitro digestibilities. Clark and Mott (1960) found discrepancies between in vitro trials made in the spring and fall with the same forage. They indicated that storing the forage promoted changes within the forage which decreased microbial activity. Bowden and Church (1962) showed differences in in vitro digestibility of forages due to time deviation in harvesting. Several studies on the effects of maturity on digestibility have been made (Phillips et al., 1939; Patton and Gieseke, 1942; Sullivan, 1955; Kamstra, 1955; Kamstra, Moxon and Bentley, 1958; Pritchard, Folkins and Pigden, 1963; Mowat, Kwain and Winch, 1969). The decreased digestibility due to maturity seemed to be related to increased lignin content. Pigden (1953) observed that lignification was the most important single factor contributing to the curing property of forages and that the distribution and extent of lignification may be more important than the quantity of lignin present in determining nutritive value of a plant.

Heinrichs, Troelsen and Warder (1969) found no significant difference between IVDMD of leaves or stems in alfalfa between species. Hosterman and Hall (1938) used timothy at two stages of maturity and found a steady increase in crude fiber with advancing maturity, just as Phillips et al. (1954) found in eight different grasses and Weir, Jones and Meyer (1960) found in alfalfa. Lloyd et al. (1961), Kivimae (1960) and Kamstra et al. (1958) agreed that individual chemical component analyses were highly correlated to nutritive value in forages of the same species, but the relationship may not be valid

when comparing chemical component analyses of forages containing two or more species. These differences between species of forages do not apply to in vivo trials (Crampton, Donefer and Lloyd, 1960) or in vitro studies (Donefer et al., 1960) because they tend to measure the total digestible energy potential of the forage and do not differentiate between species.

Studies indicating close agreement between IVDMD and in vivo digestibility are numerous (O'Shea and Wilson, 1965; Wilkins, 1966). The most recent comparison of in vivo digestibility trials and indirect in vitro digestibility estimates was made by Scales et al. (1974). They described the relationship between in vivo digestibility (Y) and the two-stage in vitro (X) method of Tilley and Terry (1963) as being $Y = 10.2 + .79X + 2.4$ ($r = 0.93$). Prediction of in vivo energy digestibility was also accurately predicted by a modified two-stage method. The nylon bag technique, the lignin ratio method and lignin determined by the permanganate method did not accurately predict in vivo digestibility. The sulfuric acid lignin procedure, however, gave accurate estimates of in vivo digestibility ($r = 0.94$).

Several factors have been found to alter IVDMD results. Tilley and Terry (1963) and Barnes et al. (1964) maintained that anaerobic conditions must persist throughout the first phase of in vitro digestion. Maintaining the pH between 6.7 and 7.0 and the temperature at 39 C was also necessary (Johnson, 1966). Noller et al. (1966) found that drying extensively at 100 C reduced digestibility, and that the degree of grinding and the moisture content at the time of grinding

gave variable results. Finer ground forages produced higher digestibilities, especially in grasses (Dehority and Johnson, 1961a). Baumgardt and Hill (1956) found dry matter losses of 65% for ladino clover and 58% for alfalfa due to such factors as length of incubation period, buffer nutrient solution used, ratio of substrate to inoculum, collection and preparation of inocula, type of roughage and losses when water was used as the inoculum. Baumgardt and Oh (1964) found no difference when using a flask or beaker as the fermentation vessel. Generally, the factors which affect IVDMD also affect IVCD and consistency in procedures for both methods is imperative if an accurate in vitro system is to be maintained.

Joshi (1972) formulated regression equations between in vivo and in vitro DMD of 32 forages and interrelationships between these two and the contents of crude fiber, ADF and ADL. He found the best correlation between IVDMD and in vivo digestibility. ADL gave highest correlations of the chemical component methods. ADF and ADL values compared similarly with in vivo digestion values but produced higher correlations when compared to IVDMD. Correlations between the chemical component analyses were significant and ADF was no better than crude fiber in measuring IVDMD of different types of forages.

Chemical Component Analysis to Estimate In Vivo Digestibility.

The nutritional quality of a forage generally increases as the crude protein content of that forage increases (Sullivan, 1963). Kjeldahl protein is thus indicative, to a certain degree, of forage nutritive value. The ruminant is not dependent on forages for its particular

amino acid requirements. Therefore, chemical methods for measuring animal digestible protein are not available, but forage protein seems consistent in availability for the animal. Thus, crude protein may have application to total nutritive value. Holter and Reid (1959) reported that protein digestibility increased exponentially with the amount of crude protein in a feedstuff, but percent digestible protein increased rectilinearly to crude protein. Metabolic nitrogen excretion also correlated well to crude protein content. This suggested the constancy of protein digestibility with crude protein. It was shown by Van Soest and Moore (1965) that the quantity of lignin in a sample does not affect the availability of the cellular protein or carbohydrates. Up to one-third of the total nitrogen may be nonprotein, with 5 to 10% of the total nitrogen bound to lignin in the cell wall (Van Soest, 1965c).

The use of proteolytic enzymes and delignifying agents resulted in increased solubility of hemicellulose (Gaillard, 1962). Therefore, hemicellulose may be divided into one fraction less lignified and associated with the protein fraction and more digestible and one fraction associated with lignin and less digestible. Hemicellulose hydrolysis with separation by chromatography (Burdick and Sullivan, 1963) or cellulose digestibility via solubility methods both indicated similar results, but these results were not adequate for estimating in vivo digestion of legume grass mixtures (Dehority and Johnson, 1963). Digestibility is related to cell contents and lignin in ADF which controls digestibility of the cell-wall constituents (Van Soest, 1965a).

Spectroscopic investigation by Bolker (1963) revealed covalent bonds between lignin and carbohydrate in wood and sulphite pulps. The point of attachment seemed to be on the hemicellulose fraction of the holocellulose. The chemical nature of lignin varies among plant groups (Towers and Gibbs, 1953; Bondi and Meyer, 1943), and protein contained in plants may affect lignin determination (Hungate, 1966). Changes in lignin composition may vary as it passes down the digestive tract (Bondi and Meyer, 1943), after it leaves the rumen or it may be altered by rumen microbial fermentation (Hale, Duncan and Huffman, 1940). During mastication, the structure of lignin may also change (Connor et al. 1963). Lignin does not seem to be a natural carbohydrate but a high-molecular weight condensation product of aromatic compounds found in the cell wall and intermixed there with cellulose and other constituents (Hansen et al., 1958).

Richards, Weaver and Connolly (1958) reported that relationships between lignin and forage digestibility varied considerably within species, and Allison and Osbourn (1970) reported that even if forages contained equal lignin values, the amounts of digestible and indigestible components differed considerably. Quickie and Bentley (1959) observed that changes in lignin content of mature forages were too similar to account for observed differences in cellulose digestibility. The lignified cell walls in forages are of inconsistent availability, therefore, the relation between lignin and quality may also vary.

Sullivan (1959, 1964) stressed a limitation to using lignin as the only indicator of forage dry matter digestibility. He observed that since the cell contents were lignified and since he assumed that lignin influenced the availability of dry matter, a limitation existed in using lignin as the sole predictor. Alfalfa has a highly lignified, but low cell wall content and grasses have a higher cell wall content and consequently higher dry matter (Van Soest, 1964). Therefore, a smaller amount of lignin in grasses has more of an effect in decreasing overall DMD than the same amount in alfalfa. This further illustrates the need for more than one analysis or measure to estimate nonnutritive sources in feedstuffs. Another important observation is that it is unwise to use the size of correlation to compare various forage evaluation procedures or relationships in forage chemical components (Van Soest, 1967).

The ADF residue described by Van Soest (1963b) consisted of cellulose, lignin, cutin and acid insoluble ash. A modification of this procedure by Sullivan (1959) using sulfuric acid (72%) treatment dissolved the cellulose. Ashing the residue then determined crude lignin plus cutin. Silica, cutin and lignin were then separated by the permanganate and acid-detergent cutin methods. One disadvantage to permanganate lignin was that large particles were poorly penetrated by the reagents and low values resulted (Goering and Van Soest, 1970). Nonenzymic browning due to drying and heating during the preparation of laboratory samples, catalyzed by moisture, also made lignin analysis more difficult especially with immature, high protein grasses

(Hodge, 1953; Van Soest, 1962; MacDougall and Delong, 1942). Drying for 16 hours at 20 to 100 C increased the lignin content from 5.5 to 14.4% and increased ADF content from 31.8 to 41.4% at a constant initial moisture. Drying at 50 C had no effect on lignin or ADF (Van Soest, 1964).

Hintz, Hogue and Loosli (1962) reported that nonenzymic browning produced a polymeric material which was essentially indigestible by ruminants, and Simkins and Baumgardt (1963) determined correction factors for feeds which contained overheating residues so that an estimate in nutritive value could be made. Van Soest (1965c) also expressed a correction for the drying effect on ADF and lignin values based on the nitrogen content of ADF. This was an advantage when samples were moved between laboratories and it was also easier to analyze dry samples.

