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Evaluation of several chemical analyses as indicators of the productive value of forages

S. V. Satyanarayanasetty

To the Graduate Council:

I am submitting herewith a dissertation written by S. V. Satyanarayanasetty entitled "Evaluation of several chemical analyses as indicators of the productive value of forages." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

J.T. Miles, Major Professor

We have read this dissertation and recommend its acceptance:

M.J. Montgomery, R.G. Cragle, H.J. Smith

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 6, 1967

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EVALUATION OF SEVERAL CHEMICAL ANALYSES AS INDICATORS OF THE PRODUCTIVE VALUE OF FORAGES

A Dissertation

Presented to

The Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

S. V. Satyanarayanasetty

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An Abstract

EVALUATION OF SEVERAL CHEMICAL ANALYSES OF FORAGES AS INDICATORS OF THE PRODUCTIVE VALUE OF FORAGES

S. V. Satyanarayanasetty

Under the Supervision of Major Professor Dr. J. T. Miles

An investigation was undertaken in which dry matter digestibility and voluntary dry matter intake data were used to compare the following chemical constituents of forages as predictors of digestibility and intake of dry matter: acid detergent fiber (ADF), lignin, lignin in ADF, cell wall constituents (CWC) and crude protein.

Two experiments were conducted: (1) with alfalfa hays harvested at six stages of maturity and (2) with three forage species: alfalfa, red clover and Lindsey 77F. The relationships of stage of maturity and forage species with forage composition, digestibility and intake were investigated.

As forage advanced in growth the fibrous fractions - ADF and CWC, increased and crude protein decreased, also in Experiment II ADF and CWC were lowest in alfalfa, intermediate in red clover and highest in Lindsey 77F. A highly significant negative correlation between the fibrous fractions and crude protein was obtained. Dry matter digestibility, dry matter intake and nutritive value index (NVI) were significantly lower with advance in stage of maturity in Experiment I and in Experiment II

were highest on alfalfa, intermediate on red clover and lowest on Lindsey 77F.

Lignin digestibility was quite variable and some negative digestibility of lignin was observed. Heat damage to feces and/or the presence of hemicellulose in lignin determination might be the factors contributory to artifact lignin values. There was a decreasing trend in CWC digestibility as influenced by stage of maturity; alfalfa was significantly higher than red clover in digestibility of CWC, while alfalfa and Lindsey 77F were similar. Digestibility of cell contents ranged from 85 to 44 per cent. Low digestibility of cell contents might be due to the non-cell-wall matter in feces, which comprises bacterial and endogenous excretions.

Increase in butyrate (rumen VFA) was associated with higher protein content of forages. Acetate:propionate ratios were significantly lower at the bud stage than at the half bloom; this ratio with red clover and Lindsey 77F was significantly higher than that for alfalfa. Dry matter digestibility (DDM) showed highly significant positive correlations with voluntary dry matter intake, digestibilities of crude protein, ADF, CWC, cell contents and per cent of lignin in ADF, crude protein content and NVI. Highly significant negative correlations between DDM and ADF or CWC, dry matter intake and ADF or CWC were observed. These results indicated that with an increase in the forage fibrous fractions digestibility and intake of dry matter

decreased significantly. In this study Availability Index and Summative Equation were poorly related to dry matter digestibility and intake and would be poor expressions of the value of feeds. NVI being dependent on energy digestibility and dry matter intake appears to be an excellent measure of feeding value. Highly significant negative correlations between ADF and CWC with either DDM or intake and NVI would indicate that the chemical components ADF and CWC, have real value for predicting the value of a feed.

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INTRODUCTION

Feed evaluation is an economic problem. Feed cost is the largest single item in the cost of milk production. Since other costs are also less variable the best opportunity for increased profits is provided by utilizing as large a proportion of nutrients produced on the farm as possible without a significant loss of milk production. Since dairy cattle are naturally forage consuming animals, a large proportion of the nutrient requirements may be furnished in the form of high quality forage. Good pastures, because of the high yield and low cost of production are frequently the cheapest source of nutrients. The greater the proportion of nutrients provided in the form of forage, the more important it is that the quality be good. Perhaps the greatest opportunity for improvement is the reduction of losses of nutrients that occur between the time the forage is at the proper stage to harvest and it is fed to cows.

The quality of forage in ruminant rations is a major factor in determining the amount and type of supplementary feeds needed and consequently has a major influence on feed cost and on the financial returns to the dairyman. However, quality in a forage crop is more commonly discussed than regulated in practice. Regulation of quality is difficult because it is influenced by plant species and variety, stage

of maturity at harvest, and method of curing and storage. Forage quality is not always easy to determine. It is most frequently measured by chemical composition, palatability and digestibility or availability of nutrients. More accurate and economical methods of measuring forage quality than those in common usage are seriously needed.

The measurement of forage intake and digestibility provides a measure of the intake of nutrients and the portion available for assimilation by the animal.

Forage crops have been much improved in recent years. Nutritive changes are constantly occurring in the herbage of forages from young to mature stages of growth. It is recognized that well conducted feeding and digestion trials give the best evaluation of the nutritive value of a forage. However, these are often not possible and are always expensive. Numerous attempts have been made to develop chemical methods for the assessment of herbage digestibility and nutritive value of forages. This study was conducted to determine the value of several chemical analyses of forages as predictors of voluntary intake and dry matter digestibility by dairy cattle.

CHAPTER II

REVIEW OF LIETERATURE

Proximate Analysis vs. Recent Chemical Methods

Chemical analyses most widely used are those of the "proximate" (Weende) system which includes crude protein, crude fiber, ether extract, ash and nitrogen free extract (determined by difference). In the Weende procedure routinely employed in the analysis of feeds and feeding stuffs the crude fiber fraction was designed to include the fibrous, poorly digested part of the feed. This was normally considered to be the skeletal portion of the plant consisting presumably of celluloses and hemicelluloses and the indigestible encrusting substances, such as lignin. The nitrogen free extract (NFE) fraction was intended to include only the easily digested starches and sugars.

Crude fiber is comprised of constituents of the structural and protective parts of the plant. During the active growth in size the cell has only a thin wall composed largely of cellulose. After growth of the cell has ceased, a secondary wall is deposited abutting the primary wall; this may be heavily thickened and consists of several microscopically differentiable layers made up of cellulose, lignin, hemicelluloses and possibly some pectic material and silica (9).

Cellulose, the chief of the cell wall constituents, is found alike in young, growing cell walls and in the mature much-

thickened walls. Highly insoluble and hydrolyzable with difficulty, it is not attacked by enzymes secreted into the digestive tract of animals, but rather by enzymes of symbiotic microorganisms, of which the bacteria are the most important. Cellulose consists of long, unbranched chains of glucopyranose units linked through one and four beta glucosidic bonds. Among the non-cellulosic polysaccharides which may occur in the cell walls of different plant species are mannans, galactans, xylans and arabans. The polyuronide hemicelluloses in the wall are made up of mixed glycosidic chains containing both pentose and uronic acid molecules. They are much less resistant than cellulose to chemical agents, for they are soluble in dilute alkali and readily hydrolyzable in hot dilute acid (9).

Lignin differs from the other major cell wall constituents in that it is not a carbohydrate, but is a condensation product of one or more aromatic nuclei into a high molecular weight aromatic complex. Lignin is a poorly digested substance and because of its intimate physical association with more digestible cell wall constituents, exerts a dominant influence on the degree of digestibility of many feeds. Norman (52) and Crampton and Forshaw (15) considered lignin to be the most important single plant fraction in limiting the digestibility of a forage.

The procedure for the determination of crude fiber is purely empirical: it consits in boiling the moisture-free,

fat-free material for thirty minutes in 1.25 per cent sulphuric acid and then for an equal period in alkali of the same strength. Theoretically this procedure removes proteins, sugars and starches, and leaves as a residue the celluloses, hemicelluloses and lignin, along with mineral material. Weight lost on ignition of this dried residue is taken as crude fiber.

The proximate scheme for crude fiber has persisted in general use for many years even though its shortcomings have been disclosed by numerous investigations. Horwitt et al. (31) found that the indigestible residue obtained was three times as great as the amount of crude fiber determined by the proximate scheme. Williams and Olmstead (93) separated the components of the indigestible residue into three fractions: cellulose, hemicellulose and lignin. They analyzed the residues obtained from various materials by the proximate scheme (crude fiber) and by enzymatic pretreatment (indigestible residue). The results showed that in the proximate scheme hemicellulose suffered the greatest loss, while the loss suffered by lignin was less and that with carbohydrate was least.

In a study of the composition of the crude fiber in a large number of agricultural products, Norman (50) noted that the proximate scheme was effective in excluding all plant constituents except cellulose and lignin. However, the lignin content of fractions from different sources was found to be quite variable. A crude fiber high in lignin was not necessarily

obtained from the more highly lignified materials. A comparison of the amounts of these constituents in the original material showed that from 60 to 80 per cent of the cellulose was recovered, but that the recovery of lignin was quite variable, ranging from 4 to 67 per cent of the lignin present. Further investigation revealed that the alkali treatment in the proximate scheme was responsible for the extensive losses of lignin and for the removel of xylan. On the basis of these results, Norman concluded that crude fiber had no definite and regular relation to any particular constituent or any group of plant constituents, or to the crude fiber fraction of any other plant material. This fraction only gave an approximate estimation of cell wall constituents and even this estimate was on the low side. Results obtained in the investigations of the crude fiber fraction by Forbes and Hamilton (26) also indicated that this fraction was not an accurate measure of the structural constituents of the plant. Nordfelt et al. (49) found that a crude fiber fraction obtained from a clover and grass hay contained 40.6 per cent of the total lignin, 17.75 per cent of the total pentosans and 62.69 per cent of the total cellulose in the original sample. The remainder of the constituents were included in the difference calculated as the NFE (nitrogen-free extract) which consisted of 9.75 per cent lignin, 20.52 per cent cellulose, 29.4 per cent pentosans and 40.32 per cent sugars, hexosans, organic acids and related compounds. These authors further observed that the

crude fiber fractions from various crops differed in composition, especially in the amount of lignin, pentosans and cellulose. There were also differences in the crude fiber fraction of the same crop at different stages of development.

Crampton and Maynard (16) indicated that at least for herbivora, a partition of the carbohydrate portion of a feed into lignin, cellulose, and other carbohydrates may have more biological significance and hence be of greater usefulness in predicting feeding values than the division into crude fiber and NFE. Numerous workers have suggested the replacement of crude fiber estimation by the determination of cellulose and lignin (23, 33, 35, 38, 68).

The methods used for cellulose and lignin were mostly modifications of those originally devised by Norman and Jenkins (54) in England or Crampton and Maynard (16) in the United States. Norman and Jenkins estimated the protein in the crude lignin residue after digestion with 72 per cent sulfuric acid and subtracted this value from the loss on ignition of the residue. Crampton and Maynard hydrolyzed the protein with pepsin -HCl prior to digestion with 72 per cent sulfuric acid. Cellullose was determined by dissolving the other constituents in hypochlorite solution (Norman and Jenkins) or in acetic acid-nitric acid mixture (Crampton and Maynard). All methods had shortcomings since their empirical nature did not permit a high degree of specificity. Moon and Abou-Raya (45) prepared a "reference

lignin" by extraction with ethyl aceto-acetate and they considered that the methoxyl content, (normally about 15 per cent of lignin) should be used as a measure of the lignin present.

Van Soest (85) investigated the use of detergents in estimating the fiber and lignin content of forages. He reported that a quaternary detergent, cetyltrimethyl ammonium bromide (CTAB) in strongly acid solution dissolved plant proteins, comparing favorably with pepsin in this capacity. The residue obtained by this reagent was designated as acid detergent fiber (ADF). This acid detergent fiber retained lignin and also had a low nitrogen content, thus overcoming two of the chief criticisms of proximate analysis for crude fiber. Further, the latter residue (ADF) was a more suitable starting material for rapid lignin analysis.

The lignin content of ADF, termed the acid detergent lignin (ADL) was determined by Van Soest (85) through a modification of the 72 per cent sulfuric acid-insoluble lignin method of Sullivan (81). The newly developed fiber and lignin methods are based on the principle that detergents can be useful in separating protein from hemicellulose and lignin in feeds. A soluble portion containing the highly digestible constituents is the cell contents (S). Cell wall constituents (W) are determined by chemical analysis and cell contents (S) are estimated by subtracting the percentage of cell walls from 100. Van Soest (88) has suggested a classification system for forage

organic matter, which appears to be of greater usefulness in predicting feeding value than the division into crude fiber and nitrogen-free extract. This system is presented schematically in Table I.

Since crude fiber contains most of the cellulose but only part of the lignin and ADF includes all the cellulose, lignin and closely allied material the ADF values are normally about 30 per cent higher than those for crude fiber on the same feed. The more correct partition of poorly digested lignin and more complete extraction of protein with soluble material probably contribute to the more accurate prediction of digestibility.

Stage of Maturity and Chemical Composition of Forage

Drapala et al. (22) have described the manner in which lignin is deposited in the stems of red clover, and Steppler (78) has made a similar study with timothy and bromegrass; the process of lignification of stems appears to be similar in these species. Drapala observed that lignification in the stems of red clover proceded regularly with maturity, the regions primarily involved being those around the vascular bundles. The percentage of lignin in the clover stems in this study increased from 7.3 per cent on May 26, to 12.2 per cent (dry basis) on July 10, with the greatest increase in lignin occurring toward the end of this period.

There is an abundance of evidence indicating that the

TABLE I

DIVISION OF FORAGE ORGANIC MATTER BY SYSTEM OF ANALYSIS USING DETERGENTS

Fraction	Components	Nutritional availability	
		Ruminant	Non-ruminant
	CATEGORY A		
Cell contents (Soluble in neutral detergent)	Lipids	Virtually complete	Highly available
	Sugars, organic acids and water soluble matter		
	Starch		
	Non-protein nitrogen		
	Soluble protein		
	Pectin		
	CATEGORY B		
Cell wall constituents (fiber insoluble in neutral detergent)			
Soluble in acid detergent	Attached protein	Complete	High
	Hemicellulose	Partial	Very low
Insoluble in acid detergent (Acid Detergent Fiber)	Cellulose	Partial	Very low
	Lignin	Indigestible	Indigestible
	Lignified nitrogen compounds	"	"
	Heat-damaged Protein	"	"
	Keratin	"	"
	Silica	"	"

main structural constituents of grasses and pasture herbage increase progressively with age. Phillips and Goss (59) have shown that there was a rapid increase in cellulose and lignin in the leaves and stalks of barley plants as they approached maturity; cellulose content increased from 19.0 per cent (dry basis) in plants twenty-one days old to 32.3 per cent in plants seventy days old and lignin content increased from 1.48 per cent in seven day-old plants to 7.74 per cent in the mature plant.

