Fiber and Nitrogen Fractions of Forages and By-product Feeds Determined by In vitro and In situ Procedures

by

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(ABSTRACT)

Objectives were to determine dry matter, fiber and nitrogen fractions, and in vitro and in situ degradability of forages and by-product feeds, and to compare in vitro methods of estimating rumen degradability with the in situ bag technique. Feeds analyzed with number of samples in parentheses included alfalfa as baled hay (23), alfalfa ensiled in conventional (43), and oxygen limiting silos (39), ammonia-treated (25), and untreated corn silage from conventional (17) and bunker silos (17), rye (25), sorghum (7), wheat (6), barley (5), and orchardgrass (4) silages, orchardgrass (19) and fescue hay (3), and dried distillers grains dark colored (2) and light (1), wet brewers grains (1), and whole cottonseeds (3). Samples were analyzed for dry matter, crude protein, buffer-soluble protein, protease insoluble nitrogen, neutral and acid detergent fiber and insoluble nitrogen, and in situ degradability of nitrogen, dry matter, and fiber.

Protease insoluble nitrogen, buffer-insoluble protein, and neutral detergent insoluble nitrogen were lowest for alfalfa from conventional upright silos. Oxygen limiting silo samples had greater dry matter, insoluble protein, and bound nitrogen compared to conventional upright silo samples. Oxygen limiting silos had 35.9% of samples with bound nitrogen greater than 15% of total nitrogen compared to 14% of conventional upright silo samples. Baled hay and oxygen limiting silo samples had similar protease insoluble nitrogen, however, ensiled samples had greater bound nitrogen.

In situ nitrogen degradability was greatest for ensiled forages compared to hays. Ensiled forages had the greatest A fraction (rapidly solubilized), alfalfa hay the greatest B fraction (slowly degraded), and orchardgrass hay the greatest C fraction (not degraded). Degradation of dry matter and fiber followed similar patterns for each forage and by-product.

Significant results were found by comparing in vitro and in situ techniques for estimating degradability. Due to differences between hay and silage, use of one technique can not be recommended at this time to predict degradability. For silage, the best measure related to in situ degradability was buffer-soluble protein; for hay, the best measure was neutral detergent insoluble nitrogen.

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INTRODUCTION

Approximately thirty to forty percent of the diet dry matter fed to dairy cows in early lactation consists of forage. As lactation progresses, forage content increases to more than 80% of the diet dry matter. Forage provides the bulk of the fiber in the dairy ration. Fiber plays an important role in regulating rate of passage, rumination and salivation, pH of the rumen, efficiency of feed utilization, and in lactating dairy cows, maintenance of milk fat percent. It has been estimated that forages also supply about 50% of the total protein fed to dairy cows (Waldo and Jorgensen, 1981). Thus, forages are an important source of nutrients for dairy cows.

Utilization of by-product feeds by the dairy industry has constantly increased. In 1981, it was estimated that by-product feeds represented approximately 20% of the feed concentrates consumed by livestock (Chase and Udedibie, 1983). Brewers and distillers grains have received much attention in recent years as feedstuffs for dairy cows because of their potential rumen escape protein.

When considering utilization of protein by ruminants, protein in forages and by-product feeds must be considered. Methods of evaluating feed proteins include in vivo (sampling of contents from the abomasum or duodenum), in situ (incubation of feeds in bags suspended in the rumen), and in vitro

(laboratory) techniques. Proposed new protein systems (Waldo and Glenn, 1984; National Research Council (Ruminant Nitrogen Usage), 1985) require the separation of dietary protein into a ruminally degraded fraction and an undegraded fraction. This division has routinely been done with the use of cannulated animals, techniques to separate bacterial and protozoal protein and undegraded dietary protein, and measurements of total protein flow. Although the in vivo method remains the reference method, because of its complexities, much research has been done to find simple, reliable, and rapid in vitro techniques for routine feed analysis. Rapid turnaround is essential for a laboratory technique to be effective in making feeding recommendations.

Another protein fraction important in ruminant nutrition is the protein in the acid detergent fiber component of feeds commonly called "bound" or "heat damaged" protein (Thomas et al., 1982). This protein is considered unavailable to the animal. Heating during ensiling or storage has been shown to increase the content of bound protein in feeds. The measurement of bound protein is especially important in hays, hay crop silages, and certain by-product feeds such as dried brewers and distillers grains. Formulating dairy rations for protein would be greatly improved by incorporating degradability and bound protein along with crude protein for feedstuffs.

Considering these previous remarks, experiments were conducted with the following objectives:

1. Determine dry matter, fiber, and nitrogen fractions of forages received at the Virginia Tech Forage Testing Laboratory and to determine if sample handling prior to analysis has an effect on fiber and nitrogen fractions.

2. Determine differences in dry matter, fiber, and nitrogen fractions, and in vitro degradability of alfalfa stored as baled hay, or ensiled in oxygen limiting or conventional upright silos and to determine relationships among analytical measurements.

3. Estimate rumen nitrogen and dry matter degradability of selected forages and by-product feeds.

4. Compare in vitro methods with the in situ bag technique in estimating rumen nitrogen degradability.

5. Determine degradability of neutral and acid detergent fiber fractions by the in situ bag technique.

LITERATURE REVIEW

I. FORAGE UTILIZATION

Waldo and Jorgensen (1981) reviewed forage utilization by dairy cattle during the last 25 years (1953-1978). During that period, concentrate feeding had increased to 154% of the amount fed in 1953, hay had decreased to 77%, pasture had decreased to 63%, and other harvested feeds (silage) had increased to 114% of the 1953 amount. Total concentrates have increased from 28 to 43% of the total feed fed to dairy cows, while forages have decreased from 72 to 57%. A major reason for the increase in milk production per cow over that time (220%) is the large increase in concentrate feeding. Although percentage of forage in the diet has decreased, forages continue to be an important source of nutrients for dairy cows. Forages have been estimated to supply about 50% of the total protein fed to dairy cows (Waldo and Jorgensen, 1981). When considering utilization of protein by ruminants, protein in forages must also be considered. Methods of storage and changes which occur during harvest and storage are important factors to be discussed.

Forage Storage Methods

Forages are stored in several ways depending on their dry matter content. Forage that is stored as field-cured hay typically has a dry matter (DM) content of 75 to 90%. Barn dried hay is stored with a DM content of 55 to 75%. Forage ensiled with a DM content between 40 to 60% is usually referred to as low moisture silage. Forage stored with a DM content between 30 to 40% is wilted silage, while forage stored with a DM content between 15 to 30% is direct cut silage. Varying amounts of storage and field losses are associated with each method. Field losses increase and storage losses decrease with drying in the field, the higher field losses usually are in the form of leaves, which represent the most valuable portion of a plant. Field cured hay has the largest field losses while direct cut silage is associated with the largest storage losses (Hoglund, 1964). The lowest total losses result with harvesting a forage as low moisture silage with a DM range of 40 to 50%. Relative differences among methods of harvesting and varying dry matter contents on field, harvesting, and storage losses are presented in Figure 1.

Noller and Thomas (1985) listed several advantages of ensiling grasses and legumes which included 1) decreased time between cutting and storage, 2) more nutrients harvested and preserved for feeding, 3) mechanized harvesting and feeding, with more flexibility in feeding program, 4) reduced field

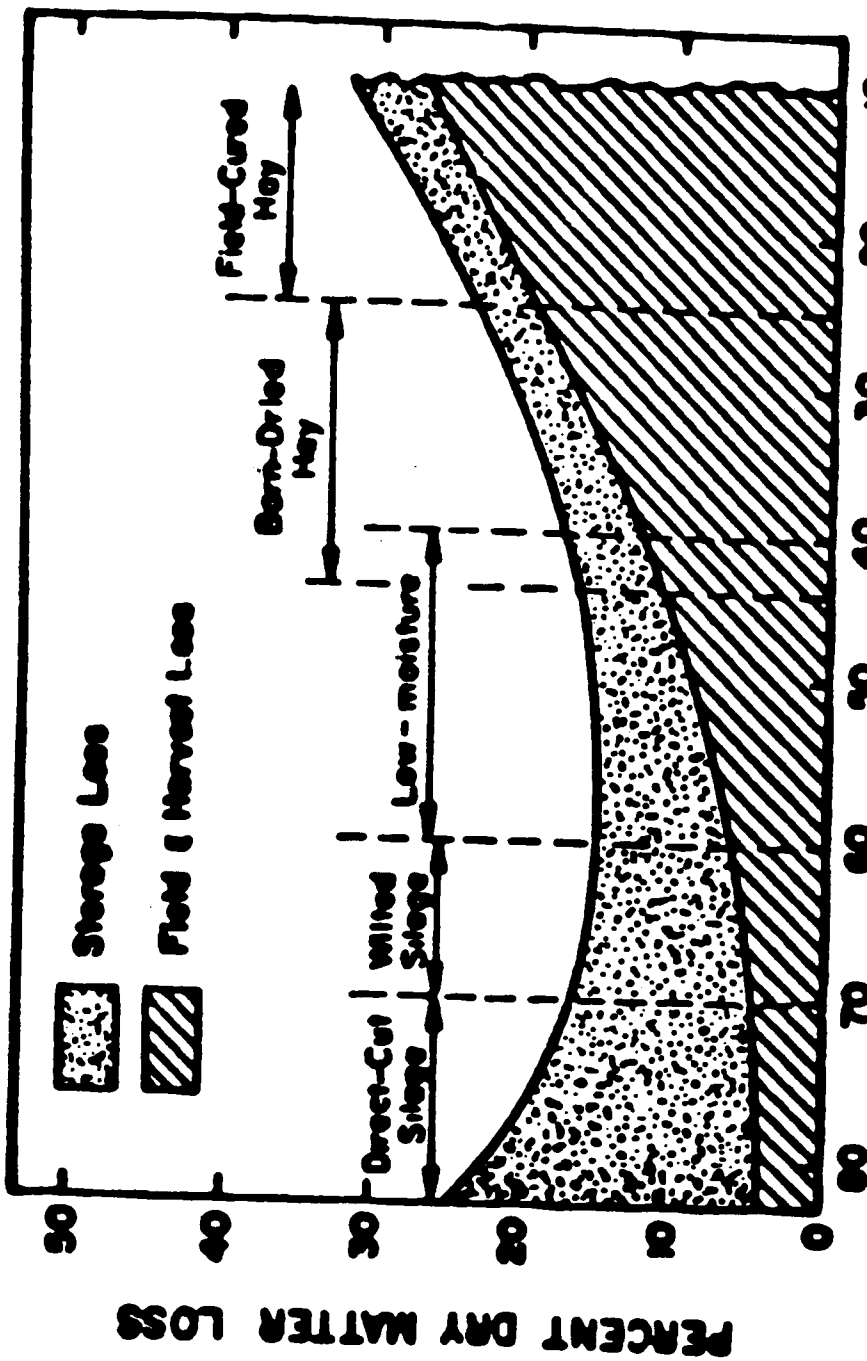

Figure 1. Estimated total field and harvest loss and storage loss when legume-grass forages are harvested at varying moisture levels and by alternative harvesting methods (Huglund, 1964).

losses, and 5) preservation for long periods, with minimum loss of nutrients.

Several types of storage structures are available for ensiled forages. These include trench, bunker, and stack silos, conventional tower silos, and sealed or oxygen limiting silos. Each of these structures used for storage have advantages and disadvantages. Trench, bunker, or stack silos are often used because of their low initial cost and suitability as temporary storage. However, DM losses tend to be higher with these because of greater surface area and difficulty of packing and excluding air. Stack silos are generally less efficient in recovery of DM than trench or bunker silos, but they can be used successfully if managed properly. Covering trench, bunker, and stack silos greatly reduces DM losses. Conventional tower silos are the most popular in the United States for storing forage. Losses are low with good silage making procedures and when covers are used. Forages can be stored with a DM content between 20 and 60%. However, losses from seepage are greatest with DM content less than 30%. Advantages of sealed or oxygen limiting silos include no visible top spoilage, refilling can be done at any time, a relatively low-moisture material can be ensiled, automated bottom unloading, and lower DM losses. Estimates of the affect of moisture level of forage to be ensiled on the DM losses in the field and in storage are shown in Table 1.

TABLE 1. Estimate of minimum dry matter losses in harvesting and storing forages at different moisture contents

Silo type	Moisture (%)	Dry matter losses (%)				
		Field	Seepage	Gaseous	Surface	Total
Conventional tower	80	2	7	9	3	21
	70	2	1	8	4	15
	65	4	...	8	3	15
	60	6	...	6	3	15
	50	8	...	5	3	16
Gas-tight tower	70	2	1	7	...	10
	60	6	...	5	...	11
	50	8	...	4	...	12
	40	11	...	4	...	15
Trench or bunker, no cover	80	2	6	10	6	24
	70	2	1	9	9	21
Trench or bunker, covered	80	2	4	9	2	17
	70	2	1	7	3	13
Stack, no cover	80	2	7	10	11	30
	70	2	1	11	19	33
Stack, covered	80	2	5	8	2	17
	70	2	...	7	4	13

Sources: Adapted from Hoglund (1964)

Changes During Ensiling

Significant changes occur in the nitrogen fractions of forages during wilting and ensiling. Chemical transformations during fermentation include production of organic acids by bacterial breakdown of soluble carbohydrates (McDonald and Whittenbury, 1973) and breakdown of protein to nonprotein nitrogen (NPN) (Hughes, 1970; McDonald, 1981; Stallings et al., 1981). Kemble and Macpherson (1954) observed during a three day wilting period, over 20% of the total plant protein was degraded to NPN. Waldo et al. (1973) found that wilting decreased insoluble nitrogen content from 63 to 53% of total N. Another problem of wilted silage is overheating and production of heat damaged protein. It has been estimated that the true protein of ensiled grass broken down during fermentation decreases from over 80% in the growing plant to about 50% in the silage. Beever et al. (1977) estimated about 80% of the crude protein of grass silage is degraded in the rumen. Merchen and Satter (1983b) reported estimated rumen degradability of 75 and 77% for baled hay and low moisture (47% DM) alfalfa silage, respectively. Prange et al. (1984) reported apparent rumen degradability of crude protein was 80% for both alfalfa silage and baled hay; indicating both forage sources were equal in ability to supply protein to the small intestine,

even though nitrogen solubility for hay and silage were 40 and 63% of total N, respectively.

Extent of proteolysis and nitrogen distribution decreases with increasing DM content of silage. Hawkins et al. (1970) found that protein in the plant had been more extensively converted to water soluble compounds during fermentation at low DM levels than at high DM levels. Alfalfa used in their study was ensiled at 22, 40, 45 and 80% DM. The water soluble N decreased from 68 to 24% of total N as DM increased. Water soluble NPN decreased as silage DM increased, with over 50% of the total N in direct cut silage (22% DM) being in this fraction. Roffler et al. (1967) also reported more extensive protein breakdown in lower DM silages during ensiling. Ammonia-N content was much greater for silage with DM content 25 to 35% compared to silage with 50% DM.

Ammonia treatment of chopped whole corn plant at ensiling to increase nitrogen content of the silage has become common practice in the United States. Milk production of cows fed ammonia-treated corn silage is equal to or greater than that of cows fed control or urea-treated silages (Huber et al., 1973; Huber and Santana, 1972; Huber et al., 1979; Heinrichs and Conrad, 1984). In addition to increasing the nitrogen content of corn silage, ammonia treatment at ensiling increases concentration of insoluble nitrogen in the material following fermentation relative to untreated con-

trols (Huber et al., 1980). The increase in insoluble N was due to inhibition of proteolysis of plant protein as well as direct incorporation of the added ammonia into the insoluble N fraction (Huber et al., 1980; Johnson et al., 1982). Kung et al. (1984) reported addition of ammonia inhibited proteolysis of alfalfa silage as indicated by increased insoluble N and lower free amino acids. Thus, ammonia may allow ensiling of wetter material to reduce field and leaf losses and also to decrease extensive degradation of nitrogen commonly found in high moisture silages.

As mentioned previously, fermentation reduces the true protein fraction in silages and increases soluble NPN. Silages, especially those of high DM, are susceptible to the browning reaction with resultant formation of nitrogen containing compounds that are largely unavailable to animals consuming them (Thomas et al., 1972; Yu and Thomas, 1975; Goering et al., 1972).

Several terms have been given to reactions occurring in feedstuffs when amino groups ($-NH_2$) of amino acids, peptides, and proteins react with other compounds, especially those containing a carbonyl group (-COH), when exposed to heat. These include the Maillard reaction, nonenzymatic browning, or the browning reaction. This reaction involves condensation of sugar residues with amino acids followed by polymerization to form brown substances of about 11% nitrogen (Van Soest, 1982). Amino acids most commonly involved in

this reaction are lysine, methionine, cystine, and the aromatic amino acids (phenyalanine, tyrosine, and tryptophan). Detailed discussion of the complex reactions have been presented by Hodge (1953), Adrian (1974), and Stallings (1979). The end result of this reaction is a decrease in the availability of protein by the formation of indigestible compounds. The reaction tends to cause development of a caramel, tobacco-like odor and a darker color.

The degree of browning a forage undergoes during ensiling is quite variable and is dependent on factors such as species, moisture content, maximum temperature developed or applied, duration of heating, pH, and method of preservation (Goering et al., 1973; Middleton and Thomas, 1983). Van Soest (1965) proposed that an assay of the nitrogen in acid detergent fiber would be a sensitive assay for heat damage caused by the Maillard reaction. Fresh forages were found to contain 7% of total N as acid detergent insoluble nitrogen (ADIN). Using this information, Goering et al. (1972) stated that twice that amount would clearly indicate heat damage. Field studies conducted in Michigan (Kung et al., 1982; Thomas et al., 1972), Minnesota (Pierson et al., 1971), and Pennsylvania (Goering and Adams, 1973) have shown from 27 to 40% of silage samples had ADIN greater than 15% of total N.

Many studies (Yu and Thomas, 1975; Goering et al., 1971; Yu and Viera, 1977; Thomas et al., 1982) have found a negative relationship between extent of heat damage and nitrogen

utilization or digestibility. Yu and Viera (1977) artificially heated alfalfa haylage (47% DM) in sealed containers at 88 degrees C for 24 or 48 h. Acid detergent insoluble nitrogen increased from 7.7% of total N in unheated haylage to 15.2% for 24 h heated haylage and 24.1% in haylage heated for 48 h. Although voluntary intake of haylages by sheep was not different, relative intake of heated haylage, in cafeteria trials, was lower than unheated haylages. Apparent digestibility of DM decreased from 61.3% in unheated to 56.5% for 24 h heated to 49.4% for 48 h heated forage. Digestibility of N decreased from 69.8% to 55.8% to 47.6% for the respective haylages. Nitrogen digestibility and N retention were negatively correlated with ADIN expressed as a percent of total N ($r = -.97$ and $-.52$, respectively). Goering et al. (1972) determined a relationship between various measurements of heat damage in 44 forage samples and in vivo digestibility. Acid detergent insoluble nitrogen (% of total N) explained 86% of the variation in N digestibility. Yu and Thomas (1976) compared 30 in vitro measurements to predict forage quality. Simple regression analysis suggested ADIN (% total N) was the most reliable predictor for in vivo N digestibility ($r^2 = .86$).

Yu and Thomas (1975) evaluated the effect of vertical positions within 10 concrete tower silos on heat development, chemical composition, and in vivo response of alfalfa haylages. With no application of special silo covers, the

greatest temperature (over 50°C) consistently occurred near the top area of silos compared to other vertical levels. The high temperature at the top was suggested to be caused by aerobic fermentation which leads to reduced DM recovery. Haylage from the top had the greatest amount of cell walls, lignin, and unavailable N and lowest digestibility of N by sheep as compared to haylages from the middle and bottom portion of the silos. The extent of heating was positively correlated with ADIN, expressed as a percent of DM or total N, ($r = .72$ and .80, respectively). The extent of heating was negatively correlated with digestibility of DM and N, and N balance ($r = -.33$, $-.81$, and $-.49$, respectively).

Several studies have been made comparing silo structure with the extent of heat damage. Shelford (1982) found greater ADIN of silages stored in oxygen limiting (25.9% of total N) compared to conventional tower silos (19.7% of total N). Thomas et al. (1972) found no differences in ADIN expressed as a percent of total N between silages stored in oxygen limiting, cement stave, sealed concrete, and bunker silos. Kung et al. (1982) found lower ADIN in alfalfa silage samples from sealed compared to concrete stave or bunker silos (10 vs. 16 and 14% of total N, respectively).

II. BY-PRODUCT FEED UTILIZATION

It has been estimated that approximately 34 million tons of by-product feed ingredients were used in the manufacture of livestock feeds in 1981, representing about 20% of the feed concentrates consumed (Chase and Udedibie, 1983). Examples of by-product feeds commonly used by the feed industry include dried and wet brewers and distillers grains, beet pulp, wheat bran, wheat middlings, molasses, corn gluten feed, citrus pulp, and cottonseed hulls. Availability and utilization of whole cottonseed as a feedstuff for dairy cattle has increased significantly during the past ten years. Since cottonseed is relatively unique in being both a high fiber and potentially a high energy feedstuff, it commonly is included in diets for cows in early lactation (Smith et al., 1981). The remaining discussion will focus on the use of distillers and brewers grains.

Brewers and distillers grains have received much interest in recent years as feedstuffs for ruminants, in part because of their potential rumen bypass (Merchen et al., 1979; Waller et al., 1980). Estimation of undegraded protein for dried distillers grains, dried brewers grains, and wet brewers grains were 55, 50, and 45% of total protein (Satter et al., 1979; Satter and Stehr, 1984). Thus, distillers feeds can be considered as a source of naturally occurring protected protein for ruminants. The basis for this classification is that protein present in distillers feeds is of grain origin which is less readily degraded in the rumen,

also heat applied to grains in drying would increase protein bypass (Klopfenstein et al., 1976, 1978).

Distillers feeds are made from the residues that remain after cereal grains (corn, rye, barley) have been fermented by yeast and the alcohol has been removed. Starch in the grain is converted to alcohol, carbon dioxide, and other carbohydrates, while the remaining nutrients (proteins, fats) are concentrated. Steps involved in processing include grinding and mixing of the grains with water, then cooking and cooling. Ground barley malt is added to convert starch to sugar, yeast is added to ferment. The fermented mash is then distilled to remove the alcohol. Wet stillage (6% solids) is then separated into a liquid portion and a coarse fibrous material by either screening or by centrifugation. The liquid is concentrated in evaporators to a syrup (condensed distillers solubles). Dried distillers grains is produced by drying the coarse material on rotary steam dryers. Dried distillers grains with solubles is the product that results when condensed syrup is combined with the coarse material and dried in either rotary steam tube dryers or air dryers (Distillers Feed Research Council, 1984). Production of distillers feeds in 1981 was over 500,000 tons.

As a result of the high energy costs associated with producing dried by-products, there has been interest in the potential of using wet or partially condensed ingredients in ruminant rations. A number of studies have compared the

relative feeding value of wet versus dried products; these reports have primarily been with either brewers or distillers grains (Porter and Conrad, 1975; Murdock et al., 1981; Firkins et al., 1984; Polan et al., 1985; Rogers et al., 1986). Studies indicate the wet by-products are at least equal to the dried product in terms of animal performance. Murdock et al. (1981) compared diets supplemented with wet brewers grains or soybean meal and found cows fed wet brewers grains produced slightly more milk and milk fat with protein levels of 100 and 118% of NRC. Polan et al. (1985) compared responses to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal on milk production and related traits in Holstein cows. Milk production (kg/d) for cows fed dried (29.4) and wet brewers grains (28.9) was higher than soybean meal (26.2) and basal diets (23.1). Rogers et al. (1986) studied the effects of feeding wet and dried brewers grains on N utilization and rumen fermentation in steers. Results showed increased N retention with wet versus dried brewers grains. There was also a reduced rumen microbial population and lower rumen ammonia concentration with dried brewers grains, due to its decreased protein solubility. Protein solubility averaged 13.4% of total N for wet brewers grains and only 3.3% of total N for dried grains. Several other studies have been made as cited by Satter and Stehr (1984). Results of five of six reported studies showed a slight advantage in milk production (2.7%) when cows were

fed dried distillers grains with solubles compared to cows fed soybean meal.

A potential problem with the use of brewers and distillers by-products is a large portion of the protein may be in the ADIN fraction and thus unavailable to the animal. It is not uncommon for the ADIN fraction to represent 15 to 30% of the total protein in many by-product feeds. Chase (1982) reported ADIN content of 15% of total N with a range 10.8 to 21.0% of total N for 11 samples of wet brewers grains. Van Soest and Sniffen (1984) reported ADIN content of 20, 31.1, and 11.8% of total N for dried distillers grains, dried distillers grains with solubles, and dried brewers grains, respectively. Another potential problem of using by-product feeds is the large variation in nutrient content. Chase (1982) analyzed 20 samples of wet brewers grains for dry matter, crude protein, and acid detergent fiber, and 11 samples for soluble N and unavailable N (ADIN). Means and ranges were 23.6 (15.2-34.2%) for DM; 28.6 (23.4-33.6% on a DM basis) for CP; 24.8 (16.7-30.2% DM basis) for ADF; 38.5 (19.2-59.8% of total N) for soluble N; and 15.0 (10.8-21.0% of total N) for ADIN. Van Soest and Sniffen (1984) reported the following means and ranges for dried distillers grains without and with solubles respectively: crude protein, 25.4 (23.1-27.2%) and 29.3 (27.1-31.7% DM basis); soluble N, 6.0 (3.0-8.0%) and 20.0 (14.0-26.0% of total N); and ADIN, 20.0 (10.0-38.0%) and 15.0 (7.0-24.0% of total N).

III. PROTEIN UTILIZATION

Digestion and Metabolism of Proteins

Requirements for protein by ruminant animals is second in quantity only to energy, thus an understanding of nitrogen metabolism and utilization is very important in ruminant nutrition. Many excellent reviews of N metabolism have been published in recent years (Chalupa, 1975; Chalupa, 1977; Clark and Davis, 1980; Huber and Kung, 1981; Satter and Roffler, 1975; Tamminga, 1979). Recent research has emphasized the importance of knowing characteristics of feedstuff proteins as they are degraded in the rumen. Dietary nitrogen available to ruminants consists of proteins, which vary in solubility, degradability, and amino acid content, and nonprotein nitrogen components. Nonprotein nitrogen includes free amino acids, peptides, nucleic acids, free ammonia, urea, nitrates, Maillard products, and other nitrogen-containing compounds. Chemically, feed nitrogen can be fractionated into several components, but their biological significance to ruminants can not be measured unless its changes in the gastrointestinal tract are known (Van Soest and Sniffen, 1984). Available feed nitrogen can undergo changes in the rumen to a variable extent through

proteolysis, deamination, and microbial protein synthesis from simple nitrogen compounds (Chalupa, 1984). To estimate the biological value of a given source of nitrogen for ruminants, it is necessary to know its potential as a source of nitrogen for rumen microbes and as a source of amino acids available post-ruminally (Van Soest and Sniffen, 1984).

