COMPARISONS OF CHEMICAL, IN VITRO AND IN VIVO METHODS FOR ESTIMATING NUTRITIVE VALUES OF NORMAL AND CHEMICALLY TREATED HAYLAGES

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ABSTRACT

COMPARISONS OF CHEMICAL, IN VITRO AND IN VIVO METHODS FOR ESTIMATING NUTRITIVE VALUES OF NORMAL AND CHEMICALLY TREATED HAYLAGES

By

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The present study was designed to gain knowledge about two problems involved in haylage preservation.

The objective of the first part of this study was to evaluate and compare several chemical and in vitro methods in estimating five animal responses (nitrogen and dry matter digestibilities, nitrogen balance and retention as a percent of absorbed nitrogen, and maximum dry matter intake) when fed hay or haylage. Chemical analyses evaluated were crude protein content, dry matter solubility in neutral detergent solution, dry matter and nitrogen (N) solubility in acid-detergent (AD), solubility in hot water, acid detergent lignin, N solubility in mineral buffer solution and degree of browning. Analyses obtained from in vitro methods were dry matter and N solubilities when incubated in: acid pepsin, pepsin + pancreatin, rumen fluid + pepsin, and rumen fluid + pepsin + pancreatin solutions. In several

cases, one analytical scheme produced two to four variables. Sources of forage samples were: 24 samples with the majority being alfalfa haylages obtained from Michigan State University (MSU), and 66 samples supplied from other U.S. experiment stations. Only limited analytical data were available for those 66 samples. Each *in vivo* parameter was regressed with (1) a single laboratory measurement using simple regression analysis technique, (2) with variables obtained from any two analytical schemes using least squares deletion multiple regression analysis technique, and (3) with selected, important variables from all analytical schemes using a least squares deletion multiple regression analysis technique.

For the 24 MSU forage samples, the best single predictors for (1) *in vivo* N digestibility was AD insoluble N as a percent of total N ($r = -.92$); for (2) dry matter digestibility was AD insoluble dry matter ($r = -.90$); for (3) N balance was pepsin soluble N as a percent of dry matter ($r = .85$); for (4) N retention as a percent of absorbed N was hot water soluble dry matter ($r = .62$); and for (5) maximum dry matter intake was rumen microbial plus pepsin soluble dry matter ($r = .82$). Forage crude protein content was a very poor predictor for *in vivo* N digestibility ($r = .19$) or digestible N content ($r = .59$).

Predictability of digestible N content was markedly improved by using N fractions determined by either AD or pepsin methods (R^2 = .91 and .89).

The effect of source of samples was evident with respect to the regression equations for *in vivo* N digestibility using AD insoluble N as predictor. Forage samples of MSU contained smaller amounts of insoluble N and had a greater depressions in ND per unit of AD insoluble N than did samples from other sources. A greater depression in ND was observed for forages containing 9% AD insoluble N or less than for forages containing greater than 9% AD insoluble N as a percent of total N.

Multiple regressions analyses using variables from two analytical methods indicated that variables obtained from the pepsin incubation were the best predictors for *in vivo* responses. Extremely high predictibility ($R^2 > .98$) were obtained for multiple regressions of *in vivo* N and dry matter digestibility using selected variables such as neutral detergent insoluble dry matter, AD insoluble N, pepsin insoluble N and rumen microbial + pepsin insoluble N.

The aim of the second part of this study was to evaluate and compare the value of propionic acid (.4 and .8%), ammonium isobutyrate (AIB, .5 and 1%) and a

mixture of AIB (.5%) and formaldehyde (1.25% of a 37% solution) in preserving nutritive value of alfalfa haylage (50% DM). Levels of propionic acid and AIB were comparable on a molar basis.

During a 42-day ensiling period, haylage treated with .5% AIB had the least quantity of heat development as expressed as degree-day above 35 C (66) as compared to other treated haylages (ranging from 119 to 203) and control haylage (322). Heat development was greatest in the upper portion of silo regardless of treatment. None of the chemical treatments were entirely effective in restricting heat development (> 35 C) in the haylage surface during the time haylage was being fed. However, results from a refermentation experiment indicated that all treatments retarded heat development (> 35 C) for at least 10 days while control haylage heated to 59 C on the second day of refermentation. The extent of top spoilage was not reduced by treatments except for .8% propionic acid and .5% AIB plus formaldehyde. These two treatments also restricted heat development in the top portion of the silos to a greater extent than for control haylage.

Treatments had no marked effect on haylage pH or acetic acid concentration, but did markedly decrease lactic acid concentration. Propionic acid and AIB reduced total fungal counts to about the same extent; a 40% reduction for the .4 or .5% levels and a 75%

reduction for the .8 or 1% levels. Treatment with .5% AIB plus formaldehyde had essentially no effect on reduction of total fungal counts.

Chemical composition was not markedly different among haylages except that high lignin and AD insoluble N values were found for control, 1% AIB and .5% AIB plus formaldehyde treated haylages. These haylages were also high in quantity of heat development during storage.

Maximum haylage dry matter intake by sheep ranged from 3.14 to 3.99 kg per 100 kg body weight with no significant differences among haylages. Dry matter digestibility was not significantly ($p > .05$) improved by treatments, however, N digestibility was significantly ($p < .05$) improved by treatments (from 55 to 60.3%). N balance and N retention as a percent of absorbed N were also significantly ($p < .05$) improved by treatment with .4% propionic acid and 1% AIB. Significant correlation coefficients were observed between N digestibility and AD insoluble N as a percent of total N ($r = -.82$, $p < .01$) and between N digestibility and degree-days above 35 C ($r = -.81$, $p < .01$). Milk production, composition and effeciency were not significantly ($p > .05$) different among treatments. Thus, propionic acid and AIB were equally effective in reducing heat development, total fungal counts and AD insoluble N of haylages and in

improving N utilization. No markedly superior results were obtained by using the higher levels of these chemicals.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

Appendix Table | Page

LIST OF FIGURES

heat damaged forages. Thus, there is a need for analytical methods which must not be too complicated to perform but can still precisely estimate the in vivo protein value of heat damaged forages.

Although the necessity of air exclusion during ensiling haylage has been recognized by agricultural researchers and farmers, instances of severe heat damage in haylage still occur frequently regardless the type of silos (including so called "oxygen-limiting" silos). On the other hand, reductions in heat development have been observed in haylage treated with various kinds of preservatives. However, more work is needed in order to define the interrelationships among application rate of the chemical, degree of preservation, and nutritive value of preserved haylage as determined by animal feeding trials.

Thus, the objectives of this study were (1) to compare the predictive values of several laboratory methods which have not been thoroughly evaluated to date for in vivo DM and nitrogen (N) digestibilities, N-balance, and maximum DM intake of haylages which were suspected of having variable amounts of heat damage and (2) to compare the effectiveness of propionic acid, ammonium isobutyrate (AIB) and mixture of AIB and formaldehyde in preserving nutritive values of alfalfa haylages as determined by sheep performances and milk yield of lactating cows.

LITERATURE REVIEW

Part 1. Regular (High-Moisture) Silages

I. Definition and General Characteristics

Silage is a succulent material produced by a process of microbial fermentation of a green crop (Watson and Nash, 1960). The anaerobic and acidic (pH 4.2 or below) condition makes a long period of storage possible. The primary object of silage making is to preserve the material with minimum loss of nutrients and with good resulting palatability. Traditionally however, more hay crops have been preserved as hay rather than silage. Only during recent years, have greater portions of hay crops been harvested for silages. Reasons for this change are: weather condition is not as large a problem when harvesting haylage as when making hay; ensilage causes less harvesting losses; recent development of automated harvesting and feeding systems minimize labor for a silage feeding program; equal or better performance of animals fed silage as compared to those fed hay in mixed practical rations (Thomas et al. 1969; Roffler et al. 1967 and Syrjala, 1972).

Three types of fermentation can occur in silage

(1) Lactic (normal or desirable) fermentation, (2) Secondary (butyric or clostridial) fermentation which is conventionally consiered undersirable and can occur both during and/or after the lactic fermentation resulting in degradation of amino acids and lactic acid and (3) A fermentation that occurs under aerobic conditions such as when a silo is opened for feeding (the so called "after fermentation"). Coli-Aerogenes bacteria, yeasts and fungi are primarily responsible for the third type of fermentation (Papendick and Singh-Verma, 1972; Beck, 1963; Gross and Beck, 1970 and Beck and Gross, 1964).

The course of a lactic acid type fermentation can be characterized by continued plant cell respiration for a time using up the oxygen and giving off CO_2 and heat. As conditions become favorable, members of acid-producing bacteria (Streptococci and Lactobacilli) increase rapidly. These organisms produce acid until the sugar is exhausted or until the pH becomes unfavorable for their further growth. The fermentation usually is complete at the end of eight days (Barnett, 1954; Conden et al. 1969; Langston et al. 1958; Langston and Bouma, 1960a,b).

Investigators are still searching for reasons to explain why some silages show predominantly lactic acid type fermentation and others show predominantly butyric acid type fermentation. Many hypothese (e.g. sugar content, ration of protein to sugar content, buffering

capacity, initial distribution of microflora on the crops, total counts of lactic acid producing bacteria during early stage of ensilage) have experimentally been found to be inconclusive (Kempton and San Clemente, 1959; Langston et al. 1958; Huhtanen and Pensack, 1963; Ohyama and Masaki, 1968a,b,c; 1969a,b; and 1971). Langston and Bouma (1960a) have listed several other possible reasons to account for the variability in acid production, they are: (1) variability in sequence changes of microorganisms, (2) antagonism among certain groups of bacteria early in the fermentation process, (3) deficient nutrients in the plant material for bacterial growth, and (4) occurrence of weakened strains of bacteria. A poor understanding concering butyric acid types of fermentation and their undersirability has caused researchers to take a new direction in silage preservation. Present work is centered on finding on agents which will selectively restrict the activity of clostridia such as mineral acids, organic acids and wilting of forages.

Numerous chemical reactions can occur during silage fermentation and this results in modification of the chemical composition of the original material. Generally, the major changes are reduction of soluble carbohydrate and true protein nitrogen; an increase in non-protein nitrogen as well as organic acids (lactic, acetic, propionic and butyric acids) (Watson and Nash,

1960). An example of chemical composition changes during ensiling is shown in the Table 1(Henderson et al. 1972).

Table 1. Chemical Composition Changes[a] of Fresh and Ensiling Perennial Ryegrass.

	Fresh	Silage
	% DM	
DM	17.75	18.35
Water soluble carbohydrates	17.70	1.22
Crude protein	14.20	14.50
Protein-nitrogen (N)	1.84	0.45
Volatile - N/total N %	0	8.15
Ether-extract	2.41	3.45
Crude fiber	26.50	30.40
Acetic acid	-	3.40
Propionic acid	-	0.17
Butyric acid	-	0.16
Lactic acid	-	10.60
Ethanol	-	1.20
pH	6.08	3.94
Ash	7.00	6.90

[a]Data from Henderson et al. 1972. J. Sci. Fd Agric. 23:1079.

Readily available carbohydrates are lower in silage than in the original forage (17.7 vs. 1.22% DM). These utilized carbohydrates are converted into various kinds of organic acids. Although ensiling usually does not result in loss of crude protein, relative proportions of the nitrogen fractions do change. For example, fresh ryegrass contains no volatile nitrogen while the resulting silage contains 8.2% volatile nitrogen calculated

on the total nitrogen basis (Table 1).

The term "silage quality" is generally used to denote not the nutritive value of the silage, but the extent to which the silage fermentation has proceeded in a desirable manner. Conventionally, good quality silages should have high levels of lactic acid (7.5-12.5 % DM), low pH values ($\leq$ 4.2); low level of butyric ($<$ 0.5% DM) and acetic acid (2.5-4.0% DM); and low level of ammonia nitrogen as percent of total nitrogen (5-8%) (Virtanen, 1933; 1952; Watson and Nash, 1960; Flieg, 1938; Breirem and Ulvesli, 1954; Nordfeldt, 1955; Wieringa, 1966 and Nilsson and Nilsson, 1956). Results from animal performance trials generally indicate that both animal digestibility and production are lower when the silage has a high pH value and/or high content of volatile nitrogen (Gordon et al. 1964; Murdoch, 1966), but any negative effect of butyric acid on silage consumption has been completely verified. Low partial correlation coefficients have been reported between content of butyric acid and silage dry matter consumption (Emery et al. 1966; Kirchgessner et al. 1972).

Silage production and preservation have undergone many changes during the last decade, and many questions have been answered but an abundance of questions remain unanswered. Recently, several workers have frequently

mentioned the significance of using aseptically grown forage or sterilized forage as ensiling material. Basic knowledge about silage fermentation can be obtained by this technique (Huhtanen and Pensack, 1963; Playne et al. 1967).

II. Reactions Occuring During Silage Fermentation

1. Carbohydrates

Glucose, fructose, sucrose and fructosans are the main water-soluble carbohydrates in grass with only glucose and fructose considered major for microbiological purposes. These sugars are fermentable by a variety of microorganisms, of which lactic acid bacteria are the most important. Two fermentative types of lactic acid bacteria are always encountered. One is the homo-fermentative type, which forms approximately two moles of lactic acid per mole of glucose fermented (Wood, 1961). The second is the hetero-fermentative type which produces one mole of lactic acid, one mole of CO_2 and one mole of ethanol per mole of glucose fermented. Prediction of the final ratio of products of a lactic acid fermentation is impossible because a mixed population always develops. In good silages, total counts of hetero-fermentative bacteria are higher than that of homo-fermentative bacteria which probably reflects the ability of hetero

fermentative organisms to withstand a lower pH than homo-fermentatives (Langston, et al. 1958).

Main products of carbohydrate fermentation by lactic acid bacteria are given in Table 2. The data indicate that hetero-fermentative lactic acid bacteria ferment fructose and glucose by slightly different pathways and produce less lactic acid than do homo-fermentative bacteria.

Table 2. Main Products of Carbohydrate Fermentation by Lactic Acid Bacteria.

Homo-fermentative:	
(a) 1 Glucose	⟶ 2 Lactic acid
(b) 1 Fructose	⟶ 2 Lactic acid
(c) 1 Pentose	⟶ 1 Lactic acid + 1 Acetic acid
Hetero-fermentative:	
(a) 1 Glucose	⟶ 1 Lactic acid + Ethanol + 1 CO_2
(b) 3 Fructose	⟶ 1 Lactic acid + 2 Mannitol + 1 Acetic acid + 1 CO_2
(c) 1 Pentose	⟶ 1 Lactic acid + 1 Acetic acid

Several investigators have found a marked disappearance of hemicellulose (polymers of glucose, xylose, arabinose, mannose and galactose plus mixed sugars and uronic acid) during ensilage, which is believed to be hydrolyzed by both microbial hemicellulases (Goering et al. 1970) and by organic acids at the low pH produced during ensilage (Dewar et al. 1963).

2. Proteins and Amino Acids

In well preserved silages or silages made from aspectically grown forages, about 50-60% of the protein is degraded (Mabbitt, 1951). Many results have confirmed the view that plant enzymes are largely responsible for the degradation of protein (Singh, 1962; Bentley *et al.* 1955; Kemble, 1956; Henderson *et al.* 1972; 1971b and Hughes, 1970a).

Clostridia are responsible for the major changes in amino acids during ensiling as stated by McDonald *et al.* (1968) (Table 3).

Table 3. Some Example of Clostridial Fermentation[a]

Organic acids
2 Lactic acid ⟶ 1 Butyric acid + 2 CO_2 + 2 H_2
Amino acids
(a) Coupled oxidation-reduction reaction
1 Alanine + 2 Glycine ⟶ 3 Acetic acid + 3 NH_3 + 1 CO_2
(b) Deamination
3 Alanine ⟶ 2 Propionic acid + 1 Acetic acid + 3 NH_3 + 1 CO_2
1 Valine ⟶ 1 Isobutyric acid + 1 NH_3 + 1 CO_2
1 Leucine ⟶ 1 Isovaleric acid + 1 NH_3 + 1 CO_2
(c) Decarboxylation
Histidine ⟶ Histamine
Lysine ⟶ Cadaverine
Arginine ⟶ Ornithine ⟶ Putreseine
Tryptophan ⟶ Tryptamine
Tyrosine ⟶ Tyramine

[a]McDonald *et al.* 1968. J. Sci. Fd Agric. 19:125.

Saccharolytic clostridia varieties multiply and increase the pH value which leads to growth of putrefactive clostridia. Destruction of amino acids is by three main pathways, namely, coupled oxidation-reduction reaction(Stickland reaction); deamination and decarboxylation (Table 3).

Many decarboxylation products of amino acids have been detected in silage such as: amines, cadaverine, putrescine, histamine, γ-amino-butyric acid, β-alanine tryamine and tryptamine (Macpherson, 1962; Macpherson and Violante, 1966; Neumark et al. 1964). These substances are of interest because of their possible effect on depression of silage intake and the health of animals (Neumark et al. 1964; Harris et al. 1966; McCullough, 1966; Thomas et al. 1961; and Okamoto et al. 1964).

3. Organic Acids (Non-Nitrogenous)

Malate and citrate are the most abundant organic acids in a wide range of plant species. These weak acids and their salts form an important buffer system in the plant (Lessard and McDonald, 1966; Playne and McDonald, 1966; Fauconneau and Jarrige, 1954; Wilson and Tilley, 1964). Both homo and hetro fermentative lactic acid bacteria will readily dissimilate malate and citrate by a number of pathways (Table 4). The products formed are either neutral (Acetoin, 2,3-butane diol and ethanol),

salts of organic acid or alkaline released cations. Because many of the organic acids are present in the plant material in salt form, their destruction by bacteria acts against preservation, as decarboxylation results in the release of cations and carbon dioxide.

Table 4. Main Products of Organic Acid Fermentation by Lactic Acid Bacteria[a]

Homo-and Hetero-Fermentative	
1) 1 Citric acid →	2 Acetic acid + 1 Formic acid + 1 CO_2
or 2 Citric acid →	2 Acetic acid + 1 Acetoin + 4 CO_2
or 2 Citric acid →	3 Acetic acid + 1 Lactic acid + 3 CO_2
2) 1 Malic acid →	1 Lactic acid + 1 CO_2 or
2 Malic acid →	1 Acetoin + 4 CO_2 or
1 Malic acid →	1 Acetic acid/Ethanol + 1 Formic acid + 1 CO_2

[a]McDonald et al. 1968. J. Sci. Fd Agric. 19:125.

4. Dry Matter and Energy

A number of workers have commented on the apparently high gross energy value of silage (Beever et al. 1971; Thomas et al. 1969; Barry and Fennessy, 1972; Alderman et al. 1971; Waldo et al. 1969; Waldo et al. 1965).

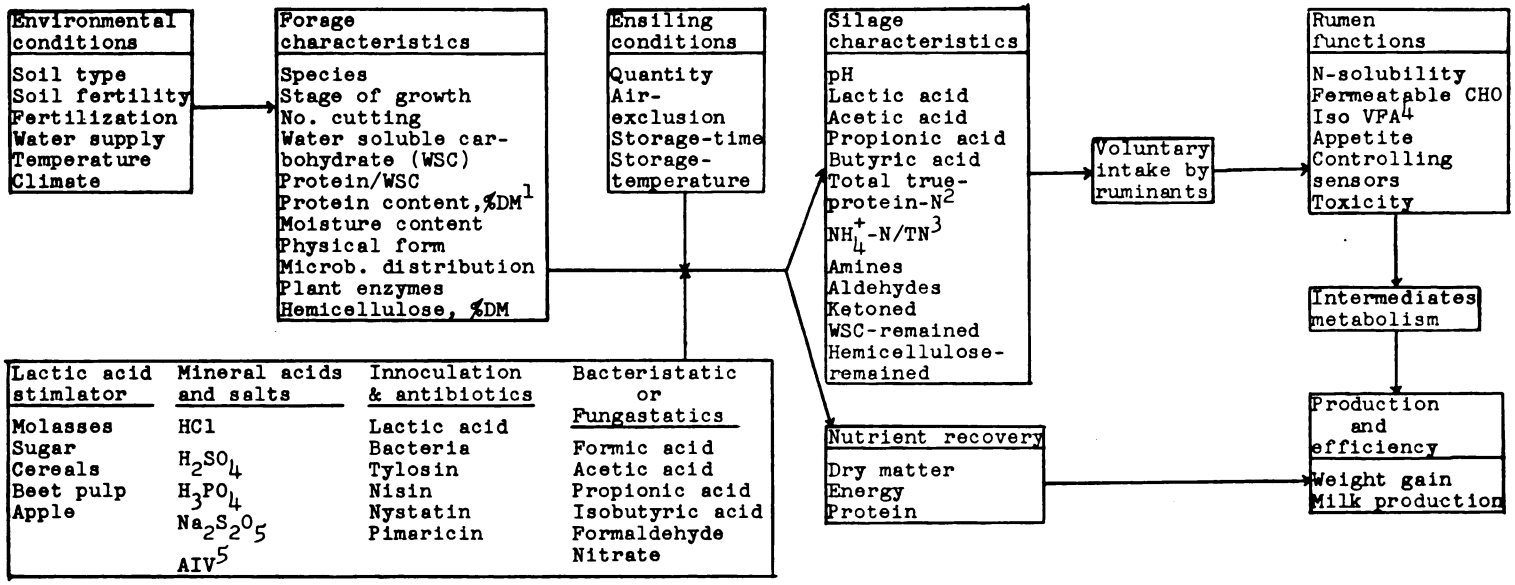
In a complete anaerobic biological system, the increase in gross energy during ensiling can be explained

biochemically, based on the known reactions. For example, fermentation resulting in high ethanol production, as in the heterolactic fermentation of glucose, will result in increased energy concentrations where as homolactatic fermentations will have little effect on the gross energy value of silages. On the other hand, in some clostridial fermentations lactate will be changed to butyrate resulting in some energy loss as hydrogen from the system but with a comparatively greater loss in DM giving an apparent increase in energy density. McDonald et al. (1973) conducted several experiments to demonstrate the high energy recovery under a complete anaerobic system. Their results strongly confirm that energy is recoverable in spite of gaseous CO_2 loss. However, if oxygen is introduced during ensiling, recovery of energy and dry matter become unpredictable based on the biochemical model designed for anaerobic fermentation. Thus, in preserving low-moisture silage or stacked-type hays energy and dry matter recoveries should not be calculated from a chemical reaction scheme but from a balance scheme involving H_2O and CO_2(Pedersed, 1971). In fact, many investigations have reported high correlation ($r > 0.7$) between CO_2 production and DM loss during storage (Honig, 1969 and Zimmer, 1969). More discussions about the actions of O_2 on silage fermentation are given in part II section III.

III. Factors Influencing Fermentation and Nutritive Value of Resultant Silage

There are several factors which can affect silage fermentation patterns. Important factors are attributed to plant characteristics e.g. content of fermentable sugar and protein, while others are related to ensiling conditions e.g. type of structure and extent of oxygen exclusion, and still others to mangement systems e.g. fineness of chop, use of additives. Many factors are interrelated. Figure 1 illustrate some interrelationships among forage, ensiling conditions and quality of resulting silage. Some important factors will be reviewed briefly here.

1. Soluble Carbohydrate Content: A wide range of values have been reported as the minimum sugar requirements for the satisfactory conservation of grass and legume crops. A general value is about 7% of dry weight (Smith, 1962) although many researchers consider this value insufficient (McDonald et al. 1964). The soluble carbohydrate content of the green crop is extremely variable depending upon species and environment. For example, the soluble carbohydrate content of orchard grass is considerably lower than that of ryegrass at any given stage of maturity, while timothy and meadow fescue have an intermediate value (Waite and Boyd, 1953). No similar data are available for grasses and legumes



[1]DM = dry matter.

[2]N = nitrogen.

[3]Ammoniacal - nitrogen as a percent of total nitrogen.

[4]Iso VFA = isobutyric acid, isovaleric acids.

[5]AIV = a silage additive (2 N HCl).

Figure 1. General Relationships Among Forage, Ensiling Conditions, Silage Fermentation and Nutritive Value of Silage.

in U.S. Temperate grasses accumulate higher concentrations of soluble carbohydrate than do the tropical grasses(Winmann and Reinhold, 1946; Ojima and Isawa, 1968; Smith 1968a; Wilson and Ford, 1971 and 1973). Barnett (1954) found that the fermentable carbohydrate content particularly that of fructosan increases with increasing stage of maturity.

Temperature also markedly affects the carbohydrate reserves of green crops. Smith (1970) showed that changing timothy plants at inflorescence emergence from a cool to a warm regime decreased water-soluble carbohydrate content in the stem bases at early anthesis. The effect of water stress on fermentable carbohydrate reserves has been studied by a number of workers with inconsistant results (Eaton and Ergle, 1948; Brown and Blaser, 1965; Blaser et al. 1966; Buckey and Weaver, 1939; Bailey et al. 1970).

The effects of nitrogen (N) fertilization on carbohydrate reserves are complex and variable (Weinmann, 1948). Generally, N applied at low to moderate rates increases soluble carbohydrate reserves while nitrogen applied at high rates decreases soluble carbohydrate reserves (Adegbola and McKell, 1966, Izumi et al. 1972; White, 1973). The physiological reasons why changes in N variably effect fermentable carbohydrate reserves are not well understood (White, 1973). Several workers have

reported that high N fertilization can produce high NH_3 levels, high pH abnormal changes in silage fermentation (Wieringa, 1966; Fox and Brown, 1969), which depress the intake of the silage (Gordon et al. 1964; Castle and Watson, 1969).

2. Mechnical Treatment - Chopping and Laceration:

Finely chopping or lacerating as compared with coarse chopping has improved silage quality as judged by pH, percentage of N degraded into ammonia, level of butyric acid, lactic acid and total volatile fatty acids (Murdoch, 1965; McDonald et al. 1965 and Dulphy and Demarquilly, 1972,1973). Silo capacity for DM is greater when forage is finely chopped (Dulphy and Demarquilly, 1972,1973). Losses of DM during storage are also lower for finely chopped forage. These effects on fermentation are at least partly due to the liberation of cell contents from the herbage by mechnical bruising, the fermentable carbohydrates in the cell contents providing an immediate substrate for bacteria (Murdoch, 1966). Finely chopped silages are not always more digestible than the coarsely chopped one, but voluntary intake of the latter is much higher (Dulphy and Demarquilly, 1973 and Murdoch, 1965).

3. Ensiling Temperature: For many years English investigators recommonded that temperature during ensiling should be allowed to rise to 32-42 C on the assumption

that this range of temperature is optimum for the growth of lactobacilli (Fry, 1885); but clostridia as well as lactobacilli show optimum growth within this temperature range. In terms of the chemical constituents in the silage no advantage is gained by allowing the temperature in the mass to rise so high, in some cases better preservation has resulted when the temperature has been held at 27 C (Murdoch, 1960a). Since the temperature rise in silage is largely the result of heat evolved in aerobic respiration of plant and oxidation processes of aerobic microorganisms any increase in temperature represents nutrients loss in silage (Murdoch, 1960). Furthermore, high ensiling temperature will seriously depress protein digestibility (Goering et al. 1972).

4. Moisture Content of Forage: Moisture content in the forage significantly influences on fermentation pattern(Wieringa, 1958). When moisture content in a forage is reduced to 65% by wilting, the resulting silage shows desirable fermentation characteristics as compared with unwilted silage: a decreased concentration of butyric, propionic, acetic, lactic acid (not always) and ammoniacal nitrogen (NH_3-N), and an increased concentration of water soluble carbohydrates hemicellulose and hot-water-insoluble nitrogen (true protein-N) (McDonald et al. 1968; Gordon et al. 1961; Roffler et al. 1967;

Thomas et al. 1969). The decreased extent of protein and sugar breakdown is due to increased osmotic pressure which has selective action upon the silage microflora particularly upon butyric acid producing organisms. Wilted silage usually has a pH value of about 5 which is considered undesirable from standards set for regular high moisture silages. This relatively high pH value is probably due to the following reasons: (1) Decreased production of lactic and other volatile fatty acids. (2) Decreased production of ammoniacal nitrogen. (3) Increased concentration of protein and cations (buffering agent) in the aqueous phase due to wilting (Wiergna, 1961). Wilting is probably the best process to use in preserving legume crops. Legume crops are low in fermentable sugars and high in protein content (buffering effect) which make these crops difficult to ensile satisfactorily as direct-cut silage when compared with grasses (Murdoch, 1960; DeVuyst et al. 1962; and Watson and Nash, 1960). In the practical situation, wilting saves transport costs of water for the farmer. In addition, as the crop becomes drier seepage nutrients are reduced. Wilted silage also is more suitable for mechanized handling especially in air-tight silos. The odor of wilted silage is not as objectionable as that of direct-cut silage and this has an aesthetic value to farm families and the general

public in contact with persons handling silage. Wilted silage, with its relatively high content of fermentable carbohydrate and true protein nitrogen should produce a more favorable rumen fermentation and nitrogen utilization by the animal than direct-cut silage (McDonald et al. 1964; Waldo, 1968). However, results from animal trials do not clearly support this concept (McDonald et al. 1968; Thomas et al. 1969; Sutten and Vetter, 1971 and Roffler et al. 1967).

Although wilted silages have frequently been evaluated as better quality silage than direct-cut silage, animal performance trials have failed to give parallel results. Animals usually consume more silage DM from wilted silage (20-40% DM) than direct-cut silage (Thomas et al. 1961, 1969; Murdoch, 1960b, 1964; Brown, 1962; Halley and Dougall, 1962 and Gordon et al. 1961, 1965), but the production data (digestibility, weight gain, milk production and efficiency) are not always most favorable for wilted silages (Thomas et al. 1969; Fisher et al. 1971; Ruszezyc et al. 1972; Forbes and Jackson, 1971; Brown, 1961 and Alder et al. 1969). Actually, Alder et al. (1969) commented on wilted silage making "wilting should be adopted if it helps the ensiling process, rather than as a necessary technique to ensure maximum voluntary intake with the doubtful anticipation of higher

animal production". The decreased digestibility, production and efficiency observed in animals fed wilted silages could be due to improper ensiling techniques. Wilting can solve some difficulties commonly encountered during ensiling, but some special precautions are needed when wilted forages are ensiled. For example, farmers should check the leaks in the silos, chop the crop reasonably short, and fill the silo rapidly, etc. (Hillman and Thomas, 1973). The degree of compaction inside a silo is usually determined by the moisture content, length of chopped forage and height of the silo. The wetter and the shorter the forage, the better the compaction. Poor compaction will entrap a significant amount of air which will allow plant cells and aerobic microorganisms to continue their wastful oxidation reactions. The heat generated during oxidation reaction can reduce the nutritive value of a resulting silage (Goering et al. 1972). By following correct procedures, Uchida et al. (1970) was able to demonstrate that the digestibility of dry matter nitrogen was higher for wilted silage than direct-cut silage (DM digestibility 61 vs. 57%, N digestibility 69 vs. 58%).

Part 2. Low-Moisture Silage (Haylage)

I. Definition and General Characteristics

Haylage is a slightly fermented wilted hay crop silage with a dry matter content of about 50%. Well preserved haylages usually have a light brown color and a very pleasant odor. One example of a comparison of the chemical composition for fresh forage, wilted silage and haylage is given in Table 5. Haylage contains a greater amount of sugar and lesser amounts of organic acids than does wilted silage indicating that less fermentation occurs in haylage than in wilted silage (Table 5). Although the crude protein content is similar between haylage and wilted silage, the ammoniacal nitrogen fraction is much lower in haylage than in wilted silage suggesting that less protein degradation occurs in haylage than in wilted silage (Table 5). The reduced extent of fermentation and protein destruction observed in haylage ensiling is probably due to the increased osmotic pressure which exerts not only a general inhibitory effect on all silage microorganisms but also a specific restrictive action on butyric acid producing bacteria (Wieringa, 1958).

II. Advantages of Haylage Making

Only in recent years, in the U.S. has a greater

Table 5. Comparison of Chemical Composition Among Fresh Crop, Wilted Silage and Haylage[a]

	Fresh[b]	Wilted	Haylage
DM[c]%	19.00	38.00	51.00
Sugar	4.20	1.50	3.60
Crude protein	18.40	19.60	19.20
Crude fiber	30.90	33.60	32.50
pH	6.00	4.80	4.80
NH_3-N/TN[d]	-	15.10	9.90
Butyric acid	-	0.79	0.06
Propionic acid	-	0.23	0.04
Acetic acid	-	2.43	0.93
Lactic acid	-	3.06	2.69
DM loss	-	10.20	15.50

[a]Gordon et al. 1965. J. Dairy Sci. 48:1062.

[b]First cutting alfalfa.

[c]Dry matter.

[d]Ammoniacal nitrogen as a percent of total nitrogen.

proportion of the hay crop been preserved as haylage than as silage. Haylage making is often recommended more favorably than is hay making because haylage does not have to be as dry as hay to be taken from the field. This reduces the dry matter losses in the field due to respiration of plant cells, leaf loss, incomplete of pick up by harvesting apparatus and possible rain-damage. Dry matter recovery of field cured hay has been estimated as 73% which is much lower than the recovery of 87% for

wilted silage (Shepherd et al. 1954; Carter, 1960). Dry matter recovery of haylages has not been sufficiently documented, but the average in addition, weather conditions necessary for good hay making are never certain; haylage offers an excellent alternative. Also, due to mechanization of the harvesting, delivery and feeding systems much less human labor is required for haylage than for hay. Chemical composition of hay and haylage is similar (Table 6), so the feeding value of well made haylage should be comparable with that of hay. Yet, several feeding trials ranked the feeding value of haylage below that of hay (Sutton and Vetter, 1971; Gordon et al. 1964 and Roffler et al. 1967).

Table 6. Proximate Composition of Hay and Haylage[a].

	Crude protein	Ether extract	Crude fiber	Nitrogen free extract	Ash
	----------	----------	% DM	----------	----------
Hay	16.18	1.48	32.65	41.18	8.51
Haylage	17.81	2.74	29.22	39.51	10.72

[a]Data from Gordon et al. 1963. J. Dairy Sci. 46(5):411.

Haylage should be superior to direct-cut or wilted silage because haylage undergoes a more desirable

fermentation pattern (Table 1 and 5). Gordon et al. (1964) observed that the volatile nitrogen content of direct-cut orchardgrass silages ranged from 8 to 37% of the total nitrogen. High volatile nitrogen in silage causes high ruminal ammonia and low nitrogen retention (Waldo and Derbyshire, 1971). Thus, the utilization of nitrogen by ruminants should be better for haylage due to smaller portion of total nitrogen as NH_4^+-N (Gordon, 1964; and Roffler et al. 1967).

Generally, animals tend to consume more dry matter from haylage than from wilted or direct-cut silage. In fact, a linear relationship between intake and silage dry matter content was observed (Thomas et al. 1961 and Gordon et al. 1965). But nutrient digestibility and animal production have not been consistently greater for animals fed haylage (Roffler et al. 1967, McDonald et al. 1968; Sutten and Vetter, 1971 and Hawkins et al. 1970). The possible reasons for this reduced efficiency in production have not been completely explained but may be partially due to the reduced digestibility of protein and energy in haylage (Gordon, 1968) and discussed in the next section.

III. Disadvantages of Haylage Making

Since solar energy is the source of energy for

removal of water from the forage in the field, there are a interrelationships between general weather conditions and good haylage making.

Relatively high dry matter losses (about 15%) during ensiling have been frequently noted when making high dry matter silages (Gordon et al. 1965; Murdoch, 1967; and Pedersen et al. 1971) representing gaseous losses (oxidation) and spoilage (mold development). The presence and penetration of air into the silage mass may be modified by the situation outlined below. (1) Forage with a high dry matter content usually has low silage density and hence more air will be entraped. (2) Variation in management factors such as degree of air tightness of the silo, fineness of forage chop, the speed of filling the silo, amount of a relatively wetter material in the top area of the silo, covering with a sheet of plastic, distribution of material when filling the silo etc. (Hillman and Thomas, 1973). (3) Variations in aeration when silo is open for feeding. (4) Air movement caused by diural temperature changes.

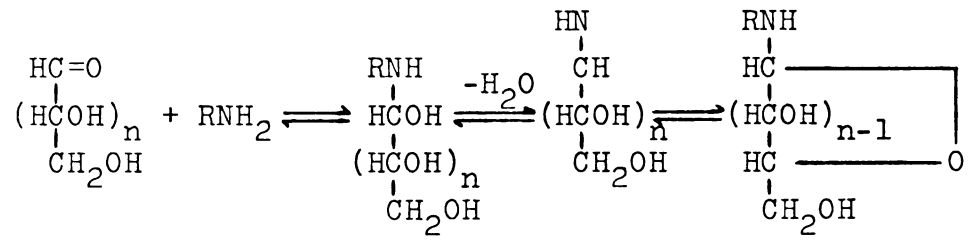
The heat generated through oxidation reactions in silos can cause two types of problems depending upon temperature. A slight amounts of temperature increase can damage nutrient availability to animals while a large temperature increase can initiate spontaneous ignition. A number of investigators (Cohn, 1890; Miehe,

1930; Festenstein et al. 1965) have enumerated the sequential factors responsible for temperature increases sufficient for spontaneous combustion as: (1) respiration of the plant material that occurs to a certain degree until all the material has "died"; (2) microbial metabolism that increases temperature as high as 71 C before the thermophilic organisms themselves are killed; (3) chemical oxidation occurs at an ever increasing rate as temperature increases. Temperatures of 70 C readily occur in a silo while spontaneous ignition rarely occurs at that temperature (Koegel, 1971). In fact, the mechnisms involved in spontaneous ignition are rather complicated and still not completely understood (Currie and Festenstein, 1971). In general, biological and chemical factors largely determine whether a sample of haylage is likely to heat, but physical factors will then decide whether the haylage develops a high temperature, whether this process continues, and what final temperature will be attained. These physical factors are : (1) the quantity of crop ensiled is sufficiently large to retain the temperature developed; (2) the crop in a certain physical state where the moisture content is low enough to markedly reduce the coefficient of thermal conductivity but still suficiently high to support microbial growth; (3) there is sufficient diffusion of gases to supply the

necessary oxygen but insufficient to transfer generated heat away from the mass (Koegel, 1971; Gordon, 1968; Currie and Festenstein, 1971; Gregory et al. 1963 and Festenstein et al. 1965).

Food scientists and organic chemists have known for a long time that sufficient heat can damage food protein during processing (Millard, 1912 and Hodge, 1953). Sufficient heat to damage proteins can result in destruction of amino acids by oxidation; modification of some of the linkages between the amino acids so that their release is delayed during digestion; formation of linkages that are not hydrolyzed during digestion, i.e. loss of biological availability (Maillard reaction); formation of brown pigments or melanoidins; and loss of palatability (Donoso et al. 1962; Bender, 1972). Because brown pigments are formed during heat-treatment of a food protein a more general term "non-enzymic browning" has been used to describe the overall reaction (Reynolds, 1963; 1965). The Maillard reaction appears to be the major course for browning development during the heating or prolonged storage of foods and the mechanism follows a common pathway for many foods (Hodge, 1953; Eillis, 1959; Reynolds, 1963, 1965). The primary step involves a condensation reaction between the α-amino groups of the amino acids or proteins and carbonyl groups of

reducing sugars known as the "carbonylamino" reaction and shown in the following reaction:



Sugar	Amino comp.	Addition product	Schiffs base	N-substituted glycosylamine

This reaction is then followed by many rather complicated reactions and finally brown pigments are formed.

