

**NATIONAL FORAGE TESTING ASSOCIATION**  
**2010 METHODOLOGY QUESTIONNAIRE**

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**NOTE: Response to this questionnaire is a requirement for Proficiency recognition.**

**DO NOT mark on and/or return the questionnaire; your responses are to be reported on the answer sheet provided. The questionnaire should be retained for future reference.**

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**2010 METHODOLOGY QUESTIONNAIRE**

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**NIRS METHODOLOGY**

Answer the following questions **ONLY** if **NIRS** results are being submitted. If chemical results are being submitted, skip to the following section.

**1. *What type of NIRS instrument was used:***

1. NIR Systems 6500
2. NIR Systems 6250
3. NIR Systems 5000
4. NIR Systems 4500
5. NIR Systems 4250
6. Other

**2. *Is the standardization file and program used to adjust spectral data to match the master instrument before making calibrations or estimating analyses:***

1. No
2. Yes
3. Don't know

**3. *Were samples re-dried before they were scanned:***

1. No
2. Yes

**4. *Do you scan a laboratory control (in-house standard) sample each time the instrument is used:***

1. No
2. Yes

**5. *Were calibration samples:***

1. Microwave-dried
2. Oven-dried
3. Both
4. Don't know

**6. Are methods used to obtain reference values for use in calibration of the NIRS known:**

1. No
2. Yes, all or some methods known (describe the method used to obtain reference values by answering the questions listed under each assay).

### **DRY MATTER DETERMINATION**

**1. Are NIRS prediction equations for DM determinations (leave blank if results are obtained from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for DM bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 9 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analyses.**

**3. Type of oven:**

1. Vacuum
2. Gravity-convection
3. Forced-air
4. Other

**4. Oven temperature in degrees Celsius ( $^{\circ}\text{C}$ ), closest to that used:**

- |                           |                           |
|---------------------------|---------------------------|
| 1. 60 $^{\circ}\text{C}$  | 4. 100 $^{\circ}\text{C}$ |
| 2. 135 $^{\circ}\text{C}$ | 5. 105 $^{\circ}\text{C}$ |
| 3. 120 $^{\circ}\text{C}$ |                           |

**5. Number of hours sample was dried (select time closest to actual time):**

1. 3 hours
2. 2 hours
3. >3 hours

**6. Type of drying vessel:**

1. Paper bags or weigh boats
2. Thin aluminum foil pans
3. Heavy aluminum pans (such as AOAC pans that have covers)
4. Glass beakers
5. Porcelain crucibles
6. Other (describe)

**7. Approximate size of sample, in grams (select weight closest to actual weight):**

- |          |                   |
|----------|-------------------|
| 1. 0.5 g | 5. 5 g            |
| 2. 1.0 g | 6. 10 g           |
| 3. 2.0 g | 7. Entire package |
| 4. 3.0 g |                   |

**8. Weighing method:**

1. Samples weighed hot
2. Samples cooled in a desiccator, then weighed

**9. Which method is closest in technique to that used in your lab:**

1. Other
2. AOAC 930.15 Drying of feeds at 135°C for 2 hours; NFTA Forage Analysis Procedures (1993) 2.1.1. or 2.2.2.1.
3. Microwaving to constant weight; NFTA Forage Analysis Procedures (1993) 2.1.3. or 2.2.2.2.
4. Oven drying 80°C or less for 12 or more hours
5. Oven drying 100—105°C for 18 or more hours
6. Oven drying 100—105°C for 6-12 hours
7. NFTA Forage Analysis Procedures (new method), 2.1.4. or AOAC 935.29 Gravimetric for malt; 103-106°C for 3 hours.
8. AOAC 967.03 Moisture in peat at 105°C for 16 hours; NFTA Forage Analysis Procedures (1993), 1.2.2. or 2.2.2.2.

## **CRUDE PROTEIN DETERMINATION**

**1. Are NIRS prediction equations for CP determinations (leave blank if results are from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for CP bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 10 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analysis.**

**3. Approximate sample size, in grams (select weight closest to actual weight):**

- |                    |                     |
|--------------------|---------------------|
| 1. Less than 0.1 g | 5. 1.0 g            |
| 2. 0.1 g           | 6. 2.0 g            |
| 3. 0.25 g          | 7. Greater than 2 g |
| 4. 0.5 g           |                     |

**4. Type of digestion:**

1. Combustion N analyzer, such as LECO or Carlo Erba (leave answers 5-6 blank)
2. Macro-block
3. Micro-Kjeldahl
4. Macro-Kjeldahl
5. Hach
6. Other