Dietary protein that passes to the abomasum has been described in (NRC (Ruminant Nitrogen Usage), 1985) as "by-pass" or "undegraded" protein. Dietary protein consists of two fractions; these are 1) protein that resists microbial attack in the rumen, and 2) protein that evades attack in the rumen and passes to the abomasum without thoroughly mixing with ruminal contents. The term "undegraded" should be used with the first fraction, while "bypass" should be used with the second fraction.

Protein degradation in the rumen occurs by hydrolysis of the peptide bonds (proteolysis), resulting in production of peptides and amino acids; peptides are degraded further to free amino acids. Free amino acids are either incorporated into microbial protein, degraded to ammonia, carbon dioxide, methane, and volatile fatty acids, or they serve as sources for branched chain fatty acids which are growth factors for a number of bacterial species including the cellulolytic bacteria. Although rumen microorganisms are very active in proteolysis, substantial amounts of ingested protein are resistant to degradation and thus pass through

the rumen unchanged (Chalupa, 1975). Conclusions of review papers (Satter and Roffler, 1975; Smith, 1969) indicate that as little as 40% or as much as 80% of the dietary protein normally might be degraded in the rumen and utilized for microbial protein synthesis. Figure 2 depicts the overall scheme of nitrogen metabolism in the ruminant.

The extent of protein degradation in the rumen will depend upon microbial proteolytic activity, microbial access to the protein, and rumen turnover (NRC, 1985). Many factors influence protein degradation in the rumen including protein structure, rumen retention time, factors which affect rumen retention time, chemical treatment of feeds, and feed processing such as pelleting, extrusion, steam rolling and flaking, and feed storage.

Classification of Feed Nitrogen

Feed nitrogen can be classified into 3 groups of biological significance: soluble NPN, true protein, and unavailable protein. These fractions have been described as fraction A (soluble), B (slowly available), and C (bound), respectively (Pichard and Van Soest, 1978). The A fraction consists of NPN or protein that is degraded very rapidly in the rumen. The B fraction consists of proteins that are degraded at a rate similar to the rate of passage (.02 to .07/h). The C fraction consists of bound or unavailable

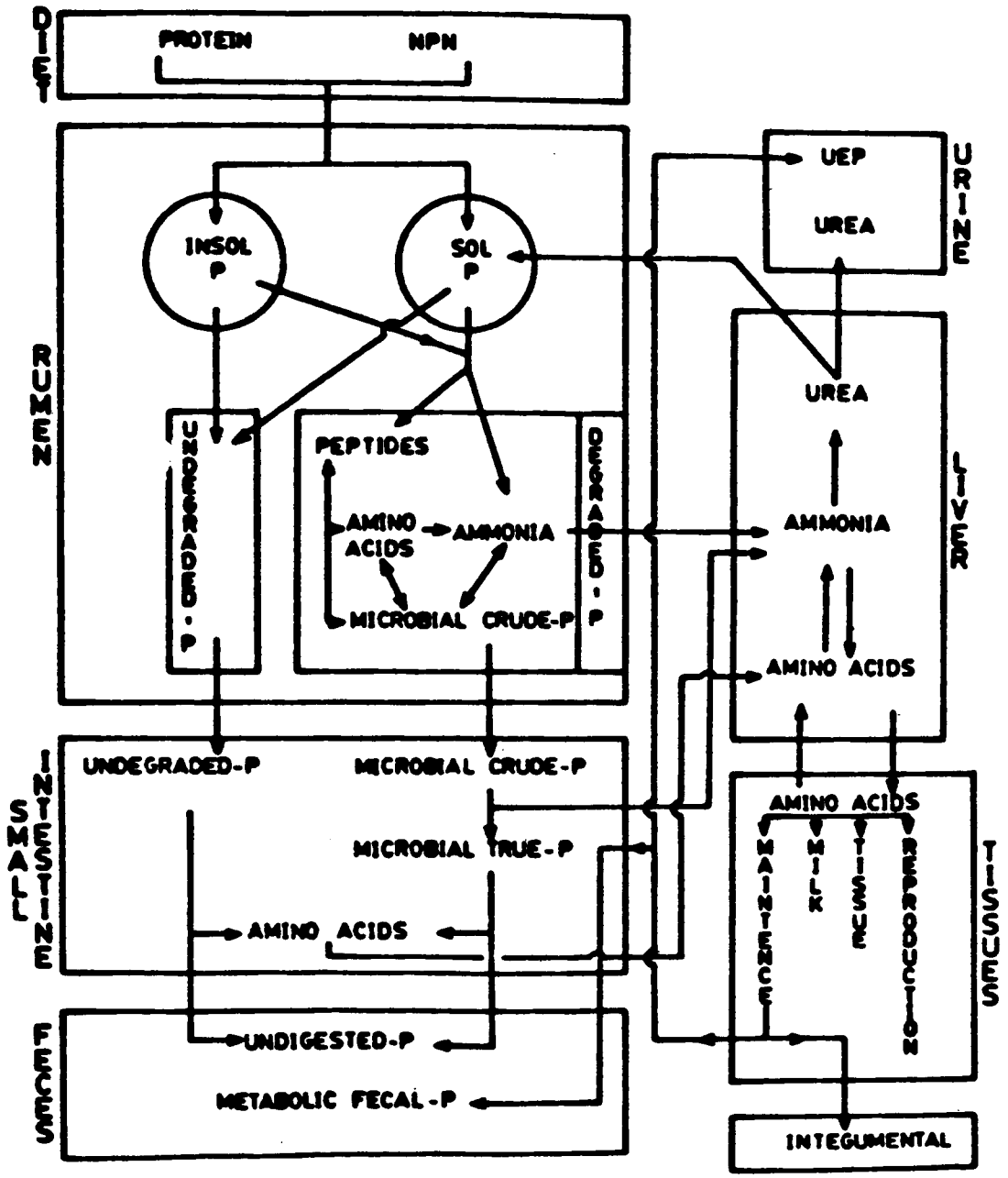

Figure 2. Protein digestion and metabolism in lactating dairy cows. Adapted from Chalupa, 1984.

protein that is not degraded in the rumen. The bound or unavailable fraction is composed of Maillard products, lignified nitrogen, and tannin-protein condensates. Of the unavailable fraction caused by heat damage, Maillard products are the most significant and occur in many processed by-product feeds and low moisture silages as previously discussed. In fermented feeds, fractions A and C are formed by depletion of protein from fraction B.

Available true protein (B fractions) are the only feed nitrogen fractions relevant to rumen nitrogen escape estimates. The B fraction has been subdivided further based on the rate of degradation. The very rapidly degraded B fraction has been labelled B_1. The B_2 fraction is intermediate in rate of degradation and are typified by the glutelin proteins found in small grains. The B_3 fraction is slowly degraded in the rumen and are typified by prolamin proteins such as zein protein found in corn. Pool sizes of these fractions in feedstuffs can be determined by slope intercepts (Pichard and Van Soest, 1978). Knowledge of pool sizes, degradation rates, and passage rates are needed to quantitate protein degradation in the rumen. Krishnamoorthy et al. (1982) found pool sizes of the various protein fractions varied considerably among feedstuffs. For example, corn showed a single slow digesting pool (B_3), soybean meal showed two faster digesting fractions (B_1 and B_2), while timothy and brewers grains exhibited B_1, B_2, and B_3 fractions.

Methods of evaluating feed proteins include in vivo (sampling of contents from the abomasum or duodenum), in situ (incubation of feeds in dacron bags suspended in the rumen), and in vitro (laboratory) techniques. Proposed new protein systems (Waldo and Glenn, 1984; NRC (Ruminant Nitrogen Usage), 1985) require the separation of dietary protein into a ruminally degraded fraction and an undegraded fraction. This division of dietary protein has routinely been done with the use of duodenally cannulated animals, techniques to separate bacterial and protozoal protein and undegraded dietary protein, and measurements of total protein flow. Although this method remains the reference method, because of its complexities, much research has been done to find simpler techniques for routine feed analysis.

PROTEIN DEGRADABILITY

IN VIVO MEASUREMENT OF PROTEIN DEGRADABILITY

In vivo estimation of ruminally degraded and postruminally digested feed nitrogen by animal feeding experiments is expensive, time consuming, and labor-intensive; although values obtained are essential in comparing in vitro and in situ measurements.

In vivo measurements require the use of surgically prepared animals with cannula in the abomasum or proximal duodenum. There is also need for a reliable technique for calculating total digesta flow to the small intestine and one which would allow an estimate of the contribution of protein from microbial origin (Stern and Satter, 1982). Procedures for estimating microbial protein involve use of markers such as radioisotopes, diaminopimelic acid (DAPA), nucleic acids, and 2-amino-ethylphosphonic acid (Stern and Hoover, 1979). Estimates of microbial protein are subject to errors inherent in the techniques used. For example, DAPA is found only in some rumen bacteria, thus protozoal protein is not included in the estimate of microbial protein.

Two methods have been used for estimating dietary protein degradation in vivo: the "regression technique" and the "by difference method". With the regression technique, the proportion of undegraded protein can be estimated from the relationship between duodenal protein flow and protein intake. This technique involves adding incremental amounts of test protein to a ration while holding dry matter intake constant. Contribution of microbial protein is considered equal across rations. Duodenal amino acid flow is then regressed on amino acid intake; the slope is representative of undegraded test protein (Stern and Satter, 1982). The "by difference method" involves measurement of dietary protein intake and total protein flow to the duodenum, estimation of

microbial and endogenous protein at the duodenum, and calculation of undegraded dietary protein (UDP) by difference. Thus, UDP = duodenal protein - (microbial protein + endogenous protein). However, estimates of endogenous N are difficult to obtain and in most instances ignored, resulting in an overestimation of undegraded dietary protein (Stern and Satter, 1982).

These two techniques are the most used to predict in vivo protein degradation in the rumen. The regression technique allows calculation of degradability values for individual feedstuffs. The by difference technique allows calculation of degradability values for complete diets.

IN SITU BAG TECHNIQUE

While the use of cannulated animals can yield estimates of protein degradability, the method is labor intensive, time consuming, and costly. An alternative technique which provides relatively rapid and reliable estimates of ruminal protein degradability for a wide variety of feedstuffs is the dacron bag technique. The bag technique has been used extensively (Orskov et al., 1981; Mathers et al., 1977; Wilson and Strachan, 1981; Stern and Satter, 1984) to estimate and characterize dietary protein degradability. Dacron bags containing feedstuffs are suspended in the rumen for different periods of time. After incubation, residues are analyzed

for chemical components. Disappearance of chemical components in the bag is then regressed on time and a rate of disappearance is determined. Earlier work used nylon bags for this procedure; however, most present research utilizes dacron polyester bags which have the advantage of containing no nitrogen (Broderick, 1982).

Mehrez and Orskov (1977) evaluated the artificial fiber bag technique for assessing the proportion of dietary DM and N which disappears in the rumen. The most important factor determining the variability in disappearance from bags incubated together was the sample in relation to bag size. Mathers et al. (1977) used the bag technique to study nitrogen disappearance and extent of protein degradation in feedstuffs. Bags were suspended in the rumen for 2, 4, 6, 8, 12, and 24 h. At short incubation times (4-6 h), the bag technique gave estimates of N disappearance similar to in vivo estimates of degradation in the rumen. Extent of degradation of protein in the rumen is important because it determines not only the amount of N available for use by the rumen microbes, but also the amount subsequently made available for digestion postruminally.

Grummer and Clark (1982) estimated degradation of protein supplements and complete diets by measuring DM and N disappearance from dacron bags suspended in the rumen of lactating, fistulated, Holstein cows. Twenty-eight dacron bags (12 x 7 cm) with pore size of 20 to 75 microns and con-

taining 2.0 g air-dry protein supplement or complete diet were placed in the rumen at the morning feeding. Bags were removed in duplicate after 0, 1, 2, 4, 8, 12, and 16 h incubation. Supplements showed three distinct rates of degradation for DM and N. A rapid initial loss of DM and N occurred from 0 to 1 h of incubation; they suggested this represented physical washout and disappearance of soluble N and carbohydrate. A second, slower rate occurred during 1 to 4 h incubation; this was termed the "lag" phase of degradation. An intermediate rate of degradation occurred from 4 to 16 h of incubation, which may represent microbial degradation of N and DM. Nocek et al. (1979) observed only two rates of degradation for DM and N. The absence of a lag phase may have been due to rinsing of the dacron bags prior to placement in the rumen or to the time and frequency that bags were removed from the rumen. Grummer and Clark (1982) suggested after rapid loss of soluble materials from bags, a lag phase followed in which rumen microbes attach to feed particles and begin to degrade them. They also stated that because the bags were placed in the rumen at feeding, the lag phase may have been due to high ammonia concentration in the rumen which normally occurs 0 to 4 h postfeeding. Complete diets showed two distinct rates of degradation for DM and N. A rapid disappearance occurred during 0 to 1 h, whereas a slower rate was evident during 1 to 16 h. The rapid rate was suggested to represent loss of soluble N and carbohydrates

and the slower rate was suggested to represent microbial degradation of the less-soluble components of dietary feedstuffs. They concluded the dacron bag technique provides useful information about rate and extent of protein degradation in the rumen with time. Mangan (1972) suggested degradation is a two-phase process; the first part is rapid and represents primarily soluble protein. The second phase represents microbial degradation of the less-soluble portion of the feed protein, which is considered to be the potentially undegraded protein.

A method of interpretation of in situ data was proposed by Orskov and McDonald (1979) where only the curve describing the slower phase of N disappearance was used to estimate protein degradation and escape. The proportion and degradation rate of the more slowly disappearing fraction (fraction B) was quantified from regressing on time the log of the fraction of N remaining in dacron bags. Slope and intercept of the regression corresponded to degradation rate (k) and proportion of the more slowly degraded fraction (B).

Stern and Satter (1984) evaluated the relationship between N disappearance from dacron bags, N solubility, and in vivo crude protein degradability for 34 total mixed rations containing various dietary N sources. Solubility of N was highly correlated with N disappearance from bags at 1 h of rumen exposure; however, as exposure time increased, the correlation progressively decreased. The relationship be-

tween in vivo CP degradability and N disappearance from dacron bags was not significant until 12 h of rumen exposure (.50) and increased to 0.68 at 24 h. The correlation between N solubility and in vivo CP degradability was 0.26, indicating N solubility was not a reliable indicator of CP degradability in the rumen.

An advantage of the dacron bag technique compared to laboratory methods is involvement of digestion processes that occur in the rumen. A number of factors in this technique may affect results and its use may lead to serious errors in interpreting data. Factors include porosity of bag material, sample weight to bag surface area ratio, particle size of sample, method of bag placement in the rumen, animal diet, and degree of bacterial attachment in the bag (Mathers and Aitchison, 1981; Stern, 1985; Nocek, 1985).

Nocek (1985) made the following conclusions in a study with soybean meal on the conditions and procedures used in the dacron bag technique:

1. Ruminal introduction of bags in reverse order decreased variation for in situ digestion profiles,
2. Use of a standard ingredient detected time related variation,
3. Bacterial N contamination did not affect N digestion rate constant,
4. Presoaking: disappearance of soluble DM and N are affected by particle size, sample weight to

bag surface area, and bag porosity,

5. Particle size: grinds of 1, 2, 5 mm or unground had no influence on DM or N digestion,
6. 12.6 mg/cm^2 feed weight to bag surface ratio compared most favorably with literature in vivo estimates for ruminal N digestion, and
7. Pore size of 40 to 120 micron were similar and higher in estimated ruminal protein availability than smaller pore sizes, and compared favorably with in vivo literature estimates.

Although the use of rumen cannulated animals for determination of rate of protein degradation is possible in a research environment; a method which can be used routinely in laboratories concerned with feed evaluation must be developed.

IN VITRO METHODS

Amino Acid Plus Ammonia Release

Broderick (1978) reported use of a new in vitro system for estimating ruminal protein degradation rate. An inhibitor of amino acid and ammonia metabolism is added to an inoculum containing strained ruminal liquor. The purpose

of the inhibitor (hydrazine) is to prevent amino acid and ammonia removal by rumen microbes. This method has not met with much success due to its complex procedure and manipulation of data.

Commercial Proteases

Pichard and Van Soest (1978) proposed the use of a commercial protease from Streptomyces griseus, which has broad specificity for cleavage of peptide bonds, to estimate ruminal protein degradation. Recent techniques developed for measurement of actual degradation of feed protein by rumen microbes are fairly complex (Broderick, 1978; Mahadevan et al., 1980), but a protease procedure using semipurified proteolytic enzymes has been evaluated and shows promise as a laboratory technique for predicting ruminal degradation of dietary protein (Krishnamoorthy et al., 1982a, 1982b, 1983; Nocek et al., 1983; Poos-Floyd et al., 1985; Poos et al., 1980; Pichard and Van Soest, 1978).

Digestion of feed protein with protease allows the measurement of rate (Krishnamoorthy et al. 1982, 1983) and maximal extent of hydrolysis (Pichard and Van Soest, 1978). Calculations from the curve of digestibility obtained by sampling at appropriate times allow the partitioning of protein into rumen degradable (fast digesting) and that likely to escape the rumen (slow digesting). Rumen proteases do not

saturate the substrate under normal feeding conditions so that too high a concentration of enzyme in vitro will overestimate rumen degradation (Van Soest and Sniffen, 1984). Estimation of the undegradable residues may be compared with ADIN, which is often lower than the protease derived value. However, the values are very highly correlated with each other. Data obtained from dacron bag studies are essentially similar, except that the residue may be contaminated with attached microbial cells, causing undigested nitrogen to be overestimated (Van Soest, 1984).

Krishnamoorthy et al. (1982, 1983) used a protease system to estimate ruminal degradation. Maximum rumen proteolytic activity at maintenance was simulated in vitro using protease from S. griseus. Feed samples whose escape N had been estimated in vivo through duodenally cannulated cows were subjected to in vitro proteolysis for 18 h (concentrates) and 48 h (forages) to reflect maximum mean retention time of those feeds in the rumen. The in vivo escape N and in vitro estimated escape N were highly correlated ($r = .84$). Results suggest potential of the in vitro protease technique to estimate rumen escape N in feedstuffs. Nocek et al. (1983) concluded that the protease enzyme from S. griseus could be used to determine percent of protein escaping degradation in the rumen. Poos-Floyd et al. (1985) found the enzyme assay was more closely related to in vivo protein escape than any of five solubility techniques evaluated.

Several different proteases have been used to estimate rumen escape N. Enzymes used were selected for maximum activity in the pH range 5 to 8 and temperature range 35 to 45 degrees C as normally found in the rumen. Enzymes used include a bacterial protease (S. griseus, Type XIV, Sigma Chemical Co.), plant proteases, including papain (Corica papaya), ficin (Ficus glabrata), and bromelain (Ananas comosus), and a neutral fungal protease (Aspergillus oryzae).

Advantages of in vitro incubation using protease enzymes include the simplicity of the technique, no requirement for cannulated animals, no sophisticated laboratory equipment is needed, yields results in a relatively short period of time, and allows routine analysis of large numbers of samples. Also, when evaluating forage proteins, the use of enzymes eliminates the problem of microbial attachment to fiber particles that would interfere with dacron bag results (Poos-Floyd et al., 1985).

Nitrogen in Neutral and Acid Detergent Fiber

An alternative to the assay of degradability using protease is to partition true protein into that soluble and insoluble in neutral (ND) and acid detergent (AD) solution (Van Soest and Sniffen, 1984; Van Soest et al., 1982). This scheme could be easily adapted by most analytical laboratories. Partition of N fractions is as follows: Buffer in-

soluble protein that is soluble in ND is a constituent of cell solubles and would have a fast rate of digestion similar to the B_1 fraction. Protein insoluble in ND but soluble in AD is completely available, but is digested at a slower rate than the insoluble non-cell wall fraction and corresponds to the B_2 fraction. This fraction is affected markedly by heating and processing (Pichard and Van Soest, 1978). Protein insoluble in AD is unavailable and represents the C fraction.

The justification of a system based on solubility in detergent solutions is based on the large range in NDIN found in feedstuffs. Further, it can be justified based on the knowledge that feedstuffs normally contain a varying mixture of protein types of differing solubility characteristics. Also, the analysis is simpler than the protease procedure although the latter has greater accuracy.

Nitrogen Solubility

An important factor influencing the rate and extent of degradation of protein in the rumen is its solubility (Bull et al., 1977; Clark and Davis, 1980; Satter et al., 1977). The degree of solubility of plant and animal protein generally is directly correlated to the rate at which ammonia is released in the rumen (Stern et al., 1978; Wohlt et al., 1973). Solubility of feed proteins is affected by several

factors. Type of protein influences solubility. Wohlt et al. (1973) demonstrated that feed proteins composed mainly of albumins and globulins had a higher solubility than those composed mainly of prolamines and glutelins. Large variation was detected in soluble nitrogen in energy feeds (3.9 to 42.6% of total N) and protein supplements (2.8 to 93.3% of total N). Other research (Crawford et al., 1978; Pichard and Van Soest, 1978) have shown soluble protein may be degraded rapidly or slowly, and insoluble proteins are degraded more slowly, and at different rates from each other.

When considering various mixtures of feedstuffs, low solubility is not "synonymous" with low degradability because degradability is not always proportional to solubility; however, a protein is assumed to be soluble if it is to be degraded (Bull et al., 1977; Sniffen, 1980; Satter et al., 1977). Solubility of barley protein is low, ranging from 17 to 31% of total N (Majdoub et al., 1978), however, degradability determined in vivo is high, ranging from 86 to 100% (Ling and Buttery, 1978; Mathers and Miller, 1981). Estimates from in situ studies range from 69 to 89% of total N (Mathers and Miller, 1977; Mehrez and Orskov, 1978). Due to the large variation in solubility among feeds and the relation to degradability, the extent of protein degradation with mixed diets varies with diet composition (Chalupa, 1977; Lichtenwalner et al., 1978).

Solubility, determined using many different methods, exhibits much variation (Wohlt et al., 1973; Crawford et al., 1978; Sniffen et al., 1979; Crooker et al., 1978; Waldo and Goering, 1979; Krishnamoorthy et al., 1982). Solvents which have been used include water, autoclaved rumen fluid, mineral buffers based on the composition of saliva or rumen fluid, and simple salt solutions. At present, there is not a uniform or "best" method for determining protein solubility. The ranking of proteins according to solubility depends somewhat on the solvent used (Waldo and Goering, 1979; Wohlt et al., 1973). In vitro determination of protein solubility may not be a good measure of degradability of feed proteins (Broderick and Craig, 1980; Crawford et al., 1978; Mahadevan et al., 1980).

Mahadevan et al. (1980), using bacterial proteases in vitro, found that soluble and insoluble protein can be degraded at similar rates. They concluded that solubility of a protein is not by itself an indication of the protein's susceptability to hydrolysis by rumen bacterial protease. Also, they suggested the structural characteristics of the protein may be important, such as the presence of crosslinking disulfide bonds which render the protein resistant to degradation. Use of in vitro nitrogen solubility as an estimate of in vivo nitrogen degradability has shortcomings; however, the correlation between nitrogen solubility in mineral buffer and extent of digestion in the rumen for a vari-

ety of feeds is high (r^2 = .99) as reported by Henderickx and Martin (cited by Wohlt et al., 1973) who used short-term batch culture incubations.

In vitro solubility is affected by the degree of agitation, length of extraction time, temperature, pH, ionic strength, and carbon dioxide tension of the solvent (Crooker et al., 1978; Waldo and Goering, 1979; Wohlt et al., 1973). Solubility of protein of a feedstuff also varies according to the source, particle size, density, and methods of processing and pretreatment (Waldo, 1977; Satter et al., 1977). Heat treatment has been shown to reduce solubility and degradability by changing the structure of proteins, resulting in lower ammonia concentration in the rumen (Folman et al., 1981; Schingoethe and Ahrar, 1979).

The difficulty with most solubility measurements is they tend to categorize feed proteins into only two classes. The soluble protein composed mainly of albumins and globulins, also include NPN; while insoluble protein, composed mainly of prolamines and glutelins, includes unavailable bound nitrogen. There are two types of unavailable protein: that which is indigenous to the feedstuff and that which is induced by heating and drying. A problem with the concept of solubility and the tendency to use heat treatment to reduce solubility is the treatment may increase risk of heat damage and formation of unavailable nitrogen. Tables 2 and 3 show the partition of nitrogen in feedstuffs using protein solu-

bility, protease incubation, and neutral and acid detergent insoluble nitrogen fractionation schemes.

Animal Response to Regulating Protein Solubility and Degradability

Selection of feeds with low degradability or solubility may lead to an increased protein supply to the intestines and to greater animal production under practical conditions (Dingley et al., 1975). The level of soluble and/or degradable protein in the diet has gained increased emphasis as a factor involved in the metabolism of protein and NPN in ruminants (Sniffen, 1974; Wohlt et al., 1976; Forster et al., 1983; Sahlu et al., 1984). The principal underlying effect of solubility is the possibility that high levels of rumen ammonia could result from rapid microbial degradation of the soluble portion of feed protein. Increased absorption of ammonia through the rumen wall would lead to decreased efficiency in energy and N utilization because of the energy cost of converting ammonia to urea, and the loss of N in the urine, respectively. In addition, when the diet contains a given percentage of crude protein and a large amount of soluble protein, the proportion and amount of less-soluble, more slowly-degraded protein is reduced. A simple method for decreasing protein degradation in the rumen would be to formulate diets from ingredients containing protein with a natural

Table 2. Partition of nitrogen and protein fractions in feedstuffs using protein solubility and detergent procedures.

Fraction	Abbr.	Estimation	Enzymatic	Class.[a]
Nonprotein N	NPN	Not precipitable	Not applic.	-
True soluble N	BSP	Buffer soluble and precipitable	Fast	A
Insoluble	INSOL	Insoluble in buffer	—	-
Insoluble non-cell nitrogen	INSOL-NDIN	Difference between INSOL and protein in neutral detergent	var.	B_1
Neutral detergent insoluble N	NDIN	Cell-wall protein	—	-
Neutral detergent insoluble, AD soluble	NDIN-ADIN	Protein insoluble in ND, but soluble in AD	slow	B_2
Insoluble in acid detergent	ADIN	Protein insoluble in acid detergent includes heat-damaged protein and N associated with lignin	indigestible	C

[a]Classification according to Pichard and Van Soest (1978), and Van Soest (1982).