Oven drying of forage or wet feces samples increases the yield of lignin, acid detergent fiber (cellulose plus lignin), and insoluble nitrogen in acid detergnet fiber (ADF-N) (Macdougall and Delong, 1942; Armitage et al. 1948; Van Soest, 1965). The increased apparent lignin and insoluble-N in the ADF was then postulated to be due to the products of non-enzymic browning reaction. Results from laboratory work revealed that the reactions involved in forages or feces probably are similar, if not identical, to the Maillard reaction (Van Soest, 1965; Gordon, 1968). Some conditions influencing the extent of formation of ADF-N have been studied in forages (Goering et al. 1973). In general, the reaction

was most rapid in the moisture range from 20 to 70% and with temperatures above 60 C but the degree of damage was species dependent. The level of insoluble protein or nitrogen content in the acid detergent fiber in the forages which were suspected to have been heat damaged was found to be highly, negatively correlated with *in vivo* nitrogen digestibility (n = 44, r = -.93)(Goering *et al.* 1972). This observation clearly suggests that increases in the acid-detergent fiber nitrogen value represent a good criterion for estimating the reduction in nitrogen digestibility. There are numerous examples of decreased nitrogen digestibility and/or animal performance from animals fed heat-damaged crops (Roffler *et al.* 1967; Zimmerman, 1952; Sutton and Vetter, 1971; Gordon *et al.* 1965; Hill and Noller, 1963; Miller *et al.* 1967; Hodgson *et al.* 1935; Wieringa *et al.* 1961 and Bechtel *et al.* 1943, 1945). Wieringa *et al.* (1961), in a field study, found that the amount of depression of *in vivo* nitrogen digestibility was highly correlated with the amount of time during which temperatures were high (number of days above 35 C) although nitrogen digestibility was not well correlated with the maximum temperature observed in a silo.

Although under farm conditions aeration and heating occur simultaneously, this does not necessarily

imply that aeration is required for peotein damage (Millard reaction). According to the reactions known to date, oxygen is not required for production of brown-pigments (Gordon, 1968). Wieringa et al. (1961) reported that, under laboratory conditions, simply heating silages in sealed jars produced little effect on protein digestibility unless heating was combined with aeration. Since then several workers have conducted many similar types of experiments (Zimmer and Gordon, 1964; Gordon, 1967 and Jorgensen et al. 1971) and noted that aeration was not required for heat damage of protein feeds as measured by increases in ADF-N (Gordon, 1967 and Jorgensen et al. 1971). Gordon (1968) suggested that although oxygen is probably not directly involved in the Maillard reaction under practical silage making operations oxygen will be important to the total effect in two ways. Firstly, presence of oxygen retards fermentation of sugars to organic acids; secondly, presence of oxygen allows greater initial heat development and the rate of browning reaction will proceed much more rapidly at higher temperatures. Thus, Browning may be greatly increased by aeration, even though it is not required for the primary reaction.

A diagrammatic sketch of interrelationships among management factors is illustrated in the Figure 2.

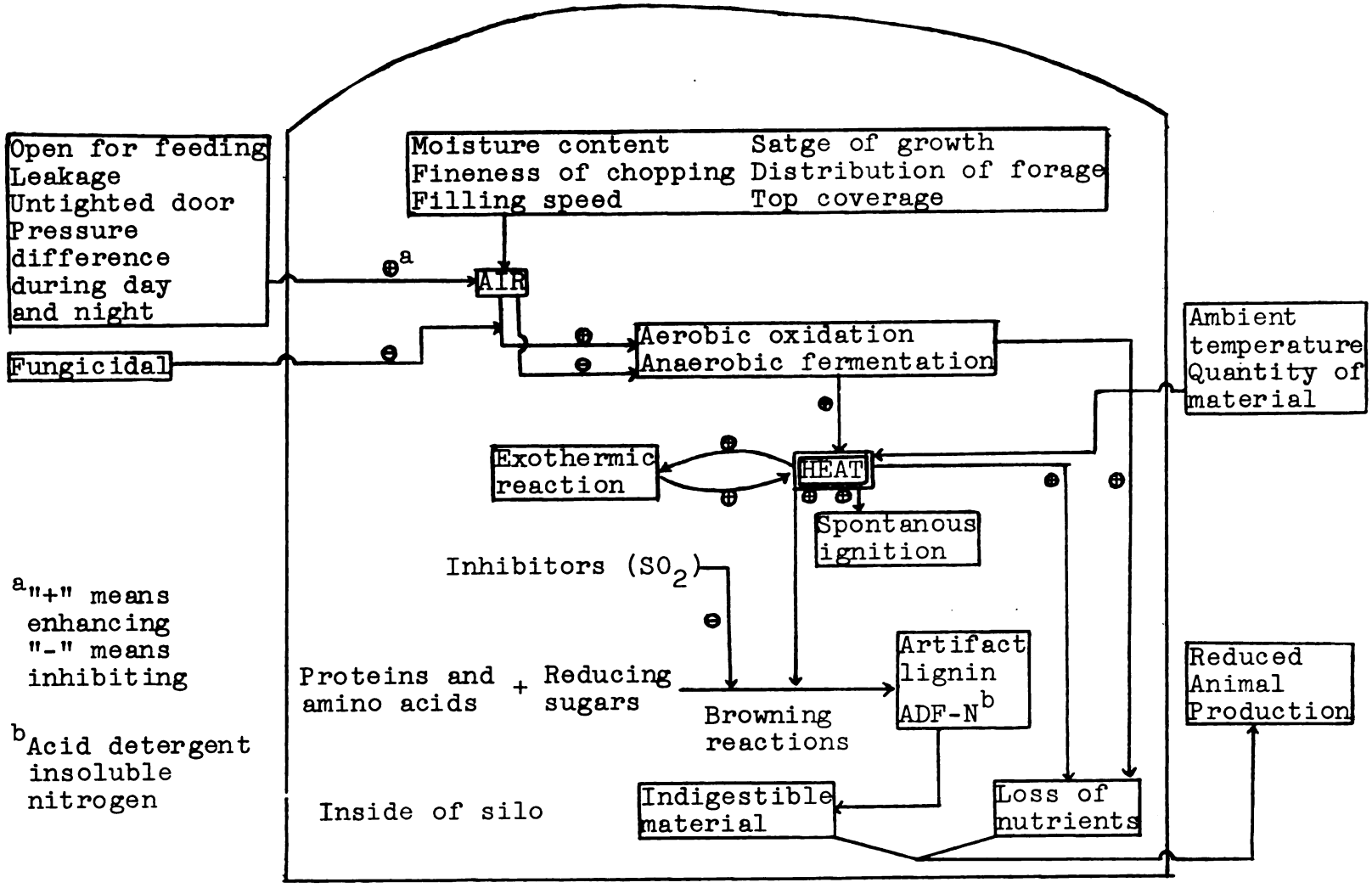


Figure 2. Development of Heat in A Silo and the Consequences of Heating.

IV. Laboratory Estimates of Nitrogen Nutritive Value in Heat Damaged Feeds

Heat damage may be of little importance with high moisture silage and properly conserved hay. The problem becomes serious with the increased use of low-moisture silage (Donosa et al. 1962; Hill and Noller, 1963; and Roffler et al. 1967) and artificially dried forages.

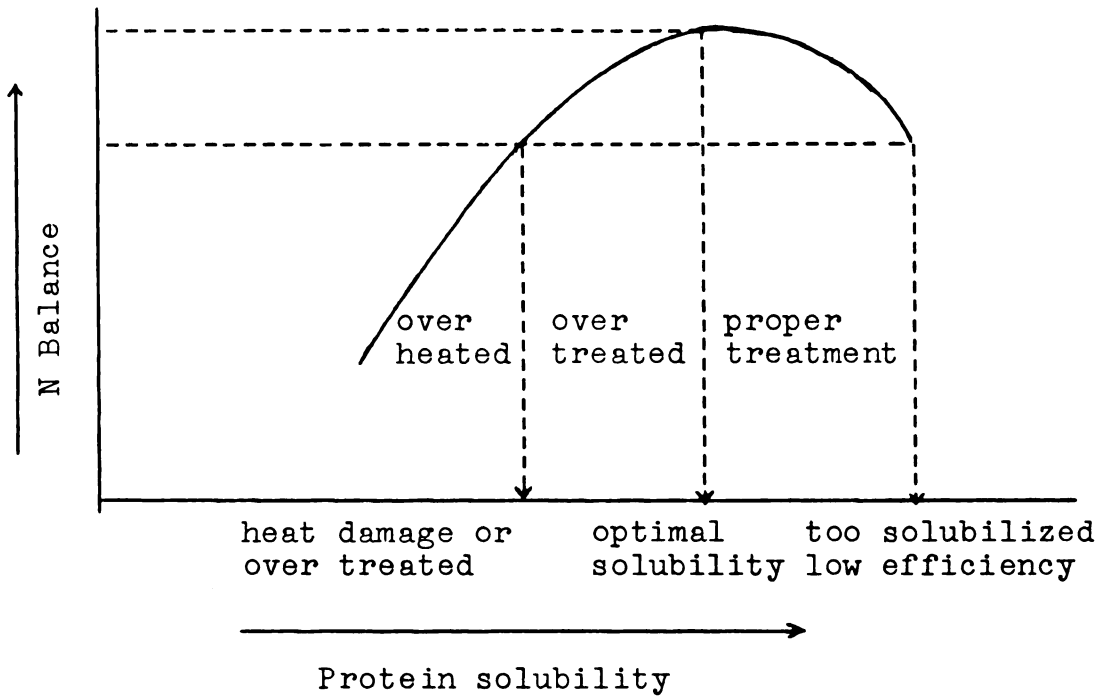
Laboratory methods used for quantitating nutritive value of nitrogen for ruminants are the solubility of general feed proteins, classification silage nitrogen fractions, and estimation of heat damaged protein (Goering, 1973). Table 7 lists some of these. The more important methods, particularly those recommended for heat-damaged feeds will be briefly reviewed.

Table 7. Laboratory Analyses Used to Evaluate Nitrogenous Feeds.

General Feeds	Silage
Water solubility	Ammoniacal N or volatile base
Live rumen fluid	Hot water insoluble N
Autoclaved rumen fluid	Rumen NH_3 production
Ionic strength	
Dilute base	
Salt solution	**Heat Damage**
Pepsin solubility	
Pepsin + Trypsin solubility	Acid-detergent solubility
Pepsin + Pancreatin solubility	Pepsin solubility
	Pepsin + Trypsin solubility
	Available lysine
	Rumen fluid + Pepsin+ Pancreatin solubility

1. Protein Solubility in Rumen Fluid and Other Related Solvents

In order to obtain maximum protein utilization by ruminants, feed protein should be hydrolyzed to NH_3 at a slower rate than rumen microbial protein synthesis (Annison et al. 1954; Chalmers, 1961). The rate of protein hydrolysis in the rumen primarily depends on the solubility of that particular protein. The solubility is, in turn related to the type of protein. For example, albumins are soluble in water while glutelins are insoluble in water, saline solutions, or alcohol, but soluble in very dilute acids and alkalies. Several researchers have shown that properly treating a readily hydrolizable protein with heat or formaldehyde will decrease the protein solubility in the rumen and improve nitrogen balance (Goering, 1973; Chalmers et al. 1954; Danke et al. 1966 and Waldo, 1973). However, when a protein feed is over heated or over treated by formaldehyde, the protein becomes practically insoluble in the rumen and also resistant to enzymatic hydrolysis in the small intestine of animals (Dinius et al. 1973; Brown and Valentine, 1972; and Waldo, 1973). Thus, the relationship between protein solubility and net protein utilization by ruminants is not a linear response, instead, it is a curvelinear response (Figure 3, Goering, 1973).



Source: Goering, 1973. Proc. Wiscon. Conf. on Use of Lab. Analy. In Feeding Programs. p. 63.

Figure 3. A Concept of the Relationship of Nitrogen Solubility of a Feed to Nitrogen Balance in A Rumiants.

Autoclaved and fresh rumen fluid, water and different salt solutions have been used as solvents of various feed protein. Generally, solubilities of proteins in these solvents are good predictors for ruminants N balance of feeds processed properly. When over treated (by heat or formaldehyde) feeds are incubated with these solvents, solubilities of proteins are good predictors of <u>in vivo</u> N digestibility but not consistantly related to N retention since <u>in vitro</u> solubility of protein is

related to solubility in rumen but does not resemble the enzymatic hydrolysis occuring in the samll intestine. Little *et al*. (1963) evaluated the nutritional significance of soluble nitrogen in protein feeds (soybean oil meal, heated soybean oil meal, linseed oil meal and corn gluten meal etc.) for ruminants. Relationships among protein solubilities in rumen fluid, water and 0.02 N NaOH, as well as *in vitro* rumen ammonia production, *in vitro* cellulose degradation, *in vivo* digestibility, nitrogen balance, and lamb performance were examined. No definite relation between nitrogen solubility and rate of ammonia production was evident; however, nitrogen solubility in rumen fluid was generally more indicative of ammonia formed that solubility in dilute sodium hydroxide or distilled water. Corn gluten meal and heat treated soybean oil meal (110 C for 24 hours) were particularly low in soluble nitrogen and the N was slowly converted to ammonia by rumen microbiota and was a poor source of nitrogen for *in vitro* cellulose digestion. However, no apparent differences in protein digestibility and nitrogen retention, feed consumption and growth were detected when lambs were fed regular or heated soybean oil meals. Protein solubility in 0.02 N NaOH was used to evaluate the optimum time for treatment of cottonseed meal by

autoclaving (Danke et al. 1966). With increasing the time of autoclaving, nitrogen solubility decreased only slightly, but in a linear fashion. But, the nitrogen utilization data obtained from sheep responed to the treatment time as positive quadratic relationships.

Recently, Wohlt et al. (173) suggested that a uniform method of determining protein solubilities is needed. This method should simulate solubility in the rumen and be readily and easily duplicated in any laboratory. The method proposed by them involves a one hour incubation of the protein feed with the Wise Burroughs (1950) mineral mixture diluted to 10% with distilled water. However, the relationship between protein solubilities and in vivo N utilizations was not stated.

The relationship of feed nitrogen solubility to nitrogen utilization is not always predictable with the present methods; however, the solubility of a given protein does give some indication of its utilization. A more exhustive study is needed (Goering, 1973).

2. Nitrogen Solubility in Acid Detergent Solution

An acid detergent solution, such as cetyl trimethylammonium bromide dissoved in 1 N H_2SO_4 is a relatively strong detergent and has been used to solubilize plant protein, other nitrogenous compounds and hemicellulose (hemicellulose becomes soluble at a low pH)

(Goering and Van Soest, 1970). The residue is called acid detergent fiber (ADF or lignocellulose) and can be subjected to further hydrolysis with 72% H_2SO_4 to yield an insoluble acid detergent lignin (ADL). Not all N compounds, however, were solubilized by the solution. About 7% of the total N was found in the residue from unheated forages (Van Soest, 1965). The amount of the acid detergent insoluble nitrogen (ADF-N) became proportionally greater when forages were heated to temperatures above 50 C. The relationship between ADF-N and *in vivo* N digestion coefficient has been evaluated by Goering *et al*. (1972). The correlation coefficient between ADF-N as percent of total N (ADF-N x 100/N) and *in vivo* N digestion coefficient (ND) was r = -.93 and the prediction equation was $\hat{Y}(ND) = 72.96 - 1.02$ (ADF-N x 100/N) for 48 forage samples. The slope in this equation indicates that every percentage unit increment in ADF-N/N will result in reduction of *in vivo* ND by one percentage unit. By using this equation, Thomas and Hillman (1972) estimated that about one-third of the haylage made in the State of Michigan was severely heat damaged regardless of silo type while Goering and Adams (1973) found that 40% of the hay crop silage has severe heat damage in the State of Pennsylvania.

An apparent advantage of measuring ADF-N for the

purpose of *in vivo* ND estimation is that *in vivo* dry matter digestibility (DMD) also can be estimated since one must obtain ADF before measuring of ADF-N and ADF is known to be a good predictor of *in vivo* dry matter digestibility (Van Soest, 1969).

One disadvantage of this laboratory method, is the time consumed in analysis which is 2 to 3 days.

3. Nitrogen Solubility in Acid Pepsin Solution

Under optimum conditions (e.g. pH$\leq$ 4, 37 C) pepsin will hydrolyze protein. The amount of nitrogen solubilized by this procedure has been adopted by many laboratories for estimating protein quality in protein rich foods. Goering *et al*. (1972) evaluated the suitability of this method for estimating protein digestibility of heat damaged forages. A correlation coefficient of -.91 between pepsin insoluble N x 100/N and *in vivo* ND was found and the prediction equation was $\hat{Y}$(ND) = 85.87 - 0.91 (pepsin insoluble N x 100/N). Marked similarities in prediction regression equations between pepsin insoluble N/N and ADF-N/N were apparent although ADF-N/N method was slightly more precise. When pepsin insoluble N/N was regressed on ADF-N/N, a slope (b_1) of 1.00 was found. This indicates that both N fractions have the same increase with each increment of heat damage. However, the intercept (b_o) was + 17. This means that

about 17% of the nitrogen is pepsin insoluble when acid detergent insoluble is 0 and no explanations were offered (Goering et al. 1972).

The extent of dry matter solubility by incubation with pepsin can also be obtained as well as N and a high correlation between voluntary intake of digestable DM and DM solubility by pepsin has been reported (Donefer et al. 1966). When DM solubility as well as N solubility is determined using pepsin then an additional advantage to the use of this procedure in forage analysis would be apparent.

4. Nitrogen Solubility in Various Combinations of Proteases Solutions

Other protease hydrolysis incubation procedures have been proposed to study and measure protein denaturation resulting from food and forage processing methods. Saunders et al. (1973) implied that a two enzyme sequence would approximately simulate the gastrointestinal tract digestion process.

The use of pepsin and pancreatin in sequence has been developed for protein quality evaluation based on the composition of amino acids released after digestion (Akeson and Stahmann, 1964). Papain hydrolysis has also been used for protein quality evaluation, but the ranking of quality by this method was not as well correlated with in vivo ND as the pepsin : pancreatin method (Buchanan

and Byers, 1969).

Recently, Saunders et al. (1973) compared the correlation coefficients for leaf protein between protein digestibility measured in rats and two sequential incubations using pepsin : trypsin or pepsin : pancreatin. A slightly higher correlation coefficient was noted for the pepsin : trypsin sequential digestion value than for the pepsin : pancreatin value (r = .91 vs. .87). Papain hydrolysis was also performed but no value reported. Generally, the digestion coefficients calculated from papain hydrolysis were low and not well correlated with the in vivo values.

5. Nitrogen Solubility in Rumen Fluid, Pepsin and Pancreatin Solutions

In vitro rumen fermentation (48 hours) followed by pepsin digestion (48 hours) is a widely used laboratory technique to estimate the in vivo dry matter digestibility (DMD) of feeds for ruminants (Tilley and Terry, 1963). This method precisely predicts DMD and has become the method of choice used to predict animal digestibility by animal nutritionists and plant breeders (Van Soest, 1973). Recently, Antongiovanni et al. (1971) evaluated this procedure and a similar procedure to predict in vivo nitrogen digestibility. They expected a closer simulation to in vivo condition by using pancreatin incubation sequential to the regular

two-stages of the *in vitro* incubation. Additional amounts of protein were hydrolyzed when pancreatin was added after the pepsin digestion, but the degree of this additional hydrolysis varied among samples, and most unfortunately, their samples did not have *in vivo* nitrogen digestibility (ND) values. Thus, evaluation of the precision of this method in predicting *in vivo* ND is impossible. The most apparent drawback for this procedure is the long time involved for three sequential incubations. A complete analysis requires about six days.

6. Hot-Water Insoluble Nitrogen

The fraction of N insoluble in hot water has been used to estimate true protein nitrogen in forages or silages. The forage sample is boiled in water, which coagulates the protein causing it to become water insoluble and remain in the cell of the sample (Goering and Van Soest, 1970). This procedure has been routinely used for silages to fractionate nitrogen into a protein and non-protein-nitrogen (NDN) fraction (Waldo and coworkers, 1973a,b,c). For example, they found that hot water insoluble nitrogen decreased during the fermentation period of direct-cut silage while in formic or formaldehyde treated silage hot water insoluble nitrogen tended to be higher. Although no correlation coefficient

was given, the N balance data were generally parellel with the amount of hot water insoluble N in direct-cut or formic acid treated silages, but in formaldehyde treated silages a less definite relationship was found (Waldo, 1973).

This procedure has a practical advantage over others in that it is rather simple, fast, inexpensive and requires little equipment.

7. Chemical Determination of Lysine Availability

Severe overheating of protein supplemental feeds results in seriously depressed availability of all amino acids with lysine apparently more heat sensitive than some of the other essential amino acids (Meade, 1972). There are several chemical method available to measure the lysine availability in proteins of animal, cereal or oil-seed origin based on quantitative reactions of the ϵ-NH_3 in the lysine molecule. Exampillary methods are: 1-fluore-2,4-dinitrobenzene (FDNB) method (Carpenter, 1960); 2,4,6-trinitrobenzenesulfonic acid (TNBS) method (Tsai et al. 1972); alkylation method (Finley and Friedman, 1973), remazol brilliant blue R method (Pruss and Ney, 1972), and modified FDNB method (Blom et al. 1967). Comparable results have been reported when different methods were used and they are all correlated with the protein utilization by young

monogastric animals (Finley and Friedman, 1973). To date these measurements and comparisons were limited only to certain typical protein sources (e.g. bovine serum albumin, casein). All these methods depend on development of a color at a specific wavelength, accurate spectrophotometric measurements on extracts of plants or plant proteins and especially heat damaged plant protein should not be expected because of interfering agents such as plant pigments, carbohydrates hexosamines and brown pigments (Maillard reactions) (Allison et al. 1973; Blom et al. 1967). At the present time, a method developed by Finley and Friedman (1973) is probably the most reliable for estimating available lysine in samples high in carbohydrate and pigments. However, this method has a serious drawback since it requires an expensive amino acid analyzer. Perhaps because of analytical difficulties, there are no data in the literature concerning the relationship between lysine availability and protein digestibility of forages by ruminants.

8. Degree of Non-Enzymic Browning

Using laboratory model systems, the brown pigments produced by the Maillard reactions can be quantitated by measuring the absorbance of an extract at about 420 nm (Reynolds, 1965; Keeney and Bassette, 1959; Eichner and Karel, 1972; Pokorny and Janicek, 1971; Janicek and

Pokorny, 1970; Pokorny et al. 1973a,b,c,d; Baloch et al. 1973; Yanagita et al. 1973). For example, Yanagita et al. (1973) reported that when mixture of casein and ethyl loinoleate was subjected to heat treatment, the degree of browning (E_{430}/16 mg N/10 ml) increased with increasing reaction temperatures while the quantity of available lysine and digestibility in vitro by pepsin and trypsin decreased with increasing reaction temperatures.

Browning pigments occur widely in processed foods, but special separation and isolation procedures are needed before the degree of browning can be accurately measured. This is because of the strong interference by various pigments. For example, carotenoid pigments in dehydrated carrot have high absorption near wavelengths of the browning products (Baloch et al. 1973; Hendel et al. 1950). Unfortunately, there is no special procedure developed for measuring the degree of browning in heat-damaged hay crops. Farmers and animal nutritionists usually estimate extent of heating by odor and sight of the sample. This is obviously not as accurate as the other laboratory methods (ADF-N or pepsin solubility). In fact, Goering and Adams (1973) found that several haylage samples with two ADF-N values were much darker in color than expected based on their ADF-N value.

V. Methods Available to Prevent Heating and Spoilage in Haylages

Under farm conditions, heating and spoilage in haylage are due to the presence of oxygen and the most effective means to solve such problems would be to exclude air. This can be partially acomplished by good management practices e.g. chop forage to reasonably short, fill silo rapidly, cover the top with a plastic sheet, (Hillman and Thomas, 1973) and by using oxygen-limiting silos. Alternatively, one can use preservatives to prevent the heating and spoilage by restricting respiration of plant cells and microbes. There are several chemicals having fungistatic and fungicidal properties such as volatile fatty acids, sorbic acid, benzoic acid, sulfur dioxide etc. that might be added to ensiled forages (Nickerson and Sinskey, 1972). Any useful chemical should have the following characteristics: (1) prevent spoilage and deterioration over a wide range of moisture and temperature conditions at application rates which are economically competitive; (2) be palatable and nontoxic to livestock and leave no residue in animals fed the material; (3) be reasonably safe to handle and require a minimum of special equipment for application, distribution and storage.

According to Sauer (1973), the best mold inhibitors, considering efficacy, cost and safety are

propionic acid and closely related compounds. These include propionic, acetic, formic and isobutyric acids, and a few salts such as sodium propionate and ammonium isobutyrate. The salts are less effective pound for pound, but are relatively non-corrosive and safer to handle compared to the acids.

The mode of action of these acids is not known for certain. The lowered pH, dissociated and undissociated molecules of the acid all play important roles in preservation. They may destroy cell membrances or inhibit some microbial enzyme systems concerned with metabolism or reproduction (Nickerson, 1972).

The effectiveness of various preservatives in preventing spoilage of silage and high moisture grain has been studied and results reported recently (Sleiman, 1972; Britt, 1973; Waldo et al. 1973a,b; Fox et al. 1972; Henderson et al. 1972; Huber, 1970; Bade et al. 1973; Jones, 1970; Papendick and Singh-Verma, 1972; Sauer, 1973; Thomas et al. 1973). However, proper comparison of effectiveness of different preservatives is difficult because lack of consistency in dosage rate, testing material, moisture level and storage conditions. The preservation value of different chemicals has inconsistant rankings under different conditions (Goering and Gordon, 1973). Thus, for illustrative and comparative

purposes an experiment which tested a large number of preservatives under the same storage condition should be selected. One experiment conducted by Sauer (1973) was choosen for this purpose although the material used was wet shelled corn grain (Table 8).

Table 8. Mold-Free Storage Time (Weeks) for Corn With 24% Moisture Content Stored at Approximately 23 C.[a]

Chemical Applied	Application Rate 0.4%
1. Methylene bis propionic acid	22 +
2. Propionic acid	22 +
3. 80% Propionic acid:20% Acetic acid	8-9
4. 80% Propionic acid:20% Formic acid	7-9
5. 80% Propionic acid:20% Isobutyric acid	6-8
6. 50% Propionic acid:50% Isobutyric acid	6-7
7. 20% Propionic acid:80% Formic acid	3
8. 50% Propionic acid:50% Formic acid	1-4
9. 50% Propionic acid:50% Acetic acid	2-3
10. 20% Propionic acid:80% Isobutyric acid	2
11. Ammonium Isobutyric acid (AIB)	2
12. Isobutyric acid	2
13. Acetic acid (glacial)	1
14. Formic acid	1
15. 20% Propionic acid:80% Acetic acid	0
16. Ammonium acetic acid	0
17. None	0

[a]Sauer, D.B., A report from U.S. grain marketing research center Agricultural research service. USDA. October, 1973.

Based on several such trials the authours conclude that: (1) salts are inferior to the acids form, (2)

propionic acid and methylene bis propionic acid are superior to other related acids, (3) there are no synergistic effects using acid mixtures. Results from this experiment are somewhat contradictory to a general believe that antimicrobial activity of the fatty acids increases with increasing chain length. Butyric acid is, for example, more effective than propionic acid (Galbraith et al. 1972). Although butyric acid was not tested in Sauer's study, the large difference in potency of mold growth inhibition between propionic acid and isobutyric acid obviousely can not be interpreted from the chain length point view.

Acetate and propionic are naturally occurring compounds in silages and in the rumen, and are a readily digestible energy source. They are intermediate products in carbohydrate and protein digestion and are produced in particularly large quantities in ruminants. The quantities consumed in acid treated forage or grain would not be sufficient to disturb normal acid levels in the digestive system (Sauer, 1973).

In a preliminary study Thomas et al. (1973) evaluated several preservatives for mold inhibition using haylage ensiled in barrels (42-61% DM). Results were comparable to Sauer's study. Propionic acid and a mixture of acids (propionic acid 80% : Acetic acid 20%)

were very effective in reducing temperature during a 55 day period. Ammonium propionate was less effective than propionic acid at the same molor application rate while formic acid was almost completely ineffective. Voluntary intake of propionic acid treated haylage was much greater than the control when fed to sheep. No marked effect of acid treatment was noted on dry matter digestibility. Similar results about the value of acids were found by Sleiman (1972) using partially wilted rye grass (28% DM) with minimum conpaction in piles and barrels.

In a recent report Goering and Gordon (1973) evaluated a number of preservatives under laboratory conditions. Their results ranked the effectiveness of chemicals in retarding mold growth of alfalfa haylage in following order: propionic acid > ammonium isobutyrate > sodium propionate > acetic : propionic (50:40) acids > formalin > sodium chloride.

There were some indications that formic acid reduced NH_3-N content in unwilted silage (18% DM) more effectively than did propionic acid. The lactobacilli counts were also higher in formic acid treated silage than in propionic acid treated silage. However, in wilted silage (38% DM) the reverse situation was found with propionic acid producing the better results (Papendick and Singh-Verma, 1972). Nevertheless, marked improvements

in intake, dry matter digestibility and nitrogen utilization have been reported when propionic acid was applied to unwilted Italian ryegrass (20% DM) (Cottyn et al. 1972).

Formic acid has been successfully used as an additive to direct-cut grass silage in Norway and Great Britain (Saue and Breirem, 1969a,b; Castle and Watson, 1970a,b; Henderson and McDonald, 1971; Carpintero et al. 1969). In the United State, thorough and systematic evaluations of formic acid as hay-crop silage additive have been conducted only by Waldo and coworkers (Waldo et al. 1966; 1971; 1973a,b,c,d; Derbyshire et al. 1971). The possible actions of formic acid on silage fermentation and animal production are: 1) inhibition of plant protease activity after cutting, thus stoping protein degradation; 2) immediate decrease in silage pH to 4.2 which is favorable only to lactic acid bacteria; 3) exertion of selective pressure on clostridia which reduces the production of butyric acid and ammoniacal nitrogen and preventing lactic acid degradation; 4) reduction in dry matter loss during ensiling; 5) slight improvement in voluntary intake; 6) slight improvement in digestibility; 7) significant improvement in efficiency of animal gain and 8) significant improvement in N balance (Waldo, 1973). However, equal milk production for cows fed formic acid treated direct-cut silage (.5%, w/w) and

control silage was reported by Thomas et al. (1969).

In general, formic acid treated unwilted silage is superior to unwilted control, wilted control, unwilted molasses treated, unwilted sodium metabisulfite ($Na_2S_2O_5$) treated unwilted silages (Carpintero et al. 1969; Waldo, 1973; Henderson et al. 1972). However, Reid (1971) reported the metabolizable energy values of formic acid treated and untreated control were not significantly different. In respiration calorimetric experiments using sheep, the proportions of the metabolizable energy stored as new body tissue were 53.4% for the untreated silage and 54.6 for the formic acid silage. Thus Reid (1971) concluded that the main advantage of formic acid treated silage was increased consumption of the treated silage. When formic acid was added to wilted silages improved recovery, gain and efficiency were found (Derbyshire et al. 1971). Since formic acid did not reduce spoilage of haylages in barrels (Thomas et al. 1973) its use as haylage additive is not promising.

Recently, formaldehyde has been reinvestigated as a direct-cut or wilted silage additive. Formaldehyde may possess two advantages over formic acid such as (1) reduced cost ($5 vs. 10/ton forage DM) and (2) improved protein utilization. Formaldehyde binds with forage protein to protect the protein from microbial degradation

in the rumen and allow subsequent digestion of the protein in the abomasum and intestine resulting in more efficient use of ingested N (Ferguson et al. 1967; Ferguson, 1970; Waldo, 1973). However, when too much formaldehyde is used, the forage protein will be over protected and even resistant to enzymatic hydrolysis in the small intestine. For example, when alfalfa (27% DM) was treated with formaldehyde at the rate of 16.6 g per 100 g crude protein when ensiled voluntary intake as well as dry matter and nitrogen digestibilities were significantly reduced as compared with values for untreated silage (Brown and Valentine, 1972). When formaldehyde was applied to a grass-legume mixture (20% DM) at a lower rate of 2 g per 100 g crude protein plus about the same amount of formic acid, the silage had less protein degradation and more net lactic acid production in the silos than did the control (Waldo et al. 1973c). Nitrogen digestibility was slightly depressed (63 vs. 65%) by treatment, but daily nitrogen retention was significantly increased (31.3 vs. 25.7 grams). Daily gain by heifers was increased from 643 to 750 grams by this treatment (Waldo et al. 1973). The authors concluded that formaldehyde at this concentration practically stopped nitrogen degradation during silage fermentation and aided protein by-pass through the rumen

producing increased nitrogen retention. On the other hand, Thomas (1964) found no significant differences in DM intake and daily gain between dairy heifers fed formaldehyde treated direct-cut alfalfa silage (2 g formaldehyde/100 g forage protein) or untreated silage. The potential use of formaldehyde as silage additive, particularly for legume crop silage, appears optimistic for direct-cut silage. However, there is no data available concerning formaldehyde for haylage preservation. However, application rate will be critical in its effect on ND.

Although ammonium isobutyrate (AIB) was classed as ineffective in preserving high moisture corn grain in a study by Sauer (1973). Goering and Gordon (1973) on the other hand, found that AIB was superior to propionic acid in inhibiting of heat and mold development in haylage. In addition to the effect on inhibiting mold development, AIB has nutritional significances to ruminants. It contains 84% crude protein equivalent which will contribute to the total nitrogen content in the ration. The carbon skeleton of AIB is a branched four carbon moiety which has been considered as one of the important growth factors for cellylulytic bacteria in the rumen (Bryant, 1973). Ammonium isobutyrate also has some convenience in field application being relatively non-corrosive and practically odorless.

Another chemical, sulfur dioxide (SO_2), theoretically should be an effective haylage preservation. Sulfur dioxide or the sulfurous acid salt (e.g. $Na_2S_2O_5$) is widely used as an anti-microbial agent to preserve various types of food (e.g. fruits and wine) (Nickerson and Sinskey, 1972). Sulfur dioxide is a multi-functional preservative acting to (1) inhibit the non-enzymic browning reaction; (2) inhibit various enzyme-catalyzed reactions, notably enzymic browning; (3) act as a anti-oxidant and a reducing agent; (4) inhibit and control growth of microorganisms (Dunn, 1956; Nickerson and Sinskey, 1972).

In practice, when $Na_2S_2O_5$ was applied at low levels ($\leq$ 4% wet basis), no significant improvements were found in silage quality, storage recovery or animal performance (Carpintero et al. 1969; De Vuyst et al. 1967a,b; Zelter, 1961; Murdoch et al. 1956; Levitt et al. 1962). However, when $Na_2S_2O_5$ was added at higher levels, silage quality was improved to some extent, but silage voluntary intake was significantly reduced (Levitt et al. 1962; Cowan et al. 1952; Allred et al. 1955).

Alderman et al. (1955) and Cowan et al. (1953) both commented on the use of $Na_2S_2O_5$ for high dry matter silage. They suspected that the instability of $Na_2S_2O_5$ in treated silage was due to the progressive decomposition of $Na_2S_2O_5$ through oxidation reactions.

There are other direct-cut silage additives which can be divided into two groups. The first group includes materials that will stimulate lactic acid fermentation such as molasses, sugar, glucose, apples or cereal products. However, these additives generally have no direct effect on clostridia and they can be beneficial to silage fermentation only under strict anaerobic condition since sugars are not only good substrates for fermentation but also an excellent energy source for oxidation by molds or fungi (Wieragne et al. 1961). The second group includes various kinds of strong mineral acids which directly increase the acidity of the silage and hence depress all microbial activity, particularly the clostridia which are sensitive tc low pH values. Strong mineral acids (e.g. 2 N HCl or 14 N H_2SO_4) were used successfully during the early 1900s (Virtanen, 1929). However, the uses of strong mineral acids and phosphoric-sulfuric mixtures has been gradually replaced by weaker organic acid for several reasons. The improvement of harvesting techniques such as fine chopping of silages made it possible to replace strong mineral acids by weaker acids, because the acid can react more easily with chopped or crushed crop. According to Virtanen (1969), mineral acids exert their silage preservation effects only by reducing pH and have no specific anti-microbial

properties. The mineral acids gradually react with the basic components of the crop and thus the final acidity of the silage depends on the original content of organic acids. On the other hand, the preservative effect of formic acid is partly due to a reduction of pH, and also to a selective anti-bacterial effect. There are several instances suggesting that animals developed metabolic acidosis when they were fed silages treated with mineral acids (McCarrick et al. 1965; L'estrange and Murphy, 1972). Also, in field situations, strong mineral acids are more difficult to handle than weaker organic acids. In addition, several organic acids possess special fungistatic or fungicidial properties which add to their use fulness thus suitable as haylage additive, while mineral acids have no such selective action.

This review has indicated that several organic acids (e.g. propionic acid) can be applied to direct-cut silage at rather low levels yet still obtain comparable or even better results than those obtained by using sugars or mineral acids or both. In fact, Saue (1968) has demonstrated that formic acid treated silage was better than molasses or mineral acid treated silages for lambs.

In conclusion, this literature review indicates that the ND of haylage is commonly lower than that of

hay or direct-cut silage. This decreased ND can not be detected by the traditional total N analysis. Although a few laboratory methods have been used successfully to estimate ND of haylages, other laboratory methods should be evaluated.

Many antifungal agents have been demonstrated to be benefial in preserving nutritive value of direct-cut silage. However, only limited experiments have been carried out using haylage. Thus, the present experiment was designed: 1) to evaluate several chemical and *in vitro* laboratory methods to estimate the nutritive value of haylages; and 2) to evaluate propionic acid, ammonium isobutyrate and formaldehyde in preserving nutritive value of alfalfa haylage.

MATERIALS AND METHODS

Part 1. Evaluation of Forage Protein Quality by Laboratory Methods

I. Forage Samples

Twenty-four forage samples that have been studied for various experimental objectives by research personnel in the Michigan State University, Dairy Science Department were selected for the present study. Among these 24 samples, eight were preserved as stacked alfalfa hays, eight were preserved as alfalfa haylages in cement silos, three were preserved as alfalfa haylages in pilot type silos (about 50 kg fresh haylage capacity) and five were preserved as sun cured alfalfa hay (Table 9).

At least 4 samples were suspected to have been heat damaged based on data of storage temperatures, unusually low voluntary intake, nitrogen(N) and dry matter (DM) digestion coefficients, while the other 10 samples were considered normal since they had normal nutritive values when fed to sheep. Each sample had values for proximate analysis (crude protein, ether extract, crude fiber, N free extract and ash), fibrous constituents analysis (cell wall constituents, acid detergent fiber, hemicellulose, cellulose and lignin) and sheep

Table 9. Analytical Values for Forages Used in Protein Solubility Study. Minimum, Maximum and Gross Mean Values Are Given For Each Item.

Total no. sample	Species	Type of Preservation & no. of samples	DM[a] as fed	crude protein	Ether extract	N-free extract
			% DM			
24	Alfalfa- or alfalfa-grass mixture	Sun-cured hay (5) Stacked hay (8) Cement silo-haylage (8) Pilot silo-haylage (3)	87-48 (72)[b]	16.5-23.6 (20.1)	3.33-0.95 (2.37)	43.8-32.5 (38.6)

Crude fiber	Ash	Cell walls	Acid-detergent fiber	Hemi-cellulose	Cellulose	Lignin
% DM						
39.6-23.1 (30.6)	10.8-6.6 (8.2)	60.7-35.0 (49.2)	25.0-51.3 (41.0)	14.1-1.2 (8.21)	38.1-16.7 (32.1)	13.8-6.5 (9.3)

[a]Dry matter. [b]Values in parenthesis are means.

performance (maximum intake, digestion coefficients of major nutrients and N utilization) as shown in Appendix Table 1,2,3, and 4. The maximum, minmum and mean values of forage constituents and sheep performance data are summarized in Table 9 and 10 respectively.

Table 10. Digestion Coefficients, Nitrogen Utilization and Maximum Intake of 24 Forages Used in Protein Solubility Experiments.