A similar rapid increase occurs in the cellulose and lignin content of ryegrass as the plant approaches maturity. Norman (51) observed that over a period of fifty-one days the cellulose content of this grass increased from 20.9 to 36.3 per cent. The greatest increase in cellulose and lignin occurred in the later stages of growth. In a later study, (55), changes in the hemicellulose of ryegrass were found to be similar to those shown by cellulose; there was a rapid increase in this constituent during the period of growth and subsequently a more gradual increase during the senescent period. Considered collectively, cellulose, lignin and hemicellulose in ryegrass increased progressively with age. Norman found that in the youngest sample the structural constituents accounted for not less than 36 per cent of the dry weight of the plant, compared to not less than 62 per cent at maturity and not less than 75 per cent after maturity. Cellulose content in the grass

increased with maturity from 26.1 to 48.8 per cent (dry basis), lignin from 3.6 to 16.4 per cent and hemicellulose from 6.2 to 17.2 per cent.

In contrast to ryegrass and barley, cocksfoot (orchardgrass) was relatively high in structural constituents throughout its development, and Norman (53) observed that in cocksfoot there was a much smaller increase with maturity in those constituents than there was in ryegrass; in samples taken from May 10 to June 28, cellulose content increased only from 38.5 to 46.4 per cent (dry basis) and lignin from 7.9 to 11.1 per cent. Cellulose plus lignin in cocksfoot increased from 46 to 58 per cent; in ryegrass the sum increased from 29 to 47 per cent.

In a study of Montana grasses, Patton (58) analyzed nine species of grasses at five stages of maturity. All species were found to increase in cellulose and lignin content at each stage of growth. Initial cellulose content of the different species varied from 18.5 to 24.8 per cent (dry basis); final content from 28.7 to 46.8 per cent. Initial lignin content of all species except blue grama ranged from 4.0 to 6.0 per cent; final lignin content from 11.4 to 20.4 per cent. The coefficient of correlation between lignin content and cellulose was greater than 0.9 for the 123 samples analyzed with the exception of blue grama which had a very high initial lignin content of 12.6 per cent.

Brown (10) observed a similar increase with maturity in the structural components of Kentucky blue grass grown in

Missouri. Herbage cut at full bloom contained higher percentages of crude fiber and lignin than that harvested at comparable dates from plots kept short by repeated mowing. Crude fiber content was from 5.5 to 12.7 per cent higher at the more mature stage, and lignin content 0.82 to 2.96 per cent higher. The greatest difference between the two stages occurred when the cuttings were made in the fall.

There was a marked increase with maturity in the cellulose and lignin content of bunch wheatgrass produced in the arid region of Utah. Stoddart (79) found that cellulose content in this species increased from 24.21 per cent (dry basis) in an early stage of growth to 31.48 per cent at a later stage of maturity. Lignin increased from 3.96 to 14.48 per cent.

An increasing crude fiber content with maturity has been observed by Fudge and Fraps (27, 28) for various species of forage plants from the Gulf Coast prairie and from the northwestern portion of Texas, by Neller (48) from mixed Everglades grass and by Staples et al. (76) for South Dakota hays (mixed species).

Sotola (73) carried out investigations with irrigated alfalfa hay that was cut at quarter-, half- and three-quarter-bloom stages. The protein content decreased and the fiber content increased as the plants matured. The hay that was cut at the half bloom stage had the highest digestible crude protein and total digestible nutrients (TDN). Van Riper and

Smith (84) observed that spring growth was generally more productive than summer growth. There was a significant negative correlation between per cent protein and per cent fiber for all forages at all growth periods of their study.

Raymond (62) has reported results of an intensive study of the digestibilities of two strains of ryegrass (S23 and S24) and one strain of cocksfoot (S37) throughout the growing season. All the grasses showed a slight decline in digestibility until flower emergence, after which the decline was more rapid. There was no appreciable difference in the digestibilities of S23 and S24 at the same stage of growth, but the flower emergence date of S23 was about three weeks later than that of S24. The digestibility of cocksfoot at any stage was six percentage units below that of S24. Murdock et al. (47) found a continuous decline in digestibility of dry matter of orchardgrass from 75.6 per cent (April 23) to 54.8 per cent (June 6), the most rapid fall occurring when the grass was advancing from the boot to the heading stage (mid-May). Data presented suggested a curvilinear relationship between date of cutting and dry matter digestibility of orchardgrass.

Because forage plants contain considerable amounts of lignin which increases with maturity, changes in this constituent have been considered as a partial explanation of the lowered feeding value of matured forages. The correlation coefficients as reported by several investigators are given in Table II.

TABLE II

CORRELATION COEFFICIENTS BETWEEN LIGNIN AND DRY MATTER DIGESTIBILITY AND LIGNIN AND ORGANIC MATTER DIGESTIBILITY

Investigator	Forage	Correlation coefficient (r)
	Lignin and dry matter digestibility	
Phillips and Loughlin (60)	Alfalfa	-.939[b]
Richards, Weaver and Connolly (67)	Alfalfa	-.744
Phillips and Loughlin (60)	Timothy	-.954[b]
Richards and Reid (66)	Pasture forage (chiefly timothy)	-.989[b]
	Lignin and organic matter digestibility	
Forbes and Garrigus (25)	Av. of three grasses and two legumes	-.950[b] (with steers)
Forbes and Garrigus (25)	Av. of five grasses and four legumes	-.930[b] (with wethers)
Lancaster (33)	Mean of a wide range of New Zealand feeding stuffs	-.978[b]

[b]Significant at 1 per cent level probability.

Voluntary Intake and Chemical Composition

The term voluntary feed intake is used to describe the amount of food eaten by an animal when food is offered ad libitum. Several factors are involved in the regulation of feed intake by animals. The amount of feed consumed, measured in terms of dry matter, increases with increasing concentration of net energy in the ration (6). Crampton (13) showed a relationship between voluntary intake and digestible nutrient content. Blaxter et al. (8) found that within limits of the quality of forage used, the amount of feed taken in by sheep was determined by the capacity of their digestive tract and physical factors; such as, digestibility of feed and rate of passage through the digestive tract. The digestibility of their forages ranged between 44.7 and 74.2 per cent of the dry matter consumed. Voluntary intake of forages by ruminants is determined chiefly by the bulkiness of digesta and the rate of its disappearance from the reticulo-rumen (4). Since rate of passage is influenced by digestibility, there is a direct, definite relationship between voluntary intake and digestibility of roughages. For a feed to be eaten in large amounts it must be highly digestible (8).

Raymond et al. (63) showed that as the level of forage intake increased, the apparent digestibility decreased and where determinations were carried out at low intakes an overestimate of digestibility was obtained. The immediate effect

of increasing the intake is to increase the rate of passage through the gut, which results in a lower total digestibility (7). Dodsworth (20) observed that the dry matter content of the herbage can influence the intake level and thereby affect digestibility. While Greenhalgh and Runcie (30) found no causative relationship between feed intake and digestibility, McCullough (39) has reported that dry matter digestibility was a highly significant factor influencing dry matter intake of direct-cut silage in dairy cows. Conrad et al. (12) suggested a changing relationship in the importance of physiological and physical factors with increasing digestibility. For roughage diets between 52 and 66 per cent digestibility, capacity limited feed intake, since feed intake was related to: (1) body weight (roughage capacity), (2) undigested residue per unit body weight per day (rate of passage), and (3) dry matter digestibility. At higher digestibility levels (67 to 80 per cent) intake appeared to be dependent upon metabolic body size and upon level of production, and decreased with increasing digestibility of dry matter. The level of concentrates in the ration has been shown to affect forage intake, with a decline in forage dry matter intake of 0.24 unit for each additional unit of concentrates consumed (36).

Ruminants are capable of adjusting voluntary intake in relation to physiological energy demand if rumen load or fill does not limit consumption (42). Spahr et al. (74) observed

that even at similar stages of maturity, there was a species difference in intake and that about 65 per cent of the variations in caloric intake associated with stage of maturity was attributable to differences in dry matter intake.

Dowden and Jacobson (21) have shown that injections of acetate and propionate drastically reduced feed intake. In dairy cows, infusion of acetic acid at levels normally produced by one half of a daily ration of hay, caused a significant reduction in voluntary intake of long hay (43).

The data of Forbes and Garrigus (25) obtained with steers showed that for each percentage unit increase in forage lignin content there was a decrease of 5.8 per cent of total organic matter and of 8.2 per cent of digestible organic matter. They reported a negative relationship ($r = -.71$) between intake of digestible organic matter and forage lignin content. As forage becomes more mature, the voluntary consumption generally decreases (65, 72).

Van Soest and Marcus (89) observed no significant relationship between cell wall constituents and voluntary intake in forages with a cell wall content of less than 60 per cent of the dry matter. Above 60 per cent cell wall contents, there was a marked decrease in voluntary intake with increasing cell wall content. They suggested that the fiber mass inhibits intake in those forages with high cell wall constituents. Reid and Jung (64) obtained a significant negative correlation

between ad libitum intake and cell wall components (r = -.60) and a significant positive correlation between ad libitum intake and acid-insoluble lignin (r = 0.62).

Dehority and Johnson (18) estimated voluntary intake of forages based on the solubility of cellulose in cupriethylene-diamine. Cellulose solubilities were found to be significantly correlated with relative intake. Similarly forage dry matter solubility in normal sulfuric acid was found to be significantly correlated with relative intake (19).

Van Soest (86) concluded that chemical composition of forages on the whole was much more closely related to digestibility than voluntary intake, further that the interrelationships among intake, digestibility and chemical composition were highly species oriented.

Predicting Nutritive Value of Forages From Chemical Analyses

Nordfelt et al. (49) studied the influence of lignin and cellulose upon the digestibility of organic matter (DOM). The correlation coefficient (r) between lignin percentage and (DOM) was -.97 and between cellulose percentage and DOM was -.96. Both values were statistically highly significant. They also reported regression or prediction equations based on the percentage of lignin or cellulose in feed. This work emphasized the value of lignin and cellulose determinations to give useful information on the nature of the carbohydrate fraction in feeds and feces.

Reid et al. (65) reported that since both the amount and digestibility of protein in forages decline as growth approaches maturity the percentage of apparently digestible protein could be predicted accurately from the concentration of protein in both first-growth and aftermath forages, thus obviating the need for its actual measurement.

Kane and Moore (32) proposed a regression equation, $Y = 74.02 - .393X$, for determination of dry matter digestibility (DMD); where Y is dry matter digestibility and X is the number of days between April 30 and harvesting date. The average difference between the calculated and known digestibilities with this formula was found to be 2.6 per cent and the standard deviation from regression was $\pm$ 2.1. Melin et al. (40) found that the regression of dry matter digestibility on the date of harvest to be linear and highly significant. The standard error of regression was 1.80. They found that between May 27 and July 22, digestible energy fell from 3.7 to 2.3 kcal./g. of dry matter and apparent digestibility of energy from 79.3 to 49.3 per cent. In this study a relatively pure stand of Climax timothy was harvested at eleven dates beginning on May 27, and at weekly intervals until August 5.

Crampton et al. (14) used the relative intake (RI), which is voluntary intake of a specific forage expressed as a per cent of the expected intake of an hypothetical "ideal" forage, together with the in vivo percentage digestibility of

energy of that forage to obtain an Effective Nutritive Value Index (NVI) for numerical description of forages. Effective Nutritive Value of a forage is a function of both total intake and efficiency of energy utilization (14).

Scholl et al. (70) have analyzed four first-growth pure grass hays (1 orchardgrass, 2 bromegrass and 1 timothy) for crude protein, acid detergent fiber and lignin. Crude protein values of the first-growth grass hays (dry basis) were: orchardgrass, 23.0, bromegrass 23.4 and timothy 14.3 per cent; acid detergent fiber: 32.2, 31.4 and 37.0 per cent and lignin values were: 3.2, 3.3 and 4.7 per cent for the three grasses, respectively. Digestibility, dry matter intake and nutritive value index (NVI) data are presented in Table III. From the data presented in this table it is observed that Sterling orchardgrass was significantly higher than Canada Common and Sac bromegrass in dry matter, crude protein and acid detergent fiber digestibility; however, there was no significant difference in dry matter intake. Bromegrass digestibilities were not significantly different. Climax (timothy) was lowest in digestibility and intake. There were no significant differences in the nutritive value indices of Sterling, Canada Common and Sac; however, the Climax NVI was significantly lower than those of the other hays.

Forbes and Garrigus (25) concluded that for purposes of predicting organic matter digestibility of pasture forage,

TABLE III

APPARENT DIGESTION COEFFICIENTS, DRY MATTER INTAKES AND NUTRITIVE VALUE INDEXES OF FIRST-GROWTH GRASS HAYS

Species and varieties	Apparent digestion coefficients			Daily dry matter intake	
	Dry Matter (%)	Crude Protein (%)	ADF (%)	($g/W_{kg}^{.75}$)	NVI
Orchardgrass					
Sterling	73.8[a*]	82.8[a]	69.2[a]	61.9[a]	57.1[a]
Bromegrass					
Canada Common	70.7[b]	79.5[b]	63.5[b]	65.4[a]	57.8[a]
Sac	70.2[b]	78.8[b]	64.2[b]	61.2[a]	53.7[a]
Timothy					
Climax	66.6[c]	72.0[c]	62.6[c]	51.1[b]	42.5[b]

$$NVI = \frac{10x\ (g.\ intake)}{80\ (W_{kg}^{.75})} \times \text{digestible dry matter (per cent).}$$

ADF = Acid Detergent Fiber.

NVI = Nutritive Value Index.

*Values followed by the same letter are not signficantly different ($P > 0.05$).

the best single measure was the lignin content of the forage. The reliability of acid-insoluble lignin in predicting the digestible dry matter (DDM) was also investigated by Sullivan (81) who proposed a simplified tentative equation: DDM = 100 - 6X, where X is the acid-insoluble lignin content. Because of the high negative correlation coefficient between DDM and acid-insoluble lignin (r = -.94), this equation may be considered a general relationship existing in some species of grass between digestibility and lignin content, and may be applied to many, if not all species of grasses.

Correlations between ADF and dry matter digestibility of eighteen forages (r = -.79) showed it to be somewhat superior to crude fiber (r = -.73) as an indication of nutritive value. Correlation between the new lignin method and dry matter digestibility was r = -.9 when grass and legume species were separated (85).

