6-1972

The use of chemical analyses in predicting nutritive value of forages as measured by an in vitro and a nylon bag method

Reuben Buford Moore

To the Graduate Council:

I am submitting herewith a thesis written by Reuben Buford Moore entitled "The use of chemical analyses in predicting nutritive value of forages as measured by an in vitro and a nylon bag method." 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

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

May 1972

To the Graduate Council:

I am submitting herewith a thesis written by Reuben Buford Moore entitled "The Use of Chemical Analyses in Predicting Nutritive Value of Forages as Measured by an In Vitro and a Nylon Bag Method." 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:

J. T. Miles

Don O. Richardson

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

THE USE OF CHEMICAL ANALYSES IN PREDICTING NUTRITIVE VALUE OF FORAGES AS MEASURED BY AN IN VITRO AND A NYLON BAG METHOD

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

Reuben Buford Moore

June 1972

ACKNOWLEDGMENTS

The author expresses sincere appreciation to his Major Professor, Dr. M. J. Montgomery for his patience, guidance and advice in conducting the research and the preparation of this manuscript. The writer is also grateful to Dr. J. T. Miles and Dr. D. O. Richardson for their helpful suggestions throughout this study and for their review and advice in the preparation of this manuscript.

Appreciation is expressed to the laboratory workers and graduate students for their help and cooperation during the investigation.

The writer greatly appreciates the help and encouragement of his parents, Mr. and Mrs. R. B. Moore throughout his educational endeavors. Most sincere appreciation is expressed to his wife, Fay, for her help and understanding throughout the period of this study.

ABSTRACT

Thirty-seven forages, used in previous performance trials, were analyzed for *in vitro* dry matter disappearance (IDMD), nylon bag dry matter disappearance (NDMD), and chemical constituents. The forages were grouped into the following classifications for comparisons: 1) grain silages, 2) legumes, 3) orchardgrass hay, 4) summer and annual hays, and 5) wheat silage. In some analyses legume silages and hays were separated. The IDMD and NDMD techniques were compared as methods of predicting nutritive value. Correlation and regression analyses were performed both across species and within classifications to evaluate chemical components as predictors of IDMD and NDMD.

A correlation coefficient of 0.85 ($P < 0.01$) was obtained when IDMD and NDMD were correlated across species. The NDMD method tended to yield higher values than the IDMD procedure. The mean dry matter disappearance values for the two methods were 74.8 and 64.9. The higher values obtained using the NDMD method along with the inaccuracies involved in handling the nylon bags, would suggest the *in vitro* procedure to be the method of choice.

Correlation coefficients were utilized to express the relationship between chemical components and digestibility estimates. Although the correlations of IDMD and NDMD with crude protein, CWC, hemicellulose, estimated cell wall digestibility and calculated digestible dry matter were highly significant, the magnitudes of these correlations were low and of little predictive value. Lignin gave the larger correlations ($r = -0.57$ to -0.85) when compared to IDMD within classifications.

Several combinations of the predictors (lignin, ADF, CWC, and crude protein) were evaluated for their accuracy in predicting IDMD and NDMD. Multiple correlation coefficients were calculated, and the combination of CWC, ADF, and lignin appeared to be the most accurate in predicting IDMD or NDMD. The multiple regression equation developed from all 37 samples using a stepwise regression analysis was as follows: IDMD = 52.38 + 0.70 crude protein - 0.71 lignin + 0.15 cell contents. The coefficient of multiple determination for this equation was 0.58.

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CHAPTER I

INTRODUCTION

Increasing animal performance is the major goal in most dairy cattle operations. Since a large portion of ruminant rations is forage, it is important to improve forage quality in order to increase animal performance. A large part of the forage requirement is grown on the farm; therefore, it is of interest to the dairyman to know the nutrient content of the forages in order to fulfill the nutrient requirements of his animals.

The most accurate method of determining the nutritive value of forages is by digestion trials. However, these trials are laborious, time consuming, expensive, and require relatively large quantities of forages. Therefore, it is desirable to use methods of estimating nutritive values that do not have these disadvantages but are still reasonably accurate. For this reason, numerous laboratory methods are being used to estimate nutritive value of forages. Many researchers have investigated the use of *in vitro* and small sample *in vivo* techniques, and found high correlations between these laboratory evaluations and *in vivo* digestibility.

With the development of new forages and the necessity to re-evaluate those already in use but produced in other environments, it becomes important to have fast and accurate methods of estimating nutritive value. Therefore, the objectives of this study were to compare two micro-methods of measuring forage digestibility and to determine how well chemical constituents predicted nutritive value.

CHAPTER II

REVIEW OF LITERATURE

I. COMPARISON OF PROXIMATE ANALYSIS TO MORE RECENT CHEMICAL METHODS

The Weende system of proximate analysis had been generally used for nutritive evaluation in all fields of nutrition - human, nonruminant and ruminant - for more than 100 years (54). This analysis divides the carbohydrates of forages into nitrogen-free extract (NFE), which is supposed to represent the highly digestible carbohydrates, and crude fiber (CF), which supposedly represents the indigestible and insoluble fraction. It is common knowledge however that the division is not realistic either chemically or nutritionally. Despite the limitations of the Weende system, many researchers continue to rely on this procedure.

The crude fiber analysis has probably received more attention than the other fractions in this system from both chemists and nutritionists. The inappropriateness of the CF analysis has been discussed in many research publications (12, 16, 24, 32, 33, 35, 48, 53, 56, 57, 63, 66). Workers have criticized the CF analysis mainly because of its variable composition and digestibility. Most researchers agree that the CF fraction consists largely of cellulose and lignin, but that a considerable portion of the cellulose and lignin of a forage is contained in the NFE fraction. Norman (35) found considerable variation in the lignin content of CF fractions and that highly lignified materials do not necessarily yield a crude fiber high in lignin.

Crampton and Maynard (12) compared the digestibility of CF and NFE in a variety of feedstuffs. They reported that in 30 percent of the dry feeds tested the CF was as digestible as the NFE. Moore (32) suggested that the principle reason for this result was that lignin was contained in the NFE fraction. Van Soest (56) attributed part of the low digestibility of NFE to the digestible hemicellulose in the fiber determination. Therefore, from the findings of these researchers, it was quite evident that the division of carbohydrates of forages into CF and NFE using proximate analysis procedures was not nutritionally realistic.

With the realization of the failure of crude fiber to adequately estimate the less digestible fraction of feedstuffs, researchers began to partition the carbohydrate portion of feeds into lignin, cellulose, and other carbohydrates. Williams and Olmstead (66) in 1935 proposed a method in which the relatively indigestible residue could be divided into three fractions: cellulose, hemicellulose, and lignin.

There has been considerable research on developing ways of determining cellulose and lignin content of feedstuffs (35, 12, 16, 66, 36, 47). Some of the older methods (13, 36) had disadvantages of being long and laborious and did not give consistent measurements. One of the major problems encountered in developing lignin methods was that of separating protein from the other feed constituents. In spite of the many efforts to develop better procedures, no system had been accepted to replace CF and NFE prior to the 1960's.

A more comprehensive approach has been developed by Van Soest et al. (52, 56, 58, 62) which was based on the observation that

detergents can be useful in separating protein from other feed constituents. The principle upon which the system was founded was that the dry matter of forages may be divided into a readily available soluble fraction and a fibrous residue of partial availability (63). The two divisions were cell contents, or the soluble, highly digestible constituents and cell wall constituents (CWC), which were the insoluble and partly indigestible constituents.

The relationships between the Weende System and these newer methods are shown in Table I. Since crude fiber contains only part of the lignin, ADF values are about 30 percent higher than those for crude fiber on the same feeds (63).

