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FORAGE ANALYSIS: THREE POINTS TO CONSIDER

PAUL K. SIROIS
Dairy One Forage Lab, Ithaca, NY

Introduction

Great strides have been made over the last few decades in our understanding of nutrient requirements for livestock. As we strive to do a better job formulating rations and meeting those nutrient requirements, better knowledge of feed composition becomes essential. Simply using average or tabular values is no longer sufficient to describe forages. Commercial feed analysis is now a routine part of much ration development. As testament to that fact, the Dairy One Forage Lab began operations in 1974 (as the NY DHIC Forage Lab) and processed 5000 samples that first year. Today, the lab processes in excess of 115,000 samples per year.

This paper will address three relevant aspects of the analysis process:

1. Representative sampling.
2. How not to submit a hay sample for analysis.
3. Near infrared reflectance spectroscopy (NIR).

Representative Sampling

Obtaining a representative sample is the first and most critical step of the analysis process. Unfortunately, it is often the most overlooked. Laboratories are staffed and equipped to do the best job possible of analysis. Quality assurance (QA) programs are employed to monitor the integrity of results. Internal QA programs usually involve analyzing daily check samples of known value and are used to insure consistency of results. Many labs also participate in external sample check programs. Sponsoring organizations submit periodic samples of unknown value to participating labs. Results are sent back to the sponsoring organization and compared to the results of other participating labs. The function of the external program is to insure that results are consistent with other labs in the industry. Thus, internal check programs serve to maintain consistency from day to day and external programs insure consistency from lab to lab.

The labs, however, have no control over the first and most important step, that of obtaining a representative sample. Labs can only analyze what they receive. They will do as good a job of analyzing a well taken sample as they will a poorly taken sample. In the latter case, you wind up with a good analysis of a poor sample. Thus, it is the responsibility of the "sample taker" to obtain a representative sample.

A paper presented by Martin et al. (1988) demonstrated the importance of multiple subsampling to form a composite sample. Twenty bales of alfalfa hay from the same lot were individually sampled. The core samples from each bale were individually analyzed by NIR. The results in Table 1 illustrate the variation in nutrient composition from bale to bale. For example, protein ranged from 18.2 – 22.4% and NDF from 33.7 – 54.1%. Relative feed value (RFV) is a forage score based on ADF and NDF reflecting the digestibility and intake potential of haycrop forages for ruminants. The RFV ranged from 103 – 184.

The individual samples were then combined to form a composite. As can be seen in Table 1, the composite analysis was equivalent to the arithmetic mean of the 20 individual samples. This clearly illustrates the importance of gathering multiple subsamples to truly reflect the quality of a lot of hay.

Table 1. Quality tests of single bales of alfalfa hay from the same lot.*

Bale No.	DM%	CP%	ADF%	NDF%	RFV
1	87.9	18.2	35.3	44.6	128
2	86.7	18.4	35.8	48.7	117
3	86.6	18.4	36.1	44.3	128
4	87.3	18.9	32.5	39.0	152
5	88.4	19.8	31.4	38.3	156
6	87.1	19.8	32.7	41.5	142
7	85.9	20.3	32.7	40.0	148
8	88.0	20.3	31.5	38.5	156
9	85.6	20.3	36.9	54.1	103
10	85.5	20.4	32.1	40.6	146
11	87.4	20.5	32.0	39.2	152
12	86.9	20.5	32.5	39.1	151
13	86.4	20.8	31.5	41.2	145
14	86.2	20.8	33.4	42.0	139
15	88.0	21.2	30.3	35.7	170
16	84.7	21.3	31.4	38.5	156
17	86.8	21.4	29.3	33.9	181
18	89.9	21.5	28.6	33.7	184
19	85.2	21.9	32.1	40.3	148
20	87.8	22.4	29.4	37.0	166
Minimum	84.7	18.2	28.6	33.7	103
Maximum	89.9	22.4	36.9	54.1	184
Average	86.9	20.4	32.4	40.5	148
Composite A	88.1	20.7	31.5	40.7	147
Composite B	88.0	20.3	31.7	41.0	146

*All results DM basis.
Adapted from Martin et al. (1988). The data were sorted by crude protein.

In practice, it is not uncommon for people to sample one to three bales. If, by chance, the poorest bale is selected, ration recommendations would result in overfeeding and vice versa. Both situations will have nutritional and economic consequences as feed cost is typically one of the largest costs on any livestock operation.

Thus, if you are willing to invest the time, effort and dollars in forage analysis to better formulate rations, it is in everyone's best interest to do the best job possible obtaining a representative sample.

In practice, the greater the number of subsamples the better. In reality, sampling 10 – 12 bales should provide a good representative sample.

Sampling Techniques

HAY

Now that you know it is important to take and composite multiple subsamples to submit for analysis, what is the proper way to collect those subsamples? The following are ***unacceptable*** when submitted for analysis:

1. A flake or slab of hay.
2. A handful of hay pulled from a bale.
3. A handful of hay grabbed from the manger.
4. A handful of hay grabbed and cut up with scissors.

