

# NIRS Forage and Feed Testing Consortium NEWS

Summer 2005



Dedicated to Increasing the Accuracy and Knowledge of NIRS Testing.

<http://www.uwex.edu/ces/forage/NIRS/home-page.htm>

## Annual Conference Update: Projects

Project Name	Status
NAMA	<p>The objective of this project is to generate program details that will establish credibility in analytical consistency within NIRS. The committee heading up this project has detailed the following tasks:</p> <ol style="list-style-type: none"> <li>1. Survey all NIRSC labs to determine which of the NIRSC equations are being used and why they might not be used or why they might be modified.</li> <li>2. Standardize NIRSC labs again.</li> <li>3. Outline the problem of variability in sample prep and cite Paolo's current program to standardize drying and grinding methods and develop calibrations that factor out intractable differences.</li> </ol> <p>Tasks #1 and #2 are currently in process.</p>
NIRSC Aggregate Spectra Use (intellectual property)	<p>The objective of this project is to protect NIRSC property and protect the certainty of ownership with existing projects. The committee working on this project has drafted a document outlining the issue of spectra and chemistry use, has drafted language to amend the NIRSC by laws, and has drafted a research agreement for use between NIRSC and those interested in using our spectra/chemistry. The NIRSC Board is reviewing the document for editing and approval.</p>
Classes of NIRSC Membership	<p>The objective of this project is to define membership categories for our membership campaign. Four membership categories have been approved by the NIRSC Board of Directors for implementation beginning of FY 05-06:</p> <ol style="list-style-type: none"> <li>1. <b>Regular Membership</b> <ul style="list-style-type: none"> <li>○ annual fee \$2500</li> <li>○ expected to participate by submitting spectra &amp; samples</li> <li>○ use NIRSC equations for commercial use</li> <li>○ voting members</li> </ul> </li> <li>2. <b>Research/Non-Profit</b> <ul style="list-style-type: none"> <li>○ annual fee \$1000</li> <li>○ a public entity, test non-commercial samples (= samples that may be from various sources,</li> </ul> </li> </ol>

	<ul style="list-style-type: none"> <li>○ but the member does not receive money for testing the samples), and do not compete with commercial labs</li> <li>○ use NIRSC equations for research and/or non-profit uses</li> <li>○ expected to participate by submitting spectra &amp; samples</li> <li>○ do not make money from the use of NIRSC equations</li> <li>○ voting members</li> </ul> <p><b>3. Sponsorship</b></p> <ul style="list-style-type: none"> <li>○ annual fee \$1500</li> <li>○ a commercial entity</li> <li>○ do not receive NIRSC equations</li> <li>○ contribute to NIRSC with expertise, collaboration, and samples, spectra, etc., and want to expand the utility of NIRSC</li> <li>○ voting members</li> </ul> <p><b>4. Collaborators</b></p> <ul style="list-style-type: none"> <li>○ no annual fee</li> <li>○ a public and non-profit entity</li> <li>○ collaborate from a research and extension standpoint or act as a reference laboratory</li> <li>○ non-voting members</li> </ul>
Unfermented Corn Silage Equation	<p>The objective of this project is to review the applicability of the Coors/Laur equation and outline liability indemnification if WARF licensing is used. After reviewing major issues related to this project such as particle size, sample handling, reference chemistry methods, and WARF licensing; the NIRSC board voted to create an independent NIRSC unfermented corn silage equation. Potential reference labs bid on the project, samples from participants have been selected, and samples are being sent to the reference lab. The equation should be ready in September.</p>
NIRSC/RFAC	<p>The objective of this project is to detail how to partner these two organizations. At this time NIRSC is waiting for RFAC to establish itself as an organization. After this is done, partnership will again be discussed.</p>
Starch	<p>The objective of this project is to receive a "recommended" procedure from Mary Beth Hall and to outline implications for other NIRSC products. Mary Beth has been working on literature review and analysis methods and has prepared an <a href="#">Update on Starch Analysis Evaluation</a>. Please see the attached document for Mary Beth's discussion, which was presented at least in part at the Dairy and Animal Sciences meeting in Cincinnati.</p>
Cincinnati Meeting	<p>The objective of this project was to put on an NIR seminar during the Dairy Science meetings in Cincinnati at the end of July, 2005 and establish NIR utility with a white paper as a product. The meeting was a success with 96 people attending. Neal Martin, Don Sapienza, Paolo Berzaghi, and Chuck Kahl spoke about issues that relate to NIR utility. All speakers are processing their talks for the white paper to be completed this year.</p>