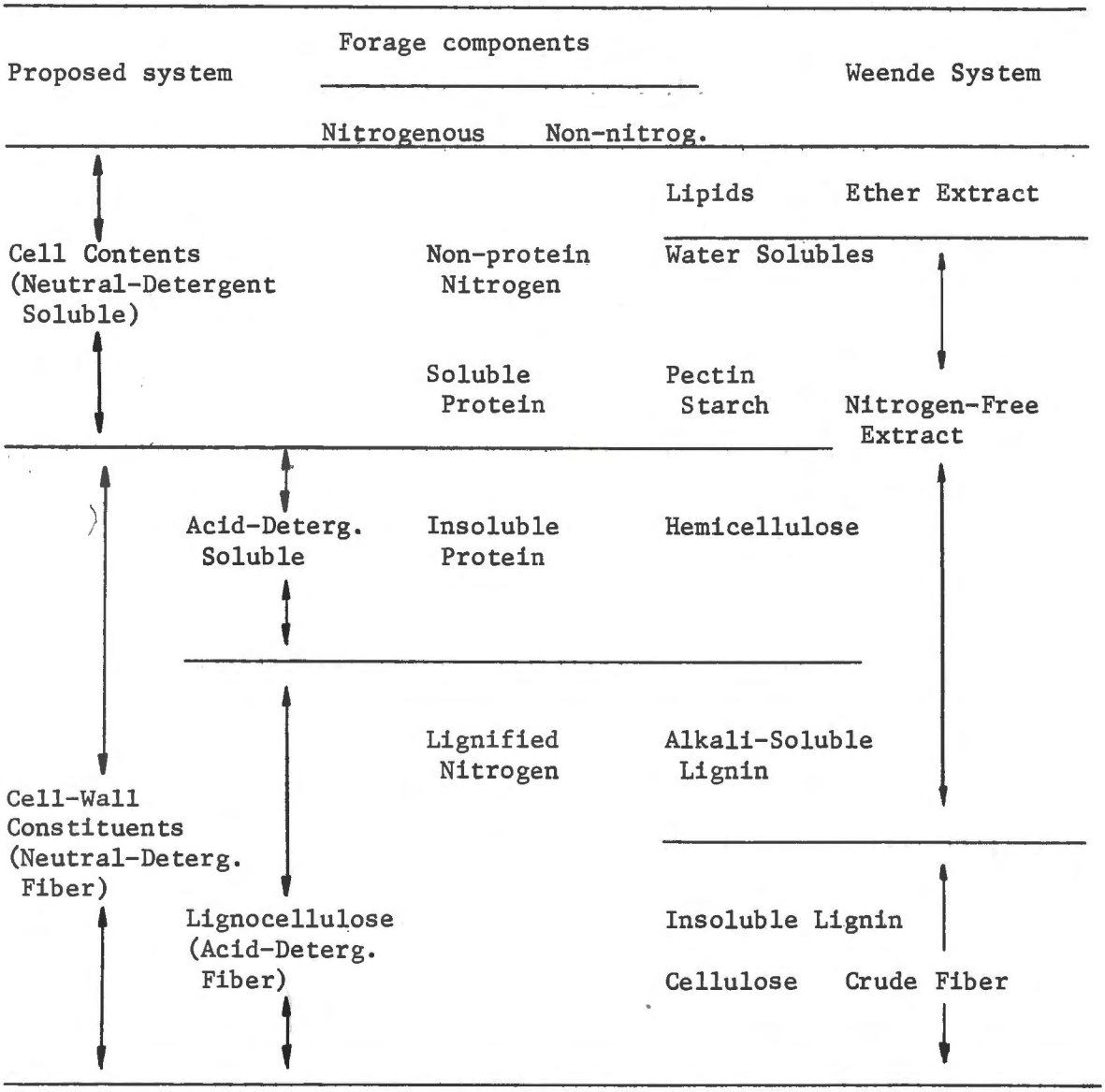
The method of separation discussed above appears to bear some resemblance to the reactions which take place in the digestive tract. The advantages of the system can readily be seen when attempts are made to estimate nutritive value.

II. *IN VITRO* ARTIFICIAL RUMEN TECHNIQUES AND THEIR USE IN PREDICTING NUTRITIVE VALUE

Much interest has developed in the use of *in vitro* methods for estimating nutritive value of feedstuffs. Many laboratory techniques have been developed in which *in vitro* dry matter or cellulose digestion was determined to estimate either *in vivo* total digestible nutrients, digestible energy, or digestible dry matter (DDM) (7, 8, 15, 49). A review and comparison of various *in vitro* techniques by Barnes (3) and Barnett and Reid (4) indicate that these techniques vary considerably in their complexity and approach. Generally the procedures employ the

TABLE I

RELATIONSHIP OF TWO DIFFERENT SYSTEMS OF DIVIDING FORAGE ORGANIC MATTER



incubation of a feed sample with conditions of pH, anaerobiosis, temperature, and darkness simulating those found in the rumen. The conditions, solutions, and length of incubation period vary between laboratories.

Numerous reports have been made on the usefulness of *in vitro* methods in estimating nutritive value of forages. Bowden and Church (9) comprised a summary of correlations between *in vitro* and *in vivo* digestibilities given in the literature before 1962. As shown in Table II, most values were highly significant. More recent investigations were also included in Table II.

Bowden and Church (9) compared correlations between *in vivo* and *in vitro* digestibility among different years of harvest. Correlations involving *in vitro* dry matter digestibility (DMD) did not vary greatly, but those involving *in vitro* cellulose digestibility varied markedly. Their data indicated that there may be some effect of storage on the *in vitro* digestibility of dried and ground forages. LeFevre and Kamstra (27) noted low correlations between *in vivo* and *in vitro* digestibility in prairie hays stored for long periods. Pigden and Bell (39) used artificial rumen procedures to obtain anthrone carbohydrate digestion. Estimates of total digestible nutrients (TDN) and digestible crude protein agreed closely with those obtained from conventional digestion trials with sheep. Baungardt *et al.* (7) found a significant relationship between TDN estimated from anthrone carbohydrate digested *in vitro* and actual TDN.

A number of researchers have compared Crampton's Nutritive Value Index (NVI) with *in vitro* data because NVI is a good indicator of

TABLE II

SUMMARY OF CORRELATIONS BETWEEN *IN VITRO* AND *IN VIVO* DIGESTIBILITIES GIVEN IN THE LITERATURE

Source of data	Substrates digested	Factors correlated	Correlations
		In vitro DMD with *in vivo*:	
Asplund *et al.* (1)	11 hays	DMD	.71*
Reid *et al.* (15)	6 hays	DMD	.82*
	Mixed pasture grasses	DMD	.98*
Clark and Mott (7)	11 Dried forages	DMD	.77*
		In vitro cellulose dig. with *in vivo*:	
Baumgardt *et al.* (3)	3 alfalfa and 8 grass forages	DMD	.81**
		Energy dig.	.80**
		Cellulose dig.	.50
	8 grass forages only	Cellulose dig.	.90**
Baumgardt *et al.* (4)	31 hays	Dig. Energy	.85**
Hershberger *et al.* (10)	35 forages of 6 spec.	Cellulose dig.	.97**
Donefer *et al.* (8)	9 forages of 5 spec.	Dig. energy	.97**
Le Fevre and Kamstra (11)	16 rations of various roughage levels	Cellulose dig.	.84**
		In vitro DMD with *in vivo*:	
Bowden and Church (9)	39 hays	DMD	.73**
Tilley and Terry (49)	148 herbages	DMD	.98**
Oh *et al.* (37)	56 forages	DMD	.88**
Johnson *et al.* (25)	11 forages	DMD	.85**

*Statistically significant at $P<0.05$.

**Statistically significant at $P<0.01$.

animal performance (10, 14, 19, 25). Nutritive value index is calculated as the relative feed intake times the digestibility of that feed compared with the intake of a standard forage (14). Johnson et al. (25) compared the in vitro digestibility of cellulose in four grasses at two stages of maturity to nutritive value indices and other measurements made by conventional in vivo digestion trials. There was a close relationship between the 12-hour in vitro cellulose digestibility and the in vivo measurements. When the data for alfalfa was included, the regression of the 12-hour in vitro cellulose digestibility on nutritive value indices resulted in a line which was described by $Y = 0.814X - 5.91$, where Y was NVI and X was the 12-hour in vitro cellulose digestibility. The correlation coefficient was highly significant. Donefer et al. (14) also demonstrated a high correlation between 12-hour in vitro cellulose digestibility of forages and their NVI. It was proposed that the Nutritive Value Index (Y) could be predicted from the 12-hour in vitro cellulose digestion (X) of that forage from the equation $Y = -7.8 + 1.314X$. Chapula and Lee (10) found a significant correlation between NVI and 18-hour in vitro cellulose digestion, along with a significant correlation between NVI and 18 X 30-hour in vitro cellulose digestion.

It was also discovered in the above study that lag-time differences between forages were evident at 6, 12, and 18 hours, whereas incubation beyond 24 hours was essentially a measure of total digestion.

A number of researchers have found discrepancies between grasses and legumes when estimating nutritive value from in vitro digestibility.

