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Prediction of animal performance from chemical analyses and in vitro digestibility data

John Roland Plummer

To the Graduate Council:

I am submitting herewith a thesis written by John Roland Plummer entitled "Prediction of animal performance from chemical analyses and in vitro digestibility data." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

M.J. Montgomery, Major Professor

We have read this thesis and recommend its acceptance:

J.T. Miles, D.O. Richardson, E.W. Swanson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

September 1, 1967

To the Graduate Council:

I am submitting herewith a thesis written by John Roland Plummer entitled "Prediction of Animal Performance from Chemical Analyses and In Vitro Digestibility Data." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

M. J. Montgomery
Major Professor

We have read this thesis and recommend its acceptance:

D. O. Richardson

Eric W. Swanson

Accepted for the Council:

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Vice President for
Graduate Studies and Research

PREDICTION OF ANIMAL PERFORMANCE FROM CHEMICAL ANALYSES AND *IN VITRO* DIGESTIBILITY DATA

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

John Roland Plummer

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CHAPTER I

INTRODUCTION

Evaluation of forages is expensive and often time consuming. Since feed cost is one of the most expensive items in milk production, the use of a good forage in the dairy ration has long been recognized as valuable and economical; therefore, it is of interest to the dairyman that accurate, fast, and economical methods of forage evaluation be available.

Well conducted feeding and digestion trials have been recognized as two of the best methods of forage evaluation; however, the time involved, facilities required, and amount of feed needed to conduct feeding and digestion trials limit the number of forages that can be evaluated.

The development of new forages, the improvement of the present ones, and the changes that occur with advancing maturity make it increasingly important that faster and more economical methods of forage evaluation be developed. This study was conducted to determine the value of several chemical analyses and *in vitro* techniques as predictors of dry matter digestibility and dry matter intake of forage.

CHAPTER II

REVIEW OF LITERATURE

Proximate Analyses Versus Recent Chemical Methods of Feed Evaluation

The chemical analyses most widely used for feed evaluation have been those of the "Proximate" (Weende) system. The Weende system was developed more than 150 years ago and has been standardized in present form for more than 100 years. It includes determinations of crude protein, crude fiber, ether extract, ash, and nitrogen free extract (NFE). The crude fiber fraction supposedly represents the fibrous, poorly digested fraction of feedstuffs which is considered to be made up of the skeletal portion of the plant, consisting of celluloses, hemicelluloses, and lignin. Nitrogen free extract supposedly represents those portions of the plant which are readily digestible, such as starches and sugars.

The procedure for determining crude fiber is purely empirical; this procedure is intended to remove proteins, sugars, and starches, leaving a residue of cellulose, hemicellulose, and lignin, along with mineral matter. Weight loss on ignition of the residue is considered crude fiber.

Many workers have observed that crude fiber is not a good measure of total fiber content or undigestible portion of a forage (15, 20, 21, 24, 25). These workers indicated that the crude fiber fraction of a forage was composed largely of cellulose and lignin, but that much of the cellulose and lignin in the forage was not retained in the crude fiber fraction but was present in the NFE fraction.

Moore (20) examined a variety of feedstuffs and reported that in 30 percent of the feedstuffs the crude fiber was as digestible as the NFE. From these results it appears quite evident that crude fiber is not a good measure of the less digestible or fibrous fraction of a feedstuff.

Crampton and Maynard (8) indicated that when considering feed evaluation for herbivore, a partition of the carbohydrate portion of feedstuff into lignin, cellulose, and other carbohydrates instead of the old partition into crude fiber and NFE may have more biological significance and be of greater value in prediction of nutritive value of forages. Many other workers (12, 38, 57) have also suggested the replacement of crude fiber estimates by the determination of cellulose and lignin.

The most commonly used methods for cellulose and lignin determinations were mostly modifications of methods originally devised by Norman and Jenkins (27) in England and Crampton and Maynard (8) in the United States. Both of these methods have shortcomings in that serious problems occurred in the determination of lignin. Research (25, 44, 46, 50) indicated that Norman and Jenkins' (27) and Crampton and Maynard's (8) determination of cellulose and lignin did not give accurate measurements of lignin in the forage.

Since the older methods (8, 27) of cellulose and lignin determinations did not permit a high degree of specificity and required many hours of laboratory work, it was apparent that a new method of laboratory forage evaluation was needed.

A major problem in lignin determination was removing the protein from the sample during pretreatments. Van Soest (48) investigated the use of a number of detergents to dissolve forage nitrogen. Results of this work indicated that a 2 percent solution of sodium lauryl sulfate in a neutral or slightly alkali solution and a 2 percent solution of cetyl trimethylammonium bromide (CTAB) in strongly acid solution appeared to be effective in the preparation of a plant fiber of low nitrogen content. The fiber prepared by the neutral solution represented the cell wall constituents in essentially undergraded form and was designated as cell wall constituents by Van Soest (48). The soluble portion contained the highly digestible constituents and was designated cell contents. Cell contents were calculated by subtracting the percentage of cell wall constituents from 100. The fiber prepared by the acid digestion gave a considerably smaller yield and probably represented the more indigestible portion of the fiber. Van Soest designated the fibrous residue from the acid digestion as acid detergent fiber (ADF). The ADF residue retained lignin and also had a low nitrogen content, thus overcoming two of the chief criticisms of the proximate analysis for crude fiber (49). Van Soest termed the lignin content of the ADF as acid detergent lignin (ADL). ADL was determined by a modification of Sullivan's (44) 72 percent acid-insoluble lignin method.

From results obtained in the experiments to separate a fiber that was low in nitrogen and still retained all the lignin, Van Soest (53) suggested a classification system for forage organic matter which

appeared to be superior to the division of feedstuffs into crude fiber and NFE for predicting nutritive value of forages. This system is presented schematically in Table I.

Effects of Stage of Maturity on Chemical Composition

The effects of stage of maturity on chemical composition and nutritive value have been studied by many researchers. Results indicated that the main structural constituents of pasture and herbage increased progressively with maturity.

Observations from research (1, 26, 28, 30, 31, 32) on stage of maturity indicated that as plants matured, the cellulose content increased rapidly during periods of active growth and the increase became more gradual during periods of slower growth.

When plants are young and actively growing, very small amounts of lignification take place, but, as the plant matures and elongation of cells ceases, the amount of lignified area increases rapidly. Research (1, 9, 30, 31, 32, 42) indicated increased lignification patterns as plants matured.