Items	Range	Mean
Digestion Coefficients (%)		
Dry Matter	43-70	57
Organic Matter	44-71	59
Cell Wall Constituents	40-62	52
Acid Detergent Fiber	42-56	48
Nitrogen (N) Utilization		
Digestibility (%)	40-77	64
N-balance (g/day)	-2.8-9.1	3.5
N-balance x 100/N absorbed	-8.9-28.3	15.5
Maximum DM Intake (%BW[a])	1.91-4.64	3.52

[a]Body weight.

These samples were deemed sufficient in number, range in analytical values and digestibility to be representative of those existing on farms and adequate to characterize protein quality.

Forage samples were ground through a 1 mm screen

of a Wiley mill and stored in sealed glass jars.

In addition to the 24 samples obtained from this Department, Dr. H.K. Goering of USDA Maryland kindly supplied data for 44 forage samples which in turn, were furnished by several experiment stations (South Dakota, Nebraska, Iowa and Utah). These samples had complete proximate analyses, nitrogen solubilities in acid detergent and acid pepsin solutions, and in vivo nitrogen digestion coefficients. Also, data for 18 forages were supplied by Dr. N.A. Jorgenson of University of Wisconsin and Dr. D.C. Pierson of University of Minnesota. Data obtained included acid detergent insoluble nitrogen fractions and in vivo nitrogen digestion coefficients. Data supplied from the other sources were combined with the data of this study and used for predicting in vivo N digestion coefficients by acid detergent insoluble N as a percent of total N.

II. Laboratory Methods Used to Evaluate Forage Protein Quality

A. Protein Solubility

1. Solubility in hot water: Two gram sample of air dry, finely ground (passed 1 mm screen, Wiley mill) forage was boiled and refluxed with distilled water for one hour and insoluble material was recovered by filtration (Whatman filter paper No.54, 15 cm). Content of

dry matter (oven drying at 90-100 C for 24 hours) and nitrogen (macro-kjeldahl procedure) in the insoluble solids were determined. Percent of hot water insoluble nitrogen (N) was calculated either as a proportion of total dry matter (DM)(Equation 1) or as a proportion of total N (Equation 2).

$$\text{Hot water insoluble N, \%DM} = \text{Gram N} \times 100/\text{oven-dry sample weight (g)} \quad (1)$$

$$\text{Hot water insoluble N, \%N} = \text{Gram N} \times 100/\text{gram N in oven-dry sample weight} \quad (2)$$

Hot water soluble N was calculated as the difference between total nitrogen and insoluble nitrogen (Equation 3).

$$\text{Hot water soluble N, \%DM} = \text{Total N, \%DM} - \text{Hot water insoluble N, \%DM} \quad (3)$$

In addition, hot water soluble dry matter was also calculated (Equation 4).

$$\text{Hot water soluble DM,\%} = 100 - (W_r - W_t/\text{oven-dry sample weight}) \times (100) \quad (4)$$

Where: W_r = Weight of filter paper plus insoluble residue;
W_t = Weight of filter paper.

2. Solubility in acid detergent solution: Two gram sample of air dry, finely ground (passed 1 mm screen, Wiley mill) forage was boiled and refluxed with acid detergent solution (1 N H_2SO_4 containing 49 g cetyl trimethylammonium bromide/liter) for one hour according to procedure outlined by Goering and Van Soest (1970). Acid detergent insoluble residue was recovered by

filtration (Whatman filter paper, No. 54, 15 cm) and analyzed for dry matter (oven drying) and nitrogen (macro-Kjeldahl procedure). Calculations of insoluble N, soluble N and soluble DM were the same as used for the hot water solubility.

3. Solubility in diluted phosphate-bicarbonate mineral buffer solution:

An aliquot of forage sample containing 25 mg N was weighed and transferred into a 200 ml centrifuge bottle. Phosphate-bicarbonate buffer solution was prepared according to Burroughs (1950) and this original buffer solution was diluted ten fold with distilled water (1 part original buffer + 9 parts distilled water). One hundred ml of this diluted buffer solution was added to the centrifuge bottle containing the forage sample. Bottles were then immersed in a 39 C incubator for one hour. After the incubation period, the solution was centrifuged at 15,000 x g for five minutes. Extracted nitrogen was determined on a 5 ml aliquot of the supernatant by the macro-Kjeldahl method. Percent total soluble nitrogen (N) was calculated as mg N extracted in the 100 ml divided by the 25 mg N.

B. In Vitro Protein Digestion

1. Acid pepsin digestion: Fifty ml of acid pepsin solution (0.01% w/v pepsin[A] in 0.1 N HCl) were

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[A]Pepsin: 1:10,000. Nutritional Biochemicals, Cleveland, Ohio.

added to a 125 ml Erlenmeyer flask containing 0.5 g air dry, ground forage sample. The mixture was incubated at 39 C for 20 hours (Goering and Van Soest, 1970). The indigestable material was recovered on Whatman filter paper No. 54, 15 cm and analyzed for dry matter (oven drying) and nitrogen (macro-kjeldahl procedure). Percent of pepsin insoluble N, soluble N, and soluble DM were calculated according to similar equations as used in the hot water solubility procedure.

2. Acid pepsin and pancreatin digestions: These procedures were outlined by Saunders et al. (1973). In a 50 ml centrifuge tube, 500 mg of air dry, ground forage sample was suspended in 15 ml of 0.1 N HCl containing 1.5 mg of pepsin and incubated at 37 C for three hours. The solution was neutralized with 0.5 N NaOH and treated with 4 mg of pancreatin[A] in 7.5 ml of 0.2 M phosphate buffer pH 8.0. The mixture was incubated for an additional 24 hours at 37 C. The solids were separated by centrifuging (20,000 x g for 5 min.) and washing with water (5 x 30 ml). The solids were finally filtered through a 1.2 μ filter (Millipore), oven dried, weighed, and analyzed for nitrogen (macro-Kjeldahl procedure). Calculations of insoluble N, soluble N and soluble DM were similar to those described for the hot water solubility procedure.

[A]Pancreatin, Grade III, Nutritional Biochemicals, Cleveland, Ohio.

3. Rumen microbial and acid pepsin digestions: Fresh rumen fluid was obtained from a fistulated cow, two hours after a morning feeding of mixed alfalfa-grass hay. Rumen fluid was filtered through four layers of cheesecloth, and the filtrate was then immediately added to tubes containing forage samples and a mineral buffer solution (modified Terry and Tilley method, 1963; outlined by Goering and Van Soest, 1970). The tubes were then capped with a rubber stoper fitted with two openings: A bunsen valve and a gassing tube connected to a common manifold. The manifold was connected to a supply of carbon dioxide. The tubes were gently gassed with CO_2 during the first 20 min of a 48 hour incubation at 39 C. At the end of this incubation period, rumen microbial digestion was stopped by adding 2 ml acid pepsin solution (.5 g pepsin in 6 N HCl) and the acid pepsin digestion continued for 48 hours at 39 C. The insoluble material was isolated by filtration (Whatman filter paper No. 54, 15 cm) and analyzed for DM and N. Calculations of insoluble N, soluble N and soluble DM were made as described for the hot water solubility procedure.

4. Rumen microbial, acid pepsin and pancreatin digestions:

Procedures involved in the first two stages for rumen microbial and acid pepsin incubations were practically the same as those described in the previous method.

after a total of 96 hours of rumen microbial and acid pepsin digestions, the incubation mixture was neutralized with 0.5 N NaOH and treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer pH 8.0. The mixture was incubated for an additional 24 hours at 39 C. Indigestible material was filtered on Whatman filter paper No. 54, 15 cm, and analyzed for dry matter (oven drying at 100 C for 24 hours) and total nitrogen (macro-Kjeldahl procedure). Percent of insoluble N, soluble N and soluble DM were calculated using equations similar to those described for the hot water solubility procedure.

5. Rumen ammonia release: Experimental procedures for quantitating the amount of NH_4^+ release were essentially those used in the rumen fluid-acid pepsin incubation except that incubation time was 3 hours and no urea was added to the mineral buffer solution. Rumen microbial digestion was stopped by addition of 1 ml saturated mercuric chloride ($HgCl_2$). The incubation mixtures were centrifuged at 25,000 x g for 10 min. and duplicate 5 ml supernatant aliquots were assayed for ammonia nitrogen by steam distillation (AOAC, 1965). Total ammonia nitrogen production was expressed as a percent of total nitrogen in the sample.

6. Degree of browning: To estimate the extent of "browning" a 500 mg forage sample was incubated with

50 ml phosphate buffer at pH 7.8 containing 3.5 mg pronase[A] at 37 C for 5 hours. The solution was then filtered through a 15 cm filter paper (Whatman No.1) and the filtrate was made to 50 ml. One ml of this diluted filtrate and 3 ml of distilled water were pipetted into a photometer tube. After thorough mixing, the optical density of this diluted solution was read in a spectrophotometer[B] at a wavelength of 440 millimicrons.

III. Statistical Analyses

A total of 31 variables were determined for each sample. These variables were also combined into 13 groups based on laboratory operations. Simple linear regressions of five in vivo parameters (DM and N digestion coefficients, N balance, N retention as a percent of absorbed N, and maximum DM intake) on each of 31 variables were calculated. Five in vivo parameters were also regressed on each of 13 groups. Multiple and linear regressions were calculated for the amount of digestible nitrogen (g/100 g dry matter) on selected various nitrogen fractions.

In vivo parameters were then regressed on variables obtained from random combinations of any two groups.

[A]Pronase. 45,000 PUK/g. B grade, Calbiochem. San Diego, California.

[B]Coleman model Junier II., Coleman Instruments Co., Maywood, Ill.

The next step was regressions selected variables derived from the combinations of more than three groups with *in vivo* responses. A least square deletion program was combined with multiple regression analyses.

Part 2. Haylage Preservation With Propionic Acid, Ammonium Isobutyrate and Mixture of Ammonium Isobutyrate and Formaldehyde

I. Ensiling Techniques

First crop alfalfa (85%) was harvested in the late bud stage of maturity. Forage was cut, crushed, and windrowed with a 4 m swath mower. When forage moisture content was reduced to about 50%, forage were picked up, chopped and blown into front unloading wagons with a field forage harvester. The chopper was set for about 1 cm theoretical length of cut. Each loaded wagon was weighed on a platform scales and empty wagons were weighed several times during the filling operation. Forage samples were obtained at the blower for DM analysis. The assignment of silos, dates, treatment and amounts ensiled are given in Table 11. The amount of additive (Table 11) for treated forages was calculated to the nearest 0.1 pound after each load was weighed. Chemicals at low application rates (i.e. 0.4% propionic acid and 0.5% ammonium isobutyrate) were diluted 1:1 with water. Chemical solutions were sprayed on the

Table 11. Harvesting Dates, Treatment and Dry Matter Content of Alfalfa Ensiled as Haylage. 1973.

Silo[a] no	Cutting date	Treatment[b]	Average DM(%)	Total Haylage loaded(lb)	Total Haylage DM loaded(lb)
3	6/15	0.8% Propionic acid[c]	63	50,900	30,771
4	6/22,23	0.5% AIB[d]+1.25% Formaldehyde[e]	52	53,530	27,568
5	6/18-20	1% AIB	56	52,865	29,235
6	6/18-20	None	54	49,790	26,387
7	6/21,22	0.5% AIB	63	53,330	32,147
8	6/21,22	0.4 Propionic acid	57	53,850	30,147

[a]Upright concrete silos. 3 x 12 m in size.

[b]Treatment level was on basis of w/w (as ensiled).

[c]Chemostor: Mixture of propionic acid and acetic acid in a ratio of 80:20. Furnished by Celanese Chemical Company, Corpus Christi, Texas.

[d]AIB: Ammonium isobutyrate, furnished in a 68% concentration solution by W.R. Grace and Company. Memphis, Tennessee.

[e]Formaldehyde was purchased from Carrier Stephens Company, Lansing, Michigan in a 37% concentrated solution.

forage as it entered the blower from the wagon by a precalibrated pump[A]. Formaldehyde and ammonium isobutyrate were mixed together after each had been weighed individually. Forage in the silo was leveled and tramped after every two loads.

Thermocouples (copper-constantan) were placed near the center of the silo at approximately 1.5 m vertical intervals after leveling and tramping so that by the end of the ensiling operation, five thermocouples were placed in each silo with two in the bottom potion. Leads from each thermocouple were extended outside the silos and attached to a 24 channel potentiometer[B] which automatically converted electrical potential in to temperature and recorded the temperature at preset time intervals. During the entire 42-day of haylage storage period temperature was recorded ten times per day for the 24 leads. Once daily recordings were obtained for the fifth or other bottom thermocouple lead for each silo and ambient using a portable potentiometer[C]. When silos were opened for feeding, temperature measurements by recording potentiometer ceased but portable potentiometer readings continued for the bottom thermocouple

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[A]Supplied from the W. R. Grace and Company, Tennessee.

[B]Supplied from the Department of Agricultural Engineering, Michigan State University.

[C]Brown portable potentiometer Model 126 W2. Minneapolis-Honeywell Regulatory Co., Philadelphia, Pa.

leads for three additional weeks. During the entire period when the six silos were being emptied, temperature 25 cm beneath the surface of the haylage was obtained about two times per week with a mercury thermometer.

All material removed from the silos as weighed and recorded as silage or spoilage. Incomplete weights were obtained on material remaining in the silo at the end of the feeding trial.

II. Feeding Trial

Forty-eight lactating dairy cows, averaging more than 18 kg of milk per day, were assigned to one of six haylage treatment groups (Table 11) in a randomized block design. Milk yields during a 2 week standarization period were used as a blocking factor. Treatment groups were also balanced for age, genetic groups, and days after parturition.

During the standardization period cows were fed haylage ad libitum (allowing 10% orts) from a general herd supply. In addition they received a grain mixture (16% crude protein) at a rate of 1 kg/3 kg milk. Refusals were weighed 5 times per week. During the experimental period (49 days) the assigned haylage was fed ad libitum. Grain mixture (16% crude protein) was

fed to cows at rate of 1 kg per 3 kg milk. Refusals of haylages and grain were weighed five times per week. Cows were weighed on two consecutive days at four days after initiation of the trial and at the end of the trial.

Milk weights were recorded five days per week during standardization and experimental periods.

III. Milk Analysis

Composite (AM and PM) samples of milk were taken from each cow at biweekly intervals during the feeding trial and twice during the standardization period. Total solids were determined by drying 2 ml for 2 hours in a forced air oven at 100 C. Butterfat was determined by a butterfat auto analyzer[A].

IV. Feed Analysis

Haylages were sampled from the feeding cart three times per week during the trial and stored in a refrigerator at 4 C. Samples were composited biweekly and used for laboratory analyses. For various kinds of analyses different aliquots were taken from the composite (Figure 4). An small aliquot was used for dry matter

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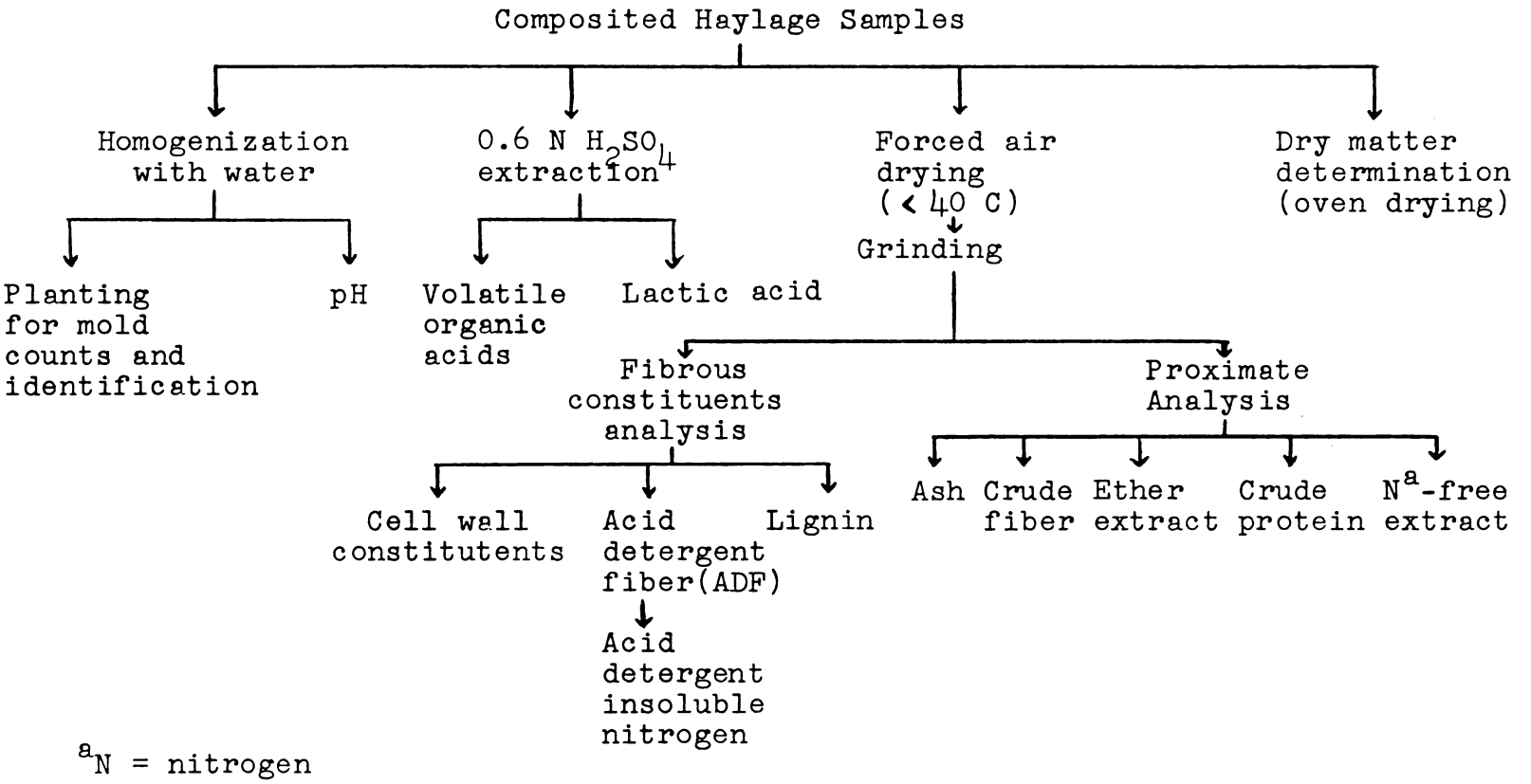
[A]Mark II. A/SN. Foss Electronic Company, Hillerod, Denmark.



[a]N = nitrogen

Figure 4. Preparations of Composited Haylage Samples for Various Kinds of Analyses.

determination by oven drying at 100 C for 24 hours. About one kg of sample was spred evenly on aluminium pans and air dried at room temperature. A fan was usually used to speed the drying process. The dried samples were then ground through a 1 mm screen in a Willey mill and used for proximate analyses (AOAC, 1965), fibrous constituent and acid detergent insoluble nitrogen analyses (Goering and Van Soest, 1970). For organic acid determinations a 10 g haylage sample was mixed with 40 ml of 0.6 N H_2SO_4 and stored for 2 or more days at 4 C. The mixture was then filtered through two layers of cheesecloth and the filtrate was further centrifugated at 12,000 x g for 20 min. The clear supernatant was used for determination of lactic acid by the procedures of Baker and Summerson (1964). Volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric and isovaleric acids) were quantified in a gas chromatograph as follows. Three µl samples were injected into a Hewlett-Packard, F and M gas chromatograph[A] using a glass column (2.4 m) packed with chromosorb 101 (80/100 mesh). The injection port temperature was set at 340 C, the column temperature at 285 C, and the flame detector at 320 C. Nitrogen was used as the carrier gas and

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[A]Hewlett-Packard, F and M Scientific Co., Model 402.

flow rate was 30-40 ml per min. Sample volatile fatty acid concentrations were calculated by comparing peak height with those of analytical grade acids made into a dilute "standard" solution.

Haylage samples were prepared for pH measurement and number and type of fungi by homogenizing 20 g of haylage and 180 ml of distilled water into a Sorvall Omni-Mixer[A] for 3 minutes at 8,000 r.p.m. with the homogenizing cup immersed in ice. The pH of the homogenized material was measured with a Sargent pH meter[B] using a combination electrode. The homogenate was then filtered through one layer of cheesecloth and the filtrate was used for the pour-plate technique for estimating the number of fungi present. Three to five serial dilutions of 1 to 1 x 10^{-4} up to 1 to 1 x 10^{-6} of filtrate with sterile water were normally required in order to have proper concentrations of fungal spores and mycelia in the plate (approximately 20-50 colonies per plate).

The growth medium used was potato dextrose agar[C]. One hundred mg of Novobiocin calcium[D] was used per liter

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[A]Ivan Sorvall, Inc., Newton, Conn.

[B]E.H. Sargent and Company, Chicago, Ill.

[C]BBL, Cockeysville, Maryland.

[D]The Upjohn Company, Kalamazoo, Michigan.

of agar. Poured plates were incubated at room temperature for approximately 9 days or until sufficient fungal growth was noted. The colonies was counted using a colony counter[A] and identified according to genus.

V. Sheep Digestion and Nitrogen Metabolism Trials

Twelve sheep averaging about 20 kg body weight were fed general supply (herd) haylage for a week before silos were open then after silos were open, two sheep were assigned to one of the six experimental haylages. Weighed amounts of these haylages were fed to sheep and refusals were also weighed daily for a 7-day preliminary period. Dry matter content of each haylage was estimated from a composite sample taken during the preliminary period and maximum dry matter intake for each sheep was thus obtained. On day 8 the amount (90% of the maximum) to be fed during the next 10 days as removed from the silo and an amount weighed out into plastic bags to be fed daily to daily each sheep and stored frozen or refrigerated. Also, haylage samples were taken during this weighing operation and handled in a manner similar to that described in the section of Feed Analyses. Sheep were transferred from calf pens into digestion crates on day 8 but no collection of feces and urine were made

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[A]Fisher Scientific Co., New York, N.Y.

during the next three days. During the last 7 days, total feces were collected daily and temporarily stored at 4 C. Urine was collected in a plastic bottle containing 20 ml of 50% H_2SO_4 (v/v), and the daily volum measured and recorded. Ten percent (by volume) of the daily urine volume was saved in a plastic bottle and stored at 4 C. This composite urine sample was thoroughly mixed and a small portion (about 20 ml) used for total nitrogen analysis. At the end of trial, feces collected from 7 days were weighed, thoroughly mixed and a small portion was used for dry matter determination (oven drying). Also, a aliquot of about 500 g were air dried ($<$ 40 C). These air dried feces samples were then ground through a 1 mm screen in a Willey mill and used for analyses of chemical and fibrous constituents.

Sheep were weighed on day 7 and day 11. The entire 17-day feeding trial was repeated two times with random reallocation of sheep to haylages. A 5-day adjustment period was allowed between trials.

VI. Statistical Analysis

All data obtained from the milk production trial were analyzed on a Central Data Corporation 6500 computer at the Michigan State University computer center. Analysis of variance and analysis of covariance programs

were used and where statistical significances were noted, differences between treatment means were tested by Duncan's multiple range tests (Steel and Torrie, 1960).

Two-way analysis of variance was used to analyze the data from sheep digestion trials. When statistical significances were noted differences between means were tested by Duncan's multiple range tests (Steel and Torrie, 1960).

RESULTS AND DISCUSSIONS

Part 1. Forage Nutritive Value Evaluation by Several Laboratory Methods

I. Relationships Among In Vivo Responses

Simple correlation coefficients among five in vivo parameters are in Table 12. Nitrogen digestion (ND) coefficients were correlated with dry matter digestion (DMD) coefficients (r = .86) and nitrogen (N) balance (r = .6) but were not well correlated with N retention calculated as a percent of absorbed N and maximum dry matter (DM) intake (r = .12 and .33) respectively. N balance was significantly correlated with DMD (r = .83) and also correrelated with maximum DM intake (r = .75) and N retention as a percent of absorbed N (r = .72). A significant but relatively low correlation coefficient (r = .65) was noted between DMD (ranging from 43 to 70%) and maximum DM intake (ranging from 1.91 to 4.64 kg/100 kg body weight).

The close relationship between DMD and ND can be expected since in normal forages, proteins are primarily associated with materials of cell contents which are also highly digestible dry matter (Van Soest, 1969). The high correlation between DMD and nitrogen balance supported

Table 12. Simple Correlation Coefficient (r) Among Five *In Vivo* Parameters Obtained From Sheep Fed Haylages.[1]

	Nitrogen digestion coefficients (%)	DMD	N-balance	Nitrogen retained as % absorbed
Dry matter digestion coefficients (DMD)%	0.86[a][2]			
N[3]-balance (g N/day)	0.60[c]	0.83[a]		
N retained as % of absorbed N (%)	0.12	0.42[e]	0.72[a]	
Maximum dry matter intake (% body weight)	0.33	0.65[b]	0.75[a]	0.74[a]

[1] n = 24 forages, each forage has been fed to three or four sheep.

[2] a = $p < 0.005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$

[3] N = nitrogen.

the concept of a significant interaction between rumen nitrogen and carbohydrates (Waldo, 1968). There are several factors which could limit the degree of correlation between ND and N balance. These factors are: protein solubility, greater experimental error involved in N balance determinations, physiological state of animal and feeding level (Chalmers, 1961).

The insignificant correlation between ND and N retention as a percent of absorbed N might be anticipated since these two determinations represented two different stages of overall N utilization by the animal, one in the gastrointestinal tract and the other within the body proper.

The significant positive correlation between DMD and maximum dry matter intake observed in this study statistically confirmed the concept that dry matter digestibility is one of the important factors which control the voluntary intake of roughage by ruminants (Van Soest, 1969), but contrary to the concept that digestibility decreases as intake of a given roughage increases (Campling, 1970).

II. True Digestion Coefficients of Forage Total Nitrogen and Other Nitrogen Fractions Estimations by Statistical Means

When digestible nitrogen contents (g total

nitrogen/100 g forage DM x apparent nitrogen digestibility) were regressed on amount of total nitrogen or other nitrogen fractions (g N/100 g forage DM), the resulting regression coefficients b_1 (slope) can be interpreted biologically as an estimate of true digestibility of the nitrogen or nitrogen fraction while b_0 (intercept) can be interpreted as the metabolic fecal nitrogen or grams of nitrogen excreted per 100 grams of feed containing no nitrogen. Generally, studies of the regressions of digestible nitrogen content of feed on total nitrogen content have resulted in R^2 (i.e. proportion of the sum of squares attributable to regression) as high as 0.99, a b_0 value of about - 0.5 to - 0.6, and b_1 values greater than 0.9 (Holter and Reid, 1959). However, Goering et al. (1972) have reported very low values of b_1 (0.79) and R^2 (0.71) when regression analyses were performed on heat damaged forages. Using the same model, true digestion coefficients of total nitrogen and metabolic fecal nitrogen for the samples in the present study are Table 13. The value of b_0 (-0.83) and b_1 (0.91) would suggest that these forage samples were probably normal but the extremely low R^2 (0.35) indicated, on the other hand, that the forages were somewhat abnormal.

Other regressions were calculated (Table 13) by

Table 13. Simple and Multiple Regressions of Digestible Nitrogen (Y) of Forage Nitrogen Fractions for 21 Michigan State University Forages.

Equation	X_1[a]	X_2	Regression Coefficients						
			b_o[b]	b_1[c]	P.C.C.[d]	b_2	P.C.C.	R^2[e]	SEE[f]
1	Total nitrogen(N)		-.83	.91				.35	.35
2	Acid-detergent sol.[g] N		-1.25	1.18				.76	.21
3	Acid-detergent sol. N	Acid-detergent insol.[h] N	-.001	.91	.89	-1.26	-.78	.91	.14
4	Pepsin sol. N		-.50	1.04				.89	.15
5	Pepsin sol. N	Pepsin insol. N	-.47	1.13	.89	-.02	-.02	.89	.15
6	Rumen[i]+pepsin sol. N		-.82	1.19				.88	.15
7	Rumen +pepsin sol. N	Rumen +pepsin insol. N	-.50	1.12	.90	-.18	-.24	.88	.15
8	Rumen +pepsin +pan.[j] sol. N		-.55	1.15				.83	.18
9	Rumen +pepsin +pan. sol. N	Rumen +pepsin +pan.insol.N	-.38	1.11	.87	-.09	-.11	.84	.18
10	Pepsin +pan. sol. N		-.14	.97				.54	.29
11	Pepsin +pan. sol. N	Pepsin +pan. insol. N	-.91	1.16	.74	.38	.29	.58	.29
12	Hot water sol. N		1.30	.39				.14	.40
13	Hot water sol. N	Hot water insol. N	-1.90	.97	.68	1.41	.73	.53	.31

[a] All values based on g/100 g dry forage.

[b] b_o= intercept (metabolic fecal nitrogen g N/100 g dry froage).

[c] b_1= slope (true digestion coefficient).

[d] P.C.C.= partial correlation coefficient.

[e] R^2= proportion of the sum of squares attributable to regression.

[f] SEE= standard error of estimate.

[g] sol.= soluble.

[h] insol.= insoluble.

[i] Rumen= Rumen microbial.

[j] pan.= pancreatin.

separating soluble and insoluble nitrogen fractions in an attempt to identify those fractions responsible for the relatively poor fit of the simple regression. Equations 2,4,6,8 and 10 imply that digestible nitrogen (Y) originates entirely within the acid detergent (AD) soluble N, pepsin soluble N, rumen microbial + pepsin soluble N, rumen microbial + pepsin + pancreatin soluble N, pepsin + pancreatin soluble N or hot water insoluble N fractions. Equations 3,5,7,9 and 11 imply that digestible nitrogen originates in the soluble fraction described above as well as in the insoluble fraction.

All equations that included a soluble nitrogen fraction gave b_1 values either above 1.0 or extremely close to 1.0. These high b_1 values (true digestion coefficients) were somewhat unrealistic based on biological basis since the maximum value of digestibility is 1 or 100% but they certainly indicated that these nitrogen fractions were not only soluble but also highly digestible. In this study the estimated true N digestibilities (b_1) of AD soluble N and pepsin soluble N fractions were all greater than 100%. This is contrary to the report of Goering *et al.* (1972). They did not find increases in b_1 values for AD soluble N and pepsin soluble N fractions greater than 1 when they changed from total N analyses to the AD and pepsin soluble N fractions.

With the exceptions of hot water and pepsin + pancreatin incubation values marked improvements of R^2 were noted when soluble N fractions were used in regressions as compared with the R^2 when total nitrogen was used (Equation 2,4,6, or 8 vs. 1). The highest R^2 value (0.89) was found for Equation 4 which is based on the pepsin soluble N fractions.

All of the b_2 values (true digestion coefficient estimates of insoluble N fractions) were negative except for the values for the pepsin + pancreatin and hot water solubility methods. The negative true digestion coefficient estimates suggested that the insoluble fractions separated by chemical or *in vitro* enzyme digestion methods were also indigestible *in vivo*. The positive coefficients for hot water and pepsin + pancreatin insoluble N fractions, on the other hand, indicated that these fractions were digestible *in vivo*. For the hot water insoluble N fraction, one should expect the positive estimates of *in vivo* digestion coefficient since the hot water insoluble N fraction consists of true protein N. The positive coefficient for pepsin + pancreatin insoluble N fraction was not anticipated, since based on theoretical consideration the action of these two enzymes should be similar to that for *in vivo* digestion. However, if the *in vitro*

incubation conditions were not optimal for enzyme digestion results could be unrelated to *in vivo* digestion.

The degree of indigestibility of insoluble N fractions varied extensively (Table 13). For example, the true digestibility of acid detergent insoluble N fraction was estimated to be -126% (Equation 3) while the value for pepsin insoluble N fraction was only -2% (Equation 5). The greater value of indigestibility from the AD insoluble N fractions suggested that this method is more sensitive to *in vivo* N digestibility than pepsin insoluble N fraction. A slight increase in insoluble N fraction would result in a large reduction of digestible protein content.

The R^2 value was markedly improved by utilizing the insoluble fractions as predictors in addition to the soluble portion for acid detergent solubility method (R^2 = 0.76 for Equation 2 vs. 0.91 for Equation 3) but improvement was not large for pepsin, pepsin + pancreatin, rumen microbial + pepsin + pancreatin, rumen microbial + pepsin and hot water solubility methods (Equation 5,7,9 and 11 vs. 4,6,8 and 10 respectively). The extent of additional improvements in R^2 due to inclusion of the insoluble N fraction are related to the partial correlation coefficients of these insoluble N

fractions. Among all insoluble N fractions only the acid detergent insoluble N and hot water insoluble N fractions possessed relatively high partial correlation coefficient values (-0.78 and 0.68 respectively).

Separation of total N into soluble and insoluble N fractions increased the R^2 value and reduced standard error of estimate (SEE) when digestible nitrogen content was predicted using both fractions. However, the value for true digestion coefficients (b_0) of these fractions are difficult or impossible to interpret. Similar statements were made by Goering et al. (1972).

Values of R^2 and SEE in Table 13 suggested that digestible nitrogen content would be estimated most precisely by Equation 3 based on acid detergent soluble and insoluble N fractions. From a practical predictive standpoint, Equation 4 based on pepsin soluble N alone would be prefered, but from an operational standpoint acid detergent solubility is a less troublesome determination than pepsin and other protein solubility methods.

III. Relationships of Various Nitrogen Fractions to Nutritive Value

(1) Total Nitrogen Digestion Coefficient

a. Nitrogen digestion coefficient and acid detergent nitrogen:

Two regression equations predicting nitrogen

digestibility from acid detergent nitrogen as percent of total N (AD insoluble N x 100/N) were calculated from two different sources of data. Equation 1 was computed based on the data of the present study (Table 14) while Equations 2 and 3 were derived from the combined data of the present study plus that of Goering (Maryland), Jorgensen (Wisconsin) and Pierson (Minnesota). An extremely high correlation coefficient (r) or adjusted r (adjusted for sample number) was found in all three cases. Similar regression coefficients (b_0 and b_1) were found for Equations 2 and 3 but both were different from the coefficients of Equation 1. Equation 1 had a greater value for b_0 (N digestion coefficient when the sample contains no AD insoluble N) than did Equation 2 and 3 (91 vs. 73 and 76). The slope (b_1) was much steeper for Equation 1 than for Equation 2 or 3 (2.2 vs. 1.1). Using equation number 1, developed from the present study, the nitrogen digestion coefficient would be 0 when the value for acid detergent insoluble N as percent of total N reached a value of 42, while the nitrogen digestion coeffieient should not be 0 until this percent reached about 72 with equations 2 and 3.

The discrepancies observed in regression analyses were probably due to the differences in the extent of heat damage (amount of AD insoluble N/N) among samples

Table 14. Linear Regression Analysis for *In Vivo* Nitrogen Coefficients ($\hat{Y}$) Using Acid Detergent Insoluble Nitrogen as A Percent of Total Nitrogen (X).

Equation no.	Source	n	r	Adj. - r[a]	Equation
1	MSU[b]	24	-.92	-.92	$\hat{Y}$ = 91.23[f] - 2.19[g] X
2	Maryland, USDA[c]	44	-.93	-.93	$\hat{Y}$ = 72.96 - 1.02 X
3	MSU + USDA + Others[d]	80	-.93	-.93	$\hat{Y}$ = 75.87 - 1.08 X
4	Normal Forages (AD-N/N[e] ≤ 9)	28	-.51	-.48	$\hat{Y}$ = 81.38 - 1.47 X
5	Abnormal Forages (AD-N/N > 9)	52	-.91	-.91	$\hat{Y}$ = 71.67 - 0.96 X

[a]Adjusted correlation coefficient. Adjusted for sample number (Steel and Torrie, 1960).

[b]Michigan State University.

[c]Values obtained from Goering *et al.* and later published. J. Dairy Sci. 55:1275. 1972.

[d]Wisconsin (N.A. Jorgnesen), Minnesota (D.C. Pierson).

[e]Acid detergent insoluble as a percent of total nitrogen.

[f]Intercept (b_o).

[g]Slope (b_1).

of different sources. Many research investigators supplied animal data and samples for analysis by Goering whereas the MSU data were from a more uniform experimental situation. In the present study, the range of AD-insoluble N/N was from 0.71 to 22.0% while the range in the data of Goering *et al.* was from 1.78 to 75%. Considering the regression equations and the amount of AD-insoluble N/N in the original samples, one is forced to conclude that Equation 2 or 3 should be used to estimate *in vivo* N digestion coefficients of forages which have been severly heat damaged (i.e. AD-insoluble N/N greater than 22%). These equations also indicate that the negative effect of AD- insoluble N on *in vivo* N digestion coefficient is variable and dependent on the absolute level of AD-insoluble N/N in the samples. Acid detergent insoluble N as a percent of total nitrogen reduced *in vivo* N digestibility to a greater extent in forages with only mild heat damage (b_1 = 2.19) than it did in forages severly heat damaged (b_1 = 1.08) where AD-insoluble N/N exceeded 22%.

Since Van Soest (1965) has indicated that normal (not damaged by heat) forages contain about 7% of AD-insoluble N/N, the combined samples were separated into "normal forage" (AD-insoluble N/N$\leq$9) and "abnormal forage" (AD-insoluble N/N>9). Regression equations

relating *in vivo* ND to AD-insoluble N/N were computed for these two sub samples and are in Table 14 as equation 4 and 5. The precision for predicting of *in vivo* ND from AD-insoluble N/N was appreciably lower for normal forages than for abnormal forages (adjusted r = -0.48 vs. -0.91). The b_0 also indicates that normal forages have greater ND than do abnormal forages (81 vs. 72%) when both of them contain no AD-insoluble N/N. Markedly different slopes (b_1) were also evident between the two equations with the normal forages having a greater slope than the abnormal forages (1.47 vs. 0.96). Statistical analysis revealed that the slopes of these eqations were significantly ($P < 0.05$) different. Results from this study clearly demonstrated that the value of AD-insoluble N/N in predicting forage *in vivo* ND will largely depend on the extent of heat damage that the forage has undergone during storage or treatment.

b. Nitrogen digestion coefficient and other nitrogen fractions:

Regressions of total nitrogen digestion coefficients on various nitrogen containing fractions excluding acid detergent insoluble N/N are in Table 15. The equation using acid detergent (AD) insoluble N as a percent of dry matter as the predictor resulted in the greatest r (-0.90) with the least standard error of the estimate

Table 15. Linear Regressions for Estimating Total Nitrogen Digestion Coefficients (Y) Using Various Laboratory Values.

