NONSTRUCTURAL CARBOHYDRATES: CHALLENGES AND PROGRESS IN FORAGE TESTING

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Introduction

Forage testing has evolved by adapting new technology but acceptance of different tests for forage quality is slow and some tests are impractical. Reliance on technology has replaced intuition and experienced knowledge in some cases. For example, alfalfa grown at high elevations was preferred as dairy hay in the 1960's and 1970's. The use of forage testing in the 1980's and 1990's appears to favor alfalfa hay grown at lower elevations. Moreover, the forage tests of acid detergent fiber (ADF) and neutral detergent fiber (NDF) do not consistently predict animal intake or performance across cuttings. For example, hot season cuttings usually have finer stems, are greener in color, and conventional tests show similar values of ADF and NDF to first (cool season) cuttings, yet animal intake is less for the hot-season cuttings. Using current forage testing to compare high versus low elevation grown hay, or to compare hays from different cuttings, is not dependable. There are some promising developments which should improve our ability to predict animal performance. The testing for nonfibrous carbohydrates (NFC) or total nonstructural carbohydrates (TNC) are additional tools you may want to use. Nonfibrous carbohydrates are defined by the National Research Council (2001) as NFC = 100 - (%NDF + % CP + %Fat + %Ash), where CP is crude protein. Total nonstructural carbohydrates are determined by a fractionation of the sample and are calculated as the sum of monosaccarhides, disaccharides, short chain polysaccharides, and starch. This paper reviews the underlying principles of forage quality and the development of testing; environmental, genetic, and harvest management effects on forage quality; and reviews diurnal cycling of total nonstructural carbohydrates and related animal preference studies.

Forage Testing Development

The "proximate analysis" procedure used in the 1960's characterized forage quality by crude fiber, ether extract, nitrogen-free extract, and crude protein. These values were adjusted by digestion coefficients and summed to total digestible nutrients (TDN). The "energy system" was developed in the 1970's and was more accurate at predicting performance, but is not practical for application because of the time and cost of analysis (oxygen bomb calorimetry). An improved analysis of fiber was developed by Van Soest which determined ADF and NDF (Goering and Van Soest, 1970). Current procedures for NDF, ADF, and lignin are described by Van Soest et al. (1991). However, ADF was developed by Van Soest as an intermediary residue to determine lignin, cellulose, Maillard products, silica, acid insoluble ash, and acid detergent insoluble N. Van Soest et al., (1991) concluded that there is no valid theoretical basis to link ADF to digestibility, even though there may be an association statistically. In the cell wall matrix interaction and degradation chapter Van Soest (1993) states "However, the use of either NDF or ADF to predict value of forages are misuses, because of the failure to consider rational models of the availability of forage fractions, in particular, the effects of climate upon forage composition and lignification."

Although intake can be predicted by NDF and digestibility by ADF, these values do not adequately represent the energy value of different cutting times. Although NDF is a predictor of dry matter intake (DMI), the digestibility of NDF varies greatly across plant species and maturities. The form of forage fed is also important for animal nutrition because finely chopped forage has a different effect on animal production than long-stemmed forage, hence the increased determination of particle size. The determination of particle size provides an estimate of effective fiber (eNDF), which relates to buffering capacity of the forage in the rumen. Regardless of the benefits of selecting forages based on NDF over ADF, much of the alfalfa hay is marketed primarily on the basis of ADF.

Summative equations were developed as an alternative for predicting forage digestibility. Forages are analyzed for energy yielding constituents and the sum of the digestible parts of each component. The "Ohio State" or "Weiss" equation (Weiss, 1994) is a refined equation based on total fiber (NDF), lignin (ADL), total protein (CP), cell wall bound protein (ADIN), fat, and ash. Forages can be analyzed for each nutrient by either wet chemistry or NIRS. Each energy yielding component is multiplied by a digestibility coefficient and the products are summed. The summative approach is more independent of population so will work across more forages than empirical equations. The standard error of prediction was 25 g/kg of TDN across several different types of forages and concentrates (Weiss et al. 1992). The disadvantages of the summative approach are the cost and time to analyze for each component, although it is less expensive than digestion analyses.