Growth and digestibility studies of Crampton and Forshaw (7) clearly indicated that marked nutritive changes do occur in only 10 days growth of a forage. Their data indicated that dry matter, nitrogen, cellulose, and NFE gradually decreased in digestibility with increased maturity of a forage. Crampton and Forshaw (7) and Patton and Gieseker (31) reported that lignin content is closely related to digestibility and that as plants matured and lignification increased, digestibility of the forage decreased. Other investigators (1, 9, 17, 19, 22, 36, 37, 40)

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	Completely	High
	Hemicellulose	Partial	Very low
Insoluble in acid detergent (Acid Detergent Fiber)	Cellulose	Partial	Very low
	Lignin	Indigestible	Indigestible
	Lignified nitrogen compounds	Indigestible	Indigestible
	Heat-damaged protein	Indigestible	Indigestible
	Keratin	Indigestible	Indigestible
	Silica	Indigestible	Indigestible

have reported that dry matter digestibility decreased as the stage of maturity increased.

Several investigators (19, 23, 32, 40, 45) reported that as the stage of maturity increased, there was a definite decrease in the crude protein content of forages. Melin et al. (19) noted that the stage of maturity not only affected the amount of crude protein but also the digestibility of the protein. His study showed that the digestibility of protein ranged from 84.9 percent for the May 27 cutting to 51.6 percent for forage harvested on July 29.

Predicting the Nutritive Value of Feeds from Chemical Analyses

Baumgardt et al. (3) and Reid et al. (36) observed that a relationship existed between total protein and apparently digestible protein of a forage. Reid et al. (36) found that the percentage of apparently digested protein (Y) could be predicted from the amount of total protein (X) by using the equation $Y = 0.929X - 3.48$, with a standard error of only .46 percent. Baumgardt et al. (3) observed that the digestible protein could be estimated from the amount of crude protein with a standard error of only .25 and a coefficient of variation of 2.26 percent. Bowden and Church (5) observed that in vivo digestible dry matter (DDM) was highly correlated with the crude protein of a forage ($r = .94$), and that a similar relationship existed between in vitro cellulose digestion and crude protein ($r = .93$). Oh et al. (29) reported that when a group of 56 forages containing different species was used to predict nutritive value, the correlation between DDM and crude protein was highly

significant (r = .37) but was low and of little predictive value. However, when a relationship between in vivo DDM and crude protein was considered within legumes, a larger correlation (r = .76) was obtained. This relationship within grasses was low and non-significant. Phillips and Loughlin (33) also noted that use of two plant classes reduced the predictive value of the equation. They observed that the relationship between crude protein and digestible energy yielded the correlation coefficients of .78 and .96 for timothy and alfalfa hay, respectively. However, the correlation coefficient (r = .24) was non-significant when the species were combined.

Patton and Gieseker (31) indicated that lignin content of a feedstuff could be useful in predicting nutritive value. Van Soest (51) analyzed forage and feces samples for cell wall constituents, ADF, lignin, and total nitrogen. Digestibility of cell wall constituents, ADF, cell contents, and nitrogen were calculated and correlated with lignin (expressed as a percentage of dry matter), cell wall constituents, and ADF. When the lignin was expressed as a percentage of the ADF, correlations of greater magnitude were obtained with cell wall constituents and ADF (r = -.95 and -.93), respectively. Most of the regressions of digestibility of fibrous components on lignin (expressed as a percentage of ADF) showed curvilinearity. The correlations were found to be improved by the conversion of the lignin percentages to logarithms.

Forbes and Garrigus (13) used correlation and regression analyses to study the effects of variation in chemical composition of a forage

on its digestible organic matter. Their study revealed that the correlation (r = .95) between digestible organic matter and lignin content was larger than for other chemical entities. Van Soest (49) observed that lignin and digestible organic matter were highly correlated for grasses and legumes (r = -.92 and -.90), respectively. Nordfelt et al. (24) also indicated a large correlation coefficient for lignin and digestible organic matter.

Oh et al. (29) studied 56 forages and indicated that ADL was more highly correlated with in vivo DDM than either ADF or protein. Other research (43, 49) also concluded that lignin was valuable in predicting DDM.

Sullivan (44) reported a high correlation between lignin and DDM. He concluded that DDM could be predicted by using the formula: DDM = 100 - 6.00X, where X is the percent of acid-insoluble lignin. Sullivan suggested that this equation could be used for many, if not all, grasses.

The use of cell wall constituents in determining the nutritive value of forages was examined by several workers. Results (4, 29, 51) indicated that the correlation coefficient between cell wall constituents and nutritive value was highly significant. Nordfelt et al. (24) observed the correlation coefficient between cell wall constituents and digestible organic matter to be -.89 and the regression equation was: Y = 95.54 - 0.60X, where Y denotes the digestibility of organic matter and X denotes cell wall constituents expressed as a percentage of dry matter. Van Soest (51) reported the following regression equation

for the prediction of cell wall constituents digestibility: $Y = 147.5 - 78.9 \log X$, where Y is the cell wall constituents digestibility and X is the percentage of lignin in the ADF.

Crampton et al. (6) developed a nutritive value index which gave numerical description to a forage. They proposed that relative intake of a forage times its percent energy digestibility be used as a Nutritive Value Index to evaluate feeds by using a hypothetically ideal forage as 100 and rating other forages by this standard.

Moore (20) reported that net energy (NE) appeared to be the most valid method of expressing the energy of a feed. Lack of sufficient numbers of determined NE values tended to hamper the use of NE in feed evaluation; however, Moore's work indicated a gradual divergence of NE values occurred as the amount of total digestible nutrients (TDN) in the feed decreased. From this relationship the following regression equation was developed: $Y = 1.45X - 38.83$, where Y represents NE and X represents TDN.

Van Soest and Moore (55) developed prediction equations based on cell contents, cell wall constituents, ADF, and lignin. The digestibility of cell wall constituents was observed to be controlled by the amount of lignin in the ADF. The cell contents were found to be highly digestible and not affected by lignification. These authors concluded that the degree of lignification and the portion of the forage free from lignin were the two main factors contributing to the determination of a forage's digestibility. Since lignification (L) was negatively related to digestibility and cell contents (S) were positively related

to digestibility, these two factors were expressed as a ratio written $\frac{L}{S}$, which is an estimate of indigestibility. This function was found to have a linear relationship with digestibility; therefore, an Index of Availability (A) was derived: A = 100 - 100 (L/S).

Most regression equations developed to predict digestibility from chemical composition were restricted in their usefulness to the evaluation of forages of the same species or to forages with similar characteristics. Van Soest (51) observed that, generally, the mixing of species or plant classes, such as grasses and legumes, lowered the accuracy of the prediction equation. In many cases where the mixing of species occurs, the results may be highly correlated but of little predictive value because the high correlation could be caused by interaction between the species (54).

A new summative equation was developed by Van Soest (51), which included consideration of cell contents, endogenous excretions of the animal and the availability of cell wall constituents for digestion. Since cell contents digestibility was determined to be 98 percent and endogenous excretion of the animal had been given the constant value of 12.9 by Van Soest (54), the only other value that required estimation was the digestibility of cell wall constituents.

