

WHAT ARE THE FIVE MOST IMPORTANT THINGS TO MEASURE IN HAY CROPS?

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ABSTRACT

The most important analytical measurements for evaluating hay crops are those that are used to establish economic value or to meet the nutritional requirements of the animals to which it is fed. The objectives of this review are to identify and discuss the most important analyses that are important for determining the economic and nutritional value of hays for dairy cows. It is proposed that the five most important nutritional measurements for hay crops are: dry matter (**DM**), ash, amylase-treated neutral detergent fiber (**aNDF**), some measure of digestibility or energy value, and crude protein (**CP**). The DM of a hay crop measures the proportion of the as-is weight that is nutrients. Each percentage-unit of DM in a ton of hay represents 20 lb of nutrients that has economic and nutritional value. In general, we purchase and feed hay crops to provide energy, roughage, and to a lesser extent CP. Ash not only measures the portion of hay crops that provides no energy to the animal, it is an excellent indicator of soil contamination, which also dilutes the energy in hays. Fiber is the major organic component of hay crops that affects intake and digestibility. Of the fiber measurements that are available, aNDF is the best method for measuring the insoluble fiber that is most important in ruminant nutrition. The simple summative equation illustrates that aNDF and its digestibility (**NDFD**) are the primary factors determining DM digestibility. Estimates of energy value (total digestible nutrients – **TDN** or net energy – **NE**), which are calculated from chemical composition, are secondary measurements that provide no additional information about hay crop quality, although they are often needed for ration formulation. Calculated TDN or NE values are nothing more than a mathematical transformation of the original chemical measurements, especially when based on one constituent. They provide no additional information and should be left to the user to estimate so that they are calculated and used in the appropriate manner. Unlike calculated values, in vitro measures of DM, OM, or aNDF digestibility provide information beyond chemical composition that is useful in ranking hay crops and assessing energy value. Although the major contribution of hay crops is to the energy in the total ration, they also can provide significant CP. The amino acid balance of ruminally undegraded protein from forages does not have the biological value of many protein supplements, but the CP in hay crops provides value in the production of microbial protein during ruminal fermentation. Mathematical models can and will be used to account for the variability in intake and digestion among high producing cows. These models need inputs for the rates and extents of digestion of fiber and non-fiber carbohydrates in forages. In the future, measurements of digestion kinetics will become more valuable in the assessment of forage quality. Because indigestible NDF provides no energy and limits intake, it will probably have the greatest and most immediate impact on evaluating hay crops.

Key Words: Forage quality, forage evaluation, fiber, digestibility, energy value

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INTRODUCTION

Selecting the most important measurements for evaluating hays is not as simple as it sounds. In practice the most important measurements are the ones that are actually used in setting economic value or in meeting the nutritional requirements of the animals to which it is fed. For example, the selection of measurements would be different if the hay is fed to horses, feedlot steers or dairy cows. Likewise the selection would be altered by the proportion of the diet that is hay crop and by the nutrient that is most limiting or crucial in the total ration. It is impossible to discuss all of these permutations in a short presentation and paper; therefore, I will restrict my discussion to the measurements of forage quality that are most important for hays when fed to dairy cows in significant proportions (>20% of total ration dry matter).

In most cases, the primary role of forages in most dairy rations is to provide energy in a form that maintains optimal fermentation in the rumen and insures that the fibrous portion of the ration is used effectively. Ruminants, such as dairy cows, can digest fiber in forages and by-product feeds that is not digested by humans or other animals. Maximizing the extraction of energy from fiber is a key factor in determining the economic and nutritional value of hays. There are times when the crude protein (CP) is important in determining value, but the value of protein in forages often depends on its conversion to microbial protein, which has greater biological value. The objectives of this report are to identify the five (or more) analytical measurements most important in the evaluation of hays for dairy cows and to describe the rationale behind their selection. The priority of the measurements discussed may vary depending on specific feeding situations, but their overall importance can be justified in almost all dairy rations.