Equation	X	n	Regression Coefficients b_o[A]	b_1[B]	r[C]	SEE[D]
1	Acid-detergent insol.[E] N[F], %DM[G]	24	90.74	-67.89	-.90[a][H]	4.54
2	Acid-detergent lignin, %DM	24	109.75	-4.92	-.90[a]	4.60
3	Pepsin insol. N X 100/N	23	88.23	-1.07	-.89[a]	4.71
4	Pepsin insol. N, %DM[J]	23	86.41	-30.63	-.86[a]	5.35
5	Rumen[I] + pepsin + pan. insol. Nx100/N	22	99.34	-1.20	-.86[a]	5.43
6	Acid-detergent fiber(ADF), %DM	24	112.89	-1.20	-.84[a]	5.79
7	Pepsin sol.[K] dry matter, %	23	6.82	1.29	.84[a]	5.66
8	Rumen + pepsin insol. N x 100/N	24	94.00	-1.26	-.84[a]	5.71
9	Rumen + pepsin insol. N, %DM	24	91.38	-36.02	-.79[a]	6.57
10	Rumen + pepsin +pan. insol. N,%DM	22	95.00	-32.82	-.79[a]	6.50
11	Rumen + pepsin + pan. sol. DM,%	22	0.50	1.15	.78[a]	6.65
12	Pepsin sol. nitrogen, %DM	23	12.35	20.78	.76[a]	6.78
13	Rumen + pepsin + pan. sol. N, %DM	22	11.67	23.18	.74[a]	7.09
14	Cell wall constituents (CWC), %DM	24	112.44	-.99	-.73[a]	7.23
15	Degree of browning (OD at 440nm)	24	88.16	-113.86	-.73[a]	7.25
16	Pepsin + pan. sol. dry matter, %	24	7.24	1.31	.70[a]	7.56
17	Rumen + pepsin sol. nitrogen, %DM	24	10.09	21.96	.70[a]	7.60
18	Rumen + pepsin sol. dry matter, %	24	-18.30	1.25	.62[b]	8.35
19	Acid-detergent sol. nitrogen, %DM	24	10.42	18.96	.57[c]	8.74
20	Pepsin + pan. sol. nitrogen, %DM	24	19.67	19.20	.56[c]	8.81
21	Pepsin + pan. insol. N x 100/N	24	84.53	-.73	-.54[d]	8.97
22	Hot water insol. nitrogen, %DM	24	39.97	12.41	.51[d]	9.15
23	Rumen NH_3 release, NH_3-N x 100/N	21	78.51	-3.23	-.49[e]	9.42
24	Pepsin + pan. insol. N, %DM	23	80.38	-8.03	-.45[e]	9.49

Table 15. (Continued)

Equation	X	n	Regression Coefficients b_o	b_1	r	SEE
25	Hot water sol. dry matter, %	24	32.31	0.95	$.40^f_f$	9.76
26	Hot water insol. N x 100/N	24	47.61	0.27	$.39^f_f$	9.82
27	Mineral buffer sol. N x 100/N	23	76.50	-.29	$-.31^f_f$	9.89
28	Hot water sol. N, %DM	24	71.32	-5.83	$-.31^f_f$	10.13
29	Total nitrogen	24	40.70	7.19	$.19^f_f$	10.44
30	ADL[L] x 100/ADF	24	75.51	-.51	$-.15^f$	10.53

[A] b_o = intercept.

[B] b_1 = slope.

[C] r = correlation coefficient.

[D] SEE = standard error of estimate.

[E] insol. = insoluble.

[F] N = nitrogen.

[G] DM = dry matter.

[H] a = $p < 0.0005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$

[I] Rumen = rumen microbial.

[J] pan. = pancreatin.

[K] sol. = soluble.

[L] Acid detergent lignin.

(4.5, Equation 1, Table 15) excluding AD-insoluble N/N from the comparison (Equation 1, Table 14). The extremely small difference in r and SEE values between regressions usingeither AD-insoluble N/N or AD-insoluble N/DM was not observed by Goering et al.(1972). They found the former much more precise in predicting nitrogen digestibility (ND) than the latter. Another high correlation was observed by using acid detergent lignin as the predictor (r = -0.90 Equation 2, Table 15). Again, this finding is contradictory to that of Goering et al. (1972) who observed a relatively low r value of -0.74 and concluded that AD lignin had no predictive value for nitrogen digestibility. According to Van Soest (1965), the acid detergent insoluble N fraction is primarily associated with AD lignin in heat damaged forages. Thus a significant negative relationship between AD lignin and ND should be expected when forages have been damaged by heat.

Other important nitrogen digestion coefficient predictors were pepsin insoluble nitrogen as a percent of total N (r = -.89, Equation 3), pepsin insoluble N as a percent of dry matter (r = -.86, Equation 4), rumen microbial + pepsin + pancreatin insoluble N as a percent of total N (r = -.86, Equation 5), acid detergent fiber (r = -.84, Equation 6), pepsin soluble dry matter

(r = .84, Equation 7), and rumen microbial + pepsin insoluble N as a percent of total N (r = -.84, Equation 8).

Moderate degrees of precision (r from -.79 to .51) in predicting N digestion coefficients were measurements obtained from rumen microbial + pepsin + pancreatin solubility, rumen microbial + pepsin solubility, pepsin + pancreatin solubility values, cell walls and degree of browning (Equation 9 to 22, Table 15). Hot water solubility, mineral buffer solubility values, rumen ammonia release and total nitrogen were correlated with N digestibility so poorly that no practical predictive value for these variables were obtained.

Saunders et al. (1973) obtained a good correlation (r = -.87) between alfalfa protein solubility in pepsin + pancreatin solutions and rat in vivo nitrogen digestion coefficients. Following the same procedures, a correlation coefficient of -.54 (Equation 21, Table 15) was found in this study. In this procedure pepsin was incubated with the sample for only three hours which might be insufficient for pepsin to digest any significant amount of forage protein. Further investigations and modifications using this procedure may be desirable.

Protein solubility in mineral buffer solutions

was highly advocated by Wohlt *et al.* (1973) as a standard procedure for evaluating protein quality although they did not present any evidence that this solubility test would be significantly correlated with *in vivo* nitrogen digestion coefficients. Results from this study do not give any credence to this hypothesis.

The simplest laboratory procedure used to predict *in vivo* N digestion coefficients was a measure of the degree of browning (an extraction-spectrophotometric procedure), but the precision of prediction by this variable was only moderately high ($r = -.73$). Apparently the degree of heat damage to forages can only be approximated by color development.

Hot water solubility measurements have been used as a measure of silage fermentation (Waldo, 1973) with increasing amounts of hot water soluble N indicating increased silage fermentation. However, hot water probably coagulates all protein regardless of its biological availability. In other words, measuring protein solubility in hot water is not a sensitive method to detect protein damage by heat. Results from the present experiment strongly supported this assumption (see Equations 22,25,26 and 28 in Table 15).

The most complicated procedures used in this study were rumen microbial + pepsin and rumen microbial + pepsin + pancreatin incubations. The predictiability

of nitrogen digestion coefficient by those measurements were high but not as great as those obtained from much simpler procedures (e.g. pepsin solubility or acid detergent solubility) (Equations 1,2,3,5,8,9,10 and 11 on Table 15). Nevertheless, one must be reminded that those *in vitro* rumen fermentations were designed mainly for predicting *in vivo* DMD not ND.

Nitrogen insolubility was believed to be positively related with degree of heat damage in forages (Goering *et al.* 1972) and because of this relationship the term N insolubility was used as the predictor for *in vivo* N digestibility in Equation 1, in Table 14 and Equations 3,5,8 and 21 in Table 15. However, if the regressions were calculated by using N solubility (100 - insolubility) as predictors, the b_0 (i.e. estimated *in vivo* N digestibility or solubility when forage protein solubility is 0) would have biological meaning. Ideally, if an *in vitro* protein digestion method had the same action on forage protein solubilization as the *in vivo* protein digestion process then the regression coefficient b_0 and b_1 would be 0 and 1 respectively. Conversely, if an *in vitro* digestion method had greater or less ability to solubilize forage protein than did the *in vivo* digestion process, the b_0 value would be a negative or a positive value respectively, and the greater the absolute

value, the greater the difference between *in vitro* and *in vivo* N digestion processes.

The b_0 values for the equations using N solubility as the predictor can be easily derived from the equations based on N insolubility and represent estimates of *in vivo* N digestion coefficients when total N was 100% insoluble. These new b_0 values are in Table 16.

Table 16. Regression Coefficients (b_0) From the Regression Equations Using N Solubility Determined by Several Solubility Methods at the Predictor.

Solubility Method	b_0
Acid detergent solutions	-127.77
Rumen microbial + pepsin solutions	-32
Rumen microbial + pepsin + pancreatin solutions	-20.66
Pepsin solutions	-18.77
Pepsin + pancreatin solutions	11.53

The b_0 values in Table 16 suggest that the extent of N solubilization by pepsin + pancreatin solutions was near but slightly smaller than *in vivo* N digestion, while the other four solutions, particularly acid detergent solution were able to solubilize forage N to a much greater extent than *in vivo* digestion. The reason for the large decrease in digestibility of AD insoluble N fraction could be due to the increase in fecal bacterial

nitrogen when forage soluble N fraction content decreases.

(2) Regressions for Nitrogen Balance

Regressions for nitrogen balance on various nitrogen containing fractions are in Table 17. Generally, nitrogen balance was not as predictable as the nitrogen digestion coefficient by these forage N fractions. Also, based on r and standard error of estimate values, the ranking for precision in predicting N balance and N digestion coefficients by these N fractions were appreciably different.

Nitrogen balance was predicted more precisely by soluble N fractions than insoluble N fractions while the reverse situation existed for predicting N digestibility. For example, the two best predictors for N balance were pepsin soluble N as a percent of DM (r = .85, Equation 1) and rumen microbial + pepsin + pancreatin soluble N as a percent of DM (r = .80, Equation 2). While the respective r values for the insoluble N fractions of these two solubility methods were -.67 and -.57 (Equations 13, and 18, in Table 17, respectively). The acid detergent insoluble N as % of total N was the best predictor for N digestibility (r = -.92, Table 14) but could predict N balance only with a moderate degree of precision (r = -.60). These results indicate that the proportion of N that is soluble is directly related to

Table 17. Linear Regressions for Nitrogen Balance Data (Y) on Various Laboratory Values.

Equation	X	n	Regression Coefficients b_o[A]	b_1[B]	r[C]	SEE[D]
1	Pepsin sol.[E] N[F], %DM[G]	23	-13.59	6.83	.85[a][H]	1.59
2	Rumen[I] + pepsin + pan.[J] sol. N, %DM	24	-15.66	7.41	.80[a]	1.84
3	Pepsin sol. dry matter, %	23	-12.14	0.35	.78[a]	1.90
4	Acid-detergent fiber(ADF), %DM	24	16.08	-.31	-.77[a]	1.91
5	Pepsin insol.[K] N x 100/N	23	9.44	-.27	-.76[a]	1.99
6	Rumen + pepsin sol. N, %DM	24	-12.65	6.61	.75[a]	1.96
7	Rumen + pepsin sol. DM, %	24	-23.83	0.42	.74[a]	2.01
8	Rumen + pepsin + pan. insol. N x100/N	24	12.00	-.30	-.72[a]	2.15
9	Hot water sol. dry matter, %	24	-12.47	0.48	.72[a]	2.05
10	Cell wall constituents, % DM	24	16.54	-.26	-.70[a]	2.12
11	Rumen + pepsin + pan. sol. DM, %	23	-13.01	0.29	.68[b]	2.29
12	Rumen + pepsin insol. N x 100/N	24	10.27	-.28	-.68[a]	2.19
13	Pepsin insol. N, %DM	23	8.54	-6.99	-.67[a]	2.26
14	Acid-detergent lignin (ADL), %DM	24	13.02	-1.02	-.67[a]	2.22
15	Rumen NH_3 release (NH_3-N x 100/N)	21	8.81	-1.26	-.65[b]	2.42
16	Acid-detergent sol. N, % DM	24	-13.25	5.96	.64[b]	2.28
17	Acid-detergent insol. N x 100/N	24	8.49	-.40	-.61[c]	2.38
18	Rumen + pepsin + pan. insol. N, %DM	23	9.93	-7.03	-.57[c]	2.54
19	Rumen + pepsin insol. N, %DM	24	8.98	-7.12	-.56[c]	2.47
20	Acid-detergent insol. N, % DM	24	8.17	-11.71	-.56[c]	2.47
21	Pepsin + pan. sol. DM, %	23	9.93	0.27	.51[e]	2.56
22	Degree of browning (OD at 440nm)	24	7.92	-20.59	-.47[e]	2.62

Table 17. (Continued)

Equation	X	n	Regression Coefficients b_o	b_1	r	SEE
23	Pepsin + pan. sol. N, % DM	23	-6.65	4.43	.46[e]	2.64
24	Total nitrogen, % DM	24	-11.43	4.65	.44[f]	2.66
25	Hot water sol. N, % DM	24	1.73	1.38	.26[f]	2.87

[A] b_o = intercept.

[B] b_1 = slope.

[C] r = correlation coefficient.

[D] SEE = standard error of estimate.

[E] sol. = soluble.

[F] N = nitrogen.

[G] DM = dry matter.

[H] a = $p < 0.0005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$.

[I] Rumen = rumen microbial.

[J] pan. = pancreatin.

[K] insol. = insoluble.

the quantity of N retained in the animal body but that ration of insoluble N to total nitrogen is not. Simple regression equations having an r value below .70 have no practical predictive value (Goering et al. 1972). Thus acid detergent lignin, rumen ammonia release as a percent of total nitrogen, degree of browning, hot water solubility measurements and total N etc. should not be used as predictors for estimating N balance (Table 17).

(3) Regressions for Nitrogen as A Percent of Absorbed Nitrogen

Some regressions for N retention as a percent of absorbed N on various laboratory measurements are in Table 18. None was sufficiently correlated to be useful for predictive purposes. The greatest relationship was between hot water soluble DM and N utilization (r = .62). Others will not be discussed or presented.

(4) Regressions for Estimating Dry Matter Digestion Coefficients

Regressions for dry matter digestion coefficients on 31 laboratory measurements are in Table 19. Several laboratory determinations were able to predict DMD with a great degree of precision. Important predictors were largely measures dry matter solubilities in various solutions and only a few were measurements of nitrogen solubilities. For example, the two highest r values

Table 18. Linear Regressions for Retained Nitrogen as % of Absorbed (Y) on Various Laboratory Values.

Equation	X	n	Regression Coefficients b_o[A]	b_1[B]	r[C]	SEE[D]
1	Hot water sol.[E] DM[F], %	24	-26.01	1.27	.62[b][G]	7.26
2	Rumen[H] + pepsin sol. DM,%	24	-45.06	0.93	.53[d]	7.79
3	Rumen NH_3 release (NH_3-N x 100/N)	21	28.47	-3.03	-.51[e]	8.44
4	Rumen + pepsin + pan.[I] sol. N, %DM	23	-15.66	13.57	.48[e]	8.35
5	Pepsin sol. N, %DM	23	-13.83	11.86	.48[e]	8.23
6	Pepsin sol. DM, %	23	-11.59	0.61	.44[e]	8.41
7	Cell wall constituents(CWC), %DM	24	40.39	-.49	-.42[e]	8.35
8	Acid-detergent fiber(ADF), %DM	24	37.34	-.52	-.42[e]	8.36
9	Hot water sol. N, %DM	24	7.32	6.78	.41[e]	8.41
10	Rumen + pepsin sol. N, %DM	24	-11.01	11.09	.41[f]	8.41
11	Hot water insol.[J] N x 100/N	24	30.37	-.24	-.39[f]	8.47
12	Total nitrogen, % DM	24	-22.34	11.96	.37[f]	8.56

[A] b_o = intercept.

[B] b_1 = slope.

[C] r = correlation coefficient.

[D] SEE = standard error of estimate.

[E] sol. = soluble.

[F] DM = dry matter.

[G] b = $p < 0.001$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$

[H] Rumen = rumen microbial.

[I] pan. = pancreatin.

[J] insol. = insoluble.

Table 19. Linear Regressions for Dry Matter Digestion Coefficients (Y) on Various Laboratory Values.

Equation	X	n	Regression Coefficients b_o[A]	b_1[B]	r[C]	SEE[D]
1	Acid-detergent fiber(ADF), % DM[E]	24	93.79	-.90	-.94[a][F]	2.42
2	Pepsin sol.[G] dry matter, %	23	12.60	.99	.94[a]	2.54
3	Rumen[H] + pepsin + pan.[I] sol. DM%	24	5.07	.93	.90[a]	3.29
4	Pepsin insol.[J] N x 100/N	23	73.34	-.73	-.88[a]	3.46
5	Cell wall constituents (CWC), %DM	24	95.82	-.79	-.88[a]	3.44
6	Pepsin sol. N, %DM	23	15.50	16.60	.88[a]	3.50
7	Rumen + pepsin + pan. insol. Nx100/N	22	81.53	-.85	-.87[a]	3.68
8	Acid-detergent lignin (ADL), %DM	24	86.45	-3.17	-.87[a]	3.57
9	Rumen + pepsin + pan. sol. N, %DM	22	14.22	18.69	.85[a]	3.87
10	Rumen + pepsin insol. N x100/N	24	77.00	-.84	-.84[a]	3.89
11	Acid-detergent insol. N x 100/N	24	73.20	-1.30	-.82[a]	4.09
12	Rumen + pepsin sol. N, %DM	24	15.16	17.06	.81[a]	4.17
13	Rumen + pepsin sol. DM, %	24	-14.63	1.09	.81[a]	4.22
14	Pepsin insol. N, %DM	23	71.39	-19.89	-.80[a]	4.29
15	Acid-detergent insol. N, %DM	24	72.24	-38.67	-.77[a]	4.56
16	Rumen + pepsin + pan. insol. N, %DM	22	77.15	-21.84	-.75[a]	4.90
17	Pepsin + pan. sol. % DM	22	17.02	.93	.74[a]	4.80
18	Rumen + pepsin insol. N, %DM	24	74.14	-22.53	-.73[a]	4.84
19	Hot water sol. % DM	24	19.16	1.14	.71[a]	5.00
20	Acid-detergent sol. N, %DM	24	15.09	14.85	.67[a]	5.32
21	Rumen NH_3 release NH_3-N x 100/N	21	69.54	-2.95	-.64[c]	5.83
22	Degree of browning (OD at 440nm)	24	70.28	-62.57	-.60[c]	5.70

Table 19. (Continued)

Equation	X	n	Regression Coefficients b_o	b_1	r	SEE
23	Pepsin + pan. sol. N, %DM	24	25.74	13.56	.59[c]	5.75
24	Pepsin + pan. insol. N x 100/N	24	68.05	-.39	-.43[e]	6.43
25	Nitrogen	24	27.20	9.24	.37[f]	6.63

[A] b_o = intercept.

[B] b_1 = slope.

[C] r = correlation coefficient.

[D] SEE = standard error of estimate.

[E] DM = dry matter.

[F] a = $p < 0.005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$.

[G] sol. = soluble.

[H] Rumen = rumen microbial.

[I] pan. = pancreatin.

[J] insol. = insoluble.

were observed for Equations 1 and 2 based on acid detergent insoluble dry matter (acid detergent fiber) and pepsin soluble dry matter respectively. The regression coefficient b_1 of Equation 2 based on pepsin soluble dry matter was 0.99, indicating the action of the pepsin solution in solubilizing forage dry matter was remarkably similar to that of *in vivo* dry matter digestion. However, the value of b_0 indicated that the *in vivo* digestion system was able to solubilize 12.6 percentage units more dry matter than did pepsin. This 12.6 percentage units dry matter could presumably consist of fibrous constituents that rumen microbiota could solubilize but that were not solubilized by the pepsin incubation.

Actually, this assumption was supported by the fact that action of *in vitro* rumen microbial + pepsin + pancreatin solutions (Equation 3) was even closer to actual *in vivo* dry matter digestion processes than that of the pepsin solution (Equation 2) alone since the b_0 value was smaller for Equation 3 (5.1) than for Equation 2(12.6).

Dry matter solubility in a rumen microbial + pepsin solution has been widely used as one of the most reliable predictors for *in vivo* dry matter digestion coefficients of normal forages (Tilley and Terry, 1963; Oh *et al*. 1962; Van Soest, 1973) but in the present

study the r value was only 0.81 (Equation 13). The regression coefficient b_1 was approximately 1.0 and would suggest a close similarity of action for this *in vitro* method with that of the *in vivo* process. The negative value of b_0 indicated that rumen microbial + pepsin solutions solubilized more dry matter than occurred in the *in vivo* digestion processes. This difference probably can be explained by the fact that some undigested microbial dry matter is associated with the indigestible forage dry matter (Van Soest, 1969).

When forage DM is partitioned into soluble and insoluble fractions by certain kinds of solvents, estimates of *in vivo* digestibility of each dry matter fraction can be obtained from regression coefficients. For example, the acid detergent method separates forage dry matter into acid-detergent soluble dry matter (cell contents and hemicellulose) and insoluble dry matter (cellulose and lignin or acid detergent fiber). The regression equation for *in vivo* DMD with AD insoluble DM (ADF) was calculated and given in Table 19 (Equation 1). The actual digestibility of ADF can be estimated from that prediction equation by extrapolating the level of ADF to 100. In other words, if forage dry matter was all ADF then the estimated *in vivo* DMD would also be *in vivo* ADF digestibility. Using values from the present study, the *in vivo* ADF digestibility was 3.79% (100 x

0.90 (b_1) - 93.79 (b_0), Equation 1, Table 19). Similarly, *in vivo* digestibility of the AD-soluble DM can be estimated by using the same equation and extrapolating the ADF level to 0% and the value thus obtained for this study was 93.79 (93.79 + (-0.90)(0%) Equation 1, Table 19). Following this method of calculation, *in vivo* digestibility estimates of various forage dry matter fractions were calculated and are presented in Table 20. Data obtained from other sources were also calculated and presented in Table 20. For the present study the estimated soluble forage dry matter fractions ranged from 133 (hot water soluble DM) to 86 (AD and 72% H_2SO_4 soluble DM). Van Soest (1963) reported an estimated *in vivo* digestion coefficient of 98% for cell contents of normal forages, the value of 96% obtained in the present study suggests that our samples are probably normal. On the other hand, *in vivo* cell content digestibility was estimated as only 73% by using combined samples. The reduced digestibility probably was due to heat damage since a certain portion of potentially digestible protein in the cell contents would be converted to indigestible material (e.g. acid detergent insoluble N). Van Soest (1973) has stated that the action of acid pepsin solution is probably very similar to that of neutral detergent solution, but in the present study

Table 20. *In Vivo* Digestion Coefficients of Various Forage Dry Matter Fractions Estimated by Statistical Means.

Solubility Method	Soluble fraction			Insoluble fraction			
	Constituents	MSU[a]	Combined[b]	Constituents	MSU[a]	Combined[b]	MSU[c]
		------- % -----			--------- % ---------		
Neutral detergent solution	Cell contents	96	73	Hemicellulose Cellulose Lignin	17	42	52
Acid detergent solution	Cell contents	94	83	Cellulose Lignin	4	20	48
Acid detergent solution + 72% H_2SO_4	Cell contents Hemicellulose Cellulose	86	75	Lignin	-3	-123	
Acid pepsin solution	Cell contents Hemicellulose	112		Cellulose Lignin Hemicellulose	13		
Pepsin + pancreatin solution	Cell contents Hemicellulose	110		Cellulose Lignin Hemicellulose	17		

Table 20. (Continued)

Solubility Method	Soluble fraction			Insoluble fraction		
	Constituents	MSU	Combined	Constituents	MSU	Combined MSU
		------- % ----			---------- % --------	
Rumen microbial + pepsin solution	Cell contents Hemicellulose Cellulose	94		Cellulose Lignin	-15	
Rumen microbial + pepsin+ pancreatin solution	Cell contents Hemicellulose Cellulose	98		Cellulose Lignin	5	
Hot water	NPN[d] Simple sugars Lipids	133		Proteins Cellulose Lignin Hemicellulose	19	

[a]MSU = Michigan State University, n = 22.

[b]Combined = MSU + Goering (Maryland) + Jorgensen (Wisconsin) + Pierson (Minnesota), n = 50.

[c]Mean *in vivo* digestion coefficients determined using sheep.

[d]NPN = non protein nitrogen.

digestibility of pepsin soluble DM was more than that of neutral detergent soluble DM (112 vs. 96%).

Similarity in estimated in vivo digestibility of soluble dry matter was observed among similar analytical methods. For example, digestibility for acid pepsin soluble dry matter was close to that for pepsin + pancreatin soluble dry matter, and the digestibilities of soluble dry matter were similar between two in vitro rumen microbial + enzyme digestion methods. Using data of the present study, estimated in vivo digestion coefficients for insoluble DM fractions ranged from -15 to 19%. Lignin digestibility is zero or nearly so (Van Soest, 1969) and the digestion coefficient of -3 estimated from the present study would support the concept that lignin is rather indigestible material. However, this negative coefficient could also be due to the effect of heat damage since lignin undergoes qualitative and quantitative changes when forage is heat damaged (Cymbaluk et al. 1973; Van Soest 1965). In fact, the estimated lignin digestibility was -123% using combined sources of samples which include a large number of heat damaged forages. The digestion coefficients estimated from the present study for cell walls and lignocellulose were 17 and 4 (Table 20). However, the actual determined digestibilities for cell walls (neutral detergent

insoluble DM) and lignocellulose (acid detergent insoluble DM) were about 50% which is much higher than the value estimated by statistical means (Table 20). This difference suggests that in the *in vivo* condition, the negative effect of lignocellulose on dry matter digestibility probably is not a linear type response.

Dry matter digestibility was more related to acid detergent insoluble N as a percent of total N (r = -.82, Equation 11, Table 19) than to the term acid detergent insoluble N as a percent of dry matter (r = -.77, Equation 15). This is opposite to that in the report of Goering *et al.* (1972) where acid detergent insoluble N as a percent of dry matter was superior to acid detergent insoluble N as a percent of total N (r = -.90 vs. -.70) for predicting energy digestibility. Furthermore, they found that the pepsin insoluble N fraction was less related to energy digestibility than was the acid detergent insoluble N fraction (r = -.79 vs. -.90) which is opposite to that of this study (r = -.88, Equation 4 vs. -.82, Equation 11, Table 19).

Results from the present study indicated that *in vivo* dry matter digestibility is primarily correlated with dry matter solubility determined either by chemical methods (acid-detergent solution), or *in vitro* enzyme digestion methods (acid pepsin solution), or *in vitro*

rumen microbial + enzyme methods (rumen microbial + pepsin + pancreatin). Nitrogen solubility determined by acid pepsin is also significantly correlated with *in vivo* dry matter digestibility. Considering the values of r and standard error of estimate the best predictor for *in vivo* dry matter digestibility was acid detergent insoluble dry matter (ADF).

(5) Regressions of Maximum Dry Matter Intakes

Linear regressions and correlations for maximum dry matter intakes with various laboratory measurements are in Table 21. From the total of 31 variables only 11 are shown in Table 21 and only rumen microbial + pepsin soluble dry matter showed a reasonably high correlation with maximum dry matter intake (r = .82, Equation 1). Presumably other factors not evaluated in this study (e.g. dry matter content, leaf-stem ratio, organic acids, pH etc.) were more closely related to voluntary intake of forage dry matter by ruminants than were the 31 items studied.

IV. Relations Among Nitrogen Containing Fractions

Correlatation coefficients among nitrogen containing fractions are in Table 22. In general, high correlations were observed among fractions obtained from similar analytical methods. For example, high correlation coefficients were noted between the following

Table 21. Linear Regressions and Correlation Coefficients of Maximum Dry Matter Intakes (% Body Weight Determined on Sheep) (Y) on Various Laboratory Values.

Equation	X	n	Regression Coefficients b_o[A]	b_1[B]	r[C]	SEE[D]
1	Rumen[E] + pepsin sol.[F] DM[G], %	24	-4.69	.13	.82[a][H]	0.46
2	Hot water sol. DM, %[I]	24	-1.08	.14	.77[a]	0.51
3	Rumen + pepsin + pan. sol. N[J], %DM	23	-.28	1.63	.69[b]	0.57
4	Pepsin sol. N, %DM	23	-.04	1.41	.66[b]	0.61
5	Rumen + pepsin + pan. insol.[K] Nx100/N	23	5.44	-.07	-.66[b]	0.59
6	Cell wall constituents(CWC), %DM	24	6.71	-.06	-.64[b]	0.62
7	Rumen + pepsin sol. N, %DM	24	-.13	1.49	.63[b]	0.62
8	Pepsin + pan. sol. % DM	23	-.25	.09	.62[c]	0.63
9	Rumen + pepsin insol. N x 100/N	24	5.16	-.07	-.61[c]	0.64
10	Acid-detergent lignin (ADL), %DM	24	5.82	-.25	-.60[c]	0.64
11	Acid-detergent fiber (ADF), %DM	24	6.18	-.06	-.60[c]	0.64

[A] b_o = intercept.

[B] b_1 = slope.

[C] r = correlation coefficient.

[D] SEE = standard error of estimate.

[E] Rumen = rumen microbial.

[F] sol. = soluble.

[G] DM = dry matter.

[H] a = $p < 0.0005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$.

[I] pan. = pancreatin.

[J] N = nitrogen.

[K] insol. = insoluble.

Table 22. Simple Correlations Among the 31 Measurements Studied.

Group no.	Variable Identification	Simple Correlation Coefficient															
1	Crude protein, %DM[a]	1.00															
2	Mineral buffer sol.[b] Nx100/N	0.39	1.00														
3	Rumen NH_3 release(NH_3-Nx100/N)	0.10	0.32	1.00													
4	Degree of browning(OD 440nm)	0.25	0.29	0.56	1.00												
5	Cell wall constituents, %DM	-.56	-.13	0.37	0.33	1.00											
6	Acid-detergent fiber, %DM	-.39	0.19	0.60	0.49	0.91	1.00										
7	Acid-detergent lignin, %DM	-.18	0.19	0.51	0.78	0.79	0.85	1.00									
8-1	Acid-detergent insol.[c] Nx100/N	-.22	0.08	0.41	0.74	0.74	0.77	0.86	1.00								
8-2	Acid-detergent insol. N, %DM	-.02	0.16	0.47	0.82	0.63	0.71	0.85	0.98	1.00							
8-3	Acid-detergent sol. N, %DM	0.90	0.28	-.13	-.14	-.77	-.66	-.54	-.63	-.46	1.00						
9-1	Pepsin sol. DM, %	0.38	-.07	-.59	-.45	-.90	-.94	-.78	-.82	-.75	0.68	1.00					
9-2	Pepsin insol. N, %DM	0.12	0.22	0.69	0.93	0.54	0.68	0.84	0.84	0.90	-.29	-.70	1.00				
9-3	Pepsin insol. N x100/N	-.07	0.15	0.67	0.88	0.65	0.76	0.88	0.88	0.90	-.47	-.78	0.98	1.00			
9-4	Pepsin sol. N[d] %DM	0.65	0.12	-.46	-.50	-.82	-.81	-.77	-.78	-.67	0.89	0.82	-.67	-.80	1.00		
10-1	Pepsin + pan. sol. DM,%	0.28	-.20	-.29	-.43	-.79	-.80	-.83	-.61	-.54	0.49	0.68	-.51	-.57	0.63	1.00	
10-2	Pepsin + pan. insol. Nx100/N	0.05	0.37	0.11	0.43	0.44	0.47	0.65	0.41	0.48	-.13	-.32	0.40	0.40	-.27	-.73	1.00
10-3	Pepsin + pan. insol. N, %DM	0.36	0.48	0.14	0.49	0.24	0.32	0.56	0.31	0.37	0.16	-.19	0.42	0.36	-.06	-.60	0.95
10-4	Pepsin + pan. sol. N, %DM	0.61	-.06	-.04	-.19	-.71	-.63	-.64	-.47	-.34	0.69	0.50	-.25	0.37	0.63	0.77	-.76
11-1	Hot water sol. DM, %	0.60	0.47	-.31	-.13	-.86	-.68	-.54	-.47	-.35	0.69	0.73	-.35	-.48	0.74	0.59	-.18
11-2	Hot water insol. N x100/N	-.49	-.84	-.31	-.34	0.09	-.20	-.25	-.22	-.32	-.29	0.08	-.24	-.14	-.21	0.27	0.45
11-3	Hot water insol. N, %DM	-.14	-.82	-.17	-.27	-.12	-.39	-.35	-.33	-.36	0.03	0.25	-.23	-.19	0.02	0.41	-.48
11-4	Hot water sol. N, %DM	0.62	0.83	0.18	0.34	-.19	0.11	0.19	0.15	0.27	0.43	0.00	0.24	0.11	0.31	-.18	0.41
12-1	Rumen[e]+ pepsin sol. %DM	0.30	0.04	-.55	-.53	-.82	-.80	-.82	-.69	-.65	0.55	0.77	-.65	-.72	0.74	0.73	-.50
12-2	Rumen + pepsin insol. Nx100/N	-.13	0.26	0.57	0.87	0.65	0.75	0.91	0.81	0.88	-.48	-.67	0.89	0.92	-.76	-.71	0.60
12-3	Rumen + pepsin insol. N, %DM	0.15	0.35	0.60	0.95	0.49	0.64	0.86	0.75	0.88	-.23	-.56	0.92	0.90	-.58	-.63	0.60
12-4	Rumen + pepsin sol. N, %DM	0.74	0.08	-.35	-.44	-.80	-.77	-.74	-.70	-.58	0.91	0.71	-.54	-.69	0.95	0.67	-.36
13-1	Rumen+pepsin+pan. sol. %DM	0.39	-.25	-.56	-.35	-.84	-.89	-.72	-.68	-.61	0.62	0.88	-.59	-.67	0.73	0.72	-.50
13-2	Rumen+pepsin+pan. insol.Nx100/N	-.09	0.24	0.64	0.80	0.68	0.77	0.88	0.79	0.79	-.44	-.74	0.90	0.90	-.75	-.72	0.58
13-3	Rumen+pepsin+pan. insol. N,%DM	0.22	0.35	0.68	0.87	0.49	0.64	0.81	0.72	0.78	-.16	-.61	0.91	0.88	-.54	-.62	0.57
13-4	Rumen+pepsin+pan. sol. N, %DM	0.68	0.06	-.42	-.41	-.85	-.82	-.76	-.71	-.59	0.87	0.79	-.59	-.72	0.95	0.69	-.39
		1	2	3	4	5	6	7	8-1	8-2	8-3	9-1	9-2	9-3	9-4	10-1	10-2

Table 22. (Continued)

Group no.	Variable Identification	Simple Correlation Coefficient													
10-3	Pepsin +pan. insol.N,%DM	1.00													
10-4	Pepsin +pan. sol. N, %DM	-.52	1.00												
11-1	Hot water sol. DM,%	0.02	0.53	1.00											
11-2	Hot water insol. Nx100/N	-.58	0.04	-.50	1.00										
11-3	Hot water insol. N,%DM	-.50	0.29	-.33	0.93	1.00									
11-4	Hot water sol. N,%DM	0.58	0.08	0.57	-.98	-.86	1.00								
12-1	Rumen + pepsin sol. %DM	-.38	0.60	0.77	-.06	0.05	0.11	1.00							
12-2	Rumen + pepsin insol.Nx100/N	0.53	-.57	-.45	-.23	-.30	0.17	-.74	1.00						
12-3	Rumen + pepsin insol. N,%DM	0.62	-.39	-.27	-.37	-.34	0.34	-.66	0.96	1.00					
12-4	Rumen +pepsin sol. %DM	-.12	0.77	0.69	-.16	0.11	0.29	0.70	-.77	-.56	1.00				
13-1	Rumen+pepsin+pan. sol. %DM	-.36	0.65	0.63	0.17	0.36	-.09	0.74	-.68	-.56	0.73	1.00			
13-2	Rumen+pepsin+pan. insol.Nx100/N	0.53	-.53	-.51	-.19	-.26	0.16	-.76	0.95	0.91	-.73	-.76	1.00		
13-3	Rumen+pepsin+pan. insol. N,%DM	0.61	-.33	-.30	-.35	-.32	0.36	-.66	0.91	0.95	-.50	-.64	0.95	1.00	
13-4	Rumen+pepsin+pan. sol. N,%DM	-.17	0.75	0.74	-.17	0.10	0.27	0.74	-.75	-.55	0.97	0.80	-.79	-.57	1.00
		10-3	10-4	11-1	11-2	11-3	11-4	12-1	12-2	12-3	12-4	13-1	13-2	13-3	13-4

[a]DM = dry matter.

[b]sol.= soluble.

[c]insol. = insoluble.

[d]pan. = pancreatin.

[e]Rumen = rumen microbial.

analytical fractions: Hot water insoluble or soluble N and mineral buffer soluble N (r = > -.82), rumen microbial + pepsin insoluble or soluble N and rumen microbial + pepsin + pancreatin insoluble or soluble N (r = .55 to .97). Nevertheless, there were some high correlation coefficients among fractions derived from completely different analytical methods. For example, a r value of .90 was found between pepsin insoluble N and acid detergent insoluble N both expressed as a percent of dry matter; a correlation coefficient of .93 was found between pepsin insoluble N and the degree of browning (brown color); and a correlation coefficient of .95 was found between rumen microbial + pepsin insoluble N as a percent of dry matter and the degree of browning (Table 22).

Goering et al. (1972) also observed high correlation coefficient (r = .92) between pepsin insoluble N as a percent of total N and acid detergent insoluble N as percent of total N but the regression coefficients (b_0 = 17.02, b_1 = 1.00) from their study were markedly different from that calculated from the present study (b_0 = .18, b_1 = 1.72). Their study indicated equal increases of both with additional increments of heat damage and that about 17% of the nitrogen was pepsin insoluble when acid detergent insoluble N content was 0.

They concluded that there was a major fraction of N insoluble with pepsin but soluble in acid detergent. Regression coefficients of the present study suggested that both analyses measured similar fractions (b_0 = .18) and that amount of pepsin insoluble N would increase 72% (b_1 = 1.72) more than would acid detergent insoluble N with every additional increment of heat damage.

The correlation coefficients observed between the following variables: AD lignin and AD insoluble N, r = .85; ADF and AD insoluble N, r = .71; and cell wall constituents and AD insoluble N, r = .63 confirmed the concept of Van Soest (1965) that the additional N in the acid detergent insoluble fraction of heat damaged forages is primarily associated with lignin but is less associated with acid detergent fiber or cell wall constituents.

V. Relations Among Various Dry Matter Solubility Measurements

Correlation coefficients among various dry matter solubility measurements are scattered throughout in Table 22. Generally, dry matter solubilities measured by different methods were rather well correlated with each other (r ranged from .94 to .59).

Dry matter solubility in pepsin was highly correlated with acid detergent soluble DM (r = .94) and neutral detergent soluble DM (r = .90) but the linear

regression coefficients indicated that the function of pepsin more resembled that of neutral detergent (b_0= 9.44, b_1 = 1.06) than that of acid detergent (b_0 = 12.50, b_1 = 1.04). Similar statements were made by Van Soest (1973).

The correlation between acid detergent soluble DM and rumen microbial + pepsin + pancreatin soluble DM was .89. The regression equation had b_0 and b_1 values of 4.99 and .97 respectively indicating that the functions of these two different analytical methods were similar. This may be related to the high negative correlation coefficient between acid detergent insoluble DM (ADF) and *in vivo* DM digestibility.

VI. Regressions of Five In Vivo Parameters on Thirteen Selected Laboratory Determinations Grouped from An Operational Standpoint

Although a total of 31 different laboratory analyses were performed on each forage sample, some analytical values were grouped together based on laboratory operations. For example, values for pepsin soluble dry matter, soluble N % DM, insoluble N %DM, and insoluble N x 100/N can be obtained from one analysis scheme on one sample. Regression coefficients for the five *in vivo* parameters using each of these 13 groups are in Table 23. Because the number of variables in

Table 23. Regression Coefficients of Five In Vivo Parameters Determined From Sheep on 13 Laboratory Determinations Which are Grouped From An Operational Standpoint.