Spectral reflectance properties of plant tissues can be measured by Near Infrared Reflectance Spectroscopy (NIRS). The instrument detects bonds of C-H, O-H, and N-H. Thus, the limit of NIRS technology is the chemical calibration procedures it depends on. Norris et al. (1976) first reported that NIRS predicted forage quality *in vivo*. They reported coefficient of determination values of 0.78, 0.64, and 0.72, respectively, for dry matter digestibility, dry matter intake, and digestible energy intake of grass and legume forages by sheep. The development of NIRS (Shenk et al., 1981) has allowed fast, low cost, and reasonably accurate estimation of ADF and NDF. Eckman et al. (1983) predicted animal intake and digestible energy by NIRS. He reported prediction errors of 11 g $W^{-0.75}$ d^{-1} for intake and 0.2 kcal g^{-1} for digestible energy. Recently, Fisher et al. (2002) and others have used wet chemistry to calibrate the NIRS for total nonstructural carbohydrates in forages.

The estimation of digestibility by kinetic *in vitro* analysis is a direct approach and has the advantages of deriving a digestion coefficient. The disadvantages are that it is time, equipment, and labor intensive, hence expensive. Few commercial labs perform this method. However, more research in calibrating NIRS may make the approach more cost-effective.

Changes are being proposed to provide better estimates of animal performance across forages. Oba and Allen (1998) proposed determination of digestible NDF because of the wide variation in forage digestibility in the rumen. They concluded that digestibility of NDF *in vitro* or *in situ* might be a better indicator of DMI than NDF digestibility *in vivo* because a shorter rumen retention time of high digestible forage would allow for greater DMI. Undersander and colleagues (University of Wisconsin, personal communication) adapted changes in National Research Council (2001) terminology, the benefits of determining digestibility of the NDF fraction, and applied dNDF use in the Milk 2000 spreadsheet. But he also notes there is considerable lab differences and week-to-week variation for *in vitro* digestion of NDF and lignin, even though NRC suggests either can be used. There is discussion on whether 24, 30, or 48-h *in situ* or *in vitro* should be the standard. Commercial labs would like a 24 h test but the error term is about twice that of a 48 h test (Rowher, Bar Diamond Inc., Parma, Idaho, personal communication). Milk 2000 produces a combined value for yield and quality--negatively related variables.

Changes in the calculation of TDN for maintenance of dairy cows are recommended by NRC 2001:

$$TDN_{maint}\ (\%) = tdNFC + tdCP + (tdFA*2.25) + tdNDF - 7$$

where tdNDF = (digestible g NDF)/(g DM) [before and after digestion]
tdNFC = NFC*0.98
tdCP = CP*0.93
tdFA = fatty acids = (ether extract -1)

Environmental Effects

Environmental factors such as climate, soil type, and soil moisture availability affect forage growth and quality. Anti-quality factors of alfalfa (NDF and ADF) have been successfully predicted (Fick and Janson, 1990) and are useful in harvest planning to restrict the fiber components. Hintz and Albrecht (1991) developed predictive equations for alfalfa quality (PEAQ) based on Wisconsin data. However, models developed based on plant morphology or weather data in one environment produce biased results when applied in another environment (Sanderson, 1992). He concluded that calibration for specific environmental regions and frequent recalibrations are necessary for the alfalfa anti-quality models.

High growth temperatures decrease stem diameter and increase rate of maturation and lignification (Fick et al., 1988; Marten et al., 1988). Van Soest (1982) noted that forage quality is often higher in fall, when temperatures are low, than in summer. Alfalfa was lower in digestibility and had a faster rate of decline in summer harvests compared to spring or fall harvests (Collins, 1983; Onstad and Fick (1983). The decrease in stem digestibility (Vough and Marten, 1971) may explain the reduced quality.

