

MANAGING FOR NUTRIENT VARIABILITY: HOW DO YOU MEASURE IT?

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Key Points:

- Feed variation has significant economic impact.
- One must be able to characterize variation in order to manage it.
- Feed variation has multiple components:
 - Farm sampling variation
 - Lab sampling variation
 - Lab analytical variation
 - Compositional variation
- Sampling variation at the farm provides the greatest source of error, followed by lab sampling, and grinding of the sample for analysis.
- Understanding the source and amount of potential laboratory variation is important in understanding feed analysis results and total feed variation.
- NIRS provides a less expensive opportunity to characterize variation in feeds through intensive analysis and quick turnaround.
- Forage laboratories can assist clients in defining variation by providing information and tools.

Introduction:

Feed and forage nutrient composition always varies to some degree. But, limiting ration variability can improve cow health and productivity. Feed costs are generally higher when variability of farm forages and feed ingredients is high. This is because rations must be balanced for higher nutrient concentrations (over-formulated) for insurance against nutrient deficiency in the event that unrecognized ingredient nutrient content changes occur.

Forage analysis is not a perfect science. If you send samples of the same forage to three different labs, you will probably get slightly different results from each. If you send the same sample to the same lab three different times, you may also get slightly different results each time. Why is this? The key is to understand that there will be variation, understand the sources of variation, and understand how much variation should be normally expected.

With today's high grain prices and tight margins, dairy producers need to better understand and control nutrient variation in their rations. Whereas in the past, a producer routinely sampled all forages once or twice per month, now a more analytical approach should be taken with each feed and forage on the farm to decide how frequently to send in samples for analysis. If the nutrient analysis of a forage or feed ingredient changes, consideration should be made as to whether this new analysis should be used for ration balancing or because of random variation, it might be better to use the average of a number of nutrient analyses of that ingredient for ration balancing purposes (Weiss and St-Pierre, 2008). With high grain prices, it may also be more important to look closely at the nutrient variation of commodity feeds and put a higher value on consistency.

Different terms are often used to describe variation. Standard deviation (SD) helps us to get an idea of how close individual numbers in a set of data are to the mean (or average). Naturally, the smaller the SD is, the better. The mean $\pm$ one standard deviation would include 68% of all measurements in a dataset. Two standard deviations would include 95% of all measurements. The standard error (SE) adjusts the SD for the sample size since you would expect a larger dataset to give you a more precise estimate of the mean. Standard error does this by dividing SD by the square root of the number of samples in a dataset. Thus, a dataset with 100 observations would have a smaller SE ($SD/10$) than one with just 25 observations ($SD/5$). The coefficient of variation (CV) or relative standard deviation (RSD) is the standard deviation as a percentage of the mean. The CV helps us to weigh sample variation properly when the mean may vary, such as when you want to compare the variation around 40% NDF vs. 20% CP.

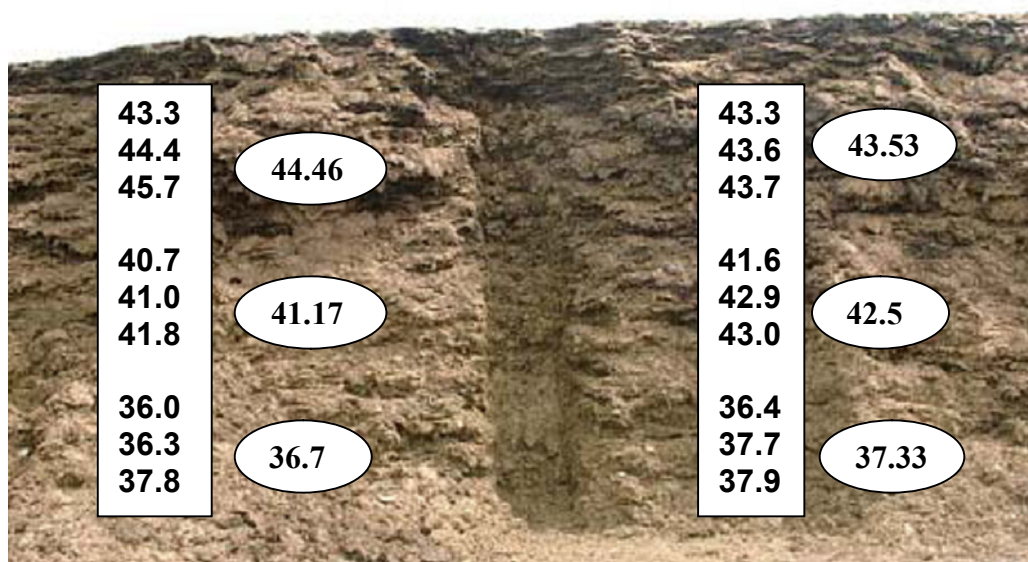
Sources of Variation

1. Sampling Variation

Many times you may think that you are sending in a sample of the same forage to a lab because it came from the same lot of hay or same bunk of haylage. In reality though, depending on sampling technique and inventory variation, your samples may be significantly different. Sampling variation is usually greater than lab variation.

