UNIFORM SAMPLING PROTOCOLS FOR SINGLE- AND DOUBLE-COMPRESSED HAY AND DETECTION OF A GMO TRAIT

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ABSTRACT

Forage quality has large economic influences on the price of alfalfa hay as well as animal performance. Although visual inspection for weeds, mold, and texture remain important to judge quality, laboratory analysis is a critical component of quality prediction. Thus, there is a need for uniform and standardized sampling protocols for testing for forage quality. Long-distance transport, use of large bales, increased import-exports, and introduction of Genetically-Engineered (GE) crops have provided increased emphasis on sampling and testing, especially for highly-compressed bales. Experiments were conducted to examine the ability to test highly compressed bales, and to measure the differences between pre- and post- compression hay. We found little to no differences between pre- and post-compression tests at three commercial hay press facilities in the US. However, individual probe-to-probe differences were large, similar to the differences observed in non-compressed bales, emphasizing the need for multiple composite samples. Principles for standardized sampling include 1) Proper identification of hay lots, 2) Random method of bale sampling, 3) selection of a proper coring device, 4) Correct sampling procedure, 5) Taking sufficient numbers of cores for a composited cored hay sample, and 6) correct handling of samples before analysis. Sampling for a low level presence (LLP) of a GE trait distributed throughout the crop mass requires methods that utilize similar principles as for quality sampling, but with additional samples required, depending upon desired level of detection. Practitioners should consider the large effect of sampling on both hay quality determination as well as detection of LLP in hay masses.

INTRODUCTION

Hay sampling principles have been worked out over decades of experience by university scientists and industry members. The objective has been to obtain a representative sample for lab analysis (e.g. ADF, NDF, CP, NDFD) to estimate feeding value and economic worth. The concept is that the sample must fairly represent the average quality of the hay mass. The principles of proper sampling have been promoted by representative hay groups, National Forage Testing Association (NFTA, National Alfalfa and Forage Alliance, National Hay Association and American Forage and Grasslands Council, as well as state hay groups, and a 'certification of methods' protocol is available on-line (see www.foragetesting.org. and http://alfalfa.ucdavis.edu, and Putnam, 2002). Sampling must represent the variation in leaf, stem, weeds, as well field variation due to soil and environmental effects. Proper sampling technique is generally the most important determinant of accuracy and repeatability of hay quality tests.

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Long-distance transport, use of large bales, increased import-export activity, and introduction of genetically-engineered (GE) crops have provided new challenges to hay testing, especially for highly-compressed bales. There have been a series of questions about the reliability of compressed vs. non-compressed hay sampling methods. Further, sampling for detection of a GE trait may require different considerations. In this paper, we report on a study of sampling of double compressed hay bales, and we discuss the implications for sampling for forage quality as well as sampling for Low Level Presence (LLP) of a genetically modified trait.

SAMPLING DOUBLE-COMPRESSED BALES

In the past 10 years, international trade in alfalfa and grass hays has become commonplace on the west coast of the US, with exports exceeding 11% of production of alfalfa hay and over 30% of grass hays in the 7 western US states (N. Gombos, 2011; Putnam et al., 2015). Long distance trade in other regions (e.g. EU, Middle East, Australia, Argentina, and China) is also becoming more common. Long-distance sales of hay within countries have increased, with buyers often purchasing based upon test alone.

Bales that are exported frequently go through an additional compression step (Double Compression) before being loaded for export or for domestic long-distance transportation (Figure 1). These bales are sufficiently dense that sampling is difficult. In addition, buyers are unsure whether the sample that is generated from stack sampling before compression (utilizing the standard NFTA protocol) matches the hay as delivered after compression and shipping. Since most hay is generally ONLY sampled in the stack, it would be useful to know whether that sample matches samples taken after compression.

Figure 1. Single compressed, baled hay (left) is often cut and re-packaged to a more compressed bale for long-distance transport. These bales present challenges for sampling.

We conducted several experiments on double-compressed hay to determine:

1. Adequacy of techniques for sampling double-compressed alfalfa hay.
2. Whether forage quality pre-compression generally matched post-compression quality analysis.
3. To describe the level of variation in double compressed alfalfa hay.

Hay was sampled at each of three locations: HayDay Farms in Blythe, CA, and ACX and Anderson Hay located in Long Beach, CA. The authors are grateful for the cooperation of these businesses in assisting in this project. Two alfalfa lots per compression unit (high quality and medium quality) were chosen. Two large bales (approximately 3500 lbs. total) were used as starting material as the sample from each of these lots to minimize the range of variation for the before vs. after compression comparison. Hay probes tested at ACX and Anderson Hay were Star Quality drill-driven hay sampler with 16" stainless steel probe, and two push-type probes (long probe with a length of 18" and short probe at approx. 12-14").

In each trial, 20 individual cores plus 1 additional composite sample made up of 20 cores combined were taken from the high and medium quality lots both before and after compression. A composite of 10 cores were used to compare forage quality between the push-driven and drill-driven hay samplers. Samples were then ground in a Cyclotec grinding machine with a 1.0 mm screen, and analyzed using NIRS for crude protein, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Digestible NDF at 30 hrs. (dNDF30), Relative Feed Value (RFV) and Total Digestible Nutrients (TDN).