In research by Johnson *et al*. (25), when 24 and 48 hour *in vitro* cellulose digestibilities were considered, the correlations with *in vivo* measurements were quite high for grasses alone, but much lower when alfalfa data were included in the analyses. Quicke *et al*. (41) obtained no significant differences in the digestibility of cellulose obtained *in vitro* and *in vivo* with grass hays, but in some of the legume hays, cellulose digestibilities as measured by the two methods were significantly different. Hershberger *et al*. (21) evaluated 35 forages of both grass and legume origin. A correlation coefficient of 0.97 was obtained between cellulose digestion *in vitro* and *in vivo*. The regression equation $Y = 30.7 + 0.769X$ explained the relationship where Y represented *in vivo* cellulose digestibility. Oh *et al*. (37) obtained a highly significant correlation ($r = 0.97$) when *in vivo* DMD was compared to *in vitro* DMD in 24 legumes.

The procedure of Tilley and Terry (49) appears to be used more frequently than other *in vitro* methods. Oh *et al*. (37) observed that the Tilley and Terry procedure provided the most reliable prediction of forage DDM when compared to other prediction methods.

In a trial by Tilley and Terry (49), a total of 148 herbages of known *in vivo* digestibility were evaluated. When *in vivo* digestibility was compared to *in vitro* digestibility, a correlation coefficient of 0.98 was obtained. The linear regression equation $Y = 0.99X - 1.01$ has been fitted to the data, where Y represented percent *in vivo* DMD and X percent *in vitro* DMD. Oh *et al*. (37) obtained a correlation coefficient of 0.88 when comparing in vivo DMD in 56 forages using the Tilley and

Terry (49) procedure. The regression equation derived from the data was Y = 16.7 + 0.74X where Y represented *in vivo* DMD and X *in vitro* DMD.

Van Soest and Wine (64) modified the Tilley and Terry (49) procedure by replacing the second stage with CWC determination using neutral detergent. The new method was shorter and simpler in that two days in the unmodified procedure were eliminated. Using this method, Van Soest and Wine (64) obtained digestibilities numerically equivalent to true *in vivo* digestibility. They were also able to more accurately estimate apparent digestibilities by means of a regression equation (r = 0.96) than with the unmodified Tilley method (r = 0.93).

It can readily be seen that *in vitro* techniques are valuable laboratory methods for estimating forage nutritive value. They require less time and money and a smaller amount of test material than experiments with animals. They are especially applicable to forage breeders who have the problem of fulfilling the producers' requirements of high quality and high yielding forages. These workers need a method which requires only small quantities of forage. The laboratory methods will not replace animal experimentation, but they are very valuable tools that can be used to estimate the results that would be obtained from animals.

III. SMALL SAMPLE *IN VIVO* TECHNIQUES AND THEIR USE IN PREDICTING NUTRITIVE VALUE

A number of research workers have recently investigated means of obtaining an estimate of forage feeding values by small-sample methods.

As mentioned earlier, a large number of these procedures are based on the use of *in vitro* techniques; however, some workers have studied the use of small sample *in vivo* techniques.

Quin *et al*. (42) in 1938 were among the early researchers to report on techniques similar to today's small sample methods. They used silk bags and suspended them in the rumen of fistulated sheep and observed the rate of disintegration of feed. Since this early research, a number of workers have used a similar technique. Erwin and Elliston (17) placed nylon bags containing forage in tygon tubing and suspended them in the anterior dorsal sac of the rumen of cattle. With this method, they were able to detect weight loss of forage. Miles (30) suspended 20 g samples of forage in silk sacks into the ventral area and compared it to the dorsal area of the rumen. A significant increase was obtained in cellulose digestion in the ventral area as compared to the dorsal area. A highly significant difference was found in the percent of cellulose digestibility among samples of alfalfa, corn cobs, and beet pulp. It was concluded that this technique had promise for predicting the feeding value for forages.

Lusk *et al*. (29) used 3 g samples in nylon bags suspended in the rumen of fistulated cows to compare the coefficients of digestibility of cellulose obtained by this method with those obtained by conventional digestion trials. Cellulose digestibility of alfalfa could be approximated after 36 hours, but 72 hours were required for Coastal bermudagrass.

In evaluation of the dacron bag technique, Hopson et al. (23) found that there was approximately 1 percent loss in forage from dacron bags after suspending them in running water for 24 hours. It was also suggested that partially digested forage may be small enough to escape through the pores; however, this source of error was small. When compared with conventional cellulose digestion, non-significant correlations of 0.52 and 0.54 were obtained for 36 and 42-hour digestion periods for cellulose on one legume and three grass forages.

The process of cellulose determination has been a difficult and time consuming procedure that requires a great deal of laboratory equipment and reagents. Therefore, there has been considerable advantage in analyzing forages for dry matter only. Van Keuren and Heinemann (51) described a procedure using nylon bags in which forage DMD could be estimated. The amount digested was obtained by subtracting the residue weight from the original weight. The advantages of the dry matter determination described above has increased its frequency of use over cellulose digestibility.

Some researchers have found that the method of handling the nylon bags after removal from the rumen and before drying could affect the results. Lusk (28) conducted an experiment to study the best methods of handling the bags upon removal from the rumen. A 5 percent to 10 percent smaller apparent DMD was obtained when the bags were rinsed only to remove the rumen ingesta as compared to when the bags were washed until clear water could be squeezed from them. Yang et al. (67) found a 14.4 percent difference between bags which had been rinsed only and those which had been squeezed to remove water-soluble material

from within the bags. Van Dyne (50) also discovered a 10 percent lower apparent DMD when the bags had been washed until the wash water was clear. It was suggested that this was a serious problem with the DMD procedure and that the cellulose procedure was probably a better technique since it does not depend on a qualitative procedure such as rinsing of the bags.

A number of researchers have used the nylon bag technique in studies of digestion rates or studies of rates of passage (12, 22, 23, 28, 34, 43). Hopson et al. (22) studied the rates of cellulose digestibility of alfalfa, bromegrass, and timothy hay by placing dacron bags filled with forage in fistulated sheep for 6 to 42 hours. The 36 and 42-hour time periods were the only ones in which significant correlations were obtained between cellulose digestibility by conventional digestion trials compared to the nylon bag technique. In an experiment by Lusk (28), eight forages for which conventional digestion trial data were available were digested for 12, 24, and 60 hours in nylon bags and apparent DMD calculated. It was observed that alfalfa hay, grain sorghum, and corn silages require only a 24-hour digestion period. But annual grass hays require approximately 48 hours, and perennial grass hays such as Coastal bermudagrass require at least 60 hours.

Researchers have found the nylon bag technique to be a valuable tool that can be used with reasonable accuracy in estimating the nutritive value of forages. In an evaluation of 124 forages, Reid et al. (43) found that dry matter loss at 72 hours was highly correlated with DMD in vivo. Lusk et al. (29) obtained a positive correlation of 0.83 ($P < 0.05$) for cellulose digestibility of eight forages as compared to

cellulose digestibility of the same forages by conventional trials. Kercher et al. (26) determined the digestibility of alfalfa, oat, crested wheatgrass, and native grass hays by the use of artificial rumen, nylon bag, and conventional digestion trials. They found that the nylon bag technique ranked the four types of hays in the same order for dry matter and cellulose digestion. Yang et al. (67) compared dry matter digestibilities obtained from conventional trials with sheep to dry matter disappearance values obtained from the use of the nylon bag technique. A correlation coefficient of 0.79 was determined between the two methods using samples of alfalfa, brome, reed canary, trefoil, and timothy.

Monson et al. (31) used 159 forage samples to compare the two techniques of in vivo nylon bag and two stage in vitro. A modification of the Tilley and Terry (49) technique was used as the in vitro method. A highly significant correlation ($r = 0.81$) was obtained between the two methods with all forages. In comparing the nylon bag and in vitro DMD of 55 Coastal bermudagrass samples, the correlation coefficient was 0.92. When comparing the two methods using the three legumes as samples, the correlation was even higher ($r = 0.97$, $P < 0.01$).

Barth et al. (5) compared the nylon bag method with the Tilley and Terry artificial rumen procedure. When compared to in vivo total collection data, they found that the nylon bag method over evaluated DDM up to 10 percent. Digestible dry matter estimates from the nylon bag method were significantly higher than the in vitro estimates. They concluded that the artificial rumen method yields better estimates of total collection DDM than the nylon bag procedure.

