

**UNIVERSITY OF GEORGIA***AGRICULTURAL and ENVIRONMENTAL SERVICES LABORATORIES*

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## Standard Operating Procedure

Subject:

**APPLICATION OF PROPER CALIBRATION EQUATION, PROOFING PREDICTED VALUES, CALCULATION OF TOTAL DIGESTIBLE NUTRIENTS AND RELATIVE FORAGE QUALITY, AND REPORTING PROTOCOL IN NEAR INFRARED (NIR) SPECTROSCOPIC ANALYSIS OF FORAGE QUALITY****Revision No. 5.0****Effective Date: July 27, 2020**Credits:Prepared  
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## 1.0 SCOPE AND APPLICABILITY

Near Infrared Reflectance (NIR) Spectroscopy uses the spectral properties of a forage samples in the NIR regions (wavelength range from 1100 nm to 2500 nm) to determine forage quality. But application of the appropriate calibration equations for the type of sample being analyzed is a prerequisite for correctly predicting various forage quality attributes. Based on the predicted basic attributes such as CP, FAT, ADF, NDF, dNDF48, and Ash etc, other quality attributes such as NFC, TDN, DMI, RFQ and others are calculated. NRC (2001) provides the guidelines for these calculations for the grasses and legumes. Use of appropriate calculation procedure according to sample type must be ascertained. This standard operating procedure (SOP) describes the guidelines to ascertain that appropriate calibration equation and calculation procedure are utilized.

## 2.0 METHOD SUMMARY

Preserve dry, ground, and screened (through 1 mm sieve) samples in a desiccator. Pack dry, ground (pass through 1mm screen) forage samples into ring cups, close and label, and place them back into a desiccator until they are scanned by the NIR spectrometer. Check the sample type and determine whether it is a grass hay, legume hay, grass+legume mixed hay, haylage/baleage, green chop, or fermented corn silage. Using the FOSS NIR Systems, Model 6500 with WINISI software version 1.5, begin the quality control measures of running a check cell (perform daily) and perform diagnostic testing (weekly). Report the outcomes of check cell run and diagnostic test to the QA officer of AESL. Once no change is observed in check cell runs, samples may be analyzed. Scan the sample with NIR system (SOP 644) and predict the quality attributes using the appropriate calibration equation for the sample type as described in the scheme outlined in Table 1. Once the predicted attributes are in the database, calculations of NFC, TDN, DMI, RFQ, etc. will be performed within the database using appropriate formulas (given in Appendix 1) according to the sample type as described in the scheme outlined in Table 2.

## 3.0 Coding System and Appropriate Equation for NIRS Calibration

3.1 Upon receipt of sample, the employee responsible for sample log-in shall use the appropriate code for the sample type and then send the paperwork for the request for analysis along with a copy of the submission form to the analysis station. Use **Table 1** below as the guideline. Any mistake in doing this will result inappropriate application

of calibration equation, erroneous calculation, and wrong reporting.

3.2 The NIRS Research Professional-I will apply the appropriate calibration equation in accordance with the type of sample and code that appear on the paperwork for the request for analysis. *It is required that NIRS Research Professional-I will verify the code with the submission form and become sure about the type of sample before applying the calibration equation.* Use **Table 1** below and revised Feed and Forage Submission Form (with codes) on **page 16** as the guideline. Any mistake in doing this will result in inappropriate application of calibration equation and wrong reporting.

