### **Understanding and Using Forage Test Results**

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# Introduction

There are many new advancements in the analytical evaluation of forages. Despite new advancements, the world of "forage testing", as we commonly refer to it, is sometimes difficult to understand. A plethora of information exist. This paper will attempt to address new advancements in forage evaluation as well as address concerns and myths with analytical procedures and utility of forage evaluation systems.

#### **Innovations : Summative Energy Equations**

The amount of energy in a ruminant diet is arguably the single most important factor in predicting animal performance. It is the author's impression that nutrition consultants and dairy producers have lost confidence in the ability of feed testing systems to predict energy content of a forage or ration. In the past this perspective was somewhat valid. Empirical equations (Rohweder et al., 1978) were used for many years to predict forage energy content from a single analyte such as acid detergent fiber (ADF). Empirical equations to predict forage energy content by and large were accurate but imprecise. The aforementioned statement simply means that when examining a large data base of forage energy contents predicted by an empirical equation, the empirical equation accurately predicts the average of the data base but cannot precisely predict the energy content of any single forage in the data base. To be of real value, feed testing systems should be able to precisely predict the energy content of any single forage, feed, or diet.

Weiss, 1996 proposed using a summative approach to predict energy content of feeds. The concept of a summative approach is simple: measure the principal components in the feed that contribute energy, give each component a digestion coefficient, multiply each component by its respective digestion coefficient, and add the products together. The greater utility of a summative energy system is that it can be used on any forage, grain, commodity, or even total mixed rations. The major drawback of summative equations is extensive laboratory measurements are needed. Seven principal nutrients need to be accurately and precisely measured in the laboratory: crude protein (CP), neutral detergent fiber (NDF), fat, ash, acid detergent fiber crude protein (ADF CP), and neutral detergent fiber crude protein (NDF CP) to facilitate the final determination of NFC. The digestion coefficients assigned to CP, fat, and NFC are well defined by research (Weiss, 1993); however, the digestion coefficient for NDF (NDFD, % of NDF) is not well defined by research and thus requires measurement in the laboratory.

A complete discussion of summative energy equations is available (Weiss, 1996; NRC, 2001) and is beyond the scope of this paper. An example of a summative energy equation adopted by the NRC, 2001 to predict the energy content of legume-grass silage is presented in

Table 1. The reader should be aware the summative equation concept presented in Table 1 has been modified for corn silage (Schwab and Shaver, 2001).

### **Innovations: NDF Digestibility**

Accurately and precisely predicting the NDFD content of the feed or forage NDF is extremely important in generating a quantitative summative forage energy prediction. Unfortunately NDFD is one of the more difficult assays to conduct in the laboratory. Most laboratories cannot conduct the assay because an in vitro NDFD laboratory procedure requires rumen fluid from a live cannulated cow.

Forage NDFD can be measured in one of two ways. First, forages can be placed in small dacron bags and inserted into the rumen of a cow via a ruminal cannula. The amount of NDF prior to ruminal incubation is compared to the amount of NDF remaining after ruminal incubation and NDFD is calculated. This is called an in situ method. The in situ method is a very viable method to estimate NDFD of forage NDF and is often used in research and other forage evaluation programs. Because of the lack of a large uniform database, the 2001 NRC, however, does not recommend the in situ method as its basis for NDFD of feeds and forages.

The 2001 NRC uses lignin as a base to predict potential NDF digestibility or advises the use of a 48 h in vitro NDFD as the basis for direct determination of the NDF digestibility coefficient. Again, advised use of a 48 h in vitro NDFD was not made based on analytical superiority over the in situ system, rather the in vitro NDF digestibility data base was larger and more uniform, making interpretation easier. An in vitro NDFD determination (Goering and Van Soest, 1970) is conducted as follows: 1) feed is weighed into a glass flask, 2) buffers, macro-and micro-minerals are added along with rumen fluid extracted from a cow fit with a ruminal cannula, 3) the forage, buffers, and rumen fluid are incubated in a water bath in an anaerobic environment (carbon dioxide) at a cow's body temperature (102° F) for 48 hours, 4) the flask containing the forage, buffers, and rumen fluid is removed from water bath and the remaining solution is refluxed in NDF solution for 1 hour, 5) after refluxing in NDF solution for 1 hour the remaining solution is filtered and the NDF that resisted digestion by rumen bacteria is retained on the filter, and 6) digestible NDF is calculated by difference.