Table 3. Biological significance of chemical fractions of feed nitrogen (N).[a]

N fraction	Biological significance
Acid detergent insoluble nitrogen (ADIN)	Estimation of unavailable N
100 - ADIN	Est. of total available N
Protease insoluble N (PIN)	Est. of total rumen escape N
PIN - ADIN	Est. of available rumen escape N
100 - PIN	Est. of total rumen degraded N
Buffer insoluble N	Pooled est. of rapid and slow rumen degradable, available rumen escape, and unavailable N
100 - Buffer insoluble N	Est. of rapid soluble N (includes soluble NPN and soluble true proteins)
Buffer insoluble N - PIN	Est. of slow rumen soluble N (mainly protein requiring proteolytic enzymes for solubilization)

[a]From Krishnamoorthy, U., C. J. Sniffen, and P. J. Van Soest. 1982. Nitrogen fractionation in ruminant feedstuffs for feed evaluation. Proc. Cornell Nutr. Conf. P. 95.

resistance to ruminal breakdown. Other methods include processing, heat treatment, or treatment with chemical agents. Treatment with chemical agents creates a reversible, pH-dependent chemical change that will inhibit breakdown of the protein at the pH in the reticulorumen, but still enable proteolysis to occur at a much lower pH as occurs in the abomasum and proximal duodenum (Tamminga, 1979). Formulating diets for ruminants by selecting feed ingredients with low degradability or solubility has proven successful (Sniffen, 1974; Majdoub et al., 1978; Oldham et al., 1979; Kung and Huber, 1983; Orskov et al., 1981; Wohlt et al., 1976) in some studies, but not in others (Erdman and Vandersall, 1983; Grummer and Clark, 1982; Crawford and Hoover, 1984).

Dingley et al. (1975) found that increasing soluble protein intake in Holstein cows in early lactation significantly decreased supply of essential and total amino acids to the udder. This work showed that supply of amino acids to the udder and overall N utilization by ruminants are influenced by the solubility of dietary protein. Using urea as a completely soluble source of N, Aitchison et al. (1976) formulated diets differing in solubility by substituting varying amounts of urea and corn for soybean meal in the concentrate portion of a corn silage based diet. Three N balance trials were conducted in which diets of 11.7, 12.6, and 15.1% CP (DM basis) were fed in early lactation (30 to 45 d postpartum). Rations ranged in solubility from 31.5 to

48.7% of total N. At 15.1% CP, highest milk (39.1 kg) and intake of DM (22.4 kg) were for low solubility diets. Highest milk yield at 12.6 and 11.7% CP were for diets with 40 and 45% soluble protein, respectively, suggesting that lower solubility resulted in insufficient rumen ammonia. Braund et al. (1978) reduced protein solubility of the diet with the use of corn products, brewers grains, and some oil seeds. Results of a feeding trial with 20 cows showed an increase in milk yield of 2.8 kg/d in cows fed the low solubility rations. This research led to the development of controlled solubility concentrates by Agway, Inc. Majdoub et al. (1978) used 20 Holstein cows in early lactation (8 to 10 wk postpartum) to study the effects of protein solubility on protein utilization. Two levels of protein (12.6 and 15.3%) and solubility (22 and 42% of total N) were formulated by selecting from natural feedstuffs. Protein solubility had no effect on intake of DM, CP, and net energy for lactation. Highest yield of milk (27.7 kg) was obtained by feeding the high protein, low solubility diet. Kung and Huber (1983) fed cows 22 to 91 days postpartum diets of 40% corn silage (without or with ammonia treatment), 10% ground alfalfa, and 50% concentrate containing soybean meal (without or with heat treatment); diets contained 11, 14, or 17% CP, and treated silage and soybean meal were used only at the two higher CP levels. Highest milk yield was for the 17% CP diet containing ammonia-treated silage and heated soybean meal. Their

findings suggest that rumen bypass protein from heated soybean meal resulted in a milk response and ammoniated silage provided NPN necessary for microbial function. Oldham et al. (1981) compared milk production in cows given rations containing urea, soybean meal, formaldehyde-treated soybean meal, or fish meal as the major source of supplemental N, at 4 levels (10.3, 12.3, 14.3, 16.3%) of CP in the ration DM. Yield of milk and milk protein was least with urea rations and greatest with the less-degradable supplemental protein source of fish meal.

Holter et al. (1982) examined the effects of percentages of CP in diets having low or high protein solubility on feed intake, milk yield, digestibility of nutrients, and energy and protein balances in high producing cows in early lactation. Two 4 x 4 latin squares, balanced for carryover effects, were used to examine responses of cows to four equicaloric diets containing on a DM basis 11.1, 13.7, 15.7, and 19.2% CP (trial 1) or 13.8, 16.3, 18.8, and 20.9% CP (trial 2) during four consecutive 36-day periods starting 2 to 3 wk postpartum. Solubility of protein was low in trial 1 (19 to 22% of total N) and high in trial 2 (44 to 47% of total N, due to urea added to corn at ensiling). In trial 1, yields of milk were less for the diet with 11.1% CP than for diets with higher CP. Milk yields increased with increasing CP up to 15.7%. Milk yields were not significantly affected by level of CP in trial 2. In trial 1, where solu-

bility was low, DMI, intake of free water, and proportion of dietary N lost in urine were not affected by treatments. There was no evidence that excess N was flushed by the cows and wasted. In trial 2, where solubility was high, intake of free water and proportion of dietary N lost in urine was high when more than 16.6% CP was in the ration, suggesting additional water was required for urinary excretion of excess nitrogen. Results suggested there was no advantage in milk yield from feeding a high solubility, corn-based diet containing more than 13.8% CP in ration DM during the first 22 wk of lactation. However, only four cows were used and performance during the entire lactation was not measured. Janicki et al. (1985) used 34 pluriparous Holstein cows in a follow-up study to examine effects of CP (15.3 vs. 13.6%, DM basis) and soluble protein (39.7 vs. 47.9% of total N) on digestibility, and energy and protein balances during early lactation. Measurements were taken during wk 6, 10, 14 postpartum. Diets were protein supplements (varying in protein content and solubility), low-protein concentrate, corn silage treated with urea at ensiling, and wilted grass silage fed individually for ad libitum intake. Reducing ration solubility improved energy intake and productive energy factors, but resulted in no significant increase of milk yield or body tissue balances. There was no effect on overall partition of N in the body. Digestibility of carbohydrates and protein was higher for 15.3 than for 13.6% CP diets, re-

sulting in higher total digestible nutrients and metabolizable energy of DM. Concentrations of rumen ammonia and blood urea N were not influenced by protein solubility; increasing dietary CP increased concentrations of both, although not enough to increase consumption of free water. It was concluded that best digestive efficiency was for animals fed the ration of medium CP (15.3%) and low protein solubility (39.7% of total N). In a companion study, Holter et al. (1985) studied the same effects over the entire lactation. Cows fed the medium-CP diets produced 196 kg more milk than those receiving low-CP diets. Cows receiving rations with reduced solubility produced 347 kg more milk than those fed the high solubility diets. Income above feed costs for the lactation were highest and postpartum loss in bodyweight was least for cows receiving medium CP and low protein solubility rations in early lactation, but no differences were significant.

Sahlu et al. (1984) found an increase in milk production when heat-treated soybean meal (SBM) was fed to high producing cows with most of the increase in production from 4 to 8 wk postpartum. Soluble protein (14.8, 9.3, and 7.0% of total N for regular, heat-treated, and extruded SBM) and degradable protein (71.0, 68.7, and 58.7% of total N) were reduced by heat treatment. Forster et al. (1983) found a significant linear increase in milk production of cows fed a ration with 14% CP of low degradability compared to medium and high

degradability rations. Corn gluten meal was substituted for SBM to reduce protein degradability. Other studies have also reported increased milk production when feedstuffs of low degradability or solubility were fed (Netemeyer et al., 1982; Davis, 1977; Lane et al., 1978; Ahrar et al., 1977; Bull et al., 1976; Ruegsegger and Schultz, 1985).

Wanapat et al. (1982) studied the effects of varying levels of protein solubility with low quality roughages on protein metabolism in sheep. Diets with the higher proportion of soluble protein in the form of urea resulted in the greatest urinary N excretion. Higher protein solubility also resulted in lower N retention as also found in other studies (Bull et al., 1976; Wohlt et al., 1976; Majdoub et al., 1978).

Spears et al. (1985) studied the influence of level of formaldehyde-treatment of SBM on N utilization and ruminal fermentation by growing steers. Treated SBM resulted in decreased ruminal protein degradation as measured by the dacron bag technique. After 12 h rumen exposure, 56% of the N in untreated SBM had disappeared compared with only 10% for SBM treated with .3% formaldehyde. Feeding trials resulted in an increased utilization of N for steers fed treated SBM.

Erdman and Vandersall (1983) fed rations containing two levels of protein degradability and 14.5% CP in diet DM to 24 Holstein cows in early lactation. Protein degradability of concentrates used to achieve these levels were 52.9 and

72.8%. Solubility of the low and high degradability diets were 31.6 and 40.3% of total N, respectively. Diets had no effect on DMI, milk production, or milk fat content. They concluded there was no advantage to selecting feeds based on degradability in early lactation (4 to 16 wk postpartum). Hawkins and Strength (1977) found no differences in milk yield in cows fed rations which varied in soluble protein from 29 to 42% of total N. Grummer and Clark (1982), using heat-treated SBM to decrease soluble protein, fed diets varying in solubility from 24.6 to 34.1% of total N to lactating Holstein cows for a 20 wk period starting 2 wk postpartum. All cows were fed to supply 85% of their CP requirement (13.4% CP on a DM basis) and at least 100% of their energy requirement (NRC, 1978) in order to minimize the use of amino acids for energy or glucose synthesis. Milk yield and composition did not differ among treatments. These results indicate that altering the solubility of SBM supplements by heat treatment did not sufficiently improve lactation performance when protein was fed at 85% of the cows requirement and other nutrient intakes were balanced across treatments. Other studies using either heat-treated (Ahrar et al., 1979) or formaldehyde-protected protein (Lundquist et al., 1986; Crawford and Hoover, 1984) have shown no milk production response to decreasing protein solubility or degradability. Other studies have shown no effect on growth in cattle or N-metabolism in sheep when solubility was re-

duced (Burris et al., 1973; Faichney and Davis, 1972; Trotta et al., 1984).

There is much debate about the reasons for such variable results in responses seen with regulating solubility or degradability in diets. Grummer and Clark (1982) suggest the following factors may be involved: 1) more dietary N escaping degradation, 2) improved pattern of amino acids reaching the small intestines, 3) decreased NPN intake, and 4) increased soluble carbohydrate intake (which will increase microbial protein synthesis).

Crooker et al. (1983) suggested the failure to increase performance through protection of dietary proteins from ruminal degradation could result from 1) factors other than absorption of essential amino acids limit productivity, 2) protected protein is of low biological value, 3) inadequate protection, 4) overprotection of protein, 5) protected protein is naturally resistant to microbial degradation, and 6) microbial production in the rumen is decreased.

As shown in the previous discussion, protein degradability and solubility in the rumen are important factors that need to be considered when formulating rations for ruminants. An in vitro procedure that estimates rumen degradation would be useful to the nutritionist in balancing rations.

IV. FIBER UTILIZATION

The fiber or roughage content of the ruminant diet plays an important role in regulating rate of passage, rumination and ensalivation, pH of the rumen, efficiency of feed utilization, and in lactating dairy cows, the percent fat in milk (Welch, 1982; Van Soest and Mertens, 1984). Fiber refers to the residue of a feed that is resistant to acid and alkali treatment and consists of cellulose, hemicellulose, and lignin (Van Soest, 1966).

Crude fiber analysis was invented more than 150 years ago to represent the indigestible part of feedstuffs. The procedure isolates cellulose with varying amounts of pectin, hemicellulose, lignin, nitrogenous artifact lignin, silica, and cutin. Crude fiber is supposed to represent the truly indigestible portion of feedstuffs, however, in some studies crude fiber digestibility was greater than the soluble carbohydrate fraction (nitrogen-free extract) (Van Soest, 1975). Due to the problems associated with variability in recovery of hemicellulose and lignin, other methods of fiber determination have been developed using detergents (Van Soest and Wine, 1967; Van Soest, 1966).

The detergent system allows partition of plant fiber into hemicellulose, cellulose, and lignin. Extraction of feedstuffs with neutral detergent determines total cell-wall. Extraction with acid detergent solution measures cellulose, lignin, lignified N compounds, heat-damaged proteins,

keratin, and silica. Hemicellulose is estimated by NDF - ADF, and ADF - lignin estimates cellulose (Van Soest, 1966). Neutral detergent fiber consists of hemicellulose, cellulose, lignin, and insoluble ash. Neutral detergent fiber gives a close estimate of the fiber constituents of feeds and is highly correlated to intake. Van Soest (1965) found NDF was highly correlated to intake based on 41 alfalfa and 230 grass observations ($r = -.65$ and $-.79$, respectively). Neutral detergent fiber separates those feed components that are indigestible or slowly digestible from those that are essentially completely digestible (Goering and Van Soest, 1970).

Neutral detergent fiber is the most useful predictor of energy availability because it not only is related to digestibility but also is related to the decline in digestibility of slowly digested compounds that are associated with changes in rate of digestion and passage through the digestive tract (Van Soest, 1975; Van Soest and Mertens, 1984). Neutral detergent fiber has also been shown to be related to all the factors which are associated with the intake limiting characteristics of the diet, including bulk density, digestibility, roughage to concentrate ratio, rumination, total chewing time, and rate of passage (Jorgensen et al., 1981). Since NDF is related to all of these factors, it may provide the most accurate and easily measured feed characteristic for formulating dairy rations (Mertens, 1980). A large difference between legumes and

grasses is greater content of hemicellulose in grass. The greater content accounts for higher cell-wall content of grasses and probably to some extent for their lower intake relative to legumes (Van Soest, 1977).

The relationship of feed intake to NDF content of the feed is related to several factors as noted by Mertens (1983). These include 1) NDF is related to the space occupying or bulk density of feeds, 2) NDF measures feed components that have the slowest rate of disappearance (both passage and digestion) from the tract, and 3) NDF is related to the rate of particle size reduction that must occur before feed can escape the rumen. Thus, for low quality diets, intake decreases as NDF content increases due to the restriction associated with gut fill. However, with high quality diets, the opposite effect on intake is apparent, because of the inverse relationship to digestibility. Thus over a range of feed quality, the relationship between NDF content and DMI is curvilinear (Mertens, 1983).

Mertens (1980, 1983) conducted experiments to study the effect of total ration NDF concentration on lactating cow performance and to determine if there is an optimum NDF content that is constant between forage quality and result in maximum feed intake and milk production. Forages used were alfalfa hay, corn silage, and coastal bermuda grass hay. Optimum milk production was obtained at 34 to 36% NDF in the total ration with all diets. Feeding more grain to give

lower NDF reduced fat corrected milk due to lower fat percent and reduced feed intake. The fiber requirement for maintaining milk fat percent has often been quoted as 15% crude fiber or 17 to 19% ADF (Mertens, 1980). Such values only refer to the composition and intake of a specific forage and concentrate in which the measurements were made. At 36% NDF, which achieved maximum milk production in the 3 forage diets, the alfalfa ration contained 26% ADF, corn silage was 19% ADF, and bermuda grass was 15%. These differences occur because the content of hemicellulose varied in the respective forages. Hemicellulose is an important part of fiber which is overlooked by ADF or crude fiber determinations. That intake of alfalfa diet was greater at equal NDF content shows the differences in quality of the forages. As far as a fiber requirement (in dietary concentration), all three forages were equal; but in supporting milk production, alfalfa ranked above corn silage and both over bermuda grass.

The extent of digestion of fiber is determined by the rate of digestion and the time the residue remains in the digestive tract. Time is determined by rate of passage and the capacity of the digestive tract. Thus, extent of digestion is set by the competing rates of digestion and passage. Rate of digestion is predetermined by the chemical and physical constituents of the feed, leaving passage or intake the only variables that can be set by the animal in response to the diet. The result is a decline in digestibility with

increasing intake of a fibrous feed. Since fiber is the slowest digesting fraction in feed it largely accounts for the depression (Van Soest, 1977; Mertens, 1985).

Waldo et al. (1972) proposed a model for cellulose digestion which assumed cellulose could be divided into potentially digestible and indigestible fractions. Mertens and Ely (1979, 1981) and Mertens and Loften (1980) proposed a dynamic model of fiber digestion and passage. In the model, NDF is divided into three fractions: fast-digesting (>.02/h), slow-digesting (<.02/h) and indigestible. While the indigestible fraction can escape the digestive tract by passage only, the digestible fraction can disappear by both passage and digestion.

Mertens (1977) suggested that the kinetics of fiber digestion of forages is divided into four components: digestion lag, rate of digestion, rate of passage, and potential extent of digestion. Smith et al. (1971) and Mertens (1977) concluded the rate of digestion of the potentially digestible cell-wall fraction can be quantitated by first-order kinetic rate constants.

With the use of models of fiber digestion, Varga and Hoover (1983) studied the rate and extent of NDF degradation for 22 feedstuffs. Polyester bags were incubated in the rumen of fistulated cows up to 48 h. Major differences were found among feeds in both extent and rate of NDF degradation. Forage results indicated that legumes in general, had faster

NDF degradation than grasses; however, grasses were degraded more extensively than legumes. Other studies have been conducted (Miller and Muntifering, 1985; Mertens and Loften,1980) using models of fiber digestion to explain depressions in digestibility by revealing which factors in the digestive process are affected most with increasing concentrates or starch in the diet.

MATERIALS AND METHODS

EXPERIMENT I.

Experiment I was divided into two parts. Objectives of experiment Ia were to determine dry matter, fiber, and nitrogen fractions of forages received at the laboratory. Objective of experiment Ib was to determine if sample handling prior to analysis had an effect on fiber and nitrogen fractions.

Forage samples submitted for analysis to the Virginia Tech Forage Testing Laboratory from September 1984 to April 1985 were randomly selected and analyzed for dry matter (DM), fiber, and nitrogen fractions. Forages selected were thoroughly mixed, sampled, dried in a convection oven at 50°C to 88-90% DM, then ground in a Wiley Mill fitted with a 1-mm screen. Separation of appropriate forages by storage structure was based on information on the Forage Testing Feed Analysis Request Form submitted by the farmer or county extension agent (see Appendix Figure 1). Samples were taken Tuesday through Friday to reduce the time from sampling on the farm to arrival at the laboratory and to avoid receiving samples which remained in the mail room over the weekend.

Forages collected were as follows: alfalfa (Medicago sativa L.) silage from conventional upright silos (43), alfalfa silage from oxygen limiting silos (39), alfalfa hay

(23), ammonia-treated corn (Zea mays) silage (25), corn silage from conventional upright silos (17), corn silage from bunker silos (17), rye (Secale cereale L.) silage (25), orchardgrass (Dactylis glomerata L.) hay (19), sorghum (Sorghum bicolor (L.) Moench) silage (7), wheat (Triticum aestivum L.) silage (6), barley (Hordeum vulgare L.) silage (5), orchardgrass silage (4), and fescue (Festuca arundinacea Schreb.) hay (3).

Laboratory analysis included DM (105^{o}C overnight), total nitrogen by the Kjeldahl method modified by trapping ammonia in 4% (wt/vol) boric acid solution and titrating with 0.1N HCl (AOAC, 1975), insoluble nitrogen (INSOL) in a borate phosphate buffer (Krishnamoorthy et al. 1982), neutral (NDIN) and acid detergent insoluble nitrogen (ADIN) and neutral (NDF) and acid detergent fiber (ADF) fractions by the methods of Goering and Van Soest (1970). Modified NDF procedure described by Mertens (1985) was used on forage samples which were difficult to filter. These included corn, sorghum, wheat, and barley silages. Filter paper (Whatman 541, 12.5cm) was substituted for crucibles in the fiber procedures to collect the residue. Filter paper plus residue was digested to determine N in neutral and acid detergent fiber fractions. Cell contents (100 - NDF), hemicellulose (NDF - ADF), soluble nitrogen (NSOL) (100 - INSOL), nitrogen insoluble in neutral detergent but soluble in acid detergent (NDIN

- ADIN) and insoluble nitrogen soluble in neutral detergent (INSOL - NDIN) were determined by difference.

Average time from sampling at the farm to arrival at the laboratory for alfalfa silage was 2.7 d with a range from 0 (same day) to 8 d. To determine if sample handling (length of time and sample temperature) had an effect on fiber and nitrogen fractions of alfalfa silage, a second study was conducted (Experiment Ib). Ensiled alfalfa samples from the Virginia Tech Dairy Cattle Center were sealed in plastic bags and placed in a forced-air draft oven at 34°C or left at room temperature (22°C) from 1 to 5 days prior to analysis. After setting for the designated period of time, the samples were dried in a convection oven at 50°C and ground through a Wiley Mill fitted with a 1-mm screen. Samples were then analyzed for DM, CP, NSOL, NDF, ADF, NDIN, and ADIN as previously described. Fractions determined by difference were also calculated as previously described.

EXPERIMENT II

A total of 39 alfalfa silage samples from oxygen limiting silos received from throughout the state, 43 silage samples from conventional upright silos, 12 alfalfa silage samples from the Virginia Tech Dairy Cattle Center, and 23 samples of baled alfalfa hay received at the Virginia Tech Forage Testing Laboratory were used in this experiment. Ex-

periment II is divided into three sections (IIa, IIb, IIc). Objectives of IIa were to evaluate differences in DM, fiber, and nitrogen fractions of alfalfa stored in oxygen limiting and conventional upright silos and to determine relationships among analytical measurements. Objectives of IIb were to compare DM, fiber, and nitrogen fractions of alfalfa silage stored in oxygen limiting silos received at the forage testing laboratory from throughout the state with those silage samples from the Virginia Tech Dairy Cattle Center (oxygen limiting silo). Objectives of IIc were to determine variations in N fractions and in vitro degradability of alfalfa stored as baled hay, or ensiled in oxygen limiting or conventional upright silos.

Forages were dried, ground, and analyzed for DM, CP, INSOL, NDIN, ADIN, NDF, and ADF as in experiment I, and protease insoluble nitrogen (PIN). Pattern of solubilization of feed nitrogen was determined by incubating with a protease solution for 0, 2, 12, 24, and 48 h. The PIN procedure was that described by Krishnamoorthy et al. (1983) and used a non-specific protease enzyme from Streptomyces griseus. Air-dry samples (0.5g) were added to 125 ml Erlenmeyer flasks in duplicate, 40 ml borate phosphate buffer was added, and flasks were placed in a water bath at 39^{o}C for 1 h to allow hydration. After 1 h, 10 ml protease enzyme solution (330 x 10^{-3} units/ml) were added and flasks were incubated for 0, 2, 12, 24, and 48 h. Residue remaining after incubation was

collected on filter paper (Whatman 541, 12.5 cm), dried at 100°C overnight, then analyzed for nitrogen content (AOAC, 1975). Blank filter papers were included with each incubation and the residual N corrected for N content of the filter paper. Nitrogen fractions determined in this experiment are those described in Tables 2 and 3.

EXPERIMENT III.

Objectives of this experiment were to estimate rumen nitrogen and dry matter degradability of selected forages and by- product feeds, to compare in vitro methods with the in situ dacron bag technique, and to determine degradability of neutral and acid detergent fiber by the bag technique.

Sampling

Farm visits were made from July 1985 to January 1986 in order to collect a large enough sample size (approximately 300 g DM) to run both in situ and in vitro procedures. Forages collected included alfalfa silage from conventional upright silos (5 samples), alfalfa silage from oxygen limiting silos (5), alfalfa hay (4), corn silage (5), ammonia-treated corn silage (5), and orchardgrass hay (5). Several by-product feeds were also collected for this study, includ-

ing dried distillers grains, dark colored (2) and light colored (1), wet brewers grains (1), and whole cottonseed (3).

In vitro analysis

Forages and by-product feeds were dried in a forced-air draft oven at 50°C to 88-90% DM, then ground in a Wiley Mill fitted with a 1-mm screen. Whole cottonseed samples were ground through a 6-mm screen due to difficulty encountered in grinding through a 1-mm screen. In vitro analysis of samples included DM, total N, buffer insoluble N, PIN at 0, 2, 12, 24, and 48 h, NDIN, and ADIN by methods previously described.

Cow Data

Six rumen fistulated lactating Holstein cows were used in this experiment for in situ estimation of rumen nitrogen and dry matter degradability. Four cows were in early lactation (<100 d in milk) and two cows were in mid-lactation (100-200 d in milk). Cows were fed a total mixed ration containing corn silage, alfalfa silage, high moisture corn, soybean meal, and mineral mix twice daily at 0630 and 1300 h. Feed intake was recorded daily and body weight weekly. Milk production was recorded twice daily. Dietary ingredients and diet composition are shown in Table 4.

Table 4. Dietary ingredients and diet composition of ration fed to fistulated cows during the in situ study.

Item	% dry matter
Ingredient	
Corn silage	53.0
Alfalfa silage	27.0
High moisture corn	15.0
Soybean meal	4.0
Mineral mix	1.0
	100.0
Composition, total mixed ration	
Dry matter	53.5
Crude protein	15.5
Acid detergent fiber	23.0

In situ bag technique

Rumen degradability of nitrogen and dry matter of forages and by-product feeds were determined using the in situ bag technique. Bags were made of spun polyester with a defined pore size of 59 micron and bag size was 10 x 20 cm. Approximately 9 to 12 g of feedstuffs were placed in each bag giving a feed exposure of 16 to 22 cm^2/mg feedstuff (Zerbini and Polan, 1985).