**TABLE 1: CODING SYSTEM AND APPROPRIATE EQUATION FOR NIRS ANALYSIS IN FEWL**

Use	Crop	Code to be Used	Equation to be Used
01 SILAGE	01 CORN	01-01	16cs50b2.eqa
01 SILAGE	02 SORGHUM (SILAGE)	01-02	16hg50b2.eqa
01 SILAGE	03 SMALL GRAINS:	<i>See 4 rows below</i>	
01 SILAGE	21 Wheat	01-03-21	16hg50b2.eqa
01 SILAGE	22 Rye	01-03-22	16hg50b2.eqa
01 SILAGE	23 Barley	01-03-23	16hg50b2.eqa
01 SILAGE	24 Oats	01-03-24	16hg50b2.eqa
01 SILAGE	04 ALFALFA	01-04	16hg50b2.eqa
01 SILAGE	05 OTHER LEGUME	01-05	16hg50b2.eqa
01 SILAGE	06 GRASS	01-06	16hg50b2.eqa
01 SILAGE	07 MIXED	01-07	16hg50b2.eqa
01 SILAGE	08 SORGHUM (GRAIN)	01-08	16hg50b2.eqa
01 SILAGE	99 OTHER	01-99	16hg50b2.eqa
02 GREEN CHOP	01 CORN	02-01	16UCS50.eqa
		<ul style="list-style-type: none"> <li>• <i>Currently we do not have this equation.</i></li> <li>• <i>Analyze by wet chemistry until the equation is made available.</i></li> </ul>	
02 GREEN CHOP	02 SORGHUM (SILAGE)	02-02	16gh50b2.eqa
02 GREEN CHOP	03 SMALL GRAIN	<i>See 4 rows below</i>	
02 GREEN CHOP	21 Wheat	02-03-21	16gh50b2.eqa
02 GREEN CHOP	22 Rye	02-03-22	16gh50b2.eqa
02 GREEN CHOP	23 Barley	02-03-23	16gh50b2.eqa
02 GREEN CHOP	24 Oats	02-03-24	16gh50b2.eqa
02 GREEN CHOP	04 ALFALFA	02-04	16ah50b2.eqa
02 GREEN CHOP	05 OTHER LEGUME	02-05	16mh50-2.eqa
02 GREEN CHOP	06 GRASS	02-06	16gh50b2.eqa
02 GREEN CHOP	07 MIXED	02-07	16gh50b2.eqa
02 GREEN CHOP	36 Grass/Legume Mix:		

TABLE 1: CODING SYSTEM AND APPROPRIATE EQUATION FOR NIRS ANALYSIS IN FEWL (Cont'd.)

02 GREEN CHOP	Grass+Legume mix with >40% legume	02-36 (>40%L)	16mh50-2.eqa
02 GREEN CHOP	Grass+Legume mix with <40% legume	02-36 (<40%L)	16gh50b2.eqa
02 GREEN CHOP	Grass+Legume mix with legume proportion unknown	02-36 (Unkn%L)	16gh50b2.eqa
02 GREEN CHOP	99 OTHER	02-99	Consult Program Coordinator or Supervisor of FEWL
03 HAY	03 SMALL GRAIN	<i>See 4 rows below</i>	
	21 Wheat	03-03-21	16gh50b2.eqa
	22 Rye	03-03-22	16gh50b2.eqa
	23 Barley	03-03-23	16gh50b2.eqa
	24 Oats	03-03-24	16gh50b2.eqa
03 HAY	04 ALFALFA	03-04	16ah50b2.eqa
03 HAY	05 OTHER LEGUMES	03-05	16mh50-2.eqa
03 HAY	06 GRASS or MIXED GRASSES	03-06	16gh50b2.eqa
03 HAY	09 BAHIA	03-09	16gh50b2.eqa
03 HAY	10 BERMUDAGRASS		16gh50b2.eqa
03 HAY	01 Alicia	03-10-01	16gh50b2.eqa
03 HAY	03 Coastal	03-10-03	16gh50b2.eqa
03 HAY	04 Common	03-10-04	16gh50b2.eqa
03 HAY	05 Tifton-44	03-10-05	16gh50b2.eqa
03 HAY	06 Tifton-78	03-10-06	16gh50b2.eqa
03 HAY	07 Mixed	03-10-07	16gh50b2.eqa
03 HAY	08 Tifton-85	03-10-08	16gh50b2.eqa
03 HAY	10 Coastcross	03-10-10	16gh50b2.eqa
03 HAY	11 Russell	03-10-11	16gh50b2.eqa
03 HAY	00 Other	03-10-00	16gh50b2.eqa
03 HAY	11 FESCUE/ORCHARDGRASS	03-11	16gh50b2.eqa
03 HAY	12 MILLET	03-12	16gh50b2.eqa
03 HAY	13 PEANUT	03-13	16mh50-2.eqa
03 HAY	36 GRASS/LEGUME MIX:		
	Grass+Legume mix with >40% legume	03-36 (>40%L)	16mh50-2.eqa
	Grass+Legume mix with <40% legume	03-36 (<40%L)	16gh50b2.eqa
	Grass+Legume mix with legume proportion unknown	03-36 (Unkn%L)	16gh50b2.eqa
03 HAY	37 PERENNIAL PEANUT	03-37	16mh50-2.eqa
03 HAY	38 ANNUAL RYEGRASS	03-38	16gh50b2.eqa
03 HAY	99 OTHER	03-99	Consult Program Coordinator or Supervisor of FEWL