Few changes have been made to the in vitro NDFD assay over the years, but some researchers and laboratories have reduced the incubation times from 48 hr to 30 or 24 hr, opting that shorter incubation times better describe the digestion potential of NDF in high producing lactating dairy cows. Reducing the incubation time of the in vitro NDFD assay to 30 or 24 hr is logical because feed is not retained in the rumen of a high producing dairy cow for 48 hr. In the larger sense, however, this issue is somewhat clouded because changing the incubation time of the assay reduces the amount of NDF digested; therefore, NDF digestibility values obtained from 30 or 24 hr digestions cannot easily be compared to available NDF digestibility data bases (NRC, 2001). The recommendation of a 48 hr in vitro NDFD by the NRC, 2001, is also designed to facilitate calculating TDN content of forages at maintenance intakes (which is TDN). The most important issue with NDF digestibility at this time is for laboratories to report forage NDF digestibilities that have a common scale and reference. Because the NRC, 2001 advises the use

of a 48 hr in vitro NDF digestibility procedure to calculate TDN contents of forages at maintenance intakes, it is most logical to identify with the 48 h NDFD reference and scale. Listed in Table 2 are 30 and 48 h NDFD (% of NDF) of many common feeds and forages. The NDFD values from 30 h in vitro evaluation systems typically yield lower NDFD values. With caution, these values can be substituted into summative energy equations (NRC, 2001) to calculate TDN at maintenance, but the user should be aware that low TDN predictions can occur if 30 h NDFD procedures are compromised. Substituting wet chemistry in vitro 48 h NDFD values into summative energy equations of forage energy estimates if done correctly, but may slightly over-estimate the TDN content of the feed.

The NDFD content of forages can be predicted using NIRS, but generally there is some loss of precision because IV NDFD wet chemistry techniques have greater laboratory errors than other laboratory assays such a crude protein. Combs (1998) used NIRS to successfully predict in vitro 48 h NDFD contents of legume grass forages. Development of accurate and precise NIRS equations for the NDFD content of corn silage has proven more challenging because of the narrow range of NDFD in corn silage and the heterogeneous nature of corn silage (Lundberg, et al., 2003). Development of improved NDFD NIRS equations is ongoing and ultimately, prediction of NDFD in forages by NIRS is preferred because laboratories using NIRS prediction systems can be standardized. Large data bases of forage NDFD contents will be required to facilitate accurate and precise measures of forage NDFD.

# **Understanding Forage Test Reports:**

Receiving a forage test report with 50 lines of information on it can be daunting. Despite the complexity of recent innovations in forage testing such as described above most forage test reports are still relatively simple. Shaver (2004) developed a simple scheme to aid producers and nutritional consultants by defining forage nutrients into specific categories. Those categories are as follows; nutrients commonly used to 1) predict dry matter intake 2) predict energy 3) direct use in ration balancing 4) nutritional diagnostics 5) supplementation strategies 6) quality indexes and 7) agronomic trials. The categorical definitions of forage test parameters as defined by Shaver 2004 and expanded by the author are presented in table 3. These data are a slight over simplification by the author as some supplemental nutrients are used in ration balancing programs such as the Cornell/Penn/Miner Model.

In addition to defining forage test parameters by use category producers and nutrition consultants often require some guidelines as to what desired values should be. Using database information from the Marshfield Soil and Forage Testing Laboratory the author has attempted to define possible ranges of nutrient parameters available on forage test reports and give general direction as to what maybe a desired value for lactating dairy cows and dry cows (Table 4).

### **Other New Developments in Forage Evaluation**

### **Evaluation of Total Mixed Rations**

Summative energy prediction systems have great utility in the evaluation of total mixed rations. One of the greatest concerns with laboratory evaluation of TMRs is sampling error. Recently, Hutjens, 2002 appropriately addressed TMR sampling error and suggested evaluating TMRs via wet chemistry for DM, CP, and ADF to determine accuracy of mixing. The recommendation of Hutjens, 2002, is logical but overlooks the potential to use summative equations to estimate of TMR energy content as compared to relying on commonly empirical generated ration energy contents. Evaluation of energy contents of TMRs is relatively simple with CP, NDF, ash, fat, NDF CP and 48 h in vitro NDF digestibility of the TMR evaluated in duplicate via wet chemistry procedures, thus minimizing potential lab error. The energy content of the TMR is then estimated using NRC, 2001 summative models and precision estimates are achieved. Excellent sampling and laboratory procedures are required to conduct summative TMR analysis.

