

# Recommendations and Critical Factors in Determining Moisture in Animal Feeds

AAFCO's Laboratory Methods and Services Committee

Moisture Best Practices Working Group

January 23, 2018

The Moisture Best Practices Working Group was directed by AAFCO's Laboratory Methods and Services Committee to review the various methods for determining moisture in animal feeds. The working group was tasked with making recommendations on methods to be used to determine moisture and to document critical factors that laboratories need to be aware of. Recommendations for pet food are currently under review and should be completed within a year.

## Acronyms

AAFCO – Association of American Feed Control Officials

AOAC – Association of Official Analytical Communities

AOCS – American Oil Chemists Society

KF – Karl Fischer

LOD – Loss on Drying

NFTA – National Forage Testing Association

NIRS – Near Infrared Reflectance Spectroscopy

NIST – National Institutes of Standards and Technology

## Terminology

*Moisture*: the water content of a feed.

Note: While most clients believe that a moisture determination is equal to water content; they often fail to comprehend that a result determined by loss on drying may also include other volatiles (such as fatty acids) that are driven off during the drying operation. Only when a method is used that directly measures water (such as Karl-Fischer or toluene distillation) can "moisture" be assumed to be equal to the water content.

*Loss On Drying (LOD)* is a laboratory test that determines the percentage of a specified test portion lost by heating at specified temperature for a specified time. For some materials, such as grains, most of mass lost on drying is water; however, for ensiled materials or high urea products, the mass lost on drying will include volatile compounds other than water. The term "loss on drying" should replace "moisture" whenever a drying operation is conducted to determine the "water" content.

*Dry Matter* or *Total Solids* refers to the percentage of material remaining after removal of water. The animal feed, pet food, and forage industry uses the term “dry matter.” The human food, water, manure and compost industry uses the term “total solids.”

*Partial Dry Matter* (also known as *Partial Moisture*) is the dry matter content (or moisture) remaining (or lost) after a material is heated (usually in a forced air oven) at 55 to 60°C to less than complete dryness, followed by equilibrating at room temperature before weighing. After these processes, the material contains more than 90% dry matter (less than 10% water), and is suitable for comminution in a laboratory mill without significant loss or gain of moisture during comminution.

*Laboratory Dry Matter* (also known as *Residual Moisture*) is the amount of dry matter (or moisture) remaining (or lost) after a partially dried material has been heated in an oven to remove all residual moisture. Since this operation is performed in the laboratory as a step to calculate total dry matter, this dry matter value is referred to as laboratory dry matter (or residual moisture).

*Total Dry Matter* (also known as *Total Moisture*) is the total dry matter (total moisture) content of the material which is determined by one of two methods:

- 1) the mathematical combination of partial dry matter and laboratory dry matter or
- 2) drying the material at a temperature that will remove free water

Moisture or dry matter in animal feeds and forages can be determined as a single measurement process or as a two-step process. Generally, for materials containing  $\leq 15\%$  moisture ( $\geq 85\%$  dry matter), moisture is determined in a single step since the entire laboratory sample can be easily comminuted. Since it is more difficult to comminute materials containing  $\geq 15\%$  moisture ( $\leq 85\%$  dry matter), moisture is generally determined as a two-step drying process: first drying to approximately 10-15% moisture at a relatively low temperature, then a second drying step at a higher temperature, with the total moisture calculated from the results of the two steps. The first step of the two-step process is generally labeled “partial dry matter” or “partial moisture”. The second step of the two step process is often called “laboratory dry matter” or “residual moisture.”

## DISCUSSION OF METHODS

### Direct Water Methods

There are two methods for determining water directly. These are *Karl Fischer titration* (AOAC 2001.12 for animal feed, grain & forage; AOAC 991.02 for semi-moist pet food; & AOAC 966.20 for molasses) and *toluene distillation* (AOAC 925.04 for animal feed) (1). Since these methods are time consuming and require trained analysts, they are not routinely used in all laboratories.