IV. THE USE OF CHEMICAL ANALYSES IN PREDICTING NUTRITIVE VALUE

A number of chemical constituents have been used by researchers to estimate the nutritive value of forages. Forbes and Garrigus (18) found the best correlation between chemical composition and organic matter digestibility was obtained with lignin. The regression of organic matter digestibility on lignin content of the forage was expressed by the equation $Y = 100 - 4.71X$ for steers. After evaluating five species of grasses, Patton and Gieseker (38) stated that lignin was of definite value in predicting the feeding value of forage plants. Sullivan (46) obtained a highly significant correlation between DMD and lignin percentage ($r = -0.94$). Using their newer procedure of lignin determination, Van Soest and Moore (63) examined the relationship between lignin and digestibility of five forage fractions: CWC, CWC soluble in acid detergent, ADF, ADF soluble in neutral detergent and nitrogen. Correlations were computed expressing lignin concentration in different ways: as a percentage of the dry matter, of the CWC, and of ADF, and as the logarithm of lignin concentration in ADF. The results showed highly significant correlations of all forms of lignin expression with digestibility of the fibrous fractions. Correlations of greater magnitude were obtained when lignin was expressed as percentage of the ADF. The correlation coefficients of lignin with CWC and ADF were -0.95 and -0.93, respectively. The highest correlations were obtained when lignin concentrations were converted to logarithms.

Oh *et al*. (37) used 32 grasses and 24 legumes and compared *in vivo* DDM with acid detergent lignin. A significant correlation was obtained between the two variables, but correlation coefficients of higher magnitude were obtained when grasses and legumes were separated when making the comparison. Allison and Osbourn (1) reported significant correlations between: acid detergent lignin and DMD; voluntary feed intake and cellulose digestibility. The correlation coefficient between lignin content and DMD when grasses and legumes were combined was -0.79. Higher correlations were obtained when the forages were separated to lower subgroups.

Reid *et al*. (44) conducted a study and found a definite relationship between the concentrations of total protein and apparently digestible protein in forages. These variables were highly correlated ($r = 0.99$). The percentage of apparently digestible protein could be predicted from total protein by use of the equation, $Y = 0.929X - 3.28$, where Y was apparently digestible protein and X was total protein. Bowden and Church (10) obtained a highly significant correlation between *in vivo* DMD and crude protein content, ($r = 0.79$) and between *in vitro* cellulose digestibility and crude protein content. Oh *et al*. (37) reported a highly significant correlation between DDM and crude protein ($r = 0.37$) but concluded that the magnitude of the coefficient was too low to be of much predictive value. When the relationship was considered within legumes, a higher correlation ($r = 0.76$) was obtained. Van Soest (57) concluded that crude protein was not likely to be a very reliable predictor because it is much affected by nitrogen fertilization and relative differences in content among legume and grass species.

The use of chemical composition to predict voluntary intake has been reported by several researchers (18, 38, 40, 45, 54, 55). Forbes and Garrigus (18) observed that voluntary intake of a forage decreases as lignin content increases. Plummer (40) reported that dry matter intake and CWC of the forages evaluated were highly significantly negatively correlated ($r = 0.74$), while dry matter intake and ADF were significantly correlated ($r = -0.60$). Van Soest (55) suggested that CWC were a good indicator of voluntary intake if the percent of CWC was above 55 or 60 percent of the dry matter of the forage. It was cautioned by Goering and Van Soest (20) that the relationships between chemical compositions and intake are fairly consistent in some forage species but unpredictable in others.

Van Soest and Moore (63) used cell contents, CWC, ADF, and lignin to develop prediction equations. Cell contents were found to be highly digestible and unaffected by lignin. The amount of lignin in ADF was observed to control the digestibility of cell wall constituents. It was deduced that two factors present in the forage contribute to a determination of the resultant digestibility: the degree of lignification and the portion of forage free from lignification. Lignification (L) was found to be negatively related to digestibility and the neutral detergent solubles (S) are positively related to digestibility. The ratio was written L/S which was an estimate of indigestibility. This function was found to regress linearly with digestibility. An index of availability (A) was devised as: $A = 100 - 100(L/S)$. The availability index equation for DDM was given: $DDM = 78.2(1 - L/S) + 12.7$ where L

represented the percentage of lignin in ADF and S the cell contents obtained by subtracting the percentage of cell walls in the dry matter from 100.

Van Soest (54) conducted an experiment in which forages were analyzed for cell wall constituents, acid detergent fiber and lignin, and total nitrogen. Digestibilities of CWC, ADF, cell contents, and nitrogen were calculated and correlated with lignin expressed as a percentage of dry matter, cell walls, and ADF. The digestibility of CWC and the amount of lignin (expressed as a percent of the ADF) in a forage were found to be highly correlated. The regression equation for the prediction of digestibility of cell walls is $Y = 147.5 - 78.9 \log X$, where X was the percentage of lignin in ADF. Van Soest (54) combined the above equation with the constant values previously obtained for cell content digestibility (98 percent) and the estimate of endogenous excretion (12.9) to form the Summative Equation. The equation was: $DDM = 0.98S + W(147.3 - 78.9 \log L) - 12.9$, where S was the percentage of cell contents; W, the percentage of cell walls; and L, the percentage of lignin in ADF.

A comparison was made of the availability index and summative equation by Van Soest (54). A group of 30 forages was used to compare the ability of the two systems to predict digestibility of forages from a population not used in the development of either of these systems. The results are shown in Table III. The summative equation showed definite superiority over the availability index. The availability index had larger increases in standard deviation from regression and standard deviation of differences. The availability index also tended to give

TABLE III

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 19 forages, was used to derive equations; Group 2, composed of 30 forages, was used to compare equations.

erratic values for forages of very high digestibility, and it tended to underestimate the digestibility of poor forages and forages where the ratios of lignin to cell contents may be unusual. One of the major reasons for the better performance of the summative equation was that it places less emphasis on lignin in forages where lignin was low and where analytical precision may be a problem.

Studies were conducted by Van Soest and Wine (65) to disclose factors other than lignin which may have depressing effects on the nutritive value of forages. Silicification was observed to have a significant influence. Van Soest and Jones (61) found silica to be an important factor in the reduction in digestibility of CWC in some species. Results of the study showed an average decline of 3.0 units of digestibility per unit of silica in the dry matter. The summative equation was changed to the following to include the silica correction. Digestibility = $0.98(100 - W) - 12.9 + W(1.473 - 0.789 \log L) - 3.0(SiO_2)$, where W was cell walls, SiO_2 was silica on a dry matter basis and L was the lignin content of ADF. The digestibility of alfalfa and perhaps other legumes did not appear to be affected a great deal by silica.

Van Soest (59) outlined two basic problems involved in the evaluation of feedstuffs: the estimation of nutritive value from feed chemical composition, and the establishment of more accurate yardsticks of nutritive value. The yardsticks used most are digestibility measurements (often calculated as TDN) because of their ease and reproducibility under controlled feeding conditions (59). The discrepancies in the use of TDN when compared to net energy (NE) are well recognized (60), but it is also known that there is a lack of information on NE feed values.

Van Soest (60) outlined a system by which various ways of estimating digestibility and TDN from chemical composition may be used sequentially in the estimation of net energy. The system consists of three major steps. The first step was the estimation of digestibility which may be accomplished in several alternative ways. Some of the alternative digestibility measurements have been discussed earlier in this review. In the second step, digestibility was converted into an estimate of TDN or other measure of digestible energy. The equation given was TDN = DDM - total ash + silica + 1.25 (ether extract) + 1.9. The third step was the conversion of TDN to estimated net energy. The equation developed as: $NE_{lact} = 0.01\ TDN(2.86 - 35.5)/(100 - CWC)$. Equations were also suggested for the conversion of NE_{lact} to NE_{gain} or NE_{main}.

Problems are involved when the appropriate laboratory methods and equations are applied to the feed industry and farmers, because of the errors in estimations from laboratory analysis (60). It was suggested by Van Soest (60) that if digestibility and TDN were known, CWC and protein would suffice for ration balancing. A summary of the analysis required to predict nutritive evaluations is shown in Table IV, as presented by Van Soest (60).

From a review of the literature, it can be seen that additional studies are needed on the newer methods of feed evaluation and how they can be used more effectively in predicting the nutritive value of feeds.

TABLE IV

ANALYSES REQUIRED TO PREDICT NUTRITIVE EVAUATIONS

Prediction	Chemical methods	Biological methods	Other
Digestibility	Cell wall, ADF, Lignin, SiO_2	Tilley-Terry *in vitro* rumen dig.	Tables, date of cut
TDN	Ash, ether extract	--	Tables
Metabolizable energy ruminants	--	--	Calculate
non-ruminants	Cell wall	Enzymatic assays	Fat, ash, protein quality
Net energy	Cell wall	--	Est. TDN or dig.
Protein avail.	ADF-nitrogen	pepsin dig.	

