Variation in Nutrient Composition of Ingredients and TMR and Does it Matter to a Cow?

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Introduction

Regardless of the sophistication of the nutritional model or software used to formulate a diet, good feed composition data is essential, and the foundation of feed composition data is a feed sample. Nutrient composition of feeds is not constant; feeds must be sampled repeatedly. 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 compositional changes may be expected (for example, a new load of hay 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, provide some advice on good sampling techniques and discuss effects of diet variation on cows.

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 lbs 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 corn cob, corn grain, corn leaves and corn stalks. The different plant components are in particles of different size and shape and have different nutrient composition (Table 1).

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

	Proportion of plant DM,%		
	% 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

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 two 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.

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

	Representative sample[1]	Biased Sample[2]	
		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.

To determine whether sampling error was 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 allowed us to partition the overall variation into that caused by farm, sampling, and analytical. Any variation remaining was assumed to be true day to day variation.

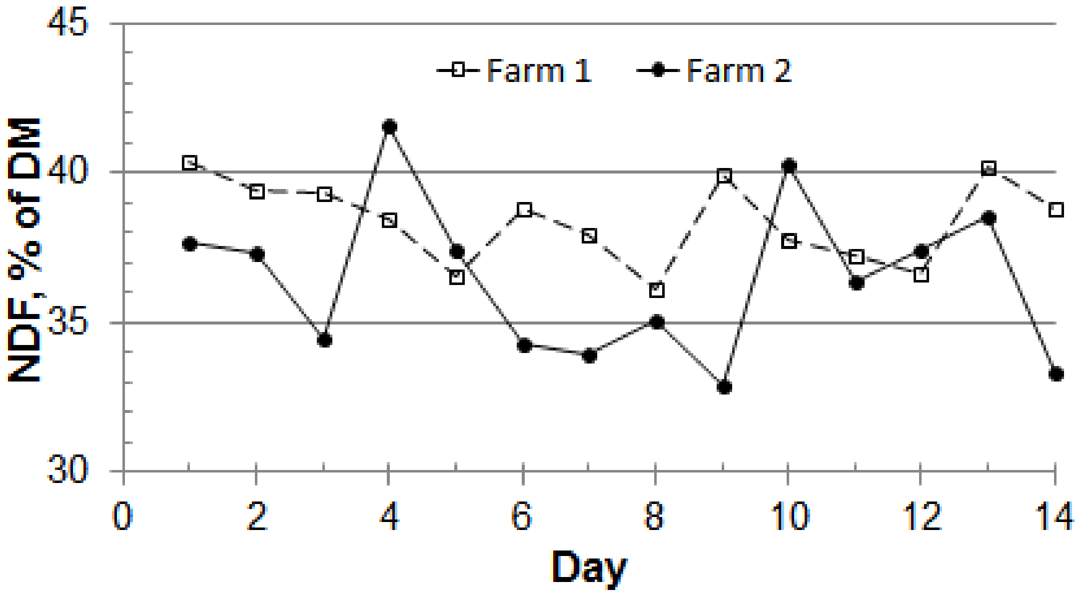

Figure 1. Concentrations of NDF in corn silage from two different dairy farms over a 14 day period. Each data point represents the value from a single assay of a single sample. The coefficient of variation (CV) for Farm 1 is 3.7% and 7.1% for Farm 2.

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, this data suggest that analytical (or lab)