The ***Karl Fischer titration method*** was developed by German chemist Karl Fischer in 1935. In the method the test material is dispersed in methanol and titrated with a mixture containing sulfur dioxide, iodine and a base. The methanol esterifies the sulfur dioxide to an ester which is then oxidized to an alkyl sulfate by the iodine and water. The reaction reaches its endpoint when all of the water in the test material has been consumed as evidenced by no change in electrical potential. A base is necessary for the complete neutralization of acids produced during the reaction (2).

Improvements in the KF method have been made over the years. One is replacing the original base pyridine (which produced a sluggish titration and has an obnoxious odor) with better bases such as imidazole. The addition of special solvents to the methanol aids in the extraction of water from certain test materials. Formamide dissolves sugars and helps to distribute protein. Octanol aids in the dissolution of long chain hydrocarbons. Heating of the titration vessel speeds up the reaction. A homogenizer probe inserted in the titration vessel is used to rupture cellular structures of the test material to release internally trapped water. In recent years, systems have been developed to replace solvent extraction with thermal extraction of water. Test material is placed in a sealed container and heated to drive off the water which is then trapped and titrated in the solvent.

The ***toluene distillation method*** was refined and made official in 1925 (3). The test material is placed in a liquid immiscible with water and refluxed. An azeotropic distillation of the test portion (toluene boils at 110°C) removes the water without decomposing the test material. The water is collected under a layer of toluene in the bottom of a narrow diameter calibrated tube enabling the volume of water to be directly measured.

Both the Karl Fischer and toluene distillation methods have been validated with solid and liquid test materials containing high fat or high sugar content.

### **Loss on Drying Methods**

Loss on drying (LOD) methods are Codex Type I methods or empirical methods. The conditions of the method determine the fraction that is volatilized. Any change in conditions (drying temperature, drying time, type of oven, type of drying vessel) result in different results on the same material. The Codex definition, “a method which determines a value that can only be arrived at in terms of the method per se and serves as the only method for establishing the accepted value of the item measured” suggests that different LOD methods must have different test names.

Loss on drying (oven methods) are routinely used methods due to their simplicity. A review of the early literature (4) shows that a variety of ovens were used (vacuum, electrically heated, gas heated, water-jacketed, and steam heated); drying times varied from 2 – 20 hours and drying to constant weight; and temperatures used varied from 65 - 135°C.

The vacuum oven was used by most laboratories in the 1920s and 1930s. The vacuum oven method (AOAC 934.01) was made official in 1934 by establishing the temperature to be between 95-100°C and the time to be 5 hours (4).

The other official LOD method for feeds is AOAC 930.15 which dries materials at 135°C for 2 hours. The matrices that were evaluated to make this method official in 1930 were oilcake meal, fish meal, meat meal, bran cereal products and (wheat) shorts (5, 6). This method was adopted tentatively as an **alternative** method to be used when a vacuum oven was not available (5). Research reported in 1931 concluded that 930.15 cannot be used on feeds with sugar content since caramelization will occur (6). The 1935 *JAOAC* added this statement: “This method is not to be used when a fat determination is to be made on the same test portion” because oxidation of fat will occur (7). The current method’s applicability statement also states that this method is not applicable for feeds containing urea since urea starts to decompose at 132°C. (This paper’s authors could not find a literature citation for this statement but lab experience has substantiated it.) While 930.15 is an official method for animal feeds, obviously, it is not applicable to **all** animal feeds. A review of the AAFCO proficiency test program data show that 930.15 is the most widely used LOD method.

This moisture working group conducted a survey in the summer of 2017 that included 13 regulatory laboratories, 7 research laboratories and 16 private laboratories. The survey results show that a variety of LOD methods using various temperatures and times are in use. Some laboratories use the same method for all matrices and other laboratories use several different methods depending upon the matrix.

