FIBER
Best Practices
Survey Results
AAFCO
August 2, 2016
Surveys were distributed via

Aglabs

AAFCO LMSC list

NFTA

Ankom customer list

Individual contacts
11 government labs
11 university research labs
28 private labs
50 total responses

International participation:
Italy government lab
Italy private lab
Shanghai China private lab
Harbin China private lab
New Zealand private lab
<table>
<thead>
<tr>
<th></th>
<th>Crude Fiber</th>
<th>ADF</th>
<th>NDF</th>
<th>Dietary Fiber</th>
</tr>
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<tbody>
<tr>
<td>Government</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>0</td>
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<tr>
<td>University</td>
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<td>11</td>
<td>10</td>
<td>1</td>
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<tr>
<td>Private</td>
<td>23</td>
<td>30</td>
<td>30</td>
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<td>34</td>
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CRUDE FIBER METHODS BEING USED

Ceramic fiber filter AOAC 962.09, AOCS Ba6-84, ISO 5498  5 private labs

Fritted glass crucible AOAC 978.10  1 government, 1 university, 2 private labs = 4 labs

Tecator Fibertec  1 government lab

Ankom filter bag  8 government, 7 private labs = 15 labs

Fibertherm filter bag  1 private lab

NIR  1 private lab

ISO 6865:2000  1 private lab
1 Private Lab Single Filtration Method

Reflux 0.5 g in 100 mL 0.255N H₂SO₄ for 30 minutes
Add 100 mL 0.879N NaOH
Reflux for 30 minutes
Filter thru Pyrex crucible
Rinse w/ 100 mL hot 1% H₂SO₄
Soak and rinse w/ 100 mL hot H₂O
Soak w/ 20-30 mL acetone
Vacuum dry and ash
Fat Extraction Pretreatment for CF

Extract fat on all samples – 20 labs

Extract fat only when fat content is >
  10% - 3 labs
  5% - 2 labs
  3% - 2 labs
  1% - 1 lab

Solvent used
  Pet ether – 17 labs
  Acetone – 7 labs
  Ethyl ether – 3 labs
  CHCl3-MeOH – 1 lab
Hot Water Washes on CF Residue

after acid digestion: 50% of labs 3X others from 1-4X

after base digestion: 83% of labs 3X others from 1-5X

water temperature: 53% of labs 95-100C
30% of labs 80-90C 17% of labs ~50C

soaking time: 63% of labs 5 min 27% of labs 0.5-3 min
10% of labs do not soak
Preheating of Acid & Base Solutions

Use Ankom system – 12 labs
Coffee pot or flask on hot plate (heat to simmer) – 9 labs
Container w/ condenser on top – 3 labs
Heat exchange unit whereby solutions pass thru – 2 labs
No preheating – 3 labs
Drying of CF Residue
76% of labs dry at 100-105C
24% of labs dry at 110-135C
47% of labs dry for 1-4 hours
24% of labs dry overnight

Ashing of CF Residue
60% of labs ash at 600C
23% of labs ash at 550C
Others were 525C, 480C, 530C, 500C

47% of labs ash for 2 hours
13% ash for 3 hours
13% ash for 0.5 hour
Other ashing times were 6 hr, 15 hr, 4 hr, 8 hr
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<th>Crude Fiber</th>
<th>Government</th>
<th>University</th>
<th>Private</th>
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<tr>
<td>Feeds</td>
<td>72%</td>
<td>1 lab</td>
<td>40%</td>
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<tr>
<td>Forages</td>
<td>12%</td>
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<td>27%</td>
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<tr>
<td>Grains include DG</td>
<td>13%</td>
<td>1 lab</td>
<td>17%</td>
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<tr>
<td>Oilseeds</td>
<td>4%</td>
<td>0</td>
<td>12%</td>
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<tr>
<td>Pet Food</td>
<td>8%</td>
<td>1 lab</td>
<td>46%</td>
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ADF Methods

Berzelius beaker, crucible  AOAC 973.18, ISO 13906  --
  1 government, 2 university, 6 private labs = 9 labs

Tecator Fibertec – 1 government, 2 private labs = 3 labs

Ankom filter bag – 7 government, 9 university, 17 private
  labs = 33 labs

Fibertherm filter bag – 1 government lab

NIR – 2 government labs, 2 private labs = 4 labs

Some labs use several methods
NDF Methods

Berzelius beaker, crucible, amylase treatment AOAC 2002.04, ISO 13906 -- 1 government, 2 university, 5 private labs = 8 labs