Scholl et al. (69) observed that of the chemical constituents studied in fifty-six dried forages, lignin gave the most satisfactory correlation (r=-.66 to -.95) with in vivo digestible dry matter (DDM). Oh et al. (56) reported that acid detergent lignin was more highly correlated (r = -.46 to -.95) with in vivo DDM than was acid detergent fiber (ADF) (r = -.39 to -.84), or protein, especially when considered within species. Scholl et al. (70) reported highly significant negative correlations between in vivo digestible dry matter (DDM) and cell wall constituents (W). Correlation coefficients were: for all forages,

r = -.47; grasses, r = -.48 and legumes, r = -.74. Van Soest and Marcus (89) reported the correlation of W with DDM to be -.65 on eighty-three samples containing eleven legumes. They further observed that the correlation tended to decline as more legumes were included. Thus, from a subgroup of thirty forages consisting of seventeen legumes and thirteen grasses, the correlation between W and DDM was -.44. Oh et al. (56) obtained a correlation of -.47 between W and DDM, but failed to observe any decline in correlation between W and DDM, as more legume samples were included in the analysis for cell wall constituents.

The percentage of crude protein (Table IV) has been used to predict three entities: digestion coefficient of dry matter (DDM), digestion coefficient of crude protein (DCP) and per cent digestible crude protein (per cent DCP). Predictions of the per cent digestible protein were more accurate than for the others (82).

Baumgardt et al. (5) also reported that crude protein and digestible protein content of hays were highly correlated (r = 0.999) and that the latter could be estimated from the former with a resultant standard error of 0.25 and coefficient of variation of 2.26 per cent.

Digestible energy (DE) represents apparent digestibility without correction for fecal constituents of metabolic origin. It represents the total potential energy available. The

TABLE IV

COEFFICIENTS OF CORRELATION (r) OF CRUDE PROTEIN WITH DIGESTION COEFFICIENTS FOR DRY MATTER AND PROTEIN AND WITH PERCENTAGE OF DIGESTIBLE CRUDE PROTEIN

Population	Predictant	r	CV
101 grasses	Dig. coeff. of Dry Matter	0.61[a]	8.7
54 alfalfa	--Do--	0.40[a]	6.3
76 grasses	Dig. coeff. of Crude Protein	0.85[a]	7.9
50 alfalfa	--Do--	0.61[a]	4.6
76 grasses	Per cent Dig. Crude Protein	0.99[a]	4.6
50 alfalfa	--Do--	0.95[a]	4.4

[a]Significant at 1 per cent probability level.

theoretical significance of total digestible nutrients (TDN) and digestible energy (DE) are identical. Overman and Gaines (57) advocated the use of digestible energy in place of the indirect total digestible nutrients procedures. Lofgreen (34) has stressed the desirability for the use of DE as a simple direct method of obtaining TDN equivalent.

Moore (46) observed that the DE values are useful in predicting approximate net energy (NE) values. Moir (41) reported that the digestible energy content of a wide range of foodstuffs for ruminants could be accurately estimated from the percentage dry matter digestibility by the regression equation, $Y = 0.0462 x - .158$, where Y is the digestible energy content (Cal./g.) and x was the per cent dry matter digestibility. From this it follows that dry matter digestibility (DDM) is a simple and accurate description of DE content of foodstuffs for ruminants.

Of the six common forages tested by Swift et al. (83) Kentucky blue grass was highest in digestible energy; the DE and nutritive value content of the forages (orchardgrass, bromegrass, timothy hay) decreased as the season progressed from about 76 per cent DE at first cut to about 47 per cent DE at past full bloom.

Butterworth (11) could not demonstrate any correlation between content of fiber or crude protein and DE of some tropical forages. However, he reported highly significant correlation between DE and DDM or DOM (digestible organic matter).

Stallcup and Davis (75) reported that the factor most highly correlated with DDM was digestible energy (r = 0.97).

Flatt et al. (24) reported that the relationships between TDN, DE and metabolizable energy (ME) were constant at all levels of intake regardless of ration composition.

Prediction equations for ruminants have been developed by Van Soest and Moore (90), based on cellular contents, cell wall constitutents, acid detergent fiber and lignin. Digestibility of cell wall constituents was found to be controlled by the concentration of lignin in ligno-cellulose (viz. acid detergent fiber). Cell contents were found to be highly digestible and unaffected by lignin. An equation to predict energy digestibility developed from these parameters gave a correlation of 0.96 for thirty-nine feeds consisting of legumes, grasses and mixtures. It was deduced that the degree of lignification and the portion of forage free from lignification were the two factors involved in the determination of the resultant digestibility of forages. Lignification (L) was negatively related to digestibility and the neutral detergent (ND) solubles (S) were positively related to digestibility, the ratio L/S gave an estimate of indigestibility. Thus, an Availability Index (A) was derived: $A = 100 - 100\ (L/S)$. This index was found to regress linearly with digestibility. Nutritive value estimated from the Availability Index (A) using the regression equations and correlations for digestible dry matter and digestible energy

on a group of thirty-nine feeds are presented in Table V.

Van Soest (87) predicted digestibility of cell walls by the regression equation $Y = 147.5 - 78.9 \log X$, where X was the percentage of lignin in acid detergent fiber and Y, the digestibility of cell walls.

The Availability Index equation for digestible dry matter (DDM) was given: $DDM = 78.2 (1 - L/S) + 12.7$, where L represents the percentage of lignin in acid detergent fiber and S the cell contents obtained by subtracting the percentage of cell walls in the dry matter from 100 (87).

A Summative Equation has been developed by Van Soest (87), combining the above equation with the cell content digestibility and the endogenous matter excreted as per cent of intake. The Summative Equation for digestible dry matter (DDM) was:

$$DDM = 0.98S + W (147.3 - 78.9 \log L) - 12.9$$

where S was the percentage of cell contents; W, the percentage cell walls and L, the percentage of lignin in acid detergent fiber.

The Summative Equation was used to predict the digestible dry matter and to compare it with the Availability Index equation. Table VI shows the results of comparison of the two equations tested on a group of thirty forages used to compare the equation.

The Summative Equation appeared quite superior to the Availability Index, showing smaller increases in standard deviation from regression and standard deviation of differences. The

TABLE V

PREDICTION EQUATIONS FOR THE ESTIMATION OF NUTRITIVE VALUE FROM THE AVAILABILITY INDEX (A)

Evaluation	Equation	Correlation	No. of feeds
Digestible Dry Matter (%)	= 0.782A+12.7	+0.94	39
Digestible Energy (%)	= 0.732A+13.7	+0.96	39

TABLE VI

COMPARISON OF TWO EQUATIONS FOR PREDICTING DIGESTIBILITY OF DRY MATTER

Digestibility predicted by	Correlation		Standard deviation from regression		Standard deviation of differences	
Group:	1(a)	2(b)	1(a)	2(b)	1(a)	2(b)
Summative Equation	0.96	0.93	2.8	2.9	2.7	3.7
Availability Index	0.97	0.81	2.5	4.6	2.4	7.5

(a) Group 1 composed of nineteen forages, used to derive equations.

(b) Group 2 composed of thirty forages, used to compare equations.

Availability Index tended to give erratic values for forages of very high digestibility, to underestimate the digestibility of poor forages and to underestimate forage species where the ratios of lignin to cell contents may be unusual. The Summative Equation had the theoretical advantage in recognizing different factors contributing to apparent digestibility, viz. amounts of digestible cell contents, cell walls and endogenous factors (87).

Based on these findings, a new Summative Equation for estimating digestibility from chemical composition of forages was developed by Van Soest (87) which appeared to be superior ($r = 0.93$) to that based on the Availability Index ($r = 0.82$) of Van Soest and Moore (90).

Ademosun et al. (1) found that the Summative Equation (SE) was a better estimate than the Availability Index (AI) for the in vivo dry matter, protein, fiber, cell wall and cellulose digestibility, as shown in Table VII.

Sullivan (82) pointed out that the differences between grasses and alfalfa were more distinct and the same relationships do not hold in many predictions of digestibility from chemical composition.

Summary

Many attempts have been made at fractionation of forage as a means of separating the more digestible from the less digestible carbohydrates. The existing knowledge on the composition and digestibility of forages does not produce a complete

TABLE VII

SIMPLE CORRELATION COEFFICIENTS BETWEEN IN VIVO VARIABLES, AVAILABILITY INDEX (AI) AND SUMMATIVE EQUATION (SE)

In vivo variable	AI	SE
Digestible Dry Matter (DDM)	0.929	0.958
Digestion Coefficient of Protein	0.864	0.910
Digestion Coefficient of ADF	0.799	0.850
Digestion Coefficient of CWC	0.807	0.866
Digestion Coefficient of Cellulose	0.760	0.827

$r > 0.708$, significant at $P < 0.01$.

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

and coherent account. The application of modern analytical techniques has thrown considerable light on the true chemical composition of forages and the new methods have revealed the shortcomings of the generally adopted "proximate" scheme of analysis. It has been established that there is a negative association of digestibility of a forage with its lignin or fiber content. So the determination of lignin appears to be more satisfactory than that for a crude fiber as a criterion of digestibility of forages. The detergent lignin is the most rapid of the methods available for its determination. There are undoubtedly differences in chemical composition among different species of forages. It is well recognized that digestibility of forages decreases as plants mature and that the rate of this decrease depends on the type of forage and stage of morphological development. In terms of chemical composition, the only consistent effect that could be observed for all forages is that of the total fibrous fraction, cell wall constituents. There is a negative relationship between voluntary intake and cell wall constituents in forages containing above 60 per cent cell wall constituents. When intake and digestibility are closely related, most of the chemical constituents are also related with these measures of nutritive value.

Another means for obtaining digestibility data without recourse to digestibility trials is the use of formulae equating digestibility or digestible constituents with chemical composition

of forages for which digestibility data exist. There have been varying reports on the accuracy of such methods.

New knowledge of nutrition, of plant composition and of improved management practices which result in forages of better quality demand finer distinctions in evaluation. New chemical methods for the fractionation of carbohydrates have been developed which are nutritionally realistic and from which digestibility and nutritive value of forages can be predicted with increased accuracy.

CHAPTER III

EXPERIMENTAL PROCEDURE

A. GENERAL

Object of Experiments

The chief objective of the study was to evaluate the nutritive value of forages based on the chemical analyses and to use some of the chemical components as predictors of forage nutritive value. The influence of the stage of maturity of a forage and forage species on chemical composition, digestibility, voluntary intake and rumen volatile fatty acid concentration were also studied. Two experiments were conducted in the investigation.

B. MATERIALS AND METHODS

Sampling

All the forages used in the present investigation were artificially dried hays. Samples of forages fed were obtained during the intake period and collection period. Dry matter determinations were made on the fed and weighback samples of the individual forages. All analyses were made on the fed and weighback samples of each hay.

Rumen samples were taken once during each experimental period. Samples of rumen fluid were taken about three hours post feeding, by means of a vacuum pump and a polyethylene stomach tube. Coarse feed material was removed by centrifugation. To each 25 milliliters of the rumen fluid one-half milliliter

of saturated mercuric chloride solution was used as a preservative and the samples stored in a refrigerator until analysed for volatile fatty acids.

Fecal collections were made using the collection bag technique (29). Feces collection was carried out for five days at approximately twelve hour interval between the two daily collections. At the end of the five-day collection period a composite sample of the feces was obtained. On this composite sample, the dry matter content of the wet feces was determined on the same day of sampling. Sufficient quantity of the composite fecal sample was dried in a force draft oven (at 96-98° C. for the first and 48-50° C. for the second experiment) for three to four days to ensure proper drying.

Chemical Analyses.

Chemical analyses were conducted on the dried, uniformly ground composite forage (fed and weighback) and feces samples. Dry matter was determined by drying in a vacuum oven overnight at 100° C.

Rumen volatile fatty acids (VFA) were determined by gas-liquid chromatography. A five milliliter aliquot of rumen fluid was acidified with one milliliter of 25 per cent metaphosphoric acid and centrifuged to remove the precipitate. The clear supernatant fluid was used for VFA analysis. Gas chromatographic analysis for VFA was conducted utilizing an F and M Model

810-19 Analytical Gas Chromatograph equipped with a hydrogen flame detector. The column was one-fourth inch coiled stainless steel and was packed with 15 per cent Carbowax 20 M and terephthalic acid on 80/100 mesh Chromosorb W(AW-DMCS). Nitrogen was used as the carrier gas.

Energy determinations on the forage samples and feces were made with a Parr Plain (isothermal jacket) Bomb Calorimeter. Acid detergent fiber (ADF) and detergent lignin (L) were determined by the method of Van Soest (85). Cell wall constituents and cell contents were determined by the method of Van Soest and Wine (91). Ash in cell wall constituents was obtained by ashing the cell walls in a muffle furnace at 500-550° C. for two hours. Cellulose was estimated by the method of Crampton and Maynard (16) as modified by Matrone (37). Crude protein was determined by the conventional Kjeldahl method for nitrogen (3).

Statistical Analyses

Statistical analyses of the data were based on the methods outlined by Steel and Torrie (77) for the designs of both the experiments of the study.

C. EXPERIMENT I

Object of Experiment

The objective of this study was to determine the value of chemical composition, digestibility and voluntary intake data

in predicting the nutritive value of forages. The concentration and proportion of rumen volatile fatty acids and the energy changes as influenced by stage of maturity were also investigated.

Experimental Procedures

Twelve non-pregnant Holstein heifers were used in a completely randomized design, with two groups of six heifers per group. Each experimental period lasted twenty-one days. Conduction of the tiral for twenty-one days was as follows:

Days 1-7 . . . . . Ration Adjustment period

Days 8-14 . . . . . Intake Measurement period

Days 15 and 16 . . . . . Harness Adjustment period

Days 17-21 . . . . . Feces Collection period

All animals were weighed at the beginning of the digestion trial. Animals were weighed on days ten, eleven and twelve and rumen samples collected on day eleven of each period. Forage samples for dry matter determinations were taken during the mid-part of the intake period and the collection period.

The twelve heifers were divided into two groups on the basis of age and body weight and were assigned at random to different treatments. The experimental plan and treatment sequences are presented in Table VIII.

Forage used in this experiment consisted of six alfalfa hays harvested at different stages of maturity. These stages are described in Table IX.

TABLE VIII

EXPERIMENTAL DESIGN AND TREATMENT SEQUENCES
EXPERIMENT I

Period	Group 1	Group 2	Hay fed	
			Group 1	Group 2
Aug. 23-29	Adjustment	Adjustment	1	
Aug. 30 - Sept. 5	Intake	Adjustment		3
Sept. 6-12	Collection	Intake		
Sept. 13-19	Adjustment	Collection	2	
Sept. 20-26	Intake	Adjustment		6
Sept. 27-Oct. 3	Collection	Intake		
Oct. 4-10	Adjustment	Collection	4	
Oct. 11-17	Intake	Adjustment		5
Oct. 18-24	Collection	Intake		
Oct. 25-31		Collection		

TABLE IX

STAGE OF MATURITY OF ALFALFA HAYS, EXPERIMENT I

Hay	Stage of maturity
1	First cut bud
2	First cut half bloom
3	Second cut bud
4	Second cut half bloom
5	Second cut half bloom plus nine days
6	Second cut half bloom plus sixteen days

All experimental animals were fed ad libitum during the adjustment and intake measurement periods. Intake was limited to 100 per cent of ad libitum during the collection period. Animals were fed individually, twice daily, at approximately 7:30 A.M. and 5:00 P.M. Weighback of the previous days refusal was weighed each morning. Feed and refusal weights were recorded daily.

