New Methods of Determining Energy Content and Evaluating Heat Damage in Forages for Dairy Cattle

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Improved System of Forage Analysis

The crude fiber method of feed analysis has been used for more than 100 years. Although this method was an important first attempt to determine energy content of feeds, a number of shortcomings have become evident:

- The crude fiber method assumes that crude fiber is the same for all forages. This is not true. The crude fibers of alfalfa, orchard grass and cottonseed hulls have different digestibilities and therefore can not be considered the same for calculating feed energy.
- The crude fiber value for the same feed may be quite different from laboratory to laboratory. This is due to the varying conditions under which chemists measure crude fiber. For example, the strength of acid and base used and the length of time feed is boiled in acid and base can affect crude fiber value.
- Crude fiber increases as forages mature, but this increase often does not accurately reflect the simultaneous decrease in energy content. Using the crude fiber method, energy content of good quality forages often is underestimated while it often is overestimated in poor quality forages.
- The crude fiber method often does not separate the highly digestible parts of the plant from the less digestible parts.

A new analytical approach for estimating energy content of forages was developed in the 1960's (by Van Soest) at the U.S.D.A. Beltsville Nutritional Research Facility. These techniques, detergent fiber analyses, give more accurate estimates of forage energy values and now are used for forage analysis.

Detergent Fiber Analysis

The detergent fiber analytical method separates a forage into two parts: (1) the cell solubles which include starches, proteins, sugars and other compounds which are highly digestible and (2) detergent fiber, which provides structural support for the plant and is lower in digestibility.

Different types of detergent fiber also are determined. Figure 1 is a schematic diagram of the plant cell illustrating the cell solubles, cell wall and types of fiber found in the cell wall.

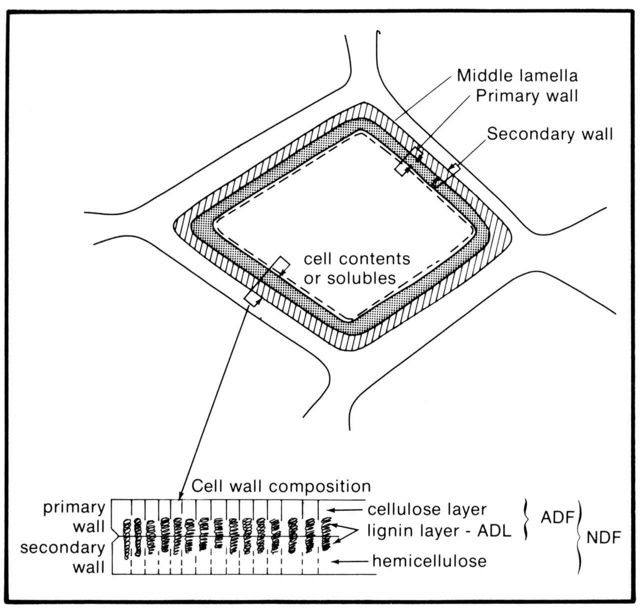

Figure 1. Schematic diagram of plant cell shows cell solubles, cell walls and fibrous parts (unscaled).

Neutral detergent fiber (NDF), also called cell wall, is measured by boiling a sample of forage in a special detergent (soap) under a neutral (pH = 7) condition and filtering the boiled sample through filter paper. The liquid that passes through the filter paper contains starch, sugar, protein and other compounds that were dissolved.

The part of the feed sample that does not dissolve remains on the filter paper; this residue is called NDF or cell wall. After drying, the NDF is calculated as a per cent of the original forage sample analyzed. Figure 2 shows how NDF is determined.

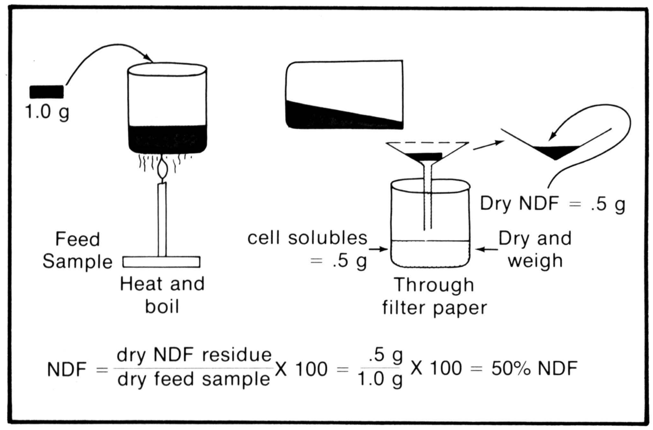

Figure 2. These steps are used to determine neutral detergent fiber.