Group	n		Regression Coefficients				
			N digest.[A]	DM digest.[B]	N balance[C]	N retained as % N absorbed	Max.[D] intake
1	21	R^2	0.03[f]	0.12[f]	0.21[f]	0.15[f]	0.12[f]
		Constant	41.75	26.81	-13.08	-27.63	0.26
		x-crude protein,%DM	1.15	1.15	0.83	2.16	0.16
2	21	R^2	0.10[f]	0.01[f]	0.02[f]	0.03[f]	0.03[f]
		Constant	78.10	60.55	1.81	9.41	2.94
		x-mineral[E] sol.[F] x100/N	-.33	-.09	0.04	0.15	0.01
3	21	R^2	0.24e[G]	0.41c	0.42[b]	0.26[e]	0.24[e]
		Constant	78.51	69.54	8.81	28.47	4.47
		x-rumen NH_3 release (NH_3 -N x100/N)	-3.23	-2.95	-1.26	-3.03	-0.24
4	21	R^2	0.57[a]	0.36[c]	0.23[e]	0.01[f]	0.16[f]
		Constant	88.52	70.16	7.86	18.19	4.40
		x-degree of browning (OD at 440nm)	-111.30	-61.68	-20.62	-12.05	-4.48
5	21	R^2	0.59[a]	0.80[a]	0.54[e]	0.20e	0.51[a]
		Constant	114.13	97.25	17.26	41.66	6.87
		x-CWC[H], %DM	-1.00	-.82	-.28	-.53	-.07
6	21	R^2	0.73[a]	0.89[a]	0.61[a]	0.20[e]	0.46[b]
		Constant	112.02	93.63	16.19	37.88	6.25
		x-ADF[I], %DM	-1.16	-.90	-.31	-.55	-.07
7	21	R^2	0.88aK	0.76[a]	0.47[b]	0.07[f]	0.44[b]
		Constant	110.28	86.72	13.12	27.12	5.85
		x-ADL[J], %DM	-4.87	-3.18	-1.04	-1.23	-.26

Table 23. (Continued)

Group	n		Regression Coefficients: Digestible Nitrogen, Before deletion	Digestible Nitrogen, After deletion	DM Digestibility, Before deletion	DM Digestibility, After deletion	Nitrogen Balance, Before deletion	Nitrogen Balance, After deletion	Nitrogen retained as % absorbed, Before deletion	Nitrogen retained as % absorbed, After deletion	Maximum Intake, Before deletion	Maximum Intake, After deletion
8	21	R^2	0.86[a]	0.86[a]	0.72[a]	0.72[a]	0.66[a]		0.25[f]		0.29[f]	0.24[f]
		Constant	84.66	91.26	44.16	42.08	-30.99		-89.57		-0.33	-0.08
		8-1 AD insol. Nx100/N	-1.65(-.19)	-2.15	-0.20(-.02)	(I)	2.16(0.47)		6.11(0.32)	(II)	0.13(.09)	(I)
		8-2 AD insol. N, %DM	-13.10(-.05)	(I)	-24.52(-.1)	-29.95	-66.70(-.51)		-161.63(-.30)	(I)	-4.78(-.12)	(II)
		8-3 AD sol. N, %DM	2.10(0.06)	(II)	8.84(0.28)	9.47	12.94(0.64)		33.02(0.45)	(III)	1.43(0.26)	1.26
9	21	R^2	0.87[a]	0.86[a]	0.95[a]	0.94[e]	0.78[a]	0.75[a]	0.29[f]		0.47[e]	0.45[b]
		Constant	126.67	56.09	39.36	33.13	-28.21	-13.86	67.05		1.64	0.001
		9-1 Pep. sol. %DM	0.56(0.47)	0.55	0.61(0.80)	0.69	0.09(0.22)	(III)	0.33(0.15)	(II)	0.02(0.10)	(II)
		9-2 Pep. insol. N, %DM	85.10(-.28)	(I)	13.15(0.10)	(II)	-21.13(-.18)	(II)	127.01(0.20)	(III)	1.11(0.03)	(I)
		9-3 Pep. insol.Nx100/N	-4.26(-.33)	-0.73	-0.81(-.15)	-.31	0.82(0.17)	(I)	-4.97(-.19)	(IV)	-.06(-.03)	(III)
		9-4 Pep. sol. N,%DM	-21.12(-.28)	(II)	-.48(-.01)	(I)	9.72(0.33)	6.96	-18.49(-.12)	(I)	0.64(.06)	1.38
10	21	R^2	0.57[d]	0.56[a]	0.59[c]	0.55[a]	0.38[f]	0.25[e]	0.27[f]		0.47[e]	0.42[b]
		Constant	-37.70	7.90	-23.71	17.35	-15.51	-7.83	92.10		-1.10	-.27
		10-1 Pep. + pan. sol., %DM	1.18(0.52)	1.32	1.30(0.59)	0.92	0.29(0.39)	0.26	0.72(0.30)	(IV)	0.10(0.54)	0.09
		10-2 Pep. +pan. insol.N/N	1.80(0.13)	(II)	0.97(0.10)	(III)	-.14(-.03)	(II)	-4.69(-.28)	(II)	-.04(-.03)	(II)
		10-3 Pep. +pan. insol.N,%DM	-43.24(-.14)	(III)	-15.83(-.07)	(I)	8.11(0.07)	(III)	122.63(0.32)	(III)	1.61(0.06)	(III)
		10-4 Pep. +pan. sol. N,%DM	17.42(0.13)	(I)	10.81(0.12)	(II)	1.35(0.03)	(I)	-37.40(-.23)	(I)	-.18(-.02)	(I)
11	21	R^2	0.68[b]	0.65[a]	0.79[a]	0.77[a]	0.62[c]	0.58[a]	0.44[e]	0.39[c]	0.67[b]	0.63[a]
		Constant	-3.35	-19.44	36.58	10.62	15.69	-13.96	71.33	-28.66	7.45	-1.19
		11-1 Hot water sol. %DM	1.83(0.73)	1.54	1.62(0.86)	1.69	0.56(0.72)	0.53	1.27(0.53)	1.35	0.17(0.79)	0.14
		11-2 Hot water insol. N/N	-.01(-.002)	(I)	-.50(-.15)	(I)	-.49(-.26)	(II)	-1.41(-.2)	(III)	-.12(-.26)	(II)
		11-3 Hot water insol.N,%DM	9.45(0.14)	16.99	8.63(0.23)	(II)	6.17(0.28)	(III)	12.59(0.16)	(I)	0.75(.15)	(I)
		11-4 Hot water sol. N,%DM	-8.19(-.07)	(II)	-15.2(-.21)	-7.33	-10.12(-.25)	(I)	-28.01(-.19)	(II)	-2.96(-.30)	(III)
12	21	R^2	0.82[a]	0.82[a]	0.84[a]	0.81[a]	0.69[b]	0.62[a]	0.42[e]	0.27[e]	0.73[a]	0.68[a]
		Constant	49.13	52.03	11.79	-13.75	-28.24	-14.33	45.57	-45.08	0.58	-4.47
		12-1 Rumen+pep. sol. %DM	0.15(.15)	(II)	0.53(0.51)	0.70	0.22(0.39)	(III)	1.23(0.51)	0.93	0.11(.67)	0.12
		12-2 Rumen +pep. insol.N/N	-.23(-.03)	(I)	-.26(-.05)	(II)	0.25(0.08)	(II)	-5.89(-.43)	(II)	-.25(-.32)	(II)
		12-3 Rumen +pep. insol.N,%DM	-19.19(-.1)	-25.87	-1.03(-.01)	(I)	-5.77(-.08)	(I)	156.29(0.47)	(III)	6.34(0.34)	(III)
		12-4 Rumen +pep. sol. N,%DM	10.60(0.18)	13.39	7.45(.19)	10.32	6.49(0.27)	7.31	-35.95(-.37)	(I)	-1.36(-.24)	(I)
13	21	R^2	0.77[a]	0.76[a]	0.90[a]	0.90[a]	0.67[a]	0.65[a]	0.37[f]		0.55[d]	0.44[d]
		Constant	52.49	46.33	10.94	21.64	-15.71	-13.57	226.56		12.49	5.46
		13-1 Rumen+pep.+pan. sol.%DM	0.44(0.32)	0.68	0.49(0.64)	.50	-.01(-.01)	(I)	0.06(0.03)	(I)	-.01(-.1)	(I)
		13-2 Rumen+pep.+pan.insol.N/N	-.38(-.03)	(I)	0.40(0.06)	(I)	0.22(0.05)	(II)	-9.31(-.41)	(IV)	-.41(-.27)	-.07
		13-3 Rumen+pep.+pan.insol.N,%DM	-11.82(-.04)	-21.43	-17.23(-.12)	-8.35	-6.96(-.06)	(III)	210.81(.41)	(III)	8.31(0.25)	(III)
		13-4 Rumen+pep.+pan. sol.N,%DM	3.91(0.03)	(II)	9.88(0.18)	6.35	8.53(0.2)	7.45	-61.41(-.33)	(II)	-1.81(-.14)	(II)

Table 23. (Continued)

[A]Nitrogen digestion coefficients, %.

[B]Dry matter digestion coefficients, %.

[C]Nitrogen balance (g N/day).

[D]Maximum dry matter intake (% body weight).

[E]Mineral buffer.

[F]sol. = soluble.

[G]significance level: a = $p < 0.0005$; b = $p < 0.001$; c = $p < 0.005$; d = $p < 0.01$; e = $p < 0.05$; f = $p > 0.05$.

[H]CWC = cell wall constituents.

[I]ADF = acid detergent fiber.

[J]ADL = acid detergent lignin.

[K]values underlined are the highest R^2 values among 13 determinations.

[L]AD = acid detergent.

[M]insol. = insoluble.

[N]values in parentheses are partial correlation coefficients.

[O]Pep. = Pepsin.

[P]Roman numberal represents the sequence of variables deleted from the regression equation when their significance was greater than 0.05.

[Q]Pan. = pancreatin.

[R]Rumen = rumen microbial.

each of these 13 groups ranged from one to four, both simple linear and multiple regression equations were obtained. In addition, least square stepwise deletion multiple regression was computed so that variables not significantly ($P < 0.05$) regressed with *in vivo* parameter were removed from the final equation. The greatest correlation for N digestibility was obtained with lignin as a percent of dry matter (Group 7). Slightly lower R^2 values were noted for Group 9 - the pepsin solubility measurements ($R^2 = .87$) and Group 8 - the acid detergent solubility measurements ($R^2 = .86$). Protein content (Group 1), protein solubility in mineral buffer (Group 2) and *in vitro* rumen ammonia release had no value for predicting the *in vivo* N digestibility of a forage (Table 23).

The greatest R^2 values for DM digestion coefficient and N balance were for the pepsin solubility measurements ($R^2 = .95$ and .78 respectively). None of the 13 groups was able to produce a high R^2 for the regression of N retention as a percent of absorbed although the highest R^2 (.49) was found for rumen microbial + pepsin solubility measurements (Group 12). This group also gave the highest R^2 for the regression of maximum intake ($R^2 = .73$).

Among these 13 groups of determinations only three

(rumen microbial + pepsin solubility measurements, acid detergent fiber and hot water solubility measurements- # 12,6 and 11) had reasonable correlations with all five *in vivo* responses. Four groups (pepsin solubility, rumen microbial + pepsin + pancreatin solubility, acid detergent solubility measurements and acid detergent lignin) were highly correlated with the three more predictable *in vivo* parameters of N digestibility, DM digestibility and N balance.

Partial correlation coefficients for variables in each group containing more than two variables are also given in Table 23. A variable could have a high partial correlation coefficient with one *in vivo* response but have a rather low coefficient for another *in vivo* response. For example, among four pepsin solubility measurements (Group 9) pepsin soluble dry matter possessed the greatest partial correlation coefficient for *in vivo* N and DM digestion coefficients but not for N balance. Pepsin soluble N% DM was more related to N balance than was pepsin soluble DM.

Least square stepwise deletion programs were used to delete out insignificant ($P < .05$) variables in those groups having more than two variables. Generally, insoluble N as a percent of forage total N was relatively unimportant in predicting *in vivo* parameters as compared

with other solubility measurements (Groups 10,11,12 and 13) except for AD/N and pepsin soluble N/N for DN. In most groups, two out of four variables were deleted and the sequences of deletion were negatively related to the partial correlation coefficients. For example, when DM digestibility was regressed on the four pepsin solubility values (Group 9), pepsin soluble N, %DM was the first variable deleted and it also had the lowest partial correlation coefficient. Pepsin insoluble N, %DM was the second variable deleted (Table 23). The new equation included only two variables and possessed essentially the same R^2 as the equation using four variables (.95) and this was also true for many other groups (such as acid detergent solubility Group 8, and rumen microbial + pepsin solubility measurements - Group 12).

Thus in vivo responses can be predicted by using fewer variables with about the same degree of precision with these equations from the least square deletion program as when using all variables.

In practical situations the selection of laboratory measurements to predict in vivo forage nutritive value will be based not only on precision of prediction but also on other factors such as time, specific equipment and labor required. When all these factors are

taken into consideration measurements for acid detergent solubility (ADF, Group 6) and pepsin solubility (Group 9) become the most desirable items to predict N and DM digestibility and N balance of forages.

VII. Multiple Regression of Five In Vivo Parameters With Measurements of Two Laboratory Determinations

A second sequential process to select the most appropriate multiple regression equations is to randomly use combined measurements from any two of the 13 groups of laboratory measurements and then to use a least square deletion program. In this process a total of 78 regression equations were generated but only important regression equations will be presented and discussed. Table 24 gives three equations which gave the highest R^2 values for predicting in vivo N digestibility. Equation 1 and 2 used variables of pepsin solubility (Group 9) plus either hot water solubility (Group 11) or rumen microbial + pepsin solubility (Group 12) measurements and gave remarkably high R^2 values of .96 and .95. These multiple regressions improved R^2 by only about .08 units when comparing these R^2 values to the highest R^2 (.88) obtained from regressions using only one group of measurements (AD-lignin, Table 23). Regressions in Table 24 are certainly much more complicated using many more variables and laboratory analyses.

Table 24. Three Multiple Regressions Calculated by Using 2 Groups of Laboratory Measurements That Gave High R^2 Values for *In Vivo* Nitrogen Digestion Coefficients (n = 21).

Equation no.	Group[a] no.		Regression coefficient		Regression[b] coefficient	
1		R^2 and SEE[c]	0.96	2.74	0.94[d]	2.75[e]
		Constant		56.42		69.79
	9-1	Pepsin sol.[f] %DM[g]		0.10(0.14)[h]		-
	9-2	Pepsin insol. N,[i] %DM[j]		67.39(0.36)		62.06(0.76)
	9-3	Pepsin insol. Nx100/N		-3.34(-.42)		-3.02(-.87)
	9-4	Pepsin sol. N, %DM		-8.56(-.17)		-
	11-1	Hot water sol. %DM		0.26(0.19)		-
	11-2	Hot water insol. Nx100/N		0.52(0.23)		0.29(0.82)
	11-4	Hot water sol. N,%DM		6.85(0.10)		-
2		R^2 and SEE	0.95	2.80	0.95	2.84
		Constant		126.11		80.32
	9-1	Pepsin sol. DM%		1.07(0.81)		1.08(0.79)
	9-2	Pepsin insol. N,%DM		258.47(0.63)		186.30(0.60)
	9-3	Pepsin insol. Nx100/N		-8.17(-.62)		-5.75(-.59)
	12-1	Rumen[k]+ pepsin sol. %DM		-.53(-.53)		-.57(-.55)
	12-2	Rumen + pepsin insol.Nx100/N		4.15(0.56)		4.09(0.53)
	12-3	Rumen + pepsin insol. N,%DM		-184.57(-.67)		-169.51(-.62)
	12-4	Rumen + pepsin sol. N, %DM		-14.55(-.32)		-
3		R^2 and SEE	0.93	3.26	0.91	3.34
		Constant		-3.14		16.62
	4	Degree of browning(OD 440nm)		-91.60(-.88)		-80.69(-.87)
	11-1	Hot water sol. %DM		0.90(0.72)		1.26(0.86)
	11-2	Hot water insol. Nx100/N		0.10(0.07)		-
	11-3	Hot water insol. N, %DM		15.10(0.45)		12.18(0.86)
	11-4	Hot water sol. N, %DM		10.20(0.17)		-

Table 24. (Continued)

[a]Group no.: Laboratory measurements were combined into 13 groups, detailed information is given in Table 23.

[b]Regression coefficients given in this colum are for the equation resulting from the least squares deletion program. Variables deleted out were not significantly ($p > 0.05$) regressed with *in vivo* N digestion coefficients.

[c]SEE = standard error of estimate.

[d]R^2 for the equation after deletion program.

[e]SEE for the equation after deletion program.

[f]sol. = soluble.

[g]DM = dry matter.

[h]Values in parentheses are partial correlation coefficients.

[i]insol. = insoluble.

[j]N = nitrogen.

[k]Rumen = rumen microbial.

Equation 3 (Table 24) is less complicated than equation 1 and 2 requiring only three laboratory measurements (degree of browning, hot water soluble DM and insoluble N, % DM), but this prediction equation still has to be considered the second choice when compared with the equation using only one laboratory measurement - acid detergent lignin (Group 7, Table 23). The partial correlation coefficients shown in Table 24 indicated the relative importance of variables in that equation.

Three multiple regression equations with high R^2 for *in vivo* dry matter digestibility are presented in Table 25. Although all three equations gave extremely high R^2 (.97), they represented only slight improvements as compared to the R^2 of .94 obtained by using only two of the pepsin solubility measurements (Group 9, Table 23). Pepsin solubility measurements are apparently important for predicting *in vivo* dry matter digestibility since all three equations in Table 25 included this set of measurements. Furthermore, a combination of the variables pepsin solubility and hot water solubility gave the highest R^2 for *in vivo* DM digestibility (Equation 1, Table 25) and also for *in vivo* N digestibility (Equation 1, Table 24). Therefore, when predicting both these *in vivo* responses pepsin and hot water measurements should be those of choice.

Table 25. Three Multiple Regressions Calculated by Using 2 Groups of Laboratory Measurements That Gave High R^2 Values for *In Vivo* Dry Matter Digestion Coefficients (n = 21).

Equation no.	Group[a] no.		Regression Coefficient		Regression[b] Coefficient	
1		R^2 and SEE[c]	0.96	1.70	0.96[d]	1.73[e]
		Constant		47.15		48.23
	9-1	Pepsin sol.[f] %DM[g]		0.30(0.42)[h]		-
	9-2	Pepsin insol.[i] N;[j] %DM		5.95(0.06)		-
	9-3	Pepsin insol. Nx100/N		-.36(-.08)		-
	9-4	Pepsin sol. N, %DM		6.74(0.22)		13.43(0.90)
	11-1	Hot water sol. %DM		0.55(0.54)		0.88(0.84)
	11-2	Hot water insol. Nx100/N		-.31(-.23)		-.47(-.53)
	11-4	Hot water sol. N,%DM		-12.46(-.29)		-19.66(-.67)
2		R^2 and SEE	0.97	1.57	0.96	1.61
		Constant		24.80		32.03
	9-1	Pepsin sol. %DM		0.28(0.41)		-
	9-2	Pepsin insol. N,%DM		-37.06(-.17)		-16.85(-.80)
	9-3	Pepsin insol. Nx100/N		0.86(0.12)		-
	13-1	Rumen[k]+pepsin+pan.[l] sol. %DM		0.38(0.51)		0.58(0.85)
	13-2	Rumen +pepsin+pan.insol. Nx100/N		-.84(-.14)		-.51(-.50)
	13-3	Rumen +pepsin+pan. insol. N,%DM		28.12(0.16)		21.15(0.66)
	13-4	Rumen +pepsin+pan. sol. N,%DM		1.64(0.05)		-
3		R^2 and SEE	0.96	1.73	0.96	1.63
		Constant		28.18		31.60
	8-1	Acid-detergent insol.N/N		-.78(-.21)		-1.16(-.49)
	8-2	Acid-detergent insol. N,%DM		40.03(0.31)		50.88(0.60)

Table 25. (Continued)

Equation no.	Group no.		Regression Coefficient	Regression Coefficient
	8-3	Acid-detergent sol. N,%DM	1.32(0.04)	-
	9-1	Pepsin sol. %DM	0.68(0.84)	0.70(0.86)
	9-2	Pepsin insol. N,%DM	-14.34(-.09)	-16.10(-.79)
	9-3	Pepsin insol. Nx100/N	-.06(-.01)	-

a,b,c,d,e,f,g,h,i,j and k see Table 24.

[1]pan. = pancreatin.

Three multiple regressions having high R^2 values for *in vivo* N balance are given in Table 26. Equation 1 used variables of acid detergent solubility (Group 8) and pepsin solubility (Group 9) measurements and produced the highest R^2 (.88) among all equations. After a least squares deletion of insignificant ($P > .05$) variables the R^2 was reduced only by .06 units for Equation 1. No reduction in the R^2 value was observed for Equation 2 after the least square deletion program. However, Equation 3 (after deletion) is the simplest equation with respect to the laboratory analyses required (pepsin solubility measurements and rumen ammonia release). Since pepsin solubility measurements would also be required to predict *in vivo* dry matter and N digestibility (Equation 1 of Table 24 and 25), only one additional laboratory analysis would be required to predict N balance (*in vitro* rumen ammonia release).

Two multiple regressions having relatively high R^2 for *in vivo* nitrogen retention as a percent of absorbed nitrogen are given in Table 27. Maximum R^2 for single group measurements was .49 (Group 12, Table 23) and the use of two group gave an increased R^2 of .75 (Equation 1, Table 27). Even the improved R^2 is still unsatisfactory from a practical predictive standpoint and some of the analytical measurements required in the equation are

Table 26. Three Multiple Regressions Calculated by Using 2 Groups of Laboratory Measurements That Gave High R^2 Values for *In Vivo* Nitrogen Balance (n=21).

Equation no.	Group[a] no.			Regression Coefficient		Regression[b] Coefficient	
1		R^2 and SEE[c]	0.88	1.28		0.82[d]	1.44[e]
		Constant		-40.23		-18.92	
	8-1	Acid-detergent insol.[i] Nx100/N[j]		1.63(0.52)[h]		0.58(0.61)	
	8-2	Acid-detergent insol. N, %DM[g]		-27.93(-.29)		-	
	8-3	Acid-detergent sol.[f] N, %DM		11.15(0.48)		8.41(0.80)	
	9-1	Pepsin sol. %DM		0.20(0.53)		-	
	9-2	Pepsin insol. N, %DM		-18.70(-.16)		-11.86(-.78)	
	9-3	Pepsin insol. Nx100/N		0.33(0.09)		-	
2		R^2 and SEE	0.85	1.44		0.85	1.35
		Constant		-43.45		-41.50	
	8-1	Acid-detergent insol. Nx100/N		2.89(0.66)		2.84(0.72)	
	8-2	Acid-detergent insol. N,%DM		-84.98(-.66)		-84.01(-.74)	
	8-3	Acid-detergent sol. N, %DM		10.21(0.60)		10.00(0.74)	
	11-1	Hot water sol. %DM		0.46(0.70)		0.45(0.75)	
	11-2	Hot water insol. Nx100/N		0.01(0.01)		-	
	11-4	Hot water insol. N,%DM		0.02(.0004)		-	
3		R^2 and SEE	0.83	1.49		0.82	1.37
		Constant		-15.71		-8.33	
	3	Rumen NH_3-N x100/N		-.66(-.48)		-.61(-.55)	
	9-1	Pepsin sol. %DM		0.02(0.06)		-	
	9-2	Pepsin insol. N,%DM		-6.01(-.05)		-	
	9-3	Pepsin insol. Nx100/N		0.29(0.07)		-	
	9-4	Pepsin sol. N,%DM		7.55(0.29)		5.79(0.83)	

a,b,c,d,e,f,g,h,i and j see Table 24.

Table 27. Two Multiple Regressions Calculated by Using Variables of 2 Groups of Laboratory Measurements That Gave Relatively High R^2 for *In Vivo* Nitrogen Retention as A Percent of Absorbed Nitrogen (n=21).

Equation no.	Group no.[a]		Regression Coefficient		Regression[b] Coefficient	
1		R^2 and SEE[c]	0.75	5.55	0.73[d]	5.57[e]
		Constant		362.19		354.52
	3	Rumen[k] NH_3-Nx100/N[j]		0.34(-.26)[h]		-
	13-1	Rumen+pepsin+pan.[l] sol.[f] %DM[g]		-5.40(-.77)		-5.09(-.75)
	13-2	Rumen+pepsin+pan. insol.[i] Nx100/N		-13.72(-.71)		-13.82(-.70)
	13-3	Rumen+pepsin+pan. insol. N,%DM		328.58(0.73)		331.94(0.72)
	13-4	Rumen+pepsin+pan. sol. N,%DM		-92.61(-.63)		-98.31(-.64)
2		R^2 and SEE	0.72	6.04	0.70	6.02
		Constant		-13.56		-87.40
	8-1	Acid-detergent insol. N/N		9.80(0.63)		10.39(0.65)
	8-2	Acid-detergent insol. N,%DM		-296.78(-.62)		-292.44(-.60)
	8-3	Acid-detergent sol. N,%DM		-18.18(-.25)		-
	12-1	Rumen + pepsin sol. %DM		1.45(0.67)		1.40(0.69)
	12-2	Rumen + pepsin insol. Nx100/N		-7.79(-.62)		-5.74(-.69)
	12-3	Rumen + pepsin insol. N,%DM		242.94(0.61)		176.89(0.65)

a,b,c,d,e,f,g,h,i,j and k see Table 24.

[l]pan. = pancreatin.

rather time-consuming and complicated.

Two multiple regressions gave relatively high R^2 values for maximum dry matter intake and are in Table 28. Both equations included *in vitro* rumen microbial +pepsin solubility measurements, particularly, the soluble dry matter fraction. This may suggest that dry matter solubility in the rumen is related to the forage dry matter intake. In other words, forage voluntary intake of ruminants is probably somewhat regulated by the physical capacity of the rumen. This was in agreement with the concept of Crampton (1957) and Weston (1966, 1967 and 1968) that voluntary intake of forage dry matter by ruminants was limited primarily by rate of cellulose and hemicellulose digestion in the rumen. Species differences with regard to forage voluntary intake has been reported (Conrad, 1966), thus, the prediction equations developed from the present study probably should be used with caution for other ruminant species.

VIII. Multiple Regressions of Five In Vivo Parameters Using Selected Variables That Produced High R2 Values

Fourteen combinations of selected variables obtained from all 13 laboratory measurements were used in a multiple regression least square deletion analysis.

Table 28. Two Multiple Regressions Calculated by Using 2 Groups of Laboratory Measurements That Gave Relatively High R^2 Values for Maximum Dry Matter Intake (n = 21).

Equation no.	Group[a] no.		Regression Coefficient		Regression[b] Coefficient	
1		R^2 and SEE[c]	0.85	0.37	0.80[d]	0.38[e]
		Constant		-5.18		-6.55
	8-1	Acid-detergent insol.[i] N^j x100/N		0.27(0.35)[h]		0.12(0.62)
	8-2	Acid-detergent insol. N,%DM[g]		-4.92(-.21)		-
	8-3	Acid-detergent sol.[f] N, %DM		-.16(-.04)		-
	12-1	Rumen[k]+pepsin sol. %DM		0.14(0.82)		0.15(0.81)
	12-2	Rumen +pepsin insol. Nx100/N		-.22(-.34)		-.05(-.46)
	12-3	Rumen +pepsin insol. N,%DM		5.18(0.26)		-
2		R^2 and SEE	0.83	0.41	0.78	0.39
		Constant		6.70		-3.51
	11-1	Hot water sol. DM%		0.01(0.06)		-
	11-2	Hot water insol. Nx100/N		-.06(-.19)		-.02(-.56)
	11-3	Hot water insol. N,%DM		1.33(0.13)		-
	12-1	Rumen+pepsin sol. %DM		0.10(0.53)		0.12(0.87)
	12-2	Rumen+pepsin insol. Nx100/N		-.24(-.35)		-
	12-3	Rumen+pepsin insol. N,%DM		4.08(0.23)		-
	12-4	Rumen+pepsin sol. N,%DM		-2.56(-.29)		-

a,b,c,d,e,f,g,h,i,j, and k see Table 24.

Selection of combinations were generally those with greatest r values. However, factors such as time, techniques and instruments, involved in practical operations and inter-correlations were also considered. These 14 combinations gave 70 multiple regression equations but only a few representative equations will be presented and discussed. Three multiple regression equations giving high R^2 values for *in vivo* ND are given in Table 29. An extremely high R^2 of .99 was noted for Equation 1 which used seven variables from six analytical groups. The standard error estimate was also smaller for Equation 1 than for the other two equations. Partial correlation coefficients of Equation 1 indicated that crude protein (Group 1) and rumen microbial + pepsin soluble DM (Group 12-1) were negatively correlated with *in vivo* nitrogen digestibility. Opposite relationships were found for these variables in simple correlation analyses. These complete complete changes in relationships suggested the existence of interrelationships among variables that were not revealed in simple correlation analyses. Partial correlation coefficients of Equation 1 also indicated that variables obtained from chemical, pepsin and *in vitro* rumen microbial + pepsin digestions were all highly correlated with *in vivo* ND although pepsin solubility measurements had slightly lower partial

Table 29. Multiple Regressions Calculated by Using Selected Variables That Produced High R^2 for *In Vivo* Nitrogen Digestion Coefficients (n=21).

Equation no.	Group no.[a]		Regression Coefficients
1		R^2 and SEE[b]	0.99 1.38
		Constant	259.06
	1	Crude protein, %DM[c]	-3.95(-.76)[d]
	6	Acid-detergent fiber, %DM	-.52(-.82)
	7	Acid-detergent lignin, %DM	-2.70(-.85)
	8-1	Acid-detergent insol.[e] N/N[f]	-.99(-.86)
	9-2	Pepsin insol. N, %DM	120.06(0.75)
	9-3	Pepsin insol. Nx100/N	-4.08(-.76)
	12-1	Rumen[g]+pepsin sol.[h] DM, %	-.78(-.87)
2		R^2 and SEE	0.98 1.71
		Constant	38.30
	3	Rumen NH_3-Nx100/N	1.72(0.80)
	9-1	Pepsin sol. %DM	0.51(0.82)
	9-3	Pepsin insol. Nx100/N	-.88(-.95)
	11-3	Hot water insol. N, %DM	7.91(0.89)
3		R^2 and SEE	0.97 1.97
		Constant	178.91
	6	Acid-detergent fiber, %DM	-.46(-.68)
	7	Acid-detergent lignin, %DM	-3.22(-.87)
	8-1	Acid-detergent insol.N/N	-1.02(-.79)
	12-1	Rumen+pepsin sol. %DM	-.81(-.75)

[a]Group no.: Laboratory measurements were combined into 13 groups, detailed information is given in Table 23.

[b]SEE = standard error of estimate.

[c]DM = dry matter.

[d]Values in parentheses are partial correlation coefficients.

[e]insol. = insoluble.

[f]N = nitrogen.

[g]Rumen microbial.

[h]sol.= soluble.

correlation coefficients. Equation 2 required measurements from only three analytical groups (Group 3, rumen NH_3-N x 100/N; Group 9, pepsin soluble DM plus pepsin insoluble N x 100/N; and Group 11, hot water insoluble N, % DM), yet had a very high R^2 of .98. Equation 3 used variables from four analytical groups and these variables result from common analysis presently performed in many laboratories. For example, acid detergent fiber, acid detergent lignin and *in vitro* rumen microbial + pepsin soluble dry matter have been routine analyses for forage samples in many university laboratories for many years. Recently, the analysis of acid detergent insoluble N x 100/N has also become a routine analyses. Thus, Equation 3 could be used for practical predictive purposes. Partial correlation coefficients of Equation 3 indicated that microbial + pepsin soluble dry matter was again negatively correlated with *in vivo* ND.

Three multiple regression equations producing high R^2 values for *in vivo* dry matter digestion coefficients are given in Table 30. Multiple regressions computed by using selected variables slightly improved the R^2 and standard error of estimate (SEE) compared with the values obtained by using variables from only two groups of measurements (R^2 = .98, SEE = 1.07, Table 30 vs. R^2 = .96, SEE = 1.61, Table 25). Equations listed

Table 30. Multiple Regressions Calculated by Using Selected Variables That Produced High R^2 for *In Vivo* Dry Matter Digestibility (n = 21).

Equation no.	Group no.[a]		Regression Coefficients
1		R^2 and SEE[b] 0.98	1.25
		Constant	64.30
	5	Cell wall constituents,%DM[c]	-.36(-.72)[d]
	8-1	Acid-detergent insol.[e] N/N[f]	0.48(0.62)
	9-1	Pepsin sol.[g] %DM	0.36(0.59)
	9-3	Pepsin insol. Nx100/N	-.51(-.87)
2		R^2 and SEE 0.98	1.07
		Constant	51.15
	4	Degree of browning(OD440)	93.48(0.82)
	9-1	Pepsin sol. %DM	0.37(0.71)
	9-3	Pepsin insol. Nx100/N	-.64(-.78)
	12-3	Rumen[h]+pepsin insol. N,%DM	-21.36(-.81)
3		R^2 and SEE 0.98	1.26
		Constant	85.01
	5	Cell wall constituents,%DM	-.68(-.94)
	7	Acid-detergent lignin,%DM	1.09(0.52)
	8-1	Acid-detergent insol. Nx100/N	0.55(0.66)
	9-3	Pepsin insol. Nx100/N	-.76(-.90)
	11-3	Hot water insol. N,%DM	2.81(0.60)

a,b,c,d,e and f see Table 29.

[g]sol. = soluble.

[h]Rumen = rumen microbial.

in Table 30 have the same R^2 value but Equation 2 is the least complicated in terms of laboratory operations, and the standard error of estimate is least for this equation.

Acid detergent insoluble N as a percent of total N (Group 8-1) was used in both equation 1 and 3. In both equations a positive relationship between this variable and the *in vivo* dry matter digestion coefficient was indicated by the partial correlation coefficients. This finding is definitely contradictory to the idea that AD insoluble N is highly negatively correlated with *in vivo* dry matter or energy digestibility (Goering *et al.* 1972). However, one also should be reminded that the samples used in the present study contained less AD insoluble N than in the Goering *et al.* (1972) study.

Two multiple regression equations calculated by using selected laboratory measurements that produced relatively high R^2 for *in vivo* nitrogen balance are in Table 31. Variables used in these two equations are practically the same except that Equation 1 includes the additional variable, crude protein content. The R^2 for multiple regression equations in Table 31 were markedly above those for simple regressions obtained from Table 17 (.92 vs. .72) and the standard errors of estimate were also improved (1.04 vs. 1.59). More

Table 31. Multiple Regressions Calculated by Using Selected Variables That Produced High R^2 for *In Vivo* Nitrogen Balance (n = 21).

Equation no.	Group no.[a]		Regression Coefficients
1		R^2 and SEE[b]	0.92 1.04
		Constant	-32.19
	1	Crude protein, %DM[c]	1.38(0.76)[d]
	8-1	Acid-detergent insol.[e] N/N[f]	2.24(0.72)
	8-2	Acid-detergent insol. N,%DM	-64.29(-.67)
	9-3	Pepsin insol. Nx100/N	-.23(-.67)
	11-1	Hot water sol.[g] %DM	0.33(0.71)
2		R^2 and SEE	0.91 1.03
		Constant	-31.49
	8-1	Acid-detergent insol. N/N	1.76(0.77)
	8-2	Acid-detergent insol. N,%DM	-47.05(-.77)
	9-4	Pepsin sol. N,%DM	8.59(0.85)
	11-1	Hot water sol. %DM	0.31(0.69)

[a]Group no.: Laboratory measurements were combined into 13 groups, detailed information is given in Table 23.

[b]SEE = standard error of estimate.

[c]DM = dry matter.

[d]Values in parentheses are partial correlation coefficients.

[e]insol. = insoluble.

[f]N = nitrogen.

[g]sol. = soluble.

analytical values will be required for multiple regressions than for simple regressions. Nevertheless, N balance is a sensitive measure for differences in value of forage nitrogen for animal productivity and maintenance (Chalmers, 1961) and animal producers and research personel may more seriously consider greater emphasis on estimating the term N balance in order to more properly evaluate feeds for most efficient production.

Since multiple regressions calculated using selected laboratory measurements did not result in any significant improvement in R^2 for *in vivo* N retention as a percent of absorbed N and maximum dry matter intake as compared to those R^2 obtained by using two groups of measurements (Tables 27 and 28), equations will not be presented and discussed.

IX. Comparisons of Multiple Regressions Developed From Different Sources of Forage Samples for Estimating In Vivo Nitrogen Digestion Coefficients

Multiple regressions by the stepwise least square deletion analyses were calculated for estimating *in vivo* ND using data from 44 samples from Dr. H.K. Goering of USDA, Maryland plus 18 samples from the Michigan State University plus some samples from University of Wisconsin and Minnesota. Variables used in the regressions were mainly acid detergent solubility

measurements. Two representative equations are given in Table 32 along with equations using the same variables on 21 samples of the present study. When variables of crude protein, acid detergent insoluble N x 100/N, and acid detergent insoluble N, % DM remained in the regression analysis, a R^2 of .89 was found for the combined samples and none of the variables were deleted in the least square deletion analysis. However, none of the variables had a high partial correlation coefficient. This suggested that these variables were not highly nor directly related to the in vivo ND. On the other hand a slightly lower R^2 (.86) was observed for the regression based on 21 samples from the present study and two of the variables (crude protein and acid detergent insoluble N, % DM) were deleted by the analysis due to insignificant ($P > .05$) regression coefficients. The standard error of estimate was somewhat smaller for the equation using MSU samples than for the equation using the combined samples. Consequently, the regression coefficients for the two resulting equations are markedly different. These inconsistencies may be due to sample size, species, type of preservations, geographical locations, variation in laboratory analytical techniques and digestion trial procedures.

Regression analyses of Equation 2 also indicated discrepancies between the two equations in that lignin

Table 32. Comparisons of Multiple Regressions for In Vivo Nitrogen Digestion Coefficient Developed by Using Different Sources of Samples Forage.

Equation no.		Regression Analyses			
		MSU[a]+ USDA[b]+ others[c]		MSU	
1	n_2	80		21	
	R^2 and SEE[d]	0.89	6.43	0.86	4.04
	Adjusted R^{2e}	0.89		0.85	
	Constant	60.13		91.26	
	Crude protein	1.05(0.35)[f]		-	
	Acid-detergent insol.[g] N[h]x100/N	-.41(-.48)		-2.15(-.93)	
	Acid-detergent insol. N, %DM[i]	-34.44(-.28)		-	
2	n_2	70		21	
	R^2 and SEE_2	0.89	6.82	0.92	3.20
	Adjusted R^2	0.89		0.92	
	Constant	49.79		105.16	
	Crude protein	1.72(0.77)		-	
	Acid-detergent insol. N,%DM	-8.74(-.91)		-27.68(-.56)	
	Acid-detergent lignin, %DM	-		-3.17(-.73)	

[a]MSU = Michigan State University. [b]USDA = Dr. H.K. Goering, USDA. Maryland.

[c]others = Dr. N.A. Jorgensen, University of Wisconsin and Dr. D.C. Pierson, University of Minnesota.

[d]SEE= standard error of estimate. [e]Adjusted for sample number and variables used in the multiple equations(Steel and Torrie, 1960).

[f]Values in parentheses are partial correlation coefficients.

[g]insol. = insoluble. [h]N = nitrogen. [i]DM = dry matter.

was deleted ($P > .05$) from the combined samples but not the MSU samples. However, in both equations and both groups of samples the term AD-N as percent of N or DM had a important relationship to *in vivo* nitrogen digestibility.

SUMMARY AND CONCLUSIONS

Part 1

The predictability of five animal responses was evaluated using data from 31 laboratory measurements on the forage fed. The five responses were nitrogen and dry matter digestibilities, nitrogen balance and retention, maximum dry matter intake. These data were obtained from sheep fed preserved forages at Michigan State University (24 samples) and at other experimental stations (66 samples). A large proportion of samples were heat damaged. Data from three groups of laboratory measurements were collected for most samples. These three analytical groups were chemical determinations, enzymatic incubations, and sequential rumen microbial plus enzymatic incubations.

Among these 31 measurements, acid detergent (AD) insoluble nitrogen (N) as a percent of total N, AD insoluble dry matter (ADF), pepsin soluble N as a percent of dry matter, hot water soluble dry matter and rumen microbial plus pepsin soluble dry matter were the best single predictors (had the highest correlation coefficient(r) and the lowest standard error of estimate)

for *in vivo* N or dry matter digestibilities, N balance, N retention as a percent of absorbed N, and maximum dry matter intake. However, the r values for the latter three parameters were lower than that for the former two (r = .80 vs. .93). Forage N content was insignificantly ($P < .05$) correlated with N digestibility (r = + .19).