**TABLE 1: CODING SYSTEM AND APPROPRIATE EQUATION FOR NIRS ANALYSIS IN FEWL (Cont'd.)**

03 HAY	39 Sorghum hybrids	03-39	11-gh50-2.eqa
09 HAYLAGE/BALEAGE <i>(See note at the end of this table on the next page for coding fermented and unfermented samples and application of appropriate equation)</i>	03 SMALL GRAIN		<i>See 4 rows below</i>
	21 Wheat	09-03-21	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
	22 Rye	09-03-22	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
	23 Barley	09-03-23	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
	24 Oats	09-03-24	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	04 ALFALFA	09-04	16hg50b2.eqa (16ah50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	05 OTHER LEGUMES	09-05	16hg50b2.eqa (16mh50-2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	06 GRASS or MIXED GRASSES	09-06	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	09 BAHIA	09-09	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	10 BERMUDAGRASS		16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	01 Alicia	09-10-01	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	03 Coastal	09-10-03	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	04 Common	09-10-04	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	05 Tifton-44	09-10-05	16hg50b2.eqa (16gh50b2.eqa, if unfermented))
09 HAYLAGE/BALEAGE	06 Tifton-78	09-10-06	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	07 Mixed	09-10-07	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	08 Tifton-85	09-10-08	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	10 Coastcross	09-10-10	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	11 Russell	09-10-11	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	00 Other	09-10-00	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	11 FESCUE/ORCHARDGRASS	09-11	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	12 MILLET	09-12	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	13 PEANUT	09-13	16hg50b2.eqa (16mh50-2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	36 GRASS/LEGUME MIX:		
	Grass+Legume mix with >40% legume	09-36 (>40%L)	16hg50b2.eqa (16mh50-2.eqa, if unfermented)
	Grass+Legume mix with <40% legume	09-36 (<40%L)	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
	Grass+Legume mix with legume proportion unknown	09-36 (Unkn%L)	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	37 PERENNIAL PEANUT	09-37	16hg50b2.eqa (16mh50-2.eqa, if

unfermented)

TABLE 1: CODING SYSTEM AND APPROPRIATE EQUATION FOR NIRS ANALYSIS IN FEWL (Cont'd.)			
09 HAYLAGE/BALEAGE	38 ANNUAL RYEGRASS	09-38	16hg50b2.eqa (16gh50b2.eqa, if unfermented)
09 HAYLAGE/BALEAGE	99 OTHER	09-99	Consult Program Coordinator or Supervisor of FEWL
09 HAYLAGE/BALEAGE	39 Sorghum hybrids	09-39	16hg50b2.eqa (16gh50b2.eqa, if unfermented)

**NOTE:** Fermentation status of the samples checked as “Haylage/Baleage” on the submission form by the clients should be indicated during coding. For example, “09-09 (ferm)” will be used for fermented Bahia grass haylage/baleage and “09-09 (unferm)” will be used for unfermented Bahia grass haylage/baleage.

#### 4.0 Sending NIRS Predicted Data Files to the Database

The NIRS Research Professional-I will proof the predictions on the screen using the appropriate data checks (given below) for the various calibration applied. Once data checks confirm that appropriate calibration was applied, the data will be exported to a filename based on the calibration equation, as follows:

Equation	Filename	Data checks
16gh50b2.eqa	1hFY[a-z]_gh.txt	Lab numbers, Sugars
16hg50b2.eqa	1hFY[a-z]_hg.txt	Lab numbers, ADP
16mh50-2.eqa	1hFY[a-z]_mh.txt	Lab numbers, IVTDMD48H
16ah50b2.eqa	1hFY[a-z]_ah.txt	Lab numbers, RUP
16cs50b2.eqa	1hFY[a-z]_cs.txt	Lab numbers, Starch

Where:

- **FY** is the fiscal year.
- **[a-z]** is an optional letter between **a** and **z**.

For example, **1h21\_gh.txt** and **1h21d\_gh.txt** are valid filenames for fiscal year 14, for grass hay calibration. *Maintenance of consistent format in file naming is required.*

The sample ID should contain the lab number only. *It should not include the sample type (use, crop, or variety).*

After exporting, the NIRS Research Professional-I will proof the data by reviewing all data checks cited in the table above.

#### 5.0 Appropriateness of Applied Calibration Equations, and NFC, TDN, DMI, & RFG Calculation Protocol

Once the predictions are received, the database manager will verify whether the appropriate calibration equation was used for the specified sample type as instructed in **Table 1**. If any error is detected, the following protocol will be followed:

5.1 Database Manager will notify that error to the Coordinator and Supervisor by e-mail.

5.2 The coordinator (or the supervisor with consent from the coordinator) will send an e-mail to the NIRS Research Professional-1 with a copy to the Database Manager and the Supervisor (or Coordinator, if the Supervisor is sending the e-mail) requesting a quick verification and feedback on the detected error.