RESULTS OF COMPRESSED BALE TESTS

One of the key questions in this set of trials was to compare pre- with post-compression forage quality results. Forage quality results were obtained from two different quality lots before and after compression at three locations in California. These represent a large number of samples – 20 samples (10 in each of 2 bales) taken before compression and 20 samples taken after compression at each of three locations, with two types of bales (high and medium quality) at each location. Bales were marked carefully so that we sampled the same bales that were sampled previously (before and after compression).

Table 1. Location 1-HayDay Farms, Blythe, CA. Forage Quality of Non-compressed and Double Compressed Alfalfa

High Quality Bales	Crude Protein	ADF	aNDF	dNDF30	TDN	RFV
Non Compressed	22.3	27.1	33.0	12.4	69.7	191.8
Double Compressed	22.3	26.9	32.6	12.6	69.9	194.3
Mean	22.3	27.0	32.8	12.5	69.8	193.1
CV%	2.7	3.4	3.4	2.9	1.4	4.5
LSD (p=0.05)	ns	ns	ns	ns	ns	ns
Medium Quality Bales	**Crude Protein**	**ADF**	**aNDF**	**dNDF30**	**TDN**	**RFV**
Non Compressed	18.3	34.4	43.0	15.2	61.9	134.8
Double Compressed	18.8	32.4	40.9	14.2	64.0	145.5
Mean	18.5	33.4	42.0	14.7	63.0	10.8
CV%	6.1	5.5	5.3	5.8	3.1	7.7
LSD (p=0.05)	ns	1.1	1.3	0.5	1.2	6.5

Effect of Compression: In most cases there was little or no effect of double compression on forage quality at these three sites, utilizing two types of bales (higher and lower quality bales) – see Tables 1,2, and 3. Where there were small (statistically significant) differences in measured

values, there was often a very minor improvement in quality due to compression (Location 1, high and medium quality bales, Location 2, high quality bales, Location 3 high and medium quality bales). In one case there was a minor decline in quality (Location 2, medium quality bale). We do not know of a mechanism which may improve forage quality in double compressed bales, in fact we would generally expect that compression may result in some leaf loss, so that decline in forage quality would be hypothesized. Since all of these differences were very small (from a practical perspective), and there were as many or more incidences of positive effect on quality than negative effect, we must come to the conclusion that compression had little or no effect on quality, as measured before and after compression.

Table 2. Location 2 - ACX, Long Beach, CA. Forage Quality of Non-compressed and Double Compressed Alfalfa

High Quality Bales	Crude Protein	ADF	aNDF	dNDF30	TDN	RFV
Non Compressed	21.5	30.0	38.2	12.2	66.6	159.6
Double Compressed	21.9	29.9	38.3	12.6	66.7	160.0
Mean	21.7	30.0	38.3	12.4	66.6	159.8
CV%	4.2	4.6	4.5	5.5	2.2	5.9
LSD (p=0.05)	ns	ns	ns	0.4	ns	ns
Medium Quality Bales	**Crude Protein**	**ADF**	**aNDF**	**dNDF30**	**TDN**	**RFV**
Non Compressed	19.4	30.1	37.1	11.8	66.5	165.3
Double Compressed	19.3	31.7	38.9	12.9	64.7	154.3
Mean	19.4	30.9	37.9	12.3	65.7	160.2
CV%	7.5	7.5	6.8	5.6	3.8	9.8
LSD (p=0.05)	ns	1.3	1.4	0.4	1.4	8.8

Table 3. Location 3 - Anderson Hay, Long Beach, CA. Forage Quality of Non-compressed and Double Compressed Alfalfa

High Quality Bales	Crude Protein	ADF	aNDF	dNDF30	TDN	RFV
Non Compressed	**20.0**	**30.4**	**37.5**	**13.5**	**66.2**	**162.8**
Double Compressed	**20.4**	**29.9**	**37.1**	**13.2**	**66.7**	**165.6**
Mean	20.2	30.1	37.3	13.4	66.4	164.0
CV%	5.9	6.6	7.1	6.2	3.2	9.1
LSD (p=0.05)	ns	ns	ns	ns	ns	ns
Medium Quality Bales	**Crude Protein**	**ADF**	**aNDF**	**dNDF30**	**TDN**	**RFV**
Non Compressed	**17.2**	**35.8**	**43.4**	**14.3**	**60**	**131**
Double Compressed	**17.5**	**34.7**	**42.2**	**14.2**	**62**	**137**
Mean	17.3	32.3	42.8	14.3	61.0	134.0
CV%	4.3	4.8	4.7	4.5	2.9	6.7
LSD (p=0.05)	ns	1.0	1.2	ns	1.0	5.2

In the types of compression processes in these plants, utilizing these methods, tests obtained before compression should adequately predict forage quality results after double compression process.

Caveats and discussion. A few caveats should be added here. These compression methods were different from each other in that different equipment was used—they were different machines—but generally were similar in their handling and processing of the bales. Each one of these machines resulted in wrapped 'large' bales. In these cases, it appears as if minimal disruption in the leaf-stem ratio occurred. One would normally expect that if double compression occurred, that some leaf loss would lead to reductions in quality due to the loosening, cutting, and re-cutting and packaging that occurred. However, this did not occur, at least the data does not reflect that. To the contrary, if there was a trend, sometimes it was towards an improvement in quality. Since these differences were so minor, these slight trends appear to be simply random trends, we come to the conclusion that the effects of compression were negligible.

