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SUBJECT: Minutes, 1st Annual NIR Workshop

TO: Participants in National NIR Research Project and Workshop

Enclosed is a copy of the minutes of the 1st Annual NIR Workshop, held at El Reno, OK February 11-13, 1981. Sam Coleman and Woody Barton prepared the minutes and got them to me quite some time ago. A few minor changes appeared necessary, and, unfortunately, I took far too long to get that done.

Please take the time to review the minutes and consider any action items for which you are responsible. Some matters have been acted upon or are in process of being resolved. Others still need attention, so this will serve as a reminder to "move ahead."

Plans are essentially completed, for the 2nd Annual Workshop to be held at Athens, GA December 3-4, 1981, near the end of the ASA-Annual Meeting in Atlanta. If you have not done so, get in touch with Jim Robertson, FTS 250-3318, and advise him of your plans so he can accommodate the group with least effort. You should have received a memo (Sept. 16) from him giving detailed information on housing and transportation.

Also, send me by November 15 any ideas for topics which merit discussion at the Athens meeting so they can be incorporated into a tentative agenda. My apology for the long delay in circulating the minutes.

*William C. Templeton, Jr.*

William C. Templeton Jr.  
Coordinator, NIR Research Project  
on Forages

Enclosure

Minutes  
1st Annual NIR Workshop  
El Reno, OK  
February 11-13, 1981

Call to Order: The meeting was opened informally by Dr. William C. Templeton, Jr. Introductions were made. Those present were:

R. F Barnes	Gordon Marten	Jim Halgerson
Melvin Anderson	Karl Norris	Bill Templeton
Robert Meyer	Bill Powell-Neotec	Bob Windham
Jim Robertson	Jim Elgin	Herb Chapman --
Sam Coleman	Woody Barton	Univ of Florida
Floyd Horn	Mark Westerhaus	Khagi Khaleeludin --
John Shenk		Woodward, OK

Following introductions by Dr. Templeton, Dr. Horn welcomed everyone and presented an overview of the research at the Southwestern Livestock and Forage Research Station. Dr. Templeton then discussed briefly the proposed AFGC hay standards and use of NIR for quality evaluation of hays. The agenda was discussed and included the following items:

1. Reports of activities by location.
  2. Statistical results of chemical and IR analyses of collaborative samples (Westerhaus).
  3. IR output interpretation, including terminology (Shenk).
  4. Software-experiences, need for new programs, etc. (Hoover).
  5. Calibration procedures (Barton).
  6. Hardware modifications (Norris).
    - a. Rotating sample holder.
    - b. Scanning indicator lamp.
    - c. User trouble shooting
    - d. Others.
  7. New applications (Marten).
  8. Monochromator maintenance (Norris).
  9. IR interest and plans at Ona and Woodward (Chapman and Khaleeluddin).
  10. Work plans for 1981.
- I. Reports by location.
- A. St. Paul (Gordon Marten). Minnesota gave a report of four studies including small grains, corn stover, the effect of sample grind, and cool-season grasses. Briefly, calibrations were fairly good for the small grains and cool-season grasses, but were somewhat poor for the corn stover, especially for ADF and ADL content. The grind study indicated variable results. The selection of short wavelengths was primarily due to reflectance properties

for coarser grinds. It was evident that one should use the same grind for calibration samples that will be used for prediction samples. For the National NIR project, standard grinding should be used. Since most participants have a Udy mill, this is probably the one that should be adopted as a standard. Several questions came out of these studies which may be of benefit for future studies. For example, can a programmed equation selection formula be developed? How many wavelengths should one use? How can one determine reproducibility of measurements of new samples?

Special problems were noted in trying to predict ADL with several grass species or forage types.

- B. El Reno (Woody Barton). El Reno has had quite a few hardware problems, especially with the hard disks. It was reported that other locations have had some of these same problems. Dr. Barton discussed calibration and prediction of the 30 samples which were circulated for our network, utilizing laboratory data from both the Athens and El Reno locations. Indications were that calibration equations could be obtained, but that they may be biased when trying to predict data from another laboratory. Some of these biases could be explained, though others could not. Also, the biases were not always in the same direction, although with crude protein they were consistent. Prediction errors suggested that within a laboratory and for relative differences, the infrared instrument was more precise than analytical wet chemistry.