Great improvement in predicting N digestibility was observed when total N was separated into soluble and insoluble N fractions by one of the following four methods (1) acid-detergent, (2) acid pepsin, (3) rumen microbial plus pepsin, and (4) rumen microbial plus pepsin plus pancreatin.

Simple regression equations for N digestibility using AD insoluble N as a percent of total N as the single variable were different depending on the absolute amount of AD insoluble N as a percent of total N. Acid detergent insoluble N had greater negative effect for forage samples containing a relatively low level of AD insoluble N as a percent of total N ($\leq 9\%$) than for samples containing high levels of AD insoluble N ($> 9\%$) presumably due to heat damage.

When the 31 laboratory measurements were combined into 13 groups based on analytical schemes and used as multiple predictors for *in vivo* responses, those measurements obtained with pepsin incubations had the

greatest precision. Some improvements were found in predicting animal responses when variables of the two groups were jointly used.

Finally, multiple regressions with extremely high degree of precision ($R^2 > .98$) were obtained for *in vivo* N and dry matter digestibilities using selected variables (no restrictions as to analytical groups).

Regression coefficients along with partial correlation coefficients were useful not only in defining relative importance of variables in predicting animal responses but also in revealing the true (unconfounded) relationships among variables and animal responses. Large differences were observed for variables obtained from different sources of samples with regard to the relative importance in predicting *in vivo* N digestibility.

Part 2. Haylage Preservation With Various Chemicals

I. Effect of Chemicals on Alfalfa Haylage Temperatures

(A) Effect of Chemicals on Haylage Temperatures During Storage

During the entire 42-day storage period, each silo was equiped with four thermocouples at four different levels. Temperature was measured ten times per day in an attempt to discover the diurnal pattern of haylage temperature. Representative hourly temperature of haylages and ambient temperature during the storage period are given in Table 33. According to the ten hourly readings of this study, the lowest and the highest ambient temperatures were generally observed near daily hours 3 or 4 and hours 18 to 20, while no consistant changes were observed in hourly haylage temperatures. For example, on June 30, haylage temperatures of silo 3,6 and 7 (treatments of .8% propionic acid, control and .5% AIB) were slightly higher during hour 14 to 18 than during hour 0 to 6 but this trend was not clearly observed for the other three silos.

Haylage temperatures were generally related to the ambient temperature on July 10, but no clear relationships were observed on July 15 to 20. These inconsistant findings indicated that no clear diurnal pattern was found in haylage temperatures. Thus, ten

Table 33. Selected Hourly Haylage and Ambient Temperatures(C) During the Storage Period[a](June 24 to August 2, 1973).

Date and time (Hour)	Treatments of haylages and silo number						Ambient
	Propionic acid		AIB[b]		AIB(0.5%) +HCHO[c]	Control	
	0.4%	0.8%	0.5%	1%			
6/30	8[d]	3	7	5	4	6	
12	32.8	40.0	30.0	38.4	39.0	42.3	13
14	32.8	40.6	30.0	38.4	40.0	42.3	13
16	33.9	40.6	31.7	38.4	40.0	43.9	13
18	33.5	40.9	32.2	41.7	40.6	44.4	15
20	32.8	41.1	32.2	41.7	40.6	44.4	19
0	30.6	41.1	32.2	39.5	40.6	44.4	19
2	29.4	41.1	32.2	39.5	36.7	44.4	19
4	29.4	41.1	32.2	37.2	40.6	44.4	21
6	30.0	40.6	32.8	39.5	39.5	43.9	20
8	32.0	42.3	35.0	41.7	39.5	45.6	22
7/5							
12	38.4	42.3	39.2	41.1	45.0	45.6	26
14	36.7	41.7	36.7	42.3	43.9	43.9	19
16	36.1	41.1	35.6	42.3	42.8	43.9	18
18	36.1	41.1	35.6	42.3	42.3	43.9	26
20	36.1	41.1	35.6	39.2	42.8	43.9	27
0	36.7	41.7	36.1	42.8	43.4	44.5	24
2	36.7	41.7	37.2	40.0	43.9	43.9	25
4	36.7	41.7	37.2	42.8	44.4	43.9	27
6	36.7	41.7	37.2	41.1	44.5	43.9	27
8	37.2	42.3	38.4	43.4	45.6	44.5	30

Table 33. (Continued)

Date and Time (Hour)	Treatments of haylages and silo number						Ambient
	Propionic acid		AIB		AIB(0.5%) + HCHO	Control	
	0.4%	0.8%	0.5%	1%			
7/10	8	3	7	5	4	6	
12	35.0	39.2	37.2	41.1	46.1	42.3	26
14	36.7	40.6	46.7	42.3	46.7	42.8	24
16	37.0	35.6	39.5	37.2	42.3	39.5	18
18	32.0	36.1	40.1	33.3	41.7	39.5	18
20	31.7	36.1	40.0	30.6	41.7	39.5	19
0	31.7	36.1	39.5	36.7	42.3	39.5	22
2	31.1	36.1	39.5	37.2	42.8	40.0	24
4	31.7	38.4	41.1	40.0	43.4	41.1	26
6	33.9	38.4	41.1	39.5	44.5	41.7	27
8	36.7	41.1	43.4	40.6	46.7	42.8	26
7/15							
12	30.6	35.6	31.1	31.7	46.7	37.8	19
14	30.6	35.6	31.1	28.3	46.7	37.8	16
16	30.6	35.6	31.1	28.3	45.6	37.8	12
18	30.6	35.6	31.1	25.6	45.6	37.8	12
20	30.6	35.6	31.1	31.1	38.4	37.8	14
0	30.0	35.6	31.7	31.7	38.4	37.8	19
2	30.0	35.6	31.1	29.4	39.2	37.8	20
4	30.0	35.0	31.1	25.6	39.2	37.8	21
6	30.0	35.0	31.1	27.8	39.2	37.8	21
8	30.0	35.0	31.1	32.8	39.2	37.2	22

Table 33. (Continued)

Date and time (hour)	Treatments of haylages and silo number: Propionic acid 0.4%	Propionic acid 0.8%	AIB 0.5%	AIB 1%	AIB(0.5%) + HCHO	Control	Ambient
7/20	8	3	7	5	4	6	
12	28.9	33.9	30.0	37.2	41.7	37.8	28
14	30.0	33.9	30.0	35.6	41.7	37.8	28
16	30.0	33.9	30.6	37.2	41.7	37.8	28
18	30.0	33.9	30.0	35.6	41.7	37.2	28
20	30.0	33.9	30.6	37.2	41.7	37.8	29
0	30.6	33.9	30.6	37.2	40.0	38.4	22
2	30.6	33.9	29.4	35.6	40.0	37.8	20
4	30.6	35.0	28.9	32.8	40.0	37.8	19
6	30.0	34.4	29.4	31.7	40.0	37.8	19
8	30.0	36.1	29.4	31.7	39.5	37.8	19

[a]Each haylage temperature represents the average of three temperature measured at three silo positions (middle near top, middle near bottom and bottom). Temperatures measured from the top position were excluded from calculation, because these temperatures were consistently higher than 40 C regardless of ambient temperatures.

[b]AIB = ammonium isobutyrate.

[c]HCHO = formaldehyde (-1.25% of a 37% solution).

[d]silo number.

hourly interval temperatures were averaged and these average daily temperatures of six haylages are shown in Figure 5. Generally, daily temperatures of control haylage was somewhat higher than temperatures of the other five haylages during the major part of the entire storage period. Patterns of temperature development in haylages were somewhat influenced by that of ambient temperature both decreasing to a low on June 29 and increasing to a pleateau afterwards. Haylage temperature apparently did not become stable until approximately 18 days after filling (July 5). During the first 10 days of the storage period, the pattern of haylage temperature development was similar for .8% propionic acid and 1% AIB treatments. Temperature development for the other three treatments (.4% propionic acid, .5% AIB and .5% AIB + 1.25% formaldehyde) were similar to some extent and temperatures for these three were somewhat lower than for those with the higher acid levels. Differences among daily average temperatures of the six haylages gradually decreased after about 10 days filling and reached the narrowest difference of 4 C,(from 39 to 43 C) on July 10. After this date, temperatures were at about 38 C except for the 1% AIB treated haylage.

For further comparisons, daily temperatures were averaged by weeks and these weekly average temperatures

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Figure 5. Temperature of Haylage During Storage Period.
Temperature Recording Started on June 22, 1973 but Filling Dates Were Five, Zero, Three, Three, One and One Day Before June 22 for Silos 3,4,5,6,7 and 8 Respectively.

3 = 0.8% Propionic Acid.
4 = 0.5% Ammonium Isobutyrate + 1.25% Formaldehyde.
5 = 1% Ammonium Isobutyrate.
6 = Control.
7 = 0.5% Ammonium Isobutyrate.
8 = 0.4% Propionic Acid.

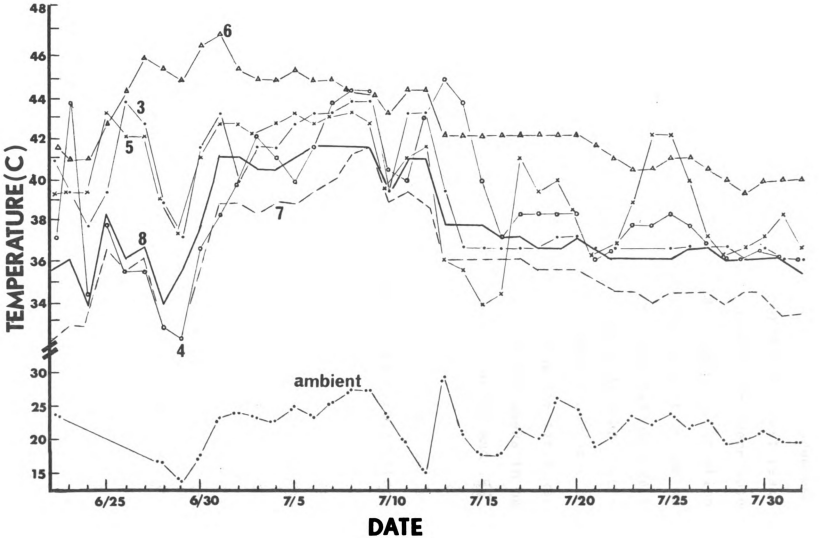

TEMPERATURE(C)
48
46
44
42
40
38
36
34
30
25
20
15
6
3
5
8
4
7
ambient
6/25
6/30
7/5
7/10
7/15
7/20
7/25
7/30
DATE

Figure 5.

are presented in Table 34. All chemical treatments reduced the overall mean and maximum temperatures. The lowest overall and maximum temperatuers were noted for haylage treated with .5% AIB. Higher application rates of propionic acid or AIB did not result in the greatest reductions in temperature. Average haylage temperatures of the first two weeks indicated that control, .8% propionic acid and 1% AIB treated haylages developed a appreciable amount of heat during the first week while heat development was retarded for two weeks in haylages treated with .4% propionic acid, .5% AIB and .5% AIB + 1.25% formaldehyde.

Several workers have suggested that the amount of time involved in heat development during storage was more related to the nutritive value depression of the resulting silage than the maximum or overall mean temperature (Gordon et al. 1963; Wieringa et al. 1961; Goering et al. 1973). Thus, the number of days above 35 C during the entire storage period was also computed and are shown in the bottom line of Table 34. These data indicated that AIB (.5%) treated haylage had the least days with high temperature. The other four treatments had similar number of days above 35 C. Huber et al. (1972) observed an improved correlation coefficient between haylage storage temperatures and in vivo

Table 34. Average Weekly Temperatures of Haylages During A 42-Day Ensiling Period. Haylages Were Treated With Propionic Acid, Ammonium Isobutyrate (AIB) and Mixture of AIB and Formaldehyde.

	Propionic Acid		Ammonium Isobutyrate (AIB)		AIB (0.5%) + HCHO[a]	Control	Mean of week	Ambient
	0.4%	0.8%	0.5%	1%				
	(8)[b]	(3)	(7)	(5)	(4)	(6)		
Period (Week)	---------- C ----------							
1st	36[c]	41	34	41[d]	37	43	39	21
2nd	40	41	37	42	39	46	41	22
3rd	41	43	40	42	43	44	42	23
4th	37	37	36	37	40	42	38	21
5th	37	34	34	40	38	41	37	22
6th	36	34	34	37	36	40	36	21
Mean	38	38	36	40	39	43		22
Days above 35 C	39	41	25	39	38	41		
Degree-days above 35 C	119	154	66	203	161	322		

[a] Formaldehyde - 1.25% of a 37% solution. [b] Silo number.

[c] Each temperature represents the mean temperatures recorded from four levels in the silo, ten recordings per day and seven days a week.

[d] Temperatures underlined represent maximum temperature.

nitrogen digestion coefficients when the haylage temperatures were calculated as "degree days above 35 C" than as mean storage temperature. Degree days above 35 C were calculated by summation of daily haylage temperatures which were above 35 C during storage. These temperatures were calculated for the six haylages and are in the bottom line of Table 34. Although the ranking of these degree-days above 35 C was the same as that for the mean haylage temperatures i.e. control > 1% AIB treated > .5% AIB + formaldehyde (1.25% of a 37% solution) treated > .8% or .4% propionate treated > .5% AIB treated haylages, the magnitude of difference in temperature was much greater for the expression of degree-days above 35 C than that of mean temperatures. For example, the mean storage temperature of control haylage was only 1.08 fold higher than that of haylage treated with .5% AIB while the former was 4.88 fold higher in degree-days above 35 C than the latter.

Weekly mean temperatures were also calculated on the position of the thermocouple in the silos (Table 35). Five thermocouples were placed in each silo. Their distribution was two near the bottom; one in the lower-middle; one in the upper middle; and one more than 1.33 m below the top of the silo. Both the overall mean and maximum temperatures were positively related to thermocouple position in the silo. Haylage temperature was

Table 35. Mean Weekly Temperatures of Haylages Ensiled in Concrete Silos for A 42-Day Storage Period. Temperatures Are Presented Based on the Level or Position of Thermocouple in the Silo.

	Position of Thermocouple				Mean
	Bottom[a]	Middle-1[b]	Middle-2[c]	Top[d]	
Period (Week)	---------- C ----------				
1st	36[e]	36[f]	38	45	39
2nd	36	37	42	48	41
3rd	36	38	45	49	42
4th	32	33	40	49	39
5th	30	32	39	48	37
6th	29	30	37	49	36
Mean	33	34	40	48	
Degree-days above 35 C	21	42	217	546	

[a]Approximately 1.2 m above ground.

[b]Approximately 3 m above ground.

[c]Approximately 5 m above ground.

[d]Approximately 7 m above ground.

[e]Values underlined represent maximum temperatures.

[f]Each temperature represents the mean temperatures recorded ten times per day and seven days per week.

always the greatest at the upper-most thermocouple. The top area had 26 times greater sum for degree-days above 35 C than did the bottom portion and even the upper middle are (> 3 m. below the surface) had 10 times greater sum for degree-days above 35 C than did the bottom portion. This relationship was probably caused by the degree of compaction and of exposure of haylage mass to the air. Gordon et al. (1961) did not observe this type of relationship between the position in the silo and haylage temperature when the top was covered by plastic sheets in similar silo structures. Temperature in bottom area of silo remained the same during the first 3 weeks while maximum temperature was attained during week 3 for other silo heights.

(B) Effect of Chemicals on Haylage Surface Temperature After Silos Were Open for Feeding

After the silos were opened for feeding, haylage temperatures were measured by mercury (Hg) therometer inserted 25 cm below the surface about two times per week. Mean and maximum haylage surface temperatures measured during the feeding period of 49 days are presented in Table 36. Maximum ambient temperature as well as haylage surface temperatures were observed during the first 10 days of the feeding period (July 11 to 23). With the exception of the .4% propionic acid treatment, the other four treatments reduced mean and maximum

Table 36. Mean and Maximum Temperatures Measured 25 cm Below the Surface of Haylages During A Period of 69 Days When Silos Were Open.

	Propionic acid		Ammonium Isobutyrate (AIB)		AIB(0.5%) + HCHO[b]	Control	Ambient
	0.4% (8)[a]	0.8% (3)	0.5% (7)	1% (5)	(4)	(6)	
	----------			C		----------	
Mean Temperature	44	39	40	39	41	45	26
Maximum Temperature	55	42	48	47	49	57	34

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

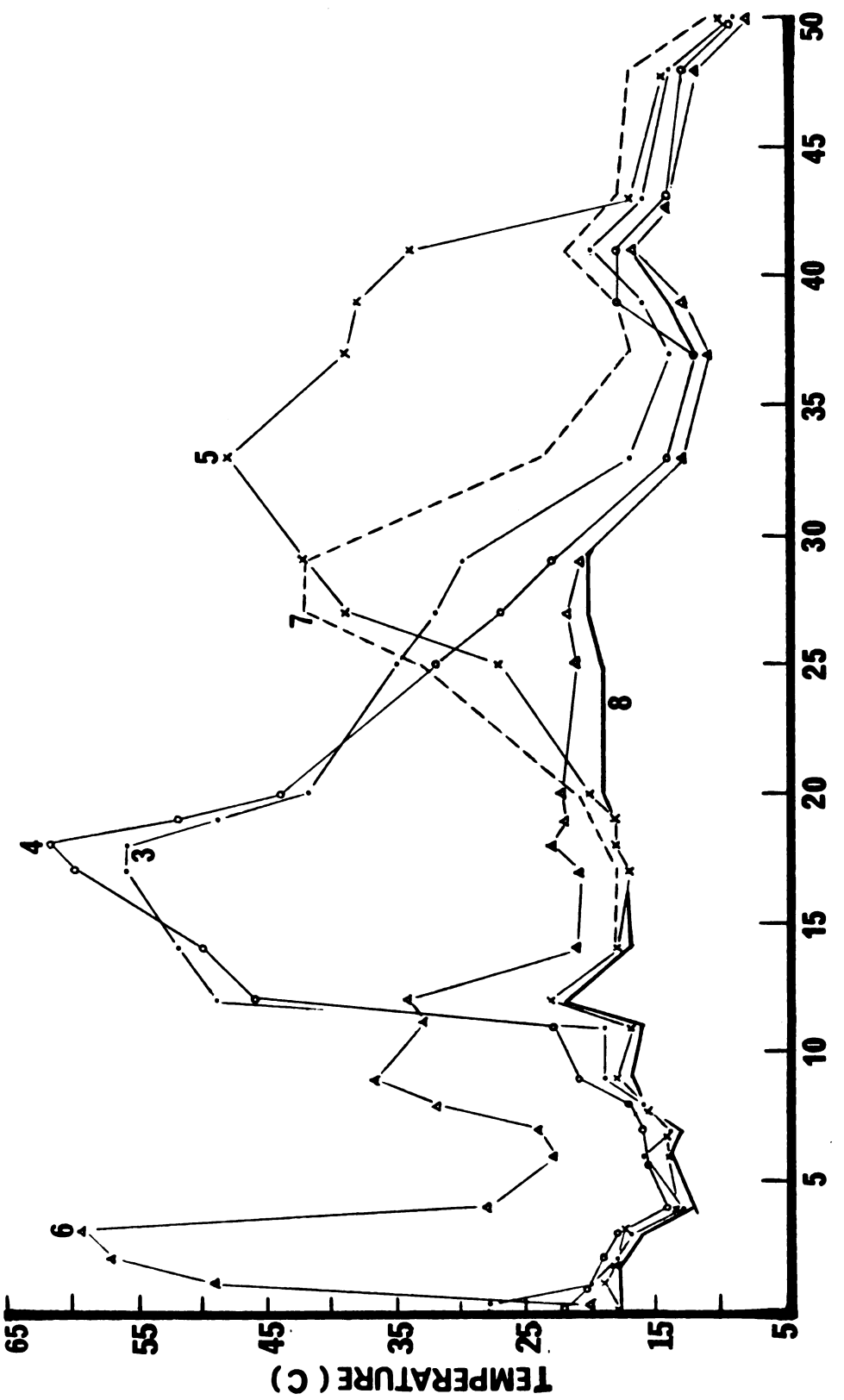
surface temperatures by approximately 5 and 10 C respectively. Treatment with .4% propionic acid had practically no effect on surface temperature of the resulting haylage. Contrary to the findings described in the previous section, chemicals applied at higher rates generally had increased effectiveness in reducing surface temperatures. No sound explanations are evident.

(C) Effect of Chemicals on Haylage Temperature During Refermentation

Approximately 30 kg of haylage taken from the lower middle portion of each silo were loosely packed into 55 gal. barrels. Temperatures during a 50-day refermentation period were measured about three times per week by thermocouple and are shown in Figure 6. A sharp increase in temperature was found in control haylage on the second day of the refermentation period. Maximum temperature (59 C) was reached by day three then haylage temperature declined rapidly. A second peak was observed by about day nine (Sept. 23), but the second maximum temperature was only about 37 C. Haylages treated with .8% propionic acid and .5% AIB + 1.25% formaldehyde did not develop a high temperature until day sixteen with a maximum temperature of 57 C attained on day eighteen. Temperatures then declined gradually. Treatment with .5% or 1% AIB was able to retard refermentation until day twenty when the rate of temperature increase

Figure 6. Temperature Developments During Refermentation of Haylage Treated With Various Chemicals.

3 = 0.8% Propionic Acid.
4 = 0.5% Ammonium Isobutyrate + 1.25% Formaldehyde.
5 = 1% Ammonium Isobutyrate.
6 = Control.
7 = 0.5% Ammonium Isobutyrate.
8 = 0.4% Propionic Acid.


TEMPERATURE (C)
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55
45
35
25
15
5
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10
15
20
25
30
35
40
45
50
DAYS
3
4
5
6
7
8

Figure 6.

was rather slow. About 10 days elapsed between initial increase and maximum temperature (about 43 C) after which temperature declined gradually. The most striking finding was that haylage treated with .4% propionic acid did not develop any significant amount of heat (i.e. above 35 C) for the entire 50 days of refermentation. In fact, this haylage was all good at day 50 when the haylage drums were emptied and separated into good and moldy portions. All other haylages were completely moldy when emptied at this time. Results obtained from this experiment were not in complete agreeable with observations reported in the previous two sections where treatment with .4% propionic acid ranked inferior to .5% AIB treatment in reducing haylage temperature during storage and surface temperature during emptying time. Samples used in the refermentation experiment represent only one area of the silo and thus, mighy not be representive.

In any event, results from all these measurements suggested that the AIB solutions (.5 and 1%) were superior to propionic acid in reduction of haylage temperature. An opposite ranking has been reported by Goering and Gordon (1973) when both chemicals were evaluated under laboratory and pilot conditions.

II. Effect of Chemicals on Haylage Dry Matter Losses During Storage

Amount of top spoilage for the six haylages is shown in Table 37. With no addition of chemicals top spoilage amounted to 4.4%. This amount of spoilage is normal when low moisture crops are ensiled (Gordon et al. 1965). No significant improvement in reduction of top spoilage was found for the two AIB and the .4% propionic acid treatments and only slightly improvement for the AIB + formaldehyde treatment. The most effective treatment in reducing top spoilage was .8% propionic acid. This treatment reduced the extent of top spoilage to about 1.8%. Zero top spilage has previously been reported for this treatment (Yu et al. 1973).

Silos with the greatest amount of top spoilage were not those with the greatest temperature. In fact, extremely low correlation coefficients were found between extent of top spilage and temperature for upper silo thermocouple ($r = -.13$) or days above 35 C for the entire silo ($r = -.43$). However, top spoilage was somewhat but not significantly ($P > .05$) correlated with degree-days above 35 C for the upper thermocouple ($r = .61$). Extent of top spoilage was positively but not highly related to measured temperature during storage.

The term "other spoilage" in Table 37 implies amount of spoilage other than top portion. Haylage

Table 37. Amount of Dry Matter Recovered and Spoiled After 42-Day Storage Period in Concrete Silos of Control and Treated Haylages.

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(0.5%) + HCHO[b] (4)	Control (6)
Top Spoilage (kg DM[c])	665	250	664	721	378	526
Top Spoilage (% DM stored)	4.86	1.79	4.56	5.43	3.02	4.40
Other Spoilage (kg DM)	267	286	585	262	247	235
Other Spoilage (% DM stored)	1.96	2.05	4.02	1.98	1.98	2.11
Good Haylage Fed (kg DM)	9,162	9,862	6,894	8,181	7,245	9,147
Good Haylage Fed (% DM stored)	67	71	47	62	58	76
Gaseous Loss (% DM stored)[d]	26	25	44	31	37	18

[a]Silo number.

[b]Formaldehyde -1.25% of a 37% solution.

[c]DM = Dry matter.

[d]Percent dry matter lost as gas = 100 - % good haylage fed - % other spoilage - % top spoilage. The term gaseous loss in this trial is larger than actual due to loss of records on silage take from silo.

treated with .5% AIB had a greater portion of other spoilage than did control (4.02 vs. 2.11%) while the other four treated haylages had comparable or slightly smaller portions of other spoilage than did control. Amount of "good" haylage fed is given in Table 37 however the value does not represent the exact amount of good haylage since haylage taken from silos after the feeding trial was not accurately recorded. Similarly, the value for percent of dry matter lost as gas which is calculated by difference is given in the bottom of Table 37 but only for approximation purposes. Furthermore, the amount of dry matter lost as gas is not necessarily related to the amount of dry matter lost due to top spoilage (Gordon et al. 1965; Yu et al. 1973). Under normal haylage processes, the extent of dry matter lost as gas ranges from 1 to 12% (Gordon et al. 1963, 1965). When haylage was treated with .8% propionic acid, Yu et al. (1973) observed a 0% gaseous loss. Unfortunately, the effect of chemicals on gaseous loss or dry matter can not be accurately evaluated in the present study.

III. Effect of Chemicals on Haylage Characteristics

When silos were opened for feeding, haylages were sampled three times per week and composited biweekly. Thus, four composites were made during the entire feeding experiment (42 days). These four composites were

also considered as representative samples from four different positions in the silo. Average values of several determinations obtained from composites are presented in Table 38. All six haylages had similar pH values of near 5 which is a typical value for low moisture silages (Gordon et al. 1963, 1965; Thomas et al. 1969; Owen and Senel, 1963). Concentrations of volatile fatty acids (VFA) in these composites were rather low but were in the usual range found for low moisture silages (Gordon et al. 1963, 1965). The propionic acid and AIB treated haylages contained appreciably greater amounts of these respective acids than did control haylage. The application rates of these two chemicals were generally reflected by the relative concentrations in the resulting haylages. Concentrations of butyric acid were extremely low and isovaleric acid was absent in all haylages. This may be considered desirable since high levels of butyric acid have been related to high levels of protein degradation in silage and to low silage intake by ruminants (Murdoch, 1966). Some lactic acid was produced in the control haylage (1.8% DM), but little or no lactic acid was found in treated haylages. Lactic acid is normally the major organic acid in silages. Results from the present study indicated that propionic acid and AIB greatly influenced the silage fermentation pattern by

Table 38. Dry Matter Content, pH, Organic Acids, Fungal Counts and Recovery of Additives to Haylages.

	Propionic acid		Ammonium Iso-butyrate (AIB)		AIB (0.5%) + HCHO[b]	Control
	0.4%	0.8%	0.5%	1%		
	(8)[a]	(3)	(7)	(5)	(4)	(6)
Dry Matter as fed (%)	54.7	60.1	53.9	47.6	49.2	50.84
pH	4.93	4.90	5.04	4.91	5.23	5.09
Organic acids (%DM)						
Acetic acid	1.411	1.145	1.380	1.629	1.373	1.559
Propionic acid	0.562	0.728	0.037	0.050	0.070	0.064
Isobutyric acid	0.032	0.019	0.743	1.526	0.487	0.042
Butyric acid	0.047	0.031	0.033	0.041	0.063	0.052
Valeric acid	0	0	0	0	0.010	0
Total VFA[c] (%DM)	2.052	1.923	2.193	3.246	1.993	1.717
Lactic acid	0	0	0	0.328	0.548	1.819
Total counts of fungal colonies ($1x10^6$/g haylage)	3.1	1.1	2.5	1.05	4.93	4.5
Recovery of chemical[d] (%)	70	52	85	82	46	

[a]Silo number. [b]Formaldehyde -1.25% of a 37% solution.

[c]VFA = Volatile fatty acid.

[d]Recovery of chemical(%) = (g chemical in treated haylage/g treated haylage - g chemical in control haylage/g control haylage dry matter) ÷ (g chemical applied to fresh forage at ensiling time/g fresh forage dry matter) x 100.

depressing lactic acid production but having no effect on acetic acid production (Table 38).

The antifungal properties of these chemical additives were evaluated based on mold counts. The control haylage contained 4.5×10^6 fungi per gram of fresh haylage. Marked reductions in total fungi counts were observed for propionic acid and AIB treated haylages. Low levels were not as effective as high levels for reducing number of fungi. Although formaldehyde solution is generally used as an antifungal agent, no reduction in mold counts was observed when 1.25% formaldehyde solution was applied with AIB. The major genera of fungi isolated from haylages were Saccharomyces (yeast), Geotrichum, Penicillium, Scopulariopsis and Mucor. Genus Geotrichum was not found in composites of .8% propionic acid treated haylage while other genera were frequently isolated and not related to treatments.

Percent recovery of chemicals were calculated based on the concentration of chemical used at ensiling and the concentration in the resulting haylage (Table 38). The percent recovery of propionic acid ranged from 52 to 70% which are somewhat lower than the recovery of AIB (82 - 85%). When AIB was applied with formaldehyde, the recovery of AIB was 46% which equals only about half the recovery when AIB was used singly. These relatively low recoveries suggest that certain

amounts of chemicals were degraded or lost.

Several values for each composite of each haylage are in Table 39. There were considerable variations in dry matter content within each treatment (silo), probably due to variation in dry matter content of various loads when harvested as well as moisture migration. Some variations in concentration of organic acids were found within treatment but these variations were not consistently observed among treatments. For example, concentration of total VFA was greater in composite three than in composite one, for the .8% propionic acid treated haylage but the reversed situation was found in the .4% propionic acid treated haylage. Silages containing dry matter content above 30% have a negative relationship between dry matter content and concentrations of various organic acids (Gordon et al. 1965). This negative relationship was also apparent for acetic acid concentration of the present study. The correlation coefficient was -.83 ($P < .01$).

Within each treatment, large variations in fungal counts were evident among composites. These variations were probably due to insufficient mixing of additives, samples and variable sized clumps of fungi formed in silages.

Table 39. Dry Matter Content, pH, Organic Acids and Mold Counts of Control and Treated Haylages.

	Propionic Acid							
	0.4% (8)[a]				0.8% (3)			
Composite No.[c]	1	2	3	4	1	2	3	4
Dry matter as fed (%)	47.8	50.3	64.8	56.0	64.8	60.8	59.4	55.6
pH	5	4.9	5	4.8	4.9	4.9	4.8	4.9
Organic acids (%DM)								
Acetic acid	1.64	1.52	1.05	1.42	0.79	1.13	1.42	1.22
Propionic acid	0.44	0.47	0.51	0.81	0.69	0.68	0.80	0.72
Isobutyric acid	0.02	0.03	0.02	0.04	0.01	0.02	0.02	0.02
Butyric acid	0.04	0.08	0.02	0.03	0.04	0.03	0	0.04
Valeric acid	0	0	0	0	0	0	0	0
Total VFA[d]	2.15	2.11	1.62	2.31	1.53	1.87	2.25	2.02
Lactic acid	0	0	0	0	0	0	0	0
Total counts of fungal colonies ($1x10^6$/g)	5.0	5.6	0.9	0.8	1.8	2.0	0.3	0.2

Table 39. (Continued)

	Ammonium Isobutyrate (AIB)							
	0.5% (7)				1% (5)			
Composite No.	1	2	3	4	1	2	3	4
Dry matter as fed (%)	50.2	46.0	58.4	61.0	41.6	46.5	50.6	51.7
pH	5	5.1	5.1	4.9	4.9	4.9	4.9	4.9
Organic acids (% DM)								
Acetic acid	1.36	1.78	1.38	0.98	1.87	1.51	1.69	1.44
Propionic acid	0.02	0.05	0.03	0.03	0.07	0.05	0.02	0.03
Isobutyric acid	0.85	0.73	0.60	0.78	1.38	1.55	1.47	1.69
Butyric acid	0.03	0.09	0	0	0.07	0.06	0	0.02
Valeric acid	0	0	0	0	0	0	0	0
Total VFA	2.27	2.66	2.02	1.79	3.40	3.19	3.18	3.19
Lactic acid	0	0	0	0	0	0.53	0.78	0
Total counts of fungal colonies ($1x10^6$/g)	0.1	8.5	0.9	0.4	0.1	3.1	0.3	0.7

Table 39. (Continued)

	AIB(0.5%) + HCHO[b] (4)				Control (6)			
Composite No.	1	2	3	4	1	2	3	4
Dry matter as fed (%)	45.6	45.9	47.9	55.5	40.9	49.5	53.4	59.5
pH	5.7	5.5	4.9	5.1	5.1	4.9	5.2	5.0
Organic acids(% DM)								
Acetic acid	1.30	1.52	1.41	1.24	1.73	1.47	1.64	1.38
Propionic acid	0.08	0.07	0.06	0.05	0.04	0.07	0.04	0.08
Isobutyric acid	0.03	0.62	0.65	0.62	0.02	0.05	0.03	0.04
Butyric acid	0.06	0.07	0.06	0.04	0.04	0.06	0.05	0.03
Valeric acid	0.03	0	0	0	0	0	0	0
Total VFA	1.53	2.29	2.21	1.97	1.85	1.67	1.77	1.55
Lactic acid	0	0	0.82	0.26	2.66	2.60	1.02	0.99
Total counts of fungal colonies ($1x10^6/g$)	5.20	7.60	1.00	5.90	1.80	12.1	3.10	1.00

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

[c]No. of composite haylage sample, also level in the silo; 1 = top, 4 = bottom.

[d]VFA = Volatile fatty acids.

IV. Effect of Treatments on Chemical Composition of Haylage

Representative haylage samples taken from sheep feeding trials 1 and 3 were analyzed for chemical and fibrous components. These samples were not composites but relatively small portions of haylages near the top (sheep trial 1) and the bottom (sheep trial 3) of the silo. Proximate chemical composition of these samples are in Table 40. Generally, chemical composition was similar among haylages although AIB treated haylage contained slightly higher levels of nitrogen than other haylages. The AIB contains the NH_4^+ moiety which would increase the nitrogen content of the treated haylages.

Bottom area(sample of period 3) were generally the lowest in crude fiber content and consequently values of other constituents particularly nitrogen free extract (NFE) and crude protein were increased. All haylages were similare in NFE content indicating similar degrees of fermentation since NFE is normally the primary source of fermentable carbohydrates. However, proper evaluation of changes in chemical composition for each treatment are possible only when the composition of the original crop is known and compared with that of the silage on a quantitative basis. Unfortunately, insufficient and non-representative samples of original forages were collected and no analyses were performed. Thus,

Table 40. Chemical Composition of Control and Haylage Treated With Propionic Acid, Ammonium Isobutyrate and Mixture of Ammonium Isobutyrate and Formaldehyde.

Treatment and Silo no.	Ash		Crude Fiber		Ether Extract		Crude Protein		N-Free Extract[a]	
	P_1	P_3[b]	P_1	P_3	P_1	P_3	P_1	P_3	P_1	P_3
	% DM									
Propionic acid										
0.4% (8)	7.39	6.98 (7.19)[c]	36.89	35.61 (36.30)	1.32	2.34 (1.83)	17.37	17.51 (17.62)	36.58	37.57 (37.08)
0.8% (3)	6.73	7.21 (6.97)	33.62	33.56 (33.59)	2.33	2.09 (2.21)	15.40	16.74 (16.07)	41.91	40.49 (41.20)
AIB[d]										
0.5% (7)	6.49	7.21 (6.85)	37.95	35.94 (36.95)	1.54	2.29 (1.92)	16.73	18.35 (17.54)	37.28	36.20 (36.74)
1% (5)	6.85	7.96 (7.41)	35.08	29.74 (32.41)	2.25	3.19 (2.72)	17.88	21.37 (19.63)	37.94	37.74 (37.84)
AIB(0.5%)+HCHO[e] (4)	7.65	7.23 (7.44)	38.28	32.91 (35.60)	0.99	2.62 (1.81)	19.24	20.71 (19.98)	33.84	36.53 (35.19)
Control (6)	8.41	7.09 (7.75)	38.97	34.81 (36.89)	1.39	2.77 (2.08)	15.69	16.11 (15.9)	35.52	39.22 (37.37)

[a]Nitrogen. [b]Period in sheep digestion trial.
[c]Values in parentheses are means. [d]Ammonium Isobutyrate.
[e]Formaldehyde - 1.25% of a 37% solution.

data in Table 40 can only be interpreted from a qualitative aspect. To obtain quantitative changes in composition, dry matter recoveries must also be accurately determined.

For each haylage, fibrous consituents and acid detergent insoluble nitrogen (AD-N) were analyzed on all three samples taken during each of three sheep feeding trials. Again, these samples represented approximately the three positions in the silo (top, middle and bottom). Values in Table 41 are comparable among treatments. Again, quantitative effects of chemical additives on fibrous constituents could not be accurately determined due to lack of analyses on original forage. Under laboratory and farm conditions, acid-detergent insoluble nitrogen as a percent of dry matter and as a percent of total nitrogen(AD-N/N) was increased in forages heated artificially or naturally (Van Soest, 1965; Goering et al. 1972; Huber et al. 1972). In the present study, close relationships between haylage temperature and these acid detergent insoluble nitrogen fractions were evident (Table 42). Haylage temperature computed as mean storage temperature or as degree-days above 35 C was significantly ($P < .05$) and positively correlated with all four analytical fractions although the correlation coefficients were slightly higher for degree-days above 35 C.

Table 41. Fibrous Constituents and Acid Detergent Insoluble Nitrogen in Control and Treated Haylages. Values are Means of Three Determinations From Three Composite Samples.

	Propionic Acid		AIB[b]		AIB + HCHO[c]	Control
	0.4% (8)[a]	0.8% (3)	0.5% (7)	1% (5)	(4)	(6)
Cell walls (% DM)						
P-1	54.2	56.4	52.8	53.9	55.6	59.3
P-2	52.7	53.2	48.6	47.3	48.7	52.9
P-3	52.5	56.4	53.8	50.3	49.1	60.0
$\bar{X}$	53.1	53.3	51.7	50.5	51.1	57.4
Hemicellulose (% DM)						
P-1	7.8	13.4	6.7	10.0	5.2	8.0
P-2	9.0	9.5	5.7	6.7	5.9	10.0
P-3	8.3	11.5	8.6	6.3	5.7	12.1
$\bar{X}$	8.4	11.5	7.0	7.7	5.6	10.0
Cellulose (% DM)						
P-1	35.2	33.8	35.0	33.5	37.7	40.2
P-2	34.2	35.1	33.6	31.7	33.3	33.1
P-3	34.8	35.7	35.4	34.6	33.8	38.2
$\bar{X}$	34.7	34.9	34.7	33.3	34.9	37.2
Lignin(%DM)						
P-1	11.2	9.2	11.1	10.4	12.7	11.2
P-2	9.5	8.7	9.3	8.9	9.5	9.8
P-3	9.4	9.1	9.8	9.3	9.7	9.6
$\bar{X}$	10.1	9.0	10.1	9.5	10.6	10.2
ADF[d](%DM)						
P-1	46.4	43.0	46.1	43.8	50.4	51.4
P-2	43.7	43.8	42.9	40.6	42.7	42.9
P-3	44.2	44.8	45.2	44.0	43.4	47.9
$\bar{X}$	44.7	43.9	44.7	42.8	45.5	47.4

Table 41. (Continued)

	Propionic Acid 0.4% (8)	Propionic Acid 0.8% (3)	AIB 0.5% (7)	AIB 1% (5)	AIB + HCHO (4)	Control (6)
ADF-N[e] (%DM)						
P-1	0.36	0.29	0.32	0.44	0.62	0.50
P-2	0.32	0.29	0.29	0.36	0.32	0.37
P-3	0.27	0.26	0.30	0.38	0.34	0.33
$\bar{X}$	0.32	0.27	0.30	0.39	0.43	0.40
ADF-N/N[f] (%)						
P-1	12.7	11.7	12.1	15.4	20.3	19.8
P-2	11.3	11.0	10.0	12.8	10.8	13.7
P-3	10.0	9.6	10.1	11.2	10.2	12.8
$\bar{X}$	11.3	10.8	10.7	13.1	13.7	15.4

[a]Silo number.