**5. Type of digestion catalyst (only indicate the catalyst (or oxidizer) used, not the salt):**

1. Peroxide (Hach and others)
2. Copper (Cu)
3. Copper + titanium (Ti)
4. Selenium
5. Mercury
6. Other (includes combustion N analyzer)

**6. Ammonia measurement:**

1. Direct, manual colorimetric, Hach method
2. Direct, automated colorimetric (including Auto-analyzer)
3. Distillation + automated titration
4. Distillation + manual titration
5. Combustion N analyzer
6. Other

**7. If a combustion analyzer method was used, were samples introduced into the instrument as (leave blank if using Kjeldahl):**

1. Non-pelleted, twist foil cups
2. Non-pelleted, open tin capsules
3. Non-pelleted, open gelatin capsule
4. Pelleted in open tin capsules
5. Pelleted in open gelatin capsules
6. Pellets, no container
7. Boat
8. Other

**8. If a combustion analyzer method was used, what was used for atmospheric blank (leave blank if using Kjeldahl):**

1. Instrument blank used, no sample
2. Sucrose
3. Micro crystalline cellulose
4. Powdered cellulose
5. Other

**9. Were blanks and laboratory quality control (or check) samples or reference standards of known N content analyzed with the samples:**

1. No blank and no standard
2. No blank, but used a standard
3. Blank and standard used
4. Blank, but used no standard

**10. What laboratory quality control (or check) sample or reference standard of known N content was analyzed with the samples? Indicate only materials that are used to check results after calibration, not materials used for standardization.**

1. No quality control sample used
2. Combination of quality control samples
3. Ammonium sulfate, oxylate or glycine
4. Lysine
5. In-house feed standard
6. Certified sample (AACC or NIST)
7. Other

**11. What was the percentage recovery of the check sample or known standard:**

1. Less than 80%
2. 80—95%
3. 95—99%
4. 99.1—103%
5. Greater than 103%
6. None used

**12. Which AOAC method is closest in technique to that used in your lab:**

1. AOAC 988.05 Macro-Kjeldahl with mixed copper + titanium catalyst; NFTA Analysis Procedures (1993), 3.1
2. AOAC 976.06 Macro-block digestion, colorimetric determination using Auto-analyzer; NFTA Analysis Procedures (1993), 3.2
3. AOAC 976.06H Macro-block digestion, distillation-titration determination; NFTA Analysis Procedures (1993), 3.2
4. AOAC 984.13 Macro-Kjeldahl with copper catalyst; NFTA Analysis Procedures (1993), 3.1
5. AOAC 990.03 Generic combustion method; NFTA Analysis Procedures (1993), 3.3
6. Other

**ACID DETERGENT FIBER DETERMINATION**

**1. Are NIRS prediction equations for ADF determinations (leave blank if results are from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for ADF bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 13 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analyses.**

**3. Was the normality of the acid checked by titration to ensure it was between 0.95 and 1.05:**

1. No
2. Yes

**4. Which refluxing apparatus was used:**

1. Kettle reflux system (such as ANKOM)
2. Fleakers with reflux condenser (such as Hach)
3. Fibertec
4. Hotplate + condenser
5. Other

**5. What filtration vessel was used:**

1. System designed in-house (hand-blown glass or hand-made screen vessels)
2. Sealed bag system (such as ANKOM)
3. GF/D glass fiber disks using Whatman filter holder (such as Hach) or in Gooch crucibles
4. Whatman 4/41/54/541 filter paper in tapered funnels
5. Whatman 4/41/54/541 filter paper using Whatman, Buchner or Oklahoma CF filter funnels
6. Fibertec P2 crucibles
7. Coarse Gooch crucible (also called fritted glass disk crucible "C").
8. Other

**6. Approximate sample size, in grams:**

- |          |                      |
|----------|----------------------|
| 1. 0.5 g | 5. 5.0 g             |
| 2. 1.0 g | 6. 6.0 g             |
| 3. 2.0 g | 7. 10.0 g            |
| 4. 3.0 g | 8. Greater than 10 g |

**7. Approximate amount of acid detergent solution that was used per sample (in mls):**

- |                    |                        |
|--------------------|------------------------|
| 1. Less than 50 ml | 3. 100 ml              |
| 2. 50 ml           | 4. Greater than 100 ml |

