

Minutes
Second 1981 NIR-Network Workshop
Athens, Georgia
December 3-4, 1981

Introduction

The meeting was opened by Bill Templeton, who called for introductions of those present. Attendees listed as Appendix 4.

Program of Forage Work at RRC

Jim Robertson, Research Leader at Athens, GA, introduced Dave Zimmer, Area Director, who welcomed the participants to Athens. Jim Robertson introduced the forage program at Athens and the hierarchy of the Russell Research Center (RRC). Woody Barton presented his and Danny Akin's work on identifying the botanical tissues in forages that have been extracted by fiber analysis procedures (i.e., NDF, ADF), and the extent of tissue removal by action of microorganisms and reagents. Bob Windham presented the findings of the hemicellulose analysis by HPLC and its relationship to animal performance and animal studies as well as the NIR. Dave Himmelsbach discussed the use of ^{13}C -NMR for structure elucidation and quantitative measurements. The utility of solid phase NMR was discussed and its applications to complement the NIR project.

John Shenk praised the direction and effort of the forage research at RRC and stressed the need for help to continue and expand the program. Karl Norris and Dave Himmelsbach discussed some aspects of quantitation of the NMR method.

Bill Templeton then put the minutes of the February 1981 meeting out for acceptance. The minutes were accepted. Following this, an agenda was formulated.

Report of Locations

St. Paul, MN. Gordon Marten reported on four studies at his location. These were in some cases continuations of studies from last year with new samples from this year's harvest. The studies were on (1) corn stover for ADF and ADL, (2) cool season grasses for chemical analysis and IVDMD, (3) Reed Canarygrass, a plant breeding project, and (4) legume - grass mixtures for chemical analysis and IVDMD. Gordon reported that in all cases, "last year's" equations needed to be updated for "this year's" samples. The Reed Canarygrass results were quite good and the NIR system can effectively screen breeder material.

El Reno, OK. Sam Coleman reported on data from a study with Old World Bluestems to show the size needed of a calibration set and the effect of the smoothing routine. The 30 network samples were rerun on a semi-automated Fibertec. Results showed this to be faster and more precise than the "boil and stir" method. These results were confirmed by the Athens reports.

University Park, PA. John Shenk's report was somewhat lengthy and will be outlined here. John reported on new locations with NIR interest, new hardware, new software, and new procedures.

1. Ian Murray of Aberdeen, Scotland is using NIR for their forage research program.
2. Reed Shaw (unsure of having correct name) in Edmonton, Alberta, Canada, has the same equipment as the network.
3. Clara Liebman at Ona, FL, is representing the University of Florida here today which has the network instrumentation,
4. Don Nichols who operates a commercial lab in Pennsylvania has network equipment and is providing NIR analysis to producers.

5. Jim Marlan at CSIRO in Sydney, Australia, has 6100, 6350, Technicon, and other NIR equipment and is interested in the network and our progress.

6. The Forage Testing Laboratory at Pennsylvania State University has a 6100 and is using it for producer samples with network software and John's calibration procedures.

7. Rohweder at Wisconsin has purchased two Neotec FQA-51A and DEC PDP 11 equipment for Wisconsin forage quality program.

8. The FQA-51A with DEC Computer is now part of the mobile van system. The Wisconsin group will use one FQA-51A in a mobile configuration.

9. Calibration procedures are continuing to be the main focus for research. A suggested outline for calibration is given below:

- a. Select 70 samples from a broad population of hays, grasses, legumes, and silages.
- b. Re-analyze all samples for calibration data.
- c. Assess results of all three math treatments (note -- new software may require modification of this step.)
- d. Validate by using 10% of the sample population in separate file.
- e. Calibrate (MWS) with 63 samples and predict with 7 samples,
- f. Select the appropriate number of wavelengths (a test in itself).

g. Recalibrate (MWS) on all 70 samples force in the 63 sample wavelengths.

h. Use starred values to check calibration data. Reanalyze all starred samples.

i. Repeat q-h until stars disappear.

j. Eventually add another 70 samples to validate the original set.

k. Periodically update with samples which expand the range of calibration.

10. John brought some Mylar bags which he feels are adequate for the storing of the reference or universal set of samples. They are moisture and light proof and will maintain the sample for a indefinite period. As such, the instruments could be checked for changes with known samples. The calibrations based on these samples would be consistent since they would remain unchanged, and updates to all the instruments would be possible through one sample set.

