Proper Sampling and Sample Scheduling Can Prevent Reduced Milk Yields

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Summary

Nutrient composition data of ingredients are a requisite component of ration formulation. Because of extreme farm to farm variation, book or table values for nutrient composition are not adequate for home grown forages, such as corn silage, haycrop silage, and hay. These feeds should be sampled, analyzed by a reputable lab, and the individual farm data used to formulate diets, especially if they account for a significant proportion of the dry matter **(DM)** fed. This process has been used for years, but too often the importance of good sampling is ignored. For corn silage and haycrop silage, sampling variation comprised between 30 and 70% of the total within farm variation for DM, neutral detergent fiber **(NDF)**, starch, and crude protein **(CP)**. In our studies, sampling variation was greater than true day-to-day variation for haycrop CP, and corn silage starch and NDF concentrations. High sampling variation means that you should not have great confidence that a single sample of silage actually reflects the composition of what you are feeding, and using data from a single sample greatly increases the chances of formulating a poorly balanced diet. Taking the mean of composition data from multiple samples reduces the likelihood of making a large formulation error. The use of proper sampling techniques can reduce sampling variation. When sampling a feed, consider how your technique may bias the sample. Does it allow loss of small particles? Are heavy, dense particles over or under represented in the sample? For wet feeds, has the liquid phase separated from the solid phase and does your sample adequately reflect both? Suggested sampling protocols are presented in this paper. Once good sample techniques are developed and used, optimal sampling designs should be developed for each farm. A sampling design consists of: 1) how often should a forage be sampled; 2) how many samples should be taken when sampling; and 3) how much does the analytical data have to change before an intervention (i.e., ration reformulation). A software program is described that aids in determining optimal sampling designs.

Introduction

Regardless of the sophistication of the nutritional model or software used to formulate a diet, good feed composition data are essential, and the foundation of feed composition data is a feed sample. Nutrient composition of feeds is not constant; feeds must be sampled and composition data adjusted. The nutrient composition of diets can change because of changes in the nutrient composition of the ingredients or because of formulation changes by the nutritionist. At times, ingredient composition will change unknowingly (for example, the silage being fed today came from a weedy part of the field), but at other times,

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compositional changes may be expected (for example, a new load of hay was purchased or a new truckload of distillers grain was delivered). Ideally, a change in diet formulation results in a planned change in diet composition or the change was designed to maintain the nutrient profile while changing the ingredient make-up of the diet. However, if a diet is reformulated based on bad feed composition data, the nutrient composition of the diet will change and the diet will not have the expected nutrient profile. This paper will discuss the importance of good sampling in diet formulation and provide some advice on good sampling techniques and proper sampling design.

Is Sampling Error an Issue?

An ideal sample perfectly reflects the population from which it was taken. If you ground and analyzed an entire 1000 lb bale of hay and it was 19% CP, you would know the exact protein concentration of the hay (assuming the analysis was perfect), but you would have nothing left to feed. On the other hand, if you took a perfect 0.25 lb sample of hay from a 1000 lb bale and assayed it, you would know the hay contained 19% CP and still would have about 1000 lb of hay left to feed. However, if the sample was not perfect, you could obtain a CP concentration of 17 or perhaps 23%. If either of those values were used to formulate the diet, the resulting diet would not contain the desired concentration of CP.

The heterogeneity of the nutrient composition of the physical components of a feed is a major factor (probably the most important factor) related to the ability to obtain a representative sample. If a feedstuff is comprised of nutritionally uniform particles, obtaining a biased sample would in fact be extremely difficult. For example, suppose that you are sampling a container of salt (sodium chloride) that is a blend of large salt crystals and fines (salt dust), if your sample contained only large crystals or only salt dust, upon assay both samples would have about 39% sodium and 61% chloride because the individual particles of salt are nutritionally homogeneous. Many common feedstuffs, however, are comprised of physical components that are extremely heterogeneous with respect to nutritional composition. Corn silage has particles of cob, grain, leaves and stalks. The different plant components are in particles of different size and shape and have different nutrient composition (Table 1). If your sample contained a similar proportion of particles from the various plant parts as did the silage, your sample should reflect the nutrient composition of the silage as a whole. However, if your sample contained more or less stalk than the actual population (for example, small pieces of silage fell out of your hand before you put the sample in the bag, enriching the stalk portion of the sample), concentrations of starch and NDF and in vitro NDF digestibility values could change substantially (Table 2).

The concentrations of NDF in corn silage on 2 commercial dairy farms over a 14 day period are shown in Figure 1. Each data point represents a value from a single analysis of a single daily sample. From Figure 1, one could reach the conclusion that the corn silage on Farm 1 is relatively consistent with respect to NDF because its range was only 4 percentage units or about $\pm$ 2 percentage units from the mean. Corn silage from Farm 2 appears much more variable (range of 10 percentage units). An alternative and just as plausible explanation to the data in Figure 1 is that the day-to-day variation is not caused by the silage actually changing but rather by unrepresentative samples. Perhaps the person taking the samples from Farm 1 was just a better sampler than the person taking samples from Farm 2. The usual way we sample forages does not allow separating sampling variation

from real day-to-day variation. If you were formulating diets for Farm 2 (Figure 1), and you sampled on day 4, you would formulate a diet assuming the corn silage had 42% NDF. If you sampled again on day 14, you would reformulate the diet assuming the silage had 33% NDF. The silage may have actually changed; however, just as plausibly, the silage never changed and actually contains about 38% NDF.