Van Soest (51) observed that cell wall constituents digestibility and the amount of lignin (expressed as a percent of the ADF) in a forage were highly correlated ($r = -.95$). The following regression equation for the prediction of cell wall digestibility was developed: $Y = 147.5 - 78.9 \log X$, where Y is digestibility of cell walls and X

is the percent lignin in ADF. Combination of the regression equation for prediction of cell wall constituents digestibility with the constant values previously obtained for cell contents digestibility (98 percent) and endogenous excretion of the animal (12.9) yielded the following equation: $DDM = .98S + W(147.3 - 78.9 \log X) - 12.9$, where S is the percent of cell contents, W the percent cell wall constituents, and L the percent lignin in ADF. This equation was termed the Summative Equation.

The availability index for DDM was predicted by the equation: $DDM = 78.2(1 - L/S) + 12.7$, where L represents the percent of lignin in the ADF and S represents the percent cell contents in the forage (51). When Van Soest compared the Summative Equation and the Availability Index Equation for the prediction of DDM, the results presented in Table II were obtained. The Availability Index displayed similar ability to predict digestibility in the group of forages used to derive the two equations; however, the Summative Equation appeared to be superior to the Availability Index Equation for the prediction of DDM in a group of forages not used to develop the regression equations. The Availability Index tended to give erratic values for forages of very high digestibility (51). The explanation for the better results obtained from the Summative Equation is in the way the factors of lignification and cell contents were combined mathematically. The Summative Equation places less emphasis on lignin in forages where lignin content is low, and in these cases greater weight is given to the value of cell

TABLE II

COMPARISON OF TWO EQUATIONS FOR PREDICTING DIGESTIBILITY OF DRY MATTER

Digestibility Predicted by	Correlation		Standard Deviation from Regression		Standard Deviation of Differences	
	Group[a]					
	1	2	1	2	1	2
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, was used to derive equations; Group 2, composed of thirty forages, was used to compare equations.

contents which are not affected, in respect to digestibility, by lignification (51).

Several workers have reported that chemical composition of a forage is related to voluntary intake. Forbes and Garrigus (13) and Patton and Gieseker (31) reported that as lignin content increases in a forage, voluntary intake of the forage decreases. Forbes and Garrigus' (13) results indicated that for each percentage unit increase in forage lignin there was a decrease of 5.8 percent of maximum intake with a negative correlation ($r = -.71$) between intake and forage lignin content. Satyanarayanasetty (39), however, observed a positive correlation between voluntary intake of dry matter and lignin as a percent of ADF. Van Soest (51) warned that the relationship between voluntary intake of dry matter and lignin content of forages was quite variable and may be confounded if grasses and legumes are mixed, due to a grass-legume interaction. Satyanarayanasetty (39) reported that ADF and cell wall constituents were better indicators of voluntary intake than was the percent lignin in ADF. Recent work by Van Soest (52) indicated that cell wall constituents were a good indicator of voluntary intake if the percent of cell wall constituents was above 55 or 60 percent of the dry matter of the forage. He observed that for forages with a cell wall constituent above 55 percent, a marked decrease in voluntary intake was noted with an increase in cell wall constituents. Satyanarayanasetty (39) reported a highly significant negative correlation between voluntary dry matter intake and percent ADF in the forage ($r = -.81$) and cell wall constituents ($r = -.83$);

however, it should be noted that one of the forages used in Satyanarayana-setty's study contained over 70 percent cell wall constituents.

Predicting Nutritive Value of Feeds from In Vitro Studies

The artificial rumen has been used in determination of nutritive value of feedstuff in a large number of laboratories. The artificial rumen procedure employs the incubation of a feed sample under conditions similar to those found in the rumen. The conditions and solutions vary between laboratories, but all involve the use of a buffer-mineral solution, inoculum from the rumen of an animal, and anaerobic conditions. These conditions are combined with the feed sample and incubated at 39° to 40° C. for varying lengths of time.

Donefer et al. (11) observed that the dry matter disappearance obtained from in vitro studies was highly correlated with the Nutritive Value Indices for fourteen forages (r = .92). Bowden and Church's (5) results from in vitro studies indicated a high correlation between in vitro DDM and in vivo DDM (r = .93). Tilley and Terry (47) also observed close correlations between in vitro and in vivo DDM. From Tilley and Terry's (47) data the following regression equation was developed: $Y = .99X - 1.01$, where Y is the percent in vivo DDM and X is the percent in vitro DDM. These authors concluded that this equation could be applied to all species of forages with a resultant standard error of $\pm$ 2.31. The reason this equation could be used for all species is explained by results of Van Soest's (54) work, which indicated that the two-stage Tilley and Terry (47) in vitro determination is actually a

measure of indigestible cell wall constituents. Since it had been shown by Van Soest (54) that forages could be divided into two fractions--cell contents which are 98 percent digestible and cell wall constituents which vary in digestibility--the use of a method which measured the indigestibility of cell wall constituents gave close approximations of DDM.

Several workers (3, 5, 29, 35) indicated that a highly significant correlation existed for crude protein and *in vivo* DDM.

Digestion of cellulose in the artificial rumen was reported to be highly correlated with *in vivo* DDM (2). Johnson *et al.* (16) reported that *in vitro* cellulose digestibility was generally related to all *in vivo* digestibilities for grasses, but the correlation coefficients were considerably lower for alfalfa and mixed forages. Quicke *et al.* (34) compared *in vivo* cellulose digestibility with *in vitro* results and concluded that within grass hays no significant differences were obtained; however, significant differences were found within some of the legume hays. Hershberger *et al.* (14) compared *in vivo* and *in vitro* cellulose digestibility of thirty-five forages consisting of legumes and grasses. The correlation coefficient ($r = .97$) indicated a close linear relationship between *in vivo* and *in vitro* digestibility of cellulose. From this high correlation the following regression equation was developed: $Y = 30.7 + 0.769X$, where Y represents *in vivo* cellulose digestibility and X represents *in vitro* cellulose digestibility. The predicted cellulose digestibility calculated by this equation had a standard deviation of 2.05 with a coefficient of variation of

2.66 percent (14). High correlations for *in vitro* and *in vivo* cellulose digestibility have also been reported by other researchers (3, 10). Van Soest (54) concluded that a forage should be divided into two major fractions--the cell contents which were 98 percent digestible and the cell wall constituents which varied in their digestibility. These two fractions of the dry matter are controlled by unrelated sets of factors. Since cellulose is a variable constituent of only one of the factors--the cell wall constituents--the use of cellulose digestibility is invalid as an indicator of DDM. Further observations by Van Soest (54) indicated that the combining of forages of different species may give highly significant correlations, but the interaction between the two may be responsible for the high correlations; therefore, Van Soest (54) concluded that the size of the correlation is an inadequate measurement for comparing various procedures as to their value in the evaluation of forages.