NDF contains all the fiber found in the forage and consists of the following fiber components—hemicellulose, cellulose and lignin. NDF is partially digestible, ranging from 20-80 per cent, depending upon forage species and stage of maturity.

NDF also maintains the original bulkiness of the feed before it was boiled in detergent. Research indicates NDF is responsible for rumen fill and consequently may be related to forage intake.

Acid detergent fiber (ADF) is determined in the same way, except a different detergent is used under an acid (pH = 2) condition. The forage sample is boiled and filtered just like the NDF procedure. Because of the different detergent and the acid conditions, hemicellulose as well as cell solubles dissolve and are filtered away. The residue left is ADF and consists mainly of cellulose and lignin.

Acid detergent lignin (ADL) is measured by further treating ADF with strong acid, which dissolves cellulose, or with permanganate (salt of permanganic acid), which oxidizes (removes) the lignin. Either approach allows calculation of amount of lignin.

ADF is partially digestible, ranging from 20-80 per cent, while ADL is low in digestibility, from 0-30 per cent. The ADF fraction is closely related to digestibility of the forage sample because it contains cellulose and lignin.

Cellulose is the major fiber fraction to be digested; there is less lignin. Lignin, however, ties up cellulose—the higher the concentration of lignin, the greater the amount of cellulose tied up and made indigestible.

Two forages may have similar ADF content, 25 per cent for example. Forage 1 may be 20 per cent cellulose and 5 per cent lignin; forage 2 may be 15 per cent cellulose and 10 per cent lignin. Forage 1 would be much more digestible.

Therefore, information about the amount of lignin and cellulose as well as ADF content of a forage is important in predicting energy content.

The last step in forage analysis is measuring the digestibility using laboratory techniques. The test for dry matter digestibility (DMD) simulates digestibility in the cow but the test is much less expensive and less time consuming.

A sample of forage, some rumen fluid and certain chemicals are put into a flask and allowed to digest for a standard period, usually 48 hours. Then NDF determination is made on the contents of the flask. The residue left on the filter paper is undigested fiber, mostly lignin and cellulose.

This measures how much forage was not digested in the 48-hour period. Little additional fiber digestion would occur past that period. DMD, or the amount of digested material, is 100 minus the NDF residue (undigested fiber). This is an improved method of estimating total digestible nutrients (TDN). TDN is estimated as DMD - 10 per cent. Table 1 summarizes the parts of the forage plant, how the detergent fiber analysis segregates these parts and the digestibility of these parts.

The amount of fiber in a forage depends upon: (1) the species and (2) the stage of maturity. Table 2 lists usual fiber values for some Missouri forages. From the values in Table 2 several important facts are evident:

- Grasses have higher fiber and lower energy than alfalfa cut at similar stages.
- Grasses and alfalfa increase in fiber content from early to late stages; the increase is greater for grasses than alfalfa.
- Corn for silage does not show an increase in fiber nor a decrease in energy from early to late stages; because the corn plant is producing a large amount of starch, the concurrent increase in fiber is not evident.

Net Energy Terminology

The term net energy (NE) is sometimes misunderstood and needs to be clearly defined. In this report, NE is used in the same context as in National Research Council (NRC) publications and in UMC Guidesheet 3104 "Calculating Rations for Dairy Cattle."

For example, a lactating cow weighing 1,430 pounds and producing 65 pounds of 3.5 per cent butterfat milk needs 10.9 Mcal or therms of NE for maintenance and 20.4 Mcal or therms of NE for production, which totals 31.3 Mcal or therms of NE. Because a certain weight of forage allows a cow to produce a given quantity of milk, forages are estimated to contain a certain amount of net energy. Separation of forage energy into the amount used for maintenance and the amount used for production has not been clearly determined by research yet. The amounts probably vary with level of intake. Therefore, how much forage energy was used for maintenance and how much was used for production is of little concern.

Table 2. Comparison of Detergent Fiber and Energy Content of Different Forages Cut at Early and Late Stages

Forage and Stage	NDF	ADF	ADL	Cellulose	*Calculated NE
Alfalfa 1/10 bloom	40	30	10	20	65
Alfalfa full head	60	45	15	30	45
Fescue boot	50	40	5	35	60
Fescue full head	75	60	10	50	35
Orchard grass boot	55	45	5	40	60
Orchard grass full head	80	65	10	55	30
Corn silage tassel	50	25	5	20	60
Corn silage dent	50	25	5	20	60

*Calculated using NDF equation for NE

NE values used here are not calculated the same as the estimated net energy (ENE) values of Morrison's Feeds and Feeding, although some values may be similar. Morrison's tables of ENE underestimated energy content of high quality forages and overestimated the energy of low quality forages.