Animals were weighed for three consecutive days during the intake measurement period at about 9:00 A.M. on the days specified (ten, eleven and twelve) and weights recorded. Rumen samples were taken approximately three hours post feeding, during the period of intake measurement. A vacuum pump and polyethylene stomach tube were used to take the rumen samples for volatile fatty acid assay.

Digestibilities of the hays were determined during the last five days of the experiment. Fecal collections were made using the collection bags. Amount of feces voided were recorded twice daily at twelve hour intervals during which feces samples were also taken for analyses. Daily feces sample collections were made in polyethylene bags and at the end of the five-day collection period, a composite sample of the feces for each heifer was obtained for laboratory analyses and dry matter determination.

Based on the amount of hay eaten by an individual heifer and her body weight, relative intake was calculated. Relative

intake values were used in computing the Nutritive Value Index (14) for each of the six alfalfa hays.

Laboratory analyses for acid detergent fiber, detergent lignin, cell wall constituents, cellulose, crude protein and energy were made on the fed and weighback forage samples and on feces samples. Rumen volatile fatty acids were determined by gas chromatography.

D. EXPERIMENT II

Object of Experiment

The objectives of this experiment were to ascertain the relationship between chemical components and animal digestibility data in evaluating forages, to find out the differences, if any, among different forage species, with regard to chemical composition, digestibility and voluntary intake and to determine the influence of different forage species on the rumen volatile fatty acids.

Experimental Procedure

Six non-pregnant Holstein heifers (different from the ones used in Experiment I) were used in a 3 by 3 Latin Square design (77) with two heifers per treatment in each of the three periods of the experiment. Each experimental period lasted twenty-one days. Conduction of the trial was as detailed under Experiment I. Three heifers formed a square on the basis of age and body weight and each animal was assigned at random

to different treatment sequences within squares. The experimental plan and treatment sequence are presented in Table X.

The forages used in this experiment were:

Alfalfa - first cutting pre-bloom, Red clover - mid bloom, Lindsey 77F - about 45-50 inches height, had light shower on it prior to baling. The alfalfa and red clover forages contained considerable amounts of orchardgrass which was past the desirable stage of maturity.

Methods of feeding, recording the amount of fed and weighback hays, rumen fluid sampling, digestion trial procedures and recording body weights on days ten, eleven and twelve of the experimental period were similar to Experiment I. Laboratory analyses on fed and weighback samples and feces were as in Experiment I. Rumen fluid was analysed for volatile fatty acids by gas chromatography. Energy determinations were made using a Parr Bomb Calorimeter.

TABLE X

EXPERIMENTAL DESIGN AND TREATMENT SEQUENCE
IN A 3 BY 3 LATIN SQUARE

Period	Date	Animal number					
		Group 1		Group 2		Group 3	
		354	362	363	367	364	370
1	Sept. 26-Oct. 16	Alfalfa		Lindsey 77F		Red clover	
2	Oct. 17-Nov. 6	Lindsey 77F		Red clover		Alfalfa	
3	Nov. 7-Nov. 27	Red clover		Alfalfa		Lindsey 77F	

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I

Results of Experiment I are summarized in Tables XI through XIV. Individual data are presented in Appendix Tables XXV through XXVIII. All results are reported on dry matter basis and all determinations were done in duplicate. Analyses of Variance on each constituent are given in Appendix Tables XXI through XXIV.

It is well known that stage of maturity accounts for marked changes in the chemical composition of forage plants. A study of the chemical composition (Table XI) of the six alfalfa hays used in Experiment I reveals that the crude protein content generally decreased with advancing maturity although the second cut half bloom was slightly higher than the second cut bud. This difference from the expected pattern could have been due to sampling variation or the effects of heavy rain on leaf loss of second cut bud hay or rapid new growth on second cut half bloom hay. However, the second cut- bud and -half bloom were considerably higher in crude protein than the post half bloom stages. Protein content of the first- and second-cut bud stages was quite similar.

Cellulose, acid detergent fiber (ADF), lignin and cell wall constituents increased from the bud to the half bloom and generally to post half bloom stages in both the first and second

TABLE XI

CHEMICAL COMPOSITION OF ALFALFA HAYS, EXPERIMENT I

Hay	Dry matter	Dry matter constituents								
		Crude protein	Cellulose	ADF	Lignin	Lignin in ADF	CWC	Cell contents	Ash in CWC	Gross energy (kcal/g)
	%									
1 Fed	94.38	18.94	28.93	30.25	4.86	16.07	43.28	56.72	2.40	4.32
Weighback	94.52	13.57	33.67	38.12	5.92	15.53	57.10	42.90	2.13	4.39
2 Fed	93.47	14.45	34.62	37.15	5.59	15.05	57.55	42.45	2.27	4.44
Weighback	93.87	11.82	36.02	46.00	9.06	19.70	62.52	37.48	1.46	4.51
3 Fed	92.63	18.81	30.23	31.45	5.35	17.01	50.04	49.96	2.32	4.47
Weighback	91.94	15.15	33.67	40.81	8.42	20.63	56.21	43.79	1.70	4.52
4 Fed	93.63	19.21	32.15	37.53	7.33	19.53	53.73	46.27	3.24	4.38
Weighback	93.40	16.15	36.19	47.14	9.51	20.17	70.06	29.94	4.65	4.56
5 Fed	94.84	15.13	33.92	39.99	6.48	16.20	61.64	38.36	3.86	4.54
Weighback	95.26	12.29	39.25	47.56	9.45	19.87	65.18	34.83	1.93	4.51
6 Fed	93.68	12.27	35.54	40.98	6.90	16.84	60.50	39.50	2.41	4.46
Weighback	94.17	7.72	41.61	51.71	8.71	16.84	73.51	26.49	1.47	4.32

Hay 1, First cut bud; Hay 2, First cut half bloom; Hay 3, Second cut bud; Hay 4, Second cut half bloom; Hay 5, Second cut half bloom plus nine days; Hay 6, Second cut half bloom plus sixteen days.

ADF = Acid Detergent Fiber. CWC = Cell Wall Constituents.

TABLE XII

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT I

Hay	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility (%)
1	3.31^{b*}	135.40^{c}	169.26^{b}	134.81^{c}	79.62^{d}
2	2.63^{a}	108.66^{b}	135.82^{ab}	94.49^{b}	69.52^{bc}
3	2.59^{a}	106.05^{b}	132.56^{a}	104.29^{b}	78.63^{d}
4	2.41^{a}	100.85^{ab}	125.98^{a}	82.33^{a}	65.34^{a}
5	2.53^{a}	105.22^{ab}	131.53^{a}	93.42^{b}	71.08^{c}
6	2.39^{a}	97.84^{a}	122.30^{a}	81.14^{a}	66.20^{ab}

% B.W. = lb./100 lb. body weight.

$gm/W_{kg}^{.75}$ = grams/metabolic size (body weight in kilograms$^{.75}$)

Relative Intake (RI) = Grams daily dry matter consumed/kg B.W.$^{.75}$ x 1.25.

Nutritive Value Index (NVI) = Relative intake x per cent digestible energy.

*Values with the same superscript are not significantly different ($P > 0.05$).

TABLE XIII

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON APPARENT DIGESTIBILITY COEFFICIENTS, EXPERIMENT I

Hay	Apparent digestibility coefficients (%)						
	Dry matter	Crude protein	Cellulose	ADF	Lignin	CWC	Cell contents
1	79.68^{b*}	84.62^{c}	79.50^{de}	71.20^{c}	27.45^{c}	71.90^{bc}	85.23^{c}
2	72.07^{a}	76.79^{b}	74.90^{cd}	60.95^{ab}	-4.25^{a}	68.54^{ab}	79.01^{b}
3	80.52^{b}	85.08^{c}	81.11^{e}	69.46^{c}	24.15^{bc}	76.49^{c}	84.47^{c}
4	68.75^{a}	76.78^{b}	68.72^{a}	58.04^{a}	11.29^{b}	64.06^{a}	73.93^{a}
5	71.91^{a}	76.96^{b}	73.59^{bc}	65.43^{bc}	14.46^{bc}	71.86^{bc}	72.57^{a}
6	68.12^{a}	70.60^{a}	70.08^{ab}	60.53^{ab}	14.10^{bc}	65.14^{a}	72.33^{a}

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

*Values with the same superscript are not significantly different ($P > 0.05$).

TABLE XIV

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON RUMEN VOLATILE FATTY ACIDS, EXPERIMENT I

Volatile fatty acid	Hay 1	2	3	4	5	6
Acetic (mg/100 ml)	317.30^{c*}	105.80^{a}	196.00^{abc}	222.50^{bc}	128.40^{ab}	156.80^{ab}
Propionic (mg/100 ml)	98.45^{b}	30.68^{a}	58.90^{a}	62.48^{ab}	37.68^{a}	45.07^{a}
Butyric (mg/100 ml)	62.23^{b}	15.82^{a}	32.83^{a}	33.65^{a}	18.70^{a}	24.40^{a}
Isovaleric (mg/100 ml)	10.42^{bc}	5.22^{ab}	7.93^{abc}	12.80^{c}	4.42^{a}	5.93^{ab}
Valeric (mg/100 ml)	6.70^{c}	1.47^{ab}	3.25^{abc}	4.88^{bc}	0.63^{a}	2.98^{ab}
Total VFA (mg/100 ml)	495.07^{c}	158.98^{a}	298.93^{ab}	336.33^{bc}	189.98^{ab}	235.20^{ab}
Acetic (% of total)	63.95^{a}	66.37^{bc}	65.43^{ab}	66.38^{bc}	67.76^{c}	66.48^{bc}
Propionic (% of total)	19.75^{b}	18.98^{ab}	19.62^{b}	18.28^{a}	19.96^{b}	19.68^{b}
Butyric (% of total)	12.80^{b}	9.98^{a}	11.20^{ab}	10.05^{a}	9.68^{a}	10.25^{a}
Isovaleric (% of total)	2.17^{a}	3.25^{ab}	2.71^{a}	4.15^{b}	2.40^{a}	2.53^{a}
Valeric (% of total)	1.31^{ab}	1.45^{b}	1.03^{ab}	1.17^{ab}	0.18^{a}	1.83^{b}
Acetate: Propionate ratio	3.24^{a}	3.51^{bc}	3.34^{ab}	3.65^{c}	3.40^{abc}	3.39^{abc}

*Values with the same superscript are not significantly different ($P > 0.05$).

cutting. The one exception was that lignin content of post half bloom hays was lower than for second cut half bloom hay. Weigh-back samples of all six hays were lower in protein and higher in fibrous fractions than the fed samples. This indicated that the animals consumed the less fibrous, higher protein fraction of the hay. The increase in the fibrous fractions with increased stage of maturity of alfalfa observed in the present study is in agreement with the results reported by Phillips and Goss (59) Drapala et al. (22), Norman (51), Patton (58), Brown (10), and Stoddart (79). A decrease in protein content and an increase in fiber content with advancing maturity supports the work of Sotola (73), Porter et al. (61), and Scholl et al. (70).

Results of voluntary intake, relative intake, nutritive value index (NVI) and energy digestibility are presented in Table XII. NVI was calculated from the data according to the method of Crampton et al. (14). NVI, which is a numerical description of the digestible energy intake of a forage, is a function of both total dry matter intake and digestibility of energy. It is evident from the data that as the alfalfa forages advanced in stage of maturity, there was a decrease in the voluntary intake of dry matter, relative intake, NVI and energy digestibility of the forages. First cut bud was significantly different ($P < 0.05$) from first cut half bloom in dry matter intake computed either as per cent of body weight (per cent B.W.) or on the basis of metabolic size ($gm/W_{kg}^{.75}$).

There were no significant differences in dry matter intake of the second cutting forages. However, there was a trend toward lower intake of the more mature forages. There was a significant difference ($P < 0.05$) in dry matter intake between the first and second cut bud stages. The lower content of cellulose, ADF, lignin and cell wall constituents of the first cut bud stage was probably the reason for the difference in intake. Likewise, the NVI of the first- and second-cut bud was significantly different ($P < 0.05$), although the energy digestibility between the two bud stages was quite similar. NVI between first cut bud and half bloom and between second cut bud and half bloom or half bloom plus sixteen days differed significantly ($P < 0.05$). However, the difference between second cut bud and second cut half bloom plus nine days was not significantly different. Although the digestibility of energy of the second cut bud hay was significantly higher than in the second cut half bloom plus nine days ($P < 0.05$),dry matter intake, relative intake and NVI were not significantly higher. The general decrease in voluntary intake and other associated entitites as alfalfa becomes more mature is similar to the results obtained by Reid et al. (65), Smith et al. (72), and Spahr et al. (74). The significant decrease in energy digestibility (from 79.6 per cent at first cut-bud to 69.5 per cent at -half bloom and from 78.6 per cent at second cut-bud to 65.3 per cent at -half bloom) with advance in maturity of alfalfa is in agreement with the findings of

Swift et al. (83) and Melin et al. (40).

Table XIII shows the effect of stage of maturity on apparent digestibility coefficients of dry matter, crude protein, cellulose, ADF, lignin, cell wall constituents and cell contents. It is evident that though the dry matter digestibilities (DDM) between the first and second cut bud stages and between the first cut half bloom and second cut half bloom stages were similar, the bud stages did differ significantly ($P < 0.05$) from the half bloom and post half bloom.

Digestibility of crude protein (DCP) of alfalfa hays at first and second cut bud stage was similar, but the bud stage differed significantly ($P < 0.05$) from half bloom or post half bloom stages. Both the protein content and digestibility of protein in alfalfa forages were adversely affected with advancing stage of maturity. Sotola (73) reported that alfalfa hay at the half bloom stage had the highest digestible protein as compared to quarter- and three quarter-bloom alfalfa, but the present study indicated that the first or second cut bud stages ranked highest in digestibility of protein.