To determine whether sampling error is a major issue in the field, we undertook a project in which corn silages and haycrop silages were sampled over 14 consecutive days on farms located near Wooster, OH (5 for corn silage and 4 for haycrop) and Ferrisburgh VT (3 for corn silage and 4 for haycrop). Every day, 2 independent samples of each silage were taken on each farm. Those samples were sent to the OARDC Dairy Nutrition Lab and analyzed in duplicate using standard wet chemistry methods for DM, NDF, starch (corn silage only), and CP (haycrop only). This resulted in 4 values for each analyte per farm per day (2 farm duplicates x 2 lab duplicates x 14 days x 8 farms = 448 analyses per silage type). This design (multiple farms, multiple days, duplicate samples, and duplicate assays) allowed us to partition the overall variation (within a silage type) into that caused by farm, sampling, and analytical. Any variation remaining was assumed to be true day-to-day variation.

As expected, farm-to-farm variation for all measured nutrients in both corn silage and haycrop silage was the greatest contributor to overall variation (Figure 2). Farm contributed between about 70 and 90% of the total variation. Although farm is by far the greatest contributor to variation, it really is not that important. Large farm-to-farm variation means that you should not take data from corn silage or haycrop silage collected on one farm and use it to formulate diets on another farm. Most nutritionists are well aware of that. Because farm-to-farm variation was not of major importance, we expressed analytical, sampling, and day-to-day variation as a percent of total within farm variation (Figure 3). With the exception of corn silage DM, analytical variation usually comprised 10% or less of the total within farm variation. Because the same procedure is used to measure DM in all feeds, the high analytical variation for corn silage DM was likely caused by subsampling error. The DM concentrations of the components of corn silage are extremely different. The average DM concentration of the ear (cob, husk, and grain) portion of corn silage is about twice as high as the DM concentration of the stover portion of silage (Hunt et al., 1989). Overall, these data suggest that analytical (or lab) variation is not a major contributor to within farm variation. However, only one lab (a research scale lab) was evaluated. Lab variation may be more or less with other labs. Sampling variation ranged from about 30 to 70% of the total within farm variation, and it was the major source of within farm variation for NDF and starch in corn silage and CP in haycrop silage. True day-to-day variation ranged from about 20 to 65% of total within farm variation, but it was especially important for DM concentraitons in both corn silage and haycrop silage and for NDF concentration in haycrop silage (Figure 4). True day-to-day variation in haycrop silage and corn silage is expected. The DM concentration of haycrop silage at the time of harvest can change over very short periods of time because of drying conditions. Multiple fields (with different drying rates) could be represented and moisture content can change because of precipitation during storage for both haycrop and corn silage depending on storage method. The proportion of within farm variation caused by day-to-day changes also was high for haycrop NDF concentration. This could be caused by multiple fields or cuttings being represented over the sampling period. Within field variation of NDF concentrations also could

be high because of changing proportions of grass and legume within the field that the silage was grown.

The very large contribution that sampling makes to within farm variation has important ramifications for ration formulation. First, high sampling variation means that a single sample of a silage is probably not a good representation of the actual silage; multiple samples are needed to obtain an accurate nutrient description of the silage. Second, high sample variation means that very often what appears to be a change in silage composition (e.g., comparing data from a sample of corn silage taken in May to one in April) actually did not occur. A nutritionist may reformulate a diet because of an apparent change in forage composition when the silage actually did not change. This reformulation based on bad data could result in a poorly balanced diet and a loss in milk yield or perhaps an increase in health problems, such as ruminal acidosis.

What Can Be Done About Sampling Error?

Sampling error could be eliminated by using a sampling protocol that always results in perfectly representative samples. Although this is likely an unobtainable goal, sampling techniques often can be improved which should reduce sampling error. We sample physical components of a feed (e.g., a piece of corn cob); we do not sample specific nutrients (e.g., a piece of CP). Therefore sampling procedures that allow for segregation of different particles will increase sampling variation if the different particles have different nutrient composition. Corn silage is arguably the most difficult feedstuff to sample properly. It is comprised of particles that differ greatly in shape, size, density, and nutrient composition. Sampling techniques that can result in an enrichment of specific types of particles include pulling a handful of silage from a face of a bag or bunker silo. Not only should the face of a bunker silo never be sampled because of the real risk of getting killed by a silage avalanche, it also can result in a biased sample. Longer pieces (usually leaves and stalks) can be stuck in the silage mass and the handful of silage you pull away will be enriched with smaller particles (likely higher starch particles) and contain fewer large pieces (likely high in NDF). Removing a sample with your palm facing down allows smaller particles to drop away, which could reduce the starch concentration of the sample and enrich its NDF concentration. Because of size and density, with movement, larger particles tend to rise to the top of a pile and small particles migrate to the bottom. Not sampling all the vertical strata of a pile could result in a biased sample.