CHAPTER III

EXPERIMENTAL PROCEDURE

I. OBJECTIVES

The objectives of this study were as follows: (1) to evaluate the use of chemical components in predicting nutritive value as measured by *in vitro* and nylon bag dry matter disappearance data; (2) and to compare an *in vitro* method of determining digestibility to a nylon bag technique. The use of *in vitro* digestibility as a measure of nutritive value was based on the observation of numerous researchers that there is a high correlation between *in vivo* and *in vitro* digestibilities (Table I, page 5).

II. FORAGES

The forages used in this study consisted of 37 samples that were used in performance trials conducted at the Dairy Experiment Station at Lewisburg, Tennessee. The 37 samples included 9 grain silages, 16 legumes, 6 orchardgrass hays, 3 summer annual hays, and 3 wheat silages. The grain silages included 6 samples of corn and 3 samples of RS610 grain sorghum. Six of the legume forages were preserved as silage and the remainder were preserved as hay. All of the legume forages were alfalfa except one sample of red clover. The alfalfa and orchardgrass forages varied in stage of maturity at harvest and in number of the cutting.

All samples had been dried and ground through a 40 mesh screen and stored in air tight bottles.

III. IN VITRO FERMENTATION STUDY

A two-stage in vitro fermentation technique described by Tilley and Terry (49) was used to determine dry matter disappearance. The first stage involved rumen liquor digestion and the second stage employed pepsin digestion.

Duplicate determination of in vitro dry matter disappearances (IDMD) were made at three different times for each sample. Because of limited laboratory equipment, the analysis was divided into two different periods. Eighteen samples were analyzed in the first period and the remainder in the second period. The data were analyzed statistically for differences in day of determination, classifications of forage, interaction between day and classifications, and samples within classifications.

IV. NYLON BAG IN VIVO DIGESTION STUDY

Two rumen fistulated dairy cows were employed in this study. These animals were fed medium quality alfalfa hay ad libitum for two to three weeks prior to and during the experiment. The nylon bags used in this experiment were hand made from nylon cargo parachute material. The finished size of the bags was approximately 2 X 4 inches.

Approximately 3 g of each sample was placed in the bags. The bags were tied at the top using nylon fishing lines. Six bags were attached to the cap of a 250 ml polyethylene bottle previously filled with water, and the bottles were placed in the ventral portion of the rumen of the two rumen fistulated cows.

The bags and bottles were gently removed from the rumen after 48 hours. The bags were detached from the bottles and washed in a circulating water bath until clear water could be squeezed from the bags. They were then dried for 12 hours at 105 degrees centigrade.

The apparent nylon bag dry matter disappearance (NDMD) was calculated from the weight of dry matter present in the bags before and after they were placed in the rumen.

Bags were placed in each cow on four different days. Samples were randomly assigned to cows and days in an attempt to eliminate confounding between cows and days. Each sample was run in duplicate and placed in each cow on two different days.

In 12 of the 37 samples, there was an insufficient amount of the feedstuff for the procedure above; therefore, only three NDMD determinations were made. The data were analyzed statistically for differences in species, samples within classifications, days of determinations, cow, and interactions between classification, day, and cow.

V. CHEMICAL ANALYSES

It is well accepted that the proximate analysis scheme of evaluating forages is inadequate (12, 32, 53, 56). Therefore, the newer and more desirable scheme of Van Soest (52, 56, 58, 62) was used in this study. Acid detergent fiber (ADF), acid detergent lignin (LIG) and cell wall constituents (CWC) analyses were all performed in duplicate. The other chemical constituents, which included cell contents (CELLCO), cellulose (CELL) and hemicellulose (HEMI) were obtained by subtraction

according to the procedure outlined by Van Soest and Moore (63). Crude protein (CP) determinations were made in duplicate by AOAC procedures (2).

VI. STATISTICAL ANALYSES

All statistical analyses were made with the aid of an IBM 360/65 computer system. The mean of multiple determinations of each variable was used in the correlation and regression analyses. In vitro dry matter disappearance (IDMD) and nylon bag dry matter disappearance (NDMD) were used as measures of nutritive value or as Y variables in the regression analysis. Least squares analysis was used to adjust IDMD and NDMD for the major sources of variation found in the analysis of variance of each procedure. The values obtained, adjusted in vitro dry matter disappearance (AIDMD) and adjusted nylon bag dry matter disappearance (ANDMD), were used in comparing these two procedures for estimating nutritive value.

The determined and calculated chemical constituents, which were used in the correlation and regression analyses, included ADF, LIG, CWC, CELLCO, CELL, HEMI, CP, estimated cell wall digestibility (ECWD), and calculated digestible dry matter (CDDM). Estimated cell wall digestibility and CDDM were calculated as described by Goering and Van Soest (20).

Regression and correlation analyses were made both within classifications and across species. A stepwise regression procedure was used in developing the regression equations. In the statistical

analyses within classifications, the summer annual and wheat forages were omitted because there were only three samples of each. The within classifications analysis included three groups: grain silages, legume forages, and orchardgrass hays. In some analyses, the legume hays and silages were separated.

CHAPTER IV

RESULTS AND DISCUSSION

I. *IN VITRO* EVALUATION

The mean IDMD of the multiple analysis for each sample are presented in Table XIV of the Appendix. The alfalfa and orchardgrass forages had much higher IDMD values than the other forages tested. However, there was a considerable range within species among all forages (Table V). This range was possibly due to the variation in stage of maturity and number of cutting in some of the forages.

The analysis of variance for both periods of the *in vitro* procedure is presented in Table VI. Most of the total variation in both periods was accounted for by differences in classifications and samples within classifications. In period 1, there was a significant difference among days; however, the difference was not significant in period 2 ($P > 0.05$). This difference could have been due to variation in the rumen liquor. The interaction between day and classification was highly significant in period 2 although it was not significant in period 1. The probable cause of this difference was due to the variation in chemical content of the samples between the two periods. The coefficient of determination for period 1 and 2 was 0.86 and 0.85 respectively; therefore, most of the variation was accounted for in the regression.

TABLE V

FORAGES USED IN THIS STUDY

Forage	No. Samples	Range In *In Vitro* DMD Values
Grain silages	9	57.35-69.10
Corn	6	57.93-69.10
Grain Sorghum	3	57.35-58.24
Legumes	16	60.65-73.40
Alfalfa	15	60.65-73.40
Red Clover	1	65.46
Orchardgrass	6	62.53-73.08
Summer Annuals	3	52.43-58.98
Wheat	3	63.03-67.21

TABLE VI

ANALYSIS OF VARIANCE FOR IN VITRO DRY MATTER DISAPPEARANCE

Source	Degrees of Freedom	Square	F Value
Period I--18 samples			
Day	2	44.45	7.79**
Classification	2	932.67	163.37**
Day x Classification	4	12.58	2.20
Samples/Classification	15	40.96	7.18**
Regression	23	113.87	19.95**
Error	83	5.71	
R^2 = 0.86			
CV = 4.09			
Period II--19 samples			
Day	2	0.25	0.13
Classification	3	51.52	14.85**
Day x Classification	6	23.28	6.71**
Samples/Classification	15	71.27	20.54**
Regression	26	52.46	15.12**
Error	86	3.47	
R^2 = 0.85			
CV = 2.86			

**Statistically significant at P<0.01.

II. NYLON BAG EVALUATION

The analysis of variance for NDMD data is presented in Table VII. The difference in NDMD between cows was not significant. The significant difference found in day of determination ($P < 0.01$) was caused primarily by the difference in samples analyzed between days. The largest amount of variation was found in classifications and samples within classification; however, other sources were significant. The mean values of the multiple determinations of NDMD are shown in Table XIV of the Appendix for each sample.

III. COMPARISON OF NYLON BAG AND *IN VITRO* TECHNIQUES

The values obtained for NDMD was much higher than the IDMD values. As shown in Table XIV of the Appendix, the mean IDMD for all forages was 64.92, and 74.76 for all NDMD determinations. *In vitro* values ranged from 52.43 to 73.40 percent and the NDMD ranged from 66.71 to 86.05 percent. Other researchers have found higher NDMD values than *in vitro* or *in vivo* digestibilities (5, 26, 28, 31).

The correlation between *in vitro* and nylon bag dry matter disappearance for the 37 forage samples was high ($r = 0.85$, $P < 0.01$). Monson *et al.* (31) obtained a similar correlation ($r = 0.81$) between the two methods using 159 forage samples. The adjustment of the IDMD and NDMD for the differences found in the analysis of variance had little effect on the correlations between the methods except in the grain silage comparison (Table VIII). (Hereafter only unadjusted values will be discussed). The highest correlations were found in

TABLE VII

ANALYSIS OF VARIANCE FOR NYLON BAG DRY MATTER DISAPPEARANCE

Source	Degrees of Freedom	Mean Square	F Value
Day	3	56.40	23.08**
Classification	4	993.87	406.76**
Cow	1	0.21	0.21
Samples/Classification	32	59.34	24.29**
Day x Cow	3	28.56	11.69**
Classification x Day	12	6.05	2.47**
Classification x Cow	4	6.24	2.56*
Regression	59	105.54	43.19**
Error	165	2.44	

*Statistically significant at $P<0.05$.

