Sampling Protocol for Alfalfa Hay, Greenchop, and Silage (Haylage)
Shannon Mueller & Dan Putnam

Background

Alfalfa is used by dairies in the form of hay, greenchop, and silage (also termed haylage). Each of these products has specific sampling requirements. Cubing or pelleting alfalfa is not widely practiced on California dairies, and is therefore not addressed here.

In May 2007, the Central Valley Regional Water Quality Control Board (Regional Board) adopted Waste Discharge Requirements General Order R5-2007-0035 for Existing Milk Cow Dairies (the General Order). The General Order requires that plant tissue samples be taken at harvest for each land application area (field) identified in the Nutrient Management Plan (NMP). Sampling must begin within 12 months of the adoption of the General Order (no later than May 3, 2008), and then at each crop harvest during coverage under the General Order. If you are covered under the General Order, you will need to comply with plant tissue monitoring requirements. Results must be submitted to the Regional Board as part of the annual report (due July 1 of the following calendar year).

Although these sampling procedures are designed primarily for compliance with the General Order, they can be integrated with other sampling activities and used for crop fertility management (fertilizer) and/or for forage quality (balancing rations).

Part I. Identification of Sampling and Analytical Requirements

1. Table 1 outlines the constituents and frequency of sampling requirements specified under the General Order. Additional nutrients may be desirable for formulating animal diets.

2. Contact your analytical laboratory to obtain sample containers, labels and chain of custody forms.

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Table 1. Nutrient Monitoring-Plant Tissue (Minimum Regulatory Requirements)

<table>
<thead>
<tr>
<th>At Harvest</th>
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<tbody>
<tr>
<td>Record the total weight (tons) and percent wet weight (% moisture) OR</td>
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<tr>
<td>volume (cubic yards) and density (grams per liter) of harvested material</td>
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<td>removed from each land application area.</td>
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<th>Laboratory Analysis</th>
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<tr>
<td>Laboratory analyses for total nitrogen, phosphorus, and potassium (expressed on a dry weight basis), and percent wet weight (if weight of harvested material is reported) or density (if volume of harvested material is reported).</td>
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**SAMPLING PROCEDURES SPECIFIC TO ALFALFA FORAGE**

A 'lot' is defined as one cutting from an individual field planted to a single variety. The lot identity should be retained throughout the record-keeping system and be included in the label identifier for individual samples. Never sample alfalfa from windrows.

**Part I. Sample Collection: Alfalfa Hay Protocol**

1. Choose a sharp, well-designed coring device with a 3/8-3/4” diameter. The corer should have a tip 90° to shaft, not angled. The probe length should allow probing to a depth of 12”-24”. It is important that the tip be sharp (and maintained sharp), and not create ‘fines’ during the cutting action, but cleanly cut across a cross-section of hay. Probes may be power, hand-brace, auger driven, or push-type.
2. Walk around the stack and sample bales at random. Both ends of bales should be sampled. For example, the sampler may walk 15 steps, sample, walk 20 steps, sample, walk 5 steps, sample, while walking around the stack—trying to represent all areas of the stack. Don’t avoid or choose bales because they look especially bad or good, because that introduces bias.
3. Take a minimum of 20 cores for a composite sample to represent a hay lot. This is the same for large (e.g. 1 ton bales), or small 2-tie or 3-tie bales. Sampling a large number of locations and bales throughout the stack to create a composite sample is a key aspect of representing the full variation contained in a hay lot. It is recommended that more than 20 cores (e.g. up to 35) be taken with very large lots (100-200 tons), or with highly variable lots (e.g. lots that may have non-attached leaves or are from very weedy fields). With small bales, sample 1 core per bale, more than 20 bales; with larger (e.g. 1 ton) bales, take 2-3 cores per bale in the center of the ends, sampling more than 10-12 bales.
4. Use proper technique. Sample the butt ends of the hay bale, between strings or wires, not near the edge. The probe should be inserted at a 90° angle, 12”-18” deep. Do not sample in the same spot twice. Do not use any technique that is likely to misrepresent the leaf-stem ratio.
5. Use the 20-core method to obtain a composite sample of approximately ½ pound (200 g) of cored hay material from either large or small bales. Never split samples without grinding and make sure that the entire sample is ground by the lab. Do
not use flakes or hand-grab samples. Handle samples carefully to retain representative leaf and stem fractions.

6. Place the composite 20-core sample in a well-sealed plastic bag and protect from exposure to sun and heat. Double bagging is beneficial, especially for dry matter (DM) measurements. Refrigeration of hay samples is helpful, however, dry hay samples (about 90% DM) are considered fairly stable. Hay samples may be stored at room temperature in a sealed bag for up to 72 hours prior to delivery to the analytical laboratory.