DISCUSSION

Dry Matter (DM). Although it may seem anticlimactic to select DM as one of the most important laboratory analyses of hays, it has a major impact on determining the economic value of feeds. The water in feeds essentially dilutes other nutrients. There appears to be no nutritional or economic value for water in feeds above that of drinking water. It is the DM in hays, cubes or pellets that contains the valuable nutrients needed by animals. However, we buy, sell and feed forages on an “as-is” basis that contains the water that is in them. Measuring DM tells us the proportion of what we buy or feed that is nutrients. Normally, “air-dry” hays contain about 10% water or 90% DM, and 90% DM is often referred to as “air-dry” basis. Depending on the forage and ambient humidity, the actual DM of a specific lot of hay, cubes or pellets can vary ± 2 %-units from the average “air-dry” DM of 90%. Given that each 1 %-unit of DM equals 20 lb per ton of hay, it contains a significant amount of nutrients and is often the daily feed intake of one or two cows. One ton of hay at 92% DM contains 80 lb more DM than a ton of hay containing 88% DM. There is no question that DM measurement has important economic value.

Expressing nutrient content or intake on a DM basis allows more direct and accurate comparisons among feeds and diets. It is possible to compare feeds and diets on a 100%, 90% or any other constant percentage of DM. When rations consisted of hay and concentrates, it was easy and convenient to adjust composition and make comparisons on a 90% DM basis. However, the feeding of silages and wet by-product feeds makes the 90% DM, or “air-dry,” basis much

less useful and it is recommended that feed and diet composition be described on a 100% DM basis. A hay that is 36% fiber on a 90% DM basis is equivalent to a hay that is 40% fiber on a 100% DM basis. The large discrepancy between the two fiber values illustrates the confusion that can occur by using a basis other than a 100% DM. By removing water, we can compare feeds, diets, and intakes directly.

Ash. Ash is important because it contributes no energy to the animal. Like water, it dilutes the energy value of the feed. Although ash contains some required minerals, they comprise only a small proportion of ash in most hays. In addition to the ash that is contained in the plant, forages can contain significant soil contamination from splashing soil on plants during rains and mixing soil into the windrow during harvesting. Alfalfa typically contains 9-11% ash and grasses contain 7 to 9% ash (NRC, 2001). In mixed grass-legume forages, the ash, and specifically calcium, concentration can be used to estimate the proportion of grass and legume in the mixture. When there is no soil contamination of hay, the regression equations used for predicting energy value takes into account the naturally occurring ash in the feed. For example, Mertens (1985) developed the following equations to predict net energy of lactation (NEL_{3X}) based on neutral detergent fiber (**NDF**):

$$\text{Legumes: } NEL_{3X} = 2.323 - 0.0216 * (\text{NDF}) \text{ or}$$

$$\text{Grasses: } NEL_{3X} = 2.860 - 0.0262 * (\text{NDF}).$$

The intercept (energy value of a hay containing no NDF) of the legume equation (2.323) is lower than that of the grass equation (2.860). The lower intercept of legumes is related to their higher ash concentration and quantitatively indicates the average effect of ash on a hay's net energy value.

Although ash is measured, the important nutritional component is organic matter (**OM** = 100 - ash). With the exception of required minerals, it is the OM of hays that contains nutrients, and only the digestible OM (**dOM**) of feeds contributes to absorbed energy. European feed evaluation systems have a long history of measuring dOM (% of DM), which is sometimes called "d value," by a variety of in vitro methods (AFRC, 1993). The relationship of between dOM and energy value is very high ($R^2 > 0.80$; Barber et al., 1984), and the main factor that alters the relationship among feeds is their fat content (because fat contains 2.25 times the energy of carbohydrates). In the United States, we could remove significant error in estimating the energy value of feeds by measuring ash. The reporting of OM or dOM as a percentage of DM should not be confused with reporting data on an OM basis. There is no value in reporting analyses on an OM basis in practical nutrition because we buy and feed forages on a DM or as-is basis.