Feeds other than corn silage also present sampling challenges. The liquid and solid phases of wet byproducts, such as wet brewers and wet distillers grains, can separate during storage. The liquid phase is obviously enriched in water compared with the solid phase, but the 2 phases also differ in NDF and total, soluble, and undegradable CP concentrations. For these feeds, using sampling techniques that ensures the sample contains similar proportions of liquid and solid as the feed is essential. Smaller, less dense particles of ground hay, especially legume hay, are enriched in CP and nonfiber carbohydrate. Rolled high moisture corn and cob meal have particles of cob (high fiber, less dense) and particles of grain which can segregate if the meal is removed from the silo and piled prior to sampling and feeding.

To our knowledge, a scientific study comparing the accuracy (i.e., how well the sample reflects the feed) and sampling variation of various sampling techniques for silages and other feeds has not been conducted. Although we do not have data showing that our method is better than other methods, we think that it

reduces many potential sampling biases. The protocols may seem laborious (and some of them are), but obtaining a good sample is absolutely essential to ration formulation.

Sampling from Bunker Silos

1. Do not sample directly from the face because of risk of a cave-in. All sampling should be done from a distance from the face of at least twice the height of the face (if the silo is 15 ft tall, stay at least 30 ft away from the face).

2. If silage is removed from the face by an end loader and directly put into the mixer wagon, the best approach would be to put several hundred pounds of silage into a clean mixer, mix for several minutes, and then discharge the contents. Take a clean 5 gal bucket and collect 10 to 15 handfuls of silage from the discharged pile, making sure to withdraw your hand from the pile with your palm facing upward. Put the handfuls into the bucket. Mix the samples within the bucket and dump the contents on a clean, smooth surface (e.g., sheet of plastic on the ground). Divide the pile into 4 or 5 sections similar to cutting a pie (the number of slices depends on the amount of sample in the bucket), and then remove all the contents of one of the slices and put it into a sample bag to send to the lab. Make sure to collect all the fines from the slice. The smaller the subsample, the less likely it will represent the feed. The sample sent to the lab should be at least the size of a softball (increase or decrease the number of slices to obtain an appropriately sized subsample). Also, be leery of putting a subsample into small sample bags. Forcing a sample into a small bag could easily enrich the sample with large pieces while smaller pieces drop to the ground.

3. If you are unwilling or unable to use the mixer wagon to blend the silage, the silage should be sampled from the loader bucket. Take a clean 5 gal bucket and collect about 5 handfuls of silage from across the loader making sure to withdraw your hand from the pile with your palm facing upward. Put the handfuls into the bucket. Repeat the same process on at least 2 or 3 loader buckets, putting all the samples in the same bucket. Then, follow the protocol outlined in Step 2 above.

4. If silage from a bunker silo is removed from the face and piled before it is put into a mixer wagon. Use the loader wagon to mix the pile prior to sampling (or ideally use a clean mixer wagon). Because vertical segregation within the pile is likely, grabbing handfuls of silage from the top of the pile may not represent the silage. Take a spade or scoop and dig into the pile at 4 or 5 locations of the pile (around the diameter and up and down within the pile). Put the contents of the shovel into a bucket, mix, and follow the procedures outlined in Step 2 above.

Sampling from Bag Silos

Sampling directly from the face is not recommended because of the very limited horizontal strata that can be sampled and because of the potential of a biased sample because of larger particles being stuck in the silage mass. It is better to sample during the feeding process. Follow the process outlined under step 2 (sampling from the bucket of the loader). As stated above, the best option would be to use a clean mixer wagon to blend the silage prior to sampling.

Sampling from Upright Silos

If silage is delivered directly from the silo into the mixer wagon, samples should be taken before and after filling the mixer. Put a container under the outlet that is large enough to collect everything, run the unloader to get 2 or 3 gallons of silage. Move the container and fill the mixer wagon. Repeat the process and collect another 2 or 3 gallons of silage. Mix the two subsamples and follow the subsampling procedure in Step 2 above.

Sampling Baled Hay

Use a sampling tool specifically designed to sample bales and make sure the teeth are sharp. Based on a statistical study of large (approximately 1000 lb) rectangular bales of alfalfa hay, 12 randomly selected bales needed to be sampled to adequately represent a single truckload (approximately 20 tons) of hay (Sheaffer et al., 2000). Each bale should be sampled once from the small end of the bale. The location of the core within the bale did not affect variation, but avoid probing within about 2 inches of the edges because the density of the bale may not be adequate to ensure representative sampling of stems. The 12 cores should be directly placed into a single plastic bag and the entire bag sent to the lab. Avoid subsampling hay cores because loss of small particles is highly likely.