However, in some systems, compression steps may involve additional chopping or mixing steps (for example to blend different forages), or repackaging methods which have additional impacts on leaf-stem ratio. In those cases, we would expect that pre-testing would not reflect the forage quality of post-compression steps and that sampling the compressed hay after compression would be warranted.

An additional caveat is the difference between the effects of compression and the (more random) effects of sampling large lots. In this experiment, we deliberately chose a limited number of bales (2 bales for each quality category, high and medium). This was done to isolate the effects of compression, vs other sources of random variation which reduced the need of comparing large number of non-compressed bales to an equally large number of compressed bales.. Before compression and after compression samples may differ to a greater degree in that case simply due to the random sources of variation sampling large lots.

COMPARING PROBES

One of the questions in this project was the actual physical ability to sample double compressed hay which is typically hard and resistant to penetration. In our preliminary attempts to sample

Table 4. Comparison between probe types - Location 2 - ACX. Long Beach, CA.

StarQuality sampler w/drill	**Ave. crude protein%**	**Ave. ADF %**	**Ave. NDF%**
Composite (10 cores)	**20.4**	**30.6**	**38.2**
Compressed	20.2	31.8	39.7
Non-compressed	20.6	29.4	36.7
Indiv. Core	**20.4**	**30.5**	**38.2**
Compressed	20.6	30.8	38.6
Non-compressed	20.2	30.3	37.8
StarQuality long probe	**20.8**	**29.0**	**36.2**
Composite (10 cores)	**20.8**	**29.0**	**36.2**
Non-compressed	20.8	29.0	36.2
StarQuality short probe	**20.9**	**29.6**	**36.9**
Composite (10 cores)	20.9	30.3	37.9
Compressed	21.0	28.8	36.0
Non-compressed	**20.4**	**30.5**	**38.2**

*There were no significant differences between short and long probes or the drill-type probe

double compressed hay, several types of probes did not work, and so we selected several probes of the 10 commercially available . The 'Penn State' probe heated quickly and became quickly ineffective. The 'Colorado' slanted-tip probe effectively penetrated the double compressed bales, but has the disadvantage of a slanted tip which may result in a non-representative leaf-stem ratio.

At all three locations we tested the power-driven spiral assist type of probe (Star Quality samplers). This proved to be effective in penetrating both non-compressed and compressed bales. If a power-assist type of probe is used, we highly recommend a gas-powered type of drill, which has considerably more torque than electric-type drills.

At two locations we compared a short-probe and a long probe with the drill-type spiral assist probe. The long probe was unable to penetrate the double compressed bales. The short type of probe (12-14") was able to penetrate the double compressed bales as was the drill-type probe. We found no significant differences between probe-types in the forage quality results at the two locations (Table 4 and 5). This suggests that probe selection may be a purely practical issue – as to which probes are most effective at penetrating bales and correctly representing the leaf-stem ratio. It is possible that other 'long' probes could work to adequately penetrate large compacted bales, but this may depend upon the sharpness of the tip and other design features.

Table 5. Comparison between probe types - Location 3 - Anderson Hay, Long Beach, CA

StarQuality sampler w/drill	**Ave. crude protein%**	**Ave. ADF %**	**Ave. NDF%**
Composite (10 cores)	**18.5**	**33.4**	**41.0**
Compressed	18.8	32.7	40.0
Non-compressed	18.1	34.2	41.9
Indiv. Core (20 cores)	**18.8**	**32.6**	**40.0**
Compressed	19.0	32.2	39.5
Non-compressed	18.6	33.0	40.4
StarQuality long probe	**18.7**	**32.9**	**40.4**
Composite (10 cores)	**18.7**	**32.9**	**40.4**
Non-compressed	18.7	32.9	40.4
StarQuality short probe	**18.8**	**32.7**	**40.0**
Composite (10 cores)	**18.8**	**32.7**	**40.0**
Compressed	18.8	33.0	40.4
Non-compressed	18.8	32.4	39.7

*There were no significant differences between short and long probes or the drill-type probe

CHARACTERIZING VARIATION IN HAY LOTS

One of the key issues is to try to understand the level of variation that occurs in hay lots. Variation comes from several sources. First, the leaf-stem ratio within individual cores is an important source of variation, since leaves and stems are very different in quality. Secondly, there is usually bale-to-bale variation which may reflect across-field variation in soil, irrigation, etc. Thirdly, the random distribution of weeds creates variation in quality across and within bales.