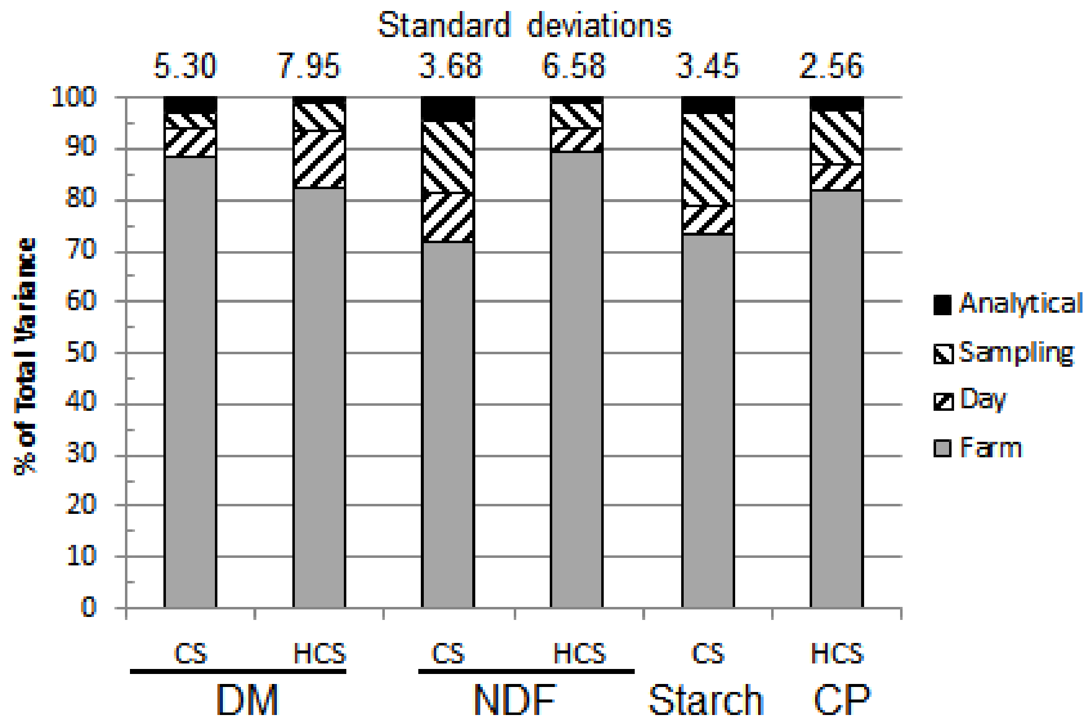

Figure 2. Partitioning total variation from sampling corn silage (CS) and haycrop silage (HCS) from multiple farms over a 14 day period.

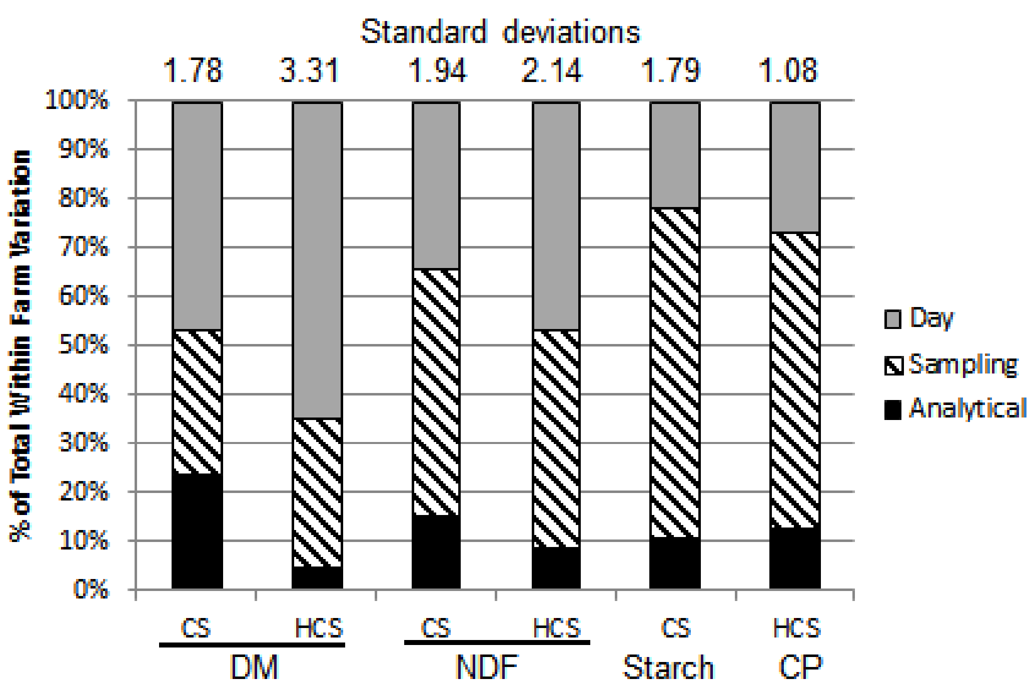

Figure 3. Partitioning within farm variation for corn silage (CS) and hay crop silage (HCS) with 14 daily samples and each assay duplicated by a single lab.

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. It was the majority source of within farm variation only for haycrop DM concentration, but the proportion of within farm variation from day to day variation was also high for corn silage DM. True day to day variation in haycrop silage and corn silage DM 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 was also 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 could also be high because of changing proportions of grass and legume within the field that the silage was grown.

The very large contribution 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 increases health problems such as ruminal acidosis.

What Can Be Done About Sampling Error?

Sampling error can be eliminated by using a sampling protocol that always results in perfectly representative samples. Although this probably is an unobtainable goal, sampling techniques often can be improved which should reduce sampling error. Mix what you going to sample as much as possible before sampling. If you take a grab sample from the face of a bag of corn silage, the sample represents that specific site in the silo. However if you take several loader buckets of the silage, put it in a mixer wagon and sample that, your sample represents a substantially larger amount of silage. 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 allow 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 two 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.

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 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 mean samples of the same silage taken over a short period of time (days or a few weeks). Independent means that the repeated samples are not subsamples. 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 dashed 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 solid 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.