Evaluating Sampling Techniques

A good sampling technique should reduce sampling error (i.e., the nutrient composition of repeated samples is similar) and should be accurate (sample results are similar to the true composition of the feed). Accuracy is very difficult to determine because you never know the true composition of the feed you are sampling. Sampling error, however, can be evaluated by repeated sampling. Consider developing a written standard operating procedure **(SOP)** for sampling. Then, over a relatively short period (1 or 2 weeks), take 4 samples of the forage following your SOP, send the samples to a good lab (use a single lab), and have the samples analyzed for DM and NDF. On larger farms that are removing substantial amounts of silage, the repeated sampling could occur during the same day (e.g., sample when feeding different pens of cows). Calculate the standard deviation **(SD)** and mean (all Spreadsheet software can do these calculations), and then calculate the coefficient of variation **(CV)** by dividing the standard deviation by the mean and multiplying by 100. This process should be done on more than one of your client's farms. Based on data we collected from multiple farms, a CV of 4% or less indicates consistent sampling. If the CV you obtained is greater than 4%, make modifications to your SOP (write down the modifications) and repeat. Once you have developed good sampling techniques, occasionally test yourself by repeating this process.

The Value of Multiple Samples

Once you have developed good sampling techniques, taking multiple independent samples of the same forage still has value. For this discussion, multiple samples refers to samples of the same silage (e.g., silage is not knowingly changing such as a different cutting) taken over a short period of time (days or a few weeks). Independent means that the repeated samples are not subsamples (i.e., they are not different slices of the same pie as described above under sampling protocols). Using the average of repeated samples for diet formulation, rather than a single sample reduces the likelihood that a really bad diet will be formulated because of bad feed composition data. Figure 4 shows the NDF concentration of corn silage from a single farm over a 14-day period. The solid line represents

data from a single sample per day from a single assay. The range, mean, SD, and CV for that line are: 9 percentage units, 36.5%, 2.61, and 7.1%. The dashed line in Figure 4 represents the mean of duplicate samples taken each day (single assay per sample). The range, mean, SD, and CV for that line are: 5 percentage units, 36.7%, 1.38, and 3.8%. Duplicate sampling had almost no effect on the overall mean but reduced measures of variation by about 50%. A single sample could have been as much as 5.2 percentage units from the overall mean; whereas, the mean of duplicate samples was at most 3 percentage units from the mean. Using means of repeated samples greatly reduces the risk of a bad sample.

Optimum Sampling Design: The Columbo software

Simply stated, an optimal sampling design for forages is one that keeps analytical and sampling costs low, and at the same time, prevents major loses in income because the diet was not formulated correctly (e.g., excess supplementation costs, lost milk production, health problems, etc.). The equations underlying the optimal sampling design assume what is known as a *renewal reward process*. Simply put, this says that the forage (ration) will not fix itself unless we intervene (i.e., adjust the ration when the forage changes). To intervene implies that we must have detected a change in the nutritional composition of the forage or feed in question. The monitoring of feed composition is done using an X-bar control chart: composition results are plotted on the Y-axis and time (dates) on the X-axis. What we want to know is:

(a) how often should we sample,
(b) how many samples should we take, and
(c) how much do the lab results have to be different from the running average before we should intervene?

The Columbo software provides the optimal answer given a certain set of circumstances. The theory and details behind this software have been published previously (St-Pierre and Cobanov, 2007a, b). Here, 'optimal' is defined as the minimum total quality cost, which is the sum of all costs associated with the monitoring of feed composition and the losses incurred when the forage has changed, but we have not intervened yet (reduced milk production or greater feed costs). To determine the optimum sampling design, Columbo requires 12 inputs (Figure 5). Fortunately, not all 12 inputs have the same importance, and we provide default values that will work well in most instances. However, 3 inputs are particularly important:

1. *The number of cows in the herd*. This is because a single feed analysis costs the same whether one milks 50 or 1,000 cows, but a drop of 2 lb/cow/day in milk production has a 20-fold greater impact on herd production (and income) for the latter.

2. *Milk price*. This too is related to the economic loss due to a drop in production.

3. *Milk production loss when feed composition changes*. This input is a little more difficult to figure out. What it means is: by how much would daily production per cow change if the feed changed by the magnitude that we want to detect? For example, if the corn silage NDF was to go from 38 to 42% of DM, what would this do to milk production? One way to answer this is by changing the composition of the forage in the ration balancing software and then look at how much this affects energy and protein allowable milk. Based on previous research, we have conducted (McBeth et al., 2013; Yoder et al., 2013) a short term change (probably 1 or 2 days) in diet composition appears to have little impact on cows; however, over longer periods, an imbalanced diet will reduce milk yields.

There are instances when you are not quite sure about the correct inputs. For example, you really do not know what the price of milk will be in the next 12 months when you are setting up a yearly sampling plan. Or, you really do not know how much the composition of the forage will change over that time period. Columbo has an *Uncertainty Optimizer* module to figure out the best sampling design over a range of multiple uncertain inputs. The downside to using this function is that it requires a huge amount of calculations. Hence, it takes a couple minutes to get the answer, even on a high-speed computer.