Thiex and Van Erem at South Dakota State University conducted a research study on LOD methods in 1999 (8). Ten partially dried hay samples, 10 partially dried haylage samples and 10 partially dried corn silage samples were analyzed for moisture by three LOD methods which were 135°C for 2 hr, 104°C for 3 hr, and 104°C for 6 hr all using a forced draft oven. Ten feed products that contained no urea and six different feed products containing urea were also studied. Feed products included milk replacer, dry cat food, soybeans, meat and bone meal, and medicated feed. Feed products were analyzed by five LOD methods which were 135°C for 2 hr, 104°C for 3 hr, 104°C for 6 hr, 110°C for 3 hr (all using a forced draft oven), and 95°C for 5 hr in a vacuum oven. All forage and feed products were also analyzed for moisture by Karl

Fischer titration. The study found that drying at 135°C for 2 hr overestimated moisture in most cases. The conclusions were that drying at 104°C for 3 hr most closely matched the KF moisture values for forages, ensiled materials and feeds containing urea and that drying at 110°C for 3 hr most closely matched the KF values for nonurea-containing feeds.

Thiex and Richardson published an evaluation of various moisture studies in 2003 (9). Their conclusion was that “drying at 135°C should be eliminated or restricted to very few materials for which it has been validated.” The paper also showed that drying at 104° or 105°C for 3 hr most closely matched KF moisture values.

The National Forage Testing Association established a task force in 2000 to investigate various LOD methods. The task force concluded that drying at 105°C for 3 hr provided the most accurate moisture value and established this as NFTA 2.1.4 official moisture method (10).

In a research study conducted by South Dakota State University in 2009 and which was supported by the American Feed Industry Assn, the Renewable Fuels Assn, and the National Corn Grain Assn, 30 different materials of distillers dried grains with solubles (DDGS) were analyzed for moisture using four LOD methods and the KF method (11). The study concluded that drying at 105°C for 3 hr most closely approximated the KF moisture value. The poorest performing method was 135°C for 2 hr.

There are no “official” LOD methods specifically for pet food. Published data includes only two pet food samples. Dry cat food was included in Thiex and Van Erem’s study (8). The 110°C/3 hr LOD method most closely matched the KF moisture value. Dry dog food was included in a similar study published by Van Erem and Thiex (12). In this study the 135°C/2 hr LOD method closely matched the KF value. Obviously more data is needed on pet foods.

All of the above LOD methods are designed for low moisture matrices (<15% moisture) that can be easily comminuted to around a 1 mm particle size allowing for a fairly representative test portion of several grams.

It is nearly impossible to obtain a representative test portion of several grams of high moisture materials such as forages, ensiled materials and wet grains including wet distillers grains. The animal feed and forage industry have long established that these materials need to be first partially dried in order to comminute the entire laboratory sample. Drying is done in a forced draft oven at a temperature of <60°C (NFTA Method 2.2.1.1) (13). Drying at higher temperatures (>60°C) causes chemical changes (Maillard reactions between protein and sugars) in the forage that affects subsequent fiber, lignin and detergent insoluble nitrogen analysis.

Partial drying is sometimes accomplished using a microwave oven (NFTA Method 2.2.1.2). The partially dried material is then comminuted in order to obtain representative test portions for various chemical analyses. A second moisture analysis is conducted on the partially dried comminuted test portion to determine its residual or laboratory moisture content. The total moisture content is calculated from the partial dry matter and laboratory dry matter values.

Various commodity groups have validated and published their official moisture methods (both LOD and KF) for specific matrices. These methods can be found in the following:

- *Official Methods and Recommended Practices of the American Oil Chemists' Society* for oilseeds, oilseed products, fats and oils
- *Standard Methods for the Examination of Dairy Products* for dairy products
- *Approved Methods of the American Association of Cereal Chemists* for cereal grains and bakery products
- *AOAC's Official Methods of Analysis* also has specific methods for various commodities including meat and poultry products, seafood, and fruits and vegetables.