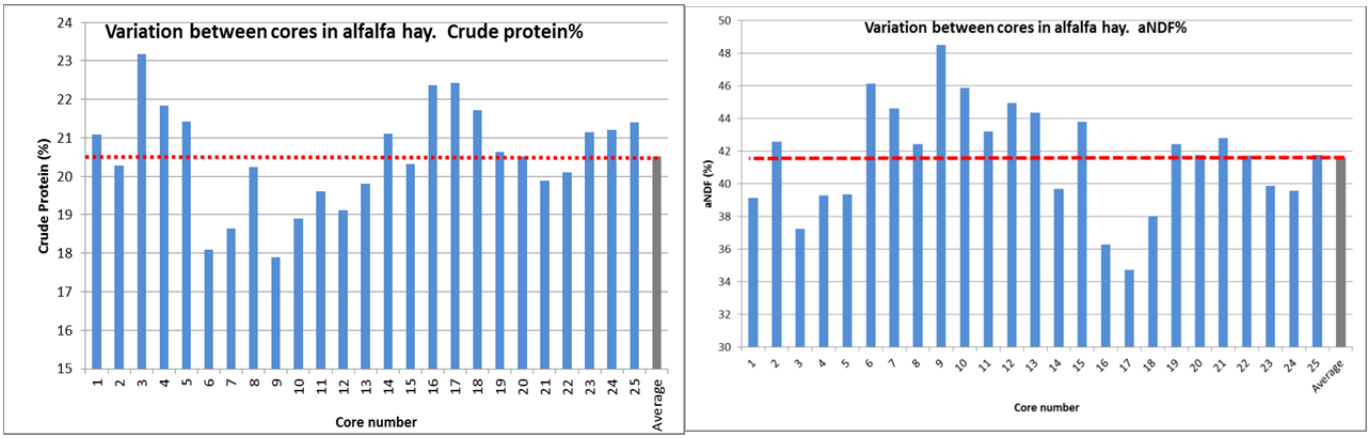

Figure 2. Variation in Crude Protein and NDF of an alfalfa hay lot from UC Davis, CA. Similar levels of variation were seen in ADF, TDN, and RFV.

The level of variation in a single-compressed typical set of samples is shown in Figure 2. Although average Crude Protein (CP) was 20.5%, the range of protein levels varied from 17.8% to 23.2%, and greater variability in NDF (34.5 to 48.2%) was observed (Figure 2). When the optimum number of samples is modelled, it is apparent that taking only a few cores as a composite sample results in a very high level of variation (Figure 3) with greater sampling significantly lowering the variability in the composite sample (2 samples results in 1.7% Standard deviation while 20 cores results in 0.25% standard deviation (Figure 3).

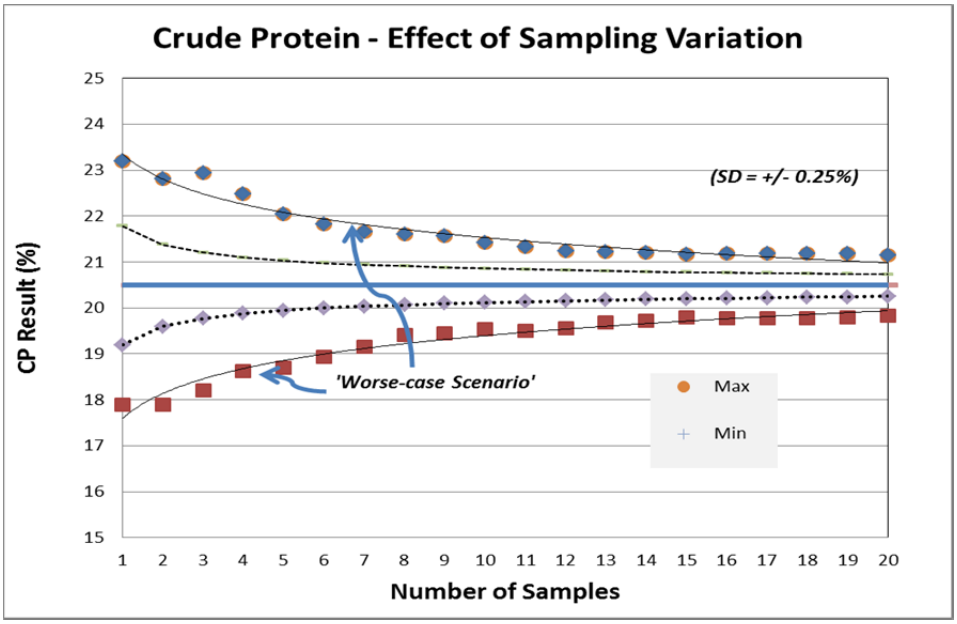

Figure 3. Effect of sample number on the variation in mean results. When fewer samples are taken, the danger of widely varied results is greater. In the case of CP, 20 cores limits variation to approximately SD of 0.25% but doesn't eliminate variation.

Such variation is also apparent in both compressed and non-compressed hay bales (Figure 4). Bale-to-bale variation can be seen in this data set, as well as probe-to-probe variation. Small trends (in this case positive) with compression should be compared with the amount of variation that is commonly seen from probe-to-probe and from bale to bale. Relative Feed Value is calculated from NDF and ADF, and the RFV results are widely divergent in this data set, with the worst case scenario of 112 to 180 RFV (max and min) when only a few samples are taken (Figure 5). Again, as sample number increased (20 cores) standard deviation is reduced to only 4.2 points, not 17 points. This illustrates that differentiating hay lots by only a few points RFV is nearly impossible, and the danger of only taking a few cores to represent a hay lot.