Oh *et al.* (29) observed that the two-stage *in vitro* digestion procedure of Tilley and Terry (47) provided the most reliable prediction of forage DDM when compared to several other prediction methods. These workers obtained a slight increase in correlation when applied to within species samples. Since all correlations were statistically significant, the following regression equation was developed: $Y = 16.7 + 0.74X$, where Y is the estimated *in vivo* DDM and X is the *in vitro* DDM. The standard error for Y was $\pm$ 2.96.

Van Soest and Wine (56) modified the Tilley and Terry (47) two-stage artificial rumen procedure by replacing the second stage--

acid-pepsin digestion--with cell wall constituent determination, using neutral detergent.

In a comparison of these two methods, results indicated that the in vitro digestion followed by determination of undigested cell wall constituents with neutral detergent yielded values nearly equal to those of true digestibility in vivo. A close linear relationship was obtained ($r = .96$) and these values gave better agreement than did the unmodified Tilley and Terry method and apparent digestibility in vivo ($r = .93$). Van Soest concluded that the difference in these two methods was that part of the bacterial residues from the unmodified in vitro method resisted the acid-pepsin digestion and caused these digestibilities to be less accurate than the ones determined by use of neutral detergents. The correlation being high for in vivo true digestibility and in vitro cell wall constituent digestibility yielded the following regression equation: $Y = 0.89X + 8.6$, where Y represents the true in vivo digestibility and X represents true in vitro cell wall constituents (CWC) digestibility. The use of this equation yielded a standard error of only 1.7.

A review of the literature indicated that more accurate methods of feed evaluation are needed and that extensive studies need to be made on the accuracy of some of the newer methods of feed evaluation.

CHAPTER III

EXPERIMENT I

Objective of Experiment

The objective of this experiment was to obtain *in vivo* digestibility data on three hays and to compare the proximate analyses with a more recent chemical evaluation of forages developed by Van Soest (53).

Experimental Procedure

A digestion trial was conducted using six non-pregnant Holstein heifers in a 3 by 3 Latin square design with two heifers per treatment in each of the three periods. Each experimental period lasted 21 days and conduction of each 21 day period was as follows:

Day 1-7 Ration adjustment period

Day 8-14 Intake measurement period

Day 15-16 Harness adjustment period

Day 17-21 Feces collection period.

All animals were weighed at the beginning of the digestion trial and also on days ten, eleven, and twelve of each period. The animals were housed in stanchion type stalls with individual feed mangers in order that measurement of forage intake could be obtained. The experimental plan and treatment sequences are presented in Table III.

Forages used in this experiment consisted of alfalfa, Lindsey 77F, and red clover. The alfalfa was third cutting and harvested at the 1/10 bloom stage of maturity. It was cut, baled, and dried overnight in a

TABLE III

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

Period	Date	Animal Number					
		Group 1		Group 2		Group 3	
		340	347	341	344	342	352
1	Jan. 10-30	Lindsey 77F		Alfalfa		Red Clover	
2	Jan. 31-Feb. 20	Alfalfa		Red Clover		Lindsey 77F	
3	Feb. 21-March 13	Red Clover		Lindsey 77F		Alfalfa	

forced air wagon drier. The red clover was second cutting, harvested in the mid-bloom stage of maturity and artificially dried. Lindsey 77F was harvested from regrowth forage at approximately 60 to 70 inches in height. The Lindsey 77F was cut, conditioned, field-cured, and baled.

The animals were fed ad libitum during the adjustment and intake measurement periods. During the collection period, intake was limited to 100 percent of ad libitum during the feed intake period. Animals were fed individually at 12-hour intervals and refused forage was weighed back each morning. Daily weights of fed and refused forage were recorded for each animal.

Samples of fed and refused forage were also taken during the intake period. These samples were used for laboratory analyses and dry matter determinations.

Digestibility of the forages was determined during the last five days of each experimental period. Feces was collected in feces collection bags and the amount voided by each animal was recorded twice daily at 12-hour intervals. Samples to be used for dry matter determinations and laboratory analyses were taken at each 12-hour interval, placed in polyethylene bags and refrigerated. At the end of the 5-day collection period the feces samplings for each animal were mixed and a composite sample was taken. A small portion of each composite sample was used for dry matter determination and the remaining portion of the feces was dried in a forced air oven at 45° C. for 5 days, ground and stored for laboratory analyses.

Laboratory analyses for crude protein, crude fiber, moisture, ash, ether extract, ADF, CWC, and detergent lignin were made on the fed and refused samples of the forage and on the feces samples of the animals. The data were analysed by the procedures outlined by Steel and Torrie (41).

Results and Discussion

Results of the digestion trial are presented in Tables IV through VII. Individual data are presented in the appendix. Data for both the proximate analyses and the more recent chemical determinations for feed evaluations are presented. All determinations were performed in duplicate and all results are reported on dry matter basis. The analyses of variance of the data are presented in the appendix.

A study of the two methods used for feed evaluation (Tables IV and VI) indicate that the total fibrous or less digestible portion of these forages was not retained in the crude fiber. The ADF values were all similar and not significantly different in digestibility. These ADF residues contained all the lignin and cellulose (the less digestible portion of the forage) and were approximately 30 percent higher than the crude fiber values, thus indicating that the crude fiber did not retain all the fibrous or less digestible portion of the forage.

The crude protein content of alfalfa and red clover were similar, while Lindsey 77F exhibited a much lower crude protein content. One would expect the crude protein of a grass to be somewhat lower than for

TABLE IV

CHEMICAL COMPOSITION OF HAYS
(PROXIMATE SYSTEM)

Forage	Dry Matter	Dry Matter Constituents				
		Crude Protein	Crude Fiber	Ether Extract	Ash	NFE
	Percent					
Alfalfa						
Fed	93.61	17.34	31.78	2.21	9.89	38.78
Weighback	93.90	11.44	47.85	1.44	10.05	29.22
Red Clover						
Fed	93.38	14.28	31.56	2.20	10.03	41.93
Weighback	93.91	11.03	38.02	1.64	10.07	39.24
Lindsey 77F						
Fed	93.05	7.31	34.37	1.78	9.95	46.59
Weighback	93.30	3.78	39.38	1.21	10.08	45.55

TABLE V

DRY MATTER INTAKE AND APPARENT DIGESTIBILITY COEFFICIENTS OF HAYS
(PROXIMATE SYSTEM)

Forage	Apparent Digestibility Coefficients in Percentages						Dry Matter Intake (% B. W.)
	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	NFE	
Alfalfa	64.11[a]	71.20[c]	53.02[a]	36.52[a]	65.70[a]	70.53[b]	2.88[b]
Red Clover	63.03[a]	54.37[b]	57.12[a]	57.24[b]	65.18[a]	70.11[b]	2.66[b]
Lindsey 77F	64.89[a]	44.20[a]	70.55[b]	63.46[b]	67.76[a]	63.66[a]	2.16[a]

Values with the same superscript are not significantly different ($P > .05$).