5.3 As soon as possible, the NIRS Research Professional-1 will verify the detected error, if necessary repredict the sample with appropriate equation, send the correct data file to the database, and *finally send an e-mail* to the Coordinator (or the Supervisor, if the Supervisor initiated the verification and feedback request) with a copy to the Supervisor (or the Coordinator, if the Supervisor initiated the verification and feedback request) and the Database Manager describing the findings of verification and action taken.

*Turn around time should be a priority for all personnel involved in completing this work.*

5.4 Once the appropriateness of the applied calibration equation is ascertained, the Database Manager will calculate NFC, TDN, DMI, RFQ, etc. using appropriate formulas given by NRC (2001) as guided in **Table 2**. **The formulas are given in Appendix 1.**

**TABLE 2.** Guidelines for calculation of TDN, DMI, and RFQ.

Applied Equation	Sample	TDN, DMI, and RFQ Calculation Protocol
16ah50b-2.eqa	Alfalfa	Use legume formulas
16mh50-2.eqa	Legumes other than alfalfa <ul style="list-style-type: none"> <li>• Peanut</li> <li>• Clover etc</li> </ul>	Use legume formulas
16mh50-2.eqa	Grass+Legume mix with >40% legume	Use legume formulas
16gh50b-2.eqa	Grass+Legume mix with <40% legume	Use grass formulas
16gh50b-2.eqa	Grass+Legume mix with proportion of legume unknown	Use grass formulas
16gh50b2.eqa	Grasses	Use grass formulas
16cs50b2.eqa	Fermented corn silage	No calculation needed
16hg50b2.eqa	Sorghum (silage)	Use grass formulas

16hg50b2.eqa	Small grains:	
16hg50b2.eqa	Wheat	Use grass formulas
16hg50b2.eqa	Rye	Use grass formulas
16hg50b2.eqa	Barley	Use grass formulas
16hg50b2.eqa	Oats	Use grass formulas
16hg50b2.eqa	Alfalfa	Use legume formulas
16hg50b2.eqa	Other legume <ul style="list-style-type: none"> <li>• Peanut</li> <li>• Clover, etc.</li> </ul>	Use legume formulas
16hg50b2.eqa	Grass	Use grass formulas
16hg50b2.eqa	Mixed: <ul style="list-style-type: none"> <li>• with &gt;40% legume</li> <li>• with &lt;40% legume</li> <li>• with proportion of legume unknown</li> </ul>	Use legume formulas Use grass formulas Use grass formulas
16hg50b2.eqa	Sorghum (grain)	Use grass formulas
16hg50b2.eqa	Other	Use grass formulas

**Note:**

- 1) None of the calibrations predicts NFC (non-fibrous carbohydrate). NFC will always be calculated as:
  - $\text{NFC (\% of DM)} = 100 - (\text{NDFn} + \text{CP} + \text{FAT} + \text{Ash})$
  - $\text{NDFn} = \text{NDF} \times 0.93$

When NDF, CP, and/or Ash are flagged and we do wet chemistry on one or more of these parameters, the NFC will be re-calculated and the re-calculated value will be reported and will be used for subsequent calculation of TDN, DMI, and RFQ as described in **section 5.0**.

**6.0 Flagging NIR Data and Trouble Shooting**

- 6.1 **Data to be Flagged:** CP, ADF, NDF, Lignin, and Ash, if they are outside the Mean  $\pm$  2SD. Mean and SD of CP, ADF, NDF, Lignin, and Ash as cited by NIRS consortium for the particular type of calibration applied for prediction will be used.
- 6.2 **Troubleshooting of Flagged Data:** Samples with flagged CP, ADF, NDF, Lignin, and Ash values will be handled in one of the following two ways as applicable:
  - 6.2.1 If any value(s) is within  $\pm$ 5% of the acceptable limits, remove the flag and report as it is.
  - 6.2.2 If any value(s) is >5% higher than the upper limit of the acceptable range or >5% lower than the lower limit of the acceptable range, re-run a wet chemistry analysis for that parameter(s) to determine the true value(s). Insert the wet chemistry data and recalculate NFC, TDN, DMI, RFQ, etc.