The terms Mcal, therm, therm/pound and therm/100 pounds need explanation. One Mcal and one therm are equal to 100 kcal, the amount of heat needed to raise 400 pounds (50 gallons) of water 10° F. Therm/pound and therm/100 pounds or cwt refer to energy concentration in a feed.

For example, if a pound of forage was analyzed and found to contain .5 of a therm, energy content would be .5 of a therm/pound or 50 therms per 100 pounds (50 therms/cwt). This also would be equivalent to .5 Mcal/pound or 50 Mcal/100 pounds.

Determining Net Energy

Net energy can be predicted reasonably well using either NDF or ADF. Forages cut at different stages of maturity have different levels of fiber and energy. Older, more mature forages have higher fiber and less energy than younger succulent forages. NDF and ADF both increase as forages mature while the DMD (or TDN) decreases. A great deal of research indicates the following relationship for net energy, NDF and DMD:

$$\text{NE (Mcal/lb or therms/lb)} = \frac{(.01) \times (\text{TDN}) \times \left(2.86 - \dfrac{35.5}{\text{cell solubles}}\right)}{2.2 \text{ lbs/kg}}$$

Where TDN = DMD - 10
cell solubles = 100 - % NDF

Table 1. Summary of Detergent Fiber Fractions

Plant part	Contains	Detergent Fiber Fraction	How Determined	Digestibility
1. Cell solubles	protein sugar starches fats pectins	Cell solubles = 100 - NDF	Released by neutral detergent extraction	90 - 100%
2. Cell wall		NDF, ADF	Filtration, leaves residue (NDF or ADF)	20 - 80%
a. Primary wall				
	Cellulose	ADF-ADL	Filtration	50 - 90%
	Some lignin	ADL	Dissolve or oxidize	0 - 30%
	Heat-damaged protein		Kjeldahl determination	
b. Secondary wall				
	Hemicellulose	NDF - ADF	Difference	20 - 80%
	Most lignin	ADL	Dissolve or oxidize	0 - 30%

Both NDF and DMD (as TDN) are needed in the equation because as a plant matures, the increase in NDF is large while the decrease in DMD is not so great. Using both NDF and DMD increases accuracy of the net energy value.

Some forages change in NDF and DMD more than others; if legumes, corn silage and sorghum silage increase one per cent in NDF, DMD and TDN simultaneously decrease by one per cent. Thus, as a legume or silage increases from 50 per cent to 60 per cent in NDF, DMD will decrease from 70 per cent to 60 per cent and TDN from 60 per cent to 50 per cent. Grasses decrease 2 per cent in DMD (or TDN) for each one per cent increase in NDF; that is as NDF goes up from 55 per cent to 65 per cent, DMD will decrease from 65 per cent to 45 per cent and TDN from 55 per cent to 35 per cent.

Two advantages of these relationships between NDF and DMD are: (1) They are more accurate than using crude fiber because both fiber and digestibility are measured and (2) stage of maturity is not necessary for estimation of energy. If only the NDF value is known, DMD can be estimated by comparing it to the same type forage which has known NDF and DMD values.

A lot of data exists for estimation of net energy from NDF. But NDF is not recognized as an official chemical method, and many commercial labs hesitate to use it until the method becomes official.

Less information is available relating ADF to forage net energy but ADF is used by most commercial feed analysis labs for estimation of net energy. Different equations are used depending upon type of forage.

1. grasses: Net energy (Mcal/lb) = 1.085 - .0150 (% ADF)
2. legumes: Net energy (Mcal/lb) = 1.044 - .0123 (% ADF)
3. mixed legume and grasses: Net energy (Mcal/lb) = 1.044 - .0131 (% ADF)
4. corn silage: Net energy (Mcal/lb) =

$$.3133 \times \left(2.86 - \frac{35.5}{100 - (1.67 \times \%\ ADF)}\right)$$

5. grains: See UMC Guidesheet 3104.

As with the NDF technique, knowing the cutting stage or date is not necessary for estimation of energy. For a paticular type of feed, a given ADF content is related to a certain amount of energy. As ADF goes up or down, energy content changes in opposite direction.

The problem with using cutting dates to estimate energy content is weather variations from year to year. The weather affects plant growth too much for cutting dates to be accurate.

Using cutting stage is more accurate than cutting date because generally a given cutting stage, such as 1/10th bloom or boot, is related quite closely to chemical composition. However, heat or drought stressed forages can have elevated fiber levels compared to the same forage cut at the same stage and not heat or drought stressed.