Cellulose digestibilities of the first cut bud and half bloom, first and second cut bud stages were similar. However, second cut bud differed significantly ($P < 0.05$) from half bloom and post half bloom stages; second cut half bloom and half bloom plus sixteen days were significantly lower in ADF, lignin, cell wall constituents and cell contents digestibilities than

at the first and second cut bud stages which were similar. Bud stages of the first and second cutting differed significantly ($P < 0.05$), in digestibilities of ADF and cell contents, from the half bloom and post half bloom stages. Apparent digestibility of lignin was quite variable and was adversely affected with advancing growth. Lignin is known to be poorly digested and often used as a "reference" material to reckon digestibility of different forages. It is surprising to note a negative digestibility (-4.25 per cent) occurring only at the first cut half bloom and not at any other stage of maturity. Van Soest (86) reported that artifact negative digestibility of lignin would sometimes appear due to heat damage to feces. The high temperature (96-98° C.) of the oven in which feces samples were dried might have caused artifact lignin increase in feces. Further, Moon and Abou-Raya (44) pointed out that the hemicellulose dissolving from the residue during treatment with 72 per cent sulfuric acid produced a precipitate upon dilution with water, and this precipitate being included in the total lignin determination may also be a source of artifact lignin. These two factors could have been responsible for negative digestibility of lignin on some samples in the study. Studies by Crampton and Maynard (16), Drapala et al. (22), Sullivan (80) have shown that lignin in plants is not only practically indigestible, but also decreased the availability of other constituents. The results of the present investigation showed that lignin at the

bud stage was much better digested than that at later stages of maturity.

Cell wall constituents at the second cut bud were digested significantly better ($P < 0.05$) than at the half bloom stage. There was a decreasing trend in digestibility of cell wall constituents as affected by stage of maturity except that the second cut post half bloom hays did not fit the general pattern, also, some of the other differences were not statistically significant.

It is interesting to note that the second cut half bloom was poorer than either the half bloom plus nine days or half bloom plus sixteen days alfalfa, in regard to digestibilities of cellulose, ADF, lignin and cell wall constituents. This observation seems odd and probably the higher lignin content (7.3 per cent) of the second cut half bloom as compared with the post half bloom stages (6.5 and 6.9 per cent, respectively), could be contributing to the low digestibility values referred to earlier.

Cell content digestibility ranged from 85.2 to 72.3 per cent. Though the bud stages were significantly higher ($P < 0.05$) than the half bloom in cell contents digestibility, the second cut half bloom and post half bloom stages were similar. The non-cell-wall materials (cell contents) are readily digested and the average digestibility of the latter fraction, as reported by Van Soest and Moore (90) was about 98 per cent,

with little variation over a wide variety of forages and digestibilites. Van Soest (88) emphasized that fecal non-cell-wall matter was composed of bacterial and endogenous excretions and that the latter were not at all constant with type of animal, percentage digestibility or as a percentage of intake. The decline in apparent digestibility of cell contents (non-cell-wall matter) of the present study may be attributed to the increase in bacterial and endogenous excretions which comprise the larger part of the fecal non-cell-wall material.

A general view of the data on apparent digestibilities, as influenced by stage of maturity of alfalfa forage showed that the first and second cut bud stages were highly digested in contrast to half bloom and post half bloom stages, which stages were also higher in the fibrous fractions: ADF, lignin and cell wall constituents, as compared to the bud stage.

Data on the effect of stage of maturity of alfalfa on rumen volatile fatty acid (VFA) concentration, distribution and acetate: propionate ratio are summarized in Table XIV. Total VFA production on first cut bud hay was significantly higher ($P < 0.05$) than on half bloom and second cut bud. Acetic acid was the major contributor to the total VFA. Sampling and individual animal variation could contribute for such differences.

When VFA distribution (per cent of total) was considered, acetate and buyrate on first cut bud were significantly higher ($P < 0.05$) than on half bloom.

Acetate:propionate ratios between first and second cut

bud stages were similar but significantly lower than at the half bloom stages. The highest (3.65:1) acetate:propionate ratio was obtained with second cut half bloom alfalfa. Acetate (per cent of total) was significantly higher ($P < 0.05$) with the half bloom and post half bloom stages than with the first or second cut bud stage; propionate was significantly lower at the half bloom stage than at bud stage.

These increases in acetate and decrease in propionate levels, which resulted in differences in acetate:propionate ratio appeared to be a reflection of a concomittant increase in fibrous fractions: ADF, lignin, cell wall constituents and an increase in butyrate was associated with a higher protein content of the forages. These results are in agreement with those of Williams and Christian (92), Davis et al. (17), Annison (2) and el-Shazly (71).

Experiment II

A summary of the results of Experiment II is presented in Tables XV through XVIII. Individual data are given in Appendix Tables XXIX through XXXVII.

Chemical composition of the three forages fed in Experiment II is given in Table XV. All determinations were in duplicate and results are expressed on a dry matter basis. Alfalfa was highest (16.6 per cent) in crude protein; red clover, though a legume, had only 13 per cent, while Lindsey 77F, essentially a grass, had about 14 per cent crude protein. This low content

TABLE XV

CHEMICAL COMPOSITION OF THE THREE FORAGE SPECIES FED, EXPERIMENT II

Forage	Dry matter constituents								Gross energy (kcal/g)
	Dry matter	Crude protein	ADF	Lignin	Lignin in ADF	CWC	Cell contents	Ash in CWC	
	%								
Alfalfa									
Fed	92.92	16.62	36.95	5.71	15.46	55.16	44.84	1.79	4.49
Weighback	92.88	17.13	38.15	6.41	16.74	54.74	45.26	2.15	4.37
Red clover									
Fed	92.96	13.44	37.43	5.53	14.74	56.18	43.82	2.18	4.33
Weighback	92.65	14.47	38.40	6.22	16.22	55.99	44.01	2.99	4.16
Lindsey 77F									
Fed	93.20	14.88	43.10	4.74	10.98	70.25	29.75	4.42	4.33
Weighback	93.51	12.34	46.11	4.79	10.38	69.90	30.10	7.00	3.95

ADF = Acid Detergent Fiber. CWC = Cell Wall Constituents.

TABLE XVI

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT II

Forage	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility (%)
Alfalfa	2.48^{b*}	102.39^{b}	127.99^{b}	87.07^{b}	68.02^{x**}
Red clover	2.24^{ab}	92.34^{ab}	115.42^{ab}	72.67^{ab}	62.53^{y}
Lindsey 77F	1.89^{a}	78.27^{a}	97.84^{a}	62.60^{a}	63.94^{xy}

*Values with the same superscript are not significantly different ($P > 0.05$).

**Values with the same superscript are not significantly different ($P > 0.10$).

TABLE XVII

APPARENT DIGESTIBILITY COEFFICIENTS OF THE THREE FORAGE SPECIES, EXPERIMENT II

Forage	Apparent digestibility coefficients (%)					
	Dry matter	Crude protein	ADF	Lignin	CWC	Cell contents
Alfalfa	70.30^{b*}	70.90^{c}	64.01^{b}	9.53^{c}	69.33^{b}	71.47^{c}
Red clover	64.00^{a}	56.99^{a}	53.92^{a}	-32.27^{b}	62.78^{a}	65.50^{b}
Lindsey 77F	62.49^{a}	63.76^{b}	60.26^{ab}	-14.50^{a}	70.18^{b}	44.13^{a}

*Values with the same superscript are not significantly different ($P > 0.05$).

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

TABLE XVIII

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON RUMEN VOLATILE FATTY ACIDS, EXPERIMENT II

Volatile fatty acid	Forage fed		
	Alfalfa	Red clover	Lindsey 77F
Acetic (mg/100 ml)	310.4^{a*}	254.6^{a}	320.3^{a}
Propionic (mg/100 ml)	97.1^{a}	72.7^{a}	90.2^{a}
Butyric (mg/100 ml)	55.8^{b}	37.6^{ab}	27.5^{a}
Isovaleric (mg/100 ml)	11.6^{a}	7.2^{a}	8.1^{a}
Valeric (mg/100 ml)	5.1^{a}	3.2^{a}	3.1^{a}
Total VFA (mg/100 ml)	480.0^{a}	375.3^{a}	449.1^{a}
Acetic (% of total)	64.6^{a}	67.8^{b}	71.3^{c}
Propionic (% of total)	20.8^{a}	19.3^{a}	20.1^{a}
Butyric (% of total)	11.5^{a}	9.9^{b}	6.2^{c}
Isovaleric (% of total)	2.3^{a}	2.0^{a}	1.8^{a}
Valeric (% of total)	0.9^{a}	1.0^{a}	0.7^{a}
Acetate: propionate ratio	3.15^{a}	3.51^{b}	3.57^{b}

*Values with the same superscript are not significantly different ($P > 0.05$).

of crude protein in red clover was due to the contamination of red clover with orchardgrass during growth and harvest. A slight increase of crude protein in the refusal of alfalfa and red clover was due to the fact that the hay got very much defoliated during the animal's feeding and the weighback sample had more of the leafy residue than the stalks.

Acid detergent fiber (ADF) appeared to have an increasing gradation from alfalfa to Lindsey 77F with red clover as intermediate. Alfalfa had about 37 per cent while Lindsey had 43 per cent ADF.

As opposed to ADF content, lignin was highest (5.7 per cent) in alfalfa and lowest (4.7 per cent) in Lindsey 77F. This trend of lignin content between legumes and grasses supports the report of Van Soest (86), who indicated that alfalfa had a higher lignin content than grasses of equal digestibility.

Cell wall constituents were lowest in alfalfa (55.2 per cent) and highest in Lindsey 77F (70.3 per cent), while ash in cell walls was highest (4.4 per cent) in Lindsey 77F and lowest (1.8 per cent) in alfalfa.

The effects of feeding different forage species on voluntary intake of dry matter, relative intake, nutritive value index (NVI) and energy digestibility are presented in Table XVI.

Voluntary intake, either as per cent of body weight or on the basis of metabolic size ($gm/W_{kg}^{.75}$), of alfalfa forage

was significantly higher ($P < 0.05$) than that of Lindsey 77F. Similarly, reltaive intake, and nutritive value index (NVI) which were highest with alfalfa were significantly different from those of Lindsey 77F. Red clover appeared to stand in between alfalfa and Lindsey 77F in voluntary intake, relative intake and NVI.

Gross energy digestibility of alfalfa was significantly higher ($P < 0.05$) than that of red clover while the energy digestibility of Lindsey 77F was not significantly different from either alfalfa or red clover.

The bulkiness of Lindsey 77F, as reported by Balch and Campling (4) might be contributory to low intake. Van Soest and Marcus (89) observed that in forages containing above 60 per cent cell wall constituents, there was a marked decrease in voluntary intake with increasing content of cell walls. In the present study Lindsey 77F had 70 per cent cell walls and this might have limited the voluntary intake of the forage.

Apparent digestibility coefficients for dry matter, crude protein, acid detergent fiber (ADF), lignin, cell walls and cell contents for alfalfa, red clover and Lindsey 77F are summarized in Table XVII.

Dry matter digestibility, digestibilities of crude protein, lignin and cell contents were significantly higher ($P < 0.05$) with alfalfa than for red clover and/or Lindsey 77F. However, alfalfa and Lindsey were similar with respect to digestibilties

of cell wall contents and although digestibility of ADF was higher in alfalfa the difference was not significant. Digestibilities of cell wall contents were significantly lower for red clover than for either of the other hays. Although the ADF digestibility was lower for red clover than for alfalfa, the difference from Lindsey 77F was not significant. The digestibilities of gross energy and dry matter on red clover and Lindsey 77F were not significantly different ($P > 0.05$), but these two values were quite close and appeared to be reversed between red clover and Lindsey 77F. The low digestibility of dry matter (about 62 per cent) with Lindsey 77F may also be a contributory factor for limiting voluntary intake and this attribute is in conformity with the observation of Blaxter et al. (8) who reported that for a feed to be eaten in large amount, it must be highly digestible.

As in Experiment I, negative digestibility of lignin was noted in Experiment II with red clover and Lindsey 77F. Since in Experiment II feed samples and feces samples were dried at about 48-49° C, a temperature at which heat damage is minimum or negligible (89), heat damage as a cause of artifact lignin as exemplified by Van Soest (86) may be ruled out. The artifact negative digestibilities encountered with lignin in red clover and Lindsey 77F might be due to some other factor as hemicellulose, which was reported by Moon and Abou-Raya (44) as a contributor to artifact lignin increase.

Although cell contents are known to be highly digestible, the poor digestibility (44 per cent) of cell contents in Lindsey 77F may be partially attributed to the non-cell-wall matter in the feces. Fecal non-cell-wall material comprises animal residues and bacterial matter from digestive processes (88), and poor quality coarse roughages such as Lindsey 77F may induce more sloughing of the digestive tract, on the part of the animal. Similar observations have also been referred to in Experiment I.

The influence of different forage species on the rumen volatile fatty acids (VFA) and the acetate:propionate ratio are presented in Table XVII. Concentration of butyric acid was highest (55.8 mg./100 ml.) with alfalfa, intermediate (37.6 mg/.100 ml.) with red clover and lowest (27.5 mg./100 ml.) with Lindsey 77F. The butyric acid level was significantly higher ($P < 0.05$) in alfalfa than in either of the other two hays. Total VFA content was highest with alfalfa (480 mg./100 ml.) but the differences in total VFA content were not significantly different.

Acetic acid was the predominate acid in the Total VFA. It was 71.3 per cent of the total for Lindsey 77F, 67.8 per cent for red clover and 64.6 per cent for alfalfa. In contrast to acetic acid butyric acid was highest (11.5 per cent) with alfalfa, intermediate (9.9 per cent) with red clover and lowest (6.2 per cent) with Lindsey 77F. For both acetic and butyric acids all differences were significant ($P < 0.05$). Acetate:propionate

ratios with red clover and Lindsey 77F were similar. However, this ratio with red clover or Lindsey 77F was significantly higher ($P < 0.05$) than with alfalfa. The increase in butyrate with alfalfa, acetate with Lindsey 77F of Experiment II confirm the findings of Williams and Christian (92), Davis et al. (17), Annison (2) and el-Shazly (71).

Correlation and Regression Analyses

Gross simple correlation coefficients among the various chemical components and related variables such as digestibility of dry matter (DDM) and voluntary intake of dry matter are presented in Table XIX. Correlation analyses were based on all forages (nine alfalfa, three red clover and three Lindsey 77F) used in the study.

Dry matter digestibility and voluntary intake of dry matter were highly correlated ($r = 0.84$, significant at $P < 0.01$). As was expected digestion coefficients for crude protein, energy, cell wall contents and cell contents were highly correlated with digestibility of dry matter (DDM). DDM showed highly significant negative correlations with content of acid detergent fiber (ADF) ($r = -.78$) and cell wall constituents ($r = -.72$). The correlation between DDM and lignin was quite small and non significant. But when lignin was expressed as per cent in ADF, a significant positive correlation ($r = 0.53$) was obtained between DDM and lignin in ADF (L/ADF). Lindsey 77F which had a low lignin content as well as the lowest intake appeared to

TABLE XIX

GROSS SIMPLE CORRELATION COEFFICIENTS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	.84	.90	.96	.83	.11	.66	.85	-.78	.05	.53	-.72	.72	.62	.35	.12	.91
1		.69	.80	.61	.58	.31	.85	-.81	.08	.55	-.83	.83	.46	.45	.15	.98
2			.86	.81	.87	.67	.70	-.57	.17	.52	-.51	.51	.72	.05	-.04	.78
3				.91	-.74	.78	.70	-.68	-.12	.34	-.59	.59	.57	.36	.25	.90
4					.85	.89	.47	-.40	-.12	.17	-.34	.34	.55	.19	.19	.75
5						.60	.58	-.36	.36	.55	-.42	.42	.63	-.12	-.22	.65
6							.19	-.22	-.37	-.15	-.03	.03	.46	.14	.30	.49
7								-.84	.35	.79	-.89	.89	.43	.34	-.12	.81
8									.70	-.54	.94	-.94	-.59	-.68	-.33	-.80
9										.80	-.19	.19	.02	-.63	-.92	.01
10											-.72	.72	.42	-.13	-.57	.49
11												-1.00	-.53	-.57	-.12	-.78
12													.53	.57	.12	.78
13														.23	.20	.54
14															-.25	.45
15																.21

Legend: 1-16 and 0-15 variables listed on following page.