A summary of total mixed rations evaluated at the Marshfield Soil and Forage Analysis Laboratory in are presented in Figures 1 - 4. The variation of TMR nutrients in Figures 1 - 4 is quite wide therefore, numerous TMR diets in Figures 1 - 4 are likely incorrectly formulated or fed. In addition, it should be noted that high group lactating cow diets containing a common 27.0 to 28.0% NDF can vary dramatically in dietary energy content. More research is needed on the normal relative sampling errors associated with TMRs. For the first time, however, the dietary energy content of a TMR can be systematically evaluated if proper laboratory procedures are used. The precision summative TMR evaluations are, however, slow (1 week minimum) and expensive to conduct ( $\cong$ \$50.00).

Finally, laboratory evaluation of TMRs for energy density using precision summative technology appears to be an excellent tool to re-check energy estimates developed from ration balancing techniques.

#### **Bypass Protein**

Recent work from our laboratory (Dorshorst et al., 2000; Hoffman et al., 1999a, b, c) has demonstrated that NIRS can predict ( $R^2 = .87$ ) bypass protein (3X maintenance) content of legume grass silages (Hoffman et al., 1999c) and legume grass hays (Dorshorst et al., 2000). The NIRS system to predict bypass protein of these forages was developed using a calibrated cow in situ technique and was then converted to NIRS techniques. The NIRS evaluation system is commercially available, but has limited use in field applications because the sample cannot be microwave dried because of protein matrix alteration due to overheating. Very good bypass protein numbers can be generated for legume/grass hays or silages if samples are dried at 55° C, then evaluated using bypass protein using NIR systems.

# pН

Some laboratories now routinely offer the prediction of pH in ensiled forages using NIRS. Reeves et al., 1989 observed that NIRS could predict silage pH, but prediction was somewhat imprecise. The actual utility of silage pH is somewhat vague, but could be used as a screening tool to conduct further silage fermentation analyses.

### **Silage Fermentation Analysis**

Similar to silage pH, silages can be evaluated for fermentation profiles which generally include pH, acetic, lactic, butyric, propionic (acids) and ammonia (NH<sub>3</sub>). Silage fermentation analyses are generally done using high pressure or gas chromatography although some labs use NIRS on undried, unground samples which has been demonstrated to be feasible (Reeves et al., 1989). Silage fermentation analysis can be used to trouble shoot silage fermentation problems, assess potential dry matter intake problems, or evaluate silage inoculant performance.

### **Starch and Starch Digestibility**

Some laboratories have begun to test corn silage and other starch containing feeds for starch and starch digestibility. Testing feeds such as corn silage for starch is relatively common although laboratory procedures differ which sometimes making interpretation of starch values between laboratories difficult. Testing forages and total mixed rations for starch digestibility is relatively new but no standard laboratory test exist for starch digestibility so producers and nutrition consultants are advised to work closely with their laboratory of choice on interpretation of results.

# Conclusions

There have been a number of new advancements in analytical evaluation of forages. To take advantage of these new analytical advancements, nutrition consultants and dairy producers should work closely with their laboratory to eliminate false expectations.

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Table 1. Example of summative calculations made to estimate the energy content of legume grass silage.

ltem	Abreviation	Unit	Value	Formula	TDN Units	
Protein Fractions						
Crude Protein Neutral Detergent Fiber Crude Protein	CP NDFCP	% of DM % of DM	21.9 4.2	CP * .93	Ecp=	20.37
Fiber Fractions						
Neutral Detergent Fiber Neutral Detergent Fiber Digestibility, 48 h	aNDF NDFD	% of DM % of NDF	40.0 48.0	((NDF)*(NDFD/100))*.75	Endf=	14.40
Carbohydrates and Fats						
Non Fiber Carbohydrate ' Fat	NFC	% of DM % of DM	29.1 3.2	(NFC*.98) ((.97*(Fat-1))*2.25	Enfc= Efat=	28.50 4.80
Macro Minerals						
Ash		%of DM	10.0			
Energy Calculations:2001 NRC						
Total Digestible Nutrients,1X Net Energy , Lactation, 3X	TDN Nel	% of DM Mcals/lb		Ecp+Endf+Enfc+Efat-7 ((.0245*TDN)012)/2.2046))		61.06 0.62

'NFC = 100-(CP+NDF+Ash +Fat-NDFCP)

\*\*\*\* Note. Not for use with corn silage.