Dent stage corn silage grown in New York and Michigan usually has NDF of about 40 per cent. Corn silage grown in Missouri usually has NDF of 50-55 per cent; some silages have been found to contain 65-70 per cent NDF. The higher NDF concentration decreases the net energy content of Missouri grown corn silage and tends to give a production response similar to sorghum silage.

As a result, New York and Michigan corn silage has a TDN value of about 65-70 per cent, whereas Missouri corn silage has a TDN value of about 55-60 per cent. Corn silage, as well as nearly all other forages grown in hotter climates, generally has a higher fiber and lower energy content.

Therefore, a much more accurate determination of energy content can be made by using fiber content and direct feed analysis rather than a cutting date or stage.

Measurement of Protein Availability

Protein is an essential nutrient for the dairy cow. However, its availability when subject to heat damage is variable and may greatly affect milk production.

Protein content of forages usually is determined by the Kjeldahl (Kell-doll) method. In this procedure a feed sample is boiled in strong acid (H_2SO4) which destroys organic matter and converts the nitrogen (N) of natural protein and N of non-protein nitrogen (NPN) compounds into ammonia, which is trapped as ammonium sulfate. Ammonium sulfate is boiled in a strong base (NaOH) to release ammonia, which is trapped in a specific chemical that allows measurement of N.

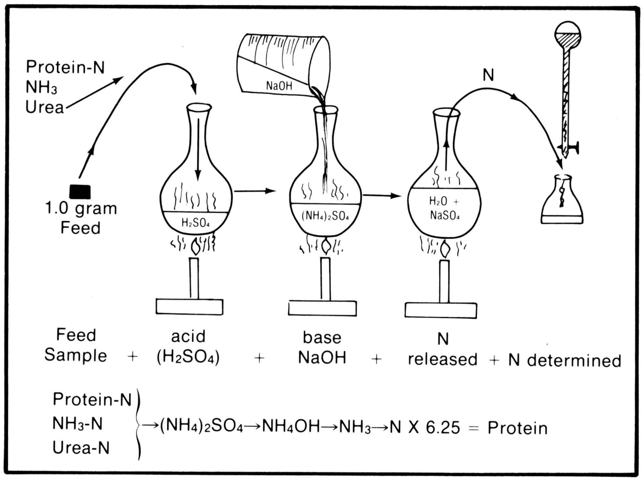

Figure 3. The Kjeldahl procedure is used to determine protein content of forages.

Plant protein usually contains 16 per cent N. Because the Kjeldahl method measures N, we calculate protein by multiplying it by a factor of 6.25. This factor is obtained by dividing 16 per cent N content in protein by 100 per cent.

$$16.0 \div 100 = 6.25$$

Two problems of the Kjeldahl method are: (1) It can not distinguish between the natural protein such as that of soybean meal and the NPN of compounds such as urea and ammonia) and (2) protein that is unavailable in the cow is still measured as N by the Kjeldahl procedure.

The Kjeldahl procedure gives no indication whether protein is available or unavailable. Therefore, a procedure must be used to measure availability of protein.

Crude protein often is assumed to be completely digested by the dairy cow, but we know a certain amount of crude protein is completely unavailable. The unavailable part is generally 25 per cent of the crude protein in the diet and is similar in most forages except extremely early or extremely late cut forage. For most forages, we consider 3 percentage units of crude protein to be normally unavailable protein.

This should not be mistaken to mean 3 per cent of the crude protein is unavailable. For example, alfalfa and grass hay usually contain 15 per cent and 10 per cent crude protein; both have about 3 percentage units of unavailable protein and 12 percentage units and 7 percentage units available protein, respectively. This fraction of protein apparently is bound to fiber and actually may not be true protein. However, for the sake of simplicity, it is calculated as N x 6.25 and is called unavailable protein.

Usually the unavailable protein content of a forage is of little concern, but in some conditions it can be a problem.

Large amounts of unavailable protein can occur in hays and haylages that become too hot during the storing process; this usually is more common in legumes than grasses.

Generally the large amounts of unavailable protein are caused by *excess moisture* in hays and *too little moisture* and *too much oxygen* in haylages. The resulting forage turns brown to black depending on severity of overheating, and it has an odor that ranges from sweet to caramel-like to tobacco-like. Cows often relish overheated forage because the sugars become condensed and turn into syrup.