Please see the Winter newsletter for a list of those on these committees and for their contact information, or contact Patty Laskowski

## ***We Need Your Spectra for Selection!***

We need to do continuing selection for all of our equation updates.

Send your spectra from all types of forage products to Alistair Carr at:  
[acarr@wisc.edu](mailto:acarr@wisc.edu) ph: 608-890-0060

## **Alistair's Report**



We'd like to keep you updated on activities in the Consortium's technical lab.

***These are some of the technical activities that we do in order to work towards our goal of accuracy in NIRS testing.***

### **Technical Support:**

In the last 3 months, Alistair has supported our labs by aiding with inquiries about our equations, helping with questions on report formats, aided with a check cell drift query, and repaired 2 of our standardization cups.

### **Standardization**

As part of our instrument monitoring program and also as part of the NAMA project, standardization of our member labs has been going on at a consistent pace this year. Alistair has standardized 11 labs' instruments, including

1 new member and 2 required after a repair of the instrument.

Also as part of our instrument monitoring program, we need to maintain our own master instrument file. After scanning our standardization samples on the ISI master instrument, the old (May 2002) and new standardization files performed similarly, with modest improvements to the new one.

### **Data Management**

A huge improvement to our equations update and sample handling process will be a sample tracking database in which we can track spectra, chemistry, and samples coming in to the NIRSC. From there we will be able to report on what happens to the spectra, chemistry, and samples and where we are in the

process of equation updating.

### **Calibration & Equation Development**

Alistair continues to work with Paolo in learning the techniques of sample selection and equation development for our commercial labs as well as our seed breeders.

### **Instrument Diagnostics**

Seven of our member labs are currently active in the weekly reporting program of instrument diagnostics. Over the last 3 months 19 failures of individual diagnostic parameters have been reported and addressed. We encourage all of our members to participate in weekly diagnostics reporting to keep your instrument in top working order.

**Contact Alistair with your questions at:**

[acarr@wisc.edu](mailto:acarr@wisc.edu)  
**608-890-0060**

# Welcome New Members

We would like to welcome 2 members to the NIRS Consortium. Stanworth Crop Consultants is located in Blythe California and does plant and soil analysis. You can learn more about them and visit their website at: <http://www.stanworth.net>. Agri Analysis Inc is located in Leola Pennsylvania and does a variety of tests for soils, plants, and agricultural products. You can learn more about them and visit their website at: <http://www.agrianalysis.com>.

## Instrument for Sale

We are listing the availability of NIRSystem 5000 instrument\*\*. This instrument is pre-owned, 3000 series with auto-gain pre-aligned lamp. Currently the instrument is being certified by an authorized engineer. Along with 30 days warranty the owner will share long and short diagnostics results for the buyer. The asking price is 24K, but negotiable. Please contact Syed Dara at [dara0012@umn.edu](mailto:dara0012@umn.edu) if you are interested.

\*\*NIRSC is providing this listing as a service to its members and in no way is involved in the sale or endorsement of any type of instrument.

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## Update on Starch Analysis Evaluation

Mary Beth Hall, 7-7-05

Have been working on both a review of literature, analytical work, and contacting other carbohydrate analysts to make most rapid progress on factors that affect starch analysis.