7. Complete a chain of custody form and record the required information in a notebook.

8. Keep a copy of the chain of custody form and records on sample collection and sample identification.

**Part II. Sample Collection: High Moisture Alfalfa (Greenchop, Silage, Haylage) Protocol Cumulative Method**

Sampling methods used to sample greenchop and silage are similar. Create one composite sample from several sub-samples collected throughout the harvest period (e.g. when harvest occurs over several hours or multiple days for a single field). It is important that the composite sample represent the field production. A sub-sample should be taken to represent each 5-acre area in the field. No more than about 40 acres should be represented by a single composite sample. For dry matter determination, composite sub-samples by field and time of day (am, pm). Designate which truck or trucks will be responsible for collecting the samples. Not every truckload needs to be sampled, but samples need to be collected throughout the harvest period to accurately reflect any variation in the field.

1. For safety purposes, greenchop or haylage samples should be taken by the truck driver from their own freshly dumped load. When a field is harvested for haylage, a single truck and driver may be designated to pull the samples as long as that truck will be making enough loads at appropriate intervals.

2. Gather sampling equipment needed (e.g., bags, permanent marker, cooler and ice or ice substitute, labels for sample identification, chain of custody forms, notebook for record-keeping, etc.).

3. Label clean, dry zipper top plastic sample bags with field identification (consistent with Nutrient Management Plan field identification), the date and time of sampling, the name of the person taking the sample and whether it is a morning or afternoon sample. You will need to label bags for sub-samples as well as bags for the final composite sample.

4. Sample greenchop immediately after it is dumped at the dairy in preparation for distribution to the animals directly or as part of the Total Mixed Ration. Sample chopped alfalfa for haylage/silage immediately after it is dumped and prior to being pushed into its storage area.

5. With clean, dry hands, collect a sample (4-6 small handfuls) of chopped alfalfa from different places around each freshly dumped load. Avoid sampling material that was at the top of a truck because silage exposed during transport is subject to large moisture losses and should not be included in the sample. This 4-6 handful sample will be referred to as a sub-sample. Place each sub-sample in the appropriately
labeled plastic bag or container (1-gallon size). Compress all air from the bag immediately and carefully and seal tightly. Store sample bags in a chilled cooler until they can be transferred to a refrigerator or freezer. See the “Sample Storage” section for recommendations to prevent water contamination of samples during storage.

6. Repeat steps 4 and 5, collecting sub-samples to represent at least every five acres up to approximately 40 acres, or four sub-samples per field or half-day, whichever is more. Fields which are more variable should be sub-sampled more frequently to improve accuracy of results.

7. Once all forage is removed from a field (greenchop), create composite samples for each 40-acre area by emptying all sub-sample bags or containers taken from the same field during the morning (or afternoon) into a large clean, dry, mixing container (plastic bucket or large pail). Composite the morning sub-samples from each 40-acre area of the field and the afternoon sub-samples separately. For haylage or silage samples, if harvesting continues into the afternoon or subsequent days, composite samples for each approximate 40 acres harvested within a field (morning or afternoon) are needed to obtain moisture results to use with corresponding forage weights. It is acceptable to conduct a nutrient analysis on each 40 acre component. It is also acceptable to make a composite of the individual 40 acre composites and submit this for nutrient analysis.

8. Thoroughly mix the sub samples together by stirring with clean dry hands, working quickly to minimize moisture loss.

9. Take 6 random handfuls from all sections of the mixed sample. Place about 1 quart or 1 liter of the mixed sample into a pre labeled tall plastic bag, compress to remove air, and knot-off close to the sample. If you will be submitting the sample to a laboratory for analysis of both DM and nutrient concentration, you need only one sample. If you will be doing your own DM determination on site, you will need to pull two samples – one for DM and one for nutrient analysis.

10. Store high moisture composite samples in a refrigerator or freezer. Deliver to an appropriate laboratory within 72 hours.

11. Complete a chain of custody form and record the required information in a notebook.

12. Keep a copy of the chain of custody form and records on sample collection and sample identification.

Part III. Helpful Recommendations Regarding Sample Timing, Handling, Storage & Frequency

1. Sample timing—It is important to sample alfalfa for analysis of dry matter and nutrient concentration at the same time yield estimates are made, since moisture content changes over time. An accurate value for the “as sampled” dry matter is critical to precisely estimate nutrient yields in each lot of forage.