Farmers often assume that because overheated forages are eaten readily by cows, nutrient composition is unaffected by overheating; some actually think that quality is improved. That is definitely not the case. Overheated forages, especially legumes, smell sweet and are dark brown to black in appearance and may contain a lot of unavailable protein.

Apparently, when forages become overheated during the curing process, some true protein becomes tied up with carbohydrates and less protein is available for use by the animal. Fortunately, the amount of protein made unavailable by overheating can be measured quite easily.

The ADF procedure removes available protein and leaves unavailable protein behind in the fiber residue. Determining the Kjeldahl protein content of the residue gives an estimate of the unavailable protein. Another procedure is to digest the feed with weak acid and pepsin, an enzyme found in the small intestine of animals. Unavailable protein cannot be digested with acid-pepsin.

Two methods of determining available protein, ADF-unavailable-protein and pepsin-unavailable-protein, agree quite closely and either one can be used to measure heat-damaged protein. Extent of heat damage is indicated by elevation of either measure of unavailable protein above the average baseline value of 3 percentage units of unavailable protein.

For example, if a clover hay had 12 percentage units pepsin-unavailable-protein or ADF-unavailable-protein, the amount of heat-damaged protein would be 12 percentage units total unavailable minus 3 percentage units normal unavailable = 9 percentage units heat damage. This means that 9 percentage units of protein are heat damaged and unavailable above the normal amount of unavailable protein.

If the clover hay originally contained 19 per cent crude protein and 9 of these 19 percentage units are heat-damaged, then the clover hay really contains only 19 - 9 per cent adjusted crude protein (not heat-damaged).

In essence, instead of feeding a 19 per cent crude protein hay, a farmer would be feeding a 10 per cent crude protein hay. The most immediate effect of heat-damaged forage is reduced milk yield—that is, cows just do not seem to produce as they should. The only practical way to overcome this is to increase the protein content of the concentrate to make up for the amount of heat damage present.

Usually, if heat-damaged forage has been fed and the net crude protein was below requirements, milk yield will increase 2-10 pounds per day within a few days after correction.

A milk yield response may not occur in some cases, even though protein content of the concentrate is increased. This can happen if the farmer is over-feeding protein, that is feeding clover hay (high in protein) and a concentrate high in protein, such as 16 per cent crude protein. Moderate heat-damage may have reduced the protein content of the hay; but because the hay was high in protein, the hay and concentrate still provided sufficient protein to meet production needs, and milk yield was not depressed. Table 3 gives three examples of adjusting crude protein for heat damage.

A crude protein determination can not distinguish if any heat damage exists, and either the pepsin-protein or ADF-protein tests must be used. Haylages, especially those that are dark-colored and/or have a sweet or tobacco smell, should be tested for heat-damaged protein.

Table 3. Examples of Adjusting Crude Protein for Heat-Damaged Forages*

1. Normal clover hay with no heat damage	
Pepsin or ADF unavailable protein	3.0%
Normal unavailable protein	3.0%
Heat-damaged protein	0.0%
Crude protein	19.0%
- Heat-damaged protein	0.0%
= Adjusted crude protein	19.0%
2. Moderately heat-damaged haylage	
Pepsin or ADF unavailable protein	12.0%
Normal unavailable protein	3.0%
Heat-damaged protein	9.0%
Crude Protein	14.0%
- Heat-damaged protein	9.0%
= Adjusted crude protein	5.0%
3. Excessively heat-damaged clover hay	
Pepsin or ADF unavailable protein	15.0%
Normal unavailable protein	3.0%
Heat-damaged protein	12.0%
Crude protein	19.0%
- Heat-damaged protein	12.0%
= Adjusted crude protein	7.0%

*Although the latter two examples are extreme cases of heat-damaged protein, the examples are actual forages from three different farmers.

In analyzing forages, spend money wisely and get chemical determinations that can be used effectively. Also, sample forages properly; UMC Guidesheet #9650 (Sampling Feedstuff for Chemical Analysis) explains how to sample. We recommend the following analyses; additional analyses such as phosphorus, magnesium and sulfur can be used but are not necessary.

	Dry Matter	Energy	Protein	Calcium	Heat Damaged ADFN or Pepsin
Grass Hay & Haylage	x	x	x		
Legume Hay & Haylage	x	x	x	x	
Legume Grass Mixtures Hay & Haylage	x	x	x	x	
Corn Silage	x	x			
Corn Silage with NPN Added	x	x	x		
Sorghum Silage	x	x	x		
Sudan Hay or Haylage	x	x	x	x	
Any high dry matter haylage with brown color or known to have heated during storage					x
Any hay, baled wet, brown in color, or suspected to have heated during storage					x

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