### **Other Methods**

The development of the ***Near Infrared Reflectance Spectroscopy*** technology and computer software systems allowed the development of NIR scanning spectrophotometers. All feed substances of plant and animal origin are composed of constituents possessing functional groups of atoms that absorb in the near-infrared region (1100 – 2500 nm). Those groups include –CH-, -OH, -NH-, and other groupings. These groups comprise the major components of plant and animal-derived foods, mainly water, protein, oil (fat), cellulose and starch. In the NIR analytical procedure, spectra of test materials are compared to spectra of a set of calibration materials to predict the compositional analysis of the test material (14). The moisture content of the calibration material has been determined by a LOD method. Because of the requirement for calibrating with similar materials, NIR is an excellent tool for quality control in a production environment where the materials are known and consistent.

NIR is routinely used by the forage industry (and other industries) to determine moisture. Established official methods are NFTA Method 2.2.2.4 (13) and AOAC 991.01 (1). These methods require that the forage be partially dried and then comminuted prior to scanning. There now exists handheld NIR scanners that will determine moisture in forages without having to do any drying.

Thiex and Van Erem, as part of their methods comparison study, also used KF values to attempt NIR calibrations and found that a robust NIR moisture calibration can be created using KF covering a variety of feed materials (8).

The ***infrared moisture analyzer*** uses an infrared lamp to heat a test material which is located on top of a balance pan. The loss in weight is measured as the moisture content. The advantage of the infrared analyzer is that it can determine moisture within minutes versus several hours by conventional LOD methods. The disadvantages are that only one test portion can be analyzed at a time and the infrared

lamp can decompose constituents and cause loss of volatile components (15). The infrared analyzer is a useful quality control tool in a production setting.

The **halogen moisture analyzer** is similar to the infrared analyzer. It uses an intense halogen lamp in combination with an integrated precision balance for the measurement and recording of test portion weights before, during, and after the drying process. The halogen technology can measure moisture content in virtually any substance (15).

## RECOMMENDED METHODS

Bidwell and Sterling stated in the *JAOAC* in 1925 “*The ideal moisture method, which will probably never be found, would be applicable to all substances, would be rapid, would require little skill, would separate and determine uncombined water and nothing else, and preferably, would determine water directly and not by difference.*” (3)

### **Karl Fischer: The gold standard method**

The method that comes the closest to Bidwell and Sterling’s ideal is Karl Fischer (KF) titration. This method directly determines water and has been found to work on a variety of animal feed and human food matrices. The KF method has been made AOAC official for

- dry animal feeds (including pet food), grains and partially dried forages (AOAC 2001.12)
- soft-moist and semi-moist pet foods (AOAC 991.02)
- liquid molasses which can be extended to liquid feeds (AOAC 966.20)
- oils and fats (AOAC 984.20)

All LOD and NIRS methods need to be validated against the KF method. The KF titration equipment is expensive compared to an oven, and requires greater expertise, so few laboratories have acquired the equipment. Laboratories without the KF capability need to assure that their LOD and NIRS methods have been formally corroborated against KF for each matrix tested. Laboratories with KF capability need to validate their LOD and NIRS methods.

### **Loss On Drying Methods**

The working group is recommending the discontinuation of the commonly used AOAC 930.15 (135°C / 2 hr) method for animal feeds, forages and grains due to gross overestimation of water in most feed materials.

It is impossible to recommend one universal LOD method due to the diverse nature of animal feeds. The working group is recommending four different methods for the following matrix types.

- Dry animal feeds, grains, partially dried forages
  - Drying in forced draft oven at **104°C for 3 hours** (NFTA Method 2.1.4, AOAC Official Method 935.29 & 945.15)
  
- Forages, ensiled materials, wet feeds and grains
  - Two-step moisture method as follows:
    - Determine **partial dry matter** by drying the entire laboratory sample in a forced draft oven at **55°C overnight** or until dry (NFTA 2.2.1.1)
    - **Comminute** the entire laboratory sample to 1 mm particle size
    - Determine **laboratory dry matter** by drying a test portion in forced draft oven at **104°C for 3 hours** (NFTA Method 2.1.4)
    - Calculate **total dry matter**:  $\% \text{ total dry matter} = (\% \text{ partial dry matter} \times \% \text{ laboratory dry matter}) / 100$
    - Calculate **total moisture**:  $\% \text{ total moisture} = 100 - \% \text{ total dry matter}$
  