High temperature stress of forages is difficult to separate from water stress because they are often concurrent. The result of temperature stress--high or low-- is usually cell wall thickening because the plant is trying to "harden" itself to survive. High temperatures usually produce water stress which results in much reduced transpiration and photosynthesis as well. Water stress generally produces higher quality forage because of lowered cell wall and lignin concentrations. Alfalfa will abscise leaves under severe water stress. If water stress occurs on alfalfa at the vegetative to bud stage, it will often recover yield when watered and maintain a good leaf to stem ratio.

Genetic Effects

A common hypothesis is that an alfalfa canopy, if stand is adequate, will develop to a point when maximum radiation is intercepted. Beyond that point, the lower leaves will senesce. Another theory is that selection for higher yields without consideration of forage quality, merely increases the internode length. Demment et al. (1986) concluded that forage quality can be increased simultaneously with forage yield. Their data suggests that larger plants did not require a higher concentration of fiber for structural support.

Harvest Management Effects

To produce premium quality hay, alfalfa should be cut at an early maturity (pre-bud stage). Harvest management such as the time of day the forage is cut and the rate of hay dry-down can also affect forage quality. Alfalfa accumulates total nonstructural carbohydrates (TNC) during daylight because photosynthesis produces TNC more rapidly than they are exported and utilized for new growth and maintenance. Total nonstructural carbohydrates are composed of starch, fructans, sucrose, glucose, and fructose. Continued plant respiration during darkness depletes TNC concentration. After hay is cut, plant and microbial respiration will continue to consume TNC until the hay reaches less than about 16% moisture. Therefore it is important to dry the hay as quickly as possible to retain as much TNC as possible, as well as avoiding rain showers and allowing the next crop to grow.

Diurnal Cycling of Total Nonstructural Carbohydrates (TNC)

A study reported by Fisher et al. (2002) documents the diurnal variation of TNC. Germain 'WL 322HQ' alfalfa was sampled at 3-hour intervals during the 24-hour period prior to cutting. A 10-acre grower's field near Kimberly, ID was sampled along a transect midway in the field and perpendicular to irrigation furrows. Sampling by compositing 10 grab-samples per plot immediately preceded first and fourth cuttings in 1997. Samples were freeze-dried and ground in a cyclone abrasive mill to pass a 1mm screen. The TNC concentrations were predicted by Near Infrared Reflectance Spectroscopy (NIRS). The calibration of TNC was determined by an adaptation (Fisher & Burns, 1987) of the wet chemistry method described by Smith (1969).

The TNC curves were sinusoidal over a 24-h period (Figure 1), but linear between 0900 (Figure 2) and about 1800 Mountain Daylight Time. On May 26 the TNC increased linearly from 5.8 % of plant dry matter (DM) at 0900 (harvest hour = 0) to 2100 MDT at the rate of 0.29 % TNC per hour. On Sept. 22 the TNC increased linearly from 5.4 % at 0900 (harvest hour = 0) to 1930 MDT at the rate of 0.5 % per hour, an increase of 193 %.