Amylase-treated Neutral Detergent Fiber (aNDF). Fiber is a major measurement for forage quality because it measures the component in feeds that is the most difficult to digest. In fact, the definition of dietary fiber for ruminants is "the fraction in feeds that is slowly or incompletely digested and occupies space the gastrointestinal tract." There are numerous fiber measurements and the selection of method for measuring fiber should be based on how closely it meets the definition of fiber. Soluble fiber cannot be digested by mammalian enzymes, but this component is rapidly fermented by ruminal bacteria and does not qualify as fiber for ruminants. The best routine method for measuring fiber in forages is aNDF because it measures the major structural components in plant cell walls (cellulose, hemicellulose and lignin) with a minimum of protein contamination. There are at least three published methods for measuring "NDF." The original

neutral detergent fiber (**NDF**; Van Soest and Wine, 1967) method used sulfite, but not amylase, and can result in high NDF values for starch-containing feeds such as corn or cereal silages and grains. Neutral detergent residue (**NDR**; Van Soest et al., 1991) modified the original NDF method by adding amylase to remove starch, but removed sodium sulfite. The NDR method generates more accurate fiber values for starch-containing feeds, but without sulfite it results in high values for any feed containing protein-carbohydrate complexes formed when heating or cooking feeds. The aNDF method (Mertens, 2002) uses both amylase and sulfite to most accurately measure fiber in all feeds.

Soil contamination not only reduces energy value, but it also contaminates fiber results because soil is not dissolved by acid or neutral detergent. This can result in aNDF values that are 2 to 5 %-units higher than the true fiber concentration. If energy values are calculated from fiber concentration, the soil contamination of fiber increases apparent fiber concentration and partially accounts for the lower energy value of feeds with soil contamination. However, soil contamination of aNDF can have significant negative consequences when feeding low fiber rations. When the ration is borderline in fiber, a 10% error in aNDF due to soil contamination can have serious consequences. In addition, when nutritionists are calculating the non fibrous carbohydrates in hay crops by difference (**NFC** = 100 – ash – CP – Fat – aNDF) there is an error when fiber contains significant ash. Nutritional models that calculate some components by difference to insure that total constituents sum to 100% DM also have errors when aNDF contains ash. These errors are encouraging some nutritionists to request that aNDF be reported as aNDF organic matter (**aNDFom**), which is ash-free fiber.

For the nutritionist, there are several reasons why aNDF is an important measurement (Van Soest, 1967). Fiber is related to intake, digestibility and meeting the minimum requirement for roughage in dairy cow diets. In the most general sense, intake is regulated by two main mechanisms. When cows are fed high energy diets they limit their intake to meet their energy demand. This mechanism is typically called the physiological intake control mechanism. The stage of lactation and production level of the cow determines the physiological need for energy, which in turn sets the physiology control point. However, when cows are fed high fiber diets, the bulk or fill of the diet limits intake. This mechanism is generally called the physical intake control mechanism. Because aNDF is related to both fill and digestibility (energy density), it can be related to both mechanisms of intake regulation (Mertens, 1992; 1994). Thus, aNDF is the measurement that provides the most consistent and useful information about the intake potential of hays. In early lactation (<120 days), high producing dairy cows are eating to the limit imposed by physical constraints, which makes the intake potential of a hay a crucial measurement. For high-forage rations, Mertens (1992) observed that cows maximize fat-corrected milk production when they consume 1.20% of body weight as aNDF per day. This is not the maximum amount of aNDF that can be consumed, rather it is the maximum amount of aNDF they can process (eat, chew and digest) and still obtain maximum milk production. The fill volume of ground by-products is much less than that of a hay of similar aNDF concentration; therefore, the aNDF of by-products must be adjusted downward to represent their filling effect when using the 1.20% of body weight per day as the fiber intake constraint.