## 7.0 Reporting Protocol

7.1 The report will **generally** have the following new parameters: NFC, dNDF<sub>48</sub>, NDFD<sub>48</sub>, DDM<sub>48</sub>, and DMI. However, the following points may be exception and merit special attention.

7.1.1 For corn silage (analyzed by 16cs50b2.eqa), no RFQ, DMI will be calculated and reported. TDN will be reported by calculation using the following equation (*source: Pennsylvania State University. Dairy Reference Manual. NRAES-63, 3rd edition, 1995*):

- $TDN = 31.4 + 53.1 \times NEL$
- $NEL = 1.044 - 0.0124 \times ADF$

7.1.2 DDM<sub>48</sub> will be estimated as:  $DDM_{48} = dNDF_{48} + NFC + CP + Fat$  and reported as “DDM (estimated)”.

7.1.3 NDFD<sub>48</sub> will be calculated as:  $NDFD_{48} = \frac{dNDF_{48}}{NDF} \times 100$

and the calculated value will be reported as long as the calculated value is ≤85% and dNDF<sub>48</sub> is within the acceptable limit of the equation (2013) used as given in the Table below.

Equation	“Mean ± 2×SD” of dNDF <sub>48</sub> (%)	Acceptable Limits (%)
GH (Grass Hay)	30.82 ± 9.02	12.78 – 48.86
AH (Alfalfa Hay)	21.46 ± 6.65	8.16 – 34.76
HG (Haylage/Baleage)	21.34 ± 5.62	10.10 – 32.58
CS (Corn Silage)	27.62 ± 5.09	17.44 – 37.80
MH (Mixed Hay)	23.09 ± 7.39	8.31 – 37.87

If the predicted dNDF<sub>48</sub> falls outside the limit of its “Mean ± 2×SD”, the system will flag the results for approval.

***In any case, if the calculated NDFD<sub>48</sub> is greater than 85%, it will be dropped from reporting.***

7.2 **Dry Matter Basis Reporting:** All parameters except DM, TDN, DMI, and RFQ will be reported as predicted by NIRS (which is on a true 0% moisture basis) or calculated using NIRS predicted parameters without any adjustment based on NIR predicted DM content.

7.3 **Dry matter (DM) and Moisture** will be reported as follows:

Suppose,

Wet chemistry moisture content (%) =  $M1$

Wet chemistry dry matter content (%),  $DM1 = 100 - M1$

**NIRS or NFTA** moisture content (%) =  $M2$

**NIRS or NFTA** dry matter content (%),  $DM2 = 100 - M2$

Reportable dry matter content (%),  $DMr = DM1 \times \frac{DM2}{100}$

Reportable moisture content (%),  $Mr = 100 - DMr$

See the following example for further clarification:

<i>M1</i>	<i>DM1</i>	<i>DM2</i>	<i>DMr</i>	<i>Mr</i>
25	75	98	<b>73.5</b>	<b>26.5</b>
45	55	91	<b>50.5</b>	<b>49.5</b>

#### 7.4 The “As-Sampled Basis” Reporting When Minerals and Nitrate (or Other Wet Chemistry Parameters) are Requested Together with NIRS

##### 7.4.1 For NIRS Predicted Parameters

*Note: All NIRS predicted values are on a true dry matter or 0% moisture based*

*As-Sampled Basis Values (%)*

$$= \text{NIRS Predicted Values (\%)} \times \frac{DMr}{100}$$

**Or**

*As-Sampled Basis Values (%)*

$$= \text{NIRS Predicted Values (\%)} \times \frac{DM2}{100} \times \frac{DM1}{100}$$

See the following example for further clarification

**Example<sup>§</sup>:**

<i>M1</i>	<i>DM1</i>	<i>DM2</i>	<i>DMr</i>	<i>Mr</i>	<b>NIRS Predicted NDF or True Dry Matter Basis NDF</b>	<b>As-Sampled Basis NDF</b>
%						
25	75	98	<b>73.5</b>	<b>26.5</b>	<b>64.25</b>	<b>47.22</b>
45	55	91	<b>50.5</b>	<b>49.5</b>	<b>68.54</b>	<b>34.61</b>

<sup>§</sup>*M1* = Wet chemistry moisture content (%); *DM1* = Wet chemistry dry matter content (%) =  $100 - M1$ ; *DM2* = NIRS dry matter content (%); *DMr* = Reportable dry matter content (%) =  $DM1 \times \frac{DM2}{100}$ ; *Mr* = Reportable moisture content (%) =  $100 - DMr$ .

