HOW TO CHOOSE A HIGH QUALITY LABORATORY AND AVOID THE COMMON ABUSES OF FORAGE QUALITY TESTING

Glenn Shewmaker and Dan Putnam[1]

ABSTRACT

High quality laboratories are distinguished by building a reputation for excellence and reliability. Internal and external monitoring measures are a key requirement to assess lab proficiency. The National Forage Testing Association (NFTA), Association of American Feed Control Officials Incorporated (AFCO), and the NIRS Forage and Feed Testing Consortium (NIRSC) are organizations facilitating accurate forage testing through education, proficiency testing, and development of better methods. Hay producers often ask "why are forage quality test results for the same hay stack different among laboratories?" There are significant sources of variation, or error, that hay producers and consumers should realize. **Hay test results are not absolute values!** Sources of variation in lab test results, how to choose a lab, and common misuses of test results are discussed below.

Keywords: Forage Quality, NDF, ADF, CP, NIR, Laboratory performance

THE IMPORTANCE OF VARIATION IN LABORATORY TESTING

Sampling Variation. The largest source of variation in quality testing is the process of adequately sampling a lot of hay. Results of a Utah State University study (Table 1) show that sampling is the largest source of error. This is a daunting task when you consider the amount of forage analyzed may be 1 to 50 grams and it should represent hundreds of tons of hay or forage. Research on large and small bales has shown that 20 cores (each from a different bale) will allow good representation of the entire lot of hay. A good average size sample should weigh from 1/3 to 1/2 lb (150 to 250 grams).

Sample Processing By the Lab. Your lab should grind the entire sample, not sub-sample before grinding. It is difficult to split a sample without grinding it first. If you wish to send subsamples that are as similar as possible to different labs, ask for return of your ground sample from a lab and send it to another lab. Be sure to talk with your lab to assure that they are grinding the whole sample.

Storage Conditions and Time Change Forage Quality. Forage quality declines with time, even if optimally stored, due to forage degradation. A sample taken from the same lot of hay the day it is baled is expected to have

Abbreviations:
ADF = Acid Detergent Fiber
NDF = Neutral Detergent Fiber
NDFD = NDF digestibility
CP = Crude Protein
TDN = Total Digestible Nutrients
IVDDM = In Vitro Digestible Dry Matter
RFV = Relative Feed Value Index
RFQ = Relative Forage Quality Index
NIRS-Near Infrared Spectroscopy
NIRSC- NIRS Consortium
NFTA = National Forage Testing Assoc.

[1] G. Shewmaker, Univ. of Idaho Kimberly R&E Center, 3806 N 3600 E, Kimberly, ID 83341 gshew@uidaho.edu. D.H. Putnam Extension Forage Specialist, Plant Sciences, MS#1, One Shields Ave., University of California, Davis 95616. (dhputnam@ucdavis.edu). In: Proceedings, 2019 Western Alfalfa and Forage Symposium, Reno, NV, Nov 19-21. UC Cooperative Extension (See http://alfalfa.ucdavis.edu for this and other alfalfa conference Proceedings.)

lower fiber values than a sample taken from the same lot six months later. Therefore, sample as close to the time of sale or utilization of the lot of hay as practical.

Certify Your Hay Sample: At this writing, 3,844 people have taken an on-line training and certification for sampling hay according to a national protocol. This is a free service, and participants can log on and read through the training materials and take the exam until you pass. This is located at: www.foragetesting.org. Thereafter, when questions or conflicts arise about hay testing, you can assert your proficiency in hay sampling, and certify that a specific sample was taken with that protocol.

Laboratory Variation. The proficiency of a laboratory depends on the methods used and the precision of laboratory techniques. The National Forage Testing Association (NFTA) certifies the proficiency of laboratories for accuracy in testing hay and corn silage for dry matter, crude protein, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Following the recommended protocol will minimize error in tests. However, growers, brokers, and livestock producers need to be aware of the limits of accuracy of forage quality tests.