Physically effective aNDF (peNDF). Although total fiber is important in determining intake and digestibility, it appears that only a portion of the fiber is important in meeting the minimum fiber

requirement of dairy cows (Mertens, 1997). Because all components in a ration have to sum to 100, it is clear that when we lower the fiber concentration in the ration it must also have higher NFC. Thus, there is debate about whether it is low fiber or high NFC that creates problems for dairy cows. However, effective fiber in the ration does more than moderate ruminal pH. Fiber is needed to cause the rumen to stratify into layers and function like a complex, multi-purpose fermentation chamber. The rumen functions best when it selectively retains large particles of fiber. The large particle mat in the rumen not only allows more time for the fermentation of slowly digesting fiber, it also traps or sequesters smaller fiber particles in the mat, which slows the rate of passage and increases the digestion of small, actively fermenting particles that are buoyant due to gases produced during fermentation.

Low fiber diets can be fed to ruminants, like the high-grain diets fed to feedlot steers, but these diets cannot be fed for longer than 90 to 120 days without being detrimental to ruminant health (low fiber diets work when the diet is terminal!). Diets that are borderline in fiber often result in lower ruminal pH, fluctuations in daily feed intake, sub-optimal fermentation of fiber, and in the case of lactating dairy cows, a depression in milk fat production. The characteristics of fiber that seem to be related to its effectiveness in meeting the minimum fiber requirement are its retention in the rumen and its stimulation of chewing activity. These responses (retention and chewing) are related not only to chemically measured fiber, but also to fiber's physical form (Mertens, 1977). In the laboratory, peNDF is measured as the portion of the forage or ration that is NDF in particles retained on a 1.18-mm sieve. The threshold of 1.18 mm is dependent on the method of particle size separation. It is only valid when using vigorous vertical shaking of a dry sample, because this separation method bounces particles on end and essentially measures their width instead of their length. The 1.18 mm threshold was chosen because particles of this size are found in the feces, which indicates they can escape the rumen without needing to be chewed. In long hay, peNDF measurement is not a concern for the hay seller because the hay buyer and feeder are responsible for processing the hay and formulating the diet in low-fiber situations. However, it can be a factor in evaluating cubed or pelleted hay crops.

Fiber and Digestibility. Traditionally, fiber has been used to estimate the digestibility or energy value of feeds because it was recognized that fiber is the fraction in feeds is the most difficult to digest. The simple summative equation for predicting total digestible dry matter (**dDM**) demonstrates the importance of fiber on overall digestibility of hays. This summative equation indicates that, for feeds and especially for hays, the dDM is the sum of digestible aNDF (**dNDF**) and digestible neutral detergent solubles (**dNDS**) minus the endogenous losses (**EL**):

$$dDM = dNDF + dNDS - EL.$$

The digestible fraction of any component in feeds is the product of its digestion coefficient (**NDFD** or **NDS**) and concentration, thus:

$$dDM = NDFD * aNDF + NDS * NDS - EL.$$

The important consequence of separating feed into aNDF and neutral detergent solubles (**NDS** = 100 - aNDF), is that NDS is almost completely digested in hays regardless of source:

$$dNDS = .98 * NDS - 12.9.$$

Thus, the variation in the dDM among hays is primarily a function of NDFD and aNDF:

$$dDM = NDFD * aNDF + .98 * NDS - 12.9 \text{ (Table 1)}.$$

Note that in Table 1, alfalfa obtains its high dDM due to low aNDF content, but that grasses have higher NDFD than that of legumes.

Table 1. Prediction of digestible dry matter (dDM) in feeds based on the simple summative equation using amylase-treated neutral detergent fiber (aNDF) analyses.

Component	Corn grain	Corn silage	Cereal silage	Grass hay	Alfalfa hay
aNDF, % of DM	9.0	45.0	50.0	55.0	40.0
Fractional NDFD	0.50	0.58	0.64	0.58	0.46
Digestible aNDF, % of DM	4.5	26.2	32.1	31.6	18.6
NDS, % of DM	91.0	55.0	50.0	45.0	60.0
Digestible NDS ^a , % of DM	89.2	53.9 ^b	49.0	44.1	58.8
True dDM	93.7	80.1	81.1	75.7	77.4
Endogenous DM loss	-12.9	-12.9	-12.9	-12.9	-12.9
Apparent dDM _{1X}	80.8	67.2	68.2	62.8	64.5

^aNeutral detergent solubles (NDS = 100 – aNDF).