You always need to start with a good sample. For hay, it is generally recommended that at least 20 core samples from different bales be taken and composited to make one sample per lot. You should sample bales randomly, perhaps sampling every 4th or 5th bale as you go around a stack of hay. With bunker silos, sample 12 to 15 sites on the silage face making sure that you get all layers. It has been estimated that the standard error increases from $\pm 1.07\%$ to $\pm 2.15\%$ when the number of core hay bale samples taken is reduced from 20 to 5. With haylage, if only 5 locations on the bunk face are sampled vs. 20 locations, standard error would be ± 2.37 vs. $\pm 1.19\%$ (Mertens as cited by Holin, 2007).

Example of NDF Variation Throughout a Haylage Bunk Sampled in Two Sections with Three Replicates per Layer (Top, Middle, Bottom) and Corresponding Means per Layer (Stone, 2003)



Stone (2003) conducted similar analyses on the top, middle, and lower sections of nine haylage and eleven corn silage bunker silos.

Table 1. Deviations between different regions (top, middle, and lower) in 9 haylage and 11 corn silage bunker silos (Stone, 2003).

Haylage	DM	CP	ADF	NDF
Minimum Deviation, %	5.2	3.3	1.1	5.4
Maximum Deviation, %	44.7	52.1	20.0	24.8
Average Deviation, %	21.0	17.6	10.7	14.7
Median Deviation, %	19.4	9.5	9.9	14.4
Corn Silage				
Minimum Deviation, %	1.3	2.5	2.3	0.5
Maximum Deviation, %	55.0	29.5	18.3	18.6
Average Deviation, %	12.3	11.0	8.4	8.6
Median Deviation, %	8.3	10.0	8.6	8.4

When marketing hay, the National Forage Testing Association (NFTA) and the National Hay Association now recommend a process where sellers and buyers of hay each have one lab test three samples from one stack of hay each made up of a different set of eight core samples (replicated analysis) (NFTA, 2006). Multiple sampling gives everyone a better idea about the variation in a stack of hay and reduces disputes. Two different stacks of hay may both have the same average NDF of 40% but one may have a range of 38-42% NDF and the other have a range of 39-41% NDF.

Table 2. Variability of Alfalfa Hay Bales (Collins, 2000)

	Average	SD between bales	Range between bales	SD within bales
NDF	40.2	2.0	36.3-44.1	2.1
CP	17.2	0.8	15.7-18.7	0.8

2. Laboratory Sampling Variation

Variation in the laboratory occurs at three primary points, those being the sub-sampling of materials sent to the lab for analysis, grinding, and in the analysis of the given nutrient. One of the most important processes occurring in the laboratory is the sampling and grinding of samples. Anything that renders a ground subsample different from the original starting material compromises all following analytical processes.

It is important that samples submitted to the laboratory are large enough to represent the material being sampled at the farm, but not so large that it creates a burden for the laboratory. Too large of a sample is not as likely to be properly sub-sampled at the lab due to the time involved. Larger laboratories may process five hundred or more samples in a day and additional prep time is problematic. Imagine the time involved to handle a sample received in a quart zip-loc bag versus a full breeder's sleeve!

The grinding of samples creates opportunity for variation. Most significant is that segregation of particles may occur through the grinding process, and if the ground sample is not thoroughly mixed, a non-homogeneous sample may result. In some samples where proper mixing of ground sample does not occur, layering can be visually evident in the sample vial. The requirement to grind larger samples adds to the importance of mixing the ground sample. It does little good to process larger samples to improve sample homogeneity but then to have segregation occur through grinding.

Certain analyses in the feed lab are defined by the size and type of grind, i.e. a cyclone mill with a 1-mm screen or a knife mill with a 6-mm screen. Samples that are ground through mills that are not properly maintained will create a particle size distribution that is not consistent for a given test. The requirement to grind larger samples can create a burden where an operator may be inclined to push a sample through the grinder too quickly. This can impact particle size and also create heating of the sample that will affect analyses such as ADF-protein, NDF-protein, and NDF.