Tecator Fibertec – 2 private labs

Ankom filter bag – 3 government, 8 university, 17 private labs = 28 labs

Fibertherm filter bag – 1 government lab

NIR – 2 government labs, 3 private labs = 5 labs

Some labs use several methods
ADF/NDF Fat Extraction Pretreatment

Extract fat on all samples – **14-10 labs**

Extract only when fat is >

- 10% - **7-8 labs**
- 5% -- **11-8 labs**
- 4% -- **1 lab**
- 3% -- **1 lab**

Oilseeds only – **1 lab**

No extraction forages only – **5-6 labs**

Solvents used:

- Acetone – **25-16 labs**
- Pet ether – **4-5 labs**
- Ethyl ether – **2-3 labs**
- CHCl3:MeOH – **1 lab**
ADF/NDF Water Washes

Number of water washes:
- 3X – 22-17 labs
- 4X – 10-12 labs
- 2X – 5-6 labs
- 5X – 3-1 labs
- Wash until rinse H$_2$O is neutral – 2-1 labs

Soaking times:
- 5 min – 26-22 labs
- 2 min – 5-2 labs
- 4 min – 3 labs
- 3 min – 3-2 labs
- 1 min – 1-2 lab
- 0.5 min – 1 lab
- 7 min – 1 lab
ADF/NDF Water Washes cont’d

Water temperature:
95-100°C – 23-20 labs
80-85°C – 8-5 labs
90°C – 4 labs
70°C – 4 labs

Acetone soak after water washes
Yes – 39-34 labs  No – 4 labs
**Drying of ADF/NDF Residue**

Temperature:
- 100-105°C – 39 labs
- 110-135°C – 3 labs
- 55-60°C (vacuum oven) – 2 labs

Drying time:
- 12-18 hrs – 12 labs
- 2 hrs – 11 labs
- 3 hrs – 9 labs
- 4 hrs – 6 labs
- 1 hr – 1 lab
- 24 hr – 1 lab

**Ashing of ADF/NDF Residue**

Ash and report on “ash-free” basis:  No – 36-32 labs  Yes – 5-6 labs

Ashing temperature:  525, 600, 500, 550°C

Ashing time:  2 hr, 3 hr, 8 hr, 12-15 hr, 0.5 hr
NDF Amylase

All labs use heat-stable a-amylase

Standardize amylase solution:
   No – 32 labs (2 labs assumed manufacturer did it)
   Yes – 4 labs

Amylase is used during:
   Digestion – 35 labs
   Rinsing – 28 labs (some labs use amylase only in 1st or 2nd rinse)

Use sodium sulfite
   Yes – 31 labs
   No – 7 labs
<table>
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<th>Private</th>
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<tr>
<td>Feeds</td>
<td>65%</td>
<td>23%</td>
<td>24%</td>
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<tr>
<td>Forages</td>
<td>49%</td>
<td>13%</td>
<td>15%</td>
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<td>Grains include DG</td>
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<td>15%</td>
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<tr>
<td>Oilseeds</td>
<td>1 lab</td>
<td>4%</td>
<td>9%</td>
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<tr>
<td>Pet foods</td>
<td>1 lab</td>
<td>6%</td>
<td>16%</td>
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</table>

1 university lab analyzing fruits & invertebrates (wildlife food)
<table>
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<th>Private</th>
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</thead>
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<td>18%</td>
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<tr>
<td>Forages</td>
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<td>70%</td>
<td>59%</td>
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<td>Oilseeds</td>
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<tr>
<td>Pet foods</td>
<td>1 lab</td>
<td>1 lab</td>
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1 university lab also analyzes feces
1 private lab also analyzes bovine feces and biogas fermentation materials
<table>
<thead>
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<th>CF</th>
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<th>DF</th>
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<tr>
<td>0.5 g</td>
<td>6 labs</td>
<td>29 labs</td>
<td>34 labs</td>
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<td>1 g</td>
<td>23 labs</td>
<td>14 labs</td>
<td>4 labs</td>
<td>8 labs</td>
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<tr>
<td>2 g</td>
<td>6 labs</td>
<td></td>
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<tr>
<td>0.4 g</td>
<td>1 lab</td>
<td>1 lab</td>
<td></td>
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<tr>
<td>0.6 g</td>
<td></td>
<td>1 lab</td>
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<tr>
<td>0.2-0.9g</td>
<td>1 lab</td>
<td>1 lab</td>
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## PARTICLE SIZE