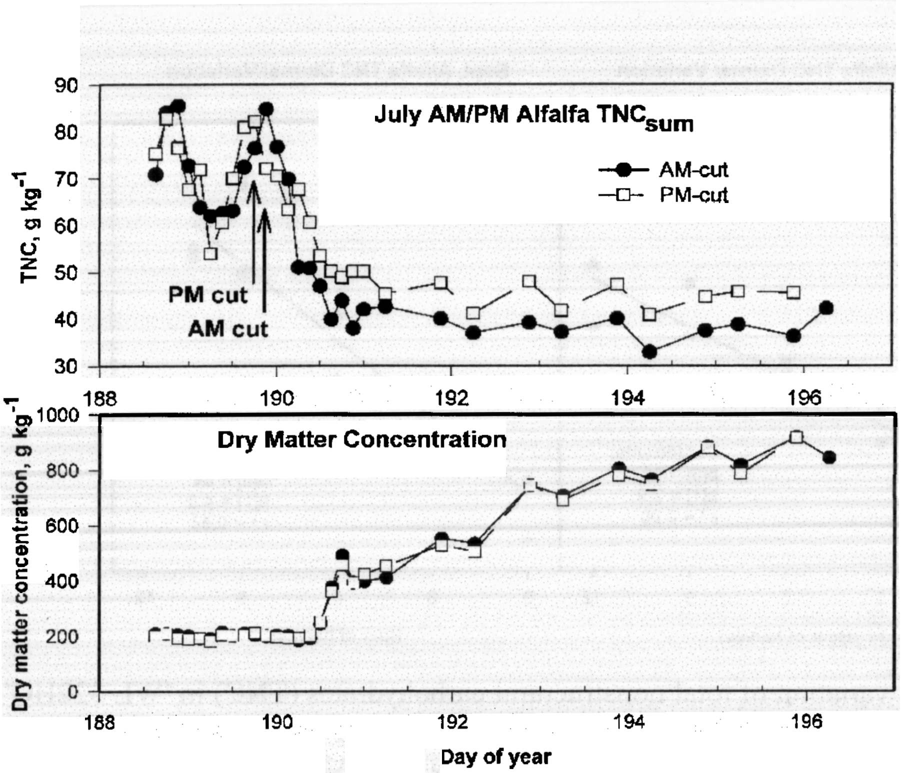

Figure 1. Daily variation in total nonstructural carbohydrate concentration and dry matter concentration and effects of PM- versus AM- cutting. The study was conducted near Kimberly, ID during July 1997.

The dry matter concentration, the reciprocal of moisture percentage, was not significantly affected by whether the alfalfa was cut in the PM or AM (Figure 1). Hay should not be cut with a heavy dew at any time of day. Producers east of the 100th meridian with only a 3-day drying period need to consider tedding or the advantage of cutting earlier in the day to maximize drying rate. In those conditions, drying forage to limit plant or microbial respiration may be more important for conserving TNC than PM-cutting.

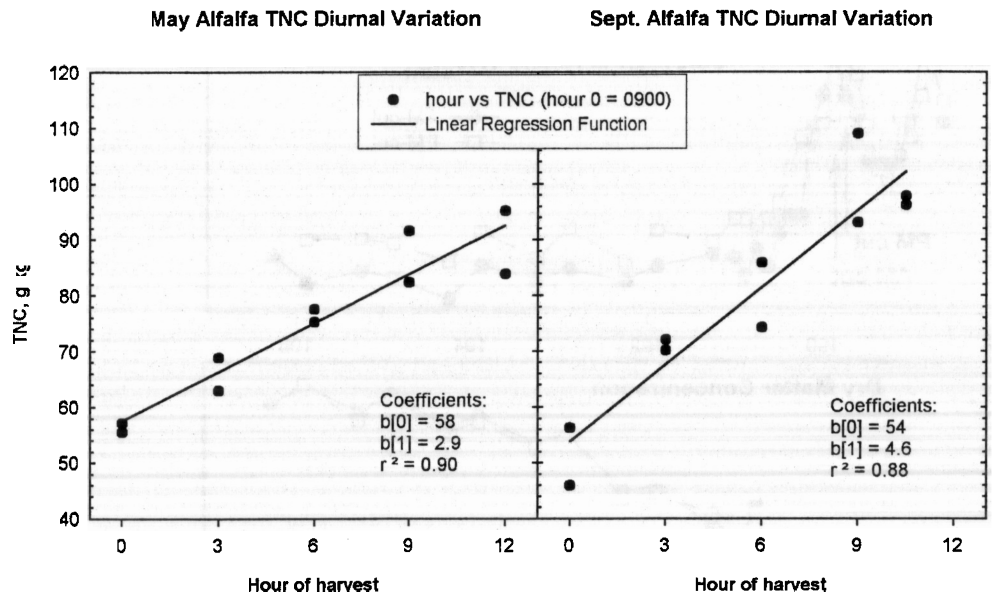

Figure 2. Daily variation of total nonstructural carbohydrates (TNC) in 'WL 322HQ' alfalfa at Kimberly, ID in May and September of 1997. The curves are sinusoidal over a 24-h period, but linear between 0900 (harvest hour = 0) and about 1800 MDT.