After certain inadequacies of the ADF and ADL methods became established, a new procedure which estimated total cell-wall constituents or fiber in feedstuffs was found. NDF associates that part of the feedstuff which is nutritively unavailable to nonruminants and insoluble in neutral detergent solution. This method can be used only for feeds with low protein and fiber contents. With the NDF method, it is assumed that fiber is insoluble vegetable matter, indigestible by proteolytic enzymes and not utilized by the ruminant except through fermentation. (Van Soest and Wine, 1967). In ruminants, however, increased fiber content was highly related to inefficiency of utilization and the indigestible portion of feces from ruminants contained largely

cell-wall constituents comprised of hemicellulose, cellulose, lignin and other insoluble residues (Van Soest and Moore, 1965). Studies have shown that NDF and enzyme digestion residues are similar (Van Soest and Wine, 1967). Work by Van Soest (1963a) showed that the noncell wall fraction was essentially completely digestible.

It was found that individual chemical components such as crude protein (Forbes, 1950), crude fiber (Minson and Kemp, 1961) or lignin (Kivimae, 1960) cannot be applied equally to all species of forage plants, even though some correlation was found between such components and in vivo forage digestibility. Kivimae (1960) used crude fiber, lignin, protein, cellulose and hemicellulose to estimate in vivo digestibility and found lignin to give the best estimate. The ADF method described by Van Soest (1963b) was more closely related to DDM and organic matter than crude fiber. McLeod and Minson (1972) and Clancy and Wilson (1966) found that the ADF method gave variable results when it was used to predict digestibility but increasing hydrolysis time and acid strength decreased variability of prediction.

The prediction of voluntary intake (VI) presents a diverse problem, especially with feeds of similar digestibility. Results by Van Soest (1962) showed that in alfalfa, timothy and tall fescue VI and DMD were unrelated, but certain chemical component digestibility measures such as ADL and ADF were shown to be positively related to VI. In live animals, the methods most consistently used to indirectly measure intake were the fecal nitrogen content or total fecal nitrogen methods (Arnold and Dudzinski, 1967). Van Soest (1964) found

differences in VI of different species of forages. This difference was attributed to the proportion of cell wall material in the digested fraction. Legumes have lower amounts of cell wall material than grasses and thus are expected to have increased movement through the tract and consequently higher intake than with grasses. Allinson and Osbourn (1970) found intake between species more closely related to cellulose digestibility ($r = 0.85$) than DMD ($r = 0.79$).

Chemical component composition of forages may indicate quite well their digestibilities. However, it is evident that VI cannot be accurately estimated by chemical component composition alone. When correlations between VI and lignin, ADF, protein, cellulose, NDF and digestibility were found in a grass and a legume, the forage component which seemed to correlate best with VI was NDF (Van Soest, 1965b; Prigge and Apgar, 1973).

In conclusion, it was found that intake of a forage can be correlated to its digestibility, but the correlation between intake and digestibility is variable between forages and especially between grasses and legumes (Blaxter, Wainman and Wilson, 1961; Blaxter, Wainman and Davidson, 1966). It was concluded in a recent study by Heaney (1970) that the factors of intake and digestibility cannot be used individually to make comparisons between species of forages or to evaluate mixtures of forages and that, to make these comparisons, a combination of both of these factors was essential.

Nutritive Value Index Studies. Nutritive value indices usually involve multiple regressions with the dependent variable (Y) set as the most relative test for forage nutritive value and a number of independent variables (X's) set as components which secondarily estimate the dependent variable. One of the first indices attempted to relate the dependent variable (DDM) to time of cutting in days after April 30 (independent variable) in first growth forage. The equation $Y = 85.0 - 0.48X$ was found to predict DDM (Y) with some accuracy (Crampton et al., 1960). It was found in this study that the indices available at the time such as federal hay grades, legume content, method of curing and feed-value tables did not accurately measure nutritive value or intake. Mellin, Poulton and Anderson (1962) found a linear and highly significant correlation between DDM and date of harvest of timothy. Several other workers have reported that cutting dates of forages were used successfully to estimate nutritive value of forages (Kane and Moore, 1959; Richards et al., 1962; Conrad, et al., 1962).

The in vitro digestibility curve levels off at 18 to 24 hours of fermentation and at this point is more comparable to in vivo digestibility. However, various substrates show various rates of in vitro fermentation (Johnson et al., 1962; Warner, 1956). Maximum level of digestion was reached sooner in legumes than in grasses (Hershberger et al., 1959). Studies such as these led to the use of in vitro digestibility measures to predict forage intake and digestible energy potential (Donefer et al., 1960). NVI studies

began by incorporating intake and digestibility using forage energy availability (Crampton et al., 1960). Twelve hour IVCD was found to correlate well with relative intake and 24-hour IVCD (Donefer, et al., 1960). High variability was associated with intake and therefore, limitations of this system were obvious. The equation $Y = y + b(X - x)$, where $Y = \text{NVI of the forage}$, $X = 12 \text{ hour IVCD}$, $y = \text{average NVI}$ and $x = \text{average of 12 hour IVCD}$, seemed to best define NVI, when the 12 hour IVCD was used. After values obtained from several experiments were substituted, the equation $Y = -7.8 + 1.314X$ seemed to define NVI more consistently. Multiplying the 12 hour IVCD by the 24 hour IVCD also closely related to NVI ($r = 0.89$).

Reid et al. (1959b) found DMD in vivo (Y) to be related to DMD in vitro (X) and expressed the relationship as $Y = 20.5 + 0.778X$ ($r = 0.98$). They also found high correlations between in vivo DMD and IVCD, energy and protein. Baumgardt et al. (1962a) indicated that percent IVCD of a forage could be used to estimate nutritive value of that forage. Repeatable estimates of forage nutritive value were also found when percent cellulose digested was compared to the percentage of TDN, digestible organic matter, DDM and digestible energy.

Jarrige, Thivend and Demarquilly (1970) developed an industrially prepared cellulolytic enzyme mixture containing cellulase enzymes, proteins and starch. This was done because in vitro methods were still somewhat irreproducible. For predictive purposes this method was very reproducible if the length of digestion was fixed at 24 hours. This

method estimated digestibility of forages quite well with a standard error of the mean of ± 0.22 for cellulase residue, ± 0.21 for ADF and ± 0.69 (digestibility unit) for in vitro digestibility.

A new method was then used to predict DMD using quantitative measurements from the ultraviolet light spectra plus chemically determined phenol and quaiacol measures in multiple regression equations (McCampbell and Thomas, 1972). These researchers found low simple correlation coefficients between digestibility and quantitative measurements. A difference was also found between these measurements in grasses and legumes. This difference was attributed to legume lignins containing a higher ratio of phenolic hydroxyl groups to methoxyl groups than grass lignins.

A single linear relationship described by the equation $IVDMD = 120.6 - (0.72 \pm 0.076) CWC$ gave a correlation of $- .80$ between IVDMD and CWC of grasses (Johnson, Guerrero and Pezo, 1973). Regressions of forages containing more than 70% CWC showed a greater decrease in DMD percent for each unit increase in CWC. The sum of cellulose, hemicellulose or silica plus lignin in predicting DMD was not as accurate as total CWC. Prediction of (DMD) using individual forage components was highest for ADF ($r = - .83$) and lowest for silica ($r = .003$). Cellulose ($r = - .82$) and lignin ($r = - .79$) were also highly correlated with percent DMD. When multiple regression using DMD as the dependent variable was calculated, lignin was the only significant contributor to the regression. Other equations found percent CWC and percent lignin to be highly significant to the

regression, but protein did not contribute to the regression. Nitrogen fertilization affects protein content in legumes and grasses, so crude protein is not likely to be a reliable indicator in a prediction equation. The factors which governed the amount of protein in a forage were not the same factors which governed the amount of other components such as lignin. For this reason using protein as a factor in estimating cellular contents was invalid.

Van Soest (1967), Goering and Van Soest (1970) and KayongoMale, Thomas and Ullrey (1972) reported that IVDMD and summative equation estimates were not useful for predicting nutritive value when using different forages. KayongoMale, et al. (1972) concluded, however, that lignin, cellulose and CWC needed to be definitely included in a prediction equation.

It has been shown that the published equations using a single composition factor such as crude protein, crude fiber or lignin as a basis for the prediction of forage digestibility did not furnish as valuable results as was first expected. However, these equations yielded very close to the most precise estimates of digestibility possible thus far through a consideration of proximate analysis. The use of other chemical entities may give better predictability than was possible with a single composition factor. The primary need for composition factors more basic than proximate nutrients has been disclosed, and numerous experimentors have indicated recently that a combination of the individual analyses may be helpful in finding an estimate of forage quality.