Below is an example of analysis from an alfalfa hay sample properly ground versus improperly ground. Note the much higher NDF-protein level and higher DM of the improperly ground sample which comes from overheating of the sample when attempting to push the sample too fast through the grinder. As well, this sample should have been dried to less than 5% moisture prior to grinding.

Table 3. Analysis of an Alfalfa Hay Sample that was properly or poorly ground

	Poorly Ground	Properly Ground
DM	92.9	90.4
CP (%DM)	25.7	26.4
ADF (%DM)	26.9	24.8
NDF (%DM)	37.6	29.5
NDF-CP (%DM)	5.2	2.7
RFV	168	220

Proper preparation of forage samples in the lab is critical for consistent results. Much of the contention that revolves around the differences in reported laboratory values for alfalfa hay occurs due to problems in sample consistency either from the farm or at the laboratory. It is the position of the NFTA that all of the sample sent to the laboratory should be ground for analysis (except what is necessary for dry matter determination). At times a gallon of material may be sent in. If all of this material has to be ground, a lot of time is involved and then mixing becomes more challenging. CVAS uses a large tumble mixer to homogenize ground samples from western sourced alfalfa, TMR, and other larger amounts of ground sample.

There have been several recently publicized blind studies of forage laboratories in evaluation of alfalfa hay. Results of these studies have been disappointing, showing some serious problems in consistency across labs in generating NIR evaluations of alfalfa hay among labs that are NFTA certified. While the problems are largely laid at the feet of NIR analysis, it is this author's contention that problems lie much more significantly with sample handling and grinding in the laboratory.

Corn silage and TMR are sample types where problems with lab sampling and especially field sampling can significantly impact results. For these feeds, typical errors in sampling will yield ADF and NDF results that are high and starch and NFC results that are low.

3. Analytical Variation

When a laboratory analyzes the same prepared and ground feed sample multiple times, it does not get the same result every time. There is normal variation around the true analytical mean for a tested nutrient. We never know the "true" value of a feed nutrient, but with good technique and replication of analysis we can obtain a value that we understand to be close to the true value. It is of value for users of a lab to have an understanding of the normal analytical variation for a given analysis. If a test has high analytical variation, interpretation of results will be different than for a nutrient with low analytical variation. Is a 53% NDF digestibility corn silage different than a 56% NDF digestibility

corn silage? If you know that the SD for 30 hour NDF digestibility is 2.4, then the answer is that they are possibly different, but not necessarily different.

There is a tendency for users of analytical information to consider results as “absolute” values. Understanding that there is analytical variation, feed test results might be more appropriately reported with ranges. This would provide the user with an understanding of potential analytical variation and provide more information for interpretation. Table 4 shows an example of a forage report that would utilize analytical ranges.

Table 4. Analysis of Second Cutting Hay Reflecting Analytical Variation

	Reported Value	SD	Range (+/- 1 SD)	
DM	48.6	0.744	47.86	49.34
CP	20.8	0.356	20.44	21.16
Soluble Protein, %CP	33.6	2.527	31.07	36.13
ADF	31.4	0.782	30.62	32.18
NDF	41.9	0.700	41.20	42.60
30 Hour NDF Digestibility	52.2	2.422	49.78	54.62
Ash	11.3	0.199	11.10	11.50
Lignin	6.14	0.238	5.90	6.38
Fat	3.44	0.121	3.32	3.56
Starch	2.1	0.074	2.03	2.17
Sugar	2.7	0.281	2.42	2.98
Ca	1.31	0.040	1.27	1.35
P	0.32	0.012	0.31	0.33
Mg	0.29	0.009	0.28	0.30
K	2.97	0.099	2.87	3.07
Fe	735	45.350	689.65	780.35
Mn	75	2.385	72.62	77.39
Cl	0.64	0.005	0.63	0.65
S	0.27	0.005	0.26	0.28
RFV	143		139	147

There is opportunity to reduce analytical variation through replicated analysis. In general, the more times that an analysis is replicated, the lower the standard deviation around the reported analysis. As an example, the standard deviation on 30-hour NDF digestibility on a quality control sample at CVAS is +/- 1.4, but if we average four replications for the analysis, the standard deviation drops to +/- 0.75. Most analysis at CVAS are run in duplicate as it is more difficult to determine error with only one analysis. Analyses that are more critical are run with more replicates, as are analysis that tend to have higher levels of analytical variation.