TABLE XIX (continued)

0 Digestion Coefficient of Dry Matter (DDM)
1 Dry Matter Intake
2 Digestion Coefficient of Crude Protein (DCP)
3 Digestion Coefficient of Energy
4 Digestion Coefficient of Acid Detergent Fiber (D/C ADF)
5 Digestion Coefficient of Lignin (D/C L)
6 Digestion Coefficient of Cell Wall Constituents (D/C CWC)
7 Digestion Coefficient of Cell Contents (D/C CC)
8 Acid Detergent Fiber (ADF)
9 Lignin (L)
10 Lignin per cent in ADF (L/ADF)
11 Cell Wall Constituents (CWC)
12 Cell Contents (CC)
13 Crude Protein (CP)
14 Availability Index (AI)
15 Summative Equation (SE)
16 Nutritive Value Index (NVI)

Values with an absolute value greater than 0.514 are statistically significant at $P < 0.05$.

Values with an absolute value greater than 0.641 are statistically significant at $P < 0.01$.

contribute to the positive relationship between DDM and L/ADF.

The lack of significant correlation between lignin and DDM observed in the present study was in contradiction to the reports of Van Soest (85), Scholl et al. (69) and Oh et al. (56), who reported highly significant negative correlations between lignin and DDM. The negative relationship between ADF and DDM, observed in the present investigation was similar to that reported by Van Soest (85), Scholl et al. (69) and Oh et al. (56). Van Soest and Marcus (89) obtained a negative correlation of -.65 between DDM and cell wall contents, while Oh et al. (56) reported a much lower correlation ($r = -.47$) between DDM and cell wall contents.

Dry matter digestibility was significantly positively correlated with crude protein ($r = 0.62$) which supports the results of Sullivan (82) who obtained a highly positive correlation ($r = 0.64$) between DDM and crude protein in forages.

The present study indicated that neither the Availability Index (AI) nor the Summative Equation (SE) was significantly correlated with DDM. In contrast Van Soest (87), Scholl et al. (70) and Ademosun et al. (1) reported highly significant positive correlations between DDM and either SE or AI. However, a highly significant positive correlation ($r = 0.91$) was obtained between DDM and nutritive value index (NVI), which supported the work of Crampton et al. (14).

Voluntary dry matter intake of forages used in the present

investigation showed highly significant positive correlations with protein digestibility (r = 0.69) and energy digestibility (r = 0.80). Significant positive correlations between voluntary dry matter intake and digestibility of ADF (r = 0.61) or digestibility of lignin (r = 0.59) were also observed.

Highly significant ($P < 0.01$) negative correlations were obtained between voluntary dry matter intake and per cent of ADF (r = -.81) and cell wall contents (r = -.83). A significant ($P < 0.05$) positive correlation between voluntary intake of dry matter and lignin per cent in ADF (r = 0.55) was observed. This positive relationship between intake and lignin in ADF was similar to that of DDM with lignin in ADF and may be attributed to Lindsey 77F which was low in lignin and had the lowest intake.

Per cent lignin in dry matter did not influence voluntary dry matter intake. However, there was a significant positive correlation (r = 0.58) between dry matter intake and lignin digestibility. This was probably due to the high dry matter intake on bud stage alfalfa in Experiment I and alfalfa in Experiment II coupled with the high digestibility of lignin in these hays. This is in agreement with Van Soest (86) that lignin in legumes is more highly digestible than in grasses. This observation was contrary to the reports of Forbes and Garrigus (25) who found that for each percentage unit in forage lignin content there was a decrease of 5.8 per cent of maximum intake and a negative relationship (r = -.71) between intake and

forage lignin content. However, Van Soest (87) observed that the relationship between voluntary dry matter intake and lignin content of forages was quite variable and confounded; further, that positive relations between intake and lignin could appear because of grass-legume interaction.

The highly significant negative relationship between voluntary dry matter intake and per cent of ADF ($r = -.81$) or cell wall content in forages ($r = -.83$), noted in the present study was in agreement with the results obtained by Van Soest (86). It can be concluded that as forages become more mature, voluntary dry matter intake generally decreases as a consequence of an increase in the total fibrous fractions of the forages.

As observed with DDM, the Availability Index and the Summative Equation had no significant relationship with voluntary intake of dry matter.

In addition to the relationship of DDM and voluntary dry matter intake with the several chemical components of forages, some interesting observations were also made in the present study. A significant ($P < 0.05$) negative correlation was observed between digestibility of crude protein and content of ADF ($r = -.59$) and between digestibility of crude protein and cell wall contents ($r = -.53$). This is in support of the observations of Sotola (73) and Van Riper and Smith (84), who reported a negative relationship between digestibility of crude protein and crude fiber in forages.

Nutritive value index (NVI) which is a numerical evaluation of a forage is a function of both total dry matter intake and energy digestibility. There was a highly significant ($P < 0.01$) negative correlation between NVI and per cent of ADF ($r = -.80$) and between NVI and cell wall contents ($r = -.78$). However, a significant ($P < 0.05$) positive correlation was obtained between NVI and crude protein content ($r = 0.54$).

Digestibility of cell contents was negatively correlated with ADF content ($r = -.84$) and with cell wall constituents ($r = -.89$); this relationship was highly significant ($P < 0.01$). However, lignin in ADF and digestibility of cell contents exhibited a highly significant positive correlation ($r = 0.79$). A diagram of the individual data indicates this could be due to low digestibility of cell contents and low lignin in ADF for Lindsey 77F.

Though the fibrous fractions in forages did influence the other variables included in the study, lignin in dry matter had only very little influence on most of the factors of the present investigation.

A highly significant ($P < 0.01$) positive correlation between per cent of ADF and lignin in dry matter ($r = 0.70$) and a significant ($P < 0.05$) negative correlation between ADF content and lignin in ADF ($r = -.54$) was observed. Between Availability Index (AI) and ADF content, there was a highly significant negative correlation ($r = -.68$) and significant ($P < 0.05$)

negative correlations between AI and lignin content (r = -.63) and between AI and cell wall contents (r = -.57) were also obtained. However, a highly significant negative correlation was observed between Summative Equation (SE) and lignin content (r = -.92) and only a significant negative correlation between SE and lignin per cent in ADF (r = -.57). The significance of these relationships among ADF, lignin, lignin in ADF, cell wall contents, AI and SE could be due to the fact that ADF and cell wall contents contain lignin and that the AI and SE values are based on the amount of lignin and cell wall contents of the forages.

Prediction equations, standard error and correlation coefficients for the various chemical constituents and voluntary intake of dry matter are presented in Table XX. The results indicated that the most reliable means of predicting DDM of all forages were: voluntary dry matter intake (r = 0.84, SE = $\pm$ 3.55), acid detergent fiber (ADF) content (r= -0.78, SE = $\pm$ 4.05) and cell wall constituents (r = -0.72, SE = $\pm$ 4.49). Crude protein content was a better predictor of DDM (r = 0.62, SE = $\pm$ 5.11) than lignin in ADF (r = 0.53, SE = $\pm$ 5.50). Although high negative correlation of lignin with dry matter digestibility was reported by Norman (50), Phillips and Loughlin (60) and Sullivan (82), the present study did not favor this opinion, instead it was found that ADF and cell wall contents were better predictors of DDM than lignin in forage dry matter. The manner in which

TABLE XX

RELATION BETWEEN CHEMICAL CONSTITUENTS OF FORAGES, DIGESTIBLE DRY MATTER AND VOLUNTARY INTAKE

Variable (X)	Prediction equation	r	SE
	Digestible dry matter (Y)		
ADF	Y = 116.25 - 1.25X	-0.78**	4.05
L/ADF	Y = 48.58 + 1.35X	0.53*	5.50
CWC	Y = 103.04 - 0.59X	-0.72**	4.49
CP	Y = 41.49 + 1.75X	0.62*	5.11
DM Intake	Y = 36.53 +13.54X	0.84**	3.55
	Voluntary intake (Y)		
ADF	Y = 5.42 - 0.08X	-0.81**	0.23
L/ADF	Y = 1.03 + 0.09X	0.55*	0.33
CWC	Y = 4.40 - 0.04X	-0.83**	0.22

r = Correlation coefficient.
SE = Standard Error
ADF = Acid Detergent Fiber.
CWC = Cell Wall Constituents.
L/ADF = Lignin per cent in ADF.
*Statistically significant at $P < 0.05$.
**Statistically significant at $P < 0.01$.

lignin affects digestibility is not completely understood and the relationship of lignin and digestibility shows marked differences in plant species, particularly between grasses and legumes. Legumes have a higher lignin content than grasses and lignin in legumes is diegested much better than in non-legumes (86). In the present study it was observed that alfalfa and red clover had a higher lignin content than it was in Lindsey 77F and the digestibility of lignin in alfalfa was much higher than that in Lindsey 77F. Lignin digestibility with red clover in the present study was lower than that with Lindsey 77F and the reason for this could not be explained within the scope of the study.

Of the four chemical predictors of voluntary dry matter intake, best predictions were obtained with cell wall content ($r = -.83$, SE = $\pm$ 0.22) and with content of ADF ($r = -.81$, SE = $\pm$ 0.23). However, lignin in ADF was less effective ($r = 0.55$, SE = $\pm$ 0.33) as a predictor of voluntary intake of forage dry matter.

Lignin in dry matter of forages could not be used in the present investigation as a possible predictor for either DDM or voluntary intake of dry matter, because lignin had no significant relationship with DDM or intake of dry matter.

Further, Sullivan (82) utilized the percentage of crude protein to predict digestible crude protein ($r = 0.997$). Baumgardt et al. (5) also reported a highly significant positive

correlation ($r = 0.999$, $P < 0.01$) between crude protein and digestible protein (DCP) in forages and that DCP could be estimated from crude protein. In the present study, crude protein and digestibility of crude protein had a highly significant positive correlation ($r = 0.72$, $P < 0.01$) and as such crude protein could be used to predict crude protein digestibility in forages.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. The fibrous fractions of forages, generally referred to as acid detergent fiber (ADF) and cell wall constituents had a significant negative correlation with crude protein. With advance in growth, the fibrous fractions increased and crude protein decreased.

2. Lignin was higher in alfalfa and red clover than in Lindsey 77F. Acid detergent fiber and cell wall constituents were highest in Lindsey 77F. Lignin content increased with advance growth of forages.

3. Voluntary intake of dry matter, computed either on the basis of body size, (per cent B.W.) or on metabolic size ($g/W_{kg}^{.75}$) showed a sharp decline with advancing growth and maturity. Voluntary intake of dry matter on first cut bud alfalfa hay was significantly higher than all the other stages of maturity studied. Voluntary intake of dry matter was also influenced by the forage species. Lindsey 77F was significantly lower than alfalfa.

4. Both relative intake and nutritive value index (NVI) were significantly lower with advance in stage of maturity; Lindsey 77F had singificantly lower relative intake and NVI than alfalfa.

5. Gross energy digestibility was affected by stage of maturity; first- and second-cut bud stages being similar but

significantly higher than at later stages of growth. Forage species had a significant effect on energy digestibility; energy digestibility was significantly ($P < 0.10$) higher in alfalfa (68.0 per cent) than in red clover (62.5 per cent) and Lindsey 77F (63.9 per cent). The difference between Lindsey 77F and red clover was not significant.

6. Dry matter digestibility (DDM), digestibilities of crude protein, cellulose, acid detergent fiber (ADF) and cell contents were similar at first- and second-cut bud stages of alfalfa, but these were significantly lower at later stages of growth (half bloom and post half bloom). Digestibility of cell wall constituents followed the same general pattern as for ADF except that values for half bloom plus nine days hay were quite high.

Alfalfa DDM, digestibilities of crude protein, lignin and cell contents were significantly higher than that of red clover and Lindsey 77F. However, alfalfa and Lindsey 77F were similar in regard to digestibility of ADF (64.0, 60.3 per cent, respectively) and digestibility of cell wall contents (69.3, 70.2 per cent, respectively), but higher than in red clover (ADF digestibility 53.9 and cell wall contents digestibility 62.8 per cent).

7. Apparent digestibility of lignin was the most variable of all the chemical constituents of forages studied. Negative digestibility of lignin encountered in the study could have been

caused by heat damage to feces and/or the presence of a precipitate due to hemicellulose in lignin determination may be considered as factors contributing to artifact lignin. The relationships of lignin and digestibility shows marked differences in plant species. Legumes have higher lignin content than grasses and lignin in legumes is digested much better than that in grasses (85). The present study showed that alfalfa and red clover had a higher lignin content than Lindsey 77F and in alfalfa lignin digestibility was higher than in Lindsey 77F. Lignin digestibility for red clover, in the present investigation was much lower than for Lindsey 77F. The reason for this observation could not be explained.

8. Digestibility of cell contents ranged from about 85 to 40 per cent. Lowered digestibility of cell contents may be attributed to the non-cell-wall matter in feces which comprises animal endogenous residues and bacterial cells from digestive processes, the magnitude of these excretions being governed by the amount of indigestible feed residue in the gut (88).

9. Per cent butyric acid from first- and second-cut bud alfalfa was similar but there was a significant decrease between the bud stage and all other stages of alfalfa hay. Alfalfa hay feeding resulted in highest butyric acid production (55.8 mg/100 ml) and significantly differed from that of Lindsey 77F (27.5 mg/100 ml). Per cent butyrate was significantly different among alfalfa, red clover and Lindsey 77F. Increase in butyrate

was found to be associated with higher protein content of the forages studied.

10. Acetate:propionate ratios were significantly lower on first cut bud and second cut bud stages than at half bloom stages. This ratio with red clover and Lindsey 77F was significantly higher than that for alfalfa.