METHODS OF PROCEDURE

Location, Management and Harvesting of Experimental Plots

Three stacks in each of three plots (nine stacks total) were used in this experiment. Plots 1, 2 and 3 consisted of 20, 17.2 and 8 hectares, respectively. Plot 2 was located on the Agricultural Experiment Station at Norbeck, South Dakota in Faulk County. Plots 1 and 3 were located on the Morrie Richards Farm, approximately 9.66 km northeast of the experiment station. Precipitation in this area averages about 43.2 cm annually. The temperature during the growing season of 1972 and 1973 averaged 28.1 and 29.1 C, respectively, and rainfall for these periods totaled 35.05 and 17.78 cm, respectively. Differences in moisture occurred primarily in the month of May, during which time 17.04 and 6.07 cm of rainfall was received in 1972 and 1973, respectively. This resulted in decreased yield in the 1973 harvest. The soil texture of this region is silt-loam. None of the plots received fertilizer or weed control in the last 5 years.

Plot 1 was seeded in 1970 with Dakota common alfalfa (Medicago sativa L.) at a rate of 2.25 kg per hectare, in combination with an oats mixture (Avena sativa L.) used as a companion crop. In 1971, plot 2 received 13.5 kg per hectare of certified pure vernal alfalfa (Medicago sativa L.) with Tridicum aestivum L. cv. cris wheat used as a companion crop. Plot 3 received 9 kg per hectare of Dakota common alfalfa in 1972 with cris wheat used as the companion crop.

All companion crops were cut and collected for feed the year prior to harvesting the alfalfa. Consequently, they did not affect

the purity of the alfalfa crop. All alfalfa was cut with a 4.89 m windrower at the first-flower stage of maturity. In order to study moisture effects at stacking, portions of plots 2 and 3 were cut on three consecutive days and stacked on the same day.

Plots 1 and 3 were harvested with a Hesston Stak Hand, Model 30, stack compressor, which chopped the alfalfa in 5 to 15 cm segments and compressed it into a 2.44 by 4.27 by 3.97 m high stack weighing approximately 2.7 metric tons and having a density equal to one-half that of baled hay. Plot 2 was harvested with a Haybuster Model 1800 Stack-Eze into approximately 7.2 metric ton round stacks 5.49 m in diameter and 4.88 m high. A packer drum compressed the relatively unaltered hay at 585 kg hydraulic pressure into a moderately compacted, large hay package.

Windrow samples were taken just prior to stacking to obtain moisture content at the time of stacking. The initial moisture content, weight and number of hectares contained in each stack are presented in table 1. Differences in moisture content were not great enough to show differences in storage effects due to moisture at the time of stacking.

All nine stacks were stored on the ground, in the field and unprotected from climatic conditions on elevated areas in their respective plots. Plot 1 stacks were positioned with a long side of the stack facing north, and plot 3 stacks were positioned with a short end facing north (figure 1).

TABLE 1. INITIAL MOISTURE, HECTARES AND WEIGHT CONTAINED IN EACH OF NINE STACKS FROM THE THREE PLOTS

Stack	Initial moisture %	Hectares	Acres	Wet wt. kg	Wet wt. lb
1	35.8	0.4740	1.185	2947.50	6550
2	31.8	0.4792	1.198	3105.00	6900
3	29.0	0.5088	1.272	2790.00	6200
4	26.6	3.8440	9.610	9745.20	21656
5	22.7	1.9600	4.900	8098.65	17997
6	23.1	1.7600	4.400	6435.45	14301
7	33.1	1.1960	2.990	3881.70	8626
8	33.4	1.0360	2.590	3767.40	8372
9	32.9	0.876	2.190	3778.20	8396

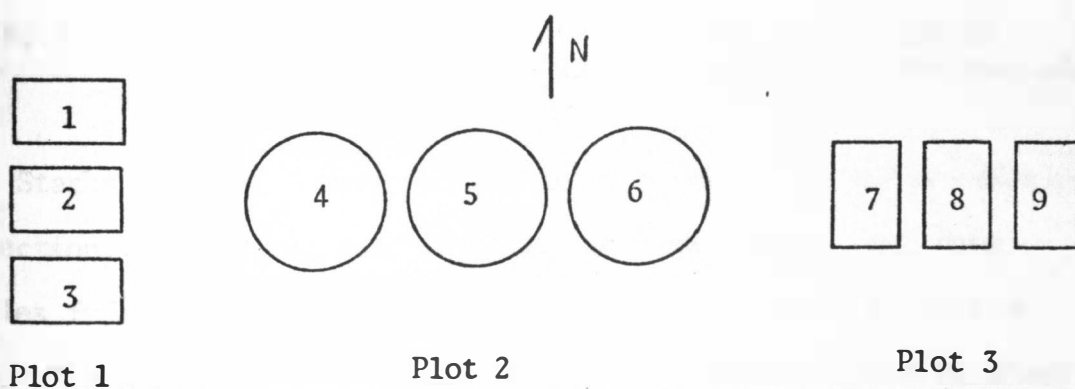


Figure 1. Position of stacks in their respective plots.

Numbers inside figures represent stack designations.

Sample Collection and Preparation

All samples were collected with an electric drill-driven hay core sampler developed by Johnson, Dowding and Turnquist (1973) for use in extracting core samples measuring 5.4 cm in diameter and 147.32 cm long from large hay packages. This sampler collected a representative sample from the exterior toward the center of each stack and parallel to the ground by a cutting action provided by relative motion between an outside tube and an inside tube. Collection of samples from the three plots were taken as shown in table 2.

TABLE 2. SAMPLE COLLECTION SCHEDULE ON PLOTS 1, 2 AND 3

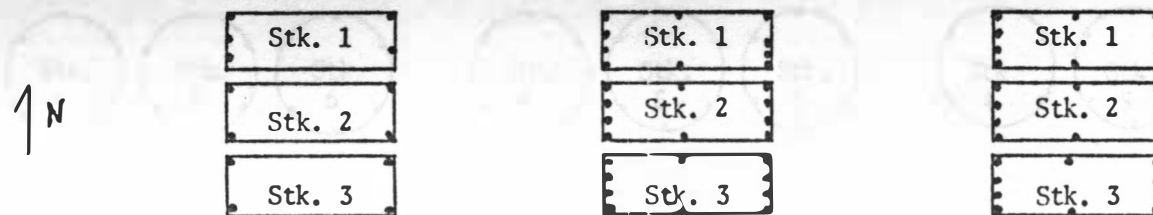
Stacks	Plot No.	Windrows	Collection dates		
1, 2, 3	1	6-15-72	6-15-72	10-10-72	5-2-73
4, 5, 6	2	6-13-73	6-13-73	7-12-73	10-19-73
7, 8, 9	3	6-14-73	6-14-73	7-12-73	10-19-73

Stacks 1, 2 and 3 were set too close together to allow efficient collection. Therefore, only five samples from stack 1, and four samples from stacks 2 and 3 were collected on the first collection date. These samples were taken from the four corners directed toward the center of the stack at a height of 1.22 meters. Due to settling, the stacks separated sufficiently, allowing more adequate collection on October 10, 1972, and May 2, 1973. Ten samples from each stack were taken on each of these dates.

Stacks 4 to 9 allowed total collection, resulting in 12 samples from each stack on each collection date. In stacks 4, 5 and 6 (round stacks), collections were made at 0.61 and 1.22 m levels at every 60 degree interval on the perimeter of each stack starting at due north. In stacks 7, 8 and 9, collections were also made at 0.61 and 1.22 m levels at the corners and in the centers of the long sides. The location and designation of all samples collected from stacks 1 to 9 are shown in figures 2, 3 and 4.

All samples were placed in brown paper sacks, transported to the Plant Science seed house and dried in a forced air, large capacity bin dryer. They were further dried in the Animal Science Laboratory at 80 C for 24 hours in preparation for grinding. All samples were ground through a Wiley mill (intermediate size) containing a coarse screen, followed by grinding through a Model 22 Weber pulverizing mill containing a 40 mesh screen. The samples were stored in tightly closed glass bottles until used for chemical analysis.

Project funds, laboratory facilities and technical assistants were limited in this study. Therefore, a method of compositing was used in order to minimize the number of analyses to be made. Plot 1 was not composited. Each composite consisted of four samples. The four samples from each of stacks 4, 5 and 6 were collected from the two areas closest to the northwest, east and southwest portions of the stack and composited. The four samples from each of stacks 7, 8 and 9 were collected directly across from each other at either end



Collection
Dates:

6-15-72

10-10-72

5-2-73

Each dot represents one collection location at 1.22 m elevation.

