

DEVELOPMENT AND USE OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)  
FOR TESTING FORAGES

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Near infrared reflectance spectroscopy (NIRS) to predict the chemical composition of feeds was developed by USDA researchers in Beltsville, Maryland. Its first use was to estimate the protein, oil, and moisture content of grains and oil seeds. More recently, NIRS has been adapted to predict the composition of forages. In simple terms, NIRS measures the reflectance of a band of light shining on finely ground samples and correlates the measurements with the composition of the samples as determined in a chemical laboratory. A simplified diagram of how a NIRS unit works is shown in Figure 1.

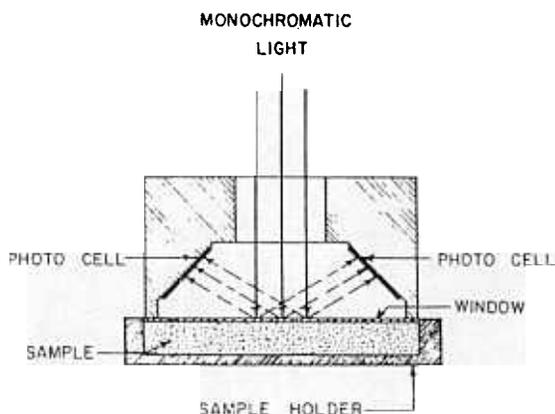


Figure 1. Optics for reflectance measurement. Four lead sulfide photo cells surround the sample; one in front and one in back are not shown.

Source: Norris, K.H. et al. 1976. Predicting forage quality by infrared reflectance spectroscopy. *J. Animal Sci.* 43:889.

Following the initial research and development of NIRS instruments at Beltsville, researchers in several states cooperated with USDA personnel in developing a National NIRS Forage Research Project Network. Objectives of the network are:

1. Relate chemical and physical properties of forages and other feedstuffs to their NIRS spectral properties.
2. Test and validate NIRS for determination of forage and other feedstuff quality.
3. Establish standards for the conduct of NIRS analyses (protocols and procedures).
4. Establish a library of reference samples for use in NIRS instrument calibration.
5. Facilitate transfer of NIRS technology.

Several privately-owned laboratories and many research institutions now use NIRS equipment for forage analysis. Universities in three states have mobile vans equipped with NIRS equipment to demonstrate the capabilities of NIRS for estimating forage composition and quality. Privately-owned mobile vans are operating in some states, also. Computer programs have been added to use results of NIRS analysis in custom formulation of dairy rations. The vans visit hay marketing centers and individual dairy farms on

request. Forage samples are analyzed and customized rations are formulated within a few minutes based on the forage analysis data.

Speed of analysis is the major advantage of NIRS. Chemical analysis of a forage sample may take one or more days before results are available. Turn around time is important when results of analyses are used for establishing selling prices for hay, or for ration formulation.

Accuracy of NIRS analysis is as good as chemical analysis if the NIRS instrument is properly calibrated for the forages being analyzed. However, this means that calibrations must be different for alfalfa grown in the western states than for alfalfa grown in the midwestern and eastern states. Differences probably are due to irrigation practices in the west, and the prevalence of alfalfa-grass mixtures outside of the western states.

Calibrations provided with current NIRS machines are based on alfalfa-grass mixtures grown in the eastern and midwestern states. Limited observations have indicated that they routinely overestimate crude protein and energy (TDN or net energy) values, and underestimate fiber values, compared with chemical analyses of the same hay samples. This has been a problem in getting acceptance of NIRS analyses in California where an extensive alfalfa testing program based on chemical analysis has been used by dairymen and hay growers for almost 30 years. Dairymen know what to expect from their cows in terms of milk production when feeding alfalfa of a certain energy (TDN) content, and have been unhappy when NIRS tests do not agree with chemical tests and over-evaluate the feeding value of the alfalfa that they purchase. This problem has been addressed by a cooperative research project between the University of California and Dairyman's Cooperative Creamery Association in Tulare, CA using NIRS machines at both locations. Calibrations based on several hundred samples of alfalfa grown in varying areas of California and Nevada are being developed and should correct the problem of over-evaluation by NIRS of alfalfa grown in the western states.