Unfortunately, laboratory error is added to sampling error in the test analysis. Normally there might be plus or minus 5% variation in results, e.g. +/- 1.5% ADF or +/- 8 RFQ. Usually a test of 31.5% ADF is not different than 30% ADF, and neither is a test of 172 Relative Forage Quality (RFQ) different than 180. Proper training and conducting of sampling and laboratory analyses will minimize errors in predicting forage quality but will not eliminate them.

Table 1. Expected lab and sampling errors from repeated coring and analysis of the same bales of alfalfa hay. The error, or variation, is expressed in units of concentration not percent difference. For example +/- 0.5 % error and a value of 20% crude protein indicates that values between 19.5 and 20.5% are considered within normal variability. Source: *Whitesides, R.E. and D.A. Chandler. 1998. The importance of hay sampling--A how to demonstration. pg. 150-158 In: California/Nevada Alfalfa Symposium, Dec. 3-4, 1998, Reno, NV.*

Constituent	Error (Percentage Points)	Source of error
Crude protein	+/- 0.5 %	By lab & sampler
Chemistry ADF	+/- 2.7 %	By sampler
Chemistry ADF	+/- 2.1 %	By lab
NIRS ADF	+/- 1.6 %	By lab

Variation of analysis from one lab to another is usually greater than variation within a lab. So it is recommended to choose a certified lab and stay with it to get consistent results. No one benefits from inaccurate forage quality testing. Forage quality is a multi-faceted attribute, the quality values are not absolute, and analysis is not adequately described by any one variable. Selecting a NFTA-certified lab should minimize variability in analyses. The lab also be capable of reporting NDF digestibility, Total Digestible Nutrients (TDN) calculated as the sum of

digestible components, and relative forage quality (RFQ) which is a better quality index than RFV, but all three of these are calculations from original lab values of NDF, ADF, and NDFD, so it's important to learn what they mean.

CHOOSING A FORAGE TESTING LAB

Most forage testing laboratories attempt to provide accurate and repeatable results to their clients. In the long term, their reputation and success depends on accurately predicting the feeding value of the forage. Price and marketability of forage is often based on laboratory test.

Standardization. In 1984, the American Forage and Grassland Council (AFGC), the National Hay Association (NHA), and forage testing laboratories combined to form the National Forage Testing Association (NFTA) to improve the accuracy of forage testing and build grower and consumer confidence. Since participation in the certification program is voluntary, not all hay testing laboratories are involved. This certification provides the producer and consumer confidence that the laboratory is proficient at certain forage quality tests, has a quality control procedure, and they know their accuracy. We recommend only using a laboratory that is NFTA certified. https://www.foragetesting.org/

Laboratories are evaluated by NFTA and graded six times every year. Unknown samples, including four alfalfa samples (one of which contains approximately 20% grass) and one sample each of corn silage and grass, are sent to each laboratory, which analyzes the samples using standard, accepted techniques (reference methods). A next step that should be implemented is to provide "blind samples" to the labs in a way that they do not know laboratories are being evaluated.

As an additional method to improve testing, The NIRS Forage and Feed Testing Consortium (NIRSC) was formed in 1992 through the efforts of several universities and commercial entities because of their interest in promoting consistent and quality forage testing results using NIRS technology. The NIRSC and many of its members participate in the NFTA certification. https://www.nirsconsortium.org/

Chemistry or NIRS? Currently accepted techniques of forage analysis include traditional chemistry methods and near infrared reflectance spectroscopy (NIRS). NIRS is a technique that uses light reflectance and is quicker and less expensive than laboratory chemistry and gives equivalent results. Some laboratories will use only one technique, while others use both. Those laboratories providing test results within the specified limits are certified. Certified laboratories located in selected states are listed on: www.foragetesting.org.

When Two Labs Produce Different Results for the Same Hay Lot. Communicate with all parties. Do something positive to resolve the situation. Ask for the ground sample back to send a proper subsample to the other lab. If a lab refuses to do this, reject this lab and consider using another NFTA-certified lab. Understand that labs cannot keep samples indefinitely but should for thirty days. If the results still vary by more than a typical error, have a joint core sampling of the lot, divide the ground sample using a laboratory sample splitter, and have each lab analyze the samples and compare results. Then it seems fair to average the test parameters of interest and negotiate a price.