<table>
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<tr>
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<th>CF</th>
<th>ADF</th>
<th>NDF</th>
<th>DF</th>
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<tbody>
<tr>
<td>0.5 mm</td>
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<td>1 lab</td>
</tr>
<tr>
<td>0.75 mm</td>
<td>8 labs</td>
<td>7 labs</td>
<td>4 labs</td>
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<tr>
<td>1 mm</td>
<td>14 labs</td>
<td>20 labs</td>
<td>21 labs</td>
<td>2 labs</td>
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<tr>
<td>1.5 mm</td>
<td>1 lab</td>
<td>1 lab</td>
<td>1 lab</td>
<td></td>
</tr>
<tr>
<td>2 mm</td>
<td>3 labs</td>
<td>4 labs</td>
<td>5 labs</td>
<td></td>
</tr>
<tr>
<td>4 mm</td>
<td></td>
<td></td>
<td>1 lab</td>
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</table>

1 lab states they do not know
MILLS

Wiley cutting mill – 17 labs
Fritsch Pulverisette cutting mill – 2 labs
Hammermills – 3 labs
Retsch ZM200 centrifugal mill – 10 labs
Tecator Cyclotec cyclone mill – 5 labs
Udy cyclone mill – 5 labs
Stein mill – 3 labs
Tecator Knifetec blending mill (for oilseeds) – 1 lab
Retsch Grind-O-Mix blending mill – 1 lab
Micro sample mill – 1 lab

Some labs use more than 1 mill depending upon matrix
Extraction Systems

Labconco – 8 labs

Homemade system of hot plates and ball condensers – 6 labs

Tecator Fibertec – 5 labs

Ankom 200 – 17 labs

Ankom 220 – 3 labs

Ankom 2000 – 11 labs

Fibertherm – 1 lab

Some labs use more than one system
Crucibles

Foss P2—4 labs
Pyrex (commercial), assorted catalog numbers – 10 labs
Pyrex (custom made Superior Glass) – 2 labs
Porcelain – 1 lab

Check flowrate of crucibles AOAC 2002.04 B(b)
Yes – 6 labs No – 6 labs
Was not aware of this – 6 labs

How often is flowrate checked
Upon receipt of new crucibles—2 labs
Once a year – 2 labs
Whenever a filtration problem occurs – 4 labs
After every cleaning – 1 lab
Use filter aid in crucibles
Yes – 11 labs    No – 7 labs
Celite – 5 labs
Sand – 5 labs
GFD glass filter – 1 lab

Ankom Filter Bags

F57 – 19 labs
F58 – 8 labs
Some labs use both
NIR

NIR Instruments:
- Foss 2500 – 3 labs
- NIRSystems 5000 – 4 labs
- NIRSystems 6500 – 3 labs
- Unity SpectraStar – 3 labs
- Bruker MPA – 1 lab

NIRS Consortium equations – 3 labs
Developed in-house equations – 8 labs

NIR is being used for
- CF – 7 labs
- ADF – 13 labs
- NDF – 13 labs
QC

AAFCO feed check samples – 20 labs
AAFCO pet food check samples – 3 labs
NFTA forage check samples – 18 labs
In-lab QC – 20 labs
Did not answer this question – 5 labs

Most labs use a combination of QC materials.

Some also stated they participate in AAFCO & NFTA check sample programs.
Dietary Fiber

AOAC 985.29 total DF – 3 private labs

AOAC 991.43 total, soluble & insoluble DF – 8 private labs

AOAC 2009.01 total DF – 4 private labs

AOAC 2011.25 total, insoluble & soluble DF – 3 private labs

Ankom dietary fiber system – 2 private labs

Prosky et. al. 1992 JAOAC & AOAC 2001.03 – 1 university lab
Dry pet foods – 3 private labs, 1 university lab
Wet pet foods – 2 private labs, 1 university labs
Human food – 8 private labs, 1 university lab
Ingredients – 7 private labs, 1 university lab
Problems/concerns encountered with fiber analysis

Distillers grains – repeatability & getting the correct answer on AAFCO samples

Finely ground samples (dried milk powder) do not work well with Ankom F57 bags

Received mixed feedback on appropriate grind size using Ankom method. Heard arguments that 1 mm Wiley grind is too fine...but blank corrections are reasonable so have stayed with 1 mm grind.

A very well trained analyst is needed for DF.

Problems with Ankom bags shifting out of place in the bag holder in the Ankom 200 unit.
Static electricity with weighing of finely ground samples in Ankom bags.

Occasionally high ADF values with Ankom 200 system.

Leaking of Fibertec extractor – requires experience

Sand causes overestimation of fiber but any systematic ashing takes the lab out of the SD of the Ring Test.

ADF can be overestimated in some sample types (brassicas & chicory) due to the presence of pectins or biogenic silica. Lab is moving towards analyzing ADF from the extract/bags remaining after NDF.