- Liquid feeds
  - Drying in vacuum oven at **60°C for 18 hours** (AOAC Official Method 925.45D)
  
- Ingredients

Most ingredients used in feeds can be analyzed for moisture using the 104C / 3 hr LOD method without introducing sources of error such as caramelization or loss of other volatiles. In cases where a specific method has been validated or published for an ingredient by a body, and the customer requires, then the matrix specific method should be used and cited. Examples of methods for ingredients commonly used in animal feeds and pet foods are:

  - Oilseeds, whole or unground – drying in forced draft oven at **130°C for 3 hours** (various AOCS methods)
  - Oilseeds, ground or oilseed meal -- drying in forced draft oven at **130°C for 2 hours** (AOCS Ba2a-38)
  - Dried milk products – drying in vacuum oven at **100°C for 5 hours** (AOAC Official Method 927.05)
  - Fish, seafood – drying in forced draft oven at **100°C for 1 hour** (AOAC Official Method 952.08)



- Meat and poultry products -- Drying in forced draft oven at **125°C for 4 hours** [AOAC Official Method 950.46B(b)]
- Recommended methods for other ingredients can be found in the methods & matrix spreadsheet which is posted alongside this paper.

The moisture working group is in the process of evaluating data on LOD methods for pet foods and will be making a recommendation in the near future.

### **Reporting of Results**

The laboratory report should provide the method used to determine moisture. This is an educational factor in letting clients know that there are a variety of moisture methods. This might help explain when a discrepancy occurs between a reported value and a guaranteed or expected value. Some reporting examples are:

- Moisture, loss on drying at 104°C / 3 hr
- Moisture, loss on drying, vacuum 60°C / 18 hr
- Moisture, Karl Fischer AOAC 2001.12
- Moisture, NIRS legume hay calibration

## **CRITICAL FACTORS**

Moisture analysis is generally assigned to a laboratory technician since it is viewed as (and is) a basic simple method. The moisture analyst needs to understand and observe the following critical factors to ensure reliable moisture values.

### **Critical Factors for Loss on Drying Methods**

LOD methods are empirical methods, i.e., the result obtained is dependent upon the conditions. If the conditions vary, the values obtained will also vary. The two most critical conditions with LOD methods are temperature and time. Other critical conditions are also discussed.

#### Temperature

The temperature used is generally that equivalent to the boiling point of water. The use of high temperatures will result in the loss of volatile constituents in addition to water and also in the possible decomposition of some constituents.

The drying temperature needs to remain consistent. Most methods require that the temperature be controlled to within  $\pm 1^{\circ}\text{C}$ . The temperature of the oven needs to be checked each time the oven is used and it should be recorded. This is done by means of a thermometer or a thermocouple that is inside the oven chamber. Most current ovens have a digital temperature display which needs to be checked to ensure it is reliable. Ovens with digital displays should also have a thermometer or thermocouple inserted into the oven chamber. Wireless temperature data recording systems can also be used.

Most forced draft ovens have a vent port on top which allows the insertion of a thermometer or thermocouple. One needs to ensure that the thermometer bulb or thermocouple probe does not come in contact with an oven shelf, the chamber wall or floor, or the drying containers inside the oven. Since vacuum ovens do not contain a port for a thermometer, one can use an Exact-Temp™ thermometer. With this thermometer the bulb is encased in a plastic bottle containing vermiculite which insulates the thermometer from instantly changing temperature when it is removed from the oven thereby allowing one to obtain an accurate reading of the temperature inside the sealed chamber.

All thermometers and thermocouples need to be calibrated against a NIST thermometer and this calibration should be done at least annually.

The oven needs to be kept closed once the test materials have been placed in it until after the drying period has ended. Opening the oven during the analyses will change the temperature.

### Time

The official methods have established an adequate drying time. Shortening the time will result in incomplete removal of water. Allowing the time to go too long (forgetting the time) may result in the loss of some volatile constituents or possible decomposition. A 10-15 minute window to remove test materials from the oven when the drying time ends and not before that should be sufficient. The drying time should start when the oven returns to its designated temperature after the test materials have been placed in it.