TABLE VI

CHEMICAL COMPOSITION OF HAYS
(MORE RECENT CHEMICAL METHOD)

Forage	Dry Matter	Dry Matter Constituents				
		Cell Wall Constituents	Cell Contents	ADF	Lignin	Lignin in ADF
	Percent					
Alfalfa						
Fed	93.61	61.12	38.88	40.18	10.15	25.26
Weighback	93.90	72.24	27.76	56.09	14.53	25.90
Red Clover						
Fed	93.38	57.45	42.55	42.53	8.65	20.34
Weighback	93.91	62.11	37.89	48.68	10.20	20.95
Lindsey 77F						
Fed	93.05	71.34	28.66	40.46	4.67	11.54
Weighback	93.30	71.92	28.08	46.59	4.90	10.52

ADF = Acid Detergent Fiber.

TABLE VII

DRY MATTER INTAKE AND APPARENT DIGESTIBILITY COEFFICIENTS OF HAYS
(MORE RECENT CHEMICAL METHOD)

Forage	Apparent Digestibility Coefficients in Percentages					Dry Matter Intake (% B. W.)
	Dry Matter	CWC	Cell Contents	ADF	Lignin	
Alfalfa	64.11^{a}	56.28^{ab}	73.88^{b}	55.87^{a}	25.08^{a}	2.88^{b}
Red Clover	63.03^{a}	50.04^{a}	78.82^{c}	54.85^{a}	0.26^{a}	2.66^{b}
Lindsey 77F	64.89^{a}	63.02^{b}	68.68^{a}	59.57^{a}	3.46^{a}	2.16^{a}

Values with the same superscript are not significantly different ($P > .05$).

CWC = Cell Wall Constituents.

ADF = Acid Detergent Fiber.

a legume; however, part of the reason for the very low crude protein of the Lindsey 77F used in this experiment was due to an advanced stage of maturity at harvest. The digestibility data indicated that the protein digestibility of red clover and alfalfa were not significantly different ($P > .05$), while the protein digestibility of Lindsey 77F was significantly lower ($P < .05$) than either red clover or alfalfa.

Crude fiber values were similar for alfalfa and red clover and only slightly higher for Lindsey 77F. There was no significant difference ($P > .05$) in the crude fiber digestibility of red clover and alfalfa. Crude fiber digestibility for Lindsey 77F was significantly higher than either red clover or alfalfa.

Cell wall constituent (CWC) values were 57.45, 61.12, and 71.34 for red clover, alfalfa, and Lindsey 77F, respectively. The only difference between acid detergent fiber (ADF) and CWC was hemicellulose content of the forage. Since the ADF values were similar for all the hays, the differences noted had to be hemicellulose. These results were in agreement with the work of Van Soest and Moore (55), which indicated that grasses have a higher hemicellulose content than legumes. Hemicellulose is more easily digested than cellulose or lignin, thus explaining the higher digestion coefficient for the CWC of Lindsey 77F. The data indicated that the legumes were much higher than the Lindsey 77F in lignin content. These results are in agreement with work reported by Van Soest and Moore (55).

There were no significant differences ($P > .05$) in the dry matter digestibilities of the three hays. Dry matter intake was not significantly

different ($P > .05$) for red clover and alfalfa; however, the dry matter intake of Lindsey 77F was significantly lower ($P < .05$) than red clover and alfalfa. The lower intake of Lindsey 77F could probably be explained by the advanced stage of maturity of the forage reflected by the high cell wall constituents and low cell contents. Van Soest's (51) data indicated that when CWC were above 55 to 60 percent, CWC were negatively related to intake. The Lindsey 77F used in this experiment contained 71.34 percent CWC.

The cell contents ranged from 38.88 for alfalfa to 28.66 for Lindsey 77F. The digestibility of the cell contents was significantly different ($P < .05$) for all hays. These results disagree with Van Soest and Moore's (55) work. They observed that cell contents were 98 percent digestible. These low values for cell content apparent digestibility may possibly be attributed to an increase in bacterial and endogenous excretions which comprise a large part of the fecal non-cell-wall materials.

CHAPTER IV

EXPERIMENT II

Objective of Experiment

The objective of this experiment was to determine *in vitro* cell wall digestibility and chemical composition and to correlate these values with known *in vivo* dry matter digestibility and dry matter intake of the hays. Regression equations were developed to predict dry matter digestibility and dry matter intake.

Experimental Procedure

The three hays used in Experiment I and nine other hays (seven alfalfas, one Lindsey 77F, and one red clover) which had known *in vivo* digestibility data available were incubated in an artificial rumen for 48 hours, using the following procedure:

A 1 gram sample of the forage that had been ground through a 40 mesh screen was incubated in a 125 ml. erlenmeyer flask, using 30 ml. of buffer-mineral solution and 25 ml. of rumen fluid.

The buffer solution used was the one suggested by McDougall (18). Before the buffer solution was added to the flasks containing the forage sample, the buffer was bubbled with carbon dioxide until the pH was approximately 6.7. Thirty ml. of the buffer-mineral solution were added to each flask, followed by glucose and urea solutions to supply .05 percent of each in the total volume of the fermentation flask. Immediately following the addition of the buffer-mineral solution,

glucose, and urea solutions, the flasks were stoppered while rumen inoculum was being prepared.

The rumen fluid was collected from a fistulated cow that was maintained on a diet of medium quality alfalfa-orchard grass hay. The rumen fluid was collected in a previously warmed jug, taken to the laboratory, and strained through four layers of cheese cloth. Twenty-five ml. of the rumen fluid was added to each fermentation flask. Immediately following the addition of the rumen fluid, each flask was flushed with carbon dioxide and closed with a rubber stopper equipped with a Bunsen valve. The flasks were placed in a 40° C. water bath and fermented for 48 hours.

At the end of the fermentation period the entire contents of each flask were transferred to a 600 ml. refluxing beaker and cell wall constituents were determined by the method of Van Soest (48). Duplicates were run in each setting and two settings were run for each forage. The results of the two settings were averaged and the cell wall digestibility was calculated as follows:

$$\frac{\text{amount of CWC in forage - amount of CWC at the end of digestion}}{\text{amount of CWC in forage}} \times 100.$$

The *in vitro* cell wall digestibility of these samples was correlated with the *in vivo* dry matter digestibility and dry matter intake. Regression equations for the prediction of dry matter digestibility and dry matter intake from *in vitro* cell wall digestibility and chemical composition were computed.