**Statistically significant at $P<0.01$.

TABLE VIII

SIMPLE CORRELATION COEFFICIENTS BETWEEN IN VITRO DMD AND NYLON BAG DMD VALUES

	No. Samples	IDMD[a] and NDMD[c]		AIDMD[b] and NDMD	ANDMD
All Forages	37	0.85**	0.82**	0.83**	0.81**
Legumes	16	0.68**	0.68**	0.69**	0.69**
Legume Silage	6	0.83*	0.88*	0.89*	0.94*
Legume Hay	10	0.71*	0.69*	0.71*	0.68*
Grain Silage	9	0.81**	0.65**	0.79**	0.63**
Orchardgrass Hay	6	0.88*	0.88*	0.89*	0.89*

[a] In vitro dry matter disappearance.

[b] Adjusted in vitro dry matter disappearance.

[c] Nylon bag dry matter disappearance.

[d] Adjusted nylon bag dry matter disappearance.

*Statistically significant at P<0.01.

**Statistically significant at P<0.05.

legume silage and orchardgrass hay. High correlations between the two methods have been reported in good quality grass forages (31). It should be noted that there were only six observations in both the legume silage and orchardgrass hay comparisons.

In vitro methods of measuring digestibility are widely used and accepted. However, both nylon bag and in vitro methods appear to give a good estimate of digestibility. Therefore, sampling procedures play an important role for either method. Since the nylon bag method required a larger sample size, (3 g vs. 0.4 g) a larger CV was expected for IDMD (31). However, in this study CV's ranged from 2.09 to 4.09 for both methods. The smaller sample size could be desirable when multiple analyses are required on each sample. More time was involved in the IDMD determination and the procedure was slightly more complicated. There was also more laboratory equipment required, compared to the nylon bag procedure. The favorable results obtained by a number of researchers using the in vitro method tend to outweight these disadvantages.

The major disadvantage of the nylon bag method lies in the handling of the bags. Extreme care must be taken when preparing the bags for placing in the rumen. It was difficult to avoid damage to the bag or spillage before the final step. It was also hard to know exactly when to remove the bags from the circulating water bath after the fermentation process.

IV. RELATIONSHIPS BETWEEN CHEMICAL ANALYSES AND ESTIMATES OF DRY MATTER DIGESTIBILITIES

A summary of all analyses on each sample is presented in Appendix Table XIV. The legumes were highest in crude protein (mean = 18.34);

however, the orchardgrass samples were almost as high (mean = 17.68). Grain silages had the lowest level of CP and the least amount of variability between samples. The range was only 8.37 to 10.23. When evaluations were compared across species, CP had the lowest standard error.

Cell wall constituents had one of the highest standard errors of any of the evaluations when compared both within classifications and across species. This was due to the wide range in forages and the difference in stage of maturity within classifications. A within classification comparison of CWC showed that summer annual hays had the highest values and the legumes had the lowest cell wall content especially the alfalfa silages. The mean alfalfa silage CWC was only 48.4 percent as compared to a mean of 70.6 percent for the summer annuals. These results were very similar to the CWC values obtained by Satyanarayanasetty (42) when alfalfa and summer annuals were compared.

The summer annual samples also had higher ADF and lignin values than any of the other classifications that were evaluated. These results contradict reports of other workers (45, 55); however, there were only three summer annual samples evaluated. The IDMD data indicates that these forages were of low quality (mean = 56.7). The legume forages were second highest in ADF and lignin despite the high IDMD values obtained. Van Soest (55) observed that alfalfa had a higher lignin content than grasses of equal digestibility. The mean lignin content for legumes was 7.7 percent whereas in orchardgrass, the content was only 4.9. The mean IDMD between the two forages was very similar.

A comparison of legume silages and hays indicated that the silages had lower CWC, ADF, hemicellulose, and lignin values. Derbyshire *et al.* (13) reported similar results when they compared orchardgrass and alfalfa-orchardgrass forages before and after fermentation. They attributed the cell wall changes to a reduction in the hemicellulose fraction. More samples are needed than were in the present study if silages and hays are to be compared.

Simple correlation coefficients between the various chemical constituents and related variables are shown in Table IX. All 37 forages were included in this analysis. Although the correlation coefficients between several constituents and DMD estimates were statistically significant, they are low and show little predictive value. The within classifications comparison (Table X) generally indicated higher correlations with some differences when compared to across species correlations.

IDMD and NDMD were highly significantly correlated with CP, CWC, HEMI, ECWD, and CDDM; however, the magnitude of the correlations was relatively low. Lignin was more highly correlated to the digestibility estimates when within classifications comparisons were made. The highest correlation between lignin and IDMD was in orchardgrass ($r = 0.85$, $P < 0.05$). A lower correlation ($r = 0.53$, $P < 0.05$) was observed within legume forages. Oh *et al.* (37) compared grasses and legumes and obtained a higher correlation within legumes when correlating *in vivo* DMD with lignin content.

The correlation coefficient of CWC with IDMD was -0.49 ($P < 0.01$) when compared across species. These results were similar to those obtained by other researchers. A correlation of -0.44 between CWC and

TABLE IX

SIMPLE CORRELATION COEFFICIENTS BETWEEN CHEMICAL CONSTITUENTS AND DIGESTIBILITY ESTIMATES

	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.00	-.39*	.20	.14	.19	-.54**	.40*	.64**	.80**	.63	.80**	-.10	.19	.11
2		1.00	.38*	.10	.45**	.65**	-.99**	-.49**	-.43**	-.49**	-.38**	.16	-.47**	-.15
3			1.00	.76**	.95**	-.45**	-.38*	-.19	-.06	-.22	-.04	-.36*	-.56**	.39*
4				1.00	.51**	-.52**	-.10	-.27	-.11	-.30	-.08	-.85**	-.82**	-.88**
5					1.00	-.34*	-.45**	-.11	-.03	-.14	-.01	-.06	-.33*	.07
6						1.00	-.66**	-.32*	-.36*	-.29	-.34*	.45**	.01	-.46**
7							1.00	.49**	.43**	.49**	.39**	-.16	.47**	.15
8								1.00	.85**	.99**	.82**	.29	.58**	-.25
9									1.00	.83**	.99**	.15	.43**	-.11
10										1.00	.81**	.31	.59**	-.27
11											1.00	.13	.39**	-.09
12												1.00	.79**	-.99**
13													1.00	-.78**
14														1.00

[a]1-14 variables listed on following page.

*Statistically significant P<0.05.

**Statistically significant P<0.01.

TABLE IX (continued)

1 Crude Protein
2 Cell Wall Constituents
3 Acid Detergent Fiber
4 Lignin
5 Cellulose (ADF-LIG)
6 Hemicellulose (CWC-ADF)
7 Cell Contents (100-CWC)
8 *In Vitro* Dry Matter Disappearance
9 Nylon Bag Dry Matter Disappearance
10 Adjusted *In Vitro* Dry Matter Disappearance
11 Adjusted Nylon Bag Dry Matter Disappearance
12 Estimated Cell Wall Digestibility (17)
13 Calculated Digestible Dry Matter (17)
14 Lignin Acid Detergent Fiber Ratio (Lignin/ADF)

TABLE X

SIMPLE CORRELATION COEFFICIENTS BETWEEN SELECTED EVALUATIONS WITHIN CLASSIFICATIONS

	1	2	3	4	5	6	7
Grain Silages							
1	1.00	.14	.29	.05	.14	.35	.40
2		1.00	.68*	.63	-.73*	-.32	-.53
3			1.00	.52	-.43	-.31	-.17
4				1.00	-.96*	-.54	-.68
5					1.00	.53	.75*
6						1.00	.81*
7							1.00
Legumes							
1	1.00	-.75**	-.92**	-.68**	.71**	.55*	.42
2		1.00	.66**	.43	-.64	-.52	-.25
3			1.00	.80**	-.76**	-.56*	-.53
4				1.00	-.94**	.44	-.57**
5					1.00	.54*	.55*
6						1.00	.68**
7							1.00

TABLE X (continued)

	1	2	3	4	5	6	7
Orchardgrass							
1	1.00	-.03	-.69	-.66	.63	.79	.54
2		1.00	.51	-.23	.17	.34	-.21
3			1.00	.50	-.43	-.82*	-.53
4				1.00	-.97**	-.81*	-.85*
5					1.00	.82*	.86*
6						1.00	.88*
7							1.00

Variables:

1 Crude Protein
2 Cell Wall Constituents
3 Acid Detergent Fiber
4 Lignin
5 Calculated Digestible Dry Matter (17)
6 Nylon Bag Dry Matter Disappearance
7 In Vitro Dry Matter Disappearance

*Statistically significant $P<0.05$.