It is very difficult for the lab to obtain a representative sample from these submissions. Any sampling of dry forages that involves grabbing a handful of material usually results in a subsample that is poorer in quality than the actual nutrient content. This is particularly true with alfalfa, because grab sampling usually results in a fistful of stems with the finer and more fragile leaves shaking off. Leaves contain most of the nutrients, being higher in both protein and digestibility than the stems. Any procedure that results in leaf loss will have a negative impact on the analyzed value. The opposite also holds true; any sampling that results in concentrating the leaves will make the sample look better than the forage actually is.

The only way to obtain a proper hay sample is by using a bale probe or corer. This is typically a metal tube from 38 – 48 cm (15 – 18 in) long and sharpened at one end. Depending upon the type of probe, it is either hand operated or may be coupled to an electric drill. Bales should be probed in the center of the small, square end. The probe takes a representative cross section as it spins and cuts its way through the bale. The resulting core sample will proportionately reflect the leaf and stem material in the bale. Typically, obtaining and combining 10 – 20 core samples will form a good composite sample.

PASTURE

The key to sampling pasture is sampling multiple sites. Randomly select 12 – 20 sites where the animals have been grazing and clip a handful of forage at grazing height. Grazing height is the level to which the animals are consuming. For example, if the grass is 25 cm (10 in) high and the animals are consuming the top 15 cm (6 in), it is only the top 15 cm that should be submitted for analysis. All subsamples should be combined and thoroughly mixed in a clean plastic bucket to form a composite (further cutting the forage into 5 – 8 cm (2 – 3 in) pieces aids in blending). Take a 0.5 kg (1 lb) sample, pack tightly in a plastic bag, seal and freeze for 12 hours prior to submission. Freezing will help prevent marked chemical changes due to respiration or fermentation.

It is also important to remember that pasture is wet. This may seem obvious, but not many people realize its significance. For example, pasture is typically about 20% dry matter. A 500 gram sample submitted for analysis will be split and half will be used for analysis. Upon drying the 250 gram split sample, 50 grams will be left. After grinding, 45 grams will be left for analysis or less than 10% of the original weight.

In several instances, we have received pasture samples of 6 – 20 blades of grass. This is not enough sample to even begin attempting an analysis.

Table 2. Weight losses from drying and grinding prior to analysis.*

Wet Weight,g	Dry Weight,g	Post Grind Weight	Post Grind Loss,g	Post Grind Loss,%	Dry Matter,%
10	1.8	1.1	0.7	38.9	18.0
20	3.7	3.0	0.7	18.9	18.5
30	5.7	4.7	1.0	17.5	19.0
40	7.4	6.6	0.8	10.8	18.5
50	9.6	8.5	1.1	11.5	19.2
60	11.1	10.0	1.1	9.9	18.5
70	12.4	11.2	1.2	9.7	17.7
80	14.5	13.6	0.9	6.2	18.1
90	17.2	15.5	1.7	9.9	19.1
100	18.9	17.2	1.7	9.0	18.9
200	34.7	33.1	1.6	4.6	17.4
300	56.5	51.5	5.0	8.8	18.8
400**	**76.5**	**59.5**	**17.0**	**22.2**	**19.1**
500	94.2	87.2	7.0	7.4	18.8

* The same grass sample was used for all original weights.

**Unexplained large sample loss post-grinding.

Table 2 illustrates starting and ending sample weights after drying and grinding. With the exception of the 400 gram samples, the average sample loss during initial preparation is 12.5%. Thus, it is important to take into account the moisture level of the sample prior to submission to insure that the lab has an adequate amount of material for analysis. The wetter the sample, the greater the amount of sample required.

As mentioned above, it is advisable to freeze or dry pasture samples prior to submission. Samples shipped internationally should be predried to avoid spoilage and marked chemical changes during shipment. Samples can easily be dried in a microwave oven. Dried samples are also less expensive to ship.

A lot of money is invested in the shipping and analysis process. Greater economic potential rides on the results. The highest return per dollar invested is realized if time is taken at the outset to follow simple collection procedures.

NIR Technology

Near infrared reflectance (NIR) spectroscopy is a sophisticated analytical technique used for determining the chemical characteristics of agricultural and food products, pharmaceuticals and beverages. It is based on the fact that each of the major chemical components of a sample has near infrared absorption properties that can be used to differentiate one component from another. Once a sample has been dried and ground, a NIR analysis can be completed in about 60 seconds, yielding up to 18 nutrients.

Advantages:

1. Accuracy – advancements in computer hard and software have provided the tools to take full advantage of the technology.
2. Speed – customers demand fast turnaround time. NIR analyses can be completed in under 24 hours.
3. Cost – analyses are typically half the price of wet chemistry.
4. Labor efficiency – more analyses can be completed in a shorter period of time with less labor.
5. Safety – eliminates the use of hazardous chemicals.
6. Environmentally friendly.