We have heard of hay buyers insisting on only using a lab they prefer and refusing to consider other certified lab results.

Unfortunately sometimes milk production does not reflect the forage test results. This may cause a hay buyer to become more conservative and discount the quality parameters or price because of the uncertainty. However, if the hay is only 7% of a total mixed ration, we question if 10 RFQ points is a significant difference.

There are philosophical differences in whether the data used in prediction formulas should be locally or globally based. A locally based formula may be more accurate for local environmental and management factors. However, a globally-based formula will better represent forage quality across states and overseas, as our exports are increasing. Without some standardization, results could not be compared at all, thus the development of the National Forage Testing Association.

Choose a Lab that Chooses Excellence: Both Chemistry and NIRS labs can return excellent results. Steps for choosing a high-quality forage testing lab: Ask Critical Questions:

1. Do you participate in NFTA Split Sample Test each year and how did you Fare? (Results are at www.foragetesting.org). Only use certified labs.
2. Do you have a Quality Assurance Program internally for your lab and routinely run QC samples?
3. Do you grind the whole sample and mix before sub-sampling? (labs should do this)
4. Do you use NFTA-approved methods for DM, ADF, NDF, and CP? (check NFTA website)
5. Do you report your data standardized on a 100% DM basis (all data should be compared this way)
6. For NIRS labs – how do you update and support your NIR calibrations and monitor machine performance? (e.g. NIRS consortium or other methods)
7. For NIRS Labs – how do you handle a sample outside your calibration (H outlier)?
8. Final step, conduct your own split-sample test of several labs using split ground samples. Documented samples are available from NFTA.

COMMON ABUSES OF HAY TESTING

There is much concern about the reliability of hay test results and how lab results impact price and sales of hay. However, some of the concern about the reliability of laboratory results is brought about not by the performance of the labs themselves, but by unrealistic expectations, abuses, or misunderstandings of the whole hay testing process. Six common abuses of hay testing results are outlined below, and potential solutions to those abuses are outlined.

Misuse 1: Failure to expect some variation in hay tests. *"My customer wanted 170 RFV hay, but my hay tested only at 165%, so they refused it."* Overemphasizing small differences in lab results for the purposes of hay marketing is quite common. However, this is clearly an abuse of the hay testing process. With current technology of sampling and lab analysis, there is no reliable way to measure a 5% difference in RFV value between two hay lots.

Solution: Each reported lab value examined for the purposes of trade should have an 'error' term associated with it. This is likely to be a minimum of 0.5% CP, 1.0% ADF and 1.5% NDF, and in practice more due to sampling variation.

Misuse 2: Problem of biased sampling. *"I brought a flake into the lab, but it didn't match another sample from the same lot"* Sometimes people will submit samples to the lab that do not fairly represent the hay lot. In some cases this is deliberate (e.g. submitting only the leafy parts), but in most cases it is due to failure to follow proper sampling procedures. Some labs have reported that customers will bring in a whole flake of hay or a small teaspoon of hay particles. This sampling is clearly inadequate. Only use proper sampling techniques using a hay probe. We recommend taking at least 20 cores taken from randomly-chosen bales around the stack to help represent a hay lot (more if it's variable), along with other parts of the protocol. See www.foragetesting.org for the protocols.

Solution: Follow proper protocols for sampling, including combining 20 cores, using good sharp coring devices, and using accepted techniques. See NFTA website for specific protocols.

Misuse 3: Encouraging lab bias. *"It seems that Lab X always gives values that are 2 points above Lab Y*" says one client. *"Since it suits my purposes, I'll just insist upon using Lab X*". While some amount of random variation (+or -) between labs is normal, a consistent difference of several points, always in one direction, is a significant problem. Some of this is the lab's problem, but part of the problem is in the willingness of customers to exploit these differences to gain economic advantage. However, it is up to the customers of the labs to insist upon unbiased hay analysis methods by their laboratory.