##### 7.4.2 For Minerals and Nitrate (or Other Wet Chemistry Parameters)

Suppose,  
Analytical value for Minerals or Nitrate = *AV*

Wet chemistry dry matter content (%) =  $DM1$  (described in **section 7.4.1**)

$$\text{As-Sampled Basis Minerals or Nitrate content} = AV \times \frac{DM1}{100}$$

- 7.5 TDN, DMI, and RFQ for all forage types except corn silage (see *section 7.1.1 for handling TDN calculation for corn silage*) will be calculated from the required parameters predicted by NIRS (no adjustment will be needed) using appropriate formulas (**Table 2 and Appendix I**) provided by NRC (2001) and the calculated values will be reported.
- 7.6 DMI and RFQ are always “Dry-Matter Basis” and will be reported on the top. No “As-Sampled Basis” values for DMI and RFQ will appear on the report.
- 7.7 The calculated “Dry-Matter Basis” TDN will be converted to “As-Sampled Basis” as described in **section 7.4** and will be reported on both bases.
- 7.8 **Handling Mineral and Nitrate Analysis (and Other Wet Chemistry Data as well) Data:**

- 7.8.1 When mineral and/or nitrate analysis are requested along with NIRS, the “As-Sampled Basis” data for both NIR and Mineral will be based on the new protocol (**section 7.4**) taking into account both wet chemistry and NIR moisture (or DM).

The “Dry-Matter Basis” **Mineral or Nitrate** content will be reported using NIR dry matter content as follows.

Suppose, NIR dry matter content (%) =  $DM2$   
Analytical value for Mineral or Nitrate =  $AV$

$$\text{The dry matter basis Mineral or Nitrate content} = AV \times \frac{100}{DM2}$$

- 7.8.2 But when **only** mineral analysis (F26), nitrate or other wet chemistry parameters is requested, a second moisture content will be determined on the dried (*partially dried*) and ground sample used for the wet chemistry analysis (**the wet chemistry lab personnel call it “NFTA moisture”, the database will take this as  $M2$** ) in addition to our ordinary wet chemistry moisture ( $M1$ ) and then the “Dry-Matter Basis” and “As-Sampled Basis” results will be calculated as follows:

7.8.2.1 "Dry-Matter Basis" Value =

$$AV \times \frac{100}{100 - M2}$$

7.8.2.2 "As-Sampled Basis" value =

$$AV \times \frac{100 - M1}{100}$$

## 8.0 Quality Assurance/Quality Control

8.1 For the machine: **Run diagnostic test every week** to check on: Response, Wavelength Accuracy & Repeatability.

8.2 **Run the purchased Check cell every day** and submit the outcome (*print the screen*) with signature and date to QA officer demonstrating "*No change in checkcell*" and the value of "T" is less than 3.0.

8.3 **In-house check cell every day.** This check is a National Forage Testing Association (NFTA) quality control sample from a previous proficiency testing round. Sample parameters should be within the acceptable limits established based on the RMA (Reference Method Average) and HSD or mHSD of a given sample obtained from NFTA. For this exercise, the following points merit special attention:

- Select NFTA samples that are representative of the routine samples to be analyzed on a given day.
- Repack NFTA samples weekly to eliminate sample deterioration.
- Test for dry matter, crude protein, ADF, NDF.
- This sample should be run once at the beginning and again at the end of each batch of analysis and results should be transmitted to database with proper ID so that the database manager can conveniently recognize this QC sample.
- Also report the results of this QC sample for each batch in the following format and submit it to QA officer for approval.

<b>FEED AND ENVIRONMENTAL LABORATORY, UGA  FORAGE ANALYSIS BY NIRS  QC BATCH REPORT</b>											
Date:	Batch#	Sample IDs included in the batch:									
<b>NFTA Sample ID</b>		<b>DM (%)</b>	<b>CP (%)</b>	<b>ADF (%)</b>	<b>NDF (%)</b>						
	True value										
	Beginning of the run										
	RPD										
	Acceptable?	Yes/No	Yes/No	Yes/No	Yes/No						
	End of the run										
	RPD										
	Acceptable?	Yes/No	Yes/No	Yes/No	Yes/No						
<table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none;">Analyst</td> <td style="width: 15%; border: none;">Signature</td> <td style="width: 15%; border: none;">Date</td> <td style="width: 15%; border: none;">Dr. Y. Yang QA Officer</td> <td style="width: 15%; border: none;">Signature</td> <td style="width: 15%; border: none;">Date</td> </tr> </table>						Analyst	Signature	Date	Dr. Y. Yang QA Officer	Signature	Date
Analyst	Signature	Date	Dr. Y. Yang QA Officer	Signature	Date						

8.4 Room Temperature should be maintained at 21°C±5°C and humidity should be maintained at <60%. No volatile chemicals should be present in area where FOSS NIR is located. Reduce air drafts as much as possible. No cell phones near the instrument.