[b]AIB = Ammonium Isobutyrate.

[c]HCHO = Formaldehyde -1.25% of a 37% solution.

[d]ADF = Acid detergent fiber.

[e]Acid detergent insoluble nitrogen as a percent of dry matter.

[f]Acid detergent insoluble nitrogen as a percent of total nitrogen.

Table 42. Linear Regressions Between Haylage Temperatures and Four Analytical Fractions.

X	AD-N[a]			AD-N/N[b]	
	N[c]	r	Equation	r	Equation
Mean storage temperature C	18	.67**[d]	$\hat{Y}$ = -.003 + .009 X	.80**	$\hat{Y}$ = -2.17 + .38 X
Days above 35 C	18	.51*	$\hat{Y}$ = .26 + .003 X	.67**	$\hat{Y}$ = 8.44 + .15 X
Degree-days above 35 C	18	.72**	$\hat{Y}$ = .30 + .0001 X	.80**	$\hat{Y}$ = 10.45 +.01 X
		ADF[e]		ADL[f]	
Mean storage temperature C	18	.47*	$\hat{Y}$ = 37.18 + .19 X	.66**	$\hat{Y}$ = 5.88 + .10 X
Days above 35 C	18	.37	$\hat{Y}$ = 42.91 + .07 X	.54*	$\hat{Y}$ = 8.84 + .04 X
Degree-days above 35 C	18	.48*	$\hat{Y}$ = 43.76 + .0001 X	.70**	$\hat{Y}$ = 9.32+.0001 X

[a] AD-N = Acid detergent insoluble nitrogen as a percent of dry matter.

[b] AD-N/N = Acid detergent insoluble nitrogen as a percent of total nitrogen.

[c] N = number of samples; haylage taken from each level of silo is considered as an independent sample.

[d] ** $p < 0.01$; * $p < 0.05$.

[e] ADF = acid detergent fiber as a percent of dry matter.

[f] ADL = acid detergent lignin as a percent of dry matter.

Number of days above 35 C was significantly but not highly correlated with AD-N/N and ADL. Acid detergent insoluble N as a percent of total N was related with haylage temperature to a greater extent than were other analytical fractions. Acid detergent fiber was generally less affected by the storage temperature than was lignin and this was probably due to the fact that acid detergent insoluble N fraction is primarily associated with ADL and not ADF (Van Soest, 1965).

Trends in composition among levels in the silo were inconsistent except that acid detergent lignin (ADL), AD-N and AD-N/N were greater in the upper portion than in lower portion (Table 41). Since haylage temperature during storage was greatest for the upper portion of the silo (Table 35) and temperature increment was positively related to acid detergent lignin and insoluble N fractions (Table 42), the greater value of AD lignin found for the haylage in the upper portion was presumably due to the higher temperature developed in that portion of the silo. In fact, the correlation coefficients were highly significant ($P < .01$) between acid detergent insoluble N fractions and haylage temperature of haylages from the three silo levels (average of the 6 silos) (Table 43). A similar effect of silo levels was also evident between temperature development and acid detergent lignin or fiber. The significance

Table 43. Linear Regressions Between Haylage Temperature Measured at Three Silo Levels and Four Analytical Fractions.

X	n[a]	Degree-days above 35 C r	Degree-days above 35 C Equation	Mean storage temperature C r	Mean storage temperature C Equation
AD[b]-N[c], % DM[d]	3	.992**[e]	$\hat{Y}$ = 0.30 + .0002 X	.98**	$\hat{Y}$ = 0.05 + 0.008 X
AD-N x 100/N	3	.999**[f]	$\hat{Y}$ = 10.41 + .009 X	.998**	$\hat{Y}$ = -.13 + 0.32 X
AD-lignin	3	.95*	$\hat{Y}$ = 9.19 + 0.003 X	.93	$\hat{Y}$ = 5.60 + 0.11 X
AD-fiber	3	.73	$\hat{Y}$ = 43.6 + 0.01 X	.69	$\hat{Y}$ = 37.76 + 0.18 X

[a] n = number of samples, average value of the same silo level for the total of 6 silos.

[b] AD = acid detergent.

[c] DM = dry matter.

[d] N = nitrogen.

[e] ** $p < 0.01$; * $p < 0.05$.

of silo level on animal performance will be discussed in the following section.

V. Effect of haylage Treatments on Digestion Coefficients and Nitrogen Utilization

Data collected from three sheep digestion trials were analyzed by two way analysis of variance. An example is given in Table 44.

Table 44. Table of Analysis of Variance Used to Analyze Data of Sheep Feeding Trials[a]

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	5	28.59	28.60**[b]
Period (silo level)	2	262.87	36.80**
Treatment x period[c]	10	11.05	1.55
Error	18	7.14	
Total	35		

[a]Using analysis of variance of nitrogen digestibility as an example.

[b]** $P < .01$

[c]Interaction between treatment and period.

Chemical treatments had no significant ($P > .05$) effects on maximum haylage DM intake or intake during sheep digestion trials although slightly higher intakes for propionic acid treated haylage and lower intakes for AIB treated haylage were found (Table 45).

Table 45. Dry Matter Intake, Digestion Coefficients, Nitrogen Utilization and Body Weight Changes of Sheep Fed Control and Treated Haylages.

	Propionic Acid 0.4% (8)[A]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(0.5%) + HCHO[B] (4)	Control (6)
No. sheep	6	6	6	6	6	6
Haylage DM[C] intake (%BW[D])						
Maximum	3.99[a][E]	3.80[a]	3.04[a]	3.28[a]	3.14[a]	3.42[a]
During digestion trials	3.22[a]	2.80[a]	2.40[a]	2.83[a]	2.54[a]	2.76[a]
Digestion Coefficient(%)						
Dry matter	53.4[a]	53.74[a]	52.79[ab]	49.80[b]	49.64[b]	51.23[ab]
Organic matter	54.89[a]	54.61[a]	52.82[ab]	50.86[b]	50.87[b]	52.41[a]
Cell wall constituents	45.4[a]	46.38[a]	41.89[ab]	38.25[b]	38.15[b]	47.60[a]
Acid detergent fiber	47.6[ab]	46.91[ab]	47.17[ab]	41.21[c]	44.49[bc]	49.46[a]
Nitrogen Utilization						
Digestibility (%)	61.8[a]	56.86[b]	61.61[a]	60.16[a]	61.18[a]	55.73[b]
N balance (g N/day)	4.11[a]	3.53[b]	1.61[b]	3.06[ab]	1.53[b]	0.60[b]
N retained as % absorbed	25.95[a]	9.00[bc]	12.86[b]	14.23[a]	1.65[b]	1.41[c]
N retained as % consumed	15.85[a]	5.59[bc]	8.25[b]	8.96[a]	7.32[b]	1.56[c]
BW changes (g/day)	-36[a]	-54[a]	-91[a]	-145[a]	-95[a]	-136[a]

[A] Silo number.

[B] Formaldehyde -1.25% of a 37% solution.

[C] DM = dry matter.

[D] BW = body weight.

[E] Values with different superscripts are significant ($p < 0.05$).

None of the chemical treatments had a significant ($P < .05$) effect on digestion coefficients for dry matter or organic matter as compared with values for untreated haylage (silo 6). Among the chemical treatments, however, N and DM digestion coefficients were significantly ($P < .05$) higher for haylages treated with propionic acid (.4 and .8%) than for haylages treated with 1% AIB and mixture of AIB and formaldehyde. All chemical treatments tended to reduce digestion coefficients of cell walls (CW) and acid detergent fiber (ADF) but the reduction was significant ($P < .05$) only for 1% AIB or AIB +formaldehyde. Apparently, the AIB treatments failed to improve fiber digestion although AIB contains an essential nutrient, isobutyric acid, for rumen cellulolytic bacteria (Bryant, 1973).

All treatments except .8% propionic acid significantly improved nitrogen (N) digestion coefficients ($P < .05$). Nitrogen balance and N retention as a percent of N absorbed or consumed were greater for all treated haylages than for control, however, only the .4% propionic acid treatment significantly ($P < .05$) improved all three responses (Table 45). Although the average values for N balance were positive, negative body weight changes would suggest that none of the haylages had a nutritive value sufficient for

sheep maintanence. Sheep fed control and 1% AIB treated haylages lost more weight than did sheep fed other haylages although the differences were not significant ($P > .05$). According to the National Research Council (1968), the DM requirement for sheep used in the present study is about 1 kg or about 4% of their body weight. Also, the daily allowance for total digestible nutrients (TDN) is 0.6 kg and that for digestible protein (DP) is 65 gram. The actual daily intakes of dry matter, TDN and DP were 0.7 kg, 0.4 kg, and 76 gram for sheep used in the present study. Although the digestible protein intake was adequate, the consumption of total dry matter and TDN were 2/3 that of requirement and this inadequate energy intake probably accounts for the negative weight gains observed in the present study.

Goering et al. (1972) reported significant relationships between ADF-N/N and digestibility of N and dry matter and for the present study these correlation coefficients were calculated (Table 46). Digestion coefficients for dry matter and nitrogen were all signigicantly ($P < .05$) correlated with acid detergent (AD) insoluble N fractions and total nitrogen (N), but the correlation coefficients were clearly higher for AD-insoluble N as a percent of dry matter. Total nitrogen content was positively related with nitrogen digestibility ($r = .54$) but was negatively related with dry

Table 46. Linear Regressions for In Vivo Dry Matter and Nitrogen Digestion Coefficients on Two Acid Detergent Insoluble Nitrogen Fractions and Three Temperature Measurements.

X	DMD[a]			ND[b]	
	n[c]	r	Equation	r	Equation
X_1 AD[d] insoluble N[e], %DM[f]	36	-.90**[g]	$Y = 70.76-54.0\ X_1$	-.82**	$Y = 86.21-75.8\ X_1$
X_2 AD insoluble Nx100/N	36	-.44**	$Y = 58.0-0.49\ X_2$	-.71**	$Y = 74.86-1.22\ X_2$
X_3 Total N, %DM	36	-.41*	$Y = 67.56-5.58\ X_3$	0.54**	$Y = 27.51+11.32\ X_3$
X_4 Mean storage temperature (C)	18	-.30	$Y = 56.08-0.11\ X_4$	-.85**	$Y = 84.47-0.64\ X_4$
X_5 Days above 35 C	18	-.12	$Y = 52.13-0.02\ X_5$	-.85**	$Y = 67.68-0.31\ X_5$
X_6 Degree-days above 35 C	18	-.33	$Y = 52.22-.001\ X_6$	-.81**	$Y = 62.86-0.01\ X_6$

[a] DMD = dry matter digestion coefficient.

[b] ND = nitrogen digestion coefficient.

[c] n = number of samples. For variables 1 and 3, sample number was total number of sheep used in three digestion trials. For variables 4 to 6, sample number was total silo levels (3 levels for each silo x 6 silos).

[d] AD = acid detergent.

[e] N = nitrogen.

[f] DM = dry matter.

[g] ** $p < 0.01$; * $p < 0.05$.

matter digestibility (-.41). This negative relationship must be considered abnormal (Holter and Reid, 1959). These calculations strongly support the idea that total crude protein analysis is inadequate in estimating nutritive value of haylage and other more specific laboratory methods such as N solubility in acid detergent solution should be used (Goering et al. 1972; Thomas and Hillman, 1972; and Goering and Adams, 1973).

A close relationship between haylage temperature and in vivo responses should be expected since temperature was significantly correlated with AD-insoluble N (Table 43). Nitrogen digestion coefficient was significantly ($P < .01$) correlated with all three expressions quantitating haylage temperature development given in Table 46 and no marked differences in correlations were noted among these three different methods of temperature computation. Dry matter digestion coefficients were negative but insignificantly related to haylage temperature. The differential significances of these negative coefficients demonstrates the detrimental effect of heat development (i.e. above 35 C) on ND of haylage exceeds the effect on DMD.

The sheep digestion trial was performed over 3 time periods of about 17 days each with 2 sheep on each haylage during each period. A significant effect of

period on several digestion parameters was noted (Table 47).

Acid detergent fiber was significantly more digestible during the first period than in the second period. Nitrogen utilization parameters steadily improved during sequential periods. Thus nitrogen digestibility values were more satisfactory for the lower portions of the silo. Sheep lost significantly less body weight during the third period than during the first two periods. Improved nitrogen utilization should partially explain the changes in sheep body weight. Previous data presented (Table 43 and 46) have indicated that haylage temperatures were positively correlated with level in the silo and with amount of AD-N or AD-N/N and to storage temperatures. Values involved in these relationships are summerized in Table 48. Total protein content of the three levels is also shown in this table and attempts to illustrate the poor relationship of total N to *in vivo* N digestion coefficients and other parameters closely related to N digestion coefficients.

Figure 7 summarizes relationships among several variables considered to be important in haylage and animal production along with their correlation coefficients from this trial. Degree-days above 35 C for the top silo level was positively correlated but

Table 47. Sheep Performance Data Which Were Significantly Influenced by the Effect of Time (Level in the Silo).

Items	Period 1	Period 2	Period 3
Digestion coefficient			
Acid detergent fiber (%)	48.24[a][1]	43.99[b]	46.17[ab]
Nitrogen (%)	54.33[c]	60.94[b]	63.38[a]
Nitrogen balance (g N/day)	0.902[b]	1.044[b]	4.105[a]
N[2] retained x 100/N absorbed	6.52[b]	6.92[b]	24.11[a]
N retained x 100/N consumed	3.86[b]	4.39[b]	15.51[a]
Body weight changes (g/day)	-132.45[b]	-112.49[b]	-30.39[a]

[1] Values with different superscripts are significant ($p < 0.05$).

[2] N = nitrogen.

Table 48. Several Measurements Related to the Levels in the Silo.

	Levels in silos		
	Top	Middle	Bottom
Temperatures			
Degree-days above 35 C	546	130	21
Average temperature during storage period C	48	37	33
Chemical composition			
Total protein (N), %DM[a]	17.11	17.45	18.47
Acid detergent insoluble N[b], %DM	0.421	0.321	0.313
Acid detergent insoluble N x100/N	15.34	11.59	10.60
Acid detergent lignin, %DM	10.98	9.28	9.51
In vivo responses			
Nitrogen digestion coefficient, %	54.33	60.94	63.38
Dry matter digestion coefficient, %	50.51	52.72	51.25
Organic matter digestion coefficient, %	52.21	53.64	52.38
Body weight changes (g/day)	-132.45	-112.49	-30.39

[a]DM = dry matter.

[b]N = nitrogen.

Figure 7. Schematic Presentation of Relationships Among Variables Considered to be Important in Haylage Evaluation and Their Simple Linear Correlation Coefficients. Number of Sample Used is Indicated by Line Connecting the Two Variables, i.e. —·—·— Representing 18 Samples; — — — Representing 6 samples and ———— Representing 36 Samples.

[a]DM = dry matter.

[b]N = nitrogen.

* $p < 0.05$.

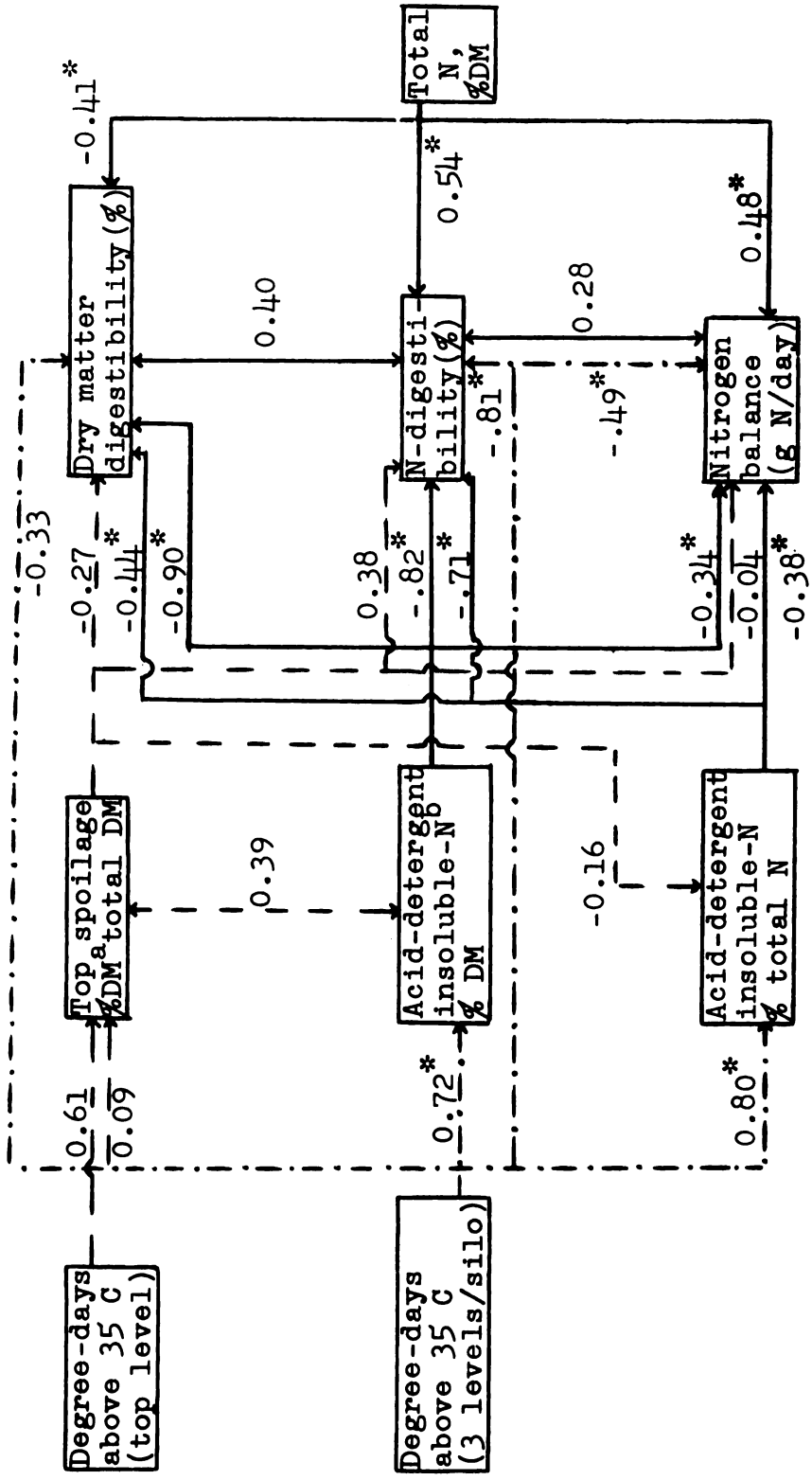
Degree-days above 35 C (top level)
Degree-days above 35 C (3 levels/silo)
Top spoilage %DM a total DM
Acid-detergent insoluble-N b % DM
Acid-detergent insoluble-N % total N
Dry matter digestibility (%)
N-digestibility (%)
Nitrogen balance (g N/day)
Total N, %DM
0.61
0.09
0.72*
0.80*
0.39
-0.16
-0.33
-0.27
-0.44
-0.90*
0.38
-.82*
-.71*
-.81*
-.49*
-0.34*
-0.04
-0.38*
0.40
0.54*
0.28
0.48*
-0.41*

Figure 7.

insignificantly ($P > .05$), with the extent of top spoilage (+ .61). No correlation was observed between the average of degree-days above 35 C measured from three silo levels and top spoilage. The extent of top spoilage was not significantly ($P > .05$) related with either acid detergent insoluble fractions or *in vivo* responses. However, when heat development was quantified as degree-days above 35 C the significant ($P < .05$) negative effect of relatively high temperatures on *in vivo* N availability of resulting haylages was evident. The reduction in haylage N availability by heat was presumably due to the increased formation of acid detergent insoluble N fraction which is indigestible by ruminents. Thomas and Hillman (1972) reported that as much as 20% of the forage N is commonly found as acid detergent insoluble N in haylage made in the state of Michigan. Analytical and sheep performance data obtained from this study were not used in the regression analyses of nitrogen digestibility on analytical fractions presented in Part 1 of this thesis. For the 21 earlier MSU samples used in Part 1, ND was significantly regressed with acid detergent insoluble N (Table 14). Similar results were found using data of this experiment. Acid detergent insoluble N as a percent of dry matter was highly correlated with nitrogen and dry matter digestion coefficients. On the other hand,

total nitrogen content was clearly inferior to AD-insoluble N % DM in estimating nutritive value of haylages for ruminants.

VI. Effect of Chemicals on Haylage Consumption, Milk Production and Composition of Milk of Lactating Cows

Haylage dry matter and total dry matter consumption by lactating cows fed the haylages for a 49-day feeding period are presented in Table 49. During the 12-day preliminary period, all cows received regular herd haylage with no differences among groups. Haylage consumption (kg DM/cow/day) increased by a factor of 1.32 to 1.70 for the treated haylages with no significant differences ($P > .05$) among groups. When haylage dry matter consumption was calculated as percent of body weight then significant differences were found among groups during the experimental period with cows received .8% propionic acid treated haylage consuming significantly ($p < .5$) more than cows fed the other haylages. Total dry matter consumption (haylage DM + grain DM, kg/day) was not significantly different among groups. However, the percent increase was significantly lower for the 1% AIB treatment group than for the other four treatment groups. Total DM consumption as a percent of body weight was not significantly ($P > .05$)

Table 49. Consumption of Haylage and Total Dry Matter and Body Weight (BW) Changes of Lactating Cows Fed Control and Treated Haylages.

	Propionic Acid 0.4% (8)[1]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(0.5%) + HCHO[2] (4)	Control (6)
Haylage DM[3] Consumption						
Preliminary period[4](kg/day)	6.03[a][5]	6.33[a]	5.52[a]	6.42[a]	5.98[a]	6.20[a]
Treatment period[6](kg/day)	9.71[a]	10.39[a]	9.21[a]	8.48[a]	9.63[a]	8.68[a]
Change(Treat.x100/Prelim.)	161[a]	164[a]	167[a]	132[a]	161[a]	140[a]
Preliminary period (% BW)	1.16[a]	1.16[a]	1.00[a]	1.27[a]	0.99[a]	1.19[a]
Treatment period (%BW)	1.68[ab]	1.77[a]	1.48[b]	1.55[b]	1.50[b]	1.53[b]
Change(Treat.x100/Prelim.)	149[a]	157[a]	157[a]	126[a]	153[a]	132[a]
Total DM Consumption						
Preliminary period(kg/day)	12.87[a]	13.07[a]	12.52[a]	13.23[a]	12.58[a]	13.09[a]
Treatment period (kg/day)	15.83[a]	16.60[a]	15.65[a]	14.29[a]	16.10[a]	14.92[a]
Change(Treat.x100/Prelim.)	123[a]	127[a]	125[a]	108[b]	128[a]	114[ab]
Preliminary period(% BW)	2.43[ab]	2.28[abc]	2.19[bc]	2.62[a]	2.03[c]	2.50[ab]
Treatment period (% BW)	2.76[a]	2.79[a]	2.56[a]	2.65[a]	2.46[a]	2.69[a]
Change(Treat.x100/Prelim.)	114[abc]	122[a]	118[ab]	103[c]	122[a]	108[bc]
Body Weight(BW) changes(kg/day)	0.885[a]	0.523[a]	0.634[a]	0.553[a]	0.617[a]	0.615[a]

[1]Silo number.

[2]HCHO = Formaldehyde, 1.25% of a 37% solution.

[3]DM = dry matter.

[4]Preliminary period was 14 days.

[5]Values on one line with different superscripts are significant ($p < 0.05$).

[6]Treatment period was 49 days but only last 42 days were used in observations.

different during treatment period, but the percent of increase was significantly ($P < .05$) greater for the .8% propionate and AIB + formaldehyde groups than that of the control and 1% AIB groups. No significant ($P > .05$) differences for average body weight changes were detected among treatment groups (Table 49).

Data on milk and fat corrected milk (FCM) yields are shown in Table 50. No significant differences ($P > .05$) were found for average daily production during preliminary period, during treatment period, adjusted daily production (adjusted by covariance analyses) and persistency (production during treatment period x 100/ production during preliminary period). Adjusted milk yields were somewhat less for the 2 AIB treated haylages than for the other four groups (17.3 vs. 18.5).

Ranges of persistency and number of cows having persistencies over 100 are given in Table 50. All but the 1% AIB group had at least two cows with persistencies over 100. Data of efficiency of production calculated as FCM milk yield (kg/day)/ total digestible energy (kg/day) are also shown in Table 50. None of the chemical treatments improved the gross efficiency of milk production as compared to untreated haylage. Among the five treatment groups, efficiencies were lower for .5% AIB and .8% propionic acid groups than the other three groups but no marked differences were

Table 50. Milk Production During Preliminary and Experimental Period of Cows Fed Control and Treated Haylages.

	Propionic Acid		Ammonium Iso-butyrate (AIB)		AIB (0.5%) + HCHO[2]	Control
	0.4%	0.8%	0.5%	1%		
	(8)[1]	(3)	(7)	(5)	(4)	(6)
Actual milk production						
Preliminary[3] period (kg/day)	19.78[a][4]	18.87[a]	20.19[a]	20.68[a]	19.01[a]	21.00[a]
Treatment[5] period (kg/day)	18.69[a]	17.37[a]	18.69[a]	17.60[a]	18.05[a]	19.87[a]
Adjusted[6] Treat. period (kg/day)	18.92[a]	18.51[a]	17.37[a]	17.33[a]	19.05[a]	19.10[a]
Persistency (%)[7]	94.00[a]	94.40[a]	89.70[a]	85.50[a]	94.70[a]	93.40[a]
Range of persistency (%)	105-69	146-56	102-77	96-74	120-81	110-82
No. cows over 100 persist.	3/8	2/8	2/8	0/8	2/8	2/8
Efficiency						
Fat-corrected milk (kg/day)/ TDN[8] (kg/day)	1.69	1.60	1.50	1.76	1.72	1.79
Fat-corrected milk production						
Adjusted Treat. period (kg/day)	16.28[a]	16.15[a]	14.15[a]	14.92[a]	16.33[a]	16.15[a]
Persistency (%)	91.90[a]	95.60[a]	81.00[a]	85.20[a]	93.10[a]	87.90[a]

Table 50. (Continued)

[1]Silo number.

[2]HCHO = Formaldehyde, 1.25% of a 37% solution.

[3]Preliminary period was 14 days.

[4]Values with different superscript in one line are significantly different. ($p < 0.05$).

[5]Treatment period was 49 days but only last 42 days were used in observations.

[6]Adjusted by covarience analyses.

[7]Production during treatment period x 100/production during preliminary period.

[8]TDN = total digestible energy.
TDN content of haylages was calculated as follow:
TDN = OMD x OM(.01 + .000125 EE) (Lofgreen, G.P. 1953. J.Anim.Sci. 12:359)
Where OMD = organic matter digestibility obtained from sheep feeding trials.
OM = organic matter as a percent of dry matter in the feed.
EE = ether extract as a percent of organic matter in the feed.
TDN content of grain mixture was assumed to be 75%.

found among the latter groups. Adjusted means of FCM and persistencies of FCM production were lower ($P > .05$) for the AIB groups than for the other groups.

Waldo (1973) commented on using daily milk production to compare the quality of silages. Daily milk production will not easily distinguish between quality of silages fed with grain, and he stated that daily body weight gain was a more sensitive criterion than daily milk production. Daily body weight gain can easily be zero with poor quality silages, and the loss of intake potential is much greater than the loss of digestibility potential with poor silages. However, the intakes of poor silages are relatively less depressed when fed with grain to milking cows then when fed alone to growing animals (Waldo, 1973). This may explain why insignificant differences of milk production between treatments occurred in the present study.

Value for milk solids and fat are shown in Table 51. Adjusted means for total solids and fat were slightly higher ($P > .05$) for the .8% propionic acid group than for the other five groups. Higher rates of application of propionic acid and AIB resulted in slightly higher adjusted total milk solids and milk fat than did the lower application rates. In any event, propionic acid and AIB added to alfalfa haylage at the

Table 51. Composition of Milk Produced During Preliminary and Experimental Period of Cows Fed Control and Treated Haylages.

	Propionic Acid 0.4% (8)[1]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(0.5%) + HCHO[2] (4)	Control (6)
Total Solids (%)						
Preliminary period[3]	11.77	11.83	12.27	11.99	11.88	12.59
Treatment period[4]	11.74	12.10	11.85	12.05	11.77	11.86
Adjusted[5] total solids	12.00	12.30	11.70	12.10	11.90	11.50
Milk Fat(%)						
Preliminary period	3.09	3.13	3.45	3.16	3.03	3.56
Treatment period	2.95	3.15	2.96	3.11	2.91	3.20
Adjusted milk fat	3.03	3.21	2.84	3.15	3.03	3.02

[1]Silo number.

[2]HCHO = Formaldehyde, 1.25% of a 37% solution.

[3]Preliminary period was 14 days.

[4]Data were recorded for entire 49 days of experimental period, but only the last 42 days were used in calculations.

[5]Adjusted by covariance analyses.

rates used in the present study had no significant effect on total milk solids or fat content. Increased rumenal production or administration of propionic acid to lactating cows has frequently resulted in reduced milk fat production (Rook and Balch, 1961; Rook et al. 1965). Balch et al. (1967) demonstrated a 7.3% reduction in daily fat production when a daily dose of 725 g propionic acid was infused into the rumen of lactating cows. The average consumption of propionic acid by cows fed 0.8% propionic acid of the present experiment was only about 180 g which is probably not sufficient to have any significant effect on milk fat production.

Effect of haylage additives on various aspects of haylage preservation is summarized in Table 52. All values for control haylage were adjusted to 100 and others expressed in relation to that value except for days that haylage temperature was below 35 C during the refermentation trial. All treatments reduced temperature development during storage, feeding and refermentation. Treatments of .4% propionic acid and .5% AIB should be considered superior to others in these aspects. Extent of top spoilage was not reduced by treatments except .8% propionic acid and AIB + formaldehyde. A positive but insignificant relationship between temperature reduction and the extent of top spoilage was found. Propionic acid treatments slightly reduced the haylage

Table 52. Comparisons of Various Effects Related to Haylage Treatments When Values for Control Haylage Were Expressed as 100.

	Control (6)	Propionic acid 0.4% (8)	Propionic acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(0.5%) + HCHO[b] (4)
Temperature Reduction						
During storage[c]						
Mean temperature	100	88	88	84	93	91
Degree-days above 35 C	100	37	48	20	63	50
During feeding-surface temperature[d]						
Mean temperature	100	98	87	89	87	91
During refermentation[e]						
Days temperature below 35 C	1	>50	10	25	25	10
DM[f] loss (% DM stored)[g]						
Top spoilage	100	111	41	104	123	69
Chemical composition[h](%DM)						
Total fungal colonies ($1x10^6$/g)	100	69	24	56	23	110
Total organic acids	100	120	112	128	189	116
Acetic acid	100	91	73	89	105	88
Lactic acid	100	0	0	0	18	30
Acid-detergent lignin, %DM	100	80	68	75	98	108
Acid-detergent insoluble N,%DM	100	73	70	69	85	89
Acid-detergent insoluble N/N	100	73	70	69	85	89

Table 52. (Continued)

	Control (6)	Propionic acid 0.4% (8)	Propionic acid 0.8% (3)	Ammonium Isobutyrate(AIB) 0.5% (7)	Ammonium Isobutyrate(AIB) 1% (5)	AIB(0.5%) + HCHO (4)
In Vivo responses						
Intake(kg/100 kg body weight)						
Sheep[i]						
Maximum intake	100	117	111	89	96	92
Intake during trials	100	117	101	87	103	92
Lactating cows[j]	100	110	116	97	101	98
Digestion coefficients[k]						
Dry matter	100	104	105	103	97	97
Nitrogen	100	111	112	111	108	110
Cell walls	100	95	97	88	80	80
Nitrogen(N) utilization[l]						
N balance	100	685	588	268	510	255
N retention, % absorbed	100	1840	638	912	1009	117
N retention, % intake	100	1016	358	529	574	469
Milk production[m]						
Actual yield(kg/day)	100	94	87	94	86	91
Adjusted yield(kg/day)	100	99	97	91	91	100
Adjusted FCM[n] yield(kg/day)	100	101	100	88	92	101
Persistency (%)	100	101	101	96	92	101

Table 52. (Continued)

	Control (6)	Propionic acid 0.4% (8)	Propionic acid 0.8% (3)	Ammonium Isobutyrate(AIB) 0.5% (7)	Ammonium Isobutyrate(AIB) 1% (5)	AIB(0.5%) + HCHO (4)
Efficiency (FCM/TDN[o])	100	94	89	84	98	96
Milk composition[p] (%)						
Adjusted fat	100	100	106	94	104	100
Adjusted total solids	100	104	107	102	105	103
Total number of relative in vivo responses over 100	0/16	12/16	12/16	6/16	8/16	9/16

[a]Silo number.

[b]HCHO = Formaldehyde, 1.25% of a 37% solution.

[c]Table 34.

[d]Table 36.

[e]Figure 6.

[f]DM = dry matter.

[g]Table 37.

[h]Table 38.

[i]Table 44.

[j]Table 48.

[k]Table 44.

[l]Table 44.

[m]Table 49.

[n]fat correlated milk.

[o]Table 50.

[p]Table 51.

acetic acid concentration while no effect was noted for AIB treatments. Lacic acid concentration was essentially zero for haylages treated with propionic acid (.4 and .8%) and .5% AIB. Extremely small amounts of lactic acid was found in haylages treated with 1% AIB and the mixture of AIB and formaldehyde. Quantities of acid detergent (AD) lignin and insoluble N fractions were lower for .5% AIB, .8% and .4% propionic acid treatments than for the other treatments. The comparison is similar to the extent of temperature development during storage. In vivo response measured from sheep were generally highly related to the quantity of AD insoluble N fractions or heat development during storage. All treatments markedly improved nitrogen utilization values. No increased responses were obtained from lactating cows fed treated haylages but propionic acid treatments gave slightly "better" responses than did AIB treatments. Values listed in the bottom line of Table 52 represent the total number of in vivo responses with a relative value over 100. None of the chemical treatments produced 16 improved in vivo responses but propionic acid treatments had 12 values above 100 while AIB treatments had six to nine. The principle effect of these acid treatments was on reducing fungal counts, storage temperature and decreasing insoluble nitrogen fractions in the haylage and increasing

nitrogen utilization in the animal. Unfortunately, the relatively action of chemicals in reducing dry matter loss during fermentation could not be evaluated quantitatively because of incomplete recording of the "good" haylage fed. Hence, no quantitative comarisons or economic evaluations with respect to animal production can be made for these chemically treated haylages. Further trials are urgently needed.

SUMMARY AND CONCLUSIONS

Part 2

The value of propionic acid (.4 and .8%), ammonium isobutyrate (AIB, .5 and 1%) and a mixture of AIB (.5%) and formaldehyde (1.25% of a 37% solution) in preserving the nutritive value of alfalfa haylage (50% DM) was evaluated by the following criteria: heat development during storage, extent of spoilage, total fungal counts, chemical composition, performance of sheep and lactating cows. Levels used for propionic acid and AIB were comparable on a molar basis.

All chemical treatments reduced haylage temperature during storage. When heat development was quantitated and expressed as degree-days above 35 C, the ranking of chemicals in preventing excessive heating was as follows: .5% AIB .4% propionic acid .8% propionic acid .5% AIB + formaldehyde 1% AIB.

None of the chemicals were completely effective in preventing heat development near the haylage surface when silos being emptied during the feeding period. The extent of top spoilage was somewhat positively related with the heat development during storage. The

lowest top spoilage was noted for .8% propionic acid treated haylage. Total fungal counts were reduced by about 30 to 70% by all treatments except AIB (.5%) + formaldehyde treatment which had no effect on total fungal counts. Chemical composition was similar among haylages except that acid detergent lignin and insoluble N fractions were greater in those haylages which had the greater quantity of heat development.

Dry matter intake determined by using both sheep and lactating cows was slightly but consistently greater for propionic acid treated haylage than for AIB treated haylages. Digestion coefficients for dry matter were not markedly affected by treatments, but nitrogen digestibility was generally improved by a factor of 1.1. Marked improvements in N utilization were observed for all treatments. Significant negative correlations were found between measures of N utilizations and both acid detergent insoluble N fractions as well as degree-days above 35 C during storage. Digestion coefficients for fibrous components of the haylages were somewhat reduced by the treatments, particularly AIB treatments. Milk production and composition were not significantly affected by the treatments. Propionic acid treatments, however, produced slightly better results than did AIB treatments. Results from this study indicated that propionic acid was only

slightly better than AIB in preserving the nutritive value of alfalfa haylage, but no marked superioety was found for higher levels of acid application. Considering the practical application situation, AIB is more acceptable than propionic acid with respect to oder and corrosive nature.

APPENDIX

Appendix Table 1. Description of Samples Used in Forage Protein Quality Evaluation Experiments.

Sample no.	Species	Type of preservation	Dry matter (%)	Laboratory Identification
1	Alfalfa-Dupuit	Hay	87	7104-Dupuit
2	Alfalfa-Vernal	Hay	85	7104-Vernal
3	Alfalfa-high nutr. value strain	Hay	87	7104-high
4	Alfalfa-low nutr. value strain	Hay	85	7105-low
5	Alfalfa grass mixture	Hay	87	7105-Boles
6	Alfalfa	Stacked hay treated with 0.5% chemostor[a]	56	7108-2
7	Alfalfa	Stacked hay treated with 1.5% chemostor	66	7108-3
8	Alfalfa	Stacked hay treated with 0.5% chemostor	48	7108-8
9	Alfalfa	Stacked hay treated with 1.5% chemostor	54	7108-9
10	Alfalfa	Stacked hay treated with 0.5% chemostor	56	7109-2
11	Alfalfa	Stacked hay treated with 1.5% chemostor	66	7109-3
12	Alfalfa	Stacked hay treated with 0.5% chemostor	48	7109-8

Appendix Table 1. (Continued)

Sample no.	Species	Type of preservation	Dry matter (%)	Laboratory Identification
13	Alfalfa	Stacked hay	85	7109-13
14	Alfalfa	Haylage	45	7102-9
15	Alfalfa	Haylage	55	7102-10
16	Alfalfa	Haylage treated with 0.4% propionic acid	55	7201-11
17	Alfalfa	Haylage treated with 0.8% propionic acid	55	7201-12
18	Alfalfa	Haylage	45	7202-9
19	Alfalfa	Haylage	55	7202-10
20	Alfalfa	Haylage treated with 0.4% propionic acid	55	7201-11
21	Alfalfa	Haylage treated with 0.8% propionic acid	55	7201-12
22	Alfalfa	Haylage treated with .65% ammonium propionate	48	7301-1
23	Alfalfa	Haylage treated with .66% chemostor	50	7302-2
24	Alfalfa	Haylage treated with 1.32% chemostor	48	7302-3

Chemostor: 80% propionic acid:20% Acetic acid.

Appendix Table 2. Approximate Analyses of Samples Used in Forage Protein Quality Evaluation Experiments.