Feed	In Vitro NDF Digestibility, % of NDF <sup>1,2</sup>								
	High	Medium	Low	High	Medium	Low			
	48	h NDF Digestibilit	30 h NDF Digestibility						
Alfalfa Hay	55.4	49.8	44.2	53.5	46.2	38.9			
Alfalfa Silage	58.2	53.1	48.0	55.9	51.3	46.7			
Grass Hay	64.8	54.2	43.6	na	na	na			
Grass Silage	62.9	53.7	44.5	na	na	na			
Legume/Grass Hay	59.4	48.0	36.6	na	na	na			
Legume/Grass Silage	59.5	54.3	49.1	na	na	na			
Ryegrass Silage	na	63.1	na	na	55.6	na			
Red Clover Silage	50.3	47.1	43.9	na	na	na			
Sorghum/Sudan Silage	na	57.2	na	na	49.2	na			
Straw	na	32.5	na	30.5	26.6	22.7			
Corn Silage	63.8	58.9	54.0	52.3	48.0	43.7			
Brown Mid-Rib Corn Silage	72.8	68.6	64.4	na	na	na			
Small Grain Silage	66.8	56.4	46.0	na	47.9	na			
Total Mixed Rations, High Group	63.0	57.1	51.2	na	na	na			
Total Mixed Rations, Prefresh	63.5	54.6	45.7	na	na	na			
Total Mixed Rations, Postfresh	61.4	55.9	50.4	na	na	na			
Total Mixed Rations, Dry Cows	64.9	59.4	53.9	na	na	na			
Total Mixed Rations, Heifer Diets	61.5	54.4	47.3	na	na	na			
Corn Gluten Feed	na	na	na	na	79.8	na			
Distillers Dried Grains	na	na	na	81.2	76.2	71.2			
Brewers Grains	na	na	na	na	49.9	na			
Wheat Midds	na	na	na	53.0	51.2	49.4			
Beet Pulp	na	na	na	89.6	83.6	77.6			
Citrus Pulp	na	na	na	na	85.0	na			
Soy Hull	na	92.0	na	na	91.6	na			
Whole Cottonseed	na	na	na	61.9	53.3	44.7			
Soybean Meal	na	na	na	90.8	87.3	83.8			
Barley	na	na	na	na	52.0	na			
Corn	na	85.0	na	na	na	na			
Steam Flaked Corn	na	na	na	81.5	73.6	65.7			

Table 2. Typical NDF digestibility values for forages, total mixed rations and byproduct feeds.

<sup>1</sup> Adapted from data bases of the Marshfield Soil and Forage Analysis Laboratory and Peter Robinson, University of California-

<sup>2</sup> High NDFD values represent the average plus 1 standard deviation. Low NDFD values represent the average minus one standard deviation. Feeds without high and low values do not contain enough samples to calculate a reliable standard deviation.

Table 3. Utility of various forage test (Adapted from R.D. Shaver, Dairy Science Dept, University of Wisconsin)

Test	Common Abreviations	Common Unit Expression	DMI Prediction	Energy Estimate, TDN, NEL	Ration Balancing	Nutritional Diagnostics	Supplement Strategies	Quality Indexing	Agronomic Trials
Crude Protein	CP	% of DM		v	v		v		
Soluble Protein	CP Sol-CP	% of CP		х	x		x		
					х	x	х		
Acid Detergent Fiber Crude Protein	ADF-CP, ADIN			X		х			
Neutral Detergent Fiber Crude Protein	NDF-CP	% of DM		X					
Rumen Undegradable Protein	RUP	% of CP			x	x	x		
Acid Detergent Fiber	ADF	% of DM		obsolete				obsolete	
Neutral Detergent Fiber	NDF	% of DM	х	х	х	х	х		
Neutral Detergent Fiber Digestibility	NDFD	% of NDF	х	х		x	х		
Lignin	Lignin	% of DM, % of NDF		х					
Fat	EE, Fat	% of DM		x	x		x		
Starch	Starch	% of DM		x		x	x		
Sugars	Sugars	% of DM				х	х		
Ash	Ash	% of DM		х					
Minerals, Ca,P,K,Mg,Na,Cl,S + micros	Ca, P etc	% of DM			x		x		
Total Digestible Nutrients	TDN	% of DM			x				
Net Energy Lacation, Maintence, Gain	NEL Nem Neg	Mcals/lb			х				
Particle Size	na	% of DM				x	x		
Relative Feed Value	RFV	na						obsolete	
Relative Forage Quality	RFQ	na						х	
Processing Score	na	na				х			
Fermentation Profile	na	% as is, % of DM				х			
In Vitro Dry Matter Digestibility	IV TDMD	% of DM							х
Milk/Ton	na	lbs/ton						х	х
Milk/Acre	na	lbs/acre						х	х