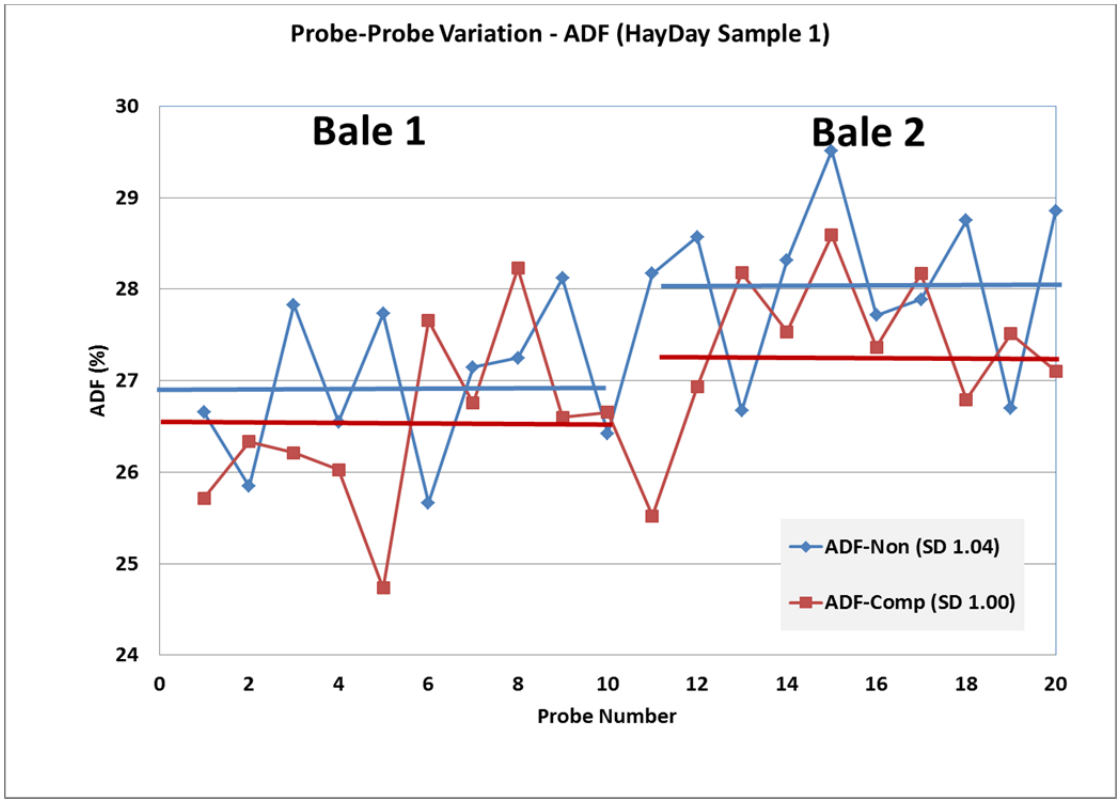

Figure 4. Probe-to-Probe and bale-to-bale variation in both compressed and non-compressed alfalfa hay for ADF, HayDay farm high-quality hay sample. Note that apparent lowering of ADF (improvement in quality) due to compression is overshadowed by the high variation from probe to probe and between bales.

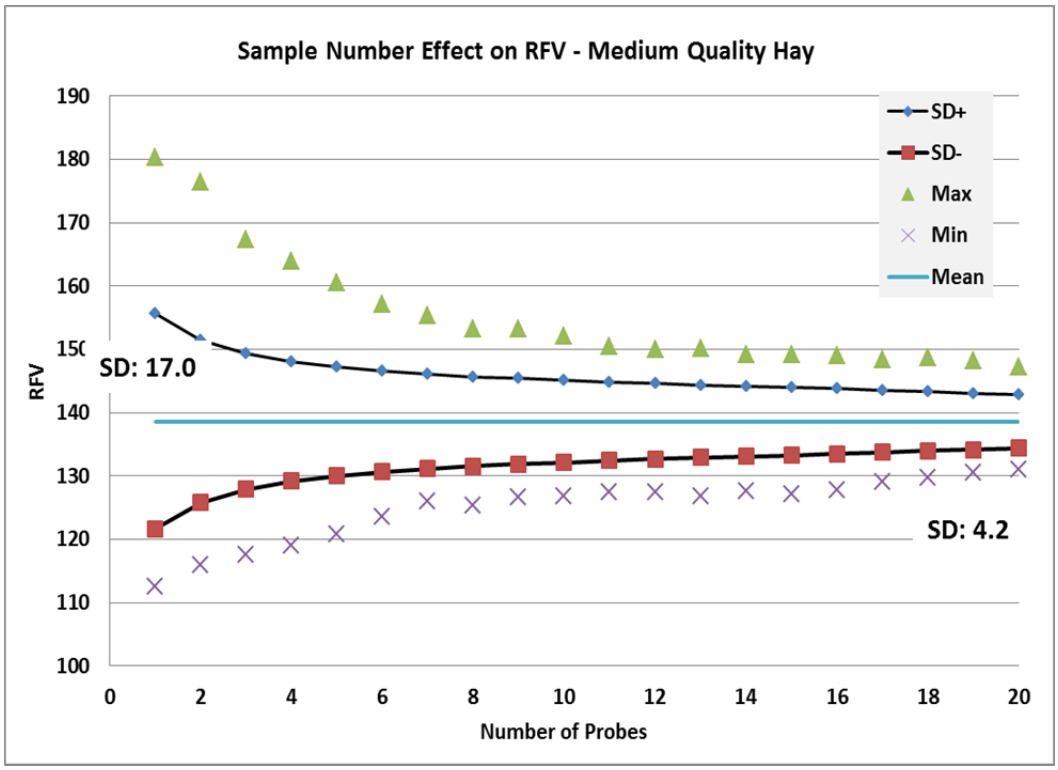

Figure 5. Influence of sample number on the Relative Feed Value (RFV) of a medium quality double compressed hay bale (HayDay Farms, Blythe, CA). Similar to non-compressed hay, 20 samples for double compressed hay lowers the standard deviation to about 4.2 percentage points, but does not eliminate variation.