Analytical variation is dependent on the nutrient and feed matrix being analyzed. The analytical error of an analysis can increase considerably with problem samples. Fiber analysis of corn distillers is a good example. Due to the high fat content and presence of Maillard products (heat damage), filtration of ADF and NDF residues can be difficult. The current effort to utilize non-traditional byproduct feeds in cattle rations has led to increased requests for laboratory evaluations of unusual materials in the

forage laboratory. CVAS routinely analyzes these types of materials. At times, procedures developed for forages and feeds are applied to these byproduct types of feeds with varying degrees of success. Dry matter evaluation is generally defined as loss of weight at a given temperature over a given period of time. Time and temperature requirements will vary by product. Products with high levels of volatile materials should be analyzed for DM by methods such as Karl Fisher (a distillation method). In general, byproducts that have higher levels of fat, sugar, or ash will be more difficult to evaluate by forage based laboratory procedures, and in some situations the application of forage procedures is inappropriate. Evaluating non-plant materials such as meat byproducts for NDF is an example.

NIRS provides opportunity to reduce analytical variation. NIRS is a secondary method based on reference method evaluation of nutrients, and by definition will never be more accurate than the reference methods on which it is based. However, as an analytical tool, NIRS is often more precise, or repeatable, than wet chemistry analysis. As with wet chemistry analyses, NIRS evaluations are never better than the quality of the sub-sample presented to the instrument for analysis.

Table 5. Average Nutrient Content and Analytical Standard Deviation (SD) of Nutrients Analyzed in a Quality Control Sample at CVAS by Wet Chemistry or NIRS.

Nutrient (%DM)	Average	SD, Wet Chemistry	SD, NIRS
CP	15.8	0.33	0.05
ADF	31.4	0.78	0.32
NDF	41.9	0.70	0.28
Ash	11.3	0.20	0.07
Fat	3.44	0.12	0.02

Defining Analytical Variation at Cumberland Valley Analytical Services

At CVAS, analytical variation is assessed by calculating coefficients of variation (CV). A coefficient of variation is the standard deviation as a percentage of the mean. As previously mentioned, the CV helps us to evaluate sample variation properly when the mean may vary, such as when you want to compare the variation around 40% NDF vs. 20% CP.

Table 6. Cumberland Valley Analytical Services, Inc. Coefficients of Variation (CV) by nutrient.

Nutrient	CV	Nutrient	CV
Dry Matter	1.53	Calcium	3.07
Crude Protein	1.71	Phosphorus	3.65
ADF	2.49	Magnesium	3.15
NDF	1.67	Potassium	3.33
Ash	1.76	Iron	6.17
Lignin	3.87	Manganese	3.18
Fat	3.53	Chloride	0.84
Soluble Protein	4.40	Sulfur	1.86
Starch	3.50	dNDF 30	2.62
Sugar	8.42	NDFD30	4.37
Crude Fiber	3.01	DNDF 48	1.64

Total Expected Sampling and Analytical Variation

Sampling variation and analytical variation should be combined to determine the expected variation for a sample. You do this by squaring each of the two standard error terms, adding them together, and

then taking the square root of that (Mertens, as cited by Holin, 2007). For example, if you sent a sample from 5 locations of a bunk ($\pm 2.37\%$) to an “A” lab ($\pm 0.60\%$) the combined SE would be 2.45 (square root of $(2.37^2 + 0.60^2)$). If you got an analyzed value of 40% NDF, then you could expect to get a value between 37.55 and 42.45% NDF 68% of the time if you sampled the same forage the same way again and again. However, if you mixed handfuls of silage from 20 locations ($\pm 1.19\%$) the combined SE would be 1.33 (square root of $(1.19^2 + 0.60^2)$) and you could expect a value between 38.67% and 41.33% NDF 68% of the time if you sampled the same forage the same way again and again.

4. Compositional Variation

Growing environment, plant genetics, and processing conditions are all sources of “fixed” variation. This is the real variation that actually affects the animal. The nutrient composition of some feeds and forages varies more than others. For example, byproduct feeds like wheat midds and distillers will vary more in their nutrient content than corn and soybean meal. Accounting for sources of “fixed” variation, such as separating forages by hybrid or cutting and sorting distillers grains by source, can help reduce the amount of variation that will otherwise be assumed to be “random” (Weiss and St-Pierre, 2008). Table 7 makes the point that total variation is the sum of compositional variation and analytical variation.