Solution: Only work with those labs that are interested in standardizing their methods, and perform well on NFTA or other programs and avoid biasing their results to please customers.

Misuse 4: Misinterpreting calculated values. ***"****My buyer said that the RFV was OK but the NDF was not good enough-as a result, the deal fell through.*" This discussion revealed a lack of understanding (or alternatively a deliberate misrepresentation) of how TDN or RFV or RFQ values are derived. These are calculations, not measurements. Let's be clear: RFV is directly calculated from ADF and NDF. TDN is most frequently calculated directly from the ADF. Additionally, RFV is almost 100% driven by NDF measurements. So if you know the NDF or ADF values, you'll also know the RFV and TDN values. RFQ includes other measurements such as NDFD and ash, but is also a calculated value.

Solution: When confusion arises between tests or hay lots over TDN or RFV, always refer to the original analyzed values (ADF or NDF or NDFD at 100% DM). Understand where your calculations are coming from – ask the laboratory to explain.

Misuse 5. Confusion over dry matter. *"My hay is high in moisture, but tested 'Supreme' in quality. My buyer said that because of the high moisture, the forage quality should be evaluated on an 'as-received' basis, and therefore should be lower in price."* The expression of any lab value at a lower DM content causes the value to appear lower (for example a 24% CP at 100% DM will be about a 20% CP at a 80% DM basis (e.g. 'as received'). Confusion over dry matter and interpretation of lab reports has been a common problem with hay testing. However, ALL comparisons between hay lots should be made on 100% DM basis, not 'as received' nor adjusted to 90% DM.

While high moisture hay contributes a greater amount of water in the tonnage, it should have no effect on forage quality, which is independently measured. However, buyer (or seller) is completely justified in adjusting tonnage of the hay based upon the as-received dry matter content.

Solution: All parameters, ADF, CP, NDF, NDFD and TDN (as well as RFV) should only be compared on a 100% standardized moisture basis. Note: The industry in California is accustomed to using TDN on a 90% DM basis – but this is actually calculated on a 100% DM basis and the 90% added back-so the calculation is based upon 100% DM.

Misuse 6. Failure to account for other quality factors. "*This alfalfa hay was only 150 RFV, (53TDN) but it fed like a premium-quality hay." Alternatively: "I purchase this hay as a "Premium" hay but it did not milk well".* The standard hay test (ADF, NDF, and CP) reveals certain things about a hay lot, but not all. Forage scientists and nutritionists for years have promoted the use of other measurements such as NDFD, nonstructural carbohydrates, lignin, and ash to help predict the feeding value of a forage. The use of ADF or TDN calculated from ADF greatly simplifies the estimation of 'feeding value', sometimes to the point that it fails to predict the actual feeding value of the hay. There are many other factors that may contribute to true feeding value. Digestibility is important, but also weeds, molds and condition (texture) can influence the feeding value. There are limitations to the way in which a standard hay test can predict milk production, and there are some aspects of hay quality that are not fully understood or are difficult to measure.

Solution: Use NDFD and Ash Analysis to help predict quality along with ADF, NDF and CP, and subjective visual analysis to determine feeding value. It is especially important to examine hay for the presence of weeds (especially toxic weeds), mold, and physical characteristics that may affect the feeding value of the hay.

SUMMARY

There is an inherent challenge to predict complex biological processes with a few chemical tests or estimates from light reflection. Additionally, the mass of forage in a lot of hay is large, making accurate sampling a daunting process. Although significant progress has been made, we are still a long way from accurately predicting forage quality and thus value with adequate confidence. Information, communication, and negotiation are the keys to agreeing on a fair price for the producer and consumer. Hay testing has proven to be an invaluable tool in predicting feeding value. However, hay analysis is meant to be an instrument, not a hammer to bludgeon prices. We can avoid abusing hay test results by: 1) understanding the range of normal variation in hay test results, 2) using proper sampling methods, 3) choosing a lab from 'NFTA Proficient" labs, 4) interpret lab values expressed at a uniform DM content and 5) understanding the limitations of hay tests, only using them in addition to subjective visual evaluation of hay quality.