## Appendix 1. How Do We Calculate Relative Forage Quality (RFQ) in Georgia?

### 1.0 RFQ calculation:

$$\text{❖ RFQ} = \text{DMI (\% of BW)} \times \text{TDN (\% of DM)} \div 1.23$$

### 1.1 How do we calculate TDN of RFQ equation?

#### 1.1.1 TDN for Legume:

$$\text{❖ TDN}_{\text{legume}} (\% \text{ of DM}) = (\text{CP} \times 0.93) + (\text{FAT} \times 0.97 \times 2.25) + [\text{NDFn} \times (\text{NDFD} \div 100)] + (\text{NFC} \times 0.98) - 7$$

Where:

- NDFn = NDF  $\times$  0.93
- CP = Crude Protein
- NDFD = 48-hour *in vitro* NDF digestibility (% of NDF) =  $\text{dNDF}_{48} / \text{NDF} \times 100$
- NFC = non fibrous carbohydrate (% of DM) =  $100 - (\text{NDFn} + \text{CP} + \text{FAT} + \text{Ash})$   
or you may have NFC directly from the prediction

#### 1.1.2 TDN for Grass:

$$\text{❖ TDN}_{\text{grass}} (\% \text{ of DM}) = (\text{NFC} \times 0.98) + (\text{CP} \times 0.87) + [(\text{FAT} - 1) \times 0.97 \times 2.25] + [\text{NDFn} \times (\text{NDFDp} \div 100)] - 10$$

Where:

- All other terms except NDFDp are as defined previously and  $\text{NDFDp} = 22.7 + 0.664 \times \text{NDFD}$
- Take 2.5% Fat (dry-matter basis) for calculation as recommended by NIRS consortium (Fat is not predicted by the calibration equation “11-gh50-2.eqa”, see note under Table 2)

### 1.2 How do we calculate DMI of RFQ equation?

#### 1.2.1 DMI for Legume:

$$\text{❖ DMI}_{\text{Legume}} (\% \text{ of BW}) = (120 \div \text{NDF}) + \{(\text{NDFD} - 45) \times 0.374 \div 1350 \times 100\}$$

#### 1.2.2 DMI for Grass:

$$\text{❖ DMI}_{\text{Grass}} (\% \text{ of BW}) = -2.318 + 0.442 \times \text{CP} - 0.0100 \times \text{CP}^2 - 0.0638 \times \text{TDN} + 0.000922 \times \text{TDN}^2 + 0.180 \times \text{ADF} - 0.00196 \times \text{ADF}^2 - 0.00529 \times \text{CP} \times \text{ADF}$$

Where:

- CP (Crude Protein), ADF (Acid Detergent Fiber), and TDN (Total Digestible Nutrient) are expressed as % of DM.

- **Note: If CP is >16%, it will be adjusted to 16% and the adjusted CP will be used for DMI calculation only. But TDN calculation will be based on the actual CP.**

1.3 For corn silage (analyzed by 13cs50b2.eqa), TDN will be calculated using the following equation (source: *Pennsylvania State University. Dairy Reference Manual. NRAES-63, 3rd edition, 1995*):

- $TDN = 31.4 + 53.1 \times NEL$
- $NEL = 1.044 - 0.0124 \times ADF$

Feed and Forage Testing Application Form – Beef Cattle and Dairy Cattle

**Client Information**

Name: \_\_\_\_\_ Date: \_\_\_\_\_  
 Address: \_\_\_\_\_ Sample No: \_\_\_\_\_  
 City: \_\_\_\_\_ State: \_\_\_\_\_ Zip: \_\_\_\_\_ Phone: \_\_\_\_\_  
 Date Received in County Office: \_\_\_\_\_ County: \_\_\_\_\_ Agent: \_\_\_\_\_

**Animal Classes (Please check only the most important class)**

**Beef Cattle:**  
 Dry Cows hd 211 wt \_\_\_\_\_ Stocker hd (steers) 221 hd (heifers) 222 wt \_\_\_\_\_ exp. ADG \_\_\_\_\_  
 Lactating Cows hd 212 wt \_\_\_\_\_ Feedlot hd (steers) 231 hd (heifers) 232 wt \_\_\_\_\_ exp. ADG \_\_\_\_\_  
 Heifers hd 213 wt \_\_\_\_\_ Other 290