Samples were added to each of 28 bags. Two bags each were used for 0 and 2-h incubations, three bags each for 12 and 24-h incubations, and four bags for 72-h incubations for a total of 14 bags in each of 2 cows. Bags were tied shut using cable ties and grouped according to incubation time period. Cable ties were also used to attach the bags to a cord extending from a weight placed at the bottom of the ventral sac of the rumen to the fistula cover. Prior to placement in the rumen, bags were soaked in warm tap water for 15 min to remove all material readily soluble in water and to reduce the lag time for wetting in the rumen (Janicki, 1983). Bags were placed in the rumen at intervals starting with the 72-h bags, begining at 2300 hours. All bags were placed at the end of the cord closest to the weight when they were introduced into the rumen. All bags were removed from the rumen at the same time and immediately placed in an ice

bath to reduce microbial fermentation. Bags were then rinsed in cold running water until the rinse water was clear. The rinsing precedure was necessary to remove ruminal contaminants from the interior and exterior of the bag. The rationale for thorough rinsing was based on the assumption that any material (except attached rumen bacteria) able to influx the bags would be removed through rinsing and any feed material removed is soluble or extensively degraded. Bags were then opened and dried at 65°C in a forced-air draft oven to a constant weight. Bags with the dry residue were weighed to determine dry matter disappearance; dried residues were then removed from the bags, composited for each incubation time, and ground through a Cyclone mill fitted with a 1-mm screen. Residues were then analyzed for DM, total nitrogen, and neutral and acid detergent fiber. Based on the composition and weight of the original samples, the percent disappearance of DM, N, NDF, and ADF for the various incubation times was calculated for each forage and by-product feed.

Degradability Calculations

Protein degradation has been described as a function of time when using in vitro or in situ fermentation or proteolytic enzymes. Most data fit a general model with three pools or fractions:

A = nonprotein nitrogen or true protein that is degraded very rapidly with a fractional disappearance rate equal infinity,

B = protein that is degraded at a measurable rate similar to the rate of passage (k_d = .02 to .07/h, and

C = bound or unavailable protein that is undegraded.

Only fraction B is usually considered to be affected by relative rates of passage and degradation at any time. Protein fractions A, B, and C were determined for forages and by-product feeds by the procedures described by Zerbini (1984). The following describes these procedures.

A monoexponential rate of decay was used to describe degradation of fraction B and to estimate its value at 0 time. Degradation constants (k_d) were obtained by regressing the natural logarithm (ln) of the percent remaining minus the C fraction (ln (percent remaining - C)) over time. Intercept and slope of the resulting straight line from regression analysis represented the value for the B fraction in the original sample and degradation constant (k_d), respectively.

Thus, the B fraction is calculated by the equation:

$$B = e^{-\text{intercept}}$$

where e = 2.71828

Once the percentage of protein in the B fraction has been derived from the above equation and percentage of protein in the C fraction is already known (72-h residual), the percentage of protein in the A fraction can be calculated by the following:

$$A = 100 - (B + C)$$

The fraction of feed remaining in the rumen at any point in time is calculated using the following equation:

$$\text{Fraction remaining} = B\ e^{-kt} + c\ (t>0)$$

$$\text{Thus, Fraction remaining} - C = B\ e^{-kt}$$

Taking the natural logarithm of each side results in:

$$\ln(\text{fraction remaining} - C) = \ln B - kt$$

where:

ln B = intercept (from regression analysis)

k = slope (from regression analysis)

t = any point in time

C = 72 h residual

A summary of the mathematical procedure of this method adapted from Zerbini (1984) is shown in Figure 3.

IN SITU BAG TECHNIQUE

TOTAL NITROGEN / TOTAL DRY MATTER = A + B + C

READILY DEGRADED	DEGRADED AT A MEASURABLE RATE	NOT DEGRADED
K = INFINITE	K = .1/h	K = 0
A = TOTAL(N,DM) − B − C	$B = e^{\text{intercept}}$	$C = \frac{72h\ \text{RESIDUAL(DM,N}}{\text{ORIGINAL(DM,N)}}$

FRACTION OF FEED REMAINING IN THE RUMEN AT ANY GIVEN POINT IN TIME $= B\,e^{-kt} + C$ (for t>0)

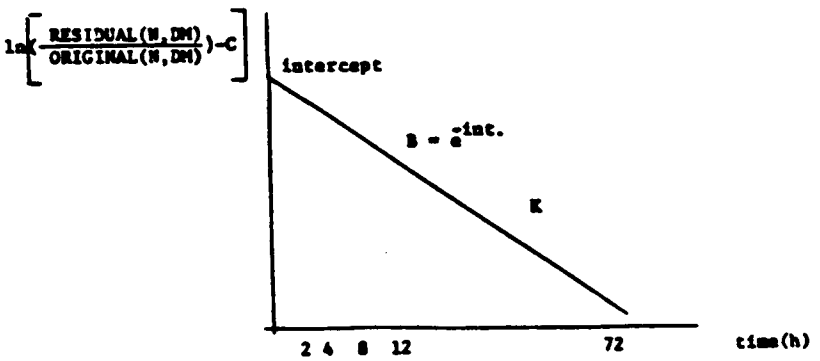

Figure 3. Summary of the mathematical model used to describe rumen degradation of nitrogen and dry matter.

Protein degradability in percent (D) was calculated after determining the % protein in the A, B, and C fractions using the equation of Orskov and McDonald (1979) as follows:

$$D = A + (B \times k_d)/(k_d + k_r)$$

where:

D = protein degradability (%),

A = fraction readily degraded (%),

B = fraction degraded at a measurable rate (%),

k_d = protein degradation constant of fraction B,

k_r = rumen turnover rate assumed to be .05/h.

Neutral and acid detergent fiber degradability were determined by the same procedures as for crude protein and dry matter.

Statistical Analysis

Statistics applied to data from experiment Ia included least squares means and ranges for each of the chemical components measured. Data from experiment Ib were analyzed by analysis of variance for a split-plot design. Data from experiment II were analyzed statistically by analysis of variance with storage method as main effect. Correlation coefficients were determined using general linear model pro-

cedures of the statistical analysis system (1982). Regression analyses were conducted to predict bound nitrogen (ADIN) from dry matter, DM^2, and DM^3.

Data from the in situ bag technique in experiment III were analyzed using linear regression to establish rates of degradation (k) of fraction B of various forages and by-product feeds. The A, B, and C fractions of forages were analyzed by analysis of variance as were the in vitro fractions and calculated degradabilities. No statistical analyses were made on by-product feeds due to the small number of samples analyzed. Treatment differences were determined using Waller-Duncan's test for paired comparisons. Statistical models are described in the Appendix.

RESULTS AND DISCUSSION

Experiment Ia.

Counties from which samples were received are shown in Appendix Figures 2 and 3. Data for alfalfa silages from conventional upright and oxygen limiting silos will be discussed in experiment II.

Number, dry matter (DM), and fiber fractions of selected forages obtained from the Virginia Tech Forage Testing Laboratory are shown in Table 5. The DM content of corn silage was 34% for untreated and 37% for NH_3-treated with ranges from 23 to 48% and 23 to 50%, respectively. Although averages were within desirable range (32-42% DM), there were some samples ensiled too wet or dry. Seepage losses tend to be greater when forages are ensiled too wet (Noller and Thomas, 1985), also, if forage is ensiled too dry, heating may occur resulting in increased unavailable protein (Thomas et al., 1982). Also, more anhydrous NH_3 may be lost through volatilization when applied to silage that is excessively dry (Klopfenstein and Owen, 1981). Rye silage averaged 36% DM indicating most samples were wilted before ensiling, however, some may have been direct cut as indicated by the lower range in DM (22%). Alfalfa and orchardgrass hays averaged 85 and 87% DM, respectively. The ranges indicate some contained excess water. Hay stored with less than 80% DM will tend to

Table 5. Dry matter and fiber fractions of forages obtained from the forage testing laboratory.

Forage	N	DM, %	Cell content	NDF	Hemi	ADF
				% dry matter		
Corn silage, horizontal	17	34.9 (25.0-45.3)	48.5 (38.2-55.7)	51.5 (44.4-61.8)	25.9 (21.1-37.6)	25.6 (21.0-30.3)
Corn silage, upright	17	34.2 (23.4-47.9)	50.2 (42.1-58.1)	49.8 (41.9-57.9)	23.6 (17.5-30.0)	26.3 (20.7-34.8)
Corn silage, NH_3 treated	25	36.9 (23.0-49.8)	49.9 (37.2-66.4)	50.1 (38.7-62.8)	24.3 (18.2-29.5)	25.8 (17.8-34.1)
Rye silage	25	35.9 (22.0-55.8)	37.0 (30.4-48.5)	63.0 (51.5-69.6)	23.0 (15.6-28.0)	40.0 (34.5-47.9)
Sorghum silage	7	36.9 (26.0-54.6)	39.0 (34.4-45.4)	61.0 (54.6-65.6)	18.9 (13.6-22.1)	42.1 (36.6-51.3)
Wheat silage	6	49.2 (39.0-59.9)	47.6 (41.6-59.0)	52.4 (41.0-58.4)	19.6 (14.2-23.6)	32.8 (26.8-37.6)
Barley silage	5	39.5 (36.0-44.1)	42.3 (35.7-47.6)	57.7 (52.4-64.3)	20.8 (16.1-26.9)	36.9 (27.7-44.7)
Orchardgrass silage	4	49.5 (34.0-70.1)	35.4 (31.4-41.2)	64.6 (58.8-68.6)	21.3 (16.6-28.5)	43.4 (40.1-50.9)
Alfalfa hay	25	85.0 (69.2-93.2)	48.1 (23.0-64.9)	51.9 (35.3-77.0)	13.8 (6.9-22.7)	38.1 (23.8-60.5)
Orchardgrass hay	19	86.6 (72.0-91.7)	28.3 (17.7-42.2)	71.7 (57.8-82.3)	29.3 (20.1-33.7)	42.2 (36.7-52.8)
Fescue hay	3	81.1 (60.1-93.1)	28.8 (24.8-35.0)	71.2 (65.0-75.2)	28.8 (24.7-31.6)	42.3 (34.8-48.6)

N = number; DM = dry matter; Cell contents = 100 - NDF; NDF = neutral detergent fiber; Hemi = hemicellulose (NDF - ADF); ADF = acid detergent fiber. Numbers in ( ) are ranges.

heat and mold if left untreated. Additives, such as propionic acid have been used to prevent molding in hay stored with a DM content less than 80% (Waldo, 1977). Sorghum silage DM (37%) was similar to corn silage. Barley silage DM (39.5%) was similar to rye, however, the range was much less (36 to 44%).

Contents of NDF and ADF of untreated and treated corn silage were similar with an average of about 50 and 26% of DM, respectively. Rye silage had NDF and ADF contents (63 and 40% of DM) greater than corn silage, however corn and rye had similar contents of hemicellulose (23-26% of DM). Hemicellulose is estimated by subtracting ADF from NDF. Orchardgrass hay had a greater content of NDF compared to alfalfa hay (72 vs. 52% of DM), but similar ADF content. Thus, orchardgrass had less cell contents and more hemicellulose compared to alfalfa. These results are typical of compositional differences between grasses and legumes as reported by Van Soest (1977). Fiber fractions of fescue hay were similar to those of orchardgrass hay. Fiber fractions of wheat and barley silages were quite similar. Ranges in fiber content of all forages were quite large indicating a very wide range in the maturity and also quality of the forages received at the laboratory.

Crude protein and nitrogen fractions of selected forages are in Table 6. Protein content of corn silage was increased 2.1 percentage units by addition of ammonia (7.4 vs. 9.5% CP

Table 6. Crude protein and nitrogen fractions of forages obtained from the forage testing laboratory.

Forage	CP	NSOL	INSOL	NDIN	INSOL-NDIN	NDIN-ADIN	ADIN
	--% DM---	----------% total nitrogen----------					
Corn silage, horizontal	7.3 (5.8-8.3)	44.2 (35.4-68.3)	55.8 (31.7-64.6)	22.3 (8.1-32.2)	37.1 (23.6-46.0)	10.4 (1.0-22.3)	11.9 (6.2-21.1)
Corn silage, upright	7.4 (5.5-11.3)	46.1 (31.6-65.9)	53.9 (34.1-68.4)	22.5 (11.7-34.4)	34.5 (21.6-49.0)	10.8 (0.7-23.0)	11.7 (6.8-16.2)
Corn silage, NH_3 treated	9.5 (7.1-11.7)	48.5 (25.4-68.0)	51.5 (37.0-74.6)	17.5 (8.6-27.7)	34.0 (4.7-47.9)	7.9 (0.0-16.7)	9.5 (6.1-14.3)
Rye silage	14.3 (8.3-21.5)	79.3 (63.4-87.8)	20.7 (12.2-36.7)	10.1 (7.2-16.6)	10.6 (2.9-21.1)	3.7 (0.7-8.2)	6.4 (4.3-11.4)
Sorghum silage	7.5 (5.4-13.3)	39.0 (27.8-54.1)	61.0 (45.9-72.2)	27.9 (16.9-36.8)	33.0 (26.2-42.4)	3.3 (0.3-5.6)	24.6 (11.3-32.7)
Wheat silage	10.4 (8.1-12.8)	69.2 (55.2-76.7)	30.8 (23.3-44.8)	12.2 (8.9-14.7)	18.6 (9.7-35.9)	4.6 (0.8-9.2)	7.6 (4.4-10.4)
Barley silage	9.3 (7.7-10.5)	67.9 (59.2-72.0)	32.1 (28.0-40.8)	18.0 (11.5-31.4)	14.1 (3.9-23.5)	6.7 (1.7-21.2)	11.3 (9.5-15.8)
Orchardgrass silage	13.3 (9.1-16.3)	59.4 (54.3-67.3)	40.6 (32.7-45.7)	26.4 (18.3-34.3)	14.2 (10.8-19.6)	14.8 (8.3-25.3)	11.6 (9.0-16.1)
Alfalfa hay	18.1 (11.2-22.8)	32.6 (23.8-43.7)	67.4 (56.3-76.2)	27.0 (11.3-53.0)	40.4 (18.6-58.7)	17.2 (4.0-36.1)	9.8 (6.1-21.4)
Orchardgrass hay	9.9 (6.1-17.4)	26.0 (13.7-35.7)	74.0 (64.3-86.3)	45.5 (31.2-61.3)	28.4 (9.2-38.8)	32.3 (17.8-50.1)	13.2 (6.6-29.4)
Fescue hay	11.6 (6.5-18.9)	33.8 (28.7-39.9)	66.2 (60.1-71.3)	31.8 (26.4-34.9)	34.4 (26.2-44.9)	17.6 (13.9-20.7)	14.2 (8.4-20.0)

CP = crude protein; NSOL = soluble protein; INSOL = insoluble protein; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen. Numbers in ( ) are ranges.

on a DM basis). Theoretically, application of anhydrous ammonia at the recommended level of 3.2 kg per ton should raise protein 4 percentage units considering an 80% retention rate. Crude protein content of treated corn silage is expected to be about 10 to 12% DM, but these results (9.5%) are slightly lower. Perhaps inadequate amounts were applied on some samples (low range in CP of 7.1% DM) or low natural CP content (5.5 and 5.8% CP on low range for untreated corn silages) are partly responsible for the lower than expected CP content of treated corn silage. Rye silage contained 14.3% CP on a DM basis. Excellent quality rye silage should contain 18% or more if harvested before heads begin to appear (NRC, 1978). Range in CP was from 8.5 to 21.5% of DM. Harvesting rye (or any other forage) at the proper stage of maturity is critical to obtain the highest quality forage possible. Alfalfa and orchardgrass hay averaged 18.1 and 9.9% CP on a DM basis, respectively, both within expected ranges. Very large ranges in CP were noted for all forages. For example, CP content of orchardgrass hay ranged from 6.1 to 17.4%, nearly a three-fold difference.

Nitrogen solubility, expressed as a percent of total N, was greater in ensiled forages than hay. Ammonia treatment of corn silage tended to increase NSOL slightly compared to untreated corn silage (49 vs. 45%), respectively. Anhydrous ammonia is a very soluble source of nitrogen, thus the increased NSOL was expected. Rye silage had the greatest per-

centage of total N in the soluble fraction (79.3%) and hays had the least.

Neutral detergent insoluble nitrogen is the total N contained in the cell wall fraction of the plant. This fraction contains protein that is digested slowly (NDIN-ADIN) and not at all (ADIN) (Van Soest and Sniffen, 1984). Treated corn silage had less NDIN when expressed as a % of total N compared to untreated corn silage (22.4 vs. 17.5% of total N) because more nitrogen was in the soluble fraction. Rye silage had an extremely low content of NDIN (10.1% of total N) indicating most of the N was in the cell contents and readily solubilized. Alfalfa hay contained less NDIN than orchardgrass hay (27.0 vs. 45.5% of total N), indicating alfalfa has more N in cell contents and less in cell walls compared to orchardgrass. Content of NDIN, INSOL-NDIN, and NDIN-ADIN of orchardgrass silage were much less than hay (26.4 vs. 45.5, 14.2 vs. 28.4, 14.8 vs. 32.3% of total N, respectively) indicating these nitrogen fractions were greatly affected by ensiling.

Insoluble non-cell wall nitrogen (INSOL-NDIN) has been classified as the B_1 protein fraction and protein insoluble in ND but soluble in AD (NDIN-ADIN) has been classified as the B_2 protein fraction in feedstuffs (Van Soest and Sniffen, 1984). The INSOL-NDIN content of alfalfa hay was greater in orchardgrass hay (40.4 vs. 28.4%) while NDIN-ADIN content was less (17.2 vs. 32.3% of total N). These results show the

protein in alfalfa hay is expected to be more rapidly degraded in the rumen while protein in orchardgrass hay is expected to be more slowly degraded. These results suggest that N in grass is more slowly digested than in legumes because of the difference in N in cell contents and more in the B_1 fraction.

The ADIN content of feeds is considered to be unavailable (Thomas et al., 1982). A large content of ADIN in forages is usually associated with heating during ensiling or storage. The least amount of ADIN was found in rye silage (6.4% of total N) and the greatest in sorghum silage (24.6% of total N). There were large ranges in the contents of the nitrogen fractions measured as previously noted with the fiber fractions.

EXPERIMENT Ib.

Average time from sampling at the farm to arrival at the laboratory for alfalfa silage was 2.7 d with a range from 0 (same day) to 8 d. Due to the large range in time, this experiment was conducted to determine if sample handling (length of time and temperature) had any effect on fiber and nitrogen measurements.

Effect of temperature and days setting prior to analysis of alfalfa silage on analytical measurements is shown in Ta-

ble 7. Results show that days setting prior to analysis had a significant effect ($P<.05$) on NSOL. Content of NSOL decreased from 64.9% of total N on day 1 (average of temperatures) to 63.2% of total N (average temperature) on day 5. No other differences were noted. The alfalfa silage used in this experiment was stored in an oxygen limiting silo at the Virginia Tech Dairy Cattle Center. Laboratory analysis of the samples indicate a very high quality forage as evidenced by the high CP (20.5% DM), low ADIN (7.5% of total N), and ADF content of 39.1% of DM. Dry matter averaged 58.0%, which is higher than recommended, however, there was no evidence of heat damage to the forage. Results from this study indicate there is very little change in chemical composition of alfalfa silage kept at 34°C vs. 21°C over a period from 0 to 5 d.

EXPERIMENT II.

Experiment II is an in depth study of alfalfa stored as baled hay, or ensiled in oxygen limiting or conventional upright silos. Experiment II is divided into three sections (IIa, IIb, and IIc). Objectives of IIa were to evaluate differences in dry matter, fiber, and nitrogen fractions of alfalfa stored in oxygen limiting and conventional upright silos, and to determine relationships among analytical meas-

Table 7. Effect of temperature and days setting prior to analysis of alfalfa haylage on analytical measurements.

	Days prior to analysis							
	0	1		3		5		
Temperature (°C)[a]	-	34	21	34	21	34	21	SE
Trait								
Dry Matter	58.0	57.7	57.7	57.5	57.6	58.0	58.0	.21
Crude protein, %DM	20.5	20.5	21.0	20.2	19.9	20.7	20.7	.15
NSOL[b], % Tot N	64.3	65.9	63.9	63.3	65.0	61.6	64.8	.55
NDIN, % Tot N	14.0	13.6	13.8	14.8	14.5	15.0	13.8	.35
INSOL-NDIN, % Tot N	21.7	20.5	22.2	21.9	20.5	23.4	21.7	.51
NDIN-ADIN, % Tot N	6.5	6.2	6.4	6.8	6.7	6.3	5.9	.40
ADIN, % Tot N	7.5	7.3	7.5	8.0	7.8	8.7	7.9	.14

[a] No significant differences in temperature ($P>.05$).
[b] Significant effect of day on NSOL ($P<.05$).

urements. Objectives of IIb were to compare dry matter, fiber, and nitrogen fractions of alfalfa silage stored in oxygen limiting silos obtained from the Forage Testing Laboratory from throughout the state with those silage samples sent for analysis from the Virginia Tech Dairy Cattle Center. Objectives of IIc were to determine variations in nitrogen fractions and in vitro protein degradability of alfalfa stored as baled hay, or ensiled in oxygen limiting or conventional upright silos.

EXPERIMENT IIa.

Comparisons of dry matter, fiber, and nitrogen fractions are listed in Table 8. Dry matter content of alfalfa silage from oxygen limiting silos (56.0%) was greater ($P<.01$) than DM of samples from conventional upright silos (44.4%) and ranged from 34.9 to 78.0% and 28.9 to 76.6% for these respective silos. Kung et al. (1982) and Thomas et al. (1972) also reported greater DM content of silages stored in sealed compared to conventional upright silos. Cell contents, NDF, hemicellulose, and ADF were not different for samples from oxygen limiting and conventional upright silos.

Acid detergent fiber content was 40.0 and 39.5% of DM (Table 8) and ranged from 27.0 to 47.7 and 31.1 to 48.2% of DM for silages from the respective silo types. These results

Table 8. Fiber and nitrogen fractions of alfalfa silages stored in oxygen limiting and conventional upright silos.

Trait	Oxygen limiting		Conv. upright	
	$\bar{X}$	SE[1]	$\bar{X}$	SE
Number	39	-	43	-
Dry Matter (DM)	56.0[b]	1.7	44.4[a]	1.6
Cell contents[2], % DM	48.1	1.0	49.9	.9
NDF[2], % DM	51.9	1.0	50.1	.9
Hemicellulose[2], % DM	11.8	.7	10.6	.7
ADF[2], % DM	40.1	.6	39.5	.6
Crude protein, %DM	20.0	.4	19.8	.4
NSOL[2], % total N	52.8[a]	1.3	59.5[b]	1.3
INSOL[2]	47.2[b]	1.4	40.5[a]	1.3
INSOL-NDIN[2]	19.7	.7	20.9	.7
NDIN[2]	27.5[b]	1.4	19.6[a]	1.3
NDIN-ADIN[2]	13.5[b]	.9	8.6[a]	.9
ADIN[2]	14.0[b]	.8	11.0[a]	.8

[a,b]Means in a row with different superscripts differ ($P<.01$).

[1]SE = standard error of mean.

[2]Cell contents = 100 - NDF; NDF (neutral detergent fiber); Hemicellulose = NDF - ADF; ADF (acid detergent fiber). NSOL = soluble nitrogen; INSOL = 100 - NSOL; INSOL-NDIN (insoluble non-cell wall nitrogen); NDIN (neutral detergent insoluble nitrogen; NDIN-ADIN (nitrogen insoluble in ND, but soluble in AD); ADIN (acid detergent insoluble nitrogen).

indicate large variation in the maturity and quality of those silage samples obtained from the laboratory.

Crude protein and insoluble non-cell wall nitrogen (INSOL-NDIN) from oxygen limiting and conventional upright silos were not different. Crude protein content averaged 20.0% of DM for silages from oxygen limiting and 19.8% of DM for silages from conventional upright silos. Ranges in CP were from 13.7 to 23.3 and 13.5 to 25.0% of DM for silage from the respective silo types. Buffer-soluble nitrogen (NSOL) was greater in silage samples from conventional upright silos compared to those from oxygen limiting silos (59.5 vs. 52.8% of total N). The increased NSOL in silages from conventional upright silos may be due to the high moisture content and greater proteolysis and deamination occurring in the wetter silages. In agreement with these findings, L. Kung Jr. (personal communication) found NSOL tended to be greater in silages from concrete stave and bunk silos compared to sealed silos. Merchen and Satter (1983b) found NSOL of 42.3% and 72.1% of total N for alfalfa ensiled in concrete stave silos at 66.0% and 29.0% DM, respectively.

The NDIN, NDIN-ADIN, and ADIN contents of silages from oxygen limiting silos were greater than those from conventional upright silos ($P<.02$). Silages from oxygen limiting silos had more nitrogen insoluble in neutral detergent but soluble in acid detergent (NDIN-ADIN) compared to silages from conventional upright silos (13.5 vs. 8.6% of total N).

However, oxygen limiting silo samples also had more ADIN than samples from conventional upright silos (14.0 vs. 11.0% of total N). Merchen and Satter (1983b) found an increase in ADIN with increased DM content of alfalfa silage. Silages which are ensiled with a high DM content may undergo excessive heating resulting in formation of compounds containing unavailable nitrogen in the plant material (Thomas et al., 1972; Yu and Thomas, 1975). Of the total N, the ADIN content of silages from oxygen limiting silos ranged from 6.6 to 37.9%; whereas, ADIN of silages from conventional upright silos ranged from 5.2 to 28.4%. Samples from both storage structure types exhibited both extremes in ADIN. Shelford (1982) also found greater ADIN of silages stored in oxygen limiting (25.9% of total N) compared to conventional tower silos (19.7% of total N). Thomas et al. (1972) found no differences in ADIN expressed as a percent of total N between silages stored in different structures, but ADIN in their study averaged 19 to 20%, much greater than 14.0 and 11.0% in this study. In contrast to these results, Kung et al. (1982) found lower ADIN in alfalfa silage samples from sealed compared to cement stave or bunk silos (10 vs. 16 and 14% of total N, respectively). Nitrogen found in ADF is affected by the DM content of forages during ensiling. Silage stored with greater DM content will usually be less densely packed and result in heating due to air entrapment; however, sealed structures are supposed to reduce this. In this study, oxy-

gen limiting silo samples had a greater DM content than samples from conventional upright silos; those samples also contained more ADIN. If DM averaged the same between the two structures it is doubtful a difference would have been observed.

The ranked distribution of ADIN and DM percentages of alfalfa silage samples by storage structure utilized in this experiment are listed in Table 9. Silage from oxygen limiting silos had 35.9% of samples with ADIN greater than 15% of total N compared to only 14% of the samples from conventional upright silos. Mean DM content of silages with ADIN content less than 10% of total N was 42.4% for conventional upright silo samples and 52.6% for oxygen limiting silos samples. Acid detergent insoluble nitrogen tended to be greater with increased DM in silage samples. Of the 43 samples from conventional upright silos, 58.1% had ADIN content less than 10% of total N compared to only 28.2% of the 39 silages from oxygen limiting silos. Field studies conducted in Michigan (Kung et al., 1982; Thomas et al., 1972), Minnesota (Pierson et al., 1971) and Pennsylvania (Goering and Adams, 1973) have observed about one-third of all alfalfa silage samples analyzed exhibited some heat damage. Thomas et al. (1972) found 33% of the silages analyzed in their study had ADIN content of 36% of total N, 32% of samples averaged 18% of total N as ADIN, and 35% of the silages had ADIN content of 10% of total N. Percentage of samples from conventional upright silos in

Table 9. Distribution of ADIN and dry matters of silages in oxygen limiting and conventional upright silos.