CONCLUSIONS: COMPRESSED HAY SAMPLING

From this work we come to the following conclusions:

- Sampling of double compressed alfalfa hay is feasible utilizing either very sharp hand-driven probes, or power-driven type probes capable of penetrating a hay bale 12-14". Such probes are available.
- We did not observe significant differences between probes tested. Requirements for optimum probe types are provided by http://foragetesting.org
- Measurements of before- and after-compression lab tests showed either very minor or non-existent differences. We conclude that compression does not change the forage quality of alfalfa hay due to compression utilizing the types of machines utilized at three sites.
- Hay quality tests taken before double compression are likely to accurately reflect post-compression tests, unless high levels of variation due to large lots or more intensive processing factors occur.
- Compression processes which significantly chop, mix, disrupt significantly or manipulate particles would likely effect quality – thus post-compression tests would be necessary, and pre-compression data is likely to be unreliable.
- The level of probe-to-probe variation in sampling is very large. Recommendations to include a minimum of 20 cores to contribute to a composite sample should be followed for both compressed and non-compressed hay.
- The principles of proper hay sampling, including identification of hay lots, and sampling protocols should be followed for compressed as well as non-compressed hay. See protocol below.

PRINCIPLES FOR PROPER HAY SAMPLING FOR DETECTION OF LOW LEVEL PRESENCE OF GE TRAITS IN ALFALFA HAY

Hay sampling for detection of a GE trait was covered by Putnam (2014), and is re-stated briefly here. The principles of sampling for a low level presence (LLP) of a GE trait are similar in many respects to sampling for forage quality (see http://foragetesting.org) Sampling must attempt to provide a subsample which truly represents the entire mass of a 'lot'. But there are some important differences. The most important difference is that the objective of GE sampling is to determine a specific (small) concentration of a gene or gene product, not the average characteristic of the hay. While representing the leaf-stem ratio, or the weed-crop mix is an

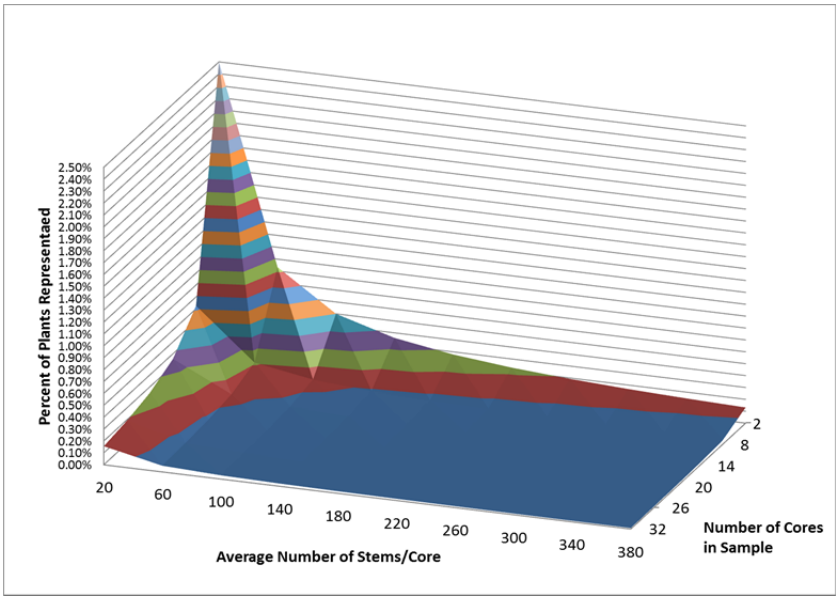

Figure 6. Effect of core number and average numbers of stems per core on the detection of a single plant represented in a sample.

important consideration for quality, this is not important for GE traits. A more important consideration is to represent a large number of plants – since LLP in an alfalfa field will be present only in a few plants in the field. Additionally, the desirable threshold level of detection must be established for GE traits, since a smaller detection threshold will require a greater number of stems for detection.

Figure 6 indicates the percentage of the sample represented by a single stem at various combinations of numbers of probes and numbers of stems per probe. We assume that a single stem per probe represents a single plant for the purposes of detection of LLP. Our research has shown that the numbers of stems present in 14" deep cored samples ranges from t 100 - 500 with an average of 269 (Figure 7). In this example using the average stems/probe, a single stem would represent approximately 0.013% of the mass collected in 30 probes given these assumptions. If PCR analysis is used, with detection limits at approximately 0.1, this sampling method should be capable of detecting about 8 stems in over 8,000 stems sampled using 30 cores, or about 0.1% of the DM of the hay. More compacted bales or deeper sampling methods (that sample a greater number of stems) would be expected to increase the total stem count in a composite sample. Keep in mind that any systematic sampling method for LLP must assume a random distribution of the trait throughout the mass.

The Impossibility of Zero Tolerance in Analysis. Some governments have not approved some GE traits, and thus have essentially zero tolerance for the importation of that trait in any agricultural products containing that trait. Likewise, some buyers or consumers wish to have 'GE-Free' crops. However, the practicality of declaring an agricultural product as containing none of a trait or 'GE Free' is an absolute impossibility. In order to assure a hay mass 'GE Free', every last gram of that mass must be tested, leaving none for its intended use! Furthermore, there is a prescribed limit of detection for any lab method, including PCR. A single stem present in a 200 ton hay crop would constitute 'contamination' in a technical sense– but it's highly unlikely that any sampling or analytical method would detect this (since the LLP is likely to be much lower than the capability of any sampling or detection method). Even 1 stem in 8,000 stems (as per the 30 core example above) would represent about 0.012% of the stems in the sample, which may or may not be detected by PCR methods (typically labs declare non-detect below 0.1% since the results are much less reliable).