Sample no.[a]	Crude protein	Ether extract	Nitrogen free extract	Crude fiber	Ash
	% DM				
1	19.65	2.71	43.75	23.06	10.83
2	19.30	2.62	42.00	25.89	10.19
3	21.28	2.47	42.29	23.85	10.12
4	20.70	2.56	41.73	24.15	10.86
5	16.51	1.70	41.79	33.41	6.60
6	18.57	1.09	32.53	39.56	8.24
7	18.45	1.31	36.78	36.03	7.43
8	20.82	0.95	33.54	35.75	8.95
9	20.36	1.24	36.17	34.89	7.34
10	20.56	-	-	-	8.52
11	18.76	-	-	-	6.96
12	20.81	-	-	-	8.46
13	17.77	-	-	-	7.24
14	18.26	1.98	39.20	33.35	7.67
15	21.79	3.28	35.24	31.43	8.22
16	19.99	2.24	38.84	31.82	6.91
17	21.88	1.98	36.79	31.86	7.80
18	20.75	2.39	33.51	35.82	7.34
19	21.68	3.33	34.35	33.10	7.60
20	19.71	3.04	35.60	34.32	7.27
21	16.99	2.89	39.21	34.07	7.28
22	23.59	3.23	40.66	23.78	8.73
23	21.70	3.11	43.76	23.25	8.17
24	21.90	3.19	43.58	23.02	8.31

[a]Detailed sample descriptions are given in Appendix Table 1.

Appendix Table 3. Fibrous Constituents Analysis of Samples Used in Forage Protein Quality Evaluation Experiments.

Sample no.[a]	CWC[b]	ADF[c]	Hemicellulose	Cellulose	Lignin	ADL/ADF[d]
	% DM					
1	41.58	32.18	9.40	25.69	6.49	20.17
2	44.38	34.28	10.10	27.09	7.19	20.97
3	43.32	33.78	9.54	27.26	6.52	19.30
4	42.50	32.96	9.54	25.72	7.24	21.97
5	49.42	40.96	8.46	31.86	9.10	22.22
6	60.50	48.75	11.75	38.07	10.68	21.91
7	55.96	46.26	9.70	36.36	9.90	21.40
8	58.30	50.28	8.02	37.21	13.07	26.00
9	53.24	49.72	3.52	37.22	12.50	25.14
10	60.69	48.16	12.53	37.44	10.72	22.26
11	57.26	46.34	10.92	36.26	10.08	21.75
12	57.74	51.30	6.44	37.54	13.76	26.82
13	53.98	44.62	9.36	35.10	9.52	21.34
14	52.08	44.73	7.35	34.69	10.04	22.45
15	42.28	41.12	1.16	32.70	8.41	20.45
16	49.38	42.24	7.14	32.78	9.46	22.40
17	50.18	42.16	8.02	32.59	9.57	22.70
18	50.48	42.01	8.47	32.77	9.24	22.00
19	44.15	41.16	2.99	32.50	8.66	21.04
20	45.82	42.44	3.38	33.82	8.62	20.31
21	57.08	42.94	14.14	33.00	9.94	23.15
22	34.95	30.43	4.52	33.00	7.61	25.01
23	36.81	25.00	11.81	16.65	8.35	33.40
24	37.86	29.19	8.67	21.75	7.44	25.49

[a]Detailed sample descriptions are given in Appendix Table 1.

[b]CWC = cell wall constituents.

[c]ADF = acid detergent fiber.

[d]ADL/ADF = acid detergent lignin x 100/acid detergent fiber.

Appendix Table 4. Sheep Performance Data of Samples Used in Forage Protein Quality Evaluation Experiments.

Sample no.	DM as fed	Maximum intake	DMD[a]	DOM[b]	DCWC[c]	DADF[d]	DN[e]	N-balance	Biological[f] value
	%	% BW[g]			%			g N/day	%
1	86.71	3.93	61.86	65.22	44.60	42.05	75.29	4.13	16.66
2	86.28	4.04	62.28	64.93	49.38	48.04	74.38	3.96	16.59
3	86.87	4.20	63.10	68.03	52.22	50.62	77.11	4.78	16.48
4	85.31	3.73	61.23	65.25	47.47	44.42	75.22	3.61	14.05
5	86.76	2.84	56.33	58.03	39.79	41.81	70.38	1.65	10.65
6	74.64	1.91	47.30	48.12	44.53	41.77	56.69	-.54	-8.90
7	82.07	2.71	56.03	57.30	55.54	53.39	62.06	0.35	-1.23
8	82.92	2.66	48.17	49.15	47.71	47.81	50.00	1.32	9.65
9	74.63	2.45	47.16	49.31	44.85	49.09	48.06	-.89	-7.44
10	75.18	2.38	48.67	49.41	47.23	44.07	62.08	3.19	18.28
11	83.47	2.74	54.58	55.59	50.29	47.49	64.77	3.64	18.08
12	82.72	2.92	43.05	43.93	41.70	46.44	40.40	-2.80	20.22
13	84.86	2.85	54.12	50.86	46.67	45.38	67.17	-.37	2.33
14	70.66	4.08	51.83	52.30	47.75	48.76	49.38	3.44	20.24
15	49.60	3.53	57.11	57.16	-	-	63.35	3.06	16.17
16	65.96	4.57	53.66	54.27	47.53	46.34	54.55	5.61	28.19
17	66.56	4.64	58.84	54.01	49.06	47.66	57.31	5.12	21.41
18	73.02	3.95	57.70	57.85	-	-	63.26	5.29	22.60
19	52.16	4.40	59.48	59.79	-	-	67.72	5.59	19.77
20	54.80	3.84	59.96	60.66	-	-	68.25	4.80	20.25
21	73.54	4.19	57.03	57.77	-	-	56.87	5.26	26.64
22	47.52	3.84	67.02	68.50	53.46	54.68	75.60	7.69	20.17
23	49.54	3.85	69.72	89.18	61.56	46.85	76.10	9.07	28.33
24	47.61	4.27	69.04	70.75	59.53	56.14	75.15	7.46	21.90

Appendix Table 4. (Continued)

[a]DMD = digestibility of dry matter.

[b]DOM = digestibility of organic matter.

[c]DCWC = digestibility of cell wall constituents.

[d]DADF = digestibility of acid detergent fiber.

[e]DN = digestibility of nitrogen.

[f]Biological value = N-balance x 100/N absorbed.

[g]BW = body weight.

Appendix Table 5. Dry Matter Solubilities of 24 Forage Samples Used in Protein Quality Evaluation Experiments.

Sample no.	In vivo DMD[a]	Hot water sol.[b] DM[c]	AD[d] sol. DM	Pepsin sol. DM	Pepsin + pan.[e] sol.DM	Rum.[f]+pepsin sol. DM	Rum.+pepsin +pan.sol.DM
1	61.86	35.27	67.82	47.65	53.05	70.02	61.93
2	62.28	31.78	65.72	47.63	47.72	73.34	64.05
3	63.10	33.62	66.22	48.04	55.76	67.87	61.94
4	61.23	34.28	67.04	50.05	51.31	68.51	58.27
5	56.33	31.10	59.04	47.16	46.50	64.21	54.44
6	47.30	24.83	51.25	35.73	35.88	60.66	45.10
7	56.03	28.39	53.74	38.89	40.48	58.92	55.05
8	48.17	29.74	49.72	35.12	36.41	54.74	49.55
9	47.16	32.82	50.28	39.52	40.94	59.51	53.17
10	48.67	26.09	51.48	40.20	34.83	58.85	48.64
11	54.58	27.87	53.66	41.38	36.35	59.54	53.11
12	43.05	29.19	48.70	34.38	36.04	58.38	47.45
13	54.12	28.75	55.38	43.60	38.28	63.02	54.49
14	51.83	34.23	55.27	-	41.93	66.96	-
15	57.11	38.60	58.88	44.42	41.99	68.62	53.29
16	53.66	35.68	57.76	41.17	43.01	68.65	50.76
17	58.84	37.12	57.84	43.17	45.21	70.51	-
18	57.70	34.00	57.99	43.08	43.17	68.13	56.22
19	59.48	38.22	58.84	46.54	43.25	65.97	49.29
20	59.96	37.45	57.56	47.64	42.71	70.20	59.45
21	57.03	30.88	57.06	42.62	39.55	65.85	52.81
22	67.02	37.70	69.57	54.90	47.85	70.42	69.55
23	69.72	40.11	75.00	58.09	45.34	70.42	67.67
24	69.04	39.47	70.81	59.44	46.74	71.41	68.05

Appendix Table 5. (Continued)

[a]DMD = digestibility of dry matter.

[b]sol. = soluble.

[c]DM = dry matter.

[d]AD = acid detergent

[e]pan = pancreatin

[f]Rum. = rumen fluid.

Appendix Table 6. Protein Solubilities of 24 Forage Samples Used in Protein Quality Evaluation Experiments.

Sample no.	N[a]	In vivo ND[b]	Hot water insol.N[c]	Hot water insol.N/N	ADF insol.DM[d]	ADF N/N	Sol.[e] in buffer	Pepsin insol. N	Pepsin insol.N/N
1	3.14	75.29	2.41	76.73	0.37	11.83	31.39	0.60	18.99
2	3.09	74.38	2.67	86.36	0.25	8.23	25.80	0.53	17.11
3	3.41	77.11	2.42	71.05	0.30	8.84	27.39	0.53	15.46
4	3.31	75.22	2.67	80.78	0.31	9.42	34.07	0.61	18.33
5	2.64	70.38	2.32	87.95	0.24	8.70	38.60	0.52	19.87
6	2.97	56.69	2.16	72.89	0.40	13.40	46.86	0.86	29.09
7	2.95	62.06	2.02	68.41	0.40	13.40	35.00	0.64	21.58
8	3.33	50.00	1.70	51.14	0.73	21.90	47.25	1.22	36.74
9	3.26	48.06	1.85	56.62	0.62	19.00	47.25	1.42	43.05
10	3.29	62.08	2.29	69.65	0.40	13.40	35.89	0.90	27.26
11	3.00	64.77	1.92	64.11	0.40	13.40	26.69	0.55	18.37
12	3.33	40.40	1.71	52.04	0.73	21.90	42.24	1.43	42.96
13	2.84	67.17	2.05	72.17	0.31	10.80	29.29	0.59	20.88
14	2.92	49.38	1.50	51.42	0.52	17.32	-	-	-
15	3.49	63.35	1.12	31.95	0.37	10.75	63.63	0.76	21.64
16	3.20	54.55	1.45	45.23	0.45	14.46	52.78	0.78	25.15
17	3.50	57.13	1.60	48.81	0.46	13.83	46.66	0.84	23.96
18	3.32	63.26	1.79	53.79	0.38	11.57	48.48	0.57	17.29
19	3.47	67.72	1.32	37.90	0.34	9.69	61.13	0.61	17.48
20	3.16	68.25	1.23	38.84	0.28	8.88	60.86	0.50	15.82
21	2.72	56.87	1.71	62.70	0.49	17.84	38.46	0.58	21.14
22	3.77	75.60	2.08	55.14	0.27	7.12	48.27	0.60	15.90
23	3.47	76.10	2.07	59.30	0.25	7.10	39.06	0.46	13.28
24	3.50	75.15	2.02	57.59	0.28	7.90	41.35	0.43	12.17

Appendix Table 6. (Continued)

Sample no.	Pepsin+pan.[g] insol. N	Pepsin+pan. insol. N/N	Rum.[h] +pepsin insol. N	Rum.+pepsin insol.N/N
1	0.27	8.60	0.51	16.15
2	0.26	8.53	0.51	16.38
3	0.63	18.50	0.51	14.92
4	0.85	25.65	0.67	20.33
5	0.61	22.96	0.59	22.11
6	0.99	33.31	0.89	31.65
7	0.89	30.06	0.64	21.84
8	1.07	32.19	1.15	34.55
9	1.01	30.88	1.32	40.63
10	1.23	37.31	0.99	30.04
11	0.81	26.88	0.65	21.73
12	1.30	38.99	1.33	39.93
13	1.01	35.56	0.63	22.18
14	0.79	27.03	0.82	28.06
15	1.11	31.72	0.83	23.88
16	1.02	30.90	0.74	23.06
17	1.00	28.63	0.66	18.89
18	0.96	28.84	0.64	19.13
19	1.08	31.03	0.77	22.24
20	0.86	27.09	0.72	22.78
21	0.97	35.73	0.74	27.11
22	0.97	25.82	0.69	18.20
23	1.21	34.98	0.69	19.97
24	1.06	30.40	0.69	19.76

Appendix Table 6. (Continued)

Sample No.	Rum. +pepsin+pan. insol. N	Rum. +pepsin+pan. insol.N/N	Rum.NH_3 N/N	Browning (OD440nm)
1	0.70	22.36	3.72	0.16
2	0.73	23.59	4.16	0.17
3	0.69	20.38	3.90	0.16
4	0.80	24.06	4.44	0.20
5	0.70	26.56	3.91	0.15
6	1.15	38.63	5.28	0.22
7	0.79	26.99	5.18	0.18
8	1.28	38.57	5.96	0.34
9	1.40	42.94	7.47	0.41
10	1.23	37.52	5.21	0.33
11	0.76	25.36	2.96	0.16
12	1.48	44.49	5.75	0.37
13	0.82	28.71	4.36	0.16
14	-	-	-	0.24
15	1.12	31.99	-	0.22
16	0.85	26.59	3.97	0.21
17	-	-	-	0.21
18	0.76	22.82	3.41	0.18
19	1.08	30.98	6.60	0.19
20	0.72	22.71	4.52	0.19
21	0.83	30.49	1.10	0.19
22	0.79	20.93	3.68	0.22
23	0.86	24.86	2.14	0.21
24	0.74	21.27	1.26	0.21

Appendix Table 6. (Continued)

[a]N = nitrogen.

[b]ND = nitrogen digestibility.

[c]insol. = insoluble.

[d]ADF = acid detergent fiber.

[e]DM = dry matter.

[f]Sol. = soluble.

[g]Pan. = pancreatin.

[h]Rum. = Rumen microbial.

Appendix Table 7. Chemical Composition of Forages Supplied by Dr. H.K. Goering, USDA.

Source Description	DM[a]	CW[b]	ADF[c]	AL[d]
Orchardgrass hay	93.1	66.9	39.9	6.39
Pelleted orchardgrass	93.5	66.3	40.1	6.66
Wafered orchardgrass hay	93.1	69.8	42.0	6.77
Grass hay	91.1	60.4	34.0	3.64
Formic acid silage	22.2	55.1	35.3	3.26
Reconstituted formic acid silage	34.1	65.1	38.3	4.91
Orchardgrass low-moisture silage	54.1	66.1	44.9	6.87
Grass silage (silo fired)	43.1	62.3	50.4	15.00
Grass silage (silo fired)	40.5	59.0	56.9	21.32
Oats silage	28.2	47.1	33.8	3.36
Grass silage	-	38.8	44.8	19.80
Bermudagrass:corn	92.0	58.8	24.1	3.62
Bermudagrass:corn	92.2	55.1	24.3	3.78
Bermudagrass:corn-autoclaved	92.7	66.3	27.1	6.45
Bermudagrass:corn-unautoclaved	92.6	67.0	28.2	8.57
Orchardgrass silage	73.6	67.0	64.0	11.54
Alfalfa silage	75.3	54.3	44.5	9.26
Low-moisture silage	73.9	65.4	51.4	15.28
High-moisture silage	34.8	43.8	33.7	4.75
Timothy hay	93.1	68.8	41.6	5.59
Timothy(autoclaved for 30 min)	93.5	72.0	46.5	9.12
Timothy(autoclaved for 60 min)	93.3	70.6	45.6	9.40
Alfalfa silage	28.2	40.9	34.4	8.05
Alfalfa silage	59.8	43.2	34.1	8.10
Alfalfa hay	89.7	36.7	29.1	6.51
Alfalfa silage	28.6	41.0	35.0	8.27
Alfalfa silage	59.8	44.9	37.9	10.21
Alfalfa hay	91.6	38.8	31.5	6.62
Alfalfa hay	73.8	43.6	32.3	7.09
Alfalfa hay	64.8	43.0	33.5	7.59
Alfalfa hay	46.6	50.7	41.5	10.31
Alfalfa hay	41.5	48.8	42.2	10.24
Native hay	80.8	70.1	46.5	7.20
Native hay	65.9	73.9	51.9	9.69
Native hay	56.5	76.3	55.7	11.34
Native hay	49.2	76.4	57.7	12.77
Alfalfa hay	94.3	38.0	31.3	5.92
Alfalfa hay-molded	95.0	48.6	36.8	8.11
Alfalfa hay-molded	94.8	50.7	37.4	7.94
Alfalfa hay	94.0	42.0	33.4	5.62
Alfalfa hay	95.0	41.2	33.9	6.87
Alfalfa hay-molded	95.4	46.2	35.8	8.15
Alfalfa hay	93.6	48.6	34.7	8.03
Alfalfa hay-molded	94.0	62.2	42.2	7.70
Alfalfa hay	94.7	44.5	34.3	7.58
Alfalfa hay-molded	93.9	61.4	42.9	7.32

Appendix Table 7. (Continued)

Source Description	AD-N[e]	Pep.-N[f]	N[g]
Orchardgrass hay	0.35	0.83	2.24
Pelleted orchardgrass	0.32	0.78	2.11
Wafered orchardgrass hay	0.39	0.82	2.04
Grass hay	0.26	0.90	3.32
Formic acid silage	0.06	0.31	3.37
Reconstituted formic acid silage	0.20	1.79	2.36
Orchardgrass low-moisture silage	0.47	1.13	1.39
Grass silage (silo fired)	0.99	1.00	1.80
Grass silage (silo fired)	1.33	1.54	2.34
Oats silage	0.05	0.47	2.78
Grass silage	1.31	1.95	3.59
Bermudagrass:corn	0.18	0.68	2.24
Bermudagrass:corn	0.18	0.68	2.20
Bermudagrass:corn-autoclaved	0.54	1.03	1.69
Bermudagrass:corn-unautoclaved	0.69	0.91	2.03
Orchardgrass silage	1.11	1.21	1.49
Alfalfa silage	0.46	0.49	2.44
Low-moisture silage	1.20	1.42	1.73
High-moisture silage	0.18	0.54	2.63
Timothy hay	0.12	0.34	1.00
Timothy(autoclaved for 30 min)	0.42	0.49	0.98
Timothy(autoclaved for 60 min)	0.55	0.65	1.01
Alfalfa silage	0.27	-	-
Alfalfa silage	0.29	0.78	3.40
Alfalfa hay	0.22	-	-
Alfalfa silage	0.29	-	-
Alfalfa silage	0.52	1.48	3.56
Alfalfa hay	0.24	0.62	3.30
Alfalfa hay	0.29	0.63	2.93
Alfalfa hay	0.36	0.62	3.10
Alfalfa hay	0.60	1.35	3.20
Alfalfa hay	0.76	1.56	2.90
Native hay	0.37	0.74	1.31
Native hay	0.61	0.94	1.31
Native hay	0.75	1.14	1.28
Native hay	0.88	1.16	1.38
Alfalfa hay	0.23	0.61	3.77
Alfalfa hay-molded	0.45	1.08	3.96
Alfalfa hay-molded	0.50	1.10	3.41
Alfalfa hay	0.20	0.52	3.22
Alfalfa hay	0.23	0.66	3.49
Alfalfa hay-molded	0.51	1.28	4.18
Alfalfa hay	0.21	-	2.74
Alfalfa hay-molded	0.27	0.64	2.35
Alfalfa hay	0.27	0.65	2.92
Alfalfa hay-molded	0.33	0.75	2.50

Appendix Table 7. (Continued)

[a]DM = dry matter.

[b]CW = cell walls.

[c]ADF = acid detergent fiber.

[d]AL = apparent lignin.

[e]AD-N = acid detergent insoluble nitrogen.

[f]Pep.-N = pepsin insoluble nitrogen.

[g]N = total nitrogen.

Appendix Table 8. *In Vivo* Digestion Coefficients of Forages Supplied by Dr. H.K. Goering, USDA.

Source Description	DM[a]	N[b]	Ene.[c]	CW[d]
Orchardgrass hay	56.0	54.1	-	-
Pelleted orchardgrass	55.2	55.6	-	-
Wafered orchardgrass hay	57.2	52.1	-	-
Grass hay	66.5	69.0	63.2	76.5
Formic acid silage	74.2	77.5	73.7	85.2
Reconstituted formic acid silage	64.9	55.6	-	-
Orchardgrass low-moisture silage	73.8	35.7	-	84.6
Grass silage (silo fired)	54.9	13.1	-	48.8
Grass silage (silo fired)	44.3	9.2	-	50.4
Oats silage	86.1	84.7	-	82.4
Grass silage	38.0	27.0	-	-
Bermudagrass:corn	73.0	69.0	-	-
Bermudagrass:corn	70.0	66.0	-	-
Bermudagrass:corn-autoclaved	65.0	47.0	-	-
Bermudagrass:corn-unautoclaved	63.0	35.0	-	-
Orchardgrass silage	55.9	6.29	-	63.3
Alfalfa silage	58.9	52.2	-	56.7
Low-moisture silage	55.0	13.8	-	62.1
High-moisture silage	65.6	59.5	-	60.2
Timothy hay	56.8	45.5	-	-
Timothy(autoclaved for 30 min)	56.9	37.5	-	-
Timothy(autoclaved for 60 min)	53.0	29.2	-	-
Alfalfa silage	59.5	72.8	-	-
Alfalfa silage	59.0	63.0	-	-
Alfalfa hay	64.5	77.5	-	-
Alfalfa silage	60.1	73.6	-	-
Alfalfa silage	54.0	48.9	-	-
Alfalfa hay	63.8	76.2	-	-
Alfalfa hay	60.0	63.0	55.1	55.7
Alfalfa hay	56.4	58.0	50.4	51.2
Alfalfa hay	51.8	40.2	47.0	52.2
Alfalfa hay	45.8	27.2	34.1	48.1
Native hay	51.6	39.7	51.2	58.8
Native hay	47.9	20.2	46.5	58.8
Native hay	45.5	10.8	44.4	57.3
Native hay	44.8	6.6	42.0	56.5
Alfalfa hay	67.4	78.5	66.4	55.5
Alfalfa hay-molded	53.8	54.1	53.9	56.5
Alfalfa hay-molded	55.0	55.5	58.1	59.9
Alfalfa hay	61.5	73.3	61.5	51.4
Alfalfa hay	62.3	77.7	61.4	52.0
Alfalfa hay-molded	52.2	51.8	51.3	52.5
Alfalfa hay	63.7	74.6	61.9	58.9
Alfalfa hay-molded	55.9	61.7	62.8	70.5
Alfalfa hay	65.3	77.4	65.6	56.3
Alfalfa hay-molded	64.7	69.2	65.1	70.8

Appendix Table 8. (Continued)

Source Description	ADF[e]	Hemi-C[f]	AD-N[g]
Orchardgrass hay	-	-	-
Pelleted orchardgrass	-	-	-
Wafered orchardgrass hay	-	-	-
Grass hay	-	-	-
Formic acid silage	-	-	-
Reconstituted formic acid silage	-	-	-
Orchardgrass low-moisture silage	80.6	93.2	68.1
Grass silage (silo fired)	47.8	52.9	22.8
Grass silage (silo fired)	35.6	-	-1.9
Oats silage	81.4	85.0	12.5
Grass silage	-	-	3.3
Bermudagrass:corn	-	-	-
Bermudagrass:corn	-	-	-
Bermudagrass:corn-autoclaved	-	-	-
Bermudagrass:corn-unautoclaved	-	-	-
Orchardgrass silage	66.9	-14.4	50.6
Alfalfa silage	56.2	58.8	54.6
Low-moisture silage	54.8	88.8	42.9
High-moisture silage	62.4	52.6	36.0
Timothy hay	-	-	-
Timothy(autoclaved for 30 min)	-	-	-
Timothy(autoclaved for 60 min)	-	-	-
Alfalfa silage	-	-	-
Alfalfa silage	-	-	-
Alfalfa hay	-	-	-
Alfalfa silage	-	-	-
Alfalfa silage	-	-	-
Alfalfa hay	-	-	-
Alfalfa hay	47.0	-	-
Alfalfa hay	47.7	-	-
Alfalfa hay	53.7	-	-
Alfalfa hay	49.1	-	-
Native hay	49.0	-	-
Native hay	53.0	-	-
Native hay	48.0	-	-
Native hay	48.8	-	-
Alfalfa hay	54.0	62.9	42.9
Alfalfa hay-molded	52.7	68.3	34.2
Alfalfa hay-molded	56.1	70.6	53.6
Alfalfa hay	49.7	58.1	20.0
Alfalfa hay	51.5	54.0	44.5
Alfalfa hay-molded	50.3	60.0	34.5
Alfalfa hay	53.5	72.4	-
Alfalfa hay-molded	64.2	83.9	34.3
Alfalfa hay	53.3	66.3	-
Alfalfa hay-molded	65.5	83.0	48.8

Appendix Table 8. (Continued)

[a]DM = dry matter.

[b]N = nitrogen.

[c]Ene. = energy.

[d]CW = cell walls.

[e]ADF = acid detergent fiber.

[f]Hemi-C = Hemi-cellulose.

[g]AD-N = acid detergent insoluble nitrogen.

Appendix Table 9. Composition and Digestion Coefficients of Forages Supplied by Dr. N.A. Jorgensen (Univ. of Wisconsin) and Dr. D.C. Pierson (Univ. of Minnesota).

	DM[a]	ADF[b]	ADL[c]	Crude protein	AD-N/N[d]	ND[e]	DMD[f]
	-%-	--	---%DM----------		----------	%	----------
Alfalfa hay	84.1	44.6	-	18.7	3.1	70.6	59.9
Alfalfa hay	71.6	47.7	-	18.4	8.6	66.8	57.6
Alfalfa hay	71.0	46.8	-	18.4	9.6	65.9	57.1
Alfalfa haylage	45.0	41.6	-	18.7	4.4	71.2	61.3
Alfalfa haylage	65.0	42.8	-	19.2	6.2	68.4	60.2
Alfalfa haylage	70.0	51.7	-	18.6	19.7	57.3	52.2
Alfalfa hay	80.3	44.6	9.7	18.7	5.2	75.7	54.2
Alfalfa hay	63.4	47.7	10.6	18.4	16.0	67.2	50.7
Alfalfa hay(treated with hay Savor)	63.0	44.8	11.2	18.4	13.5	68.2	52.1
Alfalfa hay	81.4	38.1	6.8	16.8	5.7	72.3	58.6
Alfalfa hay	74.2	37.3	6.6	17.2	6.3	74.0	58.5
Alfalfa hay(treated with hay Savor)	74.6	37.0	6.9	17.1	8.9	72.0	58.7
Alfalfa haylage	59.7	-	-	19.4	7.3	71.6	62.7
Alfalfa haylage	58.2	-	-	19.1	31.2	40.4	47.3
Alfalfa silage	36.8	-	-	19.8	6.6	72.3	63.3
Alfalfa silage	35.9	-	-	19.6	27.4	47.8	55.2
Alfalfa haylage	48.5	34.6	8.8	19.0	8.2	70.8	61.2
Alfalfa haylage	55.4	43.7	15.9	18.7	28.3	43.0	52.2

Appendix Table 9. (Continued)

[a]DM = dry matter.

[b]ADF = acid detergent fiber.

[c]ADL = acid detergent lignin.

[d]AD-N/N = acid detergent insoluble nitrogen as a percent of total nitrogen.

[e]ND = nitrogen digestibility.

[f]DMD = dry matter digestibility.

Appendix Table 10. Original Data of Sheep Digestion Trials (Dry Matter Digestibility).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	54.8	54.0	46.8	50.7	45.7	47.8
	49.8	54.9	54.6	50.6	47.6	48.9
$\bar{X}_1$.	52.3	54.4	50.7	50.7	46.6	48.4
Period 2	54.5	52.4	48.9	52.4	51.8	55.2
	51.5	56.6	52.0	53.0	52.4	52.1
$\bar{X}_2$.	53.0	54.5	50.5	52.7	52.1	53.6
Period 3	55.3	52.8	61.5	48.6	47.7	52.1
	54.7	51.8	52.9	43.6	52.7	51.4
$\bar{X}_3$.	55.0	52.3	57.2	46.1	50.2	51.7
$\bar{X}$..	53.4	53.7	52.8	49.8	49.6	51.2

[a]Silo number.

[b]Formaldehyde- 1.25% of a 37% solution.

Appendix Table 11. Original Data of Sheep Digestion Trials (Organic Matter Digestibility).

	Propionic acid 0.4% (8)[a]	Propionic acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	56.7	54.9	48.8	52.9	47.5	49.0
	51.7	55.9	56.3	52.6	49.2	50.8
$\bar{X}_{1.}$	54.2	55.4	52.5	52.8	48.4	43.3
Period 2	55.6	52.7	50.7	53.5	53.1	56.5
	52.8	56.8	53.3	52.4	53.2	53.1
$\bar{X}_{2.}$	54.2	54.8	52.0	53.0	53.2	55.3
Period 3	56.9	54.1	54.0	50.0	48.8	52.8
	55.6	53.2	53.8	43.8	53.4	52.3
$\bar{X}_{3.}$	56.2	53.7	53.9	46.9	51.1	52.5
$\bar{X}_{..}$	54.9	54.6	52.82	50.86	50.87	52.41

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 12. Original Data of Sheep Digestion Trials (Digestibility of Cell Walls)

	Propionic acid 0.4% (8)[a]	Propionic acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	45.6	49.4	29.6	42.8	40.4	45.9
	47.1	47.2	46.6	46.8	42.6	49.6
$\bar{X}_{1.}$	46.4	48.3	38.1	44.8	41.5	47.7
Period 2	45.5	41.8	36.4	34.8	38.6	48.5
	42.4	47.2	38.3	36.9	37.0	44.1
$\bar{X}_{2.}$	43.9	44.5	37.4	35.8	37.8	46.3
Period 3	47.5	46.3	56.9	40.0	32.3	49.4
	44.2	46.5	43.5	28.3	38.2	48.2
$\bar{X}_{3.}$	45.8	46.4	50.2	34.1	35.2	48.8
$\bar{X}_{..}$	45.4	46.4	41.9	38.3	38.2	47.6

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 13. Original Data of Sheep Digestion Trials (Digestibility of Acid-Detergent Fiber).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	50.5	46.0	40.7	43.8	49.0	51.8
	48.7	47.8	51.7	46.8	49.5	52.6
$\bar{X}_{1.}$	49.6	46.9	46.2	45.3	49.3	52.2
Period 2	46.7	44.9	42.7	39.4	42.9	47.8
	44.0	48.7	43.2	39.9	43.6	44.0
$\bar{X}_{2.}$	45.3	46.8	42.9	39.7	43.3	45.9
Period 3	49.5	47.9	58.5	43.1	39.5	50.8
	46.1	46.1	46.2	34.2	42.3	49.8
$\bar{X}_{3.}$	47.8	47.0	52.4	38.7	40.9	50.3
$\bar{X}_{..}$	47.6	46.9	40.0	41.21	44.5	49.5

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 14. Original Data of Sheep Digestion Trials (Digestibility of Nitrogen).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	63.9	52.6	55.2	55.5	53.4	48.7
	54.3	54.5	57.1	56.9	52.0	48.1
$\bar{X}_{1.}$	59.1	53.5	56.1	56.2	52.7	48.4
Period 2	62.7	57.4	59.5	60.7	63.2	60.2
	60.2	61.1	64.6	60.2	63.4	58.2
$\bar{X}_{2.}$	61.5	59.3	62.1	60.4	63.3	59.2
Period 3	63.9	59.7	70.4	63.5	64.9	59.5
	65.5	55.9	63.0	64.3	70.3	59.7
$\bar{X}_{3.}$	64.7	57.8	66.7	63.9	67.6	59.6
$\bar{X}_{..}$	61.8	56.9	61.6	60.2	61.2	55.7

[a]Silo number.

[b]HCHO = Formaldehyde -1.25% of a 37% solution.

Appendix Table 15. Original Data of Sheep Digestion Trials (Nitrogen Balance, g. N/Day).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	1.97	1.69	-0.17	-0.21	2.18	-0.95
	3.42	0.63	3.47	0.44	0.94	-2.59
$\bar{X}_{1.}$	2.70	1.16	1.65	0.12	1.56	-1.77
Period 2	3.40	0.10	0.44	3.40	-1.04	2.46
	1.76	1.06	1.37	-0.02	-0.04	-0.37
$\bar{X}_{2.}$	2.58	0.58	0.91	1.69	-0.54	1.04
Period 3	5.07	3.03	2.09	6.38	4.27	4.85
	9.04	0.63	2.47	8.37	2.88	0.20
$\bar{X}_{3.}$	7.05	1.83	2.28	7.38	3.58	2.52
$\bar{X}_{..}$	4.11	1.19	1.61	3.06	1.53	0.60

[a] Silo number.

[b] HCHO = Formaldehyde - 1.25% of a 37% solution.

Appendix Table 16. Original Data of Sheep Digestion Trials (Nitrogen Retained As A Percent of Absorbed Nitrogen).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	16.3	14.2	-4.8	-2.3	16.9	-8.3
	35.5	5.7	20.9	4.2	8.5	-28.5
$\bar{X}_{1.}$	25.9	10.0	8.0	0.94	12.7	-18.4
Period 2	20.3	0.7	5.3	20.0	-11.7	20.8
	15.7	6.3	10.1	-0.1	-0.3	-4.1
$\bar{X}_{2.}$	18.0	3.5	7.7	10.0	-6.0	8.3
Period 3	29.5	22.5	24.3	31.1	32.5	26.7
	38.4	4.6	21.4	32.5	24.0	1.9
$\bar{X}_{3.}$	34.0	13.5	22.9	31.8	28.3	14.3
$\bar{X}_{..}$	26.0	9.0	12.9	14.2	11.7	1.41

[a]Silo number.

[b]HCHO = Formaldehyde - 1.25% of a 37% solution.

Appendix Table 17. Original Data of Sheep Digestion Trials (Nitrogen Retained As A Percent of Nitrogen Intake).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	10.40	7.49	-2.67	1.28	9.00	-4.05
	19.26	3.12	11.92	2.37	4.41	-13.70
$\bar{X}_{1.}$	14.83	5.30	4.62	0.54	6.70	-8.87
Period 2	12.20	0.66	3.16	12.12	-7.31	12.47
	9.20	6.33	6.49	-0.07	-0.17	-2.38
$\bar{X}_{2.}$	10.70	3.50	4.83	6.03	-3.74	5.05
Period 3	18.83	13.41	17.08	19.74	21.10	15.86
	25.20	2.55	13.50	20.88	16.87	1.15
$\bar{X}_{3.}$	22.02	7.98	15.29	20.31	18.99	8.51
$\bar{X}_{..}$	15.85	5.59	8.25	8.96	7.32	1.56

[a]Silo number.

[b]HCHO = Formaldehyde - 1.25% of a 37% solution.

Appendix Table 18. Original Data of Sheep Digestion Trials (Maximum Dry Matter Intake, % Body Weight).

	Propionic Acid 0.4% (8)[a]	0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	3.57	4.26	2.24	2.91	3.26	3.66
	3.34	4.22	4.52	2.78	4.47	3.26
$\bar{X}_{1.}$	3.46	4.24	3.38	2.85	3.87	3.46
Period 2	3.91	3.49	3.24	3.81	2.52	3.23
	4.32	3.15	3.27	3.22	3.41	2.35
$\bar{X}_{2.}$	4.12	3.32	3.26	3.52	2.97	2.79
Period 3	4.03	3.76	1.63	3.61	3.06	4.29
	4.78	3.89	3.34	3.35	2.13	3.74
$\bar{X}_{3.}$	4.41	3.83	2.49	3.48	2.60	4.02
$\bar{X}_{..}$	3.99	3.80	3.04	3.28	3.14	3.42

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 19. Original Data of Sheep Digestion Trials (Dry Matter Intake During Digestion Trials, % Body Weight).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	2.93	3.18	1.38	2.25	2.94	3.14
	2.30	2.52	3.54	2.78	3.16	2.57
$\bar{X}_{1\cdot}$	2.62	2.85	2.46	2.52	3.05	2.86
Period 2	3.15	2.36	2.26	3.15	1.70	2.72
	3.43	2.60	2.67	2.23	2.61	1.93
$\bar{X}_{2\cdot}$	3.29	2.48	2.47	2.69	2.17	2.33
Period 3	3.59	3.05	1.76	3.47	2.99	3.58
	3.91	3.07	2.80	3.07	1.83	2.62
$\bar{X}_{3\cdot}$	3.75	3.06	2.28	3.27	2.41	3.10
$\bar{X}_{\cdot\cdot}$	3.22	2.80	2.40	2.83	2.54	2.76

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 20. Original Data of Sheep Digestion Trials (Maximum Digestible Dry Matter Intake, % Body Weight).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB (.5%) + HCHO[b] (4)	Control (6)
Period 1	1.96	2.30	1.05	1.48	1.49	1.75
	1.66	2.32	2.47	1.41	2.13	1.59
$\bar{X}_{1.}$	1.81	2.31	1.76	1.45	1.81	1.67
Period 2	2.13	1.83	1.58	2.00	1.31	1.78
	2.22	1.78	1.70	1.71	1.79	1.22
$\bar{X}_{2.}$	2.18	1.81	1.64	1.86	1.55	1.50
Period 3	2.23	1.99	1.00	1.75	1.46	2.23
	2.62	2.01	1.77	1.46	1.12	1.92
$\bar{X}_{3.}$	2.43	2.00	1.39	1.61	1.29	2.08
$\bar{X}_{..}$	2.14	2.04	1.60	1.64	1.55	1.75

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 21. Original Data of Sheep Digestion Trials (Digestible Dry Matter Intake During Digestion Trials, % Body Weight).

	Propionic Acid 0.4% (8)[a]	Propionic Acid 0.8% (3)	Ammonium Isobutyrate (AIB) 0.5% (7)	Ammonium Isobutyrate (AIB) 1% (5)	AIB(.5%) + HCHO[b] (4)	Control (6)
Period 1	1.60	1.72	0.65	1.14	1.34	1.50
	1.15	1.38	1.93	1.41	1.50	1.26
$\bar{X}_{1.}$	1.38	1.55	1.29	1.27	1.42	1.38
Period 2	1.72	1.24	1.11	1.65	0.90	1.50
	1.77	1.47	1.39	1.18	1.37	1.00
$\bar{X}_{2.}$	1.77	1.35	1.25	1.42	1.13	1.25
Period 3	1.99	1.61	1.08	1.69	1.43	1.86
	2.14	1.59	1.48	1.34	0.96	1.35
$\bar{X}_{3.}$	2.06	1.60	1.28	1.51	1.20	1.61
$\bar{X}_{..}$	1.73	1.50	1.27	1.40	1.25	1.41

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

Appendix Table 22. Original Data of Sheep Digestion Trials (Body Weight Change - g/Day).

	Propionic Acid		Ammonium Isobutyrate (AIB)		AIB (.5%) + HCHO[b]	Control
	0.4% (8)[a]	0.8% (3)	0.5% (7)	1% (5)	(4)	(6)
Period 1	-.18	-.36	-.45	-.27	-.09	-.18
	-.27	-.18	-.27	-.45	-.36	-.45
$\bar{X}_{1.}$	-.23	-.27	-.36	-.36	-.23	-.32
Period 2	0	-.27	-.36	-.27	-.09	-.36
	+.18	-.09	-.27	-.82	-.27	-.36
$\bar{X}_{2.}$	+.09	-.18	-.32	-.55	-.18	-.36
Period 3	-.09	+.27	+.27	-.09	-.09	-.18
	-.09	-.09	-.09	0	-.36	-.27
$\bar{X}_{3.}$	-.09	+.09	+.09	-.05	-.23	-.23
$\bar{X}_{..}$	-.08	-.12	-.20	-.32	-.21	-.30

[a]Silo number.

[b]Formaldehyde - 1.25% of a 37% solution.

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