Bound nitrogen[1]	Oxygen limiting		Conv. upright	
	N[2] (%)	DM	N[2] (%)	DM
<10.0	11 (28.2)	52.6	25 (58.1)	42.4
10.0–14.9	14 (35.9)	53.6	12 (27.9)	46.7
15.0–19.9	8 (20.5)	60.0	4 (9.3)	43.5
20.0–29.9	5 (12.8)	59.7	2 (4.7)	57.3
>30.0	1 (2.6)	78.0	0 (0)	-
Total	39 (100)	56.0	43 (100)	44.4

[1]Bound nitrogen assayed as acid detergent insoluble nitrogen (ADIN) expressed as a percent of total nitrogen.
[2]N = Number of samples.

this experiment exhibiting some heat damage (14.0%) was less than the results reported by (Goering and Adams, 1973; Kung et al., 1982; Pierson et al., 1971; Thomas et al., 1972); however, percentage of samples from oxygen limiting silos exhibiting some heat damage (35.9%) was similar.

Regression equations have been developed relating nitrogen digestibility to ADIN in forages (Thomas et al., 1982). Using these equations, nitrogen digestibility is reduced relative to silage with 10% of total N as ADIN by an average of 11 and 21% in alfalfa silages with ADIN content of 15 and 20% of total N, respectively. If an ADIN content of up to 10% of total N is typical in unheated silages, an ADIN content of 15 to 20% of total N should be considered moderately heat-damaged, and an ADIN content above 20% of total N should be considered excessively heat-damaged.

Correlation coefficients of analytical measurements of alfalfa silage samples from oxygen limiting and conventional upright silos are in Tables 10 and 11, respectively. Dry matter was negatively correlated with NSOL and positively correlated with NDIN and ADIN in both silo types. Dry matter of alfalfa silage was more positively correlated with ADIN for oxygen limiting (0.52, $P<.001$) than conventional upright silo samples (0.31, $P<.05$). Thomas et al. (1972) reported a positive correlation of 0.44 ($P<.01$) between alfalfa silage DM and ADIN. L. Kung Jr. (personal communication) found no significant correlation between DM and ADIN in silages from

Table 10. Correlation coefficients of analytical measurements of alfalfa silage samples from oxygen limiting silos.

	DM	CP	NSOL	NDIN	INSOL-NDIN	NDIN-ADIN	ADIN	NDF	ADF
DM	-	-.19	-.63***	.78***	-.33*	.70***	.52***	.44**	.16
CP		-	.37*	-.38*	.06	-.17	-.43**	-.38*	-.28
NSOL			-	-.82***	-.18	-.74***	-.55**	-.38*	-.02
NDIN				-	-.42**	.78***	.79***	.72***	.35*
INSOL-NDIN					-	-.16	-.49**	-.63***	-.56***
NDIN-ADIN						-	.24	.51***	.04
ADIN							-	.63***	.50**
NDF								-	.73***
ADF									-

*P<.05, **P<.01, ***P<.001.

DM = dry matter, CP = crude protein, NSOL = soluble nitrogen in a borate-phosphate buffer, NDIN = neutral detergent insoluble nitrogen, INSOL-NDIN = insoluble non-cell wall nitrogen, ADIN = acid detergent insoluble nitrogen, NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 11. Correlation coefficients of analytical measurements of alfalfa silage samples from conventional upright silos.

	DM	CP	NSOL	NDIN	INSOL-NDIN	NDIN-ADIN	ADIN	NDF	ADF
DM	-	.22	-.39**	.43**	-.12	.37*	.31*	-.10	-.14
CP		-	.43**	-.37*	-.16	-.21	-.42**	-.76***	-.63***
NSOL			-	-.94**	-.16	-.85***	-.64***	-.71***	-.42***
NDIN				-	-.18	.85***	.75***	.72***	.48***
INSOL-NDIN					-	.02	-.31*	-.03	-.16
NDIN-ADIN						-	.29	.59***	.15
ADIN							-	.56***	.68***
NDF								-	.72***
ADF									-

*P<.05, **P<.01, ***P<.001.

DM = dry matter, CP = crude protein, NSOL = soluble nitrogen in a borate-phosphate buffer, NDIN = neutral detergent insoluble nitrogen, INSOL-NDIN = insoluble non-cell wall nitrogen, ADIN = acid detergent insoluble nitrogen, NDF = neutral detergent fiber, ADF = acid detergent fiber.

sealed or bunk silos, however a positive correlation of 0.35 ($P<.05$) was noted for silages from cement stave silos.

As expected, buffer-soluble nitrogen was negatively correlated with insoluble nitrogen fractions (NDIN, NDIN-ADIN, and ADIN). Correlation coefficients (r) of NDIN, NDIN-ADIN, and ADIN with NSOL were -.82, -.74, and -.55 for oxygen limiting and -.94, -.85, and -.64 ($P<.01$) for conventional upright silos, respectively. These relationships indicate the lower the DM content of alfalfa silage the greater the NSOL and lesser content of slowly available (potential rumen escape) protein and unavailable nitrogen.

Crude protein was negatively correlated with ADF for samples from conventional upright silos and was not significantly correlated in samples from oxygen limiting silos. Fiber content is related to the maturity of the forage; the greater content of fiber associated with more mature forage. These results indicate that as forage fiber increases CP would decrease which is associated with changes in forages as they mature. Nitrogen solubility was negatively correlated with ADF in conventional upright silos, but not in oxygen limiting silos. Dry matter was positively correlated with NDF for oxygen limiting, but not for conventional upright silo samples. Insoluble non-cell wall nitrogen (INSOL-NDIN) was negatively correlated with DM, NDF, and ADF for oxygen limiting but not for conventional upright silo samples. These results show large differences in relation-

ship of the analytical measurements of alfalfa silage stored in oxygen limiting versus conventional upright silos.

Regression equations were formulated for DM versus ADIN expressed as a percent of total N. Dry matter, DM^2, and DM^3 accounted for only 34 and 35% of the variation in ADIN in conventional upright and oxygen limiting silos, respectively. Wide variation in ADIN was evident in silage samples as shown in Figures 4 and 5. Several silages with DM content less than 55 to 60% exhibited some heat damage (>15% ADIN), while other silage samples with DM content greater than 55 to 60% did not have ADIN content greater than 15% of total N. Results of the regression equations suggests that other factors must be considered to accurately predict ADIN.

Our results indicate storage structure did not significantly affect the total N content or fiber fractions of alfalfa silages; however, nitrogen fractions including NSOL, NDIN, NDIN-ADIN, and ADIN were significantly different between structures probably related to DM at ensiling and the resulting fermentation. Results also indicate that samples from each storage structure have some nutritional advantages and disadvantages. Alfalfa ensiled in oxygen limiting silos had greater DM and insoluble protein thus more dry matter intake may result when animals are fed higher DM silages and more potential rumen escape protein, however, it also had a greater content of bound protein which is unavailable. The greater DM, insoluble nitrogen, and ADIN in oxygen limiting

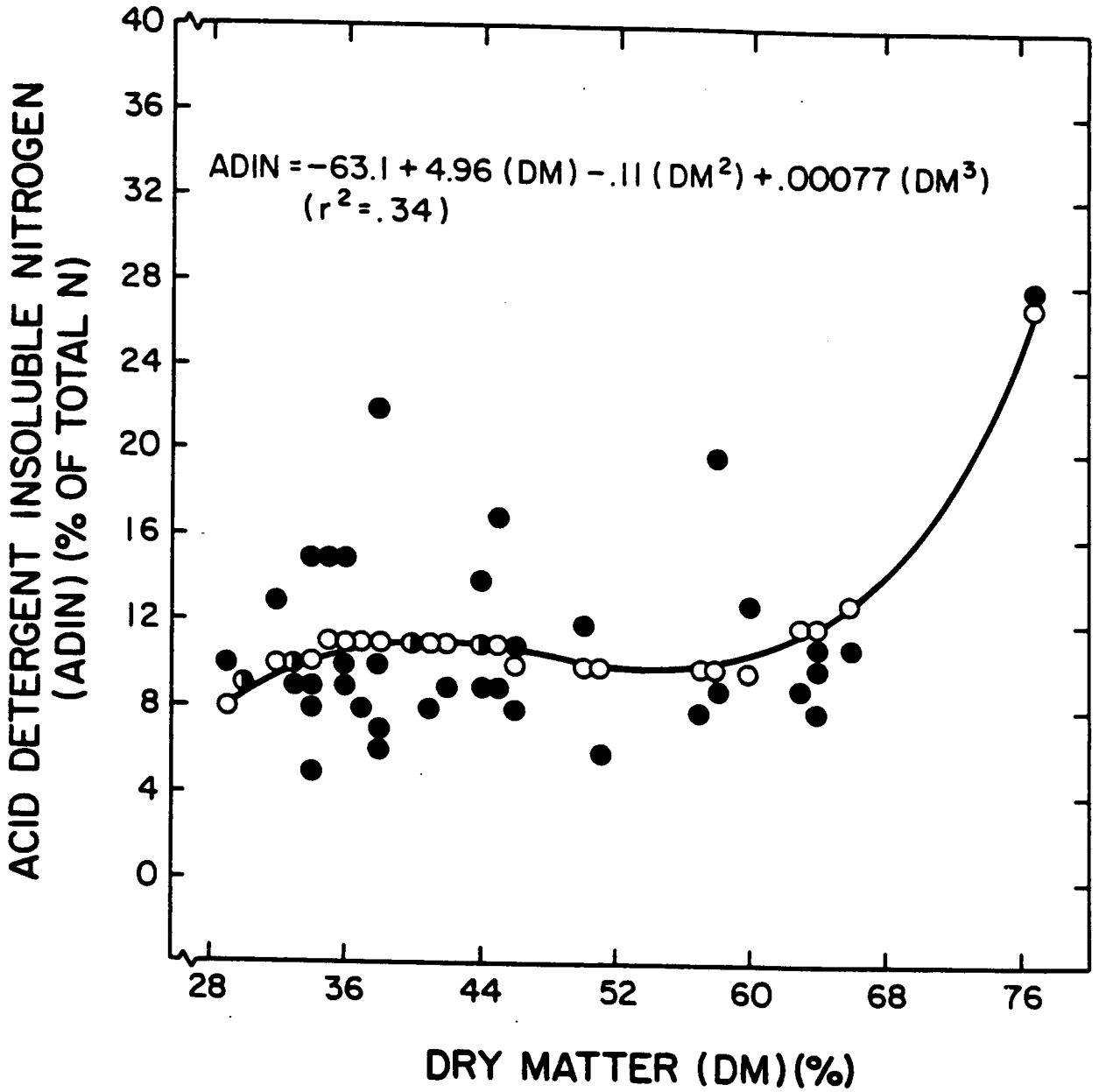

Figure 4. Relationship between acid detergent insoluble nitrogen and dry matter of alfalfa silage from conventional upright silos. (Actual data = '●'; predicted data = 'o').

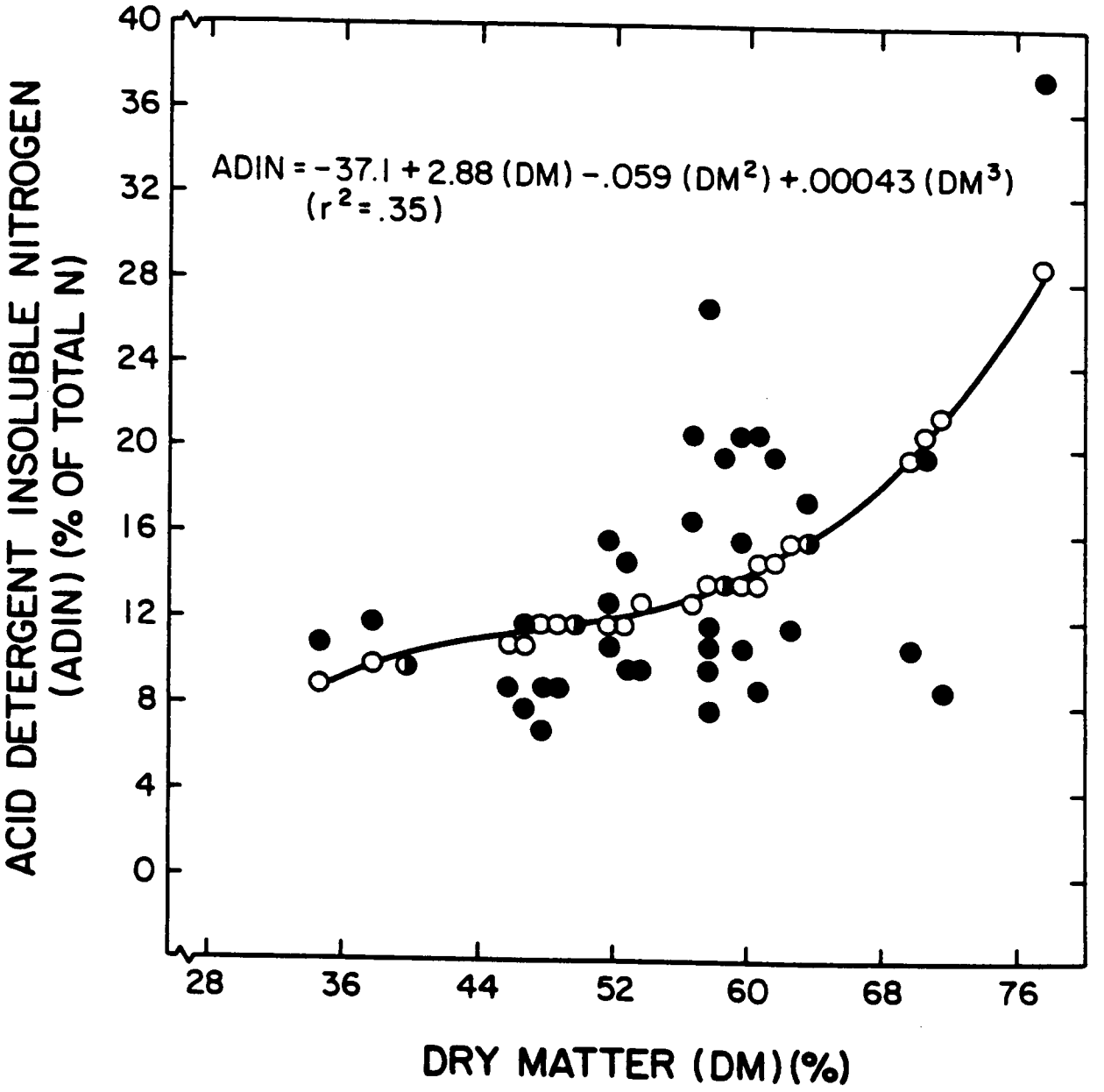

Figure 5. Relationship between acid detergent insoluble nitrogen and dry matter of alfalfa silage from oxygen limiting silos. (Actual data = '●'; predicted data = 'O').

silos suggests decreased proteolysis and deamination and increased heating during fermentation. Alfalfa ensiled in conventional upright silos had a greater content of soluble protein and less DM and ADIN, thus more protein would be degraded in the rumen.

Although DM content at ensiling is an important factor related to the quality of the ensiled forage, other factors to consider include stage of maturity, drying conditions, packing density, as well as others, all of which were not controlled in this study.

EXPERIMENT IIb.

Twelve alfalfa silage samples were collected over the period September 1984 to April 1985 from the Virginia Tech Dairy Cattle Center (VPI) oxygen limiting silo to compare DM, fiber, and nitrogen fractions with those received from throughout the state. Dry matter, fiber, and nitrogen fractions are shown in Table 12. Contents of DM and ADF were not significantly different between VPI and statewide samples. Cell contents (51.4 vs. 48.1% of DM) were greater ($P<.01$) and NDF (48.6 vs. 51.9% of DM) and hemicellulose (8.5 vs. 11.8% of DM) were less ($P<.01$) in VPI samples compared to statewide samples.

Table 12. Dry matter, fiber, and nitrogen fractions of alfalfa silage samples from oxygen limiting silos from the VPI dairy cattle center and statewide.

Trait	VPI Dairy	SE[1]	Statewide	SE
Number	12	-	39	-
Dry matter, %	54.5	2.4	56.0	1.3
Cell contents, % DM	51.4[b]	1.4	48.1[a]	.8
NDF[2], % DM	48.6[a]	1.4	51.9[b]	.8
Hemicellulose, % DM	8.5[a]	1.0	11.8[b]	.6
ADF[2] % DM	40.1	1.1	40.1	.6
Crude protein, % DM	19.9	.5	20.0	.3
Soluble N, % total N	58.1	2.4	53.6	1.3
Insoluble N, % total N	41.9	2.4	46.4	1.3
INSOL-NDIN[2] "	22.9[b]	1.6	18.9[a]	.9
NDIN[2] "	19.0[a]	2.5	27.5[b]	1.4
NDIN-ADIN[2] "	10.2[a]	1.7	13.5[b]	.9
ADIN[2] "	8.8[a]	1.6	14.0[b]	.9
Samples above 15% bound protein	0	-	14(35.9%)	-

[a,b]Means in the same row with different superscripts differ ($P<.01$).

[1]SE = standard error of mean.

[2]NDF = neutral detergent fiber, ADF = acid detergent fiber, INSOL-NDIN = insoluble non-cell wall nitrogen, NDIN = (neutral detergent insoluble nitrogen, NDIN-ADIN = nitrogen insoluble in ND, but soluble in AD, ADIN = acid detergent insoluble N.

Crude protein and NSOL contents were similar between samples received from throughout the state and those from the VPI dairy center. Neutral detergent insoluble nitrogen, (19.2 vs. 27.5% of total N), NDIN-ADIN (10.2 vs. 13.5% of total N), and ADIN (8.8 vs. 14.0% of total N) were greater ($P<.01$) and INSOL-NDIN (22.9 vs. 18.9% of total N) was less in statewide samples. As previously discussed, INSOL-NDIN represents the slowly available B_1 fraction and NDIN-ADIN represents the more slowly available B_2 fraction in feeds. Results show that VPI samples had more N in the B_1 fraction and statewide samples had more N in the B_2 and unavailable protein (ADIN) fractions. This is interesting because with increased ADIN in a forage more insoluble protein (less soluble protein) is expected. This was not found in these results. Significant changes within the insoluble fraction did occur with a shift away from the slowly available B_1 fraction to a more slowly available B_2 and an unavailable fraction.

Fourteen statewide samples (36%) had ADIN content greater than 15% of total N (indicative of moderate heat damage) compared to 0 VPI samples. These results show that oxygen limiting silos, when properly managed, can yield an excellent high quality forage. The results show that the use of oxygen limiting silos are not the answer to poor management on the farm. No matter what type of storage structure is used (bunker, conventional upright, oxygen limiting, plastic bags), the ensiled product can only be as good as

what went into the structure. Proper management of the forage before ensiling is extremely important in making high quality alfalfa silage. Although management conditions on other farms from which samples were taken are not known, results from VPI dairy center demonstrate what can be done under proper management conditions.

EXPERIMENT IIc.

Objectives of this experiment were to determine variations in N fractions and in vitro protein degradability of alfalfa stored as baled hay, or ensiled in conventional upright or oxygen limiting silos.

Dry matter and fiber fractions are shown in Table 13. As expected, DM content was significantly different between storage methods. Dry matter of alfalfa samples from conventional upright silos was lowest (44.4%), oxygen limiting silo samples were intermediate (56.0%), and baled hay samples were greatest (85.0%). Fiber fractions appear similar between alfalfa silages and hay.

The nitrogen fractions measured in this experiment were those in Table 3 as described by Krishnamoorthy et al. (1982). The protease procedure used has been evaluated and shows promise as a laboratory technique for predicting ruminal degradation of dietary protein (Krishnamoorthy et

Table 13. Dry matter and fiber fractions of alfalfa stored in oxygen limiting or conventional upright silos, and as baled hay.

	Silage					
	O_2-limiting		Conv. Up.		Baled Hay	
Trait	$\bar{X}$	SE[e]	$\bar{X}$	SE	$\bar{X}$	SE
Number	39	-	43	-	23	-
Dry matter (DM)	56.0[b]	1.7	44.4[a]	1.6	85.0[c]	1.3
Cell contents, %DM	48.1	1.0	49.9	.9	49.3	1.3
NDF[d], %DM	51.9	1.0	50.1	.9	50.7	1.3
Hemicellulose, %DM	11.8	.7	10.6	.7	13.5	.7
ADF[d], %DM	40.1	.6	39.5	.6	37.2	1.0

[a,b,c] Means in a row with different superscripts differ ($P<.05$).
[d] NDF = neutral detergent fiber, ADF = acid detergent fiber.
[e] Standard error of mean.

al., 1982, 1983; Nocek et al., 1983; Poos-Floyd et al., 1985). The system of fractionation is based on the solubility and insolubility of feed N in buffer solution, protease enzyme solution, and acid detergent solution.

Soluble, insoluble, protease insoluble nitrogen (PIN) and related nitrogen fractions are in Table 14. Soluble and insoluble nitrogen were significantly different between storage methods. Insoluble N of alfalfa from conventional upright silos was least (40.8% of total N), oxygen limiting silo samples were intermediate (47.5%), and baled hay samples were greatest (66.6%) and are directly related to the DM content. As DM of alfalfa silage increases, the insoluble N content will also increase due to decreased proteolysis and deamination during the ensiling process. Baled hay, stored at a greater DM content than silages, undergoes less proteolysis and deamination during the drying process resulting in less soluble protein and greater content of insoluble protein. These results are in agreement with Merchen and Satter (1983b) who also found decreased insoluble nitrogen in alfalfa ensiled at 40% DM compared to alfalfa ensiled at 66.0% DM and alfalfa hay (35.9 vs. 57.7 and 67.9% of total N, respectively).

Pattern of solubilization was determined by incubation in protease solution for 0 (represented by the insoluble N content), 2, 12, 24, and 48 h. After 2 h incubation in protease enzyme solution differences were still significant (0

Table 14. Soluble, insoluble, and protease insoluble nitrogen and related nitrogen fractions of alfalfa stored in oxygen limiting silos, conventional upright silos, or as baled hay.

	Silage					
	O_2-limiting		Conv. Up.		Baled Hay	
Trait	$\bar{X}$	SE[1]	$\bar{X}$	SE	$\bar{X}$	SE
Soluble N, %total N	52.5[b]	1.3	59.2[c]	1.2	33.4[a]	1.6
Insoluble N,	47.5[b]	1.3	40.8[a]	1.2	66.6[c]	1.6
2 hour PIN[2]	43.9[b]	1.1	37.9[a]	1.1	54.1[c]	1.5
12 hour PIN	38.9[b]	1.0	34.6[a]	.9	39.4[b]	1.3
24 hour PIN	35.2[b]	.9	31.6[a]	.8	35.5[b]	1.2
48 hour PIN	32.2[b]	.8	29.0[a]	.8	32.5[b]	1.1
Crude protein, %DM	20.0[b]	.4	19.8[b]	.4	18.5[a]	.5
ADIN[2], % total N	14.0[b]	.8	11.0[a]	.7	9.3[a]	1.0
Available N[2]	86.0[a]	.8	89.0[b]	.7	90.7[b]	1.0
Avail. escape N[2]	18.2[a]	.7	18.0[a]	.6	23.2[b]	.9
Tot. rumen degraded[2]	67.8[a]	.8	71.0[b]	.8	67.5[a]	1.1
Slowly solubilized[2]	15.4[b]	.9	11.8[a]	.8	34.1[c]	1.2

[a,b,c]Means in a row with different superscripts differ ($P<.05$).
[1]SE = standard error of mean.
[2]PIN = protease insoluble nitrogen (expressed as % total N), ADIN = acid detergent insoluble nitrogen, Available N = 100 - ADIN, Avail. escape N = PIN - ADIN, Tot. rumen degraded = 100 - PIN, Slowly solubilized N = Insoluble N - PIN. All expressed as % of total nitrogen (N).

h incubation or insoluble N were different as mentioned previously) between storage methods. However, at 12 h incubation, PIN of baled hay and oxygen limiting silo samples were similar (39.4 and 38.9% of total N) and conventional upright silo samples were significantly less (34.6% of total N). This trend was the same for the remaining incubation periods (24 and 48 h). Forty-eight h PIN (an estimate of total rumen escape N) was not different between alfalfa samples stored as baled hay or ensiled in oxygen limiting silos (32.5 vs. 32.2% of total N, respectively). Alfalfa from conventional upright silos was significantly less in 48 h PIN (29.0% of total N). Lower 48 h PIN of samples from conventional upright silos suggests more protein would be degraded in the rumen and less would escape the rumen compared with alfalfa samples from oxygen limiting silos and baled hay. The 48 h incubation time is used for determination of available escape N, total rumen degraded N, and slowly solubilized N as shown in Table 14. The 48 h time is used because that reflects maximum mean retention time of forages in the rumen (Krishnamoorthy et al., 1982).