Results and Discussion

Results of the *in vitro* cell wall digestibility and chemical composition of the hays with known digestibility data are presented in Table VIII. The two Lindsey 77F hays had the highest CWC and also the highest cell wall digestibility. These results are in agreement with Van Soest and Moore's (55) data, which indicated that CWC are usually higher in grasses than legumes because the grasses contain more hemicellulose. Since hemicellulose is more easily digested than cellulose or lignin, the grass hay's CWC are usually more digestible.

The *in vitro* cell wall digestibilities were lower than *in vivo* dry matter digestibility (Table VIII) and did not follow any particular pattern. It should be noted that the relationship between *in vitro* cell wall digestibility and *in vivo* dry matter digestibility was close for the two Lindsey 77F hays. These hays had much higher CWC than did the legumes. The high CWC and the chemical make-up of the CWC could possibly be the cause of the closer relationship observed in the Lindsey 77F hays. The low *in vitro* digestibilities observed for the legumes may be due to the chemical make-up of the CWC of the legumes.

The data indicated that the lowest dry matter intakes were associated with the highest *in vitro* cell wall digestibilities. The reason for this relationship is not entirely known; however, the use of legumes and grasses no doubt contributed to the results.

Correlation and regression equations for the hays that had known *in vivo* digestibility data are presented in Table IX. A highly significant negative correlation was observed for dry matter digestibility

TABLE VIII

CHEMICAL COMPOSITION, DRY MATTER INTAKE, AND IN VITRO CELL WALL DIGESTIBILITY ON SAMPLES WITH KNOWN DIGESTIBILITIES

Forage	ADF	CWC	Dry Matter Intake (% B. W.)	In Vivo Dry Matter Digestibility	In Vitro Cell Wall Digestibility
Hay 1	30.25	43.28	3.31	79.68	45.94
Hay 2	37.15	57.55	2.63	72.07	51.46
Hay 3	31.45	50.04	2.59	80.52	49.75
Hay 4	37.53	53.73	2.41	68.75	44.82
Hay 5	39.99	61.64	2.53	71.91	48.51
Hay 6	40.98	60.50	2.39	68.12	42.57
Hay 7	40.18	61.12	2.88	64.11	48.30
Hay 8	36.95	55.16	2.48	70.30	53.05
Hay 9	42.53	57.45	2.66	63.03	36.12
Hay 10	37.43	56.18	2.24	64.00	48.91
Hay 11	40.46	71.34	2.16	64.89	61.52
Hay 12	43.10	70.25	1.89	62.49	60.59

ADF = Acid Detergent Fiber.

CWC = Cell Wall Constituents.

Description of hays listed on following page.

TABLE VIII (continued)

Hay		
1	Alfalfa	First cut bud
2	Alfalfa	First cut half bloom
3	Alfalfa	Second cut bud
4	Alfalfa	Second cut half bloom
5	Alfalfa	Second cut half bloom plus nine days
6	Alfalfa	Second cut half bloom plus sixteen days
7	Alfalfa	Third cutting 1/10 bloom
8	Alfalfa	First cut pre-bloom
9	Red Clover	Second cut mid bloom
10	Red Clover	First cut mid bloom
11	Lindsey 77F	Regrowth 60 to 70 inches in height
12	Lindsey 77F	45 to 50 inches in height

TABLE IX

RELATION BETWEEN CHEMICAL CONSTITUENTS AND IN VITRO CELL WALL DIGESTIBILITY OF FORAGES, DRY MATTER DIGESTIBILITY AND DRY MATTER INTAKE

Variable (X)	Prediction Equation	r	SE
	Dry matter digestibility (Y)		
CWC	Y = 102.50 - .573X	-.73**	4.38
ADF	Y = 120.88 - 1.355X	-.88**	2.98
In vitro cell wall digestibility	Y = 73.89 - .096X	-.11	6.37
	Dry matter intake (Y)		
CWC	Y = 4.25 - .03X	-.74**	.253
ADF	Y = 4.57 - .054X	-.60*	.303
In vitro cell wall digestibility	Y = 3.84 - .027	-.53	.320

r = Correlation coefficient.

SE = Standard Error.

*Statistically significant at $P < .05$.

**Statistically significant at $P < .05$.

and ADF ($r = -.88$), and dry matter digestibility and CWC ($r = -.73$). The following regression equations were developed to predict dry matter digestibility: $Y = 102.50 - .573X_1$, and $Y = 120.88 - 1.355X_2$, where Y is dry matter digestibility, X_1 is the CWC of the forage, and X_2 is the ADF of the forage.

Dry matter digestibility showed a low negative correlation with in vitro cell wall digestibility ($r = -.11$). This correlation was in strong disagreement with work reported by Van Soest and Wine (56). Van Soest and Wine's work indicated that apparent dry matter digestibility and cell wall digestibility were highly significantly correlated ($r = .96$). Several factors may be the cause for the low negative correlation derived in the present experiment. The procedure used for the in vitro studies were slightly different with the preparation of the rumen inoculum being more extensive in Van Soest and Wine's procedure (56). There was also a difference in the two groups of hays used to arrive at the correlations. Van Soest used a group of hays consisting of twelve grasses and eight legumes. The group of hays used in the present experiment consisted of ten legumes and only two grasses.

Dry matter intake showed a highly significant negative correlation with CWC ($r = -.74$), and the following prediction equation was developed to predict dry matter intake: $Y = 4.25 - .03X$, where Y is dry matter intake and X is CWC of the forage. These results were in agreement with results reported by Van Soest (52), which indicated that as CWC rises above 55 to 60 percent of the forage, a negative correlation existed between CWC and dry matter intake.

Dry matter intake showed a significantly negative correlation with ADF ($r = -.60$) and the prediction equation was: $Y = 4.57 - .054X$, where Y is dry matter intake and X is ADF of the forage. A non-significant correlation was obtained for dry matter intake and *in vitro* cell wall digestibility. These results appeared low and may have been due to the procedure used.

CHAPTER V

EXPERIMENT III

Objective of Experiment

The objective of this experiment was to study the effects of stage of maturity on chemical composition and *in vitro* cell wall digestibility of Lindsey 77F. The prediction equations for dry matter digestibility and dry matter intake that were developed in Experiment II were applied to the Lindsey 77F stage of maturity hays.

Experimental Procedure

Hand-clipped samples of first growth Lindsey 77F were collected three times per week from a plot in a field being utilized for green chop. Sampling of the Lindsey 77F began on June 24 when the forage was 27 inches tall. Sampling continued until July 25, when approximately 75 percent of the stems were headed out and the forage was 75 inches in height. The forage was clipped, leaving approximately 6 inch stubble. To obtain a more representative sample, small samples were taken from several areas in the plot and composited. The composite sample was measured for height, chopped, and artificially dried in a forced air oven at 50° C. The samples were then ground and stored for laboratory analyses and *in vitro* fermentation studies.