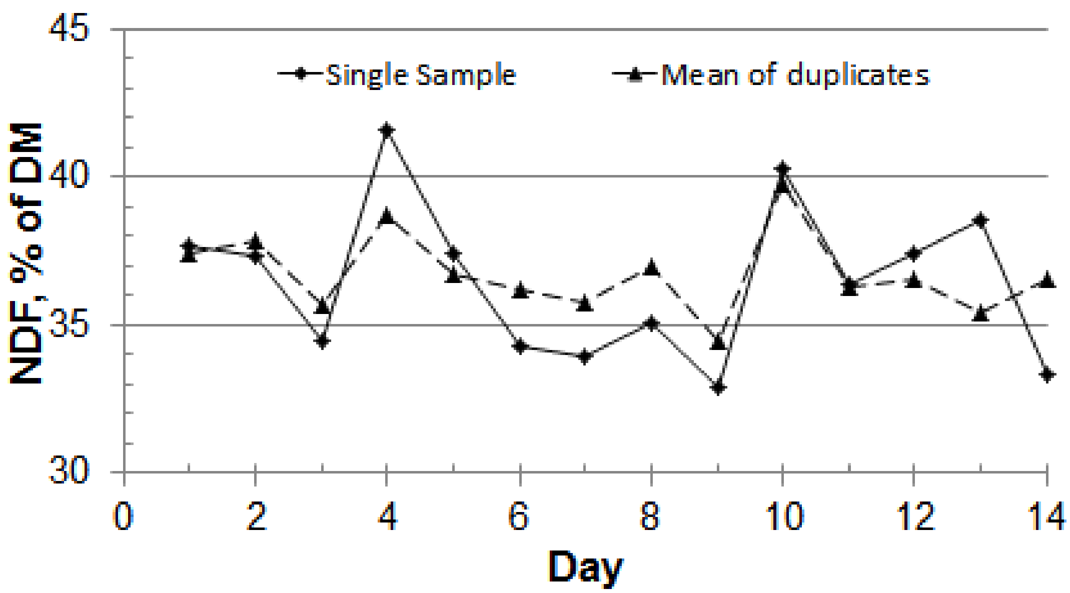

Figure 4. Effect of duplicate daily sampling on reducing variation in corn silage NDF. The solid line is data from a single assay of a single daily sample. The dashed line is the mean of the sample used in the solid line plus its duplicate sample. The coefficient of variation for the Single sample line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

Does Variation Matter to a Cow?

Although sampling error is a major cause of short term variation in composition of feed ingredients and TMR, feeds do have real variation. If you have read articles or attended conferences about dairy cattle nutrition, you have likely heard or read something to the effect, "cows do better when fed a diet that is consistent day to day". Although this seems to make sense, essentially no research has evaluated the effect of diet inconsistency on dairy cows. In the past few years we have conducted 4 studies at Ohio State to address the question, does short term variation or transient changes in diet composition affect dairy cows. We have evaluated effects of varying silage dry matter concentration (McBeth et al., 2013) and dietary concentrations of long chain fatty acids (Weiss et al., 2013), crude protein (Brown and Weiss, 2014), and forage NDF (Yoder et al., 2013). Extreme variation in concentrations of dietary fatty acids (from corn oil and distillers grains) reduced dry matter intake and milk yield but considering the degree of variation (diets changed from 4.8 to 7.0% long chain fatty acids), the effects were small. In another experiment cows were fed a diet with 16.4% crude protein (CP) or 13.4% CP every day or a diet that contained 10.3% CP for 2 days followed by a diet with 16.4% CP for 2 days over a 28 day period. The average CP concentration of the oscillating treatment was 13.4%. Concentrations of milk urea nitrogen accurately reflected the oscillation in dietary protein however it had a 1 day lag. Milk yield also followed a cyclic pattern in cows fed the oscillating treatment, but average milk yield for the entire period was not significantly different between treatments (78, 76, and 74 lbs/day for cows fed the 16.4%, 13.4% or oscillating treatments). Although not statistically different, if the experiment went longer, milk yield by cows on the oscillating treatment would likely be lower. Even though milk yield was likely reduced because of variation in dietary protein concentration, the imposed variation was extreme (10.3% to 16.4% CP).

