<< 7. Total Ash in Forages Appendix >>

8. Crude Fat (Ether Extract) in Forages

Reference:
Fat (Crude) or Ether Extract in Animal Feed. (920.29) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.

Scope:
This method is applicable for the determination of crude fat in dried forages and mixed feeds. It is not applicable for oilseeds, baked and/or expanded products (pet foods), liquid feeds, sugar products, and feeds containing dairy products. For determining fat in oilseeds, consult Official Methods and Recommended Practices of the American Oil Chemists Society.

Basic Principles:
A dried, ground sample is extracted with diethyl ether which dissolves fats, oils, pigments and other fat soluble substances. The ether is then evaporated from the fat solution. The resulting residue is weighed and referred to as ether extract or crude fat. Both the ether and the samples must be free of moisture to avoid coextraction of water-soluble components in the sample such as carbohydrates, urea, lactic acid, glycerol, etc. If water-soluble components are present in large amounts in the sample, they are washed out of the sample prior to drying. Low temperatures are used to evaporate the ether and remove residual moisture to prevent oxidation of the fat. Petroleum ether does not dissolve all of the plant lipid material, and therefore it cannot be substituted for diethyl ether.

Equipment:
Goldfisch fat extraction apparatus, 6-flask unit, equipped with glass thimble holders and ether reclaiming tubes
Extraction thimbles, 22 x 80 mm, Alundum (porous clay), coarse Fat beakers, pyrex, with ground lip, engraved with a number, 50 x 85 mm Drying oven, 102oC gravity convection Analytical balance, sensitive to 0.1 mg Desiccator and tongs
Filter paper, Whatman #1, 11 cm, or equivalent
Steambath in a hood (optional) Gloves, white nylon, lintless

Reagents:
Anhydrous Diethyl Ether, purified for fat extraction Mallinkrodt #0844 or equivalent. To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed.

Safety Precautions:

- Ether has an extremely low flash point. Have no open flames nearby. Avoid inhaling ether vapors. Store ether in metal containers. Handle open containers (reagent cans and fat beakers) in a hood. Conduct the extractions in a well ventilated area.
- Peroxides can accumulate in open containers of ether. These are explosive and shock sensitive. Check each container opened for more than 30 days for peroxides. Ether-containing peroxides must be disposed with special techniques.
- Electrical equipment is to be grounded. Extractors should be spark-proof.
- Make sure all ether is evaporated from the beakers before placing them in the oven to avoid a fire or explosion.

Procedure: Sample drying

1. Weigh 1.5 to 2 g of ground sample into a thimble recording the weight to nearest 0.1 mg (W1). Weigh a second subsample for dry matter determination.
   - Or -
   1A) If the sample contains large amounts of carbohydrates, urea, glycerol, lactic acid or water-soluble components, weigh 2 g sample to nearest 0.1 mg (W1) into a small filter cone. Extract with five 20 mL portions of deionized water allowing each portion to drain, then insert the paper and sample into thimble.
2. Dry for 5 hr at 100oC.
3. Dry beakers to be used for fat determination for at least 1 hr at 100oC. Cool the appropriate number of fat beakers in a desiccator. Weigh and record the weight to the nearest 0.1 mg (W2).
4. When the drying period is over, remove the samples from the oven to a desiccator. (This is a convenient stopping point. The samples should be stored in a desiccator if not immediately extracted.)

Extraction

1. Line the fat beakers up in front of the extractor and match the thimbles with their corresponding fat beakers.
2. Slip the thimble into a thimble holder and clip the holder into position on the extractor.
3. Add about 40 mL of diethyl ether (one glass reclaiming tube full) to each fat beaker.
4. Wearing white gloves, slip the beaker into the ring clamp and tightly clamp the beaker onto the extractor. If the clamp is too loose, insert another gasket inside the ring.
5. Raise the heaters into position. Leave about a 1/4 inch gap between the beaker and the heating element.
6. Turn on the heater switch, the main power switch, and the condenser water.
7. After the ether has begun to boil, check for ether leakage. This can be detected by sniffing around the ring clamp. If there is leakage, check the tightness of the clamp and if necessary replace the gasket(s).
8. Extract for minimum of 4 hr on a Hi setting (condensation rate of 5 to 6 drops per second), or for 16 hr on a Low setting (condensation rate of 2 to 3 drops per sec).
9. After extraction, lower the heaters, shut off the power and water, and allow the ether to drain out of the thimbles (about 30 min). This is a good stopping point.

Ether Distillation and Weighing of Fat Residue

1. Remove the thimble from the holder, and rinse the holder with a small portion of diethyl ether from the washbottle. Clip an ether reclaiming tube in place and reattach the fat beaker.
2. Reposition the heaters and turn on the electricity and water. Proceed to distill the ether using a Hi setting. Watch Closely.
3. Distill until a thin layer of ether remains in the bottom of the beaker, and then lower the heater. Do not allow beakers to boil dry. Overheating will oxidize the fat. When the last beaker has finished, shut off the power and water.
4. Wipe the exterior of the beaker clean with a Kimwipe as it is being removed from the extractor.
5. Empty the reclaiming tubes into the "USED" diethyl ether container.
6. Place the tray of beakers in an operating hood to finish evaporating the ether. If there is no hurry, air moving through the hood will be sufficient without heat. A steambath may be used to speed up the evaporation. Beakers should remain in the hood until all traces of ether are gone. Carefully sniff each beaker to determine if any ether remains.
7. Place the beakers in a 102oC gravity convection oven. Warning: If a beaker containing ether is placed

in the oven an explosion may occur.
8. Dry for 1/2 hr. No longer. Excessive drying may oxidize the fat and give high results.
9. Cool in a desiccator and weigh and record weight to the nearest 0.1 mg (W2).
10. The fat beakers are best cleaned by warming on a steambath or on a hot plate on a low setting. Add some used ether to dissolve the fat. The use of a rubber policeman is helpful. After soaking the beakers in Alconox detergent, wash them using hot water and vigorous brushing. The thimbles are best cleaned by blowing out with air.

Comments:

- If doing a proximate analysis, the residue left in the thimble may be used to determine crude fiber.

Calculations: Percent Crude Fat (Ether Extract), DM basis

% Crude Fat (DM basis) = (W3 - W2) X 100 / W1 X Lab DM / 100

- W1 = initial sample weight in grams
- W2 = tare weight of beaker in grams
- W3 = weight of beaker and fat residue in grams

Quality Control:
Include a reagent blank and one or more quality control (QC) samples in each run, choosing QC samples by matching analyte levels and matrices of QC samples to the samples in the run. Include at least one set of duplicates in each run if single determinations are being made.

An acceptable average standard deviation among replicated analyses for crude fat about ±0.10, which results in a warning limit (2s) of ±0.20 and a control limit (3s) of ±0.30. Plot the results of the control sample(s) on an X-control chart and examine the chart for trends. Results outside of upper or lower warning limits, ±2 standard deviations (95 percent confidence limits), are evidence of possible problems with the analytical system. Results outside of upper or lower control limits, ±3 standard deviations (99 percent confidence limits), indicate loss of control, and results of the run should be discarded. Two consecutive analyses falling on one side of the mean between the warning limits and the control limits also indicate loss of control.

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