Table 7. Average Content and Partitioning of CP Variation of a Variety Feedstuffs

	Average	Analytical SD	True SD	Total SD
Alfalfa Silage	20.0	0.6	2.4	3.0
Corn Silage	8.8	0.4	0.8	1.2
Alfalfa Hay	20.2	0.6	2.0	2.6
Cornmeal	9.4	0.4	0.9	1.3
Wheat Midds	18.5	0.6	1.5	2.1
Dry Distillers	29.7	0.8	2.5	3.3
Corn Gluten Feed	23.8	0.7	5.0	5.7
Soybean Hulls	13.9	0.5	4.1	4.6
Soybean Meal - 48	53.8	1.3	0.8	2.1
Canola Meal	37.8	0.9	0.2	1.1
Blood Meal	95.5	2.1	6.1	8.2

(St-Pierre and Weiss, 2006)

Defining Feed Variation on the Farm

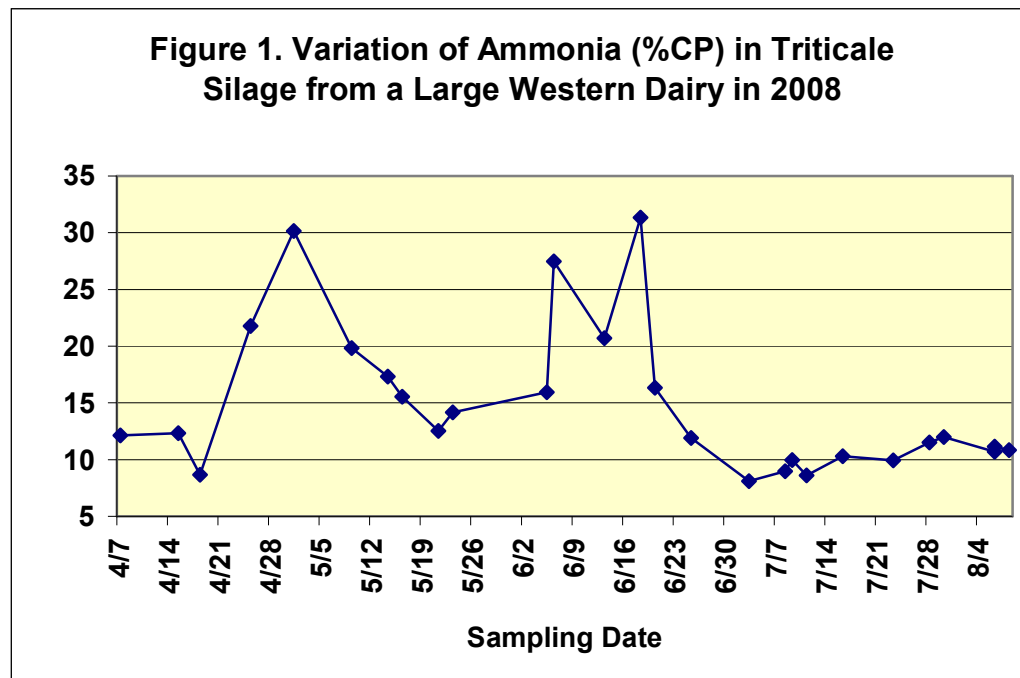
As previously mentioned, limiting ration variability can improve cow health and productivity. It can also reduce feed costs because it reduces the need to over-formulate for insurance against nutrient deficiency in the event that unrecognized ingredient nutrient content changes occur. Knowing how much particular forages or feed ingredients vary helps to reduce the risk of ration formulation error.

Characterization of TMR variation is a quality control check against all of the inputs that come together to generate the TMR that is placed in front of the cow. It is helpful to monitor the quality of the mixing process to recognize when problems occur with equipment or with labor inputs.

Ongoing characterization of feed inputs on the farm provides reference information for problem solving purposes. If there is a major health or production issue, those involved in feeding program

management have information available to assist in determining if feed inputs may have created a problem. If there is a health or production problem and forage and feed tests are executed at that point, variation that may have caused the problem is now historical and may not be determined by current testing.

Figure 1 show a means of charting nutrients over time to evaluate change. The percentage of protein in a forage sample as ammonia is a good means of inferring fermentation quality. Higher levels of ammonia are associated with clostridial fermentations and the production of amines. Clostridial forages can depress intake and elevate blood ketone levels. One may be able to correlate higher ammonia levels in the triticale silage as causative of a higher incidence of ketosis or displaced abomasums that might otherwise go unresolved.



CVAS is in the process of establishing web-based software that will allow for efficient summarization of intensively sampled forage test information. As a laboratory, we can generate a lot of quality data, but if there is not a means of summarizing data for decision analysis, the data may be of little value. One of the key objections to intensive testing (after cost) is the time necessary to manage the information in a manner that allows it to be an effective decision analysis tool.