Columbo also allows fixing some parameter values. For example, suppose that the optimum schedule would be to sample every 6 days. You might think that sampling every 7 days – always on the same day of the week – would be far more manageable. You could fix the sampling frequency parameter (using the parameter fixing tab) to 7 and re-optimize. The total quality costs will be greater when you fix a parameter, but we have found that fixing only one (and sometimes 2) does not materially change the total costs. In other words, there are many near optimal sampling designs that are just equally as good; some are more convenient than others. As for what to monitor, it really seems that monitoring the moisture and NDF contents of forages would capture nearly all significant changes in forage composition (for diets based heavily on alfalfa silage, its CP concentration also should be monitored). The Columbo software is available free of charge at http:// **dairy.osu.edu** (Click on the heading, "OSU Dairy Computer Software" located on the right size of the screen).

Conclusions

Good samples are the cornerstone of good diet formulation. If sampling technique is poor and the uncertainty surrounding feed composition data is expressed as plus or minus several percentage units, using nutritional models that formulate diets to the tenth decimal place will not result in well formulated, consistent diets. Good SOP for sampling should be developed and followed. Multiple samples of feeds should be taken to monitor sampling variation and averages of composition data should be used rather than data from a single sample to reduce the impact of improper sampling. Software is available to develop optimal sampling designs for specific farms which should help increase overall farm profitability.

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Table 1. Concentration and 30 hr in vitro digestibility (IVNDFD) of NDF in corn silage and its component parts (Thomas et al., 2001).

	% of Plant DM	NDF, % of DM	IVNDFD, % of NDF
Cob	6.5	84.0	55.8
Grain	49.8	11.0	89.7
Husk	5.6	80.3	62.2
Leaves	12.3	63.6	64.5
Stalks	25.1	76.7	39.2
Tassel	0.7	78.1	32.8

Table 2. Hypothetical effects of biased samples on concentration and 30 hr in vitro digestibility of NDF (IVNDFD) of corn silage.

		Biased Sample[2]	
	Representative sample[1]	Extra stalk	Less stalk
% of Whole plant DM	100	100	100
Cob	6.5	5.8	7.2
Grain	49.8	44.3	55.3
Husk	5.6	5.0	6.2
Leaves	12.3	10.9	13.7
Stalk	25.1	33.4	16.8
Tassel	0.7	0.6	0.8
Whole plant NDF[3], % of DM	43.0	46.8	39.3
Whole plant IVNDFD[3], % of NDF	54.6	56.3	53.0
Whole plant starch[4], % of DM	34.9	31.0	38.7

[1]Plant proportions and concentrations of NDF and IVNDFD of the components are from Thomas et al. (2001).

[2]The Extra Stalk biased sample has 33% more stalk than the representative sample (all other components were decreased proportionately) and the Less Stalk biased sample as 33% less stalk than the representative sample.

[3]Whole plant NDF and IVNDFD data were calculated from weighted means of the nutrient data (Table 1) of the plant components.

[4]Whole plant starch data are not from Thomas et al. (2001). Those values were calculated assuming grain contained 70% starch and all other plant parts contained 0% starch.