**Dairy Cattle:** Lactating Cows 311 Dry Cows 312 Heifers 313 wt. \_\_\_\_\_

Group	Num	Wt.	Milk lb.	Fat%	Days in Milk	
1	_____	_____	_____	_____	_____	
2	_____	_____	_____	_____	_____	Other: <u>390</u>
3	_____	_____	_____	_____	_____	Milk price, \$/cwt _____

**Test(s) Requested (Check all appropriate.)**

F1 Hays and Silage (NIR + nitrate + minerals)  F7 Routine Feed Analysis (Includes minerals)  
 F2 Hays and Silage (NIR + nitrate, excluding minerals)  F8 Proximate Analysis (Protein, Fat, Fiber, Moisture & Ash)  
 F3 Hays and Silage (NIR only)  F10 Crude Protein  
 F4 Hays and Silage (Wet Chemistry) includes minerals  F14 Nitrates  
 F5 Hays and Silages (Wet Chemistry) excludes minerals Other: \_\_\_\_\_  
 F6 Hays and Silages (Wet Chemistry) Other: \_\_\_\_\_

**Type of Feed and Forage**

Please check the one most appropriate type from the list below.

<p><b>Silage: <u>01</u></b>  <u>01</u> Corn  <u>02</u> Sorghum (Silage)  <u>03</u> Small grain  <u>21</u> Wheat  <u>22</u> Rye  <u>23</u> Barley  <u>24</u> Oats  <u>08</u> Sorghum (grain)  <u>04</u> Alfalfa  <u>05</u> Other legume  <u>06</u> Grass  <u>07</u> Mixed  <u>99</u> Other:</p>	<p><b>Hay: <u>03</u> Haylage/baleage: <u>09</u></b>  <u>04</u> Alfalfa  <u>38</u> Annual Ryegrass  <u>09</u> Bahia  <u>10</u> Bermudagrass  <u>01</u> Alicia  <u>03</u> Coastal  <u>10</u> Coastcross  <u>04</u> Common  <u>05</u> Tift 44  <u>06</u> Tift 78  <u>08</u> Tift 85  <u>07</u> Mixed  <u>11</u> Russell          Other (specify): _____  <u>11</u> Fescue/Orchardgrass  <u>12</u> Millet  <u>03</u> Small Grain  <u>21</u> Wheat  <u>22</u> Rye  <u>23</u> Barley  <u>24</u> Oats  <u>36</u> Grass/Legume Mix          _____ % legume (est.)  <u>13</u> Peanut  <u>37</u> Perennial Peanut  <u>05</u> Other Legumes  <u>39</u> Sorghum hybrids  <u>99</u> Other:</p>	<p><b>Grain: <u>04</u></b>  <u>01</u> Corn  <u>08</u> Grain Sorghum  <u>14</u> Wheat  <u>15</u> Barley  <u>16</u> Oats  <u>17</u> Triticale  <u>99</u> Other:</p> <p><b>Protein Source: <u>05</u></b>  <u>18</u> Soybean Meal 48  <u>19</u> Soybean Meal 44  <u>20</u> Cottonseed Meal  <u>21</u> Peanut Meal  <u>22</u> Whole Cottonseed  <u>23</u> Protein Supplement          (% Protein )</p> <p>Other not listed:          _____          _____</p>	<p><b>By-products: <u>06</u></b>  <u>24</u> Soybean hulls  <u>25</u> Cottonseed hulls  <u>26</u> Peanut hulls  <u>27</u> Citrus pulp  <u>28</u> Brewers grains, wet  <u>29</u> Poultry litter  <u>30</u> Wheat midds  <u>99</u> Other:</p> <p><b>Mineral Mixes: <u>07</u></b>  <u>31</u> Base mix  <u>32</u> Premix  <u>33</u> Trace-mineral mix  <u>99</u> Other:</p> <p><b>Mixed Feeds: <u>08</u></b>          Complete Feed <u>34</u>          (list ingredients separately)          Silage/grain mix <u>35</u>          Silage: _____ Grain: _____</p>
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Please send all samples and forms to: *For Lab Use Only*  
 Ag & Environmental Services Laboratories  
 Feed & Environmental Water Laboratory  
 2300 College Station Rd  
 Athens GA 30602-4356  
 Lab # \_\_\_\_\_  
 03/23/2012 Rick Hitchcock

For assistance in formulating a ration, please contact your local Extension office (1-800-ASK-UGA-1).