Some sample types are unsuitable for extraction using F57 bags due to fine particles. F58 bag (finer mesh size) is more expensive than F57 bag.
Results than to run lower on ADF/NDF on forages than other labs (resulting in slightly higher RFV values). Using 0.8 mm grind size instead of 1 mm.

Struggle with low results on NDF. Lab always close to bottom of passing stats on AAFCO & NFTA check samples. All techs and same equipment (Tecator Fibertec) do very well with crude fiber and ADF.

Blank values for F58 bags not as consistent as for F57 bags. Difficult to grind some matrices thru 1 mm screen.
Some sample matrices hold on to excess NDF solution and require additional rinsing

Samples that form a gel

Inhomogeneous samples

Material leaks

Negative ash values
<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawrence Novotny</td>
<td>retired from South Dakota State University Olson</td>
</tr>
<tr>
<td></td>
<td>Biochemistry Labs; consultant w/ SD Ag Labs</td>
</tr>
<tr>
<td>Sharon Webb</td>
<td>University of Kentucky Regulatory Services</td>
</tr>
<tr>
<td>Kristy Broten</td>
<td>Minnesota Dept of Agriculture - Regulatory Feed Lab</td>
</tr>
<tr>
<td>John Szpilka</td>
<td>Mérieux NutriSciences (Chicago), Scientific Affairs Director, Chemistry</td>
</tr>
<tr>
<td>Lei Tang</td>
<td>FDA Center for Veterinary Medicine, Division of Animal Feeds</td>
</tr>
<tr>
<td>Teresa M Grant</td>
<td>North Carolina Dept. of Agriculture &amp; Consumer Services - Food, Feed, Fertilizer Supervisor</td>
</tr>
<tr>
<td>Christina V Childers</td>
<td>Mississippi State Chemical Laboratory</td>
</tr>
<tr>
<td>Ken Riter</td>
<td>Nestle Purina Analytical Labs</td>
</tr>
<tr>
<td>Kathryn Phillips</td>
<td>Nestle Purina Analytical Labs</td>
</tr>
<tr>
<td>Name</td>
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</tr>
<tr>
<td>Jeff Horst</td>
<td>Agri-King, Laboratory Production Manager</td>
</tr>
<tr>
<td>Dr. George Collings</td>
<td>Nutrition Solutions</td>
</tr>
<tr>
<td>Tom Phillips</td>
<td>Maryland Department of Agriculture Acting State Chemist</td>
</tr>
<tr>
<td>Jerome King</td>
<td>Midwest Labs (Omaha) Technical Director</td>
</tr>
<tr>
<td>Lisa Ruiz</td>
<td>Eurofins (Des Moines) quality manager</td>
</tr>
<tr>
<td>Dr. Kai Liu</td>
<td>Eurofins (Des Moines) chemist</td>
</tr>
<tr>
<td>Kent Karsjens</td>
<td>Eurofins (Des Moines) chemist</td>
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<tr>
<td>Joseph Garrison</td>
<td>Eurofins (Des Moines) chemist</td>
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<tr>
<td>Name</td>
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<td>---------------------------</td>
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</tr>
<tr>
<td>Angela Carlson</td>
<td>SGS North America (Brookings SD)</td>
</tr>
<tr>
<td>Jacob Swanson</td>
<td>SGS North America (Brookings SD)</td>
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<tr>
<td>Matt Karp</td>
<td>New York State Dept of Agriculture and Markets</td>
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<tr>
<td>Brian Davidson</td>
<td>New York State Dept of Agriculture and Markets</td>
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<tr>
<td>George C. Fahey, Jr</td>
<td>Dept of Animal Sciences, University of Illinois at Urbana-Champaign</td>
</tr>
<tr>
<td>Kelly S. Swanson</td>
<td>Dept of Animal Sciences, University of Illinois at Urbana-Champaign</td>
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<tr>
<td>Maria R. C. de Godoy</td>
<td>Dept of Animal Sciences, University of Illinois at Urbana-Champaign</td>
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</tr>
<tr>
<td>Dan Berg</td>
<td>Covance Food Solutions (Madison WI)</td>
</tr>
<tr>
<td>Yannis Vrasidas, PhD</td>
<td>Eurofins Food Testing Netherlands</td>
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<tr>
<td>Nick Tedesche</td>
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<td>Brian Layton</td>
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<tr>
<td>Andy Komarek</td>
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<tr>
<td>Michelle Walter</td>
<td>Foss North America (Tecator)</td>
</tr>
<tr>
<td>Eva Lynch</td>
<td>Rock River Lab, Watertown WI</td>
</tr>
<tr>
<td>Andy Crawford</td>
<td>Crawford Consulting (statistician)</td>
</tr>
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