**Statistically significant $P<0.01$.

in vivo DDM was reported by Van Soest and Marcus (62) on a group of 17 legumes and 13 grasses. Oh *et al.* (37) obtained a correlation of -0.47 when the above comparison was made.

The correlation of ADF with IDMD was statistically significant only when compared within legumes. This is in contradiction to reports by Oh *et al.* (37), Van Soest (52), and Satyanarayanasetty (45). Oh *et al.* (37) stated that the relationship between certain chemical components and digestibility is dependent on forage species. Therefore, care must be taken when comparing correlations obtained by different researchers and on different forages.

The calculated DDM, using the summative equation of Van Soest, was highly significantly correlated with IDMD ($r = 0.58$, $P < 0.01$) when compared across species. A correlation of much higher magnitude was obtained by Van Soest (54) when comparing CDDM to *in vivo* DMD. This difference in results could have been caused by the differences in the forages used for the comparison.

Regression coefficients and constants for multiple regression equations calculated to estimate IDMD and NDMD are shown in Tables XI and XII. The variables used in the equations were selected by using a stepwise regression analysis. The three most reliable variables used to predict IDMD on all forages were CP, lignin, and cell contents. In predicting NDMD, CWC was used instead of cell contents. The coefficient of multiple determination (R^2) using all three variables was 0.58 for IDMD and 0.69 for NDMD. Different variables were selected for within classification equations in some cases. A larger R^2 value was obtained

TABLE XI

REGRESSION COEFFICIENTS AND CONSTANTS USED IN REGRESSION EQUATIONS TO PREDICT IDMD

Equation No.	All Forages			Grain Silages			Legumes		Orchardgrass		
	1	2	3	1	2	3	1	2	1	2	3
Constant	53.64	57.56	52.38	74.67	54.86	63.18	73.50	78.01	77.70	106.77	107.07
CP	0.74	0.80	0.70		2.07	1.91					
LIG		-0.79	-0.71				-0.84	-0.60	-1.87	-2.96	-4.88
CELLO			0.15								
LIGADF				-0.90	-0.87	-0.74					0.75
CWC						1.91					
ADF							-0.17				
HEMI										-0.83	-0.87
R^{2a}	0.41	0.54	0.58	0.49	0.61	0.66	0.33	0.35	0.72	0.94	0.98

[a]Coefficient of multiple determination.

TABLE XII

REGRESSION COEFFICIENTS AND CONSTANTS USED IN REGRESSION EQUATIONS TO PREDICT NDMD

Equation No.	All Forages			Grain Silages				Legumes			Orchardgrass		
	1	2	3	1	2	3	4	1	2	3	1	2	3
Constant	60.98	63.38	67.66	75.57	62.66	56.48	47.69	94.80	98.63	99.11	122.24	121.59	89.34
CP	0.91	0.94	0.90		1.41	1.77	2.35						
LIG		-0.48	-0.45	-1.35	-1.40	-1.81	-4.10						
CWC			-0.65										-0.75
ADF								-0.48	-0.51	-0.45	-1.22	-1.00	-0.57
LIGADF							0.73			-0.10		-0.51	
HEMI						0.15	0.21	-0.18	-0.21				0.57
CELLCO													
R^{2a}	0.63	0.68	0.69	0.30	0.44	0.48	0.56	0.31	0.35	0.36	0.68	0.90	0.98

[a]Coefficient of multiple determination.

on within classification equations; however, the lower number of degrees of freedom could have caused this increase.

Multiple regression and correlation analysis was performed using combinations of ADF, LIG, CWC, and CP to determine how well these variable combinations would estimate IDMD and NDMD. Multiple correlation coefficients on a within classification basis are shown in Table XIII. Higher correlations were obtained in orchardgrass than in any other classification using the predictors selected. ADF, LIG, and CWC appeared to yield the best estimate of IDMD and NDMD when comparing predictors within classifications.

TABLE XIII

MULTIPLE CORRELATION COEFFICIENTS BETWEEN IDMD OR NDMD AND VARIOUS PREDICTOR COMBINATIONS

	IDMD					NDMD				
Predictors	Grain Silages	Legume Silage	Legume Hays	All Legumes	Orchard-Grass	Grain Silages	Legume Silages	Legume Hays	All Legumes	Orchard-Grass
LIG, ADF	0.71	0.75	0.70	0.59	0.86	0.54	0.68	0.57	0.56	0.95
LIG, ADF, CWC	0.77	0.80	0.76	0.59	0.98*	0.55	0.76	0.57	0.59	0.98*
LIG, CWC	0.69	0.66	0.58	0.57	0.94*	0.54	0.39	0.42	0.57	0.98**
CWC, ADF	0.59	0.59	0.75	0.55	0.53	0.34	0.73	0.54	0.59	0.83
LIG, CP	0.80*	0.64	0.64	0.58	0.85	0.66	0.37	0.62	0.55	0.88
ADF, LIG, CP	0.81	0.79	0.71	0.60	0.87	0.68	0.77	0.62	0.56	0.95

*Statistically significant $P<0.05$.

**Statistically significant $P<0.01$.

CHAPTER V

SUMMARY AND CONCLUSIONS

Thirty-seven forages, used in previous performance trials, were analyzed for *in vitro* dry matter disappearance (IDMD), nylon bag dry matter disappearance (NDMD), and chemical constitutents. The forages were grouped into the following classifications for comparisons: 1) grain silages, 2) legumes, 3) orchardgrass hays, 4) summer and annual hays, and 5) wheat silage. In some analyses legume silages and hays were separated. The IDMD and NDMD techniques were compared as methods of predicting nutritive value. Correlation and regression analyses were performed both across species and within classifications to evaluate chemical components as predictors of IDMD and NDMD.

A correlation coefficient of 0.85 ($P < 0.01$) was obtained when IDMD and NDMD were correlated across species. The NDMD method tended to yield higher values than the IDMD procedure. The mean dry matter disappearance values for the two methods were 74.8 and 64.9. The higher values obtained using the NDMD method along with the inaccuracies involved in handling the nylon bags, would suggest the *in vitro* procedure to be the method of choice.

Correlation coefficients were utilized to express the relationship between chemical components and digestibility estimates. Although the correlations of IDMD and NDMD with crude protein, CWC, hemicellulose, estimated cell wall digestibility and calculated digestible dry matter were highly significant, the magnitudes of these correlations were low

and of little predictive value. Lignin gave the larger correlations ($r = -0.57$ to -0.85) when compared to IDMD within classifications.

Several combinations of the predictors (lignin, ADF, CWC, and crude protein) were evaluated for their accuracy in predicting IDMD and NDMD. Multiple correlation coefficients were calculated, and the combination of CWC, ADF, and lignin appeared to be the most accurate in predicting IDMD or NDMD. The multiple regression equation developed from all 37 samples using a stepwise regression analysis was as follows: IDMD = 52.38 + 0.70 crude protein - lignin + 0.15 cell contents. The coefficient of multiple determination for this equation was 0.58.