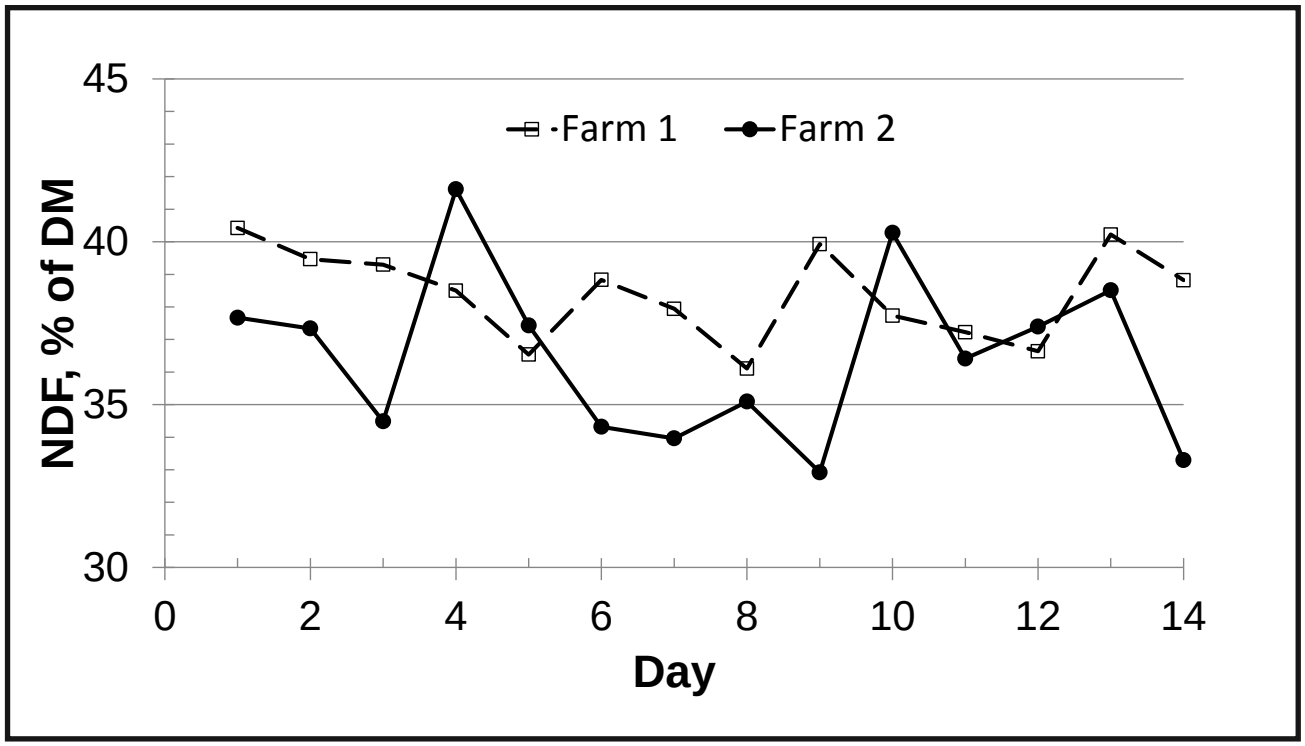

Figure 1. The concentrations of NDF in corn silage from 2 different farms near Wooster, OH. The silages were sampled daily over a 14-day period. Each data point represents the value from a single assay of a single sample. The coefficient of variation (CV) for Farm 1 is 3.7% and 7.1% for Farm 2. Based on the data shown, it is unknown whether the difference in variation between farms is caused by sampling error or true day-to-day variation.

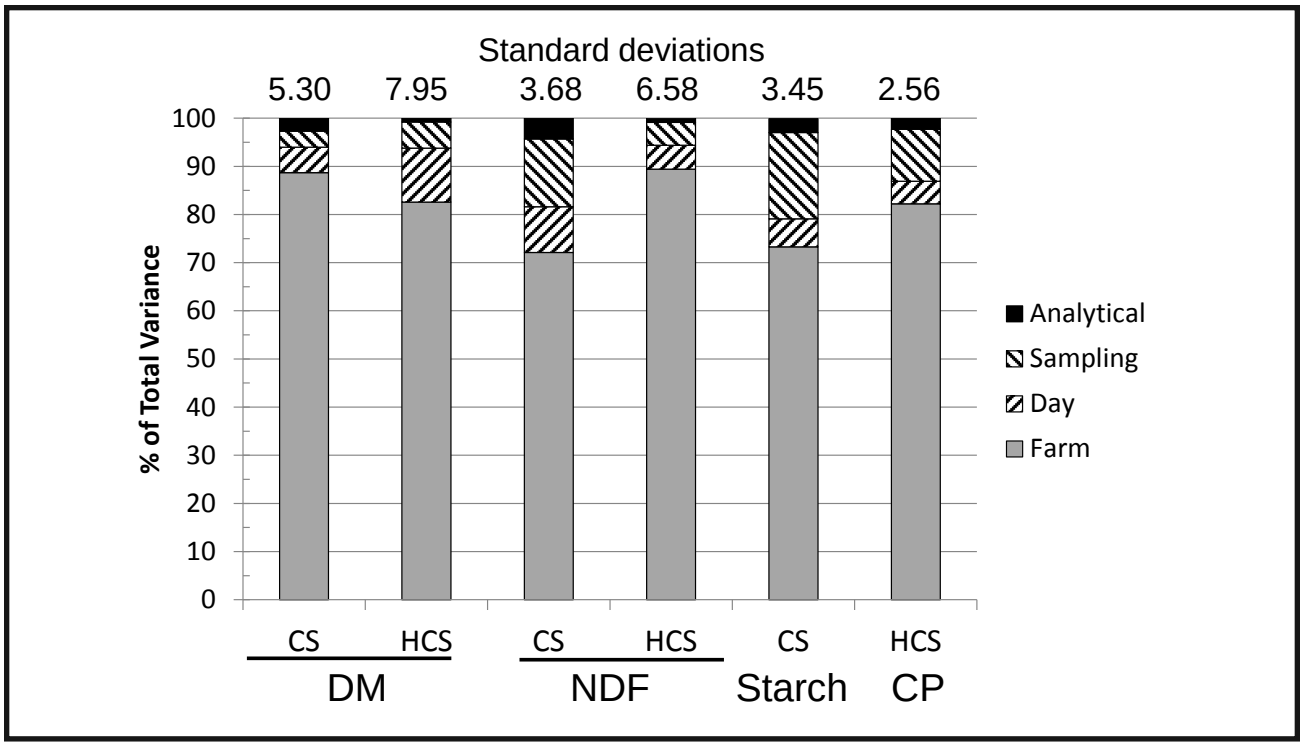

Figure 2. Partitioning total variation from sampling corn silage (CS) and haycrop silage (HCS) at multiple farms (8 farms for each silage type) with duplicate daily samples (over 14 days) and each assay duplicated by a single lab (448 samples per silage type). Farm-to-farm variation contributed 70 to 90% of the total variation.