Location	Stack 1			Stack 2			Stack 3		
	6-15-72	10-10-72	5-2-73	6-15-72	10-10-72	5-2-72	6-15-72	10-10-72	5-2-73
N.W. Corner	1-72I	1a-72I	31a-72I	11-72I	11a-72I	41a-72I	21-72I	21a-72I	51a-72I
N.Center		2a-72I	32a-72I		12a-72I	42a-72I		22a-72I	52a-72I
N.E. Corner	3-72I	3a-72I	33a-72I	13-72I	13a-72I	43a-72I	23-72I	23a-72I	53a-72I
E. Side	4-72I	4a-72I	34a-72I		14a-72I	44a-72I		24a-72I	54a-72I
E. Center		5a-72I	35a-72I		15a-72I	45a-72I		25a-72I	55a-72I
S.E. Corner		6a-72I	36a-72I	16-72I	16a-72I	46a-72I	26-72I	26a-72I	56a-72I
S. Center		7a-72I	37a-72I		17a-72I	47a-72I		27a-72I	57a-72I
S.W. Corner		8a-72I	38a-72I	18-72I	18a-72I	48a-72I	28-72I	28a-72I	58a-72I
W. Side	9-72I	9a-72I	39a-72I		19a-72I	49a-72I		29a-72I	59a-72I
W. Side	10-72I	10a-72I	40a-72I		20a-72I	50a-72I		30a-72I	60a-72I

Figure 2. Location and designation of samples collected from stacks 1, 2 and 3 in plot 1.



Collection
dates:

6-13-73

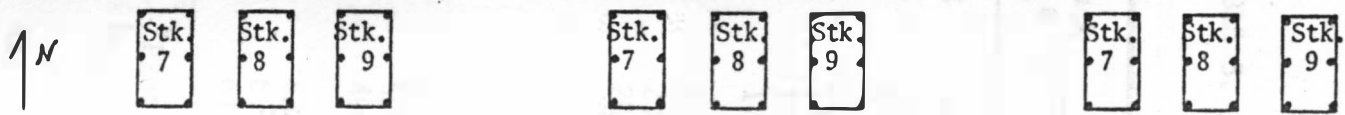
7-12-73

10-19-73

Each dot represents one collection location at 0.61 and 1.22 m elevations.

Location	Ht.	Stack 4			Stack 5			Stack 6		
		6-13-73	7-12-73	10-19-73	6-13-73	7-12-73	10-19-73	6-13-73	7-12-73	10-19-73
S. side	0.6 m	1a-73N	101a-73N	201a-73N	13a-73N	113a-73N	213a-73N	25a-73N	125a-73N	225a-73N
	1.2 m	7a-73N	107a-73N	207a-73N	19a-73N	119a-73N	219a-73N	31a-73N	131a-73N	231a-73N
S.W. side	0.6 m	2a-73N	102a-73N	202a-73N	14a-73N	114a-73N	214a-73N	26a-73N	126a-73N	226a-73N
	1.2 m	8a-73N	108a-73N	208a-73N	20a-73N	120a-73N	220a-73N	32a-73N	132a-73N	232a-73N
N.W. side	0.6 m	3a-73N	103a-73N	203a-73N	15a-73N	115a-73N	215a-73N	27a-73N	127a-73N	227a-73N
	1.2 m	9a-73N	109a-73N	209a-73N	21a-73N	121a-73N	221a-73N	33a-73N	133a-73N	233a-73N
N. side	0.6 m	4a-73N	104a-73N	204a-73N	16a-73N	116a-73N	216a-73N	28a-73N	128a-73N	228a-73N
	1.2 m	10a-73N	110a-73N	210a-73N	22a-73N	122a-73N	222a-73N	34a-73N	134a-73N	234a-73N
N.E. side	0.6 m	5a-73N	105a-73N	205a-73N	17a-73N	117a-73N	217a-73N	29a-73N	129a-73N	229a-73N
	1.2 m	11a-73N	111a-73N	211a-73N	23a-73N	123a-73N	223a-73N	35a-73N	135a-73N	235a-73N
S.E. side	0.6 m	6a-73N	106a-73N	206a-73N	18a-73N	118a-73N	218a-73N	30a-73N	130a-73N	230a-73N
	1.2 m	12a-73N	112a-73N	212a-73N	24a-73N	124a-73N	224a-73N	36a-73N	136a-73N	236a-73N

Figure 3. Location and designation of samples collected from stacks 4, 5 and 6 in plot 2.



Collection
dates:

6-13-73

7-12-73

10-19-73

Each dot represents one collection location at 0.61 and 1.22 m elevations.

Location	Ht.	Stack 7			Stack 8			Stack 9		
		6-13-73	7-12-73	10-19-73	6-13-73	7-12-73	10-19-73	6-13-73	7-12-73	10-19-73
S.W. corner	0.6 m	37a-73I	137a-73I	237a-73I	49a-73I	149a-73I	249a-73I	61a-73I	161a-73I	261a-73I
	1.2 m	43a-73I	143a-73I	243a-73I	55a-73I	155a-73I	255a-73I	67a-73I	167a-73I	267a-73I
W. Side	0.6 m	38a-73I	138a-73I	238a-73I	50a-73I	150a-73I	250a-73I	62a-73I	162a-73I	262a-73I
	1.2 m	44a-73I	144a-73I	244a-73I	56a-73I	156a-73I	256a-73I	68a-73I	168a-73I	268a-73I
N.W. corner	0.6 m	39a-73I	139a-73I	239a-73I	51a-73I	151a-73I	251a-73I	63a-73I	163a-73I	263a-73I
	1.2 m	45a-73I	145a-73I	245a-73I	57a-73I	157a-73I	257a-73I	69a-73I	169a-73I	269a-73I
N.E. corner	0.6 m	40a-73I	140a-73I	240a-73I	52a-73I	152a-73I	252a-73I	64a-73I	164a-73I	264a-73I
	1.2 m	46a-73I	146a-73I	246a-73I	58a-73I	158a-73I	258a-73I	70a-73I	170a-73I	270a-73I
E. Side	0.6 m	41a-73I	141a-73I	241a-73I	53a-73I	153a-73I	253a-73I	65a-73I	165a-73I	265a-73I
	1.2 m	47a-73I	147a-73I	247a-73I	59a-73I	159a-73I	259a-73I	71a-73I	171a-73I	271a-73I
S.E. corner	0.6 m	42a-73I	142a-73I	242a-73I	54a-73I	154a-73I	254a-73I	66a-73I	166a-73I	266a-73I
	1.2 m	48a-73I	148a-73I	248a-73I	60a-73I	160a-73I	260a-73I	72a-73I	172a-73I	272a-73I

Figure 4. Location and designation of samples collected from stacks 7, 8 and 9 in plot 3.

and at the center of the stacks and composited. The compositing schedule for plots 2 and 3 is contained in table 3.

TABLE 3. COMPOSITING SCHEDULE FOR PLOTS 2 AND 3

Stack no. ^a	Date		
	6-13-73	7-12-73	10-19-73
4	1a-2a-7a-8a ^b	101a-102a-107a-108a	201a-202a-207a-208a
	3a-4a-9a-10a	103a-104a-109a-110a	203a-204a-209a-210a
	5a-6a-11a-12a	105a-106a-111a-112a	205a-206a-211a-212a
5	13a-14a-19a-20a	113a-114a-119a-120a	213a-214a-219a-220a
	15a-16a-21a-22a	115a-116a-121a-122a	215a-216a-221a-222a
	17a-18a-23a-24a	117a-118a-123a-124a	217a-218a-223a-224a
6	25a-26a-31a-32a	125a-126a-131a-132a	225a-226a-231a-232a
	27a-28a-33a-34a	127a-128a-133a-134a	227a-228a-233a-234a
	29a-30a-35a-36a	129a-130a-135a-136a	229a-230a-235a-236a
7	39a-40a-45a-46a	139a-140a-145a-146a	239a-240a-245a-246a
	38a-41a-44a-47a	138a-141a-144a-147a	238a-241a-244a-247a
	37a-42a-43a-48a	137a-142a-143a-148a	237a-242a-243a-248a
8	51a-52a-57a-58a	151a-152a-157a-158a	251a-252a-257a-258a
	50a-53a-56a-59a	150a-153a-156a-159a	250a-253a-256a-259a
	49a-54a-55a-60a	149a-154a-155a-160a	249a-254a-255a-260a
9	63a-64a-69a-70a	163a-164a-169a-170a	263a-264a-269a-270a
	62a-65a-68a-71a	162a-165a-168a-171a	262a-265a-268a-271a
	61a-66a-67a-72a	161a-166a-167a-172a	261a-266a-267a-272a

^aPlot 1 was not composited.

^bFour samples represent one composite.

All samples from plot 1 and composites from plots 2 and 3 were analyzed for IVDMD, NDF, CMC, ADF, ADL, crude protein, ash and moisture.

Chemical Analysis Procedures

In Vitro Dry Matter Digestibility. The following procedure, based on the method of Tilley and Terry (1963), was used for the determination of IVDMD: the forage sample (0.5 g), after passing through a Model 22 Weber pulverizing mill containing a 40 mesh screen, was carefully weighed and placed into a 100 ml polypropylene test tube which acted as a fermentation vessel. A water bath incubator with a capacity of 200 test tubes was adjusted to 39 C and calibrated to maintain constant temperature. McDougall's solution (25 ml) was added to each tube and the tubes were placed into the water bath before addition of the inoculum. The rumen fluid was collected via rumen fistula 2 hours after feeding and strained through eight layers of cheesecloth into a prewarmed thermos for transport to the laboratory.