Effects of transient variation in silage dry matter

Transient changes in silage DM concentrations can occur because of weather events (e.g., unprotected silage in a bunker gets rained upon); therefore, this experiment (McBeth et al., 2013) was conducted to determine whether short terms changes in silage DM affected cows and whether as-fed rations should be adjusted to account for the short term change in silage DM. One treatment was a consistent diet over the 21 day experiment that contained 55% forage (2/3 alfalfa silage and 1/3 corn silage) on a DM basis and 45% concentrate. The second treatment was the same as the first treatment except during two 3-day bouts when wetted silage was fed. Wetted silage was made by adding enough water to the mix of alfalfa and corn silage to reduce its DM concentration by 10 percentage units. During those two 3-day bouts the wetted silage replaced the normal silage on an equal as-fed basis. Because the silage was wetter, the forage to concentrate ratio during the bouts for this treatment was reduced to 49:51 on a DM basis. During the bouts the NDF concentration was lower for this treatment and the starch concentration was higher. The third treatment was the same as the second treatment except that during the bouts the amount of as-fed forage offered was increased to maintain the same forage to concentrate ratio, and concentrations of NDF and starch (on a DM basis) as the control diet. Over the 21 day experiment, DM intake of the two wet silage treatments did not differ from the control but milk yield was higher than control for the unbalanced, wetted silage treatment (87.6 vs. 86.5 lbs/day). The increased milk yield is likely in response to the increased concentrate in the diet during the bouts. Milk yield was the same for cows fed the control or fed the diet with wetted silage that was reformulated to account for the added water. **In this experiment, cows were offered excess feed so that when the wetter diets were fed, the cows did not run out of feed.** This approach was likely the reason we did not observe any negative effects. When fed the wetted silage, as-fed intake of the cows increased immediately; this could not have happened if excess feed was not offered to the cows. As-fed intake continued to increase during the second day of the bouts and it was not until the second day of feeding wetted silage that DM intake returned to normal for those cows.

An interesting finding of this experiment, which also has practical application, is the intake pattern of cows when they switched from the wetted silage back to their normal diet. The day following each bout, DM intake was higher than control. Cows appeared to consume about the same amount of as-fed feed on the day when they returned to the normal DM silage but because the diet was drier, DM intake increased compared to control. This implies that extra feed should be offered to cows when they are switched from wet silage back to the normal silage. From our study, rebalancing diets for a short term (a few days) change in silage DM is not necessary. However, increasing the amount of feed offered is probably important to maintain production, and excess feed should be offered for a day or two after the silage DM returns to normal.

Extreme Day to Day Variation in Forage Quality

Because of variation within fields, the composition of a mixed legume-grass silage can be extremely variable. This experiment (Yoder et al., 2013) was conducted to evaluate the effects of extreme daily variation in forage quality. The experiment had 3 treatments but because of space limitations, only 2 treatments will be discussed. One treatment was the control and forage quality was as consistent as possible day to day (SD for dietary concentration of forage NDF = 0.5). The second treatment (Variable) had a constant forage to concentrate ratio (same as the control), but the ratio of alfalfa to grass varied daily in a random pattern resulting in large variation in the concentration of forage NDF in the diet (fNDF SD = 2.0). On average, over the 21 day period, treatments were equal in percent forage, alfalfa to grass ratio, forage NDF (25%), CP, and starch.

Over the 21 day experiment, cows on the Variable treatment consumed similar amounts of DM and produced similar amounts of milk compared to the Control. Daily within cow variation in milk yield and DM intake were significantly greater for cows on the Variable treatment compared with Control. Based on other measurements we made, there are two likely reasons cows were not negatively affected by extreme daily variation in forage quality in this study. First excess feed was provided to cows every day. On days when cows were fed a high forage NDF diet, dry matter intake was reduced (high feed refusal) but then on days when lower forage NDF diets were fed, the excess feed delivery allowed cows to consume additional feed. Effects of diet variation were also probably mitigated by transient mobilization of body energy. On days when cows were fed high concentrations of grass (i.e., lower quality forage), DM intake was reduced but cows mobilized energy to maintain milk yield. On days when cows were fed a better diet (more alfalfa and less grass), cows ate more and produced more milk. This suggests that over a longer time period (this experiment only lasted 3 weeks) a highly variable diet could reduce body condition which can have long term negative impacts on reproduction and production. Unquestionably, long term losses in body condition is a negative, the very modest potential effects on body condition must be put in context of the extreme variation imposed in this experiment.

Conclusions

Good samples are the cornerstone of good diet formulation; however sampling error for some feeds is large. 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. Although sampling is a major source of variation in diet composition, real variation does exist but substantial day to day variation in nutrient composition did not have large negative effects on cows. This may mean that a 24 hour day is not the correct periodicity for assessing variation. Some of our data suggest that a period of 2 or 3 days may be more appropriate. In other words, if nutrient composition differed between two successive 3-day periods, cows might be more likely to respond to that variation. We have some evidence that diet variation may have cumulative negative effects and that over a longer term (months), negative effects of variation may increase. A key management factor that appeared to reduce the effects of variation was ensuring cows had access to adequate feed on all days. If the diet changes and cows need to consume more feed (e.g., the diet becomes wetter) or the diet changes and the cow can consume more feed (e.g., diet changes from a higher concentration of NDF to a lower concentration), feed must be available to allow the cow to compensate. If this compensation cannot occur, the effects of variation would likely be exacerbated. Although providing excess feed may mitigate some negative effects of variation, it will also increase feed costs.

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Notes: