

## The Use and Maintenance of the NIRS in the Laboratory

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Testing alfalfa hay using wet chemistry procedures involves many steps and can take most of a working day. The sample is prepared by grinding and mixing well. In running modified crude fiber (m.c.f.) the hay is weighed and placed in a beaker for digestion. Samples are filtered twice and the final result is obtained by weighing a Gooch crucible containing the dried fiber. The total time required to complete a m.c.f. is approximately 5 hours. During the process there are several potential sources of error. For example, if the digestion times are altered or poor filtering techniques are used the final result will probably be inaccurate.

For protein determination, the Kjeldahl procedure is commonly used. This procedure requires the use of highly corrosive reagents and takes about 3 hours to complete, depending on the type of equipment used. As with the m.c.f. procedure, the many steps involved allow many potential sources of error.

In years past, I could perform complete analysis on only 12 hay samples in an 8 hour period. The 4250 can do the same task in less than 1 hour and at the same time test for additional constituents. This has allowed the DCCA Feed Lab to better serve the needs of our 262 milk producers. On busy days, up to 50 hay samples have been run during an 8 hour period. Hay samples delivered to the lab in the morning can be tested and results returned to the dairy, via the milk truck, by late afternoon of the same day. In past years, during early first and second cuttings, it could take a week to complete the analysis of a sample.

Testing alfalfa hay on the 4250 requires no chemicals, glassware, hot plates or weighing. However, this equipment is subject to temperature variations. The unit should be kept at a room temperature of 75-80 degrees F. If the unit gets too hot or cold it could create false readings. A sealed check sample is run each day to monitor the instrument operation. After testing the check sample, a table of the previous day's readings is displayed with the means and standard deviations listed. If the calibration is to be generated by your lab (as in our case) it is advisable to have a series of 10 sealed check samples and their spectra stored in a safe place. If the equipment does breakdown, this will insure that the calibration values are saved. However, during the past year we have tested over 1600 hay samples with the only repair being the replacement of a 99 cent "O" ring.

Due to the system's small size, some labs have installed the 4250 in a van so hay can be tested at the farm.

When testing hay, the sample must be ground to <1.00 mm. particle size. Particle size of the hay is very important. Samples with moisture levels greater than 18% should be dried first. The sample is mixed well and put in a special cup using a scoopula which is provided. A cardboard disc is then pressed on to seal the cup. I use a soft brush to clean the outside of the cup and lense paper to clean the sight glass.

Using the software provided, I start the forage program. The sampling cup is placed in the 4250 sample drawer which is then closed. The sample is rotated in the drawer for about 25 seconds. While the sample is being scanned, there is a continuous beam focused by a lense onto a set of three filters from the lamp source. These filters then rotate in front of the light beam which passes through them. As the filter turns in front of the beam, the angle changes which permits a series of single wavelengths of light to pass through the filter. Thus, a range of wavelengths is created from each filter. Different molecular bonds in the sample absorb specific wavelengths in a characteristic pattern. Everything not absorbed is then reflected back to the instrument. The 4250 has detectors at a 45 degree angle to the sample which pick up the reflectance and transform it to an electronic signal. This signal is put into numbers by a computer using the calibration equation. A beeping signal from the 4250 indicates the scanning is complete. The hay report is then printed.

The hay constituents quantified using the 4250 are moisture, dry matter, crude protein, modified crude fiber, acid detergent fiber, E.N.E. and T.D.N.

Calibration is accomplished by scanning a number of hay samples to collect NIRS data which are stored in a computer file. Lab values are obtained for the samples by performing the wet chemistry procedures on duplicate samples. The calibration program correlates the lab values with the spectra information. The computer selects the wavelengths which correlate best with known lab values. These wavelengths are then incorporated into a regression equation which is then used to predict values of the constituent to be tested. At least 150 samples are needed to develop a reliable equation. We have over 900 samples to develop our calibration equation. Samples were obtained throughout California and Nevada from different cuttings of the year.

It is my belief that anyone involved with hay testing will benefit from this quick, accurate method. With the use of this technology, there is no reason for dairymen, hay growers or truckers to wait long periods for results.