Table 4. Numeric ranges of common forage test and qualitative desired level

				Range	_		
Test	Common Abreviations	Common Unit Expression	Legume-Grass Silages	Legume-Grass Hay	Corn Silage	Desired Level Within Range - Lactating Cows	Desired Level Within Range - Dry Cows
Crude Protein	CP	% of DM	9.2-24.7	12.8-25.21	5.0-10.2	Mid-Upper	Mid
Soluble Protein	Sol-CP	% of CP	20.5-76.5	na	20.5-45.0	Mid	Mid
Acid Detergent Fiber Crude Protein	ADF-CP, ADIN	% of DM	.14-2.3	.20-1.25	.2270	Lower	Lower
Neutral Detergent Fiber Crude Protein	NDF-CP	% of DM	1.0-8.8	2.27-5.08	.5-2.3	Lower	Lower
Rumen Undegradable Protein	RUP	% of CP	16.2-39.4	13.0-45.2	na	Mid	Mid
Acid Detergent Fiber	ADF	% of DM	obsolete	obsolete	obsolete		
Neutral Detergent Fiber	NDF	% of DM	32.3-70.8	29.6-70.6	30.1-61.9	Lower	Mid
Neutral Detergent Fiber Digestibility	NDFD	% of NDF	32.5-79.4	35.8-74.5	44.0-72.0	Upper	Mid
Lignin	Lignin	% of DM	2.45-9.78	4.7-9.9	1.6-6.0	Lower	Lower
Lignin	Lignin	% of NDF	5.39-23.1	10.9-23.3	3.82-16.1	Lower	Lower
Fat	EE, Fat	% of DM	1.0-3.8	.9-3.8	1.1-4.2	Mid	Mid
Starch	Starch	% of DM	na	.0 0.0 na	7.2-38.1	Mid-Upper	Mid
Ash	Ash	% of DM	6.4-16.4	7.4-15.8	3.3-14.4	Lower	Lower
Calcium	Са	% of DM	.31-1.61	.53-1.66	.1337	Upper	Mid
Phoshorus	P	% of DM	.1653	.0840	.1523	Mid	Mid
Potassium	ĸ	% of DM	1.1-3.83	.67-3.74	.74-1.66	Mid-Lower	Lower
Magnesium	Mg	% of DM	.1940	.1841	.1226	Upper	Upper
Sodium	Na	% of DM	.0114	.0112	.0509	Mid	Mid
Chlorine	CI	% of DM	.26-1.25	.0883	.1040	Mid	Upper
Sulfur	S	% of DM	.1338	.1039	.0520	Upper	Upper
Total Digestible Nutrients	TDN	% of DM	47-72	49.0-69.6	42-76.4	Upper	Mid
Net Energy Lacation 3x	NEL	Mcals/lb	.4775	.4972	.7278	Upper	Mid
Relative Feed Value	RFV	na	obsolete	obsolete	obsolete		
Relative Forage Quality	RFQ	na	63-230	69.4-237.0	na	Mid-Upper	Mid
Milk/Ton	na	lbs/ton	1650-3801	1790-3437	1582-3901	Upper	Mid

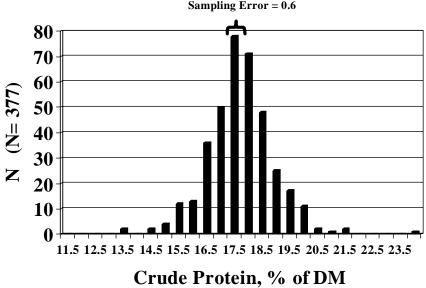


Figure 1. Distribution of CP Content in High Group TMRs Sampling Error = 0.6