Sam Coleman reported concerning some other studies conducted at El Reno. He showed that Old World bluestem samples corrected for dry matter gave larger errors of prediction than those which were uncorrected for dry matter. The reason for this is that the infrared instrument is seeing an uncorrected sample, that is, it has ambient moisture in it. Two possible solutions were suggested. Either dry the sample and quickly read it on the IR instrument, or else input the calibration data on an uncorrected basis and then adopt a software routine to correct them for dry matter as the data are printed out. Sam further reported some data in cooperation with Dr. Bill Holloway from Tennessee where they were trying to predict dry matter intake, dry matter digestibility, and fecal output from chemical characteristics of the feces. The infrared instrument in every case could predict as well or better than the many chemical variables that Dr. Holloway had studied and had attempted to correlate with these parameters.

C. Beltsville (Jim Elgin). Jim briefly discussed statistical analysis of the wet chemistry data for the IR network samples which were circulated. Other work they were doing concerned predicting perloline and other alkaloid levels in fescue. They were also working with alfalfa and found that in trying to predict crude protein, samples that were more than three years old presented calibration problems. They found that they had low  $R^2$ 's even though the possibility exists that predictions may be better than one would think, especially since the regression line averages noise differences about the regression line. Also from Beltsville, Karl Norris discussed math treatments which may overcome some of the physical problems such as temperature, grind, etc. that have been observed with some samples. He indicated sample temperature was very critical. Also, the fineness of grind created problems, especially when certain samples did not have the same particle size even though ground through the same mill. A single math treatment, called OD/OD, corrects both of these problems. He discussed the possibility that this may need to be included in our present IR software.

D. Logan (Melvin Anderson). The primary interest in Utah is concerned with alfalfa. They obtain reasonably good correlations and low standard errors in predicting NDF, ADF, crude protein, TDN and in vitro dry matter digestibility. The errors of some of the nutrients were high. The samples came from a commercial hay testing lab. Other work they were doing concerned the prediction of IVDMD, protein, and ADF on 80 samples of oat straw. With these kinds of samples they obtained poor results. In another study using 108 old samples (14-16 yrs), primarily alfalfa from digestion trials,  $R^2$  of 0.88 was obtained for in vivo dry matter digestibility. When the data were run on a within-year basis,  $R^2$ 's were as high as 0.92. A unique application was being conducted in Utah in which carotene content of forage samples was being predicted. Carotene content normally runs 300-400 ug/lb, and is therefore quite small. They have high hopes that IR would work for the samples. They also looked at effects of various drying procedures and conditions such as freeze drying, sun curing, fresh, microwave, and rain damage on the content of carotene and prediction using NIR.

E. Athens (Bob Windham). They reported data from samples of coastal bermudagrass and pensacola bahiagrass prediction of both in vivo and in vitro dry matter disappearance as well as chemical parameters. Using the default derivative options,  $R^2$ 's were low but the standard errors of

calibration and standard errors of prediction were reasonable. They were using up to 5 wavelengths for in vivo and obtained a standard error of calibration of 1.8 with an  $R^2$  of 0.76. Actual vs. predicted  $R^2$  was only 0.53.

F. Other groups not included at the present time in the IR network:

1. Ona, Florida (Herb Chapman). An overall view of the program at Ona and IFAS in general, especially their forage program was given. Their plans for NIR included its use both by extension and research in the departments of Agronomy and Animal Science as an alternative to wet chemistry. He reported their primary application would be with tropical forages and that several thousand samples per year are being collected at the Ona station alone.
2. Woodward, Oklahoma (Khagi Khaleeludin). They have 4,000 Eastern gammagrass and Old World bluestem samples at the present time in which they need to analyze for IVDMD, ADF, NDF, and crude protein. He presented a new idea for determination of IVDMD/in vivo by duodirectional in situ technique using the animal.