^bAssumes that corn kernels are thoroughly processed or chewed (at maintenance intakes) so that starch digestion is 98%.

When ash and aNDFom are routinely measured it will be possible to improve the use of the summative equation so that it calculates dOM instead of dDM per unit of DM:

$$dOM = dNDFom + dNDSom - ELOm \text{ or}$$

$dOM = NDFomD \cdot aNDFom + NDSomD \cdot NDSom - ELOm$; where **NDSom** = (OM – aNDFom); **NDFomD** and **NDSomD** are the digestion coefficients of aNDFom and NDSom, respectively; and **ELOm** is the endogenous loss of OM. It is expected that the NDSomD and ELOm coefficients would be constants like NDS and EL in the dDM summative equation, but the magnitudes might be different.

In vitro aNDF digestibility (IVNDFD). The simple summative equation demonstrates why IVNDFD is an important forage measurement. Because the digestibility of aNDF is not a constant it must be measured or predicted (Van Soest, 1967). Across maturity levels within a cutting, lignin is the fraction of aNDF that is negatively related to NDFD. However, the relationship is not as accurate within maturity or between cuttings. In addition, lignin is not the easiest hay constituent to measure. The variability in the relationship between lignin and fiber digestibility has stimulated the direct measurement of NDFD using *in vitro* “artificial rumen” procedures (Goering and Van Soest, 1970). The direct approach to the measurement of IVNDFD allows all of the characteristics of the hay and its fiber that interacts with the bacterial fermentation of fiber to be assessed.

The challenge in the measurement of IVNDFD is obtaining adequate repeatability within laboratory and reproducibility among laboratories. The *in vitro* method has all of the variability inherent in chemical methods, plus the variation in the rumen inoculum that is used to measure digestion. Currently, IVNDFD measurements vary in the size of grind (1, 2 4, or 6-mm screens), the time of fermentation (24, 30 or 48 h), the fermentation vessel (flasks, tubes or filter bags),

buffer used, inoculum donor diets, and time of collecting inoculum. The current variation in IVNDFD suggests that results are best interpreted within-laboratory, differences in IVNDFD are relative, and use of IVNDFD results in nutrition models and ration formulation should be monitored closely to insure that their use is appropriate. Nonetheless, IVNDFD is an important measurement in assessing fiber quality that will increase in usage and reliability in the future.

Energy values – Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), Total Digestible Nutrients (TDN) or Net Energy of lactation (NEL). If the IVNDFD and summative estimates of dDM or dOM are not available, there is still a need for some estimate of energy value in most systems of feed evaluation or ration formulation. At present, estimates of dMD, dOM, TDN and NEL are calculated using regression equations containing some measure of fiber as the predictor. Numerous equations are available that were generated by various sources using data sets that differed in the forages evaluated and the method used to measure in vivo digestibility (different species of animals and levels of feed intake). Many of these equations were created more than 30 years ago using sheep fed at a maintenance level of intake. The source of many equations is unknown making it difficult to interpret their usefulness.

Given that estimates of energy value for hays are derived from a fiber measurement, they are nothing more than a re-parameterization of the measured fiber value. Thus, it makes little sense to use calculated values for economic evaluation or forage selection, which can be done and comprehended more easily using the fiber measurement directly. Personally, I would prefer that no calculated values be reported on hay analysis reporting forms. Without knowing the source of the equation used to calculate them, the numbers are difficult to interpret. It would be better if reporting forms contained only measured values and it would be the responsibility of the user to calculate any energy value they need using equations they know are appropriate for their specific use.