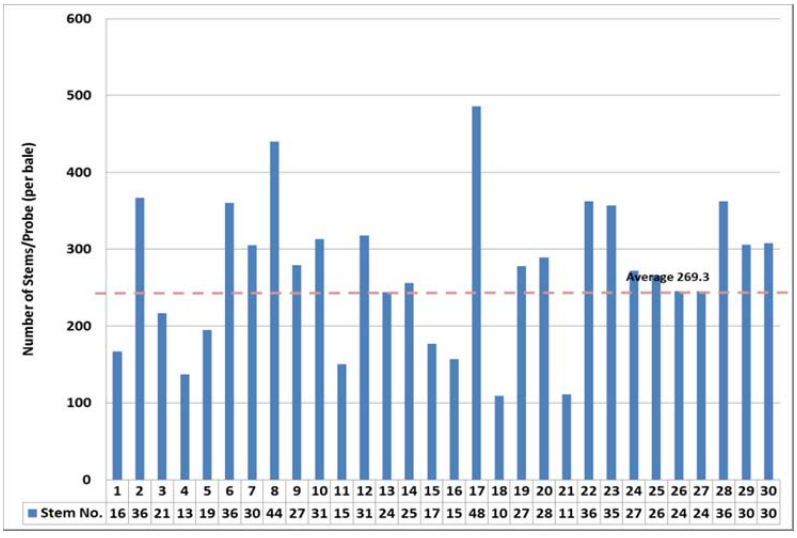

Figure 7. Approximate numbers of plants (stems) encountered per probe core in a single lot to a depth of 14" core in one bale (one lot, Davis, CA)

Thus, declaration of non-detect is made within a definition of the threshold of tolerance, analytical limits of detection, and the sampling method. Here,

sampling methods are used to supplement a declaration of "Non-GE" hay which may include other stewardship methods, such as care in labelling, management of inventory, and prevention of contamination in the field.

PROTOCOLS FOR HAY SAMPLING

The protocols for sampling hay for forage quality of either single-compressed or double compressed hay exports are remarkably similar. The major differences are related to the ability to penetrate double-compressed bales. Thus it is highly recommended that those involved with hay analysis should read the hay protocols and take the hay sampling certification offered by the National Forage Testing Association (NFTA) – and become a certified hay sampler. Over 2,000 people have done so, and improved their sampling techniques. (http://foragetesting.org)

For detection of a GE trait randomly distributed in the hay (an important distinction), sampling protocols are remarkably similar to those for forage quality. However, it is very likely that larger numbers of samples would be needed to detect low levels of an unwanted GE trait in an otherwise non-GE hay crop. Here we recommend a composite sample of 30 cores as a minimum, but the number of cores required will depend upon the level of tolerance for Low Level Presence. For example if 100% of the hay is GE, a single sample (without replication) will identify the crop as containing the GE trait (no sampling protocol is needed). If low level presence of 0.1% randomly distributed throughout the mass, a 30 core sample, with 12-14" depth should contain about 8 stems out of 8,000 stems (0.1%), so if a method is capable of detecting the trait at that level, this sampling method should suffice.

Here are the important principles of hay sampling for alfalfa for single- and double-compressed hay for quality, and for detection of a GE trait:

1. **Identify a single "lot" of hay.** This is a key first step to proper hay sampling, and one frequently ignored. Normally, a hay lot should be identified which is a single cutting, a single field and variety, and generally be less than 200 tons. For exports, a hay lot can be considered a single container or group of containers. However, in principle, the lot should consist of hay originating from a single field, single cutting, and as uniform as possible. For exports, identification of grower(s) and source of hay is important.

2. **When to Sample?** Sampling can occur in defined lots before double compression. If Dry Matter is important, sampling should take place at the point of weighing to adjust tonnage (not quality, which is determined on a 100% DM basis). Care should be taken to keep identification of these lots throughout the re-packaging process, and quality-assurance processes by exporters

Figure 8. Probes should be taken perpendicular to the butt-ends of bales, so that stems are arranged perpendicular to the probe. Spiral assists and gas-powered drills may assist in penetrating highly-compressed bales.

(including spot-checking compressed bales). This may assist confirming the determination of non-GE status in the final double-compressed product. Sampling can also occur after double compression to confirm quality results.

3. **Choose a sharp, well-designed coring device.** We generally recommend a sharp coring device 3/8-3/4" in diameter, approximately 16-24" length, which is capable of penetrating a bale 12-14" (Figure 3). Do not use flakes or grab samples. The probe should be capable of penetration (whether single- or double-compressed), and fairly represent the leaf stem ratio (*see note below for double-compressed bales). Probes larger than ¾" are acceptable for GE testing, but may inadvertently create samples which are too large to be handled by a laboratory, or make the sampler stop before the prescribed number of samples is obtained. Thus, smaller diameter probes are preferred, as long as they are capable of obtaining a cross-section of stem and leaf. The number of samples is likely more important than the diameter or depth. The principle is to sample as many stems as possible, not maximize the size of the sample – larger diameter probes are not likely to sample a greater number of stems than a smaller diameter probe at 12-14". A range of probe tip designs have been successfully used, from serrated to non-serrated tips. It is probably most important that the tip be sharp (and maintained sharp), and create a clean cut across a cross-section of hay, and not heat during the sampling process. Not all probes meet the criteria. **Note:** only a few probes can be practically used for double compressed hay. Gas-powered drills utilizing a spiral-assist probe (like the Star-Quality Sampler, Edmonton, AB) have been shown to work, as have some punch-type probes which require strength. In both cases, tips must be sharp. We have found serrated-type probes (e.g. Penn State probe) to heat too fast to be practical.