Pattern of solubilization of nitrogen in alfalfa by storage method is shown in Figure 6. Baled hay had a very rapid rate of solubilization as evident by the steep slope from 0 to 12 h. From 12 to 48 h, rate of solubilization (slope) was similar for all three storage methods although the quantity solubilized was greatest for conventional up-

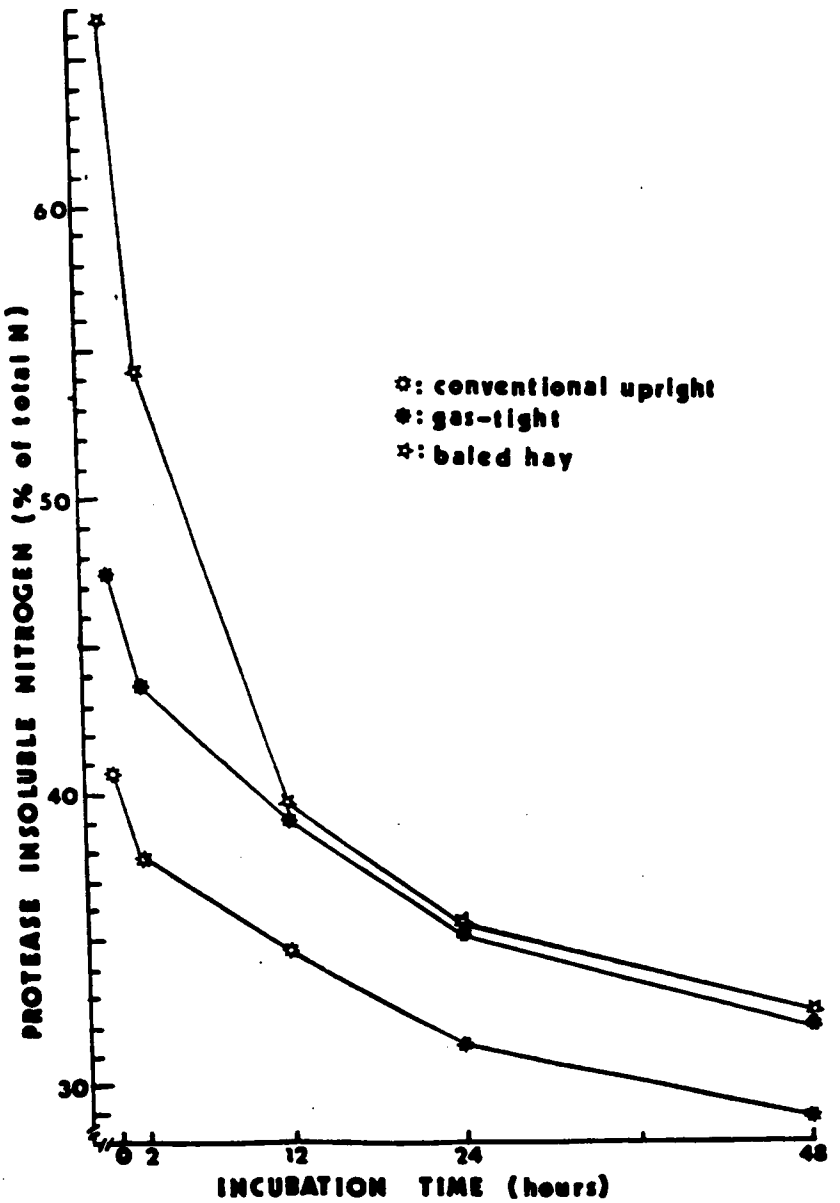

Figure 6. Pattern of solubilization of nitrogen by the protease enzyme technique for alfalfa stored as baled hay, or ensiled in conventional upright or oxygen limiting silos.

right silo samples. After 12 h incubation, oxygen limiting and baled hay samples behaved similarly as shown in Table 14 and Figure 6.

Crude protein (% DM) was significantly less for baled hay (18.5%) compared to alfalfa stored in oxygen limiting and conventional upright silos (20.0 and 19.9% DM, respectively). The lower protein content of hay may be due to leaf loss during harvest, however, ADF content of hay was slightly lower than alfalfa silages. Bound protein assayed as ADIN was greater in alfalfa ensiled in oxygen limiting compared to alfalfa ensiled in conventional upright silos and as baled hay (14.0 vs. 11.0 and 9.3% of total N, respectively). These data indicate more heating may have occurred in the oxygen limiting silos. Available N (100 - ADIN), a direct reflection of the amount of bound protein, was least for the silages from oxygen limiting silos compared to silages from conventional upright silos and baled hay. Available escape N (48 h PIN - ADIN) was greatest for baled hay samples (23.2% of total N) compared to oxygen limiting and conventional upright silo samples (18.2 and 18.0% of total N, respectively). Although 48 h PIN was similar for baled hay and oxygen limiting silos, baled hay had more available rumen escape N because these samples contained a lesser amount of bound protein. Thus, when determining available rumen escape N, the amount of unavailable N in that fraction must also be considered. These data indicate more protein would be

available in the lower gastrointestinal tract of ruminants when fed alfalfa hay compared to alfalfa silage, however, this is dependent on the amount of total protein and unavailable protein in the forage.

Estimate of total rumen degraded N (100 - 48 h PIN) was greatest for alfalfa ensiled in conventional upright silos compared to alfalfa ensiled in oxygen limiting silos and as baled hay (71.0 vs. 67.8 and 67.5% of total N, respectively). This degraded protein includes the very soluble true protein and nonprotein nitrogen and the protein that would be slowly degraded in the rumen. Merchen and Satter (1983b) reported estimated rumen degradability of 75 and 77% for baled hay and low moisture (47% DM) silage, respectively. Prange et al. (1984) reported apparent rumen degradability of crude protein was 80% for both alfalfa silage and baled hay, indicating both forage sources were equal in ability to supply protein to the intestine, even though NSOL for hay and silage were 40 and 63% of total N. Our in vitro reults slightly underestimated protein degradability in the rumen compared to the in vivo results. Protein which is slowly solubilized (INSOL - 48 h PIN) was greatest for baled hay (34.1%), intermediate for oxygen limiting silos (15.4%), and least for conventional upright silo samples (11.8% of total N) as shown in Table 14 and illustrated in Figure 6.

In summary, although crude protein content of oxygen limiting and conventional upright silos were not different,

there were significant differences in the nitrogen fractions. Crude protein of baled hay was less than that of silages. Several nitrogen fractions of alfalfa samples were similar for two of the three storage methods (baled hay, oxygen limiting silos, conventional upright silos). Conventional upright silo samples had the least insoluble protein and 48 h PIN, indicating the majority of the N would be degraded with little escaping the rumen. The reason for this is probably more extensive proteolysis in the silo due to a lower DM content. Baled hay and oxygen limiting silo samples had similar 48 h PIN, however, oxygen limiting silo samples had more unavailable N and, thus, a greater percentage of the protein in baled hay would be available postruminally. In this experiment, the in vitro protease enzyme technique in conjunction with solubility and detergent procedures were satisfactory in making comparisons between alfalfa samples from the three storage methods. These techniques can assist in evaluating an individual sample without the need for actual feeding to determine protein availability postruminally.

EXPERIMEMT III.

In vitro results

Forages used in this experiment were collected locally to get a large enough sample size for both in vitro and in situ procedures. Five samples each of alfalfa silage from conventional upright silos (AS-CU), alfalfa silage from oxygen limiting silos (AS-OL), corn silage (CS), ammonia-treated corn silage (CS-T), and orchardgrass hay (OGH), and 4 samples of alfalfa hay (AH) were collected.

Dry matter, fiber, and nitrogen fractions are shown in Table 15. As expected, DM of AH and OGH was greater than that of ensiled forages. Alfalfa from AS-OL was intermediate (57.5%) and corn silages and AS-CU were lowest. Cell contents were least (25.7%) and NDF content greatest (74.3% DM) for OGH compared to other forages. Acid detergent fiber of OGH was greatest (44.6% DM), alfalfa forages intermediate, and corn silages were lowest. Hemicellulose was greatest in OGH, intermediate for corn silages, and lowest for alfalfa forages. These results are similar to results reported in experiment Ia and agree with reported differences in composition between legumes and grasses (Van Soest, 1977).

Crude protein content was greatest for alfalfa forages; although in experiment IIc a significantly greater content of CP in alfalfa silages compared to alfalfa hay was found. Ammonia treatment of corn silage increased crude protein content 3.6 percentage units. The treated corn silage used in this experiment averaged 2.0 percentage units greater than the treated silage in experiment I and was within the ex-

Table 15. Dry matter, fiber, and nitrogen fractions using solubility and detergent procedures of forages used in the in situ study.

Trait	AS-CU[1]	AS-OL	AH	CS	CS-T	OGH	SE[2]
Number	5	5	4	5	5	5	
Dry matter, %	48.3^{a}	57.7^{b}	85.5^{c}	42.4^{a}	40.8^{a}	86.8^{c}	3.2
Cell contents[3]	52.4^{b}	52.4^{b}	52.9^{b}	53.1^{b}	49.0^{b}	25.7^{a}	2.1
NDF[3]	47.6^{a}	47.6^{a}	47.2^{a}	46.9^{a}	51.0^{a}	74.3^{b}	2.1
Hemicellulose[3]	9.3^{a}	8.7^{a}	9.6^{a}	22.7^{b}	24.3^{b}	29.7^{c}	1.1
ADF[3]	38.3^{b}	39.0^{b}	37.5^{b}	24.2^{a}	26.6^{a}	44.6^{c}	1.4
Crude protein[3]	21.2^{c}	20.5^{c}	19.3^{c}	7.9^{a}	11.5^{b}	9.8^{ab}	.8
NSOL[3]	63.3^{c}	57.5^{c}	30.4^{ab}	39.0^{b}	53.5^{c}	26.0^{a}	3.5
INSOL[3]	36.7^{a}	42.5^{a}	69.6^{bc}	61.0^{b}	46.5^{a}	74.0^{c}	3.5
INSOL-NDIN[3]	19.8^{a}	22.3^{a}	50.2^{d}	40.7^{c}	32.2^{b}	30.2^{b}	2.4
NDIN[3]	16.8^{a}	20.2^{a}	19.4^{a}	20.3^{a}	14.3^{a}	43.9^{b}	3.3
NDIN-ADIN[3]	5.8^{ab}	8.7^{ab}	11.1^{b}	11.0^{b}	3.0^{a}	29.8^{c}	2.5
ADIN[3]	11.0	11.4	8.3	9.2	11.4	14.0	1.9

^{abcd}Means in the same row with different superscripts differ ($P<.05$).

[1] AS-CU = alfalfa silage from conventional upright silos, AS-OL = alfalfa silage from oxygen limiting silos, AH = alfalfa hay, CS = corn silage, CS-T = ammonia-treated corn silage, OGH = orchardgrass hay.

[2] SE = standard error of mean.

[3] DM = dry matter, Cell contents = 100 - NDF, Hemicellulose = NDF - ADF, NDF = neutral detergent fiber, ADF = acid detergent fiber, fiber fractions expressed as % of DM, Crude protein (% DM), NSOL = soluble protein, INSOL = insoluble protein, INSOL-NDIN = insoluble non-cell wall nitrogen, NDIN = neutral detergent insoluble nitrogen, NDIN-ADIN = protein insoluble in ND, but soluble in AD, ADIN = acid detergent insoluble nitrogen. N fractions expressed as % of total nitrogen.

pected range of 10-12% CP. As expected, soluble protein of AH and OGH, expressed as a % of total N, was lower than that of ensiled forages. This is due to decreased proteolysis and deamination in hay compared to silage. Ammonia treatment of corn silage decreased the amount of insoluble protein (61.0 vs. 46.5%) when expressed as a % of total N, but not when expressed as total insoluble N (Huber et al., 1979). As reported earlier, ammonia is a source of rapidly available N, thus a lower INSOL is expected. Protein solubility of AS-CU and AS-OL were not different (63.3 and 57.5% of total N, respectively). In experiment II, differences were significant between alfalfa silages; perhaps insignificant differences in this experiment were due to small sample numbers. Neutral detergent insoluble N was grestest for OGH (43.9% of total N) and not different for the other forages, indicating much of the N in orchardgrass is in the cell wall fraction. No significant differences were found in content of ADIN. Nitrogen fractions (NDIN, NDIN-ADIN, ADIN) of CS (20.3, 11.0, 9.2, respectively) were quite similar to those values reported by Krishnamoorthy et al. (1982) of 16.4, 8.8, and 7.6% of total N, respectively. Nitrogen fractions (NDIN, NDIN-ADIN, ADIN) of CS, alfalfa silages and AH were similar to values reported by Pichard and Van Soest (1978) of 15.9, 8.4, 7.5% of total N for corn silage, 11.0, 2.2, 8.8 for alfalfa silage, and 14.7, 7.6, and 7.1% of total N, respectively for hay. The INSOL-NDIN fraction (estimate of the B_1

protein fraction), was greatest for AH (50.2% of total N) and least for alfalfa silages. The NDIN-ADIN fraction (an estimate of the B_2 protein fraction), was greatest for OGH (29.8% of total N) and least for CS-T and alfalfa silages. According to these results, total insoluble available N in OGH is divided equally into B_1 and B_2 protein fractions (30.2 and 29.8% of total N) whereas in AH, there is a 4.5 fold larger B_1 fraction compared to B_2 (50.2 vs. 11.1% of total N). These results with alfalfa hay are similar to those of Van Soest and Sniffen (1984) who reported estimated B_1 and B_2 fractions of 50 and 10% of total N, respectively. The larger estimated B_1 protein fraction (INSOL-ADIN) in alfalfa hay indicates N in alfalfa should be degraded more rapidly in the rumen compared to N in OGH. This is in part, due to the greater content of NDIN in orchardgrass hay. This N requires bacterial degradation of the plant cell wall before being degraded in the rumen as reported by Akin (1979). Results from N fractions indicate a large variation in the composition of the protein fractions among forages, especially between hay and silage as reported by Sniffen et al. (1979).

Nitrogen fractions determined using protease enzyme technique, protein solubility, and detergent procedures, and pattern of solubilization of protein by protease enzyme technique are in Table 16. The protease enzyme technique has been used successfully as an in vitro procedure to estimate rumen escape N in feedstuffs (Poos-Floyd et al. 1985;

Table 16. Nitrogen fractions determined using protease enzyme technique, protein solubility, and detergent procedures and pattern of solubilization of protein of selected forages.

Trait	AS-CU[1]	AS-OL	AH	CS	CS-T	OGH	SE[2]
INSOL[3]	36.7[a]	42.5[a]	69.6[bc]	61.0[b]	46.5[a]	74.0[c]	3.5
PIN-2h[3]	32.3[a]	32.5[a]	53.3[b]	46.6[b]	34.0[a]	49.6[b]	2.8
PIN-12h[3]	25.1[a]	29.8[ab]	35.0[bc]	42.9[c]	29.8[ab]	36.7[bc]	2.7
PIN-24h[3]	20.8[a]	28.6[b]	29.5[b]	40.6[c]	26.6[ab]	30.5[b]	2.5
PIN-48h[3]	20.4[a]	26.4[ab]	26.3[ab]	36.9[c]	24.7[ab]	28.5[b]	2.4
PIN k_d (h^{-1})[3]	.1577[d]	.0443[a]	.1226[cd]	.0744[abc]	.0555[ab]	.1075[bcd]	
Crude protein	21.2[c]	20.5[c]	19.3[c]	7.9[a]	11.5[b]	9.8[ab]	.8
ADIN[3]	11.0	11.4	8.3	9.2	11.4	14.0	1.9
Avail. N[3]	89.0	88.6	91.7	90.8	88.6	86.0	1.9
Avail. escape[3]	9.4[a]	15.0[ab]	18.0[b]	27.7[c]	13.3[ab]	14.5[ab]	2.1
Rumen degr.[3]	79.6[c]	73.6[bc]	73.7[bc]	63.1[a]	75.3[bc]	71.5[b]	2.4
Slow sol. N[3]	16.3[ab]	16.0[a]	43.3[c]	26.9[b]	21.9[ab]	45.5[c]	2.7

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

[1]AS-CU = alfalfa silage from conventional upright silos, AS-OL = alfalfa silage from oxygen limiting silos, AH = alfalfa hay, CS = corn silage, CS-T = ammonia-treated corn silage, OGH = orchardgrass hay.

[2]SE = standard error of mean.

[3]INSOL = insoluble protein, PIN-2, 12, 24, 48h = protease insoluble nitrogen after respective incubation times, CP = crude protein, ADIN = acid detergent insoluble nitrogen, Avail. N = 100 - ADIN, Avail. escape N = PIN-48h - ADIN, Rumen degraded = 100 - PIN-48h, Slow sol. N (slowly solubilized N) = INSOL - PIN-48h PIN k_d = degradation rate of slowly solubilized N. All N fractions expressed as a % of total N.

Krishnamoorthy et al. 1982). As reported earlier, INSOL was greatest for AH and OGH, followed by CS and other ensiled forages. After 2 h incubation in protease solution, AH, OGH, and CS were similar and significantly greater than PIN content of treated corn silage and alfalfa silages. This trend was similar at 12 h incubation. At 24 h incubation, PIN content of CS was greatest (40.6% of total N) and least for AS-CU (20.8% of total N) followed by CS-T (26.6), AS-OL (28.6), AH (29.5) and OGH (30.5% of total N). Forty-eight hour PIN (estimate of total rumen escape N) was greatest for CS (36.9% of total N), followed by OGH, AS-OL, AH, CS-T, and AS-CU. Rate of solubilization from 2 to 48 h was greatest for alfalfa silage from conventional upright silos (.1577/h) as shown by the largest degradation rate constant (k) in Table 16, followed by alfalfa hay (.1226/h), OGH (.1075/h), CS (.0744/h), CS-T (.0555/h) and AS-OL (.0443/h).

Although AS-CU had the greatest degradation rate constant, alfalfa and orchardgrass hays had the greatest amount of protein solubilized from 2 to 48 h, indicating much of the N in hay would be slowly solubilized in the rumen. When expressing PIN at 48 h as a percentage of PIN at 2 h; AH and OGH had the lowest percent (26.3/53.3 = 49; 28.5/49.6 = 57%); while AS-OL and CS had the greatest percent (26.4/32.5 = 81; 36.9/46.6 = 79%, respectively). Corn silage had the greatest content of protease insoluble N at 48 h (expressed as % of total N) indicating protein in corn was solubilized or de-

graded very little by the protease enzyme. Van Soest and Sniffen (1984) and Krishnamoorthy et al. (1982) reported that protein in corn (zein) is a slowly degrading protein naturally resistant to bacterial degradation in the rumen. In vitro results with the protease enzyme agree with these reports.

Available N (100 - ADIN) was not different between forages because content of ADIN was not significantly different. Available escape N is determined by subtracting unavailable N from the estimated total rumen escape N (PIN-48 h - ADIN), thus, estimating the protein which is available post-ruminally. Available escape N was greatest for corn silage (27.7% of total N), followed by AH, AS-OL, OGH, CS-T, and AS-CU. Because ADIN content of forages were not different, the differences in available rumen escape N are directly related to PIN at 48 h. Estimate of total rumen degraded N (100 - PIN-48 h) are also directly related to protease insoluble N at 48 h. Total rumen degraded N as % of total N was greatest for AS-CU (79.6% of total N), followed by CS-T, AH, AS-OL, OGH and CS. Total degraded N was significantly different between forages, and estimates are that more than 60% of the total N in all forages would be degraded in the rumen. When excluding CS, more than 70% of the total N would be degraded in the rumen. These values are similar to those reported by Merchen and Satter (1983b) for alfalfa silage and hay of 77 and 75% degradable protein and slightly lower than

the estimate of 80% for alfalfa silage and hay by Prange et al. (1984). Satter and Stehr (1984) reported tentative estimates of the fraction of degraded protein for alfalfa hay (75%), alfalfa silage (<35% DM) (80%), alfalfa silage (>55% DM) (70%), and corn silage (70%). Our in vitro results are quite similar to these. Slowly solubilized N (INSOL - PIN-48 h) was greatest for OGH (45.5% of total N) and AH (43.3%) as discussed previously. The results with AH and OGH indicate that even though much of the protein is insoluble, it would still be degraded in the rumen, but at a slower rate compared to the soluble protein fraction. Results are in agreement with Prange et al. (1984) who reported similar rumen protein degradabilities of 80% for alfalfa silage and hay even though NSOL was different (63 vs. 40% of total N, respectively). Our estimated total rumen degraded N, were similar between alfalfa forages even though NSOL of AH (30.4% of total N) was less than AS-CU and AS-OL (63.3 and 57.5% of total N, respectively).

In situ Results

Cows used in the in situ study averaged 17.6 kg/d dry matter intake (2.6% BW) and produced 19.5 kg/d milk. All cows were fed the same ration as shown in Table 4.

Recovery of DM at various incubation times, fractional description of forages, degradation rate (k_d) of dry matter

of fraction B, and rumen degradability of DM are shown in Table 17. Recovery of DM at 0 h (15 min rinsing in warm water) was greatest for OGH (80.7% recovered) and AH (67.0%). This suggests hays lose less dry matter and soluble particles than ensiled forages during the presoaking period. After 2 h incubation in the rumen, recovery of DM was still greatest for OGH followed by AH. Very little change had occurred in recovery of DM within this time period for all forages except CS. When 2 h recovery was expressed as a percent of 0 h recovery, all forages except CS were greater than 97%; CS was 84.4% (49.3/58.4). These results also suggest that a lag period may have occurred in most forages in which bacteria must adhere to forage particles before degradation can occur. Perhaps, rumen microorganisms require less time to begin to degrade DM of CS compared to the other forages. Recovery of DM from 12 to 72 h was similar for all forages except OGH which had greater recovery at each incubation time (12, 24, and 72 h). At 72 h incubation, recovery of DM of OGH (40.1%) was significantly greater than other forages and not different for the other forages. Orchardgrass hay had one of the slowest rates of degradation of DM (.0310/h); and, also, a very slow rate of degradation of NDF (.0254/h) and ADF (.0246/h) as shown in Table 22. These results indicate it takes more time for fiber fractions of OGH to be degraded compared to alfalfa.

Table 17. Recovery of dry matter (DM) at various incubation times, fractional description of DM, degradation rate of fraction B of DM, and rumen degradability of DM of forages.

Trait	AS-CU[1]	AS-OL	AH	CS	CS-T	OGH	SE[2]
Recovery of DM[3] (%)							
DM-0h	57.9^{ab}	58.6^{b}	67.0^{c}	58.4^{b}	52.2^{a}	80.7^{d}	2.1
DM-2h	56.4^{b}	57.3^{b}	65.6^{c}	49.3^{a}	51.0^{a}	79.7^{d}	1.8
DM-12h	40.7^{a}	45.2^{a}	46.4^{a}	39.9^{a}	44.7^{a}	71.1^{b}	3.0
DM-24h	28.4^{a}	33.1^{ab}	36.2^{ab}	32.7^{ab}	38.7^{b}	60.0^{c}	2.7
DM-72h	20.8^{a}	23.8^{a}	27.1^{a}	20.9^{a}	27.3^{a}	40.1^{b}	2.3
Fractions of DM[4] (%)							
A	39.0^{c}	39.1^{c}	30.8^{b}	45.2^{d}	47.3^{d}	16.8^{a}	1.9
B	40.2^{c}	37.2^{bc}	42.1^{c}	33.9^{b}	25.4^{a}	43.0^{c}	2.2
C	20.8^{a}	23.8^{a}	27.1^{a}	20.9^{a}	27.3^{a}	40.1^{b}	2.3
DM k_d[4]	.0711^{c}	.0579^{bc}	.0676^{c}	.0457^{ab}	.0344^{a}	.0310^{a}	.0067
Est. degrad.[5]							
k_r = .05	62.1^{c}	58.8^{bc}	54.6^{b}	61.3^{bc}	57.4^{bc}	33.4^{a}	2.3
k_r = .07	58.9	55.7	51.1	58.5	55.5	30.2	
k_r = .09	56.4	53.5	48.5	56.6	54.1	28.0	

^{abcd}Means in the same row with different superscripts differ ($P<.05$).

[1] AS-CU = alfalfa silage from conventional upright silos, AS-OL = alfalfa silage from oxygen limiting silos, AH = alfalfa hay, CS = corn silage, CS-T = ammonia-treated corn silage, OGH = orchardgrass hay.

[2] SE = standard error of mean.

[3] DM-0h, 2h, 12h, 24h, 72h = dry matter recovered (%) after respective rumen exposure times.

[4] Fractions of DM are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residual after 72h incubation, DM k_d = degradation rate of fraction B of DM.

[5] Estimated rumen degradability of DM (%) with respective rumen turnover rates (k_r).

Fraction A (rapidly solubilized) of DM was least for OGH in agreement with the previous finding of a greater recovery of DM from 0 to 2 h. Fraction B of DM was least for CS-T (25.4%) and greatest for alfalfa forages and OGH. Fraction C of DM was greatest for OGH (40.1%) and similar for other forages. Studies with fractionation of DM of forages are quite limited. Zerbini (1984) reported values of 20.9, 34.5, and 44.6% of total DM for A, B, and C fractions of OGH with a degradation rate of .046/h. Our results had a slightly lower A and C and slightly greater B fraction and a lower degradation rate was observed (.031/h). Of course many factors are involved which affect the fractionation including forage quality, animal factors, and fineness of sample grind.

If it is assumed that similar dynamic processes (rates of degradation and passage) occurring in the rumen affect dry matter the same as nitrogen, then the calculation used to determine crude protein or nitrogen degradability in the rumen may be used as an estimate of rumen DM degradability. Similar models of fiber digestion have been used by Mertens and Loften (1980) and Varga and Hoover (1983) to describe fiber degradation in the rumen. Using the equation of Orskov and McDonald (1979), in situ degradability of DM of OGH was least (33.4%) followed AH (54.6%), CS-T (57.4%), AS-OL (58.8%), CS (61.3%), and AS-CU (62.1%). Treated and untreated CS were not significantly different (P>.05). Klopfenstein and Owen (1981) reported increased digestibility

of DM, NDF, and ADF of wheat straw treated with ammonia compared to controls in a lamb growth trial. Modes of action of ammonia treatment include solubilization of hemicellulose, and increased rate and extent of hemicellulose and cellulose digestion (Klopfenstein and Owen, 1981). This suggests that DM degradability should have been greater in CS-T compared to CS, which does not agree with these results. Merchen and Satter (1983a, 1983b) reported in vivo DM degradability for alfalfa hay was 65.2% and 67.1% in Holstein cows and sheep, respectively. Nocek and English (1986) reported DM degradability for alfalfa hay ranging from 44.2 to 67.4% depending on the method used to describe degradation rates. Using the same method as in this experiment, alfalfa hay DM degradability was 55.9%; quite similar to the 54.6% in these results.

Recovery of CP at various incubation times, fractional description of forages, degradation rate (k_d) of N of fraction B, and rumen degradability of N are shown in Table 18. Recovery of CP at 0-h (15 min rinsing) was greatest for AH and OGH (59.3 and 58.5% of total N, respectively).

After 2 h incubation, recovery of CP of OGH (60.5% of total N) was greater than at 0-h indicating some rumen bacteria may have adhered to the forage particles and were not removed with washing and rinsing. Recovery of CP at 2-h was greatest for OGH and AH and not different for the other forages. Alfalfa hay showed a very rapid rate of degradation

Table 18. Recovery of crude protein (CP) at various incubation times, fractional description of CP, degradation rate of fraction B, and rumen degradability of CP of selected forages.