Finding the Appropriate Sampling Regimen

As discussed previously, there are multiple sources of variation that can impact the analytical information obtained by the user. Feeds, whether hay, silage, ingredients, or commodities, are non-uniform and require proper sampling for creation of a consistent sample. Variation occurs as the lab sub-samples, dries, and grinds the sample, and then as the sample is analyzed. There is one more significant source of variation that impacts accuracy, and that is time. If change is occurring in a given forage material, and the normal sampling regimen is every two weeks and it takes one week to obtain results from the laboratory (including shipment time), then there may be significant error in properly characterizing a feed material.

It has been typical for nutritionists and feed company representatives to sample farm forages every two or four weeks during regularly scheduled visits to the farm. Now, with tighter margins, more commodity ingredients, and higher numbers of cows on farms, a more analytical approach should be taken with each feed and forage on the farm to decide how frequently to send in samples for analysis. Experts are recommending that feeds and forages be sampled more frequently depending on the expected variability. They also suggest that the mean nutrient analysis of a set of samples be used unless analyses are widely different or a known change (like a change in cutting or supplier) has occurred (Weiss and St-Pierre, 2008).

Many Samples Analyzed by NIR vs. Fewer Samples Analyzed by Wet Chemistry

Using well-calibrated NIRS analysis procedures rather than wet chemistry, more samples can be tested at the same cost. Because of the extra samples, total sampling and analytical variation may be reduced with this strategy. Using corn silage with 45% NDF (%DM) as an example, Berzaghi (2005) estimated that sampling and analytical error would be 2.1% if four NIR analyses were conducted whereas, if one sample was run by wet chemistry, analytical error would be 3.6%.

Through its web-based data access system, CVAS is providing a means for clients to generate reports of replicated samples (multiple samples taken at one time). This report will allow for users to generate a mean and standard deviation on multiple samples that can be used in ration modeling. This same approach can be used to weight results of samples that have been taken over a period of time, often better reflecting the true nutrient analysis of a feed material.

Economics of Intensive Testing

Intensive testing of forages and TMR allow for a manager to be able to define variation and to use this information in decision analysis. The question quickly arises as to whether this is a cost effective approach. Below is an example utilizing potential costs:

Example of Costs of an Intensive Testing Program

- 3000 milking cow operation
- 3 key forages
- 2 milking cow TMR, 1 dry cow TMR, 1 pre-fresh TMR
- Regimen: sample every 3 days

7 analyses, 10 times per month, \$12.50 for NIRS analysis	\$875.00
10 overnight shipments per month, \$28.00 per shipment	\$280.00
10 hours labor per month, \$20.00 per hour	\$200.00
Total Cost	\$1355.00

At \$18.00 cwt milk price, it would take 0.08 lbs / cow / day to pay for the cost of the program before netting out the cost of the current testing that this approach would replace.

Role of the Laboratory

Traditionally, the laboratory has been viewed as simply a provider of analytical data for ration balancing. The forage testing laboratory can be much more of a partner in the process of managing the feeding program on the farm. Opportunities for lab service are outlined below.

NIRS Capabilities

NIRS is the backbone of an intensive testing program as it is the only technology that can deliver large amounts of analytical information, quickly and cost effectively. Where NIRS may lack in accuracy compared to reference procedures, it makes up for it in providing increased precision. NIRS is used as a tool throughout many industries as part of process control as it does an excellent job of recognizing change. NIRS can be applied to the evaluation of feed variation in the same manner.

In recent years forage laboratories have provided significant amounts of additional information by NIRS. Where tests for VFA and fiber digestibility were not routine five years ago, they are now being offered by NIRS. Laboratories are in the process of developing additional methods for fiber, starch, and NFC degradability that will be used to develop NIRS equations for routine use. These new evaluations will not only have value for ration modeling, but for management of ration variability as well.

Quality of Results

The quality of forage test results across laboratories can vary. Check testing programs that laboratories subscribe to such as the NFTA (National Forage Testing Association) do not guarantee that laboratories can routinely generate good values on commercial samples. The current NFTA program only evaluates a lab's ability to obtain appropriate values on pre-dried and ground hay and corn silage samples for dry matter, protein, ADF, and NDF. The ability of check testing programs to monitor forage laboratory performance for the wide range of nutrients and feeds types that are run by forage testing laboratories will generally be inadequate.

In the future, laboratories will need to be accountable to clients by being more transparent. Information on quality control programs, standard operating procedures, and laboratory analytical error should be available upon request. Laboratories should be willing to be involved in round robin testing for the development and verification of testing procedures, especially where established methods do not exist. Laboratories should be able to provide information on the source of NIR equations that are used (developed in-house, purchased / leased), how they are updated and biased, and the statistics of the prediction models that are used. Statistics such as the number of samples in the calibration, the standard error of calibration (SEC), the standard error of prediction (SEP), and the regression coefficient (RSQ) are used to define the quality of a prediction model (NIRS Consortium, 2008).