11. The new software package is not quite ready.

Logan, UT. Melvin Anderson reported his work with alfalfa hay and the use of NIR in a grass breeding project. He was not successful in measuring carotene and since no valid chemical method is available for nitrate, could not calibrate and predict NO_3 ,

Beltsville, MD. Jim Elgin reported that they had used the instrument to look at the level of alkaloids (Perloline) in tall fescue and protein in corn (high and low lysine). Jim stated that the temperature in the machine fluctuates during the day and an adequate warmup is required.

Karl Norris reported no answers for the poor results on corn (6.25 not appropriate to high and low lysine). Dye binding crude protein (CP) analysis gives no better calibration than Kjeldahl ($SEC=.6$, $R^2 = .9$). Math treatment not a factor since 1st derivative/1st derivative ($\Delta OD/\Delta OD$) gives no improvement. Two other math treatments, curve fitting and factor analysis were no better for corn, maybe for forage. Factor analysis uses the whole curve and seeks factors which are mutually exclusive as a means of incorporating discriminant functions. Karl reported the preliminary results of the tests of the six instruments. Essentially these six "identical" instruments are "different". They differ in (1) noise level, (2) absolute reflectance, and (3) sensitivity.

New Orleans, LA. Bob Barnes extended a challenge to the network participants to assess the status of technology and develop priorities for applying the methodology to research and extension problems.

Athens, GA. Jim Robertson reported on their efforts to measure moisture by the Karl Fisher method. The equipment arrived on December 1, 1981 and is being set up. It is hoped that a one-time calibration can be obtained and that software written to use that dry matter to correct all other equations. The Athens laboratory will continue to work on this project.

Woody Barton reported on calibration data using the Fibertec system to run the fiber analysis for ADF, NDF, and ADL. In all cases, the data from triplicate analysis on the Fibertec gave better results for calibration than triplicate analysis in his laboratory by the "boil and stir" method. The Fibertec results for calibration were as good as or better than the average of four of the network laboratories' results. The precision of analysis was always better and throughput much higher. As many as 84 samples a day can

be run with the Fibertec by one person. Previously, 40 a day with two people was considered good in this laboratory. Other studies are being undertaken cooperatively between Athens, GA, El Reno and Woodward, OK for NIR calibration and prediction using Fibertec to analyze samples.

Bob Windham reported on his work with calibrating the instrument to predict CP and CP-digestion from diet, abomasum, and fecal samples. Results were quite good. Two factors are hindering progress: (1) subtraction in "Draw" requires a set file and the difference spectra cannot be stored, and (2) particle size differences need to be corrected prior to math treatment. The difference spectra Fecal minus Abo show that little is digested in the lower tract.

This concluded the reports by locations.

Hardware Considerations

1. John Shenk recommended that each location purchase the rotating cup option with a center pivot cup. This has been found to reduce error from Penn State's experience. Also, from the experience of Athens with FQA-51A and non-central rotating cup, it has been found to improve errors of calibration and prediction.

2. Karl will have a final report on the status of the instruments after January 1, 1982.

3. There was no discussion on the addition of a meter to measure light intensity/energy to the sample as had been mentioned in February, 1981.

New Software Package

John Shenk reported on the new software package which will be sent to all locations. The new software will be more comprehensive, but at a considerable

cost of time. If 100 samples for CP take 2 hrs for calibration on the old MWS, it will take 2 days with the new version. The files will retain similar structure, but some programs will be changed. The new package will contain:

| <u>OLD</u> | <u>NEW</u> |
|------------|-------------|
| 1. DATA | 1. NEW SCAN |
| 2. FILE | 2. NEW MWS |
| 3. STAT | 3. NEW PRE |
| 4. LAMBDA | 4. NEW EQA |
| | 5. SCAN 51 |
| | 6. NOISE |

All of the set functions are now in the MWS program.

Changes:

1. Scan - The noise output for bad scans is eliminated. The program will keep the first 64 good scans out of 128 (after 128, you will get output). Scan will look at the data more closely and can recognize and count zero's as bad data.
2. MWS - Set functions are incorporated such that no restraints are imposed. It will try all math treatments and all set segments both for derivative and division terms. A brief description of how the program operates follows:
 1. Evaluate 0, 1, 2 derivative and choose 1st λ .
 2. Program now selects math and fine tunes the set parameters,
 3. Continue for second λ (and 3rd, etc.) and redo the 1st (2nd, etc.).
 4. Can use a subset of calibration set to validate equation within the MWS program, or you can identify another sample set to validate while you calibrate.

5. Can now have division terms to cancel particle size, temperature, and interfering adsorptions.
 6. Restart program and select a numerator as option (parenthesis around default on options). With division, once a denominator is picked, rechecks numerator and gives one term equation.
 7. Coefficients now have statistical importance.
 8. Single term may not always be better. The program then reruns numerator and selects for term 2.
 9. If you restrict the program to the 1st λ , you will eliminate viable better terms with the fine tuning and divisions. Thus, we retain three best wavelengths to start with for numerator/denominator. Also have a best pair and best trio option.
 10. You can lock in math or treatment parameters and speed up the process.
 11. Program will have interactive graphic output.
 12. This software will generally improve prediction by 20% and will provide F values and other items in the EQA file. It can output to terminal and/or storage device.
3. SCAN 51 - This program operates the FQA-51A, uses the drawer switches, and the new noise program for SCAN to measure noise difference between samples. Predictions are faster (FQA 51 vs. 6100), but longer warm-up needed.

Several requests for additional features were requested: (1) run the new software on RSX for those with bigger systems -- such that other things can be done while the new MWS is working: (2) work off a video terminal since the

output can go to the disk; and (3) do "Draw" from "Raw" file with provision to store the difference spectra in a "Raw" file.

Assessment from NPS

Wilda Martinez addressed the group from the NPS perspective and asked a number of questions. At this time it is necessary to determine how our original objectives have been met. Are they still valid? What needs to be done? What are the priorities? What resources are needed to accomplish stated goals? These questions and comments promoted a revision of the network research project which is included as Appendix 1.

Jerry Carlson explained his and Wilda's participation in the Strategic Long Range Planning Process and the need for the network to have a plan to implement when funding is available. Jerry outlined a process for structuring a plan. The steps in the planning process include:

1. Goal - How it is stated is important and we should look to using the goal as a part of the research program.
2. Develop strategies (specific objectives) to achieve the goal.
3. Select approaches to accomplish the specific objectives.
4. Define project areas and specific projects to support the approaches.
5. Assess priorities from the "What are we not going to do" side.
6. Don't consider resource limitations as management will assign resources as well as an implementation plan.

We are to focus on the identification of problems and their importance to the goal and be able to translate the scientific problem into an operational plan to be implemented when resources are available. As such, we will have a plan from which we can work at a number of funding/resource levels. The CRIS is used to document the Plan through the use of the project areas, projects, and progress reports. The network will, perhaps, through its Minutes collate and coordinate the progress.

The Question of How to Calibrate

Hypothesis: You have 50 samples representative of a total population of 1,000 samples.

1. 50 is a minimum number of samples as a guideline. Split the file 70/30 (35 cal; 15 verify). Get the math and # λ 's/terms and verify from MWS. Recombine and fix math and λ 's/terms and adjust coefficients.
2. Calibrate on the-50 for math and # λ 's/terms. Split 70/30, recalibrate 35 samples, split by range and standard error, and use 15 for prediction with some math and # λ 's/terms to verify original calibration of the 50. Then take every 10th or 15th from the rest of the population and analyze to verify.

Both answers have valid merit. It may be that the network should publish a method of calibration to include how to handle "stars" and define a population.

Universal Crude Protein Equation

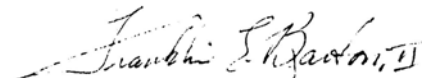
It was generally agreed that an equation for CP could be developed for all species, at least on a regional basis. Gordon Martens suggested procedure which was mailed to everyone is included as Appendix 2. Sam Coleman has divided the states and territories among the locations. This scheme is included as Appendix 3. Note John Shenk did say he would "help out in the West". He did not specify how far West. Willingness to participate in this endeavor needs to be stated in our FY 82 plans. If there are some who do not wish to participate in this project, their states will be divided among those who will. It should be recognized that this project will take an inordinate amount of time to coordinate.

Close

The network will meet at Pennsylvania State University for its next workshop. No date was specified. Mid to late October, 1982 or early spring, 1983 would be best. The October, 1982, might be best since we would have a 1982 meeting.

Each location is requested to submit their FY 82 plan of work and progress toward the original network goals by March 1, 1982 to Bill Templeton,

Respectfully submitted.


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