From these results, we conclude that TNC concentrations in alfalfa can increase linearly during the day. To maximize the TNC concentration in alfalfa, center your cutting time on 6 pm. If you need to cut 12 hours per day, begin cutting at noon and quit at midnight to capture the most TNC in the hay. If you have to cut in the morning, cut a field that is already too mature for dairy quality hay and keep the lots separate. Many dairymen are aware of the better quality of PM-cut hay and hay producers should market this advantage. The increased forage quality can be determined experimentally because of replications, but perhaps not commercially, as a 1% reduction in ADF. A direct measure of TNC by NIRS or the wet chemistry procedure of Smith (1969) will be more accurate than a computed value of non-fibrous carbohydrates calculated as 100 - (% NDF + %CP + %fat + % ash).

Review of AM-PM Cut Hay Preference Study

Daily cycling of TNC in alfalfa has been reported (Lechtenburg et al., 1971; Putnam et al. 1998), however, the application of this knowledge to producing higher forage quality and improved palatability is largely ignored. Beef cattle, sheep, and goats all preferred tall fescue and alfalfa hays harvested in the afternoon over hays harvested in the morning. Preferences and intakes were associated with the level of soluble carbohydrates in the forage. Beef steers consumed more PM-cut than AM-cut alfalfa (Table 1) in the experiment by Fisher et al. (2002). Dry matter intake was negatively correlated with lignin and nitrate-N ($R^2 > 0.99$). Cellulose had a relatively small positive coefficient and starch had a relatively large positive coefficient. Digestibility analysis for this study has been submitted for publication in J. Animal Science (Burns et al. xxxx) Thus we come back to Van Soest's interpretation and the "post-ingestive feedback" hypothesis suggested by Provenza (1995). Lignin is a difficult wet lab procedure in terms of repeatability and NIRS is not very accurate.

Although dry matter intake is negatively related to NDF and positively related to TNC, note the large difference in DMI for the PM-8 July and AM-9 July cut hays versus hays cut in PM-14 August and AM-15 August. Intake is about twice that for the July 8-9 cuts as for the August 14-15 cuts, but ADF and NDF are about the same. Our hypothesis is that higher air temperatures are associated with faster growth rates of alfalfa and higher lignification than alfalfa grown in cooler temperatures. Higher elevation hay is grown under high solar radiation--good for photosynthesis--but cooler temperatures--hence less plant respiration which conserves TNC. Idaho extension is currently building a database of alfalfa yield and quality from alfalfa grown at a series of elevations up the Snake River Plain. This information will be correlated to weather data collected from automated weather stations nearby.

Table 1. Intake by beef steers and composition of alfalfa hays as affected by cutting date and time of day (Source: Fisher et al. 2002). Steers were fed all combinations in pairs as a portion of their diet.

Hay	ADF	NDF	TNC	Intake
	--- --------- % --------- ------ -			g/meal
PM-8 July	**31.1**	**40.7**	**4.29**	**1022**
AM-9 July	32.8	42.7	3.49	842
PM-14 Aug	**32.0**	**41.9**	**5.16**	**619**
AM-15 Aug	32.5	42.0	3.97	324
PM-22 Sept	**27.9**	**36.6**	**6.55**	**1320**
AM-23 Sept	28.5	37.2	5.46	1107
PM mean	**30.3**	**39.7**	**5.33**	**987**
AM mean	31.2	40.6	4.31	758

Conclusions

- There is daily cycling in forage quality and this is important to consider when testing forage or testing animal preference or intake of forage.
- The PM-cut hay quality is greater than AM-cut.
- We estimate 136 (first cutting) and 81 lbs TNC/ac (fourth cutting) increase by PM- versus AM-cutting.
- Ruminants prefer and eat more PM- than AM-cut hays.
- A decrease of 1% ADF is worth @ $10 to 15/ton at today's prices for premium alfalfa hay.

- Although NDF is related to intake and ADF is related to digestibility, neither is a good indicator of animal intake or production between hays of different cutting season. More accurate forage tests are needed.
- Determination of NFC, TNC, or dNDF in addition to common lab tests for ADF, NDF, RFV, and CP should improve prediction of DMI and animal production.
- Use of newer summative equations should be about 90% accurate versus 70% for old equations.

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