The fluid was again strained through eight layers of cheesecloth, combined with McDougall's solution in a 2:3 ratio and mixed thoroughly. Twenty-five milliliters of the inoculum mixture was added to each tube with constant mixing. The temperature was maintained at 39 C to prevent chilling of the inoculum mixture. The tubes were then capped with bunsen gas release valves. The forage was resuspended after 2 hours and every 8 hours thereafter by shaking the tubes carefully in a circular motion so that forage particles would not remain on the sides of the tubes.

The tubes were removed from the water bath after 48 hours and placed in a refrigerator to stop bacterial action. The tubes were centrifuged after 1 hour at 1500 rpm for 10 minutes and the

supernatant drained with an aspirator fitted with a filter. The filter was rinsed with a small amount of distilled water after each use.

Fifty milliliters of Tilley and Terry's (1963) pepsin solution was added to each tube after the bacterial fermentation procedure. The fermentation residue was suspended by shaking carefully, and the tubes were incubated for 48 hours at 39 C with periodic shaking every 8 hours. The tubes were centrifuged after 48 hours at 1500 rpm for 10 minutes and the supernatant drained as described previously.

The tubes and residue were dried in a forced air drying oven at 90 C for 24 hours, placed in a desiccator for 1 hour and weighed. The tubes were then washed, dried and weighed again to determine the residue of fermentation. Six tubes which did not contain a forage sample but contained the inoculum mixture and pepsin solution were carried through the entire procedure. These tubes served as a correction factor for the residual dry matter of inoculum.

Calculation of IVDMD proceeded using the following formula:

$$\text{IVDMD} = \frac{100 \times \text{sample dry matter} - \text{residue of fermentation} - \text{residue of inoculum}}{\text{sample dry matter}}$$

Neutral Detergent Fiber. The following method was used, based on the procedure of Van Soest and Wine (1967), in determining NDF: one-half g of air dry sample, previously ground in a Model 22 Weber pulverizing mill containing a 40 mesh screen, was placed in a 600 ml refluxing flask along with 100 ml of room temperature neutral

detergent solution, 2 ml decahydronaphthalene and 0.5 g sodium sulfite in that order. The solution was refluxed at a low boil for 1 hour from the onset of boiling. After 1 hour, the solution was filtered using a previously tared Gooch crucible which had been lined with ashed asbestos. Low vacuum was used at first and was increased as it was needed for adequate filtration.

The sample was rinsed twice with hot water followed by two rinses using acetone. The crucibles were dried at 100 C for 8 hours, cooled for 1 hour in a desiccator and weighed. The yield recovered divided by initial sample weight was the estimated cell-wall constituents (NDF). Subtracting this value from 100 estimated noncell-wall material.

Cellulose Determination. The cellulose content of the forages was determined by the method of Crampton and Maynard (1938) with minor alterations in procedure. One-half g of air dry forage which had previously passed through a 40 mesh screen was weighed into a 100 ml polypropylene tube. Twelve milliliters of glacial acetic acid and 2.5 ml of concentrated nitric acid was added to each tube which was placed in a boiling water bath for 20 minutes.

The tubes were removed from the water bath and allowed to cool. The mixture in each tube was then filtered through a Gooch crucible containing an ashed asbestos pad. The residue was washed with hot water, acetone, benzene, acetone and ether in that order.

The residue and crucibles were dried for 4 hours at 100 C and weighed. They were then ashed for 2 hours at 760 C, allowed to cool

in a desiccator for 1 hour and weighed. Percent cellulose content was calculated by subtracting the ash weight from the dry weight, dividing this weight by the initial sample weight and multiplying by 100.

Acid Detergent Fiber and Lignin. The method of Van Soest (1963b) was used to determine ADF and ADL content of the forage samples. The only change made in this procedure was the use of Gooch crucibles with an asbestos filtering pad instead of sintered glass crucibles. The analyses were made in the following manner: a 2 g sample of the forage was placed in a 600 ml Berzelius beaker along with 100 ml of room temperature acid-detergent solution and 2 ml decalin. The mixture was refluxed for 1 hour at a low boil and filtered in a previously tared Gooch crucible containing an asbestos pad.

The residue was washed twice with hot water, followed by acetone, until the filtrate became colorless. The crucible and residue were dried for 8 hours at 100 C, placed in a desiccator for 1 hour and weighed. The difference between the first and second weighings determined ADF.

The residue remaining from the ADF procedure was placed in a 50 ml beaker and covered with 72% sulfuric acid. The mixture was stirred with a glass rod occasionally and filtered after 3 hours through a Gooch crucible containing an asbestos pad.

The residue was washed with hot water until it was free of acid, dried at 100 C for 8 hours and placed in a desiccator for 1 hour. The crucible plus residue was weighed, ashed at 500 C for 2 hours, cooled

in a desiccator and weighed again. The difference in weights between the crucible plus residue and crucible plus ash determined ADL.

Ash, Moisture and Crude Protein. A.O.A.C. (1960) methods of analysis were used for the determinations of ash, moisture and total nitrogen (crude protein) in the forage samples. The percent of protein was determined by multiplying the nitrogen content of the plant times the factor 6.25.

RESULTS AND DISCUSSION

Effects of Field Storage on Certain Forage Quality Parameters with Two Different Large Hay Packaging Systems

Alfalfa is the primary source of hay in the United States, and it is estimated that 28% of the total production of this crop is lost (Von Bargen, 1965). Hay loss is attributed to the number of sequential operations required to handle and harvest hay and to the hazards of weathering while hay is drying. A 35% loss in yield can be realized in alfalfa which is raked and packaged when too dry (Dobie et al., 1963). Today, "one-man operation" machines may allow harvesting at higher than normal moisture contents which reduce the time lapse between operations and therefore reduce the chance of weathering loss.

The purpose of this study was to determine if large hay package storage in the field affected certain chemical components of forage as storage time increased and to what extent this occurred. A survey of literature showed much research which related changes in chemical analyses of a feed to its storage time, but the forages studied in these reports were of different maturities. All forages in this study were harvested and stacked at the same maturity and at similar moisture levels. The averages of all chemical component analyses of each stack and collection dates used in this comparison are included in table 4. Individual component values were used for statistical analysis.

TABLE 4. AVERAGE COMPONENT ANALYSES OF NINE STACKS AND THREE COLLECTION DATES

Stk.	Date	ADF ^a	NDF	ADL	CMC	CP	IVDMD	Moisture	Ash
1	6-15-72	40.27	55.65	7.04	33.43	12.87	72.66	35.80	11.99
1	10-10-72	44.22	62.08	8.28	33.91	13.31	63.94	11.70	12.28
1	5-2-73	46.11	70.38	10.02	45.06	13.09	50.13	7.90	9.94
2	6-15-72	36.98	49.93	6.86	31.14	22.10	67.69	31.80	13.38
2	10-10-72	43.91	59.90	7.88	34.08	21.35	68.47	11.40	13.11
2	5-2-73	44.57	70.13	10.30	39.15	13.37	54.23	7.60	9.58
3	6-15-72	39.93	56.22	6.42	34.13	12.16	65.67	29.00	12.37
3	10-10-72	43.23	62.08	7.76	36.11	12.77	57.56	11.33	12.41
3	5-2-73	44.50	70.55	9.51	43.60	12.48	55.56	8.02	9.61
4	6-13-73	27.96	49.94	8.29	23.31	17.97	66.28	34.20	11.15
4	7-12-73	33.76	50.88	10.65	23.37	18.52	61.47	18.83	11.61
4	10-19-73	31.29	53.94	8.07	23.70	18.22	63.99	9.90	11.91
5	6-13-73	29.16	53.03	7.22	23.73	17.92	64.24	29.50	10.89
5	7-12-73	33.86	52.19	12.29	24.09	18.76	61.30	16.00	11.10
5	10-19-73	31.30	53.75	7.55	23.64	17.85	63.19	9.80	11.36
6	6-13-73	30.53	55.24	7.79	25.34	17.76	64.14	32.30	10.81
6	7-12-73	35.32	53.95	9.90	25.67	18.01	61.00	12.17	11.46
6	10-19-73	33.00	52.03	8.09	26.52	17.66	62.88	9.10	11.25
7	6-13-73	32.48	52.81	7.77	27.18	17.42	62.41	39.90	12.05
7	7-12-73	37.42	54.39	9.17	26.25	16.68	58.12	12.83	13.88
7	10-19-73	36.44	52.59	7.89	27.92	16.47	57.97	9.70	13.10
8	6-13-73	32.32	53.28	7.95	28.38	16.19	62.82	40.10	11.98
8	7-12-73	37.35	55.11	9.05	26.57	16.58	56.87	13.42	16.82
8	10-19-73	36.38	55.54	8.00	28.44	16.64	59.59	9.67	12.44
9	6-13-73	33.52	54.72	7.64	28.11	17.13	63.40	39.80	11.29
9	7-12-73	36.52	56.69	8.74	26.36	17.08	57.77	11.30	15.43
9	10-19-73	34.89	55.21	7.40	27.49	16.95	61.60	8.90	11.67

^aADF = acid-detergent fiber, NDF = neutral detergent fiber, CMC = Crampton and Maynard cellulose, ADL = acid-detergent lignin, CP = crude protein and IVDMD = in vitro dry matter digestibility.