2. Sample handling—Appropriate sample handling is important to ensure accurate results, especially for moisture content. Poorly handled samples (not airtight, exposed to heat) will result in inaccurate information. Because nutrients are analyzed on a dry weight basis, even a small error estimating moisture (dry matter) can lead to large errors when nutrient uptake is calculated. Chopped forage loses moisture very rapidly, especially in hot weather. Chopped samples must be
immediately placed into airtight plastic bags. Gallon sized (approximately 9 X 12 inch) zipper top bags work well for sub-samples, and 10 x 8 x 24 gusseted bags, which can be knotted, are ideal for composite samples. Remove all excess plant material from the seal area to allow for complete closure.

3. Sample storage—Completely close and seal collection bag once the sample or sub-sample is collected. Sub-samples and composite samples should be stored in a cool, dry environment until delivered to the laboratory. Ice-cooled chests are ideal for sample storage. Ice in a tightly sealed trash bag, sealed ice substitute, or 12 v cooler in the designated sampling truck are suggestions to keep the samples at or below 60ºF for up to 12 hours, after which time they must be refrigerated. If a refrigerator on the dairy is available, that is an ideal place to store the sub-samples while the rest of the field is harvested. If needed, high moisture samples may be stored in a refrigerator or freezer prior to delivery to the analytical laboratory.

4. Sample frequency—Samples to represent early, mid and late season harvests of hay are appropriate if all associated cuttings are cut at similar maturity. The results from these samples would be applied to lots removed during associated cuttings (e.g. sample cuttings 2, 4 or 5 and 7; apply lab results from cutting 2 yield weights from cuttings 1and 2; lab results from cutting 4 or 5 to yield weights from cuttings 3, 4, 5 and 6; and lab results from cutting 7 to yield weights from cuttings 7 and 8). For greenchop, use the cumulative method to obtain a sample representing each area of the field harvested at any given time, up to a maximum of about 5 acres per sub sample. These samples will later be combined into one composite sample to represent 40-acre sections of the field. For alfalfa that will be ensiled (haylage/silage) sub-samples of chopped forage should be collected to represent about 5 acres and then composited to represent approximately 40 acres. The more variable the moisture content, the more sub-samples should be taken to improve the accuracy of the composite sample. Moisture content can vary greatly across a field and within a day, especially when temperatures are high and/or conditions are windy. In other words, a separate composite sample should be created for each morning and afternoon when more than a half-day is needed to harvest a field. Refer to your sampling and analysis plan for site-specific sampling frequency needs.

5. Delivery to laboratory—Submit the sample to a laboratory meeting the requirements of the Central Valley Regional Water Quality Control Board for plant tissue analysis (plant tissue analyses shall be conducted by: methods utilized by the North American Proficiency Testing (NAPT) Program or accepted by the University of California; and laboratories participating in the NAPT Program or other programs whose tests are accepted by the University of California).

Additional information
Contact your analytical laboratory for additional information on sample collection, handling, preservation, and delivery. Contact the Central Valley Regional Water Quality Control Board for any other information requests.
http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/general_order_guidance/index.shtml (scroll down to sampling and analysis)
http://alfalfa.ucdavis.edu/+producing/forage_quality/hay_sampling/HAYSAMPLINGSTEPS.htm
Analytical requirements for laboratories analyzing plant tissue samples are available at: [http://www.waterboards.ca.gov.centralvalley.water_issues/dairies/general_order_guidance/sampling_analysis/sampling_procedures_rev_23may08.pdf](http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/general_order_guidance/sampling_analysis/sampling_procedures_rev_23may08.pdf)

Participants in the North America Proficiency Testing Program are available at [http://www.naptprogram.org/pap](http://www.naptprogram.org/pap)

Information in this document was compiled by UCCE and CDQAP to assist dairy producers in understanding and complying with the General Order Waste Discharge Requirements for Existing Milk Cow Dairies (Central Valley Regional Water Quality Control Board Order R5-2007-0035). Effort has been made to ensure accuracy, but these summaries are not official regulatory guidance and are not legal advice. Producers are advised that these summaries are not intended to be a substitute for producers reading the complete order and consulting their own legal counsel to ensure compliance with the waste discharge requirements. Should any information here conflict with the General Order and/or official information provided by the Regional Board, Board-provided information takes precedence.

Technical review provided by: Regional Water Quality Control Board 5 Confined Animal Facility staff. Financial support was provided wholly or in part by grants received from the California Dairy Research Foundation, and from the SWRCB Grant number 05-095-550-0. The contents of this document do not necessarily reflect the views and policies of the US EPA, SWRCB, or RWQCB, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.