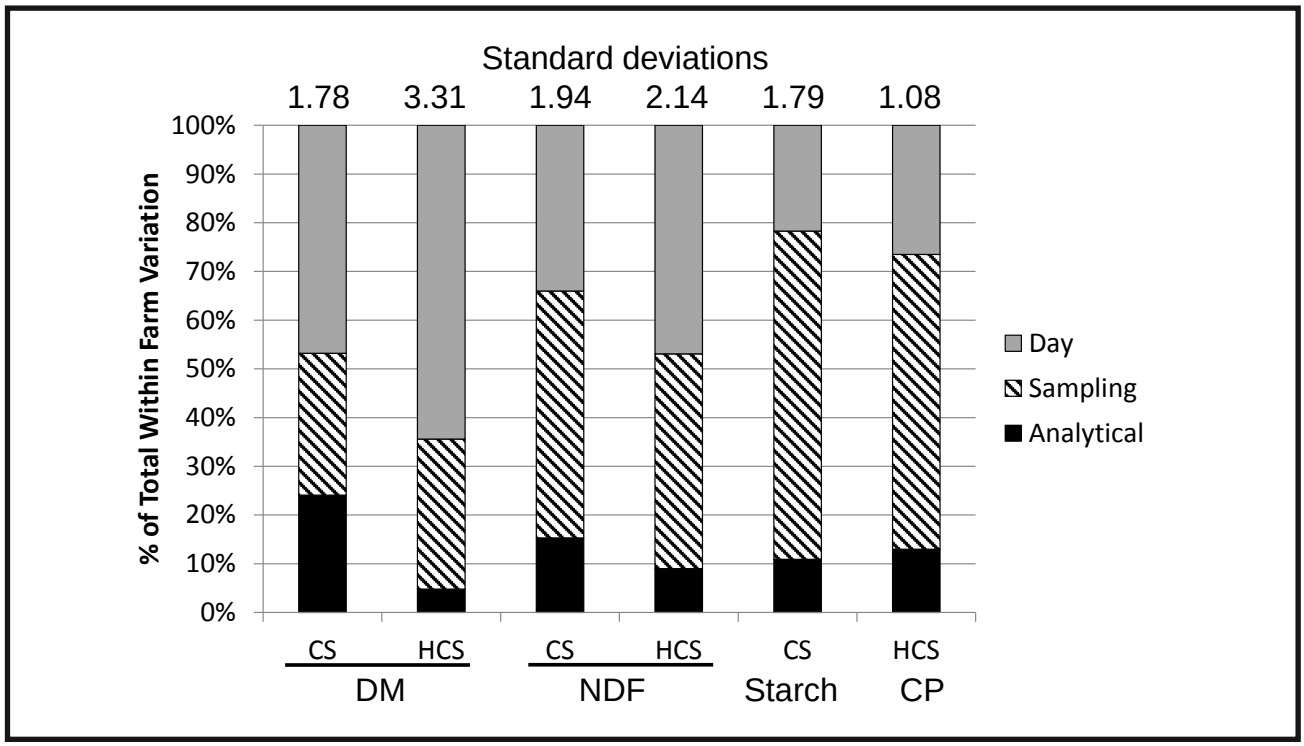

Figure 3. Partitioning within farm variation for corn silage (CS) and hay crop silage (HCS) with duplicate daily samples (over 14 days) and each assay duplicated by a single lab (448 samples per silage type). Sampling and analytical variation were the major sources of variation.

