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

	Crude Fiber	ADF	NDF	Dietary Fiber
Government	10	10	5	0
University	1	11	10	1
Private	23	30	30	9
Total	34	51	45	10

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 H_2SO_4 for 30 minutes

Add 100 mL 0.879N NaOH

Reflux for 30 minutes

Filter thru Pyrex crucible

Rinse w/ 100 mL hot 1% H_2SO_4

Soak and rinse w/ 100 mL hot H_2O

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 1ab

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

Crude Fiber	Government	University	Private
Feeds	72%	1 lab	40%
Forages	12%	1 lab	27%
Grains include DG	13%	1 lab	17%
Oilseeds	4%	0	12%
Pet Food	8%	1 lab	46%

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_2O 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:

95-100C – 23-20 labs

80-85C – 8-5 labs

90C – 4 labs

70C – 4 labs

Acetone soak after water washes

Yes – 39-34 labs No – 4 labs

Drying of ADF/NDF Residue

Temperature:

- 100-105C – 39 labs
- 110-135C – 3 labs
- 55-60C (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, 550C

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

ADF	Government	University	Private
Feeds	65%	23%	24%
Forages	49%	13%	15%
Grains include DG	5%	13%	15%
Oilseeds	1 lab	4%	9%
Pet foods	1 lab	6%	16%

1 university lab analyzing fruits & invertebrates (wildlife food)

NDF	Government	University	Private
Feeds	28%	18%	22%
Forages	32%	70%	59%
Grains include DG	2%	20%	17%
Oilseeds	1 lab	5%	11%
Pet foods	1 lab	1 lab	9%

1 university lab also analyzes feces

1 private lab also analyzes bovine feces and biogas fermentation materials

TEST WEIGHTS

	CF	ADF	NDF	DF
0.5 g	6 labs	29 labs	34 labs	2 labs
1 g	23 labs	14 labs	4 labs	8 labs
2 g	6 labs			
0.4 g		1 lab	1 lab	
0.6 g			1 lab	
0.2-0.9g		1 lab	1 lab	

PARTICLE SIZE

	CF	ADF	NDF	DF
0.5 mm	1 lab		1 lab	1 lab
0.75 mm	8 labs	7 labs	4 labs	
1 mm	14 labs	20 labs	21 labs	2 labs
1.5 mm		1 lab	1 lab	
2 mm	3 labs	4 labs	5 labs	
4 mm			1 lab	

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
- NIRSytems 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

Lawrence Novotny	retired from South Dakota State University Olson Biochemistry Labs; consultant w/ SD Ag Labs
Sharon Webb	University of Kentucky Regulatory Services
Kristy Broten	Minnesota Dept of Agriculture - Regulatory Feed Lab
John Szpylka	Mérieux NutriSciences (Chicago), Scientific Affairs Director, Chemistry
Lei Tang	FDA Center for Veterinary Medicine, Division of Animal Feeds
Teresa M Grant	North Carolina Dept. of Agriculture & Consumer Services - Food, Feed, Fertilizer Supervisor
Christina V Childers	Mississippi State Chemical Laboratory
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