Collections on stacks 1 to 3 were made for 11 months, and collections on stacks 4 to 9 were made for 4 months. Therefore, any long-term study involving these stacks was not possible at this time. Long-term storage studies using these stacks will be possible at a later date.

Least squares analysis of variance was used in this study to determine if differences existed between dates of storage and chemical composition parameters. Differences between stacks and differences between dates within individual stacks were also obtained. The component analysis values used in this study included moisture, crude protein, NDF, CMC, ADF, ADL, IVDMD and ash. These values were obtained in 239 observations of common alfalfa. Results of the least squares analyses of variance on eight chemical component parameters and two sources of variation are found in table 5. All values of each source of variation were shown to be significant ($P < .05$), which indicated a difference between stacks and between dates within the same stack.

Moisture. In this study, stacks 1 to 3 were stored at moisture levels ranging from 29 to 36%. Moisture at the time of stacking ranged from 30 to 40% in stacks 4 to 9. Moisture differences were not great enough to make comparisons of the chemical changes occurring within stacks due to moisture or to determine maximum moisture levels possible with the two packaging systems. Moisture-spoilage comparisons were not possible for the same reason.

Moisture levels at the time of stacking did not seem to affect storage quality in this study. Bledsoe et al. (1973) reported

TABLE 5. LEAST-SQUARES ANALYSIS OF VARIANCE ON EIGHT CHEMICAL COMPONENT PARAMETERS

Source	df	Mean squares							
		ADF ^a	NDF ^a	ADL ^a	CMC ^a	CP ^a	IVDMD ^a	Moisture	Ash
Stack	8	724.4*	624.8*	3.04*	980.9*	214.6*	145.2*	39.3*	18.31*
Dates/Stacks	18	59.2*	237.1*	18.85*	100.6*	39.1*	196.1*	7.6*	19.14*
Residual	239	4.7	8.8	.90	8.6	1.0	8.5	.1	.26

^aADF = acid-detergent fiber, NDF = neutral detergent fiber, ADL = acid detergent lignin, CMC = Crampton and Maynard Cellulose, CP = crude protein and IVDMD = in vitro dry matter digestibility.

*Significant at the .05 level.

satisfactory storage in compressed Hesston stacks containing 30% moisture. Von Bargaen (1965) reported similar results. Weeks, Peterson and Owen (1971) noted satisfactory storage at moisture levels of 40% in the Hesston stack. Studies pertaining to changes in chemical composition of the Haybuster type stack due to moisture differences were not available at the time of this writing, but similar results in storage quality at similar moisture levels were obtained by both machines.

Stacks 1 to 3 in this study lost 60 to 70% of their total moisture content during the first 4 months of storage. After this period, moisture loss averaged 1.5% per month for 8 months. The moisture content leveled off to a relatively constant 8.0% after 11 months. Stacks 4 to 9 followed a similar trend during 4 months of storage. Slightly faster moisture loss was observed in the 1972 stacks even though rainfall during this time was greater. The amount of rainfall did not seem to affect the rate of moisture loss. This may be related to differences which existed in leaf-to-stem ratio between stacks, due to different amounts of rainfall received prior to harvesting. Rate of moisture loss was slightly higher in the Hesston stacks than in the Haybuster stacks, however, comparisons of this type are not statistically valid because of differences which existed between plots.

Crude Protein. Gill (1973) found very slight changes in protein values in large round bales and in Hesston Stack Hand 30 stacks.

Similar results were found in this study. Generally, protein values were found to vary slightly between stacks during the storage period.

Generally, crude protein increased slightly as length of storage increased up to 11 months. Bledsoe et al. (1973) reported that a change in crude protein was related to the moisture content at the time of storing. Changes in protein content at different initial moisture levels were too small to make a similar conclusion. The increase in protein noted in some stacks may be the result of microflora solubilizing a proportion of the structural carbohydrates (Weeks et al., 1971). Increased protein values do not mean increased nutritive value in all cases, since this protein increase would probably result in decreased digestibility and palatability in actual animal trials due to its unavailability.

Renoll et al. (1972) reported higher crude protein values in samples taken from the top of the stack when compared to core samples but found no important differences in crude protein content between baled or Hesston stacks. Results of this study did not include samples obtained from the tops of the stacks. The amount of spoilage which occurs on the exterior of the stack due to weathering and relative proportionate differences between kinds of stacks is reserved for future studies.

Cell Wall Materials. The components NDF, ADF, CMC and ADL differed significantly ($P < .05$) between stacks and between dates within individual stacks with advancing storage time. Ash decreased in this

study which is contrary to expected results. Individual mineral analyses of ash samples collected for this study were not made, therefore, no explanation for this decrease is possible without further study. However, the fact that ash values remained relatively constant may indicate that no spoilage occurred in 11 months of storage. Weeks et al. (1971) reported slower initial increases in ash values of large stacks compared to small stacks. This may be related to the faster drying which seems to occur in smaller stacks. Volatilization of some minerals may have reduced the ash content observed in this study.

ADL values remained most constant of the cell wall components. During the initial 4 month period NDF increased more than any other component followed by ADF and CMC, but from 4 to 11 months CMC increased much faster than ADF. Cellulose is included in the NDF fraction, therefore, the increase in NDF should be directly related to the increase in CMC. Results of this study indicate this to be true. Weeks et al. (1971) reported that percent of cell wall components increased with storage time, but Renoll et al. (1972) reported no important differences in total cell walls and noncell-wall constituents in bales and Hesston stacks. The results of this study showed that differences did exist in all cell wall components and that in most cases an increase in cell wall components was observed.

Significant differences ($P < .05$) were found in all components between stacks and between chemical components and dates within the same stack in this study. These results indicate that the stacks were not composed of identical material or that inaccuracies in chemical analyses occurred.

Differences between stacks could have occurred because plot 1 contained some small patches of bromegrass and weeds. This foreign material may have been included in some of the samples and not in others.

The significant differences observed between dates in the same stack illustrate that forage quality changed significantly within each stack. These differences were noted within all stacks, however, differences were more pronounced in stacks 1 to 3. Results indicate that storage of a feedstuff decreases its nutritive value, especially during storing periods longer than 4 months.

The observation that differences between stacks existed was apparent. However, this difference does not invalidate the observation that differences existed between dates within the same stack. The differences which existed between dates within the same stack should be reliable because each sample was drawn from the same area within each stack. This study was intended to identify changes within each stack as time of storage increased, therefore, the differences observed between stacks should not influence the differences observed between dates within the same stack.

NDF remained generally constant in stacks 4 to 9 during the first 4 months of storage. A large increase in NDF was noted, however, in stacks 1 to 3 during the first 4 months of storage. This increase probably occurred as a result of increased rainfall received in the month prior to harvesting. A period of rapid growth along with a rapid increase in stem elongation may have resulted in an increased

stem-to-leaf ratio and therefore a rapid increase in the NDF fraction. No evident difference was observed in NDF between types of stacks in the first 4 months of storage in stacks 4 to 9. The increase in NDF in stacks 1 to 3 was linear from summer to the following spring. Additional collections from stacks 4 to 9 are needed to determine the effect of the winter season on individual components of these stacks.

ADF was found to follow a similar pattern as NDF, except that all changes in the ADF component were smaller. ADF increased quickly for 4 months in stacks 1 to 3 and increased slowly from 5 to 11 months. This initial increase was greater than during the same time from harvest in stacks 4 to 9. Increased rainfall during the 1972 harvesting season also may have caused this increase.

The CMC component differed greatly between stacks. Stacks 1 to 3 had an average increase in CMC of 0.9% per month for 11 months but most of this increase came after the fourth month of storage. Stacks 7 to 9 increased slightly in 4 months of storage and a slight decrease occurred in stacks 4 to 6 during this period. It was apparent that appreciable increases in CMC did not occur until 4 to 6 months after stacking.

Lignin increased very slowly with increased storage time and great actual differences between stacks were not found. Lignin values of samples from stacks 1 to 3 increased an average of 0.27% per month of storage. A greater increase occurred after 4 months of storage than before this time. Van Soest and Moore (1965) reported that lignin increased significantly with storage time. This study found

similar results.