### Correct Method

Many laboratories **inappropriately** use the same drying temperature and time for all of their test materials. This is acceptable only if all materials tested are similar, such as in a manufacturing facility. The analyst needs to keep in mind when encountering a different matrix that it may require the use of a different method.

### Oven

The air oven used should be either forced draft or mechanical convection (both are the same – just different terminology in use). A fan circulates heated air inside the chamber to ensure uniform temperature throughout and to aid in the rapid removal of water. Important oven considerations:

- The velocity of the air movement cannot result in material being blown out of the drying containers.
- The oven shelves need to be constructed of wire rod or be perforated to allow air circulation.
- The oven should have a short recovery time (time it takes to return to its set temperature) of around 5 minutes after the door is closed. The oven needs to be preheated to the designated temperature prior to placing the test materials in it.
- Avoid overloading the oven. Allow space around each drying container for adequate air circulation.

### Drying Containers

Drying containers need to be sufficiently large to provide a surface area that allows the test portion to be spread out to provide maximum exposure for drying. The containers need to be shallow which will bring the heated oven air more closely over the surface of the test material. Aluminum containers are ideal since aluminum has a low specific heat resulting in rapid cooling in a desiccator.

Liquid feeds and some wet materials such as fish and seafood can form a crust during drying which prevents removal of moisture. For these materials, sand should be added to the drying container prior to its initial drying. The wet test material is then incorporated into the sand which provides a larger surface area and prevents crusting. (See AOAC Methods 925.45D and 952.08.)

The earlier methods called for the use of covers on drying containers while they were cooling in the desiccator and being weighed. Weighing on the mechanical balances used in the earlier days was slow and covers prevented the test material from absorbing atmospheric moisture. Covers are not needed with electronic balances since weighing is rapid. However, covers are a good measure at preventing a spill.

Disposable aluminum baking or roasting pans are ideal containers for partially drying of forages, ensiled materials and wet feeds and grains. Paperboard containers or paper bags can be used as long leaching from the test material does not occur. One should not pack the material into the pan or over fill it (recommended maximum depth of 1.5 inches). If a large amount of

material is being dried, it should be mixed during the drying operation to bring wet material on the bottom to the top.

### Desiccation

Most LOD methods require the cooling of the test samples in a desiccator before final weighing. The exception is LOD methods that employ hot weighing.

The desiccant needs to be changed or regenerated on a periodic basis to ensure that it is effective. The use of desiccant with color indicators is a good way of knowing when the desiccant needs to be replenished. The test samples should be kept in the desiccator long enough to have cooled to room temperature for weighing. Test samples should not be left in the desiccator overnight since they may re-absorb moisture from the desiccant. Containers should be removed quickly for weighing and the desiccator closed. The desiccator should not be left open between weighings.

### Balance

The calibration of the balance should be verified each day it is used. Verification needs to be done with certified weights that bracket the mass of the drying containers and test portions. The same balance needs to be used for weighing both before and after drying. Balances should be calibrated on an annual basis.

## **Critical Factors for Karl Fischer methods**

### Dry Conditions

The Karl Fischer titration needs to be conducted under conditions where only the water that is being measured comes from the test material. The titration vessel along with the titrant and solvent reservoirs need to have air-tight seals and be fitted with drying tubes to prevent the introduction of atmospheric moisture. The solvents and titrants must be free of residual moisture. At least one blank titration (preferably several) needs to be done at the beginning of each run in order to dry and condition the system. A blank run needs to be done each time fresh solvent is added to the titration vessel.

### Standardization

The titrant needs to be standardized against a known quantity of water during each run. Two common standards are sodium tartrate dehydrate and pure water. Several standardization titrations should be done during each run – preferably using both standards.