**8. Approximate time for refluxing after samples have attained boiling temperature:**

1. Less than 55 minutes
2. 56-65 minutes
3. Greater than 65 minutes

**9. Were blanks and laboratory quality control (or check or reference) standards analyzed with the samples:**

1. No blank and no standard
2. No blank + standard
3. Blank + no standard
4. Standard and blank

**10. What was the average temperature of residue-soaking water and the time allowed for soaking:**

1. Hot water (80-90°C) + immediate rinsing/no soaking
2. 80-90°C water + less than or equal to 30 second soaking
3. 80-90°C water + greater than 30 second soaking
4. Boiling to near-boiling water (90-100°C) + immediate rinsing/no soaking
5. 90-100°C water + 15-30 second soaking
6. 90-100°C water + 0.5 to 2.0 minute soaking
7. 90-100°C water + >2.0 minute soaking
8. Other

**11. How many soakings with water and acetone were used to clean residues:**

1.  $\leq 1$  water and  $\leq 1$  acetone
2.  $\leq 1$  water and  $> 1$  acetone
3. 2 water and 1 acetone
4. 2 water and  $> 1$  acetone
5.  $> 2$  water and 1 acetone
6.  $> 2$  water and  $> 1$  acetone

**12. What were the drying times and temperatures for the fiber residues:**

1.  $< 100^\circ\text{C}$
2.  $135^\circ\text{C}$  for  $< 1$  hour
3.  $130-135^\circ\text{C}$  for 2-3 hours
4.  $100-105^\circ\text{C}$  for  $< 8$  hours
5.  $100-105^\circ\text{C}$  for 8 or more hours
6. Other

**13. Which AOAC method is closest in technique to that used in your lab:**

1. AOAC 973.18 or NFTA Forage Analysis Procedures (1993), 4.1, including ash
2. AOAC 973.18 or NFTA Forage Analysis Procedures (1993), 4.1, modified to be ash-free
3. Hach method
4. AOAC 973.18 or NFTA Analysis Procedures (1993), 4.1 modified for Fibertec extractors
5. ANKOM method
6. Other

**NEUTRAL DETERGENT FIBER DETERMINATION**

**1. Are NIRS prediction equations for NDF determinations (leave blank if results are from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for NDF bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 12 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analyses.**

**3. Was pH of neutral detergent solutions checked to ensure it was between 6.95 and 7.05:**

1. No
2. Yes

**4. Which refluxing apparatus was used:**

1. Kettle reflux system (such as ANKOM)
2. Fleakers with reflux condenser (such as Hach)
3. Fibertec
4. Hotplate + condenser
5. Other

**5. Which filtration vessel was used:**

1. System designed in-house (hand-blown glass or hand-made screen vessels)
2. Sealed bag system (such as ANKOM)
3. GF/D glass fiber disks using Whatman filter holder (such as Hach) or in Gooch crucibles
4. Whatman 4/41/54/541 filter paper in tapered funnels
5. Whatman 4/41/54/541 filter paper using Whatman, Buchner or Oklahoma CF filter funnels
6. Fibertec P2 crucibles
7. Coarse Gooch crucible (also called fritted glass disk crucible "C")
8. Other

**6. Approximate sample size, in grams (select weight closest to actual weight):**

- |          |                      |
|----------|----------------------|
| 1. 0.5 g | 5. 5.0 g             |
| 2. 1.0 g | 6. 7.0 g             |
| 3. 2.0 g | 7. 10 g              |
| 4. 3.0 g | 8. Greater than 10 g |

**7. Approximate amount of neutral detergent solution that was used per sample, in mls (select volume closest to actual volume):**

- |                    |                        |
|--------------------|------------------------|
| 1. Less than 50 ml | 3. 100 ml              |
| 2. 50 ml           | 4. Greater than 100 ml |

**8. Identify the major modifications of the NDF method used in your lab:**

1. Decalin (or anti-foamant) + no Na sulfite + no amylase
2. Decalin (or anti-foamant) + no Na sulfite + amylase
3. Decalin (or anti-foamant) + Na sulfite + no amylase
4. Decalin (or anti-foamant) + Na sulfite + amylase
5. No decalin (or anti-foamant) + no Na sulfite + no amylase
6. No decalin (or anti-foamant) + no Na sulfite + amylase
7. No decalin (or anti-foamant) + Na sulfite + no amylase
8. No decalin (or anti-foamant) + Na sulfite + amylase (amylase activity not standardized)
9. No decalin (or anti-foamant) + Na sulfite + amylase (amylase activity standardized)

**9. Method used to remove starch contamination of NDF:**

1. No amylase
2. Amylase pre-treatment before refluxing
3. One addition of heat-stable amylase at the beginning of refluxing
4. One addition of heat-stable amylase in the middle of refluxing
5. One addition of heat-stable amylase immediately before filtration and one addition during filtration
6. Amylase only used during filtration when plugging of the crucible occurs
7. One addition of heat-stable amylase at the beginning of refluxing + one addition at filtration
8. One addition of heat-stable amylase in the middle of refluxing + one addition at filtration
9. Other