In conclusion, significant increases in all cell wall materials between stacks and between dates within the same stacks were observed throughout the storing period. Additional study is necessary to establish the actual increases of cell wall materials during storage periods of one year and longer.

In Vitro Dry Matter Digestibility. Renoll et al. (1972) reported that baled hay produced higher DMD values than Hesston stacked hay. Even though statistically significant differences were observed in IVDMD between stacks, little actual difference in types of stacks and IVDMD was noted in the first 4 months of storage. This study found that IVDMD decreased an average of 18.7 percentage units in the first 11 months of storage in stacks 1 to 3 and approximately 5% in the first 4 months of storage. This would indicate that short term storage of the forage crop did not greatly affect total nutritive value. Periods of storage longer than 4 months, however, resulted in a substantial decrease in nutritive value.

In conclusion, it was found that differences existed in stacks between harvesting methods and in stacks using the same method of harvesting. Significant differences in all components were also seen between dates within the same stack. The relationships found in this study imply that great differences between storage time and the component analysis of moisture occur within the first 6 months of storage, after which time differences still occur but less noticeably. IVDMD seems to decrease at a constant rate during 11 months of storage.

The forage components NDF, ADL, ADF and CMC generally increased in 4 months of storage but increases were gradual and nearly linear through 11 months of storage.

It was not possible at this time to make conclusions regarding forage quality as it was affected by long periods of storage. It was apparent from this study, however, that forage quality was reduced, especially after a period of 4 months storage, during which time decreases in quality were nearly linear for 11 months. Longer periods of storage must be studied before all relationships between chemical parameters and nutritive value can be evaluated.

Relationships Between In Vitro Dry Matter Digestibility and Other Chemical Component Analyses Using Multiple Regression

Future nutritive value studies may depend on simple chemical component analyses in combination with IVDMD methods to establish a more efficient forage evaluation procedure. This study was conducted to determine which chemical component analyses, if any, contributed most significantly to the IVDMD method. With this information, analyses which do not explain significant variability in IVDMD can be deleted and additional selected component analyses can then be determined and studied in relation to IVDMD. Eventually, the combination of analyses which best correlate to IVDMD can be used to increase the relationship between in vivo and in vitro methods.

Stepwise-Forward Multiple Regression. The stepwise-forward regression approach was used in this study. The procedure involved

the use of a dependent variable (IVDMD) and seven independent variables (ADF, ADL, NDF, crude protein, CMC, ash and moisture). Multiple regression explains that proportion of the variability in a dependent variable which can be explained by one or more independent variables. The stepwise-forward multiple regression approach describes the component analysis (X) which explains most variability in the dependent variable (Y) followed by the component analysis which next explains most variability in Y. This proceeds with each independent variable until the total variability of all independent variables is explained.

Results of the stepwise regression of seven chemical component analyses on IVDMD are shown in table 6.

The table indicates that, of the seven chemical component analyses made, ADL best explained the variability in IVDMD, contributing 30.1% of the total variability. NDF explained 9.2% of the total variability in IVDMD. A combination of ADL and NDF explained 39.3% and the first five component analyses explained 48.7% of the total variability.

The multiple regression analysis (table 7) illustrates that the first five components consisting of ADL, NDF, moisture, crude protein and ash contributed significantly ($P < .05$) to the regression, while CMC and ADF did not.

Acid-Detergent Lignin. The chemical component analysis which best compared to IVDMD was found to be ADL. This finding is consistent with results obtained by other workers (Sullivan, 1955;

TABLE 6. STEPWISE-FORWARD MULTIPLE REGRESSION OF SEVEN CHEMICAL COMPONENTS ON IN VITRO DRY MATTER DIGESTIBILITY

Independent variable ^a	Proportion explained %	Total explained %
ADL ^b	30.1	30.1
NDF ^b	9.2	39.3
Moisture	6.4	45.7
Crude protein	1.4	47.1
Ash	1.6	48.7
CMC ^b	0.3	49.0
ADF ^b	0.0	49.0
Total for all seven variables		49.0

^aEach independent variable was regressed on IVDMD.

^bADL = acid-detergent lignin, NDF = neutral detergent fiber, CMC = Crampton and Maynard cellulose and ADF = acid-detergent fiber.

TABLE 7. MULTIPLE REGRESSION ANALYSIS

Source	df	S	MS
IVDMD	7	4016.71	573.82*
ADL	1	2467.18	2467.18*
NDF	1	756.46	756.46*
Moisture	1	522.80	522.80*
Crude protein	1	111.63	111.63*
Ash	1	129.14	129.14*
CMC	1	25.48	25.48
ADF	1	4.06	4.06
Residual	258	4182.00	16.21

* Significant at the 5% level.

Kamstra et al., 1958) who explained the decreased digestibility as a result of increased lignin content. Joshi (1972) also found good correlation between IVDMD and ADL. The results of this study did not totally agree with Richards et al. (1958) who found considerable variability within species of forage between ADL and digestibility values. Alfalfa contains a smaller amount of holocellulose than grasses. However, this fraction is more highly lignified and less digestible in alfalfa. A greater proportion of the dry matter of alfalfa is, therefore, not influenced by lignin (Van Soest, 1964). The principal difference between grasses and legumes lies in the proportion of hemicellulose present. Grasses contain a higher proportion of hemicellulose than legumes. The major discrepancy in using lignin in equations estimating nutritive value is related to the determination of its chemical makeup (Van Soest, 1964). It is difficult to obtain accurate and consistent results in lignin determination due to its diverse chemical structure and the separation of protein from the lignin fraction.

Neutral Detergent Fiber. NDF explained a significant proportion of the variability in IVDMD when combined with the lignin fraction. This is important because of the relationship between NDF and intake observed in some studies (Prigge and Apgar, 1973), and it is thus far the individual chemical component analysis which most highly relates to intake. Results of this study show that NDF and ADL used in combination predicted IVDMD to some extent. However, another report indicated better prediction of IVDMD with CMC or hemicellulose than

with NDF (Johnson and Pezo, 1973). Results of the study made in this laboratory do not agree with this report concerning the CMC fraction. Hemicellulose analyses were not made on the forage samples used in this study.

In certain grasses, silica seems to decrease the digestibility of cell-wall constituents by increasing structural strength, but this is not the case in alfalfa (Van Soest and Jones, 1968) except in certain areas of high soil silica content. Silica determination of samples used in this study was not made, therefore, any effect that silica may have had on the digestibility of these samples was not possible to determine.

Moisture. The effect of moisture on IVDMD depends upon the chemical procedure used to remove it prior to analysis. Browning and other heat damage effects are possible at temperatures above 50 C (Ely, Mellin and Moore, 1956). The samples in this study were dried at higher temperatures than 50 C. Therefore, browning may have occurred, which may account for the correlation between moisture and IVDMD observed in this study. If excess browning occurred in this study, it may have resulted in an underestimation of nutritive value.

Crude Protein. Protein is not usually considered an important contributor in regressions for IVDMD because it is variable when considering nitrogen fertilization (Butterworth and Diaz, 1970). However, narrower variations persist when nitrogenous fertilizers are not used. In this study, inclusion of protein in the regression may

have been possible only because of the absence of nitrogen fertilization.

Ash. Ash content did contribute to the regression in this study. However, inclusion of this fraction at this time is of questionable value. Ash content may indicate spoilage characteristics of a feed and in this way may estimate the overall value of the feed. In the future, ash values might be indicative of nutritive value when they relate to storage of feeds longer than 1 year.

Acid-Detergent Fiber and Crampton and Maynard Cellulose. The fibrous portions ADF and CMC did not contribute to the regression equation, probably because of the diverse nature of these components. ADF consists of cellulose, lignin and lignified nitrogenous fractions which are, in themselves, diverse entities. These diverse entities are believed to be responsible for the low correlation observed between ADF and CMC with IVDMD. These components usually represent chemical complexes associated to a greater or lesser degree with the lignification in a feed. Prigge and Apgar (1973) reported a significant correlation between ADF and CMC when compared to intake but to a lesser degree than NDF.

Regression Equations. Stepwise regression equations obtained from this study are found in table 8. The regression equation $Y = 86.4 - 0.76(\text{ADL}) - 0.34(\text{NDF}) + 1.37(\text{moisture}) + 0.31(\text{crude protein}) - 0.54(\text{ash})$ includes all values which contributed significantly to the regression and is therefore the best model relating to IVDMD found in

TABLE 8. STEPWISE REGRESSION BETWEEN IN VITRO DRY MATTER DIGESTIBILITY AND CHEMICAL COMPONENT ANALYSES

Relationship	Regression equation	r
IVDMD Lignin	$Y = 78.1 - 1.99(L)^a$	0.549
IVDMD NDF	$Y = 87.8 - 1.33(L) - 0.258(NDF)^a$	0.625
IVDMD Moisture	$Y = 85.9 - 0.66(L) - 0.36(NDF) + 1.25(M)^a$	0.673
IVDMD Protein	$Y = 78.2 - 0.79(L) - 0.28(NDF) + 1.20(M) + 0.26(P)^a$	0.682
IVDMD Ash	$Y = 86.4 - 0.76(L) - 0.34(NDF) + 1.37(M) + 0.31(P) - 0.54(A)^a$	0.692

^aL = lignin, NDF = neutral detergent fiber, M = moisture, P = crude protein and A = ash.

this study. However, if laboratory facilities and time are important, the equation $Y = 87.8 - 1.33(ADL) - 0.258(NDF)$ ($r = 0.63$) may be of practical significance.