11. Dry matter digestibility showed highly significant positive correlations with voluntary intake of dry matter, digestibility of crude protein, digestibilities of acid detergent fiber, cell wall constituents, cell contents. Lignin per cent in ADF and nutritive value index were also correlated similarly with DDM. The per cent of crude protein was significantly correlated with DDM. Highly significant negative correlations existed between DDM and the per cent of ADF or cell wall contents; Voluntary dry matter intake was similarly related to cell wall content. This observation led to the conclusion that with an increase in the fibrous fractions (ADF and cell wall constituents) in forages, there was a corresponding decrease in digestibility of dry matter and/or voluntary intake of dry matter.

12. Lignin in dry matter had no significant relationship with digestibility or intake of dry matter.

13. Digestibility of dry matter (DDM) was not related to either the Availability Index or the Summative Equation.

14. The most reliable means for predicting dry matter digestibility of forages were: voluntary intake of dry matter

(r = 0.84, SE = ± 3.55), acid detergent fiber (r = -.78, SE = ± 4.05) and cell wall constituents (r = -.72, SE = ± 4.49).

15. Voluntary intake of dry matter was most accurately predicted from cell wall constituents (r = -.83, SE = ± 0.22) and ADF content (r = -.81, SE = ± 0.23) in the forages.

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APPENDIX

TABLE XXI

ANALYSES OF VARIANCE SUMMARY FOR DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT I

Source	Degrees of freedom	Mean squares for: Dry matter intake (% B.W.)	Dry matter intake ($g/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility
Treatments	5	0.702**	1093.37**	1710.64*	2347.75*	220.58**
Error	30	0.039	42.10	656.11	68.82	11.52
CV		7.46	5.95	18.80	8.43	4.73

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

CV = Coefficient of Variation.

TABLE XXII

ANALYSES OF VARIANCE SUMMARY FOR DIGESTIBILITY COEFFICIENTS, EXPERIMENT I

Source	Degrees of freedom	Mean squares for apparent digestibility coefficients (%)						
		Dry matter	Crude protein	Cellulose	ADF	Lignin	CWC	Cell contents
Treatments	5	172.17**	181.54**	147.06**	167.32**	747.50**	131.45**	207.92**
Error	30	11.34	6.81	12.81	22.17	152.22	16.08	11.40
CV		4.58	3.33	4.80	7.33	84.93	5.76	4.34

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

CV = Coefficient of Variation.

TABLE XXIII

ANALYSES OF VARIANCE SUMMARY FOR RUMEN VOLATILE FATTY ACID CONCENTRATION, EXPERIMENT I

Source	Degrees of freedom	Mean squares for VFA concentration (mg/100 ml)					
		Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
Treatments	5	41350.46**	4082.06**	1855.05**	73.88*	29.57**	103507.2**
Error	30	9573.91	828.82	235.98	23.27	7.87	21827.5
CV		25.69	29.55	38.47	45.14	75.59	27.66

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

VFA = Volatile Fatty Acid.

CV = Coefficient of Variation.

TABLE XXIV

ANALYSES OF VARIANCE SUMMARY FOR RUMEN VOLATILE FATTY ACID DISTRIBUTION AND ACETATE: PROPIONATE RATIO, EXPERIMENT I

Source	Degrees of freedom	Mean squares for per cent of total					Acetate: propionate
		Acetic	Propionic	Butyric	Isovaleric	Valeric	
Treatments	5	856.51**	75.21**	30.21**	4.74**	1.26	2.39**
Error	30	2.78	0.96	2.19	1.09	0.93	0.05
CV		1.84	6.23	5.88	23.15	90.70	5.51

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

CV = Coefficient of Variation.

TABLE XXV

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT I

Hay	Animal	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake	Nutritive value index	Gross energy digestibility (%)
1	334	3.60	145.24	181.55	148.53	81.81
	333	3.32	134.02	167.53	136.79	81.65
	328	3.20	132.25	165.31	130.88	79.17
	327	3.32	136.91	171.14	136.78	79.92
	326	3.39	136.59	170.74	131.06	76.76
	321	3.04	127.41	159.26	124.80	78.36
	Mean	3.31	135.40	169.26	134.81	79.62
2	334	2.89	117.92	147.40	103.39	70.14
	333	2.68	108.73	135.91	92.39	67.98
	328	2.62	108.85	136.06	90.72	66.68
	327	2.54	106.30	132.88	81.38	61.24
	326	2.66	109.01	136.26	111.90	82.12
	321	2.41	101.12	126.40	87.13	68.93
	Mean	2.63	108.66	135.82	94.49	69.52
3	331	2.52	103.59	129.49	103.62	80.02
	330	2.88	114.05	142.56	113.48	79.60
	329	2.84	113.27	141.59	112.80	79.67
	325	2.43	102.04	127.55	97.67	76.57
	324	2.49	103.31	129.14	101.57	78.65
	322	2.38	100.03	125.04	96.62	77.27
	Mean	2.59	106.05	132.56	104.29	78.63

TABLE XXV (continued)

Hay	Animal	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake	Nutritive value index	Gross energy digestibility (%)
4	334	2.56	107.31	134.14	91.66	68.33
	333	2.32	95.02	118.78	77.31	65.09
	328	2.42	101.90	127.38	75.52	59.09
	327	2.47	104.50	130.06	88.41	67.98
	326	2.29	93.97	117.46	78.90	67.17
	321	2.39	102.42	128.03	82.18	64.19
	Mean	2.41	100.85	125.98	82.33	65.34
5	331	2.58	108.28	135.35	97.74	72.21
	330	2.80	112.63	140.79	95.01	67.48
	329	2.64	107.05	133.81	94.83	70.87
	325	2.33	98.14	122.68	86.81	70.76
	324	2.51	105.55	131.94	94.47	71.60
	322	2.33	99.69	124.61	91.66	73.56
	Mean	2.53	105.22	131.53	93.42	71.08
6	331	2.27	93.99	117.49	76.16	64.82
	330	2.77	109.75	137.19	89.48	65.22
	329	2.71	109.19	136.49	98.05	71.84
	325	2.19	91.06	113.83	74.70	65.62
	324	2.34	96.85	121.06	79.15	65.38
	322	2.03	86.20	107.75	69.30	64.32
	Mean	2.39	97.84	122.30	81.14	66.20

Relative Intake = gms. daily DM consumed/$kgBW^{.75}$ x 1.25

Nutritive Value Index = Relative Intake x per cent Digestible Energy.

TABLE XXVI

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON DIGESTIBILITY, EXPERIMENT I

Hay	Animal	Per cent digestibility of						
		Dry matter	Crude protein	Cellulose	ADF	Lignin	CWC	Cell contents
1	334	82.69	85.62	84.15	74.98	36.27	77.26	86.68
	333	81.88	86.63	83.11	75.48	38.18	74.71	87.01
	328	79.34	84.48	80.92	69.48	18.20	71.54	84.97
	327	79.47	85.01	80.17	71.91	29.23	71.26	85.35
	326	75.27	81.18	77.06	63.61	10.19	65.15	82.12
	321	79.42	84.77	71.60	71.73	32.62	71.46	85.27
	Mean	79.68	84.62	79.50	71.20	27.45	71.90	85.23
2	334	73.34	76.94	75.21	63.26	10.15	69.58	78.40
	333	71.51	76.33	75.41	63.01	-2.54	68.91	74.95
	328	68.82	75.48	71.95	57.95	-10.05	64.40	88.21
	327	64.84	70.42	68.40	49.92	-40.77	60.32	70.74
	326	83.18	86.02	84.39	74.36	30.13	80.65	86.53
	321	70.75	75.52	74.04	57.20	-12.44	67.40	75.22
	Mean	72.07	76.79	60.95	60.95	-4.25	68.54	79.01
3	331	82.13	86.20	83.45	72.08	29.66	78.89	85.27
	330	81.48	85.34	80.66	72.47	32.81	78.12	84.77
	329	81.28	86.09	82.11	71.59	27.16	77.53	84.95
	325	78.90	83.57	79.64	66.27	18.00	74.11	83.63
	324	80.71	85.18	81.28	68.23	20.11	76.40	84.89
	322	78.62	84.09	79.52	66.13	17.16	73.91	83.28
	Mean	80.52	85.08	81.11	69.46	24.15	76.49	84.47

TABLE XXVI (continued)

Hay	Animal	Per cent digestibility of						
		Dry matter	Crude protein	Cellulose	ADF	Lignin	CWC	Cell contents
4	334	71.93	76.81	73.51	62.34	20.00	67.48	76.91
	333	68.28	76.99	69.83	58.62	12.27	64.15	72.62
	328	63.34	73.61	64.45	50.44	-5.29	56.66	70.49
	327	70.55	78.09	69.80	61.26	18.73	67.08	74.55
	326	70.80	78.38	69.35	59.98	14.79	65.87	76.27
	321	67.62	76.82	65.37	55.59	7.26	63.14	72.75
	Mean	68.75	76.78	68.72	58.04	11.29	64.06	73.93
5	331	73.39	78.35	74.12	68.14	23.20	73.25	73.60
	330	67.97	73.70	68.46	61.80	7.10	69.42	69.05
	329	71.48	76.87	72.53	66.69	16.11	71.57	71.34
	325	71.24	76.48	74.65	63.78	9.27	71.02	71.60
	324	73.03	78.02	75.17	65.54	13.50	72.09	74.54
	322	74.35	78.34	76.62	66.60	17.57	73.78	75.28
	Mean	71.91	76.96	73.59	65.43	14.46	71.86	72.57
6	331	66.95	68.84	69.01	58.64	8.53	64.06	71.17
	330	67.50	68.49	70.60	61.27	13.65	65.88	69.83
	329	73.21	74.18	74.76	67.24	27,57	71.15	76.15
	325	67.26	70.44	68.32	58.83	12.62	62.49	73.78
	324	67.68	71.70	69.30	60.43	17.31	64.67	72.07
	322	66.13	69.97	68.47	57.34	4.90	62.57	71.01
	Mean	68.12	70.60	70.08	60.63	14.10	65.14	72.33

ADF = Acid Detergent Fiber. CWC = Cell Wall Constituents.

TABLE XXVII

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON RUMEN VOLATILE FATTY ACID CONCENTRATION
EXPERIMENT I

Hay	Animal	VFA concentration (mg/100 ml)					
		Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
1	334	354.3	113.4	68.1	14.3	11.1	561.2
	333	303.1	96.0	57.8	10.4	7.4	474.7
	328	225.3	69.2	38.6	9.8	3.7	346.6
	327	456.7	144.3	75.9	9.8	9.3	696.0
	326	244.3	70.9	72.4	7.8	2.9	398.3
	321	319.9	96.9	60.6	10.4	5.8	493.6
	Mean	317.3	98.5	62.2	10.4	6.7	495.1
2	334	19.9	5.3	3.0	1.1	1.6	30.9
	333	60.7	17.4	9.6	3.2	1.4	92.3
	328	90.1	24.1	13.3	3.2	nd	130.7
	327	87.8	27.4	13.4	3.9	nd	132.5
	326	201.2	59.7	31.5	10.7	2.9	306.0
	321	175.1	50.2	24.1	9.2	2.9	261.5
	Mean	105.8	30.7	15.8	5.2	1.5	159.0
3	331	126.6	35.5	23.3	6.0	nd	191.4
	330	69.9	22.6	14.2	2.9	1.4	111.0
	329	359.4	108.9	65.1	14.1	7.6	555.1
	325	182.7	54.4	29.5	6.4	3.8	276.8
	324	273.7	86.0	39.8	10.2	3.8	413.5
	322	163.8	46.0	25.1	8.0	2.9	245.8
	Mean	196.0	58.9	32.8	7.9	3.3	298.9

TABLE XXVII (continued)

Hay	Animal	VFA concentration (mg/100 ml)					
		Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
4	334	258.8	68.5	36.8	10.1	4.5	378.7
	333	197.4	59.1	37.2	8.1	3.0	304.8
	328	445.7	122.7	64.7	17.7	12.8	663.6
	327	31.0	7.3	4.4	2.0	nd	44.7
	326	199.5	55.4	31.4	10.6	6.0	302.9
	321	202.7	61.9	27.4	28.3	3.0	323.3
	Mean	222.5	62.5	33.7	12.8	4.9	336.3
5	331	69.3	20.4	7.8	3.0	nd	100.5
	330	273.0	79.5	40.2	9.1	3.8	405.6
	329*						
	325	85.6	25.3	13.7	3.5	nd	128.1
	324	102.9	31.0	13.7	3.0	nd	150.6
	322	111.3	32.2	18.1	3.5	nd	165.1
	Mean	128.4	37.7	18.7	4.4	0.6	190.0
6	331	77.2	23.6	11.3	2.5	nd	114.6
	330	67.9	20.5	9.3	2.4	nd	100.1
	329	308.2	79.1	46.1	10.6	6.0	450.0
	325	109.2	34.2	21.1	5.1	3.0	172.6
	324	293.5	87.2	46.6	11.6	6.0	444.9
	322	84.9	25.8	12.0	3.4	2.9	129.0
	Mean	156.8	45.1	24.4	5.9	3.0	235.2

*Sample lost by accidental breakage of test tube.

nd = not detectable.

TABLE XXVIII

EFFECT OF STAGE OF MATURITY OF ALFALFA HAYS ON DISTRIBUTION OF RUMEN VOLATILE FATTY ACIDS AND ACETATE: PROPIONATE RATIO, EXPERIMENT I

Hay	Animal	VFA (per cent of total)					Acetate: propionate ratio
		Acetic	Propionic	Butyric	Isovaleric	Valeric	
1	334	63.1	20.2	12.1	2.5	2.0	3.12
	333	63.9	20.2	12.2	2.2	1.6	3.16
	328	65.0	20.0	11.1	2.8	1.1	3.26
	327	65.6	20.7	10.9	1.4	1.3	3.16
	326	61.3	17.8	18.2	2.0	0.7	3.45
	321	64.8	19.6	12.3	2.1	1.2	3.30
	Mean	64.0	19.8	12.8	2.2	1.3	3.24
2	334	64.4	17.2	9.7	3.6	5.1	3.75
	333	65.8	18.9	10.4	3.5	1.5	3.49
	328	68.9	18.4	10.2	2.4	nd	3.74
	327	66.3	20.7	10.1	2.9	nd	3.20
	326	65.8	19.5	10.3	3.5	0.9	3.37
	321	67.0	19.2	9.2	3.6	1.1	3.49
	Mean	66.4	19.0	10.0	3.3	1.5	3.51
3	331	66.1	18.5	12.2	3.1	nd	3.57
	330	63.0	20.4	12.8	2.6	1.3	3.09
	329	64.7	19.6	11.7	2.5	1.4	3.30
	325	66.0	19.7	10.7	2.3	1.4	3.36
	324	66.2	20.8	9.6	2.5	0.9	3.18
	322	66.6	18.7	10.2	3.3	1.2	3.56
	Mean	65.4	19.6	11.2	2.7	1.0	3.34

TABLE XXVIII (continued)

Hay	Animal	VFA (per cent of total) Acetic	Propionic	Butyric	Isovaleric	Valeric	Acetate: propionate ratio
4	334	68.3	18.1	9.7	2.7	1.2	3.78
	333	64.8	19.4	12.2	2.7	1.0	3.34
	328	67.2	18.5	9.7	2.7	1.9	3.63
	327	69.4	16.3	9.8	4.5	nd	4.25
	326	65.9	18.3	10.4	3.5	2.0	3.60
	321	62.7	19.1	8.5	8.8	0.9	3.27
	Mean	66.4	18.3	10.1	4.2	1.2	3.65
5	331	69.0	20.3	7.7	3.0	nd	3.40
	330	67.3	19.6	9.9	2.2	0.9	3.43
	329*						
	325	66.8	19.8	10.7	2.7	nd	3.38
	324	68.3	20.6	9.1	2.0	nd	3.32
	322	67.4	19.5	11.0	2.1	nd	3.46
	Mean	67.8	20.0	9.7	2.4	0.2	3.40
6	331	67.4	20.6	10.0	2.2	nd	3.27
	330	67.9	20.5	9.3	2.4	nd	3.31
	329	68.5	17.6	10.2	2.4	1.3	3.90
	325	63.3	19.8	12.2	3.0	1.7	3.19
	324	66.0	19.6	10.5	2.6	1.3	3.37
	322	65.8	20.0	9.3	2.6	2.2	3.29
	Mean	66.5	19.7	10.3	2.5	1.8	3.39

*Sample lost by accidental breakage of test tube.