II. Maintenance. (Karl Norris).

Quotes have been received from Neotec concerning maintenance of the monochromator alone. The quotes were \$4,000-5,000 per year per instrument. After much discussion, it was decided that this was an excessive amount and the suggestion was made that no location accept the maintenance agreement, but that we simply call Neotec on an as-call basis when service is required and pay as we go.

III. Hardware changes (Karl Norris).

Two or three suggestions have been made: (1) a scanning indicator lamp—this would be easy to add and would notify people when the monochromator was in the scanning mode; (2) rotating sample holder—the purpose of this device is to present to the instrument more sample area than is presented at the present time, an offset rotation is used wherein 40% more sample surface can be presented to the instrument. This decreases sampling variation; however, it is quite expensive, approximately \$2,000; (3) a line conditioner—to take the spikes out of the AC line voltage reducing instrument noise, would cost about \$450. It is available from Topaz in

California; (4) noise level of the instruments should be considered--this would be used to determine if rotating sample cups are needed; (5) voltage regulator on detectors and voltage control from detectors--would allow us to essentially calibrate the instruments externally without software control.

#### IV. Statistical evaluations of chemical data (Mark Westerhaus).

The collaborative study for wet chemistry analysis among labs was conducted using a group of 30 samples of which each lab had a subsample. There were differences among labs for analyses of the same sample. Mark presented the statistical analyses for calculating the error due to any one lab and the components that contribute to the error. This error includes the variation due to lab, lab x sample interaction, and the error term. One interesting point is that for every analysis, there was a significant lab x sample interaction. For the most part, no explanation was found for this, although in some isolated cases, reasons could be given.

In the same study, each location was to run three IR scans on the same samples. One conclusion drawn from this part of the study was that instruments are different. In other words, the spectrum is vertically offset at different locations. Karl Norris suggested several ways to determine what contributed to the shift, whether sampling error associated with dividing the samples was a problem or whether it truly was the instrument. One solution would be to send a sealed sample around to all locations to be run at a standard temperature. Athens was to initiate this. Karl next suggested that we must work on software to calibrate different instruments to the X axis. Considerable work has been done on the Y axis, but now we should work on the X axis. Karl suspects that the Penn State sample cups may not be big enough or that the beam width needs to be cut down and suggested that comparisons be run at each location between the Penn State cups and the large Neotec cups. Also, OD screens are to be sent around for completion of this check.

#### V. Software (John Shenk).

Two additional programs are available for those who would like to have them. They are called Chem and GENEQA. GENEQA is very difficult to use and must be used with a fortran compiler. These can be obtained from the University Park group.

6 Output Interpretation (John Shenk and Mark Westerhaus).

$$SED = \sqrt{\sum D^2 / n}$$

$$SED(c) = \sqrt{(\sum D^2 - (\sum D)^2 / n) / (n - 1)}$$

$$\text{Repeatability} = \sqrt{b_i^2 \times \text{noise}(\lambda_i)}$$

$$\text{PRESS} = \sqrt{\sum (y_i - \hat{y}_{(i)})^2 / n}$$

where  $\hat{y}_i$  is the predicted  $i^{\text{th}}$  sample based on the  $n-1$  other samples

$$"H" = \mathbf{x}_i (\mathbf{X}'\mathbf{X})^{-1} \mathbf{x}'_i$$

where  $\mathbf{x}_i$  is the vector of NIR data (independent variables) for

the  $i^{\text{th}}$  sample and  $(\mathbf{X}'\mathbf{X})^{-1}$  is the inverse of the covariance

matrix based on  $n$  samples.

V. New applications (Gordon Marten).

1. Interaction among locations.

- a. Run NOISE - 50 scans and LAMBDA - 10 scans. Karl Norris is to circulate instructions, sample holder, and disk by way of Athens to all locations. Wait until you have these to run your scans.
- b. Chemical data collaboration. El Reno and Athens are to work on crude protein. El Reno and Minnesota are to work on fiber components using the fibertec, to see if the fibertec system will reduce errors. Everyone should examine their IVDMD procedure and see if we can come up with a standard such as the NC64 system with direct acidification instead of centrifuging in the middle stage.