#### Fiber and Energy Values

Data from NIRS instruments or from chemical analysis can be used in equations to predict the energy content of alfalfa. The California TDN system that has been used for many years is based on chemical analysis of the hay sample for Modified Crude Fiber (MCF). The equation to predict TDN is (all constituents on a 100% dry matter basis):

$$\text{TDN}\% = 81.07 - .8558 \text{ MCF}\% \quad (\text{Equation \#1})$$

A newer fiber test developed by USDA researchers is based on treating samples with detergent and acid to separate the digestible and indigestible constituents of feed ingredients. The test, known as acid detergent fiber (ADF), has been shown to be as good as MCF in estimating TDN of alfalfa, and better than MCF for grasses and other forages. The ADF test is used in most other states because it is faster and easier to run in a laboratory. Also, it is more accurate than MCF for alfalfa-grass mixtures which are common in most areas except the western states. The equation for predicting TDN from ADF is (100% DM basis):

$$\text{TDN}\% = 78.2 - .657 \text{ ADF}\% \quad (\text{Equation \#2})$$

Net energy for lactation (NEL) has replaced TDN as the most common energy value for dairy ration formulation. NEL can be calculated from TDN by the equation:

$$\text{NEL (Mcal/lb)} = .0245 \text{ TDN}\% - .12 (.4536)$$

NEL can be calculated directly from MCF% or ADF% by substitution in the above formula. The equations are:

$$\text{NEL (Mcal/lb)} = .8465 - .0095 \text{ MCF}\% \quad (\text{Equation \#3})$$

$$\text{NEL (Mcal/lb)} = 8146 - .0073 \text{ ADF}\% \quad (\text{Equation \#4})$$

Cooperative research at several eastern and midwestern states resulted in the suggestion of a National Equation to predict the digestible dry matter (DDM) of alfalfa and alfalfa-grass mixtures based on their ADF content. The equation is:

$$\text{DDM \%} = 88.9 - .779 \text{ ADF\%} \quad (\text{Equation \#5})$$

Use of the DDM equation allows the comparison of alfalfa samples from various areas of the nation. However, the equations to predict TDN and NEL from ADF or MCF are more accurate for alfalfa grown in the western states, and TDN or NEL are more commonly used in ration formulation than is DDM. Therefore, for both chemical analysis and NIRS methods, it is suggested that analysis reports for alfalfa grown in the western states list TDN values calculated from MCF using Equation #1, or from ADF using Equation #2. Similarly, NEL values should be calculated from MCF using Equation #3, or from ADF using Equation #4. If ADF values are available, DDM can be calculated using Equation #5.

In addition to fiber and energy values, crude protein should be determined separately for alfalfa samples. This can be done using the Kjeldahl method in a laboratory, or determined directly on an NIRS instrument if it is properly calibrated.

A proposed system for reporting the nutritional value of alfalfa hay is shown in Figure 2. The report form accommodates the use of either MCF or ADF for predicting the TDN and NEL values of the sample, and can be used for data from a chemical laboratory or from NIRS instruments. The Hay Quality Rating at the bottom of the form corresponds with the quality grades currently included in the California Hay Market News reports.

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<u>Lab Analyses:</u>	<u>As Received</u>	<u>90% DM</u>	<u>100% DM</u>
DM (%)	87	----	----
ADF (%)	27.0	27.9	31.0
(OR)			
MCF (%)	23.7	24.5	27.2
CP (%)	17.4	18.0	20.0
 <u>Estimated Energy Values:</u> (from ADF or MCF)			
DDM (%)	56.4	58.3	64.8
TDN (%)	50.3	52.0	57.8
NE <sub>1</sub> (Mcal/lb)	.51	.53	.59
 <u>Hay Quality Rating For This Sample:</u>			
<input type="checkbox"/> Premium (Above 54% TDN)			
<input checked="" type="checkbox"/> Good (52-54% TDN)			
<input type="checkbox"/> Fair (49-51% TDN)		>	90% DM Basis
<input type="checkbox"/> Low (Below 49% TDN)			

Figure 2. Proposed laboratory reporting procedure for the nutritional value of alfalfa hay.