Literature review/discussions: Possible issues with starch assays that could lead to reduced recovery:

- ◆ Reversion of glucose to  $\alpha$ -glucan oligosaccharides by amyloglucosidase. If the concentration of enzyme to substrate is too high (one option),  $\alpha$ -glucan can be resynthesized from glucose released from starch hydrolysis and will not then be measurable as released glucose.
- ◆ Creation of maltulose. When heat stable  $\alpha$ -amylase hydrolyzes starch to smaller oligosaccharides under neutral to basic pH (as in gelatinization in water), the glucose on the reducing end of the molecules can be converted (isomerized) to fructose, giving a molecule that is an  $\alpha$ -glucan with a fructose on the reducing end. Amyloglucosidase can hydrolyze the molecules down to the last glucose + fructose. The glucose+fructose is maltulose; it is not hydrolyzed by amyloglucosidase, and it does not analyze as starch. Key is to keep solutions with the oligosaccharides at a more acidic pH to avoid the isomerization, or do not create a great number of reducing ends (i.e., hydrolyze the starch to smaller molecules) while at neutral to basic pH.
- ◆ Expect a  $\pm$  2 percentage unit error with starch assays.

- ◆ Common starch recovery of ~96% for the two enzyme starch method (hot water+heat-stable  $\alpha$ -amylase liquefaction of starch with subsequent amyloglucosidase hydrolysis at pH 4.5).
- ◆ Phosphate groups on starch may limit enzymatic digestion. Suggestion offered that the natural phosphorylation of starch (phosphate groups esterified to the glucose in starch, amount varies) may present challenges to enzymatic hydrolysis (suggestion that the phosphate may block the enzyme). Likely that this depends on extent of phosphorylation and analysis conditions that may remove the phosphate groups.

### **Analytical work**

1. Evaluated effects on recovery of glucose and corn starch: a) gelatinization time (23 or 60 min), b) use of volumetric flasks vs. 50 ml screw cap tubes with volumetric additions, and c) starch substrate amount (0.1 to 0.2 g). Used the two enzyme method: hot water+heat-stable  $\alpha$ -amylase (source: ANKOM) liquefaction of starch with subsequent amyloglucosidase hydrolysis at pH 4.5 (Sigma A-1602; added 8.7 to 12.4 units per starch sample with 8 ml Na acetate buffer with 1 ml of sample that contained up to 1990 ug/ml of glucose).

- ◆ Recoveries 99.2% to 102.6% for glucose run through the starch assay. Suggests that we're not getting reversion of glucose to short chain  $\alpha$ -glucans with the amount of enzyme and substrate used in the assay. This also shows the approximately  $\pm$  2% error reported by others for starch assay.
- ◆ Recoveries for corn starch were 93.6 to 95.7% of sample dry matter. Recoveries differed by run ( $P < 0.01$ ), but were not affected by vessel type for amyloglucosidase hydrolysis ( $P = 0.91$ ), gelatinization time ( $P = 0.97$ ), or sample amount ( $P = 0.99$ ).

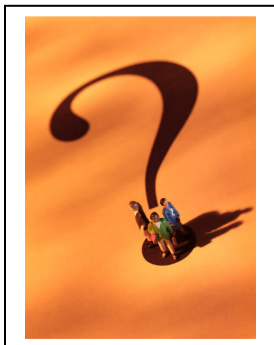
2. Evaluated glucose sources for use as standards. Glucose source for standard matters, not surprising. Use at 99.5% or greater reagent glucose if preparing your own standard solutions. Verify dry matter content of glucose and use to adjust to accurately describe glucose content.

### **Food for Thought**

The maltulose issue – converting some of the starch to material that won't analyze as starch – may help answer why 96% starch recovery is common in the two enzyme method. I do not think it is a good idea to come up with a multiplier to adjust for the recovery of a pure starch sample. This presumes that the analysis conditions were identical for all other samples, when, in fact, the feeds themselves may generate different conditions.

### **Next Steps**

- ◆ Pursue other starch analysis routes
  - Alkali gelatinization with amyloglucosidase hydrolysis (no heat stable  $\alpha$ -amylase) -- may address potential phosphorylation and maltulose issues
  - Evaluate the use of the YSI system (we now have one in the lab)
  - Evaluate gelatinization times/methods
  - Pursue other enzyme/run condition options for freeing starch for hydrolysis



**For further information on any of these topics, please contact Patty Laskowski.**

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