4. **Take enough cores.** For forage quality, 20 cores are still recommended, although greater may be needed for highly variable hay lots. For GE sampling, 30 cores, composited to a single sample is likely to provide a sample which represents LLP (if present) at about 0.1%, if the number of stems in each probe is above about 250 per probe (Figure 6). In practice we have found the range of stems to be from about 100-400 per probe. This is greater than the 20 probes recommended for forage quality testing – since in this case we are looking for low level presence, not the average quality of the sample. This is the same recommendation for larger (e.g. 1 ton), or smaller packages, as long as these group of bales fit the definition of a 'lot' (see step #1). The key aspect of sampling hay bales is number of samples composited, not necessarily the mass of the sample collected, as long as the mass is sufficient to represent discrete stems in each probed sample.

5. **Sample at random.** Cores should be taken without bias. Ideally, the sampler should sample bales at random from all sides, including both ends of the bales. This is sometimes difficult since all of the bales are not available to the sampler (they may be against walls of a barn, present only on one face of a container, or up too high for practical sampling). However, the sampler should make every attempt to sample in a random fashion. This means not to bias either for or against any bales in the stack. For example, the sampler may walk 15 steps, sample, walk 20 steps, sample, walk 5 steps, sample, while walking around stack, trying to represent all areas of the stack. Don't avoid or choose bales because they look especially bad or good--If 20 or 30 cores are

taken, they won't make much difference anyway. Avoiding or choosing bales introduces bias. Note for bales in a container: 25-30 Cores can be taken from the face presented to the sampler, even multiple samples taken from the same bale, since there is often as much variation within bales as between bales. The key is to make sure that 20-30 cores are taken, and that each core is at least 30" (0.7 meter) away from other sample cores to assure that the same plants are not sampled.

6. **Use proper technique.** Sample butt ends of hay bale, near the center in the compacted area between strings or wires, not near the edge. The butt ends of the bales should have the stems of the product perpendicular to the probe. Probe should be inserted at 90^{o} angle, 12-14" deep (Figure 8). Sample multiple bales if possible, but if sampling the same bales, assure that samples are at least 30" apart. The sides or the top of the bale should not be sampled, since these cores will only represent one flake from a single area of the field, and the stems will not be perpendicular to the probe. With round bales, sample towards middle of bale on an angle directly towards the center of the bale.

7. **Sample amount: not too big, not too small.** Sampling should be done so that about ½ lb. (226 g) to 1 lb. (454 g) of sample is produced-taking a minimum of 20 cores (for forage quality) and 30 cores for GE detection. For LLP testing, larger is better – but make sure the laboratory grinds the entire sample. Too-small samples don't fairly represent the full range of variation in the hay lot. Very big samples (common with large length or diameter probes) are excellent at representing the hay but have practical disadvantages. Large samples are difficult to handle and are often sub-sampled by the lab since only ¼ of a gram is often used for actually analysis. The sampler should ensure that the entire sample is ground by the lab. But you should also assure that you are providing a reasonable weight sample, so that it can be practically handled by the lab.

8. **Handle samples correctly.** Seal Composite 20- or 30-core sample in a well-sealed plastic bag and protect from heat. Double bagging is beneficial, especially for DM measurements. Deliver to lab as soon as possible. Do not allow samples to be exposed to excess sun (e.g. in the cab of a pickup truck). Refrigeration of hay samples is helpful, however, dry hay samples (about 90% DM) are considered fairly stable. Note: this is more important for quality analysis than for GE analysis.

9. **Choose Labs with Established Methods.** For forage quality testing, we recommend labs that participate in the NFTA proficiency certification program (www.foragetesting.org). For PCR testing, consult recommendations of the regulatory agencies for labs which have been approved.

ABBREVIATIONS: ADF = Acid Detergent Fiber, NDF = Neutral Detergent Fiber, CP=Crude Protein, TDN = Total Digestible Nutrients, RFV = Relative Feed Value.

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SUMMARY

Sampling alfalfa hay requires the following protocol:

1. Identification of a hay lot, consisting of a single cutting, from a single farm and field.
2. Sampling either at the point of sale, before or after compression for transport.
3. Use of a sharp, effective hay coring device, capable of multiple samples to a depth of 12-14" with either non-double compressed or double compressed hay.
4. Take 20 cores composited for quality analysis
5. Take 30 cores composited for detection of a GE trait.
6. Use random sampling methods – removal of bias.
7. Use of proper technique – 90^{o} probe into butt-ends center of bales, with stems arranged perpendicular to the probe.
8. Obtain approximately ½ to 1 lb. (227 to 454 g) of sample.
9. Double seal samples in zip-lock bags, protect from heat, sun.
10. Carefully choose a lab which has met quality control standards