2. Interaction within locations.

- a. Plant breeders: St. Paul will interact with plant breeders concerning reed canarygrass; Oklahoma and Texas - various range grasses, lovegrass, and bermudagrass; Pennsylvania - alfalfa and orchardgrass; Florida - Digitaria, Panicum, Hemarthria; Utah - range grasses and legumes.

- b. Animal science: Logan-alfalfa; El Reno-warm-season grasses; Florida-tropical grasses; Georgia- tropical grasses.
  - c. Extension: This is an area which may be difficult for USDA, However, some possible locations for work in this area include Pennsylvania, Minnesota, and Oklahoma. Minnesota and Oklahoma are question marks at the present time.
3. Hay marketing-Input is similar to Extension activities above.
  4. There may be a need to standardize wavelengths. This responsibility will come from the principal laboratory.
  5. Some have suggested the appointment of a chemometrician. Woody Barton is to work with Mark Westerhaus to consider software applications for the coming year.
  6. Definition of the lignin complex. The Athens group has been working on this for some time and will continue to do so.

#### VI. Calibration.

Chemical data still present the biggest problem with calibration. One thing that is needed is standardized procedures, especially for the fiber fraction to identify exactly what we are measuring. In other words, what is ADF? Duplicate samples will probably not be sufficient when trying to calibrate the 1R instrument. We need at least triplicates and maybe quintuplets. Math treatments can be used to improve calibration files. OD/OD has already been discussed. For protein it works very well. Another approach may be 2 der/2 der. Second derivative takes out some shifts due to temperature and grind, though not completely. John Shenk and Karl Norris are to work together to try to come up with a way to implement OD/OD in our software. One suggestion is to use the "best pair" option that we now have. Once this is incorporated, calibration can be run with varying temperature and particle size. Then the predictive samples will work. John Shenk suggested that sample selection was one of the more important factors in calibration. He also suggested this was probably an art rather than a science. Two choices were available, closely defined for a specific set of samples, or highly variable for predicting producer samples. Which one is used depends upon intended use of the predicted data. The real question, "How do we go about getting good sets of good calibration samples, especially if we intend to sample the world?"

A charge was given to all participants to give thought to calibration and how to set up a sample set that represents all forages known and all that we are working on. We would probably want to look at protein first. Mark Westerhaus, John Shenk, and Bob Windham were assigned to pursue this question. Moisture or dry matter continues to be a problem. Karl Norris suggested that the Karl Fisher method be used to determine moisture to calibrate the instrument. The Fisher method is a very accurate technique. We could then use this as a universal moisture prediction file and simply adjust all the data from the moisture wavelength instead of trying to adjust within each data set.

#### VII. Software.

Questions included:

1. Possibility of going below 1100 nm. Could be looked for on the reverse scan. This would take another program. We could go down as low as 1,000 in the present scan.
2. Assign the 700 available data points to a shorter spectrum. Since a band pass is 10 nm, this really holds no advantages. Therefore, the subject was dropped.
3. It was suggested that the F statistic be put back into the EQA program printout. This might be useful for helping decipher which wavelength to choose without having to go back to the MWS printout. For wavelength selection, one must consider the difference (bias), SED, SED(c),  $R^2$ , number of  $\lambda$ 's and the F statistic. All of these things make up the composite, so no definite scientific method can be obtained for selecting these. Karl, John, Mark, and Woody were to get together for guidelines concerning selection of wavelengths. Another suggestion concerning calibration consisted of drying the samples before running wet chemistry, such as Minnesota was doing. This may give better results with less variation.

VIII. Foreign visitors interested in IR should be encouraged to participate. From Alberta, Canada, Ed Redshaw in the Longman lab has indicated some interest. From Aberdeen, Scotland, the Rowett Institute, Dr. Murray has an instrument and is interested. Possibly a newsletter could be printed concerning the progress and plans of the NIR group. Another way to attract foreign visitors would be to have a meeting at the International Grassland Congress. Woody is to contact Garland Bastin in order to obtain a room close to the end of the 3-day period in which the van from University Park will be there.

Athens is to be the next host. It will be at the end of the Agronomy meetings in early December. Work plans for each location were requested by Dr. Templeton to be turned in to him by March 20. Of primary importance is how each location plans to contribute to the national objectives.