Moisture, crude protein and ash added only 0.067 to the multiple correlation coefficient. Therefore, the exclusion of these components did not affect the correlation considerably. Johnson and Pezo (1973) used least squares regression on 370 observations of chemical composition values and found the models $Y = 117.2 - 0.761(\text{cellulose})$, $Y = 117.2 - 1.926(\text{permanganate lignin})$ and $Y = 117.2 - 0.586(\text{hemi-cellulose})$ effective in predicting IVDMD (Y) of grasses.

Nutritive Value Index Using a Regression Equation

The NVI study of Crampton et al. (1960) combined relative intake of a forage with the digestibility of its energy. Relative intake of the forage was found by first multiplying the metabolic size of the animal ($W_k^{.75}$) times 80 g to get expected "standard forage" intake. Relative intake was found by dividing actual daily consumption by expected intake times 100. This value times energy digestibility gave a numerical value index for the forage which correlated well with daily gain by animals in feeding trials. Donefer et al. (1960) found a correlation of $r = 0.91$ between this index and IVCD; however, inconsistent results occurred in the measurement of intake. Likewise, a nutritive value index (Y) could be obtained using IVDMD (X), but this would also result in inaccurate results for the same reason.

It was proposed in this study that intake may be indirectly included in an index by considering the NDF value obtained in the

multiple regression model. NDF has been shown to correlate quite well with intake. This value, along with the ADL value which was found to correlate well with IVDMD, would then be indicative of nutritive value.

To illustrate this procedure the following outline is proposed:

1. Low, hypothetical analysis values of a "perfect standard forage" are given to ADL and NDF. These values would indicate the best forage possible under normal conditions. Values obtained for ADL and NDF of an immature alfalfa were used as standard forage values. The values 3.0 and 37.0% for ADL and NDF, respectively, were used.
2. The values 3.0 and 37.0 were substituted in the regression model $Y = 87.8 - 1.33(ADL) - 0.258(NDF)$. This model proved to be the most practical one obtained when all possible combinations of all analyses made in this laboratory were compared. The result of this step was:

$$Y = 87.8 - 1.33 (3.0) - 0.258 (37.0)$$

$$Y = 87.8 - 13.54$$

$$Y = 74.26$$

3. A value relative to 100 would be more appropriate in a NVI so 74.26 was divided by itself and taken times 100. The result gave a value of 100 to the "perfect standard forage."

$$\frac{74.26}{74.26} \times 100 = 100$$

4. Substituting mean values of ADL and NDF obtained from stack 1 on the first (a) and third (b) collection dates we obtain:

- a. Stack 1, collection 1:

$$Y = 87.8 - 1.33 (7.0) - 0.258 (55.7)$$

$$Y = 87.8 - 23.7$$

$$Y = 64.1$$

- b. Stack 1, collection 3:

$$Y = 87.8 - 1.33 (10.0) - 0.258 (70.0)$$

$$Y = 87.8 - 31.36$$

$$Y = 56.44$$

5. NVI values relative to the standard forage was then calculated.

a. $NVI = \frac{64.1}{74.26} \times 100 = 86.3$

b. $NVI = \frac{56.44}{74.26} \times 100 = 76.0$

IVDMD values decreased approximately 22.5, 13.5 and 10.1 percentage units during a period of 1 year storage in stacks 1, 2 and 3, respectively. NVI values for collections 1, 2 and 3 in stacks 1, 2 and 3 decreased approximately 10.3, 13.0 and 10.5 nutritive value units, respectively.

The result of the NVI calculations above indicates a reduction of 10.3 nutritive value units in the first year of storage in stack 1. IVDMD during this same period indicated a reduction of 22.5 percentage units in the same stack. Staples et al. (1951) reported that 3 years of storage did not affect nutritive value in hays cut at similar maturity in South Dakota. Results of this study showed that IVDMD,

which is a good indicator of nutritive value, decreased significantly with storage time. This would disagree with the study of Staples et al. (1951). The NVI values obtained in this study were more consistent than the IVDMD values, with a range of 2.7 NVI units and 12.4 percentage units, respectively. Apparently, the large decrease in IVDMD observed in these samples during 1 year of storage is larger than actual digestibility values in actual feeding trials would indicate. Assuming this to be the case, the NVI figure, which is relatively higher than the IVDMD figure would be more indicative of actual nutritive value. Further investigation of differences between IVDMD and NVI values and their relation to actual in vivo digestibility data is necessary to obtain information on the feasibility of the NVI indicated in this study.

In conclusion, it was found that combining several chemical component analyses using a stepwise-forward multiple regression equation significantly explained the variability obtained in the IVDMD trials conducted at this laboratory. It was found that the components ADL, NDF, moisture, crude protein and ash contributed to the regression equation, while CMC and ADF did not. It was also found that diverse chemical entities may not be valuable predictors of IVDMD due to variations which may exist due to species differences, maturity or storage of feedstuffs.

A possible NVI, which accounts for intake indirectly by inclusion of NDF and increases the relationship to IVDMD by including ADL, was reported in this study. The use of a multiple regression model was

used in addition to average component values of ADL and NDF to obtain a relative NVI of forage. The NVI seemed to be more appropriate in predicting in vivo digestibility than the standard IVDMD procedure. NVI figures were relatively higher and more consistent than IVDMD values obtained at this laboratory.

This study will enable future nutritive value predictions using a combination of simple chemical component analyses. Future progress is dependent on additional selected components which might be included in these combinations.

SUMMARY AND CONCLUSIONS

A study was made to provide information concerning effects of storage on certain chemical quality parameters in two different large hay packaging systems. The intentions of this study did not include comparisons of different packaging systems and their ability to maintain quality as storage time progressed. This would not have been possible because of differences in location of the experimental plots and because the machines were used in different plots. Statistical comparisons using a different machine in each of two different plots would produce invalid results.

Results pertaining to the rate of change in each quality component as time of storage increased were also reported. Chemical component analyses used in this study were IVDMD, ADL, NDF, CMC, Moisture, ash, ADF and crude protein. The results of all chemical analyses found in this study were used in a second study which incorporated these analyses to relative nutritive value of the forages.

The results of this study were based on 2,032 chemical component analyses made in this laboratory over a period of 11 months. Composites of samples obtained in similar areas of the same stack were made so that total analyses necessary could be reduced. Comparisons of differences in chemical composition at different locations within the same stack were not made at this time, however, the composition values obtained in this study will permit comparisons of any separation of forage parts within stacks at a later date.

It was found that significant differences ($P < .05$) in all chemical component values compared in these forages existed between stacks and also between dates within the same stack. Larger differences seemed to exist between kinds of stacks than between stacks made by the same machine. This difference was attributed to differences which existed in forages between stacks, sampling and analytical errors or non-uniform distribution of plant species within stacks.

The differences which existed between dates within the same stack showed that the chemical composition of the forages changed significantly as time of storage increased. This change occurred as a result of a percent increase in non-nutritive components which indicated a decrease in readily available nutrients. The decrease in nutritive value was partially dependent upon the moisture content at the time of harvest.

Significant increases ($P < .05$) were observed in all cell wall materials between stacks and between dates within the same stack throughout the storing period. This was indicative of decreased quality. The study showed that forage quality was reduced throughout 11 months of storage but this reduction in quality occurred especially after a period of 4 months storage. The effect of longer periods of storage will be evaluated at a future date.

The purpose of the second objective was to include one or more of the chemical component analyses found in the first study in a regression equation which would be indicative of the nutritive value of the forage studied in this report.

It was found that IVDMD could be used to estimate nutritive value of a forage but certain restrictions were imposed on this value which decreased the accuracy of the system. This value did not take into consideration the voluntary intake of the animal.

The chemical component NDF has been found to relate to voluntary intake in other studies, therefore, it was proposed that the use of this component plus the ADL component, in combination with IVDMD would indicate nutritive value of a forage more accurately than the component IVDMD alone.

Stepwise multiple regression analysis was used to indicate that 40% of the variability in IVDMD could be explained by the components ADL and NDF. These components were used, along with IVDMD in a multiple regression model, to establish a relative NVI which seemed to be more indicative of nutritive value than the IVDMD component alone.

In conclusion, it would appear that the use of individual selected chemical components in addition to, or in combination with IVDMD may in the future provide a means by which the nutritive value of a forage may be evaluated without tedious and expensive in vivo digestibility trials.

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