Trait	AS-CU[1]	AS-OL	AH	CS	CS-T	OGH	SE[2]
Recovery of CP[3] (%)							
CP-0h	31.7^{ab}	33.0^{ab}	59.3^{c}	36.7^{b}	25.6^{a}	58.5^{c}	3.3
CP-2h	27.6^{a}	30.5^{a}	56.1^{b}	27.0^{a}	24.4^{a}	60.5^{b}	2.5
CP-12h	15.4^{a}	21.8^{ab}	27.4^{b}	17.9^{ab}	19.4^{ab}	50.6^{c}	3.2
CP-24h	8.1^{a}	11.9^{ab}	13.8^{ab}	13.8^{ab}	15.9^{b}	37.0^{c}	2.6
CP-72h	5.2^{a}	7.3^{a}	9.1^{a}	8.3^{a}	10.3^{a}	22.9^{b}	2.1
Fractions of CP[4] (%)							
A	65.1^{bc}	61.8^{b}	29.4^{a}	71.6^{c}	74.5^{c}	33.4^{a}	3.7
B	29.7^{b}	30.9^{b}	61.6^{d}	20.1^{ab}	15.2^{a}	43.7^{c}	3.8
C	5.2^{a}	7.3^{a}	9.1^{a}	8.3^{a}	10.3^{a}	22.9^{b}	2.1
CP k_d[4]	.1057^{c}	.0819^{bc}	.1119^{c}	.0538^{ab}	.0423^{a}	.0445^{a}	.0104
Est. degrad.[5] (%)							
k_r = .05	84.9^{c}	80.9^{c}	71.5^{b}	82.2^{c}	81.4^{c}	54.1^{a}	2.5
k_r = .07	82.6	78.5	66.9	80.5	80.2	50.6	
k_r = .09	80.8	76.6	63.2	79.3	79.3	48.1	

^{abcd}Means in the same row with different superscripts differ ($P<.05$).

[1] AS-CU = alfalfa silage from conventional upright silos, AS-OL = alfalfa silage from oxygen limiting silos, AH = alfalfa hay, CS = corn silage, CS-T = ammonia-treated corn silage, OGH = orchardgrass hay.

[2] SE = standard error of mean.

[3] CP-0h, 2h, 12h, 24h, 72h = crude protein recovered (%) after respective rumen exposure times.

[4] Fractions of crude protein are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residual after 72h incubation, CP k_d = degradation rate of fraction B.

[5] Estimated degradability by the bag technique with respective k_r.

from 2 to 24 h incubation, while degradation of CP of OGH was very slow as also indicated by degradation rate (k_d) of N of B fraction of AH (.1119/h) and OGH (.0445/h). Alfalfa hay behaved similar to AS-OL and corn silages from 12 h to 72 h incubation. These results are similar to results reported earlier with protease incubations. After 72 h incubation, recovery of CP of OGH (22.9% of total N) was significantly greater than other forages.

Crude protein in the A fraction, which is instantaneously solubilized in the rumen, was greater for ensiled forages compared to hays. This result is typical because of the greater content of NPN in ensiled forages. Crude protein in the B fraction, which is degraded at a measurable rate, was greatest in AH (61.6% of total N) followed by OGH (43.7% of total N), and ensiled forages. In this experiment, there were very few differences between AS-CU and AS-OL. Both ensiled forages had similar amounts of N in the A, B, and C protein fractions. Crude protein in the C fraction, which is the residual at 72 h incubation and considered undegradable in the rumen, was greatest for OGH and not different for the other forages. This is in agreement with in vitro findings of greater NDIN in OGH compared to other forages. Neutral detergent insoluble nitrogen requires more time to be degraded in the rumen. Degradation rate (k_d) for N of fraction B was greatest for AH (.1119/h) and alfalfa silage from conventional upright silos (.1057/h) followed by

AS-OL (.0819/h), CS (.0538/h), OGH (.0445/h), and CS-T (.0423/h). The significance of a large B fraction and relatively small A and C fractions, as found with alfalfa hay would indicate a forage that is degraded in the rumen slowly, but with time it would be degraded to a greater extent. In contrast, N in CS-T, with a very large A fraction and small B and C fractions, would be degraded in the rumen very rapidly, with very little degraded post-ruminally.

Estimate of in situ protein degradability was calculated using the equation of Orskov and McDonald (1979) in which N in A and B fractions and rumen turnover rate are used. Rumen turnover rate was not measured in this experiment, however, literature values of .05/h have been reported and are commonly used with these equations (Hartnell and Satter, 1979; Colucci et al., 1982). Diet and rumen turnover rate greatly influence rate of protein degradation. There is a positive relationship between the size of the B fraction and the extent of protein degradation (Tamminga, 1979). Estimated in situ degradability with turnover rate of .05/h, was lowest for OGH (54.1%), intermediate for alfalfa hay (71.5%), and greatest for ensiled forages (80.9 to 84.9%). In situ degradability of ensiled forages was greater than 80%. Degradability is reduced by increasing turnover rate as shown in Table 18 for in situ degradability with turnover rate (k_r) equal to .07/h and .09/h. Zerbini (1984) reported values of 26.0, 40.8, and 33.2 for A, B, and C protein fractions of OGH

with degradation rate for B fraction of 0.082/h and estimated degradability of 51.3%. Results from Table 18 show a greater A fraction (33.4), similar B fraction (43.7) and lesser C fraction (22.9) and similar estimated degradability (54.1%) of OGH in this experiment. Poorer quality OGH or microbial contamination of 72 h residual in the study of Zerbini (1984) may have contributed to the greater C fraction in OGH; although assay for cytosine showed only 4% microbial contamination. No correction or adjustment was made on nitrogen values of residues for microbial contamination in this experiment. Residual CP at 72 h was similar to or less than content of ADIN for all forages except OGH (see Tables 16 and 18) indicating microbial contamination may have been a problem.

The estimated in situ degradabilities in these results for alfalfa silages and corn silages are similar to the results reported by (Satter and Stehr, 1984; Merchen and Satter, 1983b; Prange et al., 1984). Mathers and Miller (1981) reported in vivo rumen protein degradability of 72% for ground alfalfa in sheep fed at an intake level of 2.8% of bodyweight. Other in vivo studies have also reported similar degradability values in sheep (Kennedy et al., 1982; Pilgrim et al., 1970). Beever et al. (1981) reported rumen degradability of 70 and 46% for Italian ryegrass fed chopped or pelleted to sheep, respectively. Kennedy et al. (1982) also reported rumen degradability of bromegrass fed to sheep

at 3.0% of body weight of 51 to 60%; similar to the in situ results obtained with OGH. Cottrill et al. (1982) reported rumen degradability of 77% for corn silage fed to Freisian calves at an intake of 3.0% of bodyweight. The limited in vivo results of rumen degradability of forages are similar to or slightly lower than the in situ results found in this experiment.

Comparison of estimated in situ degradability by the bag technique with in vitro estimated total rumen degraded N by the protease technique shows that values for four out of six forages are within 10%. Degradabilities of AS-CU (84.9 vs. 79.6), AS-OL (80.9 vs. 73.6), CS-T (81.4 vs. 75.3), and AH (71.5 vs. 73.7) were similar between the two methods. Degradability of corn silage was underestimated (82.2 vs. 63.1) and OGH (54.1 vs. 71.5) was overestimated by the protease technique. Of the four forages estimated closely by both techniques, AH was overestimated and the others were underestimated. The low estimated degradability of CS in vitro may be due to the slowly degrading protein in corn not being degraded by the protease enzyme. The similar results in vitro and in situ for CS-T may be due to the effect ammonia treatment has on protein in corn silage. Ammonia treatment would raise the pH of the ensiled forage causing fermentation to last longer, this would allow more time for protein breakdown which is in agreement with results of greater NSOL

in CS-T. The reason for the overestimated degradability of OGH remains unclear.

Comparison of Methods

Correlation coefficients were determined between in vitro fractions and in situ degradability. Table 19 shows correlation coefficients after all data were combined. Four in vitro N fractions had similar correlations with in situ degradability. These were slowly solubilized N (SLOWSOLN = INSOL - PIN-48 h) ($r=-.81$; $P<.001$), NDIN ($r=-.80$; $P<.001$), NSOL ($r=.78$; $P<.001$), and NDIN-ADIN ($r=-.77$; $P<.001$). Nitrogen solubility was correlated with in situ degradability as expected because with increased NSOL more nitrogen will be available in the rumen. The three other N fractions were negatively correlated with in situ degradability. These three are associated with N fractions that are estimates of slowly degraded N in the rumen, thus it is expected these would be inversely related. The high correlation between NSOL and in situ degradability found in this experiment does not agree with results of Stern and Satter (1984) in which NSOL was only slightly correlated with in vivo measurements of degradability (.26) in 34 mixed diets. The large content of NPN in forages and NSOL measuring that fraction may be one

Table 19. Correlation coefficients for all data combined.

Trait	NSOL	NDIN	INSOL-NDIN	NDIN-ADIN	PIN-2h	PIN-48h	SLOW SOL N	RES-CP 2h	RES-CP 72h	CP-A	CP-B
In situ[1]	.78***	-.80***	-.19	-.77***	-.62***	-.21	-.81***	-.88***	-.90***	.78***	-.50**
NSOL	--	-.69***	-.62***	-.71***	-.89***	-.55**	-.90***	-.81***	-.60***	.72***	-.56**
NDIN		--	-.13	.95***	.52**	.36	.63***	.71***	.71***	-.61***	.40*
INSOL-NDIN			--	.00	.66***	.37*	.54**	.37*	.08	-.32	.33
NDIN-ADIN				--	.54**	.38	.67***	.72***	.66***	-.64***	.46*
PIN-2h					--	.61***	.74***	.76***	.39*	-.69***	.62***
PIN-48h						--	.13	.17	.17	-.10	.05
SLOW SOL N							--	.87***	.63***	-.80***	.64***
RES-CP 2h								--	.63***	-.97***	.83***
RES-CP 72h									--	-.50**	.13
CP-A										--	--
CP-B											--

*P<.05, **P<.01, ***P<.001.

[1]In situ = degradability of nitrogen by the bag technique, NSOL = soluble nitrogen, INSOL-NDIN = insoluble non-cell wall N, NDIN-ADIN = N insoluble in ND but soluble in AD, PIN-2h = protease insoluble N after 2h incubation, PIN-48h = PIN after 48h incubation, SLOW SOL N = slowly solubilized N (INSOL - PIN-48h), RES-CP 2h = residual crude protein after 2h rumen exposure, RES-CP 72h = residual CP after 72h rumen exposure, CP-A = CP in the A fraction which is rapidly solubilized, CP-B = CP in the B fraction which is degraded at a measureable rate.

explanation for the high correlation between these two traits.

Nitrogen solubility was highly correlated with many other in vitro measurements and recovery of crude protein in situ. Slowly solubilized N was significantly correlated with NSOL (-.90; P<.001) indicating greater content of NSOL results in decreased content of nitrogen that is slowly solubilized in the rumen. Residual CP recovered after 2 h incubation in situ and NSOL were significantly correlated (-.81; P<.001). These results are in agreement with Stern and Satter (1984) who found a correlation of 0.79 between NSOL and N disappearance of mixed diets from dacron bags at 1 h of rumen exposure. As exposure time increased in their study, the correlation progressively decreased to 0.33 at 24 h. In this experiment, correlation decreased to -.60 at 72 h incubation time. Crawford et al. (1978), comparing 28 feedstuffs, found the correlation coefficient between NSOL in 10% Wise Burroughs solution and N disappearance from bags following 2 h of rumen exposure was 0.66. After samples were separated into concentrates, hays, and silages, correlations were improved to 0.70, and 0.94 for concentrates and silages. Lower correlation for hays than for silages was noted with 2 h rumen exposure. Further correlations made for NSOL of hay with degradation at various intervals revealed a higher correlation after 4 h rumen exposure (.88).

As expected, NSOL and PIN at 2 h incubation were negatively correlated (-.89). Likewise, PIN-2h and residual CP at 2 h were positively correlated (.76). These are two measurements of insoluble protein after a relatively short incubation period thus the high correlation is expected.

Due to the large differences noted between hays and silages in this experiment and reported by Crawford et al. (1978), data were divided into hays (nine samples) and silages (twenty samples) and correlations were determined on each set of data. Correlation coefficients for silage and hay data analyzed separately are in Tables 20 and 21, respectively. Correlations were changed greatly after separating data due to the differences in forages. Correlation for NSOL and NDIN with in situ degradability for silages was reduced (.58; $P<.01$; -.51; $P<.05$), but still significantly correlated, while other correlations became insignificant, such as NDIN-ADIN and SLOWSOLN with in situ degradability (-.23; $P>.05$ and -.39; $P>.05$, respectively). Correlation for NSOL with PIN-2h for silages was still high (-.90);, and similar for all data combined.

Correlation for NDIN and NDIN-ADIN with in situ degradability for hays were only slightly changed compared to correlations when all data were combined (-.83 vs. -.80 and -.76 vs. -.77, respectively). Correlation for NSOL with in situ degradability for hay became insignificant (.56; $P>.05$) when data were separated. These differences are par-

Table 20. Correlation coefficients for silages.

Trait	NSOL	NDIN	INSOL-NDIN	NDIN-ADIN	PIN-2h	PIN-48h	SLOW SOL N	RES-CP 2h	RES-CP 72h	CP-A	CP-B
In situ[1]	.58**	-.51*	-.24	-.23	-.48*	-.54*	-.39	-.77***	-.71***	.60**	-.41
NSOL	--	-.54*	-.75***	-.48*	-.90***	-.88***	-.74***	-.41	-.53*	.11	.02
NDIN		--	-.15	.83***	.46*	.54*	.22	.62**	.24	-.44	.39
INSOL-NDIN			--	-.19	.70***	.61**	.65**	-.01	.49*	.23	-.34
NDIN-ADIN				--	.42	.52*	.12	.41	-.10	-.34	.35
PIN-2h					--	.89***	.52*	.48*	.31	-.20	.12
PIN-48h						--	.33	.44*	.41	-.21	.11
SLOW SOL N							--	.19	.47*	.07	-.19
RES-CP 2h								--	.16	-.90***	.84***
RES-CP 72h									--	.01	.23
CP-A										--	--
CP-B											--

*P<.05, **P<.01, ***P<.001.

[1]In situ = degradability of nitrogen by the bag technique, NSOL = soluble nitrogen, INSOL-NDIN = insoluble non-cell wall N, NDIN-ADIN = N insoluble in ND but soluble in AD, PIN-2h = protease insoluble N after 2h incubation, PIN-48h = PIN after 48h incubation, SLOW SOL N = slowly solubilized N (INSOL - PIN-48h), RES-CP 2h = residual crude protein after 2h rumen exposure, RES-CP 72h = residual CP after 72h rumen exposure, CP-A = CP in the A fraction which is rapidly solubilized, CP-B = CP in the B fraction which is degraded at a measureable rate.

Table 21. Correlation coefficients for hays.

Trait	NSOL	NDIN	INSOL-NDIN	NDIN-ADIN	PIN-2h	PIN-48h	SLOW SOL N	RES-CP 2h	RES-CP 72h	CP-A	CP-B
In situ[1]	.56	-.83***	.68*	-.76*	.12	-.41	-.38	-.68*	-.96***	-.15	.89***
NSOL	--	-.57	.10	-.56	-.20	-.04	-.94***	-.61	-.46	.41	.23
NDIN		--	-.87**	.99***	-.20	.35	.42	.54	.72*	.25	-.72*
INSOL-NDIN			--	-.86***	.37	-.44	.05	-.30	-.60	-.55	.74*
NDIN-ADIN				--	-.30	.28	.43	.41	.65	.33	-.69*
PIN-2h					--	.03	-.18	.58	-.20	.70*	.45
PIN-48h						--	-.36	.27	.30	.40	-.42
SLOW SOL N							--	.48	.33	-.52	-.07
RES-CP 2h								--	.60	-.52	-.31
RES-CP 72h									--	.14	-.92***
CP-A										--	--
CP-B											--

*P<.05, **P<.01, ***P<.001.

[1]In situ = degradability of nitrogen by the bag technique, NSOL = soluble nitrogen, INSOL-NDIN = insoluble non-cell wall N, NDIN-ADIN = N insoluble in ND but soluble in AD, PIN-2h = protease insoluble N after 2h incubation, PIN-48h = PIN after 48h incubation, SLOW SOL N = slowly solubilized N (INSOL - PIN-48h), RES-CP 2h = residual crude protein after 2h rumen exposure, RES-CP 72h = residual CP after 72h rumen exposure, CP-A = CP in the A fraction which is rapidly solubilized, CP-B = CP in the B fraction which is degraded at a measureable rate.

tially due to the differences in analytical measurements between hays and silages. Silages tend to have a greater content of NSOL compared to hays, thus you would expect NSOL to be more highly correlated to rumen degradability for silages than for hays which was found in this experiment. Likewise, for hays, with more slowly degraded protein compared to silages, you would expect measurements estimating that protein fraction to be more highly correlated with in situ degradability, which was also found in this experiment with the INSOL-NDIN and NDIN-ADIN protein fractions.

Other possible explanations for the large differences in correlations between hays, silages, and combined data are the confounding effect of alfalfa hay. Although alfalfa hay was similar to OGH in several in vitro measurements, it behaved more closely to alfalfa silages for many other measurements. Thus differences between legumes and grasses may also be partly responsible for some of the differences in correlations. The small number of hay samples (9) may also be partly responsible for some loss of significance in correlation, especially with the large variation among samples.

After comparing all three correlation tables, only a few protein fractions were related similarly for both hays and silages. These included SLOWSOLN with NSOL, NDIN with in situ degradability, residual CP at 2 h with in situ degradability, and residual CP at 72 h (C fraction) with in situ degradability.

In situ fiber degradation

Fractional description of forages, degradation rate (k_d) of NDF and ADF of B fraction, and estimated rumen degradability of NDF and ADF are in Table 22. Degradation rate of B fraction of NDF was greater for alfalfa forages compared to corn silages and OGH. Alfalfa silage from conventional upright silos and AH had the greatest degradation rates (.0596/h and .0577/h, respectively). This indicates the NDF of legumes has a faster rate of digestion compared to grasses. Mertens and Loften (1980) also reported greater rate of digestion of NDF for alfalfa hay compared to coastal bermudagrass, fescue, and orchardgrass hays. Fraction B of NDF was significantly lower for CS-T (30.1%) and not different for the other forages. Perhaps ammonia treatment of corn silage caused a shift in NDF fractions from the slowly available B fraction to the more rapidly available A fraction. Ammonia treatment of crop residues has resulted in increased digestibility of fiber fractions in vivo and increased rate and extent of cellulose and hemicellulose digestion in vitro (Klopfenstein and Owen, 1981). In contrast, these results show greater estimated degradability of NDF and ADF in situ for CS compared to CS-T and no significant differences in rate of degradation of the B fraction of NDF and

Table 22. Degradation of neutral and acid detergent fiber in situ in selected forages.

Trait	AS-CU[1]	AS-OL	AH	CS	CS-T	OGH	SE[2]
Fractions of NDF[3] (%)							
A	9.8^{a}	10.1^{ab}	4.7^{a}	16.2^{b}	23.8^{c}	6.2^{a}	2.1
B	52.4^{b}	47.5^{b}	48.3^{b}	44.6^{b}	30.1^{a}	49.5^{b}	3.0
C	37.8^{a}	42.4^{ab}	50.4^{c}	39.2^{ab}	46.1^{bc}	46.3^{bc}	2.2
NDF k_d[3]	.0596^{c}	.0462^{bc}	.0577^{c}	.0286^{ab}	.0203^{a}	.0254^{ab}	.0062
Est. degrad.[4] (%)							
k_r = .05	61.8^{b}	57.0^{ab}	52.5^{a}	60.0^{b}	53.0^{a}	54.6^{ab}	2.3
k_r = .07	61.6	56.7	52.3	59.6	52.8	54.3	--
k_r = .09	61.4	56.5	52.1	59.3	52.5	53.9	--
Fractions of ADF[5] (%)							
A	8.0^{abc}	11.5^{bcd}	3.1^{a}	14.6^{cd}	17.1^{d}	3.3^{ab}	2.6
B	55.8^{c}	47.7^{bc}	47.9^{bc}	41.6^{b}	30.2^{a}	48.9^{bc}	3.2
C	36.2^{a}	40.8^{ab}	51.0^{d}	43.8^{bc}	52.7^{d}	47.3^{cd}	2.1
ADF k_d[5]	.0665^{b}	.0504^{b}	.0564^{b}	.0254^{a}	.0161^{a}	.0246^{a}	.0065
Est. degrad.[6] (%)							
k_r = .05	63.4^{d}	58.8^{cd}	50.5^{ab}	55.2^{bc}	46.4^{a}	51.2^{abc}	2.2
k_r = .07	63.2	58.6	50.3	54.8	46.0	50.8	--
k_r = .09	63.0	58.4	50.2	54.5	45.6	50.4	--

^{abcd}Means in the same row with different superscripts differ (P<.05).
[1]AS-CU = alfalfa silage from conventional upright silos, AS-OL = alfalfa silage from oxygen limiting silos, AH = alfalfa hay, CS = corn silage, CS-T = ammonia-treated corn silage, OGH = orchardgrass hay.
[2]SE = standard error of mean.
[3]Fractions of NDF are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residue after 72 h incubation. NDF k_d = degradation rate of fraction B.
[4]Estimated degradability of NDF with respective turnover rates (k_r).
[5]Fractions of ADF (defined as in 3), ADF k_d = degradation rate of ADF.
[6]Estimated degradability of ADF with respective turnover rates.

ADF. Estimated in situ degradability of NDF was greatest for AS-CU and CS, followed by AS-OL, OGH, CS-T, and AH. Estimated degradability of NDF for AH was lower than reported by Nocek and English (1986) (52.5 vs. 63.3%, respectively), but identical to that reported by Mertens and Loften (1980). Degradation rate, fractional description, and estimated degradability of ADF followed similar trends as NDF for all forages.

Reports indicate degradation in the rumen is greatly influenced by rumen turnover rate. However, when estimated degradability of DM, CP, NDF, and ADF were determined with turnover rates of .05/h, .07/h, and .09/h, very little change had occurred (see Tables 17, 18, and 22). These results are in agreement with Stern and Satter (1984) who also found a modest effect of changing turnover time from 0.04/h to 0.06/h on protein degradation in the rumen.

Evaluation of By-product Feeds

By-product feeds analyzed included whole cottonseed (3), dried distillers grains (DDG) dark (2) and light colored (1), and wet brewers grains (WBG) (1). Dry matter, fiber, and nitrogen fractions using solubility and detergent procedures are in Table 23. Neutral detergent fiber content of DDG-light was extremely high in the sample analyzed. Al-

Table 23. Dry matter, fiber, and nitrogen fractions of by-product feeds.

Trait	WC[1]		DDG-Dark		DDG-Light		WBG	
	$\bar{X}$	SE[2]	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Number	3	--	2	--	1	--	1	--
DM[3], %	93.9	.3	95.2	.4	89.4	.6	21.7	.6
Cell contents[3]	33.3	2.5	50.2	3.1	25.9	4.3	35.0	4.3
NDF[3]	66.7	2.5	49.8	3.1	74.1	4.3	65.0	4.3
Hemicellulose[3]	14.6	2.7	22.0	3.3	54.8	4.7	38.2	4.7
ADF[3]	52.1	1.3	27.8	1.5	19.3	2.2	26.8	2.2
Crude protein	18.3	1.5	32.1	1.9	30.5	2.6	32.0	2.6
NSOL[3]	42.7	1.3	15.7	1.6	44.0	2.3	12.5	2.3
INSOL[3]	58.3	1.3	84.3	1.6	56.0	2.3	87.5	2.3
INSOL-NDIN[3]	41.1	3.6	30.5	4.4	18.3	6.2	55.0	6.2
NDIN[3]	17.2	3.1	53.8	3.8	37.7	5.4	32.5	5.4
NDIN-ADIN	4.6	2.9	14.8	3.6	24.8	5.1	16.3	5.1
ADIN[3]	12.6	1.9	39.0	2.4	13.0	3.4	16.2	3.4

[1]WC = whole cottonseed, DDG-dark = dried distillers grains, dark colored, DDG-light = dried distillers grains, light colored, WBG = wet brewers grains.
[2]SE = standard error.
[3]DM = dry matter (%), cell contents = 100 - NDF, NDF = neutral detergent fiber (% DM), hemicellulose = NDF - ADF, ADF = acid detergent fiber (% DM), NSOL = soluble protein, INSOL = insoluble protein, INSOL - NDIN = insoluble non-cell wall nitrogen, NDIN = neutral detergent insoluble nitrogen, ADIN = acid detergent insoluble nitrogen. N fractions expressed as % of total N.

though literature values of NDF range from 40 to 50% of DM (Van Soest et al., 1984; Jorgensen et al., 1981); large variations have been observed in the composition of by-product feeds as reported by Chase (1982). Acid detergent fiber contents of DDG-dark and light, and WBG were similar to reported values (NRC, 1978); while ADF of WCS (52.1% DM) was greater than values of 34 and 37% of DM reported by Smith et al. (1981) and Coppock et al. (1985), respectively.

During the process of making distillers grains, nutrient components undergo a three-fold concentration, thus, for corn by-products of the distilling industry, a CP content of at least 25 to 30% is expected. Crude protein content of DDG-dark and light, and WBG were slightly greater than values reported by Sniffen et al. (1979) and Van Soest and Sniffen (1984). Crude protein content of WCS averaged 18.3% of DM, slightly lower than reported by Smith et al. (1981) and Coppock et al. (1985) of 22.3 and 20.9% of DM, respectively. Working with whole cottonseeds was found to be quite troublesome. As mentioned previously, WCS was ground through a 6-mm screen rather than the 1-mm screen used for other by-product feeds and forages. This was due to the problems of passing the lint through the 1-mm screen. After grinding through the 6-mm screen, WCS samples separated into seed contents and lint which made getting a representative sample for each analysis very difficult. Because of this problem, all analyses were done in triplicate rather than duplicate.

Nitrogen solubility for DDG-dark and WBG were low and similar to values reported in the literature; however, NSOL of DDG-light was slightly greater than literature values, but within the range of 26 to 46% of total N reported by Van Soest and Sniffen (1984). Content of ADIN for DDG-light and WBG were similar to literature values; however, ADIN of DDG-dark, expressed as a % of total N, was extremely high (39.0%) indicative of a large content of heat damaged proteins. Van Soest and Sniffen (1984) reported a range of 10 to 38% of total N for ADIN content of DDG. The percentage of protein in the INSOL and NDIN fractions for DDG, and WBG indicates the large potential rumen escape protein content of these by-product feeds.