Laboratory Information Management

Generation of analytical information on feeds and forages has been the traditional role of the feed laboratory. With the need for more information and greater frequency of analysis, the user of this information can be overwhelmed. The laboratory is in a position to provide tools for data management and analysis. Many laboratories currently provide on-line capabilities for retrieval of information, but few provide opportunity for more involved statistical analysis and reporting of client data. Figures 2 and 3 provide examples of charting variation over time in triticale silage in a large western dairy. These graphs point out relationships between nutrients and trends over time.

Table 8 is an example report that summarizes nutrient variation in TMR between April 10, 2008 and August 11, 2008. Consistency of ration delivery can be evaluated and new analyses can be tested against the normal variation seen in these TMR analyses.

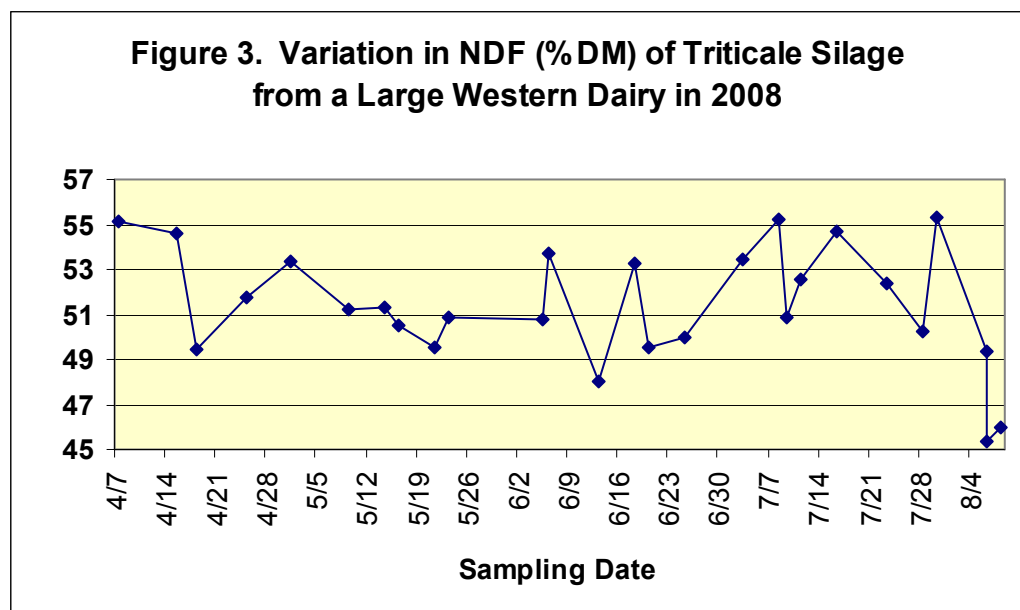
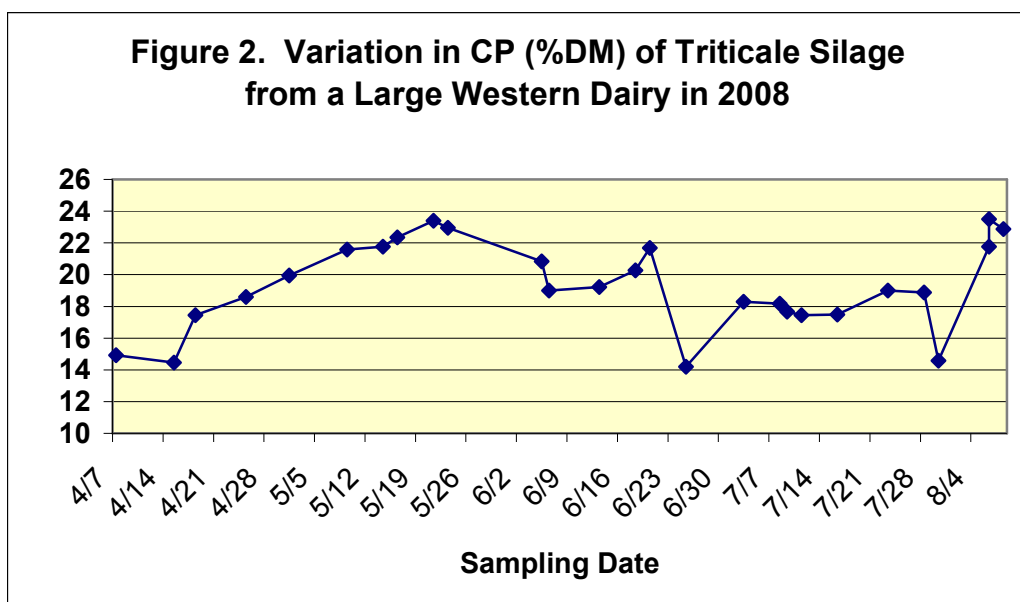