### Release of Water

One needs to ensure that all of the water is being released from the test material. The addition of special solvents to the methanol aids in the extraction of water from certain test materials. Formamide dissolves sugars and helps to distribute protein. Octanol aids in the dissolution of long chain hydrocarbons. A homogenizer probe inserted in the titration vessel is used to rupture cellular structures of the test material to release internally trapped water. Thermal extraction can also be used on solids to dry off the water which is then trapped and titrated in the solvent. To check that all water has been released, a test portion can be re-titrated, and relative percent difference between results should be negligible.

### Replication

KF with solvent extraction can use test portions up to 5 grams. KF thermal extraction systems are limited to very small test portions (generally 0.3 – 0.5 g). Therefore test materials should be analyzed several times to check on representative sampling and consistency. An alternative would be to extract a larger test portion with the solvent and titrate an aliquot of the extract.

### **Critical Factors for NIRS methods**

NIRS methods need to be calibrated using test materials with a known moisture content. The moisture should ideally be determined by KF or by a LOD method that has been validated by KF. The moisture content of some of the early calibration materials had been determined by AOAC 930.15 (135°C / 2 hr) which is over-estimating moisture and under-estimating dry matter. The moisture content of current calibration materials needs to be determined by NFTA Method 2.1.4 or AOAC Official Method 935.29 & 945.15 (104°C / 3 hr). The calibration equations should be updated periodically using recently analyzed test materials.

NIRS requires that the calibration materials need to have a matrix similar to that of the test materials. Thiex and Van Erem have shown that a robust moisture calibration based on KF can be created using a variety of animal feeds (8). One needs to assure that the global H value of the test material is within the predetermined global H value of the calibration equation.

The test materials need to be prepared using the same comminution equipment as the calibration materials since variations in particle size and shape will affect the NIR spectra.

## CRITICAL FACTORS FOR ALL METHODS

### Particle and Test Portion Sizes and Comminution

All methods require that the test materials need to be comminuted to a small particle size to provide a maximum surface area for drying, ease of water extraction, and scanning. Small particle sizes ensure a more representative test portion.

The test material is not to become heated during the comminution process. The generation of heat while comminuting will result in the loss of moisture. Some of the causes of heat generation during comminution are:

- overloading or too rapid feeding of the mill
- a very small screen size especially when comminuting very large particles (A way around this would be to first comminute the test material to a coarse particle size such as 6 mm and then re-comminute to a finer particle size such as 1 mm.)
- use of the wrong equipment (One of the authors was able to avoid heat generation by using a rotor with half the number of teeth in a centrifugal mill.)

The test material needs to be transferred to an air-tight container having minimal headspace immediately after comminution to prevent it from either losing or gaining moisture. The test material also should be delivered to the lab in a sealed container to prevent loss or gain of moisture.

The test portion size needs to have enough mass to ensure that it is representative of the test material. See the new publication *GOOD Test Portions* for information on obtaining representative test portions (16).

### Quality Assurance

Every laboratory needs to have a quality assurance/quality control plan. AAFCO's *Quality Assurance Quality Control Guidelines for Feed Laboratories* provides excellent guidance (17).

Laboratories should participate in a proficiency testing program to evaluate their moisture methods. Some recommended programs are:

- AAFCO for animal feeds and pet foods
- NFTA for forages and corn silage
- AOCS for oilseeds and oilseed meal
- U.S. Department of Agriculture Food Safety Inspection Service for meat products
- FAPAS – a United Kingdom PT program offers a variety of materials <https://fapas.com/>

A quality control (QC) material, a reference material and blank container should be included in each run. The QC material needs to be of a matrix similar to the test materials. Excellent QC materials are previously analyzed proficiency test samples since these will have moisture values established by various labs. The QC materials need to be stored in an air-tight container to prevent loss of or gain of moisture. Most QC materials can be stored at room temperature. However, those with a high moisture content should be stored refrigerated or frozen to retard spoilage. QC materials also need to be stored out of direct light. Reference materials include sodium tartrate dehydrate and materials spiked with a known quantity of water.

Control charts are used to monitor the performance of the QC material. A continuous downward or upward trend in the chart indicates that the QC material may be losing or gaining moisture and should be replaced with a fresh QC material.

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