The samples of Lindsey 77F were incubated in an identical manner to the twelve hays in Experiment II. In each fermentation setting, two of the forages with known *in vivo* digestibility data and *in vitro*

cell wall digestibility data were also incubated in order that corrections could be made for day to day variation in the rumen fluid used.

The prediction equations obtained in Experiment II were applied to the samples of Lindsey 77F harvested at different stages of maturity.

Results and Discussion

Table X presents description, chemical composition, and *in vitro* cell wall digestibility of the Lindsey 77F samples. The crude protein decreased linearly with advanced stage of maturity. There was a negative relationship between the crude protein and ADF values, with crude protein decreasing from 20.29 to 4.84, while ADF increased from 31.56 to 40.15 between June 24 and July 25. During the 14 day period from June 24 to July 8, moisture and growing conditions were good and the Lindsey 77F more than doubled in height with an 8 percentage unit drop in crude protein and an 8 percentage unit increase in ADF.

The results of the *in vitro* fermentation of the Lindsey 77F (Table X) indicated that the cell wall digestibility followed an expected pattern with the more advanced stages of maturity being less digestible. The highest *in vitro* cell wall digestibility was 74.44 for Lindsey 77F harvested on June 27 at 33 inches in height. The lowest *in vitro* cell wall digestibility was 48.99 for Lindsey 77F harvested on July 25 at 75 inches in height.

The dry matter digestibility and dry matter intake of the Lindsey 77F stage of maturity samples were predicted from CWC, ADF, and *in vitro* cell wall digestibility, using the regression equations developed

TABLE X

DATE OF HARVEST, FIELD HEIGHT, CHEMICAL COMPOSITION, AND IN VITRO CELL WALL DIGESTIBILITY OF LINDSEY 77F

Date	Field Height in Inches	Field Dry Matter	Dry Matter Constituents					Lignin in ADF (Percent)	In Vitro CWD
			Crude Protein	CWC	Cell Contents	ADF	Lignin		
			Percent						
6-24	27	13.60	20.29	64.49	35.51	31.56	2.36	7.48	72.75
6-27	33	11.22	19.41	74.50	25.50	35.46	2.82	7.95	74.44
6-29	40	11.76	16.66	73.91	26.09	36.61	2.91	7.95	72.73
7-1	45	12.02	13.53	74.94	25.06	37.56	2.95	7.85	71.75
7-4	65	16.40	11.81	60.45	39.55	38.56	4.14	10.73	62.06
7-6	68	14.10	13.08	75.44	24.56	39.81	4.30	10.80	63.15
7-8	70	14.25	11.90	67.60	32.40	37.95	3.51	9.25	66.44
7-11	70	17.08	8.01	67.77	32.23	40.48	4.24	10.47	60.32
7-13	70	16.17	8.54	69.46	30.54	42.59	4.10	9.63	60.84
7-15	71	19.67	9.86	68.66	31.34	40.04	4.84	12.09	60.94
7-18	73	18.27	7.92	70.09	29.91	40.09	4.58	11.42	57.02
7-20	73	19.74	7.23	67.33	32.67	38.75	5.48	14.14	54.34
7-22	74	28.83	6.30	70.58	29.42	41.72	4.48	10.74	53.90
7-25	75	19.84	4.84	65.56	34.44	40.15	4.99	12.43	48.99

CWD = Cell Wall Digestibility.

in Experiment II. These predicted values are presented in Table XI. Since digestibility data were available on only two grass hays, prediction equations were developed from a group of forages that contained eight alfalfas, two red clovers, and the two grass hays; therefore, it is possible that error could be introduced by using these equations to predict the dry matter digestibility and dry matter intake of the Lindsey 77F.

Dry matter digestibility and dry matter intake as predicted by CWC gave unexpected results with all values being similar and ranging between 59.27 and 67.86. The probable reason for these values being so close, even for early and late cut forage, can be easily seen when one examines the CWC of the different stages of maturity. The early and late cut Lindsey 77F had approximately the same amount of CWC. One should also remember that little work had been done on summer annuals and that it is possible that the CWC may not change very rapidly but that the cellulose and lignin (ADF) may increase while the hemicellulose decreases, thus not altering the total CWC but effecting the digestibility of the forage.

When dry matter digestibility and dry matter intake were predicted using the regression equations for ADF, expected results were obtained. This data indicated that as the forage matured, its dry matter digestibility and dry matter intake decreased; however, using the regression equations for ADF gave higher dry matter intake values than observed in vivo with Lindsey 77F hays. Since legumes are usually consumed in larger quantities than grasses and most of the hays used to develop

TABLE XI

PREDICTED DRY MATTER DIGESTIBILITY AND DRY MATTER INTAKE
ON LINDSEY 77F USING PREDICTION EQUATIONS
DEVELOPED IN EXPERIMENT II

Date	Field Height in Inches	DMD from CWC	DMD from ADF	DMD from In Vitro CWD	DMI from CWC	DMI from ADF	DMI from In Vitro CWD
6-24	27	65.55	78.12	66.91	2.32	2.87	1.88
6-27	33	59.81	72.83	66.74	2.01	2.66	1.83
6-29	40	60.15	71.27	66.91	2.03	2.59	1.88
7-1	45	59.56	69.99	57.00	2.00	2.54	1.90
7-4	65	67.86	68.62	67.93	2.44	2.49	2.16
7-6	68	59.27	66.94	67.83	1.99	2.42	2.13
7-8	70	63.77	69.46	67.51	2.22	2.52	2.05
7-11	70	63.67	66.03	68.10	2.22	2.38	2.21
7-13	70	62.70	63.17	68.05	2.17	2.27	2.20
7-15	71	63.16	66.63	68.04	2.19	2.41	2.19
7-18	73	62.34	66.56	68.42	2.15	2.41	2.30
7-20	73	63.92	68.37	68.67	2.24	2.48	2.37
7-22	74	62.06	64.35	68.72	2.13	2.32	2.88
7-25	75	64.93	66.48	69.19	2.28	2.40	2.52

DMD = Dry Matter Digestibility.

DMI = Dry Matter Intake.

the regression equations were legumes, it appeared reasonable that these predicted values were high.

The predicted dry matter digestibility and dry matter intake values using in vitro cell wall digestibility indicated that the more mature Lindsey 77F was the more digestible. The results obtained in the present experiment were unexpected and different from work reported by many researchers (1, 7, 9, 17, 19, 22). These results could be due to procedure and also from use of prediction equations developed from hays which were mostly legumes and then applied to summer annuals (grasses).

CHAPTER VI

SUMMARY AND CONCLUSIONS

Three hays used in a digestion trial were combined with nine other hays (seven alfalfas, one red clover, and one Lindsey 77F) that also had *in vivo* digestibility data available. These twelve hays were incubated in an artificial rumen and *in vitro* cell wall constituents (CWC) digestibility was determined. Correlations and regression equations for predicting dry matter digestibility and dry matter intake were developed, using *in vitro* cell wall digestibility, acid detergent fiber (ADF), and CWC of the forage.