Calculated energy values are useful when formulating rations and the animal's requirement is provided in specific units (TDN or NEL). They are also useful when they combine more than one measurement to calculate a multi-factor index. For example, the summative equation combines the measurements of aNDF and NDFD with the digestibility of non-fiber components to estimate dDM or dOM. Because two independent measures are used (aNDF and NDFD), the summative equation result contains more information than the calculated value from fiber concentration alone. Similarly, the weighting of fat for its higher energy density than carbohydrates makes the summative calculation of TDN more accurate than estimating TDN from fiber measurements alone when feeds contain significantly different fat concentrations.

Crude Protein (CP). Although the amino acid quality of the CP in most hays is not as high as that of most protein supplements, hay crops, especially alfalfa hay, can provide a significant proportion of CP in the ration. Less of the CP in hay is soluble compared to silages, which also improves its value for dairy cows. Unless it is heat-damaged or contains significant tannins, the true digestibility of CP in hays is between 0.90 and 0.98, like that of NDS. In addition, the majority of CP in hays is fermented in the rumen and converted to microbial protein when mixed in dairy rations that have adequate fermentable carbohydrates. Thus, there is little benefit for additional analytical fractionation of CP in hays. The one exception, is the measurement of heat-damaged protein using acid detergent insoluble CP (**ADICP**). When hays are harvested at

moisture levels >15%, they can heat and form Maillard products, which are complexes of protein and carbohydrates that can be indigestible. Whenever hays are brown and have a sweet caramel or tobacco aroma (a characteristic of Maillard products) they should be analyzed for ADICP.

Measurements for the Future. Digestion is a time-dependent process, especially for the fibrous constituents in hays. However, as nutrient requirements increase, cows increase intake, which slows rates of passage and decreases the retention time in the gastrointestinal tract for digestion. Increasing intake and decreasing retention time results in a depression in digestibility. Future feeding systems will have to estimate intake and its effect on digestion more accurately by taking time into account and using measurements of rate and extent of digestion (digestion kinetics) of feed components. Using mathematical models, it is possible to estimate nutrient intake and digestion based on the varying rates of passage and digestion and potential extents of digestion that occur when different feeds are combined in rations and when cows have differing levels of production.

Because fiber is the component that is digested most slowly it is the fraction that is affected to the greatest extent by increasing intake and decreasing retention times in the gut. Digestion curves have three distinct phases that define digestion kinetics (Figure 1). Initially there is a lag phase during which digestion gradually increases, this is followed by a period of rapid rate of digestion, and finally digestion ends with a plateau or asymptote that is less than 100%. These three phases are described by kinetic parameters for lag, rate, and extent (Mertens, 2005). Of these three, it appears that the potential extent of digestion, or its converse, the extent of indigestibility, is the most important (Mertens and Ely, 1979; Huhtanen et al., 2006).