Disadvantages:

1. Expense and time required to build new calibrations.

More on Accuracy

Several factors influence the accuracy of NIR measurements:

1. Does the component have NIR reflectance properties? Each of the major organic feed components has absorption characteristics (due to vibrations arising from the stretching and bending of H bonds associated with C, O, N) in the near

infrared region that are specific to that component (Marten et al., 1985). NIR is most sensitive to organic compounds.Compounds lacking the above properties will not calibrate as well.

2. Robustness of the calibration set. There must be adequate variation in the population of samples used for developing the calibration. The variation must be inherently reflective of the sample population as a whole. The more closely the calibration set resembles the sample population, the better the performance of the calibration.
3. Accuracy of the reference method. A NIR measurement can only be as accurate as the reference method used to develop the calibration. For example, crude protein is accurately measured by Kjeldahl and can be calibrated quite well. The detergent fiber methods of analysis are not as precise, and therefore are less well determined by NIR. For example, the standard error of calibration (SEC) of the Dairy One hay calibration for CP is 0.63 while for ADF is 1.52 (Table 3).
4. Calibration updates. New varieties and hybrids are introduced every year. In order to keep calibrations current, they must be continually expanded to include new genetics. Software routines ease the process of identifying new samples for calibration expansion. Each sample has its own spectral fingerprint (spectra). Software comparisons of new spectra to existing spectra in the calibration database identify samples to add for expansion.

In an ideal world, calibrations would exist for each different forage species. For example, alfalfa, timothy and tall fescue would have their own individual calibrations. In the real world, most samples are mixtures of different species and are often not well identified. Thus, typical commercial calibrations are developed to cover a broad range of samples. For example, a typical hay calibration starts with a poor grass hay (5% CP) and ends with a high quality alfalfa (28% CP). The goal is to cover a wide variety of qualities, species and mixtures.

When all of the above are taken into account, excellent calibrations can be developed. Table 3 compares the proficiency of NIR to wet chemistry. Following the evolution of software, calibration refinements will lead to further enhanced accuracy. NIR is recognized by the Association of Official Analytical Chemists (AOAC) as an official method.

Table 3. Dairy One NIR & wet chemistry standard errors of analysis for major components.

Component	RSQ	NIR SEC	Wet SE
CP%	0.99	0.63	0.26
ADF%	0.95	1.52	1.26
NDF%	0.97	2.36	1.40
Ash%	0.90	0.66	0.40
Fat%	0.81	0.31	0.15

RSQ = r squared statistic for Dairy One hay calibration.
NIR SEC = standard error of calibration.
Wet SE = standard error for repeated measures from Dairy One 1/00 QA report averaged over several feed types.

Minerals

NIR does not measure minerals directly. Minerals are indirect measurements based on relationships with other components. Predicted mineral values will be better than average tabular values, but it must be understood that they may not be the absolute values. Given these restrictions, the results are quite good. Table 4 lists the statistics for the Dairy One hay calibration.

Table 4. Dairy One NIR & wet chemistry standard errors of analysis for minerals.

Component	RSQ	NIR SEC	Wet SE
Ca%	0.89	0.169	0.068
P%	0.70	0.039	0.029
Mg%	0.73	0.041	0.010
K%	0.85	0.276	0.469

RSQ = r squared statistic for Dairy One hay calibration.
NIR SEC = standard error of calibration.
Wet SE = standard error for repeated measures from Dairy One 1/00 QA report averaged over several feed types.

NIR mineral results are routinely used by the feed industry for ration formulation. Wet chemistry minerals should be substituted when precise formulation is required for exceptional circumstances. This could be for rations where the animals are not performing as expected or for high performance rations where fine tuning to the “nth” degree is desired.

The bottom line is: if mineral concentrations and their balance with other elements is of paramount concern, wet chemistry minerals should be used for ration balancing.

The Future

The next generation of NIR software is available and will soon be in commercial use. In the past, *global* calibrations were developed for specific feed types. For example, thousands of hay samples would be collected from which 500 - 2000 would be used for calibration development. A single hay global calibration would then be used to analyze all future samples. In the next generation, databases will be built for a particular feed. When a sample is scanned, its spectra will be compared to all of the spectra in the database. The software will then select 100 samples that most nearly resemble the sample being scanned and develop a calibration specifically for that sample. Thus, a *local* calibration will be developed for each sample as it is analyzed. This will eliminate the need for broad based calibration development. The focus will shift to database expansion and increasing diversity. Thus, calibrating will no longer be an issue as it will be handled on an individual sample basis. The end result is enhanced accuracy.

Summary

Routine feed analyses are an essential component of ration development. For meaningful results, time must be invested in the sample collection process. Multiple subsamples must be taken to form a representative composite. The greatest return per dollar invested will be realized by following a few simple procedures to insure that a good sample is submitted for analysis.

NIR forms the basis for most commercial feed analyses. At Dairy One, NIR accounts for 69% of all analyses performed. Enhancements in computer hard and software have enabled the industry to make full use of this powerful technology. The next great leap will be the use of local rather than global calibrations. Individual calibrations will be developed for each sample as it is analyzed. This should result in enhanced accuracy. NIR expansion in the market will continue as new calibrations are introduced.

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