Nitrogen fractions determined using protease enzyme technique, NSOL, and detergent procedures are in Table 24. Available N was very low for DDG-dark due to the large content of heat damaged protein (ADIN). Estimated total rumen degraded N was less than 60% for all by-product feeds. Estimations of rumen degraded N for DDG-dark and light and WBG by Satter and Stehr (1984) were similar to in vitro estimates by the protease technique (45 vs. 47.0 and 46.0; 55 vs. 57.1%), respectively. Krishnamoorthy et al. (1982) reported values of 47.6 and 32.0% of total N for total rumen degraded N and slowly solubilized N for DDG-dark, almost identical to the 47.9 and 31.2% found in this study.

Table 24. Nitrogen fractions determined using protease enzyme technique, protein solubility, and detergent procedures protein solubility, and detergent procedures and pattern of solubilization of protein of by-product feeds.

Trait	WC[1]		DDG-Dark		DDG-Light		WBG	
INSOL[2]	58.3	1.3	84.3	1.6	56.0	2.3	87.5	2.3
PIN-2h[2]	57.5	8.7	70.9	10.6	82.3	15.0	83.8	15.0
PIN-12h[2]	51.2	10.1	64.2	12.3	73.0	17.4	69.0	17.4
PIN-24h[2]	45.9	8.0	61.9	9.8	67.9	13.9	55.4	13.9
PIN-48h[2]	41.8	7.0	53.1	8.6	54.0	12.2	42.9	12.2
PIN k_d[2]	.0620		.0031		.0032		.0540	
Crude protein	18.3	1.5	32.1	1.9	30.5	2.6	32.0	2.6
ADIN[2]	12.6	1.9	39.0	2.4	13.0	3.4	16.2	3.4
Avail. N[2]	87.4	1.9	61.0	2.4	87.0	3.4	83.8	3.4
Avail. esc. N[2]	29.2	7.0	14.1	8.6	41.0	12.2	26.7	12.2
Tot. degraded[2]	58.2	7.0	47.0	8.6	46.0	12.2	57.1	12.2
Slow sol. N[2]	16.5	6.9	31.2	8.5	2.0	11.9	44.7	11.9

[1]WC = whole cottonseed, DDG-dark = dried distillers grains, dark colored, DDG-light = dried distillers grains, light colored, WBG = wet brewers grains.

[2]INSOL = insoluble protein, PIN-2h, 12h, 24h, 48h = protease insoluble nitrogen after respective incubation times, PIN k_d = degradation rate of slowly solubilized N, ADIN = acid detergent insoluble nitrogen, Avail. N = 100 - ADIN, Avail. esc. N = PIN-48h - ADIN, Tot. degraded = 100 - PIN-48h, Slow sol. N = INSOL - PIN-48h. N fractions expressed as % of total N.

Recovery of DM, fractional description, and estimated rumen degradability of DM for by-product feeds is in Table 25. Recovery of DM for WCS and WBG was very high at 0 h indicating very few feed particles were solubilized or removed during the 15 min presoaking period. More than 50% of the DM was removed from DDG-dark and light during the presoaking period. After 72-h incubation, 43.2% of DM was removed for WCS, indicating fiber content was degraded slowly. Less than 10% of DM was recovered after 72 h incubation for DDG-dark and light. Estimated degradability of DM was similar for WCS and WBG (41.3 and 43.2%, respectively), and similar for DDG-dark and light (69.9 and 64.5%, respectively). Increasing rumen turnover rate from .05 to .09/h changed estimated degradability very little as also found with forages.

Recovery of CP, fractional description, and estimated rumen degradability of CP for by-product feeds is in Table 26. Recovery of CP after 15-min soaking were similar for WCS and WBG and for both DDG. After 72 h incubation, recovery of CP for DDG was less than 10%, WCS was 25.1% and WBG was 48.8%. Even though DDG-dark had a very large content of ADIN (39.0% of total N), after 72 h incubation only 8.0% of total CP was recovered indicating either some of the heat damaged protein was washed out of the bags or some was degraded by rumen microorganisms. Crude protein in the B fraction was greater than 40% for all by-product feeds. Estimated rumen

Table 25. Recovery of dry matter at various incubation times, fractional description of DM, degradation rate of fraction B, and rumen degradability of DM of by-product feeds.

Trait	WC[1]		DDG-Dark		DDG-Light		WBG	
Recovery of DM[2] (%)								
Res. DM-0h	79.7	1.5	50.4	1.8	59.3	2.6	84.3	2.6
Res. DM-2h	76.8	4.2	43.0	5.1	54.3	7.2	69.8	7.2
Res. DM-12h	57.3	5.9	31.2	7.2	38.2	10.2	60.7	10.2
Res. DM-24h	51.8	6.8	25.3	8.4	26.9	11.9	49.9	11.9
Res. DM-72h	43.2	4.6	8.7	5.6	7.6	7.9	36.2	7.9
Fractions of DM[3] (%)								
A	21.6	4.9	55.9	6.1	42.1	8.6	26.4	8.6
B	35.1	7.3	35.3	9.0	50.3	12.7	37.4	12.7
C	43.3	4.6	8.7	5.6	7.6	7.9	36.2	7.9
DM k_d[3]	.069		.032		.040		.041	
Est. degrad.[4] (%)								
k_r = .05	41.3	5.2	69.9	6.4	64.5	9.0	43.2	9.0
k_r = .07	38.5		67.2		60.5		40.2	
k_r = .09	36.5		65.4		57.6		38.1	

[1] WC = whole cottonseed, DDG-dark = dried distillers grains, dark colored, DDG-light = dried distillers grains, light colored, WBG = wet brewers grains.
[2] Recovery of dry matter after respective rumen exposure times.
[3] Fractions of DM are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residue after 72h rumen exposure, DM k_d = degradation rate of fraction B.
[4] Estimated degradability of DM with respective rumen turnover rates (k_r).

Table 26. Recovery of crude protein at various incubation times, fractional description of CP, degradation rate of fraction B, and rumen degradability of CP of by-product feeds.

Trait	WC[1]		DDG-Dark		DDG-Light		WBG	
Recovery of CP[2] (%)								
Res. CP-0h	85.4	6.1	63.0	7.5	62.6	10.6	86.0	10.6
Res. CP-2h	78.0	9.0	49.1	11.1	56.6	15.6	84.3	15.6
Res. CP-12h	37.2	5.8	34.8	7.1	38.2	10.0	80.2	10.0
Res. CP-24h	29.3	4.3	26.2	5.2	29.1	7.4	65.3	7.4
Res. CP-72h	25.1	3.7	8.0	4.6	4.9	6.5	48.8	6.5
Fractions of CP[3] (%)								
A	18.4	10.4	48.7	12.7	41.6	18.0	10.2	18.0
B	57.5	10.4	43.3	12.7	53.5	18.0	41.0	18.0
C	25.1	3.7	8.0	4.6	4.9	6.5	48.8	6.5
CP k_d[3]	.153		.037		.034		.035	
Est. degrad.[4] (%)								
k_r = .05	58.8	5.6	67.1	6.9	63.4	9.8	27.8	9.8
k_r = .07	54.6		63.7		59.2		24.5	
k_r = .09	51.3		61.3		56.4		22.3	

[1] WC = whole cottonseed, DDG-dark = dried distillers grains, dark colored, DDG-light = dried distillers grains, light colored, WBG = wet brewers grains.
[2] Recovery of crude protein after respective rumen exposure times.
[3] Fractions of CP are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residue after 72h rumen exposure, CP k_d = degradation rate of fraction B.
[4] Estimated degradability of CP with respective rumen turnover rates (k_r).

degradability of CP was similar for WCS, DDG-dark and light, and lower for WBG (27.8%).

Degradation of NDF and ADF in situ for by-product feeds is in Table 27. Recovery of NDF after 72 h incubation was 50.3% for WCS indicating rumen microbes require a long time to degrade the fiber fraction in WCS. Recovery of NDF after 72 h was 11.6 and 6.6% for DDG-dark and light, indicating the NDF in those feeds is either rapidly degraded in the rumen or removed to a large extent during presoaking as indicated by the low recovery of NDF for DDG-light (49.4%) after presoaking for 15 min. Recovery of NDF of WBG was similar to that for WCS. Trends in degradation of ADF in situ were similar to those of NDF.

Table 27. Degradation of neutral and acid detergent fiber in situ in by-product feeds.

Trait	WC[1]		DDG-Dark		DDG-Light		WBG	
Recovery of NDF[2] (%)								
Res. NDF-0h	79.8	7.0	70.5	7.0	49.4	9.9	87.2	9.9
Res. NDF-2h	67.1	9.8	61.8	9.8	48.7	13.8	76.2	13.8
Res. NDF-12h	54.1	4.5	45.8	4.5	34.5	6.4	65.3	6.4
Res. NDF-24h	52.6	4.8	36.5	4.8	22.4	6.7	55.0	6.7
Res. NDF-72h	50.3	4.2	11.6	4.2	6.6	5.9	42.4	5.9
Fractions of NDF[3] (%)								
A	31.5	9.6	36.2	9.6	46.8	13.6	19.8	13.6
B	18.3	10.0	52.2	10.0	46.6	14.1	37.8	14.1
NDF k_d[3]	.092		.031		.045		.045	
Est. degrad.[4] (%)								
k_r = .05	43.2	5.5	56.4	5.5	68.8	7.8	37.7	7.8
k_r = .07	41.7		52.5		64.9		34.6	
k_r = .09	40.6		49.8		62.2		32.4	
Recovery of ADF[5] (%)								
Res. ADF-0h	80.1	2.1	62.0	2.1	61.6	2.9	85.7	2.9
Res. ADF-2h	71.0	5.7	53.0	5.7	55.3	8.0	81.3	8.0
Res. ADF-12h	63.4	5.1	38.2	5.1	40.0	7.2	79.2	7.2
Res. ADF-24h	59.8	1.3	30.7	1.3	35.5	1.8	66.7	1.8
Res. ADF-72h	53.2	3.3	12.5	3.3	11.1	4.7	52.2	4.7
Fractions of ADF[6] (%)								
A	26.5	4.7	42.3	4.7	44.8	6.6	25.5	6.6
B	20.3	7.8	42.2	7.8	44.1	11.0	22.3	11.0
ADF k_d[6]	.065		.036		.027		.034	
Est. degrad.[7] (%)								
k_r = .05	36.3	1.6	60.0	1.6	60.2	2.2	34.5	2.2
k_r = .07	34.7		56.7		57.0		32.8	
k_r = .09	33.5		54.4		54.9		31.6	

[1] WC = whole cottonseed, DDG-dark = dried distillers grains, dark colored, DDG-light = dried distillers grains, light colored, WBG = wet brewers grains.

[2] Recovery of neutral detergent fiber after respective rumen exposure times.

[3] Fractions of NDF are A = rapidly solubilized, B = degraded at a measureable rate, C = undegraded residue after 72h rumen exposure, NDF k_d = degradation rate of fraction B.

[4] Estimated degradability of NDF with respective rumen turnover rates.

[5] Recovery of acid detergent fiber after respective rumen exposure times.

[6] Fractions of ADF are those described for NDF.

[7] Estimated degradability of ADF with respective rumen turnover rates (k_r).

SUMMARY AND CONCLUSIONS

1. Large differences were noted between forage species and large ranges occurred within the same forage specie for content of dry matter, fiber, and nitrogen fractions.

2. Nitrogen solubility of alfalfa silage was significantly affected by days setting prior to analysis. Content of NSOL decreased from 64.9% on day 1 to 63.2% of total N on day 5. No other chemical analyses were affected by temperature (34^{o} vs. 21^{o}C) or days setting (0 to 5 d) prior to analysis.

3. Storage structure did not significantly affect the total N content or fiber fractions of alfalfa silage stored in oxygen limiting and conventional upright silos. Nitrogen fractions including INSOL (47.2 vs. 40.5% of total N), NDIN (27.5 vs. 19.6% of total N), NDIN-ADIN (13.5 vs. 8.6% of total N), and ADIN (14.0 vs. 11.0% of total N) were greater in oxygen limiting silos.

4. Silage from oxygen limiting silos had 35.9% of samples with ADIN greater than 15% of total N compared to only 14% of samples from conventional upright silos.

5. Low r^2 (.34 and .35) of regression equations for DM versus ADIN for conventional upright and oxygen limiting silos, re-

spectively, suggests that other factors must be considered to accurately predict ADIN.

6. Alfalfa samples from VPI were lower in NDIN (19.0 vs. 27.5% of total N), NDIN-ADIN (10.2 vs. 13.5% of total N), and ADIN (8.8 vs. 14.0% of total N) compared to alfalfa samples received from the state.

7. Crude protein content of baled hay was less than that of ensiled alfalfa from oxygen limiting and conventional upright silos. Content of protease insoluble N at 48 h was lowest for AS-CU (29.0% of total N). Baled hay and AS-OL samples had similar protease insoluble N at 48 h (32.5 vs. 32.2 % of total N), however, AS-OL samples had greater content of ADIN (9.3 vs. 14.0% of total N).

8. In vitro protease technique, in conjunction with protein solubility, and detergent procedures were satisfactory in making comparisons between alfalfa stored as baled hay or ensiled in oxygen limiting or conventional upright silos.

9. Orchardgrass hay had significantly greater content of NDF (74.3% of DM), hemicellulose (29.7% of DM), and ADF (44.6% of DM) compared to other forages used in the in situ study. Orchardgrass hay and AH had lowest content of soluble protein compared to ensiled forages.

10. Corn silage had greatest content of protease insoluble N at 48 h (36.9% of total N) indicating that protein in CS is not degraded well by protease enzyme in a buffered water solution.

11. Slowly solubilized N was significantly greater for AH and OGH and similar for ensiled forages.

12. Estimated rumen nitrogen degradability was greater than 80% for ensiled forages.

13. Ensiled forages had the greatest estimated A fraction compared to AH and OGH. Alfalfa hay had the greatest estimated B fraction, and orchardgrass hay had greatest C fraction.

14. High correlations between in situ degradability and some in vitro measurements suggest laboratory techniques of estimating degradability of forages is feasible. For silages, NSOL had the greatest correlation (.58; $P<.01$) with in situ degradability, followed by protease insoluble N at 48 h (-.54; $P<.05$) and NDIN (-.51; $P<.05$). For hay, NDIN had the greatest correlation (-.83; $P<.001$) with in situ degradability, followed by NDIN-ADIN (-.76; $P<.05$) and INSOL-NDIN (-.68; $P<.05$).

15. Degradation of NDF and ADF followed similar patterns for each forage. Degradability of fiber of CS-T and AH were lowest, this result was related to the small B fraction of CS-T and the small A fraction of AH. Rate of degradation of B fraction of NDF and ADF was greater for alfalfa forages compared to corn silages and OGH.

16. Too few samples were analyzed for by-product feeds to make firm conclusions. In situ data show the potential of all by-product feeds as sources of rumen escape N, as indicated by the relatively low total rumen degraded N and large protease insoluble N at 48 h fractions.

CONCLUSIONS

For the most part, this study dealt with fiber and nitrogen fractions of forages and a few by-product feeds. Large differences were found in the fiber and nitrogen fractions measured in vitro and in situ between forage species as well as within forage specie.

It will be advantageous to devise a laboratory method which can be used to estimate rumen degradable protein for ration formulation purposes. It is also recommended to analyze for ADIN in forages and by-product feeds suspected of

having heat-damage, and to correct for that fraction when balancing rations.

Comparing in vitro techniques with the in situ method of estimating degradability ended with some very positive and favorable results. Because of the large differences in forages (hay vs. silage), use of only one in vitro technique can not be recommended to predict estimated rumen degradability at this time. For silage, the best method related to in situ degradability was protein solubility; for hay, the best method was NDIN. Too few samples of by-product feeds were used to even try to evaluate the in vitro methods.

Further studies are required with more by-product and concentrate feeds to evaluate each of the in vitro techniques in their ability to estimate rumen degradability.

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APPENDIX

VIRGINIA COOPERATIVE EXTENSION SERVICE

VIRGINIA TECH

Reply to
Department of Dairy Science
Virginia Tech
Blacksburg VA 24061

VIRGINIA STATE

Form 404-716

Reprinted March 1985

FORAGE TESTING LABORATORY

Feed Analysis Request

Please Print All Information

LAB NO._____
(for lab use only)

NAME_____

DATE SAMPLED_____

ADDRESS_____

DATE RECEIVED_____
(for lab use only)

_____

COUNTY_____

AGENT'S SIGNATURE_____

SAMPLE DESCRIPTION_____
(for grass-legume forages, provide cutting and date of harvest if known)

FARM SAMPLE NO._____

COMPLETE ALL INFORMATION IF POSSIBLE

SAMPLE TYPE

___Hay
___Silage
___Green-chop
___Grain
___Complete ration
___Concentrate mix
___Protein or mineral supplement

FORAGE TYPE:

___Corn
___Alfalfa
___Grass
___Barley
___Rye
___Legume____%, Grass____%
___Other_____

STORAGE OR CONDITION OF SAMPLE

___Standing forage samples

SILO

___Upright
___Horizontal
___Gas-tight
___Plastic bags

HAYS

___Large bales
___Compressed stacks
___Conventional small bales

ADDITIVES OR PRESERVATIVES INCLUDED IN SAMPLES
(Forages or high moisture grains only)

___Urea
___Ammonia
___Limestone
___Propionic acid
___Enzymatic or probiotic-type preservatives
___Commercial NPN additives
___Other

Amount Added

_____% or _____Lb/ton

Name or Product

_____

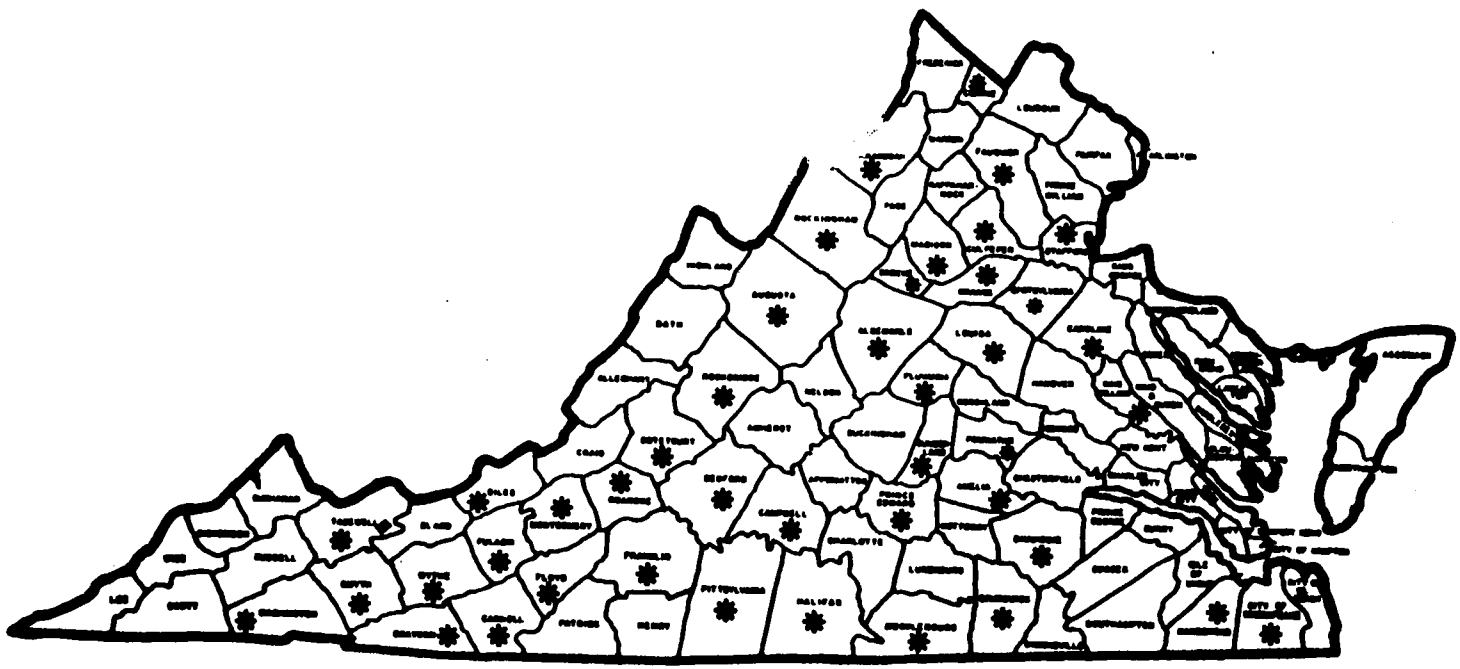

Figure 2. Map of Virginia depicting counties from which forage samples were received at the laboratory for use in experiment 1a.

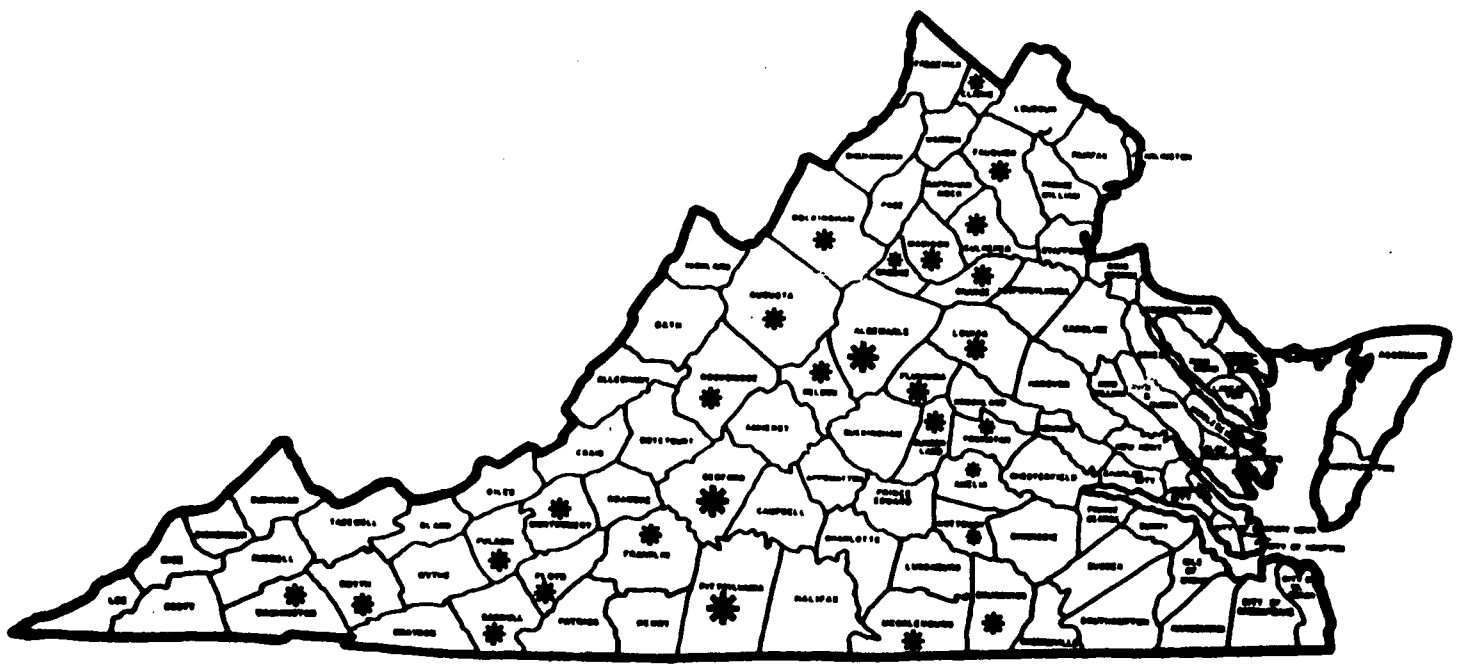

Figure 3. Map of Virginia depicting counties from which alfalfa samples were received for use in experiment II.

Appendix Table 1. Statistical models.

Experiment Ib.

$$Y_{ijkl} = u + R_i + D_j + RD_{(ij)} + T_k + RT_{(ik)} + DT_{(jk)} + e_{ijkl}$$

where:
u = overall population mean,
R_i = effect of replications, i=1,3
D_j = effect of day, j=1,5
T_k = effect of temperature, k=1,2
$RD_{(ij)}$ = interaction of replication with day
$RT_{(ik)}$ = interaction of replication with temperature
$DT_{(jk)}$ = interaction of day with temperature
e_{ijkl} = residual error

Source	df	
Rep	2	
Day	4	MS(Rep x Day) used to test effect of day.
Rep x Day	7	
Temp	1	MS(Rep x Temp) used to test effect of Temp.
Rep x Temp	2	
Day x Temp	4	MS(error) used to test other effects.
Residual	7	
	27	

Appendix Table 1. (cont'd)

Experiment IIa. (Conventional vs. oxygen limiting)

$Y_{ij} = u + T_i + e_{ij}$

Source	df		
Treatment	1	Conv. upr. 43 - 1 =	42
Residual	80*	Oxygen lim.39 - 1 =	38
			80*

Regressions

$Y = b_0 + b_1DM_1 + b_2DM^2 + b_3DM^3 + e$

Conv. upright		Oxygen limiting	
Source	df	Source	df
Treatment	3	Treatment	3
(DM)	(1)	(DM)	(1)
(DM^2)	(1)	(DM^2)	(1)
(DM^3)	(1)	(DM^3)	(1)
Residual	39	Residual	35

Experiment IIb. (Oxygen lim. statewide vs. VPI)

$Y_{ij} = u + T_i + e_{ij}$

Source	df		
Treatment	1	Oxy. lim. 39 - 1 =	38
Residual	49*	VPI 12 - 1 =	11
			49*

Appendix Table 1. (cont'd)

Experiment IIc. (Conv. upr. vs. Oxy. lim. vs. Baled hay)

$Y_{ij} = u + T_i + e_{ij}$

Source	df		
Treatment	2	Conv. upr.	43 - 1 = 42
Residual	102*	Oxy. lim.	39 - 1 = 38
		Baled hay	23 - 1 = 22
			102

Waller Duncan LSD test used for treatment differences.

Experiment III. (In vitro vs. in situ)

$Y_{ij} = u + T_i + e_{ij}$

Source	df		
Treatment	5	AS-CU	5 - 1 = 4
Residual	23*	AS-OL	5 - 1 = 4
		AH	4 - 1 = 3
		CS	5 - 1 = 4
		CS-T	5 - 1 = 4
		OGH	5 - 1 = 4
			23*

Waller Duncan LSD test used for treatments differences.

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