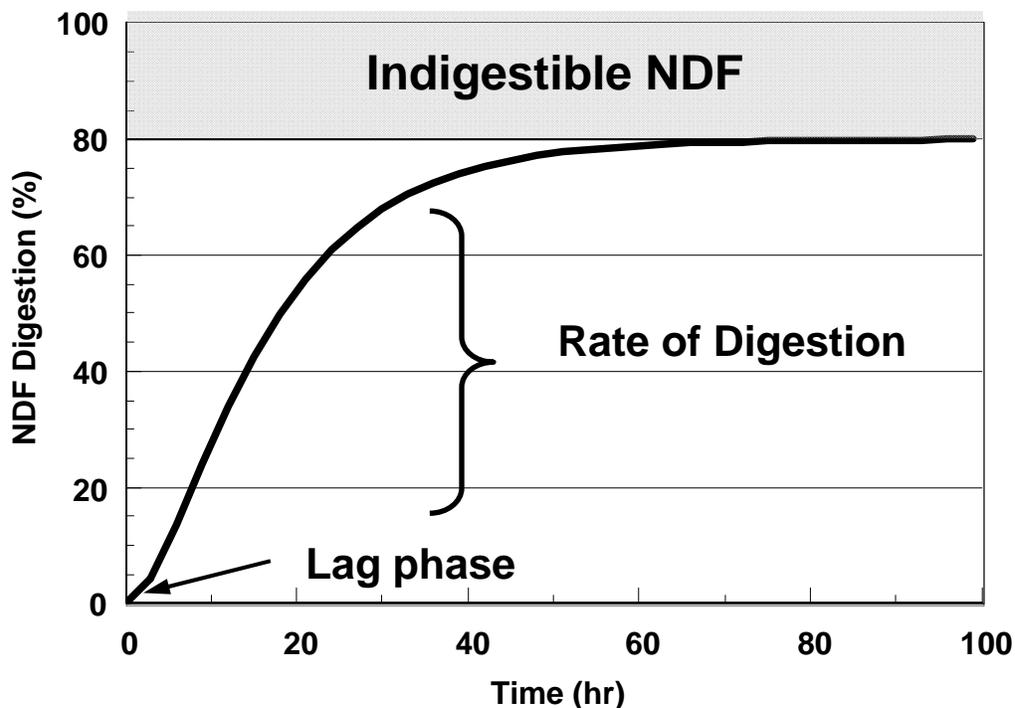


Figure 1. Digestion curve of NDF illustrating the lag, rate and extent (indigestible) phases of digestion kinetics.

The indigestible matter in forages is fiber, so for all practical purposes, the indigestible DM in hays is actually indigestible NDF (iNDF). The major constituent of iNDF is lignin, but lignin also prevents the digestion of cellulose and hemicellulose equivalent to 1 to 3 times its mass. There is a high correlation between lignin and iNDF (Smith et al., 1972), and lignin has been used to estimate iNDF (Traxler et al., 1998; Van Soest et al., 2005). Due to this relationship, lignin would be a logical measurement for hay quality that might replace estimates of DM or OM digestibility based solely on fiber. Alternatively, lignin could be used to estimate NDFD (Georing and Van Soest, 1970), which in combination with aNDF, would predict digestibility more accurately. However, lignin is not easy to measure and its relationship with fiber or total DM digestibility is variable, especially within maturity. Thus, the direct measurement of iNDF using long-term fermentations (72 to >240 h) would more accurately measure all of the factors that affect the ultimate digestion of fiber (Raffrenato and Van Amburgh, 2010). Not only does iNDF affect digestibility of hay crops and provide no energy to the animal, it also occupies space in the gut and limits intake.

CONCLUSIONS

The major contribution of hay crops to the diets of lactating cows is in providing energy. Therefore, measurements that impact the energy value of forages are the most important in determining their nutritive value. Because hay crops are bought and fed on an as-is basis, DM is an important measurement for defining both economic (actual nutrients purchased) and nutritive (water dilutes other nutrients) value. Ash contributes nothing to the energy value of hay crops, and is also an indicator of soil contamination (which adds no nutrients). Insoluble fiber is important because it is the slowest fraction of forages to digest; and therefore limits the energy value of hay crops. Of the routine methods available, aNDF provides the best measurement of insoluble fiber and provides information not only about the digestibility, but also about the intake potential of hay crops. When fed high-fiber rations, intake is often limited by the aNDF content of the forage. Conversely, intake and ruminal fermentation can be adversely affected by low fiber rations, especially when the forage provides too little peNDF. Because the digestibility of aNDF is variable, its value in estimating DM or OM digestibility can be improved by the measurement of NDFD. Alternatively, direct in vitro measurements of DM or OM digestibility can be used to estimate energy value. However, TDN or NE values calculated from a fiber measurement provide no additional information that is not indicated by the fiber measurement. The future of hay crop nutritive evaluation will be based on measurements of digestion kinetics that will allow nutritionists to account for changes in intake and digestion of cows at different levels of production. Of these, iNDF is most accurately measured and has the greatest impact on intake and overall digestion.

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