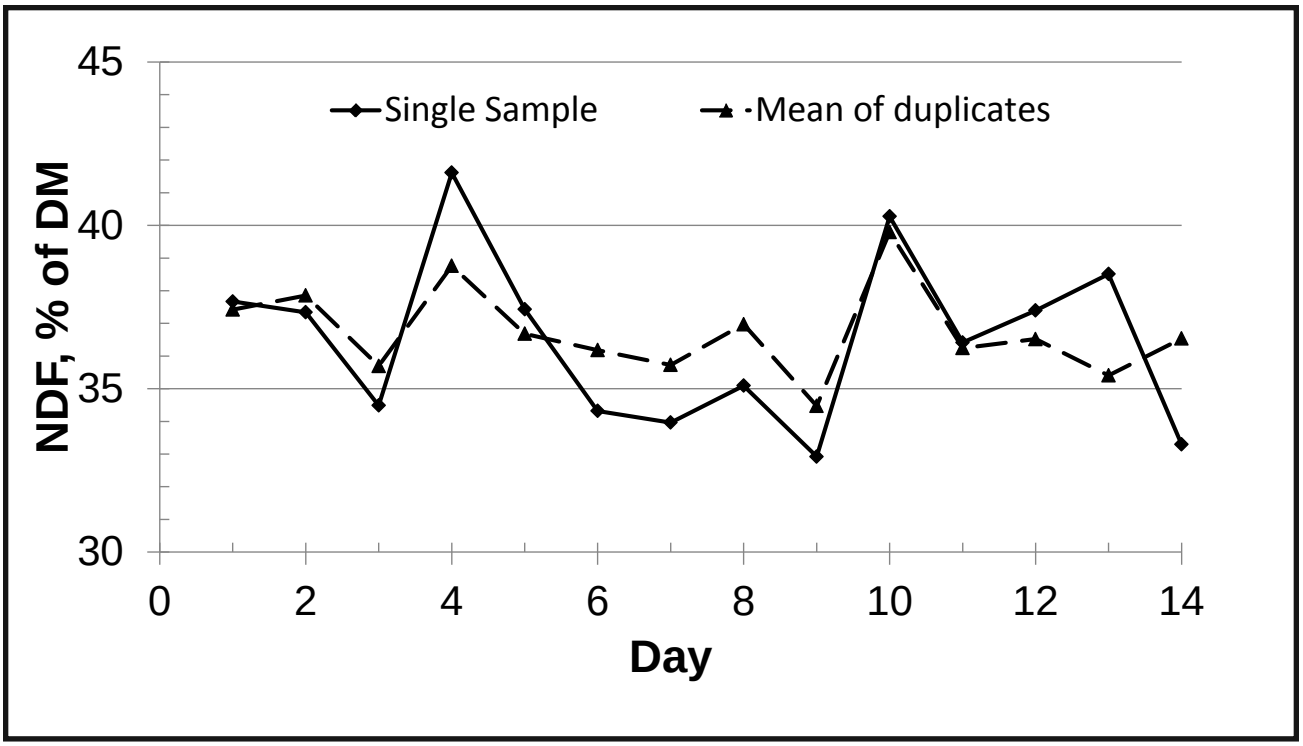

Figure 4. Effect of duplicate daily sampling on reducing variation in corn silage NDF concentration. The data are from a single farm. The solid line is data from a single assay of a single daily sample (Farm 2 data from Figure 1). The dashed line is the mean of the sample used in the solid line plus its duplicate sample. The coefficient of variation for the single sample line is 7.1% and 3.8% for the duplicate sample line.

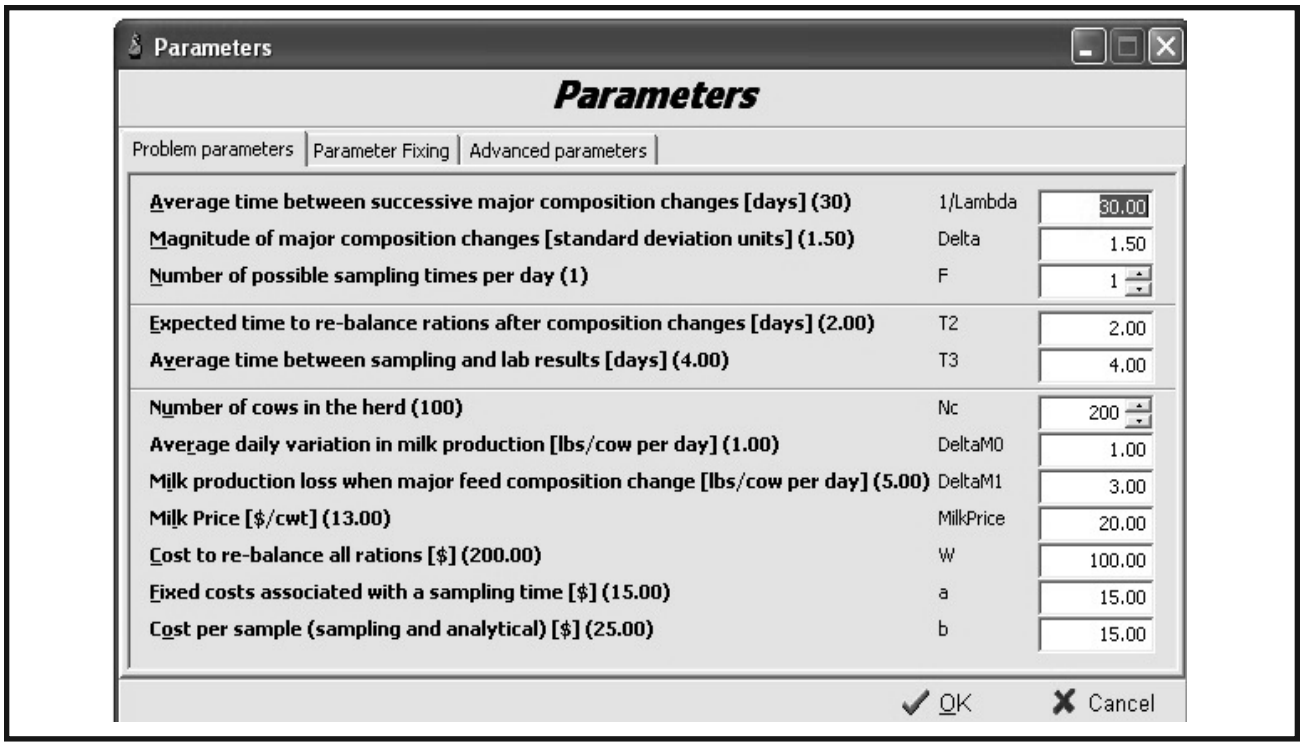

Figure 5. Snapshot of the data entry screen of Columbo, a software program to determine an optimal sampling design.