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APPENDIX

TABLE XIV

SUMMARY OF INDIVIDUAL ANALYSES ON EACH SAMPLE[a]

Sample No.		CP	CWC	CELLCO	ADF	LIG	LIGADF	CELL	HEMI	IDMD	NDMD	ECWD	CDDM
Grain Silages													
1	Corn	8.40	67.54	32.46	31.43	4.97	15.81	26.19	36.11	59.04	68.04	52.70	50.10
2	Corn	8.84	57.22	42.48	25.26	4.30	17.02	20.96	31.96	57.93	70.48	50.17	53.04
3	Corn	8.44	57.22	42.48	25.26	4.30	17.02	20.96	31.96	60.55	69.42	50.17	53.04
4	Corn	9.11	52.69	47.31	27.57	3.20	11.61	24.37	25.12	59.73	66.71	63.29	62.41
5	Corn	10.23	50.51	49.49	28.19	3.69	13.09	24.50	22.32	69.10	73.11	59.17	61.09
6	Corn	9.70	62.55	37.45	28.27	2.91	10.29	25.36	34.28	65.46	73.85	67.41	61.56
7	RS610	9.54	68.62	31.38	30.66	5.55	18.10	25.11	37.96	57.35	67.22	48.07	46.44
8	RS610	9.92	62.42	37.58	29.75	5.52	18.55	24.22	32.67	57.99	67.60	47.22	49.00
9	RS610	9.92	62.42	37.58	29.75	5.52	18.55	24.22	32.67	58.24	69.60	47.22	49.00
	Mean	9.34	60.12	39.80	28.46	4.44	15.56	23.99	31.67	60.60	69.56	53.94	53.96
	Standard Error	0.23	2.07	2.06	0.73	0.34	1.04	0.61	1.66	1.34	0.85	2.50	2.05
Legumes													
10	Alfalfa Silage	19.44	47.86	52.14	35.00	7.87	22.49	27.13	12.85	68.15	79.70	40.63	53.24
11	Alfalfa Silage	19.70	48.11	51.89	34.17	7.11	20.81	27.06	13.94	68.02	81.08	43.29	54.38
12	Alfalfa Silage	19.70	48.11	51.89	34.17	7.11	20.81	27.06	13.94	62.57	77.71	43.29	54.38
13	Alfalfa Silage	20.97	46.85	53.15	32.81	6.21	18.93	26.60	14.04	67.99	78.58	46.54	56.59
14	Alfalfa Silage	17.09	51.31	48.69	34.70	5.14	14.81	29.56	16.61	69.17	79.12	54.94	58.60
15	Alfalfa Silage	18.07	47.93	52.07	36.19	5.02	13.87	31.17	11.74	73.40	82.25	57.19	61.14
Alfalfa Silage Mean		19.16	48.36	51.64	34.51	6.41	18.62	28.10	13.85	68.22	79.74	47.65	56.39
	Standard Error	0.56	0.62	0.62	0.46	0.47	1.43	0.75	0.66	1.41	0.68	2.78	1.23
16	Alfalfa Hay	12.52	62.10	37.90	45.63	13.04	28.58	32.59	16.47	67.26	73.59	32.42	39.97
17	Alfalfa Hay	17.77	54.76	45.24	36.53	8.28	22.67	28.25	18.23	66.47	72.28	40.36	49.14
18	Alfalfa Hay	20.33	54.36	45.64	35.05	4.97	14.18	30.08	19.31	69.26	77.23	56.43	58.10
19	Alfalfa Hay	21.27	46.03	53.97	31.75	8.03	25.29	23.72	14.28	67.31	77.71	36.31	52.44

TABLE XIV (continued)

Sample No.		CP	CWC	CELLCO	ADF	LIG	LIGADF	CELL	HEMI	IDMD	NDMD	ECWD	CDDM
20	Alfalfa Hay	21.41	45.26	54.74	33.01	5.43	16.45	27.58	12.25	71.73	81.36	51.35	59.58
21	Alfalfa Hay	17.05	55.60	44.40	39.21	6.62	16.88	32.59	16.39	63.07	70.09	50.45	54.26
22	Alfalfa Hay	18.17	51.07	48.93	35.47	6.74	19.00	28.73	15.60	67.02	75.85	46.40	54.35
23	Alfalfa Hay	19.49	59.90	40.10	32.45	5.08	15.65	27.37	27.45	72.26	81.98	53.04	53.77
24	Alfalfa Hay	15.72	57.81	42.19	39.25	7.05	17.96	32.20	18.56	64.43	73.79	48.33	51.99
25	Red Clover Hay	14.75	56.48	43.52	45.53	12.39	27.21	33.14	10.95	60.65	76.53	34.10	44.61
	Legume Hay Mean	17.85	54.34	45.66	37.39	7.76	20.39	29.63	16.95	66.93	76.04	44.95	51.82
	Standard Error	0.92	1.74	1.76	1.59	0.90	1.63	0.97	1.45	1.15	1.20	2.68	1.87
	All Legumes Mean	18.34	52.10	47.90	36.31	7.26	19.72	29.05	15.79	67.42	77.43	45.96	53.53
	Standard Error	0.62	1.32	1.32	1.05	0.60	1.07	0.68	0.99	0.88	0.90	1.95	1.35
26	Summer Annual Hay	12.25	70.60	29.40	51.84	9.38	18.09	42.46	18.76	52.43	68.68	48.08	45.46
27	Summer Annual Hay	15.93	70.25	29.75	47.68	8.76	18.37	38.91	22.57	58.54	67.61	47.56	45.26
28	Summer Annual Hay	15.21	70.85	29.15	50.41	11.11	22.04	39.34	20.40	58.98	71.11	41.32	40.54
	Mean	14.46	70.57	29.43	49.98	9.75	19.50	40.24	20.58	56.65	69.13	45.65	43.75
	Standard Error	1.13	0.18	0.18	1.22	0.71	1.27	1.12	1.10	2.12	1.03	2.17	1.61
29	Orchardgrass Hay	14.51	62.71	37.29	37.21	7.65	20.56	29.56	25.50	62.53	74.01	43.70	46.65
30	Orchardgrass Hay	16.23	59.91	40.09	32.16	5.20	16.17	26.96	27.75	69.64	80.77	51.93	53.10
31	Orchardgrass Hay	22.08	61.82	38.18	32.07	2.92	9.11	29.15	29.85	73.08	86.05	71.61	64.39
32	Orchardgrass Hay	15.66	63.80	36.20	38.20	5.10	13.35	33.10	25.60	70.40	78.07	58.50	55.50
33	Orchardgrass Hay	22.97	66.10	33.91	33.63	4.09	12.16	29.54	32.47	68.47	80.74	61.69	56.71
34	Orchardgrass Hay	14.61	68.49	31.51	37.31	4.16	11.15	33.15	31.18	67.53	76.74	64.67	57.87
	Mean	17.68	63.80	36.20	35.10	4.85	13.75	30.24	28.72	68.61	79.40	58.68	55.70
	Standard Error	1.56	1.26	1.26	1.14	0.65	1.67	0.99	1.19	1.44	1.69	4.01	2.38

TABLE XIV (continued)

Sample No.		CP	CWC	CELLCO	ADF	LIG	LIGADF	CELL	HEMI	IDMD	NDMD	ECWD	CDDM
35	Wheat Silage	13.00	59.69	40.31	41.49	4.90	11.81	36.59	18.20	65.89	72.01	62.70	59.63
36	Wheat Silage	11.26	56.29	43.71	32.46	4.02	12.38	28.44	23.83	63.03	73.74	61.07	59.91
37	Wheat Silage	10.91	53.32	46.68	31.65	4.77	15.07	26.88	21.67	67.21	71.57	54.34	57.42
	Mean	11.72	56.43	43.57	35.20	4.56	13.09	30.64	21.23	65.38	72.44	59.37	58.99
	Standard Error	0.65	1.84	1.84	3.16	0.28	1.01	1.74	1.64	1.24	0.67	2.56	0.79
All Samples Mean		15.19	57.80	42.19	35.22	6.17	17.19	29.05	22.59	64.93	74.76	51.03	53.64
Standard Error		0.74	1.25	1.25	1.06	0.40	0.75	0.80	1.29	0.85	0.84	1.51	1.00

[a]All analyses are expressed as percent and are on a dry matter basis.

CP - Crude Protein
CWC - Cell Wall Constituents
CELLCO - Cell Contents (100 - CWC)
ADF - Acid Detergent Fiber
LIG - Lignin
LIGADF - Lignin ADF ratio (LIG/ADF)
CELL - Cellulose (ADF-LIG)
HEMI - Hemicellulose (CWC-ADF)
IDMD - *In vitro* Dry Matter Disappearance
NDMD - Nylon Bag Dry Matter Disappearance
ECWD - Estimated Cell Wall Digestibility
CDDM - Calculated Digestible Dry Matter

VITA

Reuben Buford Moore was born August 1, 1947 in Philadelphia, Mississippi. He was reared on a dairy farm in a rural area near Philadelphia. In June 1965, he graduated from Philadelphia High School. He attended Mississippi State University for four years where he was awarded a Bachelor of Science degree in June, 1969. While at Mississippi State University, he was selected for membership in Who's Who in American Colleges and Universities. After graduation he served a term of active duty in the United States Air Force for membership in the Air National Guard.

In the spring of 1970, he accepted a research assistantship at the University of Tennessee in the Department of Dairying. He began study in the area of dairy nutrition under the guidance of Dr. M. J. Montgomery.

He is a member of the American Dairy Science Association, Alpha Zeta, and Gamma Sigma Delta.

He is married to the former Fay Pilgrim of Union, Mississippi, and they presently have a son Reuben Bartley who was born May 23, 1971.