A plot of Lindsey 77F was sampled at different stages of maturity to determine the effects of stage of maturity on chemical composition and *in vitro* cell wall digestibility. The prediction equations developed from the hays with *in vivo* digestibility data were applied to the different stages of maturity of the Lindsey 77F.

The digestibility data for the digestion trial are presented. Correlations and regression equations were developed from the twelve hays that had *in vivo* digestibility data available and are presented. These results indicated that there was a highly significant negative correlation for dry matter digestibility and CWC and ADF ($r = -.73$ and $-.88$), respectively.

Dry matter intake and CWC of the forages were also highly significantly negatively correlated ($r = -.74$), while dry matter intake and

ADF were significantly correlated (r = -.60). *In vitro* cell wall digestibility gave low non-significant (P> .05) negative correlations with both dry matter digestibility and dry matter intake. These negative results were unexpected and may be due to the procedure used or possibly caused by using a group of hays that consisted of both grasses and legumes to obtain the correlations.

The chemical composition data on the Lindsey 77F hays indicated that the crude protein decreased from 20.29 percent for the June 24 sampling to 4.84 percent for the July 25 sampling. The acid detergent fiber increased with advancing maturity, changing from 31.56 percent for the June 24 sampling to 40.15 percent for the July 25 sampling.

The results of these experiments indicated that ADF was the best chemical component to use in developing regression equations for predicting the nutritive value of Lindsey 77F. The values predicted by the regression equations for ADF were higher than the *in vivo* data indicated. The reason for the high values was probably due to the use of a group of hays which consisted largely of legumes to develop the regression equations.

The regression equations developed in this investigation to predict dry matter digestibility and dry matter intake from *in vitro* cell wall digestibility and cell wall constituents lacked accuracy and were of little value in predicting the nutritive value of the Lindsey 77F.

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APPENDIX

TABLE XII

APPARENT DIGESTIBILITY COEFFICIENTS, EXPERIMENT I
(PROXIMATE SYSTEM)

Period	Forage	Animal	Apparent Digestibility Coefficients in Percentages					
			Dry Matter	Crude Protein	Ether Fiber	Extract	Ash	NFE
I	Alfalfa	341	67.84	72.26	60.32	42.61	69.72	72.97
		344	65.68	70.94	59.00	38.64	66.94	69.82
	Red Clover	342	54.54	43.01	48.07	46.85	57.05	63.06
		352	64.65	52.30	59.11	63.98	66.20	72.43
	Lindsey 77F	340	63.95	45.42	70.51	66.96	67.78	61.28
		347	64.93	46.19	69.78	62.73	67.18	64.14
II	Alfalfa	340	67.28	74.17	58.23	42.00	68.05	72.85
		347	64.19	71.40	51.50	33.54	65.82	70.95
	Red Clover	341	74.79	70.50	71.04	69.64	76.45	78.95
		344	57.00	48.77	48.10	48.73	59.26	66.11
	Lindsey 77F	342	67.50	46.44	73.05	62.73	70.37	66.41
		352	65.84	45.80	72.83	61.68	68.76	63.45
III	Alfalfa	342	62.89	70.71	50.97	29.15	65.20	69.30
		352	56.75	67.72	38.24	33.18	58.46	67.27
	Red Clover	340	61.92	52.77	56.30	58.21	64.63	68.73
		347	65.36	58.86	60.10	56.02	67.51	71.38
	Lindsey 77F	341	62.87	40.39	67.72	62.12	65.55	62.76
		344	64.23	40.93	99.39	64.54	66.89	63.91

TABLE XIII

APPARENT DIGESTIBILITY COEFFICIENTS, EXPERIMENT I
(MORE RECENT CHEMICAL METHOD)

Period	Forage	Animal	Apparent Digestibility Coefficients in Percentage				
			Dry Matter	CWC	Cell Contents	ADF	Lignin
I	Alfalfa	341	67.84	61.49	74.11	64.95	27.33
		344	65.68	60.11	74.24	58.96	19.24
	Red Clover	342	54.54	42.57	70.43	42.87	-32.66
		352	64.65	54.83	77.50	56.65	2.92
	Lindsey 77F	340	63.95	56.58	68.56	59.57	24.49
		347	64.93	62.80	70.52	60.41	25.09
II	Alfalfa	340	67.28	56.58	77.29	58.41	36.00
		347	64.19	55.37	76.48	53.83	27.85
	Red Clover	341	74.79	63.98	83.99	70.07	37.93
		344	57.00	37.26	79.95	48.23	-13.81
	Lindsey 77F	342	62.89	56.26	72.51	53.87	25.50
		352	65.84	64.94	68.04	60.92	12.36
III	Alfalfa	342	62.89	56.26	72.51	53.36	25.50
		352	56.75	47.88	70.37	45.21	14.53
	Red Clover	340	61.92	52.24	74.80	53.36	0.80
		347	65.36	49.33	86.25	57.89	6.38
	Lindsey 77F	341	62.87	61.47	66.35	55.45	-15.57
		344	64.23	64.22	68.05	60.79	- 6.00

TABLE XIV

ANALYSES OF VARIANCE FOR DRY MATTER INTAKE AND DIGESTIBILITY COEFFICIENTS (PROXIMATE SYSTEM)

Source	Degrees of Freedom	Mean Squares for Apparent Digestibility Coefficients in Percentages						
		Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	NFE	DMI (% B. W.)
Periods/squares	4	32.38	47.11	54.95	46.63	24.78	22.39	0.03
Animals/squares	4	20.96	32.88	35.63	61.72	18.29	13.21	0.19
Treatments	2	5.24	1116.07	503.26	1193.72	5.30	88.98	0.82
Squares	1	12.58	9.04	44.05	16.49	5.64	2.61	0.00
Error	6	20.83	35.85	65.26	16.25	14.45	7.72	0.03
CV		7.12	9.67	12.13	7.71	7.48	4.07	7.00

TABLE XV

ANALYSES OF VARIANCE FOR DRY MATTER INTAKE
AND DIGESTIBILITY COEFFICIENTS
(MORE RECENT CHEMICAL METHOD)

Source	Degrees of Freedom	Mean Squares for Apparent Digestibility Coefficients in Percentages					
		Dry Matter	CWC	Cell Content	ADF	Lignin	DMI (% B.W.)
Periods/squares	4	32.38	44.99	21.23	40.98	133.98	0.03
Animals/squares	4	20.96	26.13	16.75	50.65	530.81	0.19
Treatments	2	5.24	253.17	154.27	27.00	1102.69	0.82
Squares	1	12.58	28.28	11.68	14.08	20.19	0.00
Errors	6	20.83	46.90	9.33	49.14	386.01	0.03
CV		7.12	12.13	4.15	12.35	204.69	7.00