VFA = Volatile Fatty Acid. nd = not detactable.

TABLE XXIX

ANALYSES OF VARIANCE SUMMARY FOR DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT II

Source	Degrees of freedom	Mean squares for				
		Dry matter intake (% B.W.)	Dry matter intake ($g/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility
Periods/squares	4	0.08	97.65	156.7	142.7	17.6
Animals/squares	4	0.02	24.83	48.9	68.9	26.5
Treatments	2	0.53*	880.7*	1376.1*	907.6*	48.7
Squares	1	0	8.5	26.0	1.0	0.3
Error	6	0.10	155.1	230.7	145.2	21.5
CV		14.00	13.7	13.4	16.3	7.2

*Statistically significant at $P < 0.05$.

CV = Coefficient of Variation.

TABLE XXX

ANALYSES OF VARIANCE SUMMARY FOR DIGESTIBILITY COEFFICIENTS, EXPERIMENT II

Source	Degrees of freedom	Mean squares for Apparent digestibility coefficients (%)					
		Dry matter	Crude protein	ADF	Lignin	CWC	Cell contents
Periods/squares	4	19.0	23.8	30.0	407.5	22.6	39.9
Animals/squares	4	23.1	31.5	20.1	229.0	9.5	56.6
Treatments	2	103.0*	290.5*	156.3*	2640.3*	98.4	1239.9**
Squares	1	2.9	6.4	4.9	328.8	2.3	5.4
Error	6	12.8	11.0	28.2	132.0	14.6	12.5
CV		5.5	5.2	8.9	92.5	5.7	5.9

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

CV = Coefficient of Variation.

TABLE XXXI

ANALYSES OF VARIANCE SUMMARY FOR RUMEN VOLATILE FATTY ACID CONCENTRATION, EXPERIMENT II

Source	Degrees of freedom	Mean squares for VFA concentration (mg/100 ml)					
		Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
Periods/squares	4	15091.2	1497.2	395.5	16.8	17.5	35316.1
Animals/squares	4	16467.3	1046.5	512.2	24.8	7.6	35764.3
Treatments	2	7520.0	951.3	1237.3*	32.8	7.4	17379.5
Squares	1	15167.0	832.3	563.4	13.7	0.5	32461.5
Error	6	5746.7	656.0	240.2	16.3	8.3	14464.7
CV		53.1	52.8	50.0	62.9	84.6	52.7

*Statistically significant at $P < 0.05$.

VFA = Volatile Fatty Acid.

CV = Coefficient of Variation.

TABLE XXXII

ANALYSES OF VARIANCE SUMMARY FOR DISTRIBUTION OF RUMEN VOLATILE FATTY ACIDS AND ACETATE: PROPIONATE RATIO, EXPERIMENT II

Source	Degrees of freedom	Mean squares for VFA (per cent of total)					Acetate: propionate ratio
		Acetic	Propionic	Butyric	Isovaleric	Valeric	
Periods/squares	4	8.6*	6.4	0.5	0.1	0.5	0.3*
Animals/squares	4	1.5	3.0	0.3	0.5	0.2	0.1
Treatments	2	67.7**	3.2	45.1**	0.3	0.2	0.3*
Squares	1	1.9	5.2	1.6	0.02	0.1	0.2
Error	6	1.6	1.6	0.3	0.2	0.6	0.04
CV		2.6	5.2	14.2	37.1	93.5	6.6

*Statistically significant at $P < 0.05$.

**Statistically significant at $P < 0.01$.

VFA = Volatile Fatty Acid.

CV = Coefficient of Variation.

TABLE XXXIII

CHEMICAL COMPOSITION OF THE THREE FORAGE SPECIES, EXPERIMENT II

Forage		Dry matter constituents								
		Dry matter	Crude protein	ADF	Lignin	Lignin in ADF	CWC	Cell contents	Ash in CWC	Gross energy (kcal/g)
		%								
Period I										
Alfalfa	Fed	91.85	16.12	37.39	5.65	15.11	55.88	44.12	1.43	4.52
	Weighback	92.16	18.78	34.82	5.68	16.31	51.11	48.89	1.62	4.42
Red clover	Fed	92.42	13.45	36.03	5.19	14.40	54.86	45.14	2.01	4.42
	Weighback	91.98	14.61	36.00	6.13	17.03	52.89	47.11	2.10	4.27
Lindsey 77F	Fed	93.25	15.16	42.21	4.43	10.50	68.77	31.23	4.21	4.41
	Weighback	93.33	12.76	46.97	5.03	10.71	69.03	30.97	8.87	3.93
Period II										
Alfalfa	Fed	93.80	17.07	35.97	5.62	15.62	55.28	44.72	2.41	4.49
	Weighback	93.97	14.66	42.78	7.62	17.81	59.55	40.45	2.88	4.31
Red clover	Fed	93.93	13.22	36.60	5.14	14.04	55.56	44.44	2.61	4.27
	Weighback	93.41	15.32	39.43	6.59	16.71	55.33	44.67	3.39	3.87
Lindsey 77F	Fed	94.05	15.30	42.04	4.44	10.56	70.56	29.44	5.53	4.24
	Weighback	94.20	13.45	45.25	4.69	10.36	70.94	29.06	7.54	3.90

TABLE XXXIII (continued)

Forage		Dry matter	Crude protein	ADF	Lignin	Lignin in ADF	CWC	Cell contents	Ash in CWC	Gross energy (kcal/g)
		Dry matter constituents								
		%								
Period III										
Alfalfa	Fed	93.10	16.68	37.48	5.86	15.64	54.32	45.68	1.53	4.47
	Weighback	92.52	17.95	36.84	5.93	16.10	53.55	46.45	1.96	4.38
Red clover	Fed	92.54	13.66	39.66	6.26	15.78	58.12	41.88	1.93	4.30
	Weighback	92.57	13.47	39.77	5.93	14.91	59.75	40.25	2.49	4.33
Lindsey 77F	Fed	92.31	14.18	45.05	5.35	11.88	71.41	28.59	3.53	4.33
	Weighback	92.99	10.80	46.12	4.65	10.08	69.74	30.26	4.60	4.02

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

TABLE XXXIV

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON DRY MATTER INTAKE, RELATIVE INTAKE, NUTRITIVE VALUE INDEX AND ENERGY DIGESTIBILITY, EXPERIMENT II

Period	Forage	Animal	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility (%)
I	Alfalfa	354	2.47	103.09	128.86	92.99	72.16
		362	2.86	120.56	150.70	101.45	67.32
	Red clover	364	2.53	95.83	119.79	77.60	62.12
		370	2.34	98.31	122.89	76.34	64.78
	Lindsey 77F	363	1.94	79.55	99.44	68.51	68.90
		367	1.79	73.39	91.74	61.36	66.89
II	Alfalfa	364	2.10	87.46	109.33	75.08	68.67
		370	2.53	101.82	127.28	83.34	65.48
	Red clover	363	2.40	99.82	124.78	82.77	66.33
		367	2.50	103.33	129.16	86.85	67.24
	Lindsey 77F	354	1.85	81.04	95.39	50.48	52.92
		362	1.89	76.53	101.30	65.08	64.24

TABLE XXXIV (continued)

Period	Forage	Animal	Dry matter intake (% B.W.)	Dry matter intake ($gm/W_{kg}^{.75}$)	Relative intake (RI)	Nutritive value index (NVI)	Gross energy digestibility (%)
III	Alfalfa	363	2.55	104.17	130.21	92.16	70.78
		367	2.34	97.23	121.54	77.41	63.69
	Red clover	354	2.10	87.56	109.45	63.24	57.78
		362	1.58	69.16	86.45	49.21	56.92
	Lindsey 77F	364	1.89	76.53	95.66	62.12	64.94
		370	1.97	82.79	103.49	68.06	65.76

Relative Intake = gms. daily dry matter consumed/$kgBW^{.75}$ x 1.25.

Nutritive Value Index = Relative Intake x per cent Digestible Energy.

TABLE XXXV

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON APPARENT DIGESTIBILITY COEFFICIENTS, EXPERIMENT II

Period	Forage	Animal	Apparent digestibility coefficients (%)					
			Dry matter	Crude protein	ADF	Lignin	CWC	Cell contents
I	Alfalfa	354	72.96	70.37	69.70	17.18	73.61	72.14
		362	67.94	65.87	62.99	-2.51	67.84	68.07
	Red clover	364	62.43	58.57	49.27	-50.20	58.70	67.03
		370	66.15	56.04	54.52	-46.50	65.22	67.27
	Lindsey 77F	363	66.62	67.61	63.51	-11.00	73.26	52.06
		367	65.84	68.17	63.80	-14.00	72.22	51.79
II	Alfalfa	364	70.06	71.02	59.23	-13.40	68.76	71.63
		370	73.15	77.96	65.64	11.80	71.38	75.33
	Red clover	363	67.71	59.33	56.67	-30.80	65.68	70.25
		367	68.66	60.69	60.89	-12.10	67.93	69.57
	Lindsey 77F	354	52.68	53.14	48.04	-44.40	61.68	31.26
		362	63.94	66.03	60.01	-12.30	71.20	46.60

TABLE XXXV (continued)

Period	Forage	Animal	Apparent digestibility coefficients (%) Dry matter	Crude protein	ADF	Lignin	CWC	Cell contents
III	Alfalfa	363	71.37	72.91	66.83	20.60	69.77	73.29
		367	66.32	67.27	59.68	23.50	64.61	68.36
	Red clover	354	59.78	52.36	52.44	-23.30	60.67	58.59
		362	59.24	54.92	49.70	-30.70	58.46	60.31
	Lindsey 77F	364	63.15	64.25	64.20	-18.00	71.44	42.14
		370	62.72	63.34	62.01	12.50	71.26	40.93

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

TABLE XXXVI

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON RUMEN VOLATILE FATTY ACID CONCENTRATION
EXPERIMENT II

Period	Forage	Animal	VFA concentration (mg/100 ml)					
			Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
I	Alfalfa	354	193.3	55.9	29.3	7.2	nd	285.7
		362	184.6	55.1	34.1	6.9	3.4	284.1
	Red clover	364	243.5	67.0	33.6	6.2	4.8	355.1
		370	216.9	60.7	31.6	5.8	nd	315.0
	Lindsey 77F	363	332.2	87.1	28.0	9.3	4.4	461.0
		367	306.6	74.2	24.7	7.9	nd	413.4
II	Alfalfa	364	121.8	51.7	21.5	2.4	0.7	198.1
		370	392.4	127.5	75.4	14.8	8.4	618.5
	Red clover	363	242.2	70.9	39.7	7.0	4.8	364.6
		367	398.9	111.2	58.9	10.1	nd	579.1
	Lindsey 77F	354	257.7	87.7	26.0	9.4	nd	380.8
		362	346.4	102.5	30.8	7.8	4.8	492.3

TABLE XXXVI (continued)

Period	Forage	Animal	VFA concentration (mg/100 ml) Acetic	Propionic	Butyric	Isovaleric	Valeric	Total
III	Alfalfa	363	520.6	160.2	89.7	17.2	8.4	796.1
		367	449.4	132.4	84.9	21.1	9.6	697.4
	Red clover	354	146.3	41.4	19.2	6.2	4.8	217.9
		362	279.7	85.0	42.5	7.8	4.8	419.8
	Lindsey 77F	364	336.7	97.0	25.3	7.8	4.8	471.6
		370	341.9	92.7	30.1	6.2	4.8	475.7

nd = not detectable.

TABLE XXXVII

EFFECT OF FEEDING DIFFERENT FORAGE SPECIES ON DISTRIBUTION OF RUMEN VOLATILE FATTY ACIDS AND ACETATE: PROPIONATE RATIO, EXPERIMENT II

Period	Forage	Animal	VFA (per cent of total)					Acetate: propionate ratio
			Acetic	Propionic	Butyric	Isovaleric	Valeric	
I	Alfalfa	354	67.7	19.6	10.3	2.5	nd	3.46
		362	65.0	19.4	12.0	2.4	1.1	3.35
	Red clover	364	68.6	18.9	9.5	1.7	1.4	3.63
		370	68.9	19.3	10.0	1.8	nd	3.57
	Lindsey 77F	363	72.1	18.9	6.1	2.0	1.0	3.81
		367	74.2	17.9	6.0	1.9	nd	4.13
II	Alfalfa	364	61.5	26.1	10.9	1.2	0.4	2.36
		370	63.4	20.6	12.2	2.4	1.4	3.08
	Red clover	363	66.4	19.4	10.9	1.9	1.3	3.42
		367	68.9	19.2	10.2	1.7	nd	3.59
	Lindsey 77F	354	67.7	23.0	6.8	2.5	nd	2.94
		362	70.4	20.8	6.3	1.6	1.0	3.38

TABLE XXXVII (continued)

Period	Forage	Animal	VFA (per cent of total)					Acetate: propionate ratio
			Acetic	Propionic	Butyric	Isovaleric	Valeric	
III	Alfalfa	363	65.4	20.1	11.3	2.2	1.1	3.25
		367	64.4	19.0	12.2	3.0	1.4	3.39
	Red clover	354	67.1	19.0	8.8	2.8	2.2	3.53
		362	66.6	20.2	10.1	1.9	1.1	3.29
	Lindsay 77F	364	71.4	20.6	5.4	1.7	1.0	3.47
		370	71.9	19.5	6.3	1.3	1.0	3.69

nd = not detectable.