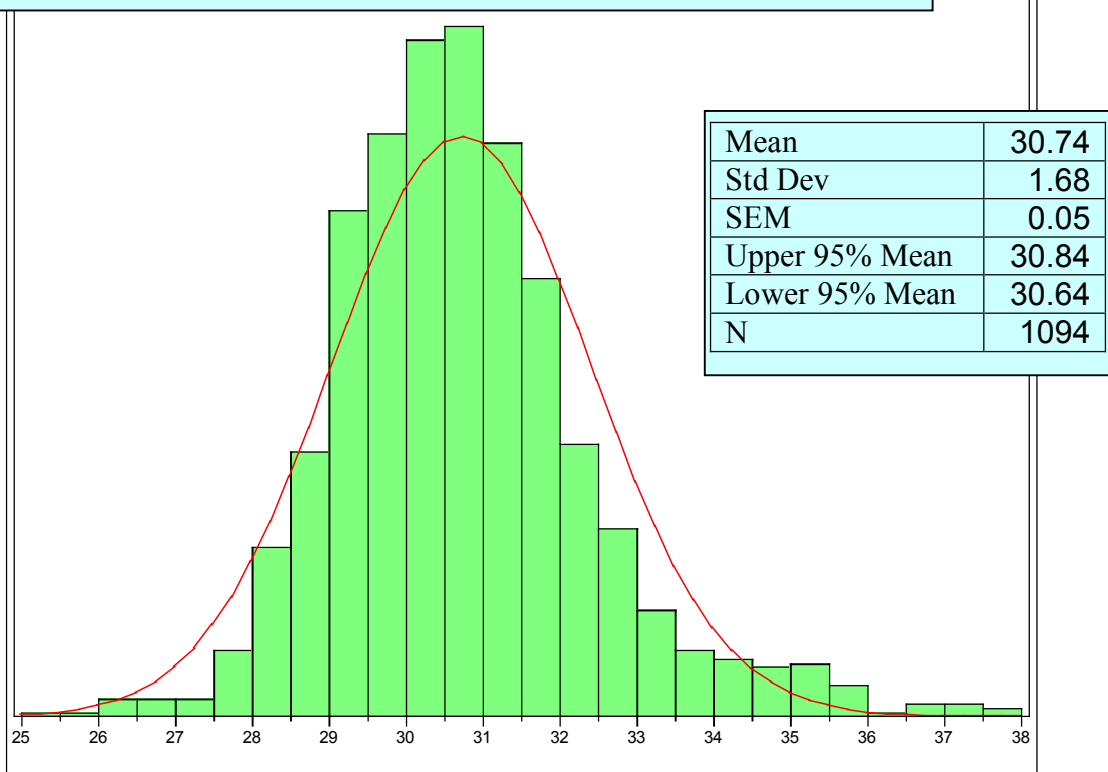
Table 8. Summary of 43 High Group TMR Analyses from a Large Western Dairy from April 10, 2008 to August 11, 2008

Nutrient (%DM)	Mean	SD	Nutrient (%DM)	Mean	SD
Dry Matter	50.2	3.21	Ash	8.2	0.52
Protein	17.5	0.59	NFC	39.8	1.31
ADF	21.7	0.96	Ca	1.04	0.37
NDF	31.9	1.19	P	0.41	0.02
Lignin	3.75	0.29	Mg	0.38	0.11
Fat	4.73	0.28	K	1.74	0.10
Starch	22.5	0.90	Na	0.38	0.10
Sugar	4.7	1.08			

Population Statistics

Defining variation on the farm requires at some level an understanding of the expected mean and ranges for nutrients by feed category. These will change depending on how the feed category is defined, by region, and by season. Laboratories can provide service to their clients by offering the means to produce summary statistics on client data as well as aggregate data. Figure 4 is an example of the range in corn distillers crude protein as observed by CVAS in 1094 samples. This frequency distribution chart visually communicates much more than a mean and standard deviation.

Figure 4. Variation in CP (%DM) of Distillers Grains Analyzed at CVAS



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