**10. What was the average temperature of residue-soaking water and the time allowed for soaking:**

1. No washing of residues
2. Hot water (80-90°C) + immediate rinsing/no soaking
3. 80-90°C water + <30 second soaking
4. 80-90°C water + >30 second soaking
5. Boiling to near-boiling water (90-100°C) + immediate rinsing/no soaking
6. 90-100°C water + 15-30 second soaking
7. 90-100°C water + 0.5-2.0 minute soaking
8. 90-100°C water + >2.0 minute soaking
9. Other

**11. How many soakings with water and acetone were used to clean residues:**

1. No water and no acetone
2.  $\leq 2$  water and no acetone
3.  $> 2$  water and no acetone
4. 1 water and  $\geq 1$  acetone
5. 2 water and 1 acetone
6. 2 water and  $> 1$  acetone
7.  $> 2$  water and 1 acetone
8.  $> 2$  water and  $> 1$  acetone

**12. What were the drying times and temperatures for the fiber residues:**

1. <100°C
2. 135°C for <1 hour
3. 130-135°C for 2-3 hours
4. 100-105°C for <8 hours
5. 100-105°C for 8 or more hours
6. Other

**13. Which method is closest in technique to that used in your lab:**

1. Van Soest and Wine (JAOAC 50:50, 1967) or Goering and Van Soest (USDA Handbook 379, 1970)—0.5-1.0 g sample/100 ml ND, decalin (or anti-foamant), Na sulfite, no amylase, filtered on coarse Gooch crucibles
2. Robertson and Van Soest (The Analysis of Dietary Fiber in Food, p. 123, 1980)—0.5-1.0 g sample in 50 ml + 50 ml/100 ml ND, no decalin (or anti-foamant), no Na sulfite, one addition of heat-stable amylase in the middle of refluxing after second addition of 50 ml ND and one during the first hot water soak, filtered on coarse Gooch crucibles
3. NFTA Forage Analysis Procedures (1993), 5.1, 0.5 g sample/50 ml ND, no decalin (or anti-foamant), Na sulfite, one treatment with heat-stable amylase at the beginning of refluxing and one during the first hot water soak, filtered on coarse Gooch crucibles
4. 0.5-1.0 g sample/100 ml ND, amylase only used during filtration when plugging of the crucible occurs
5. Hach method
6. NFTA Forage Analysis Procedures (1993) 5.1, modified for Fibertec extractors
7. ANKOM method
8. Other

**CALCIUM DETERMINATION**

**1. Are NIRS prediction equations for Ca determinations (leave blank if results are from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for Ca bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 5 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analyses.**

**3. Were calibration standards:**

1. Mixed from reagents on-site
2. Purchased as stock solutions that are diluted to make standards on-site

**4. What type of sample is used as the in-house control (or check or standard) sample:**

1. None
2. In-house sample of known composition
3. AAFCO check sample
4. NIST Standard Reference Material
5. Other

**5. Which AOAC method most closely resembles the one used in your lab:**

1. Ca determined colorimetrically on Kjeldahl digest
2. AOAC 910.01 titrimetric macro method or AOAC 921.01 titrimetric micro method
3. AOAC 953.01 emission spectrographic method
4. AOAC 980.03 direct reading spectrographic method
5. AOAC 968.08 atomic absorption spectrophotometric method
6. AOAC 975.03 atomic absorption spectrophotometric method in plants
7. AOAC 985.01 inductively coupled plasma (ICP) spectroscopic method
8. Other

## **PHOSPHORUS DETERMINATION**

**1. ARE NIRS prediction equations for P determinations (leave blank if results are from chemical analysis):**

1. Purchased
2. Developed on-site

**2. Were NIRS prediction equations for P bias-corrected after calibration (leave blank if results are from chemical analysis):**

1. No
2. Yes, using data from in-house chemical methods
3. Yes, using data from an outside source

**Answer Questions 3 through 5 ONLY if chemical methods were used to obtain results or if NIRS was calibrated using in-house analyses.**

**3. *Were calibration standards:***

1. Mixed from reagents on-site
2. Purchased as stock solutions that are diluted to make standards on-site

**4. *What type of sample is used as the in-house control (or check or standard) sample:***

1. None
2. In-house sample of known composition
3. AAFCO check sample
4. NIST Standard Reference Material
5. Other

**5. *Which AOAC method most closely resembles the one used in your lab:***

1. P determined colorimetrically on Kjeldahl digest
2. AOAC 965.17 photometric method
3. AOAC 985.01 inductively coupled plasma (ICP) spectroscopic method
4. Other