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CHEMICAL AND BIOLOGICAL METHODS

FOR FEED ANALYSIS

Compiled and Edited

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Section II

CHEMICAL AND BIOLOGICAL METHODS

The chemical and biological methods of analyses of feeds and tissues in this section have been prepared for use in an Animal Nutrition Laboratory. They have been found satisfactory with both time and accuracy for the analyses of biological material.

There are many ways to do analyses. Methods are offered as guides. In some cases they will need to be modified to fit conditions in your laboratory or the conditions of the experiment.

The Weende proximate analysis system for analyzing food and animal feed includes the determination of moisture (dry matter), ether extract, crude protein, ash, crude fiber and nitrogen free extract (NFE).

In the past, these analyses have been the starting point for more detailed analysis of specific nutrients. If the feed and feces are analyzed for the proximate analyses, total digestible nutrient (TDN) may be calculated.

Expanded knowledge of nutrient requirements of animals and their nutrient utilization from feeds has revealed serious errors in the methods and assumptions underlying the crude fiber, nitrogen-free

extract and ether extract components. It is recommended that the TDN system be discarded for more modern methods of partitioning the nutrients of feeds.

In modern nutrient requirement standards, the calorie system is used (see page 701).

For evaluating forages the procedures of Van Soest are being used in preference to the Weende system. Other important analyses are voluntary intake and the *in vitro* dry matter digestion coefficient.

For making up feed composition tables the important analyses are dry matter, protein, amino acids, energy values, according to the calorie system, minerals and vitamins.

WEIGHING SAMPLES BY DIFFERENCE FOR CHEMICAL ANALYSES

Principle

The sample is kept covered so that moisture is not lost while weighing.

Equipment

(a) Analytical balance to weigh to an accuracy of four decimal points (x.xxxx)

(b) 50 ml weighing bottles

(c) Small spoon made from aluminum or plastic

(d) 10 to 25 ml weighing bottles

(e) Pipettes

Procedure

Solid samples

Fill a 50 ml weighing bottle about half full of the sample. Place a small spoon made out of a piece of aluminum or plastic in the weighing bottle. Place the lid on the weighing bottle. Weigh the correct amount of sample by difference for each determination.

Arrange to weigh out samples for several analyses at the same time. For example, samples for dry matter, nitrogen, ash, cell walls, etc., could be weighed from one weighing bottle. This would mean that only one weigh-

ing would need to be made for each sample for a single analysis or two weighings for duplicate analyses.

After the samples have been weighed for the various determinations, empty out the weighing bottle, wipe it and the spoon with a clean cloth. The bottle is then ready for the next sample. If contamination is a problem between samples such as when working with trace minerals or insecticides, use a separate weighing bottle and spoon for each sample.

Do not weigh exact amounts of sample. This takes more time to weigh and may result in errors in sampling.

Liquid samples

Stir sample well if sediment is on bottom. While stirring, pipette sample into a tared weighing bottle. Weigh the sample plus the weighing bottle. The difference in weight is the weight of the sample delivered by the pipette. Use the same pipette to pipette a separate sample into the container used for chemical analysis.

This procedure is helpful if a large amount of material such as urine needs to be weighed and sampled. If the above procedure is used results are reported on a weight basis.

Reference

Harris, Lorin E. 1970. Nutrition research techniques for domestic and wild animals. Published by L.E. Harris (in press).

DRY MATTER AND PARTIAL DRY MATTER

Principle

The moisture (water) of the sample is removed by volatilization caused by heat or freeze drying. The percentage of sample left after removing this moisture is known as the percent dry matter or percent partial dry matter.

The aim is to:

- obtain "as fed" dry matter or "as collected" dry matter,
- prepare the samples for chemical analyses, and
- report the chemical analyses on a dry basis.

The following definitions are in the glossary, but are repeated here for discussion:

Dry matter content of feed samples. (general term) The dry matter content of feed samples and other materials is expressed on three dry matter bases: as fed; partially dry; and dry. Definitions of these terms follow:

> As fed refers to the feed as it is consumed by the animal; the term "as collected" is used for materials which are not usually fed to the animal, i.e. urine, feces, etc. If the analyses on a sample are affected by partial drying, the analyses are made on the as fed or as collected sample. Similar terms: Air dry i.e. hay; As received; Fresh; Green; Wet.
>
> Partially dry refers to a sample of "as fed" or "as collected" material that has been dried in an oven (usually with forced air) at a temperature usually about 60° C or freeze dried and has been equilibrated with the air; the sample after these processes would usually contain more than 88% dry matter (12% moisture); some materials are prepared in this way so they may be sampled, chemically analyzed and stored. This analysis is referred to as "partial dry matter % of 'as fed' or 'as collected' sample." The partially dry sample must be analyzed for dry matter (determined in an oven at 105° C) to correct subsequent chemical analyses of the

samples to a "dry" basis. This analysis is referred to as "dry matter % of partial dry sample." Similar term: Air dry (sometimes air dry is used for as fed).

Dry refers to a sample of material that has been dried at 105° C until all the moisture has been removed. Similar terms: 100% dry matter; moisture free. If dry matter (in an oven at 105° C) is determined on an "as fed" sample it is referred to as "dry matter of as fed sample." If dry matter is determined on a partial dry sample it is referred to as "dry matter of partial dry sample". It is recommended that analyses be reported on the "dry" basis (100% dry matter or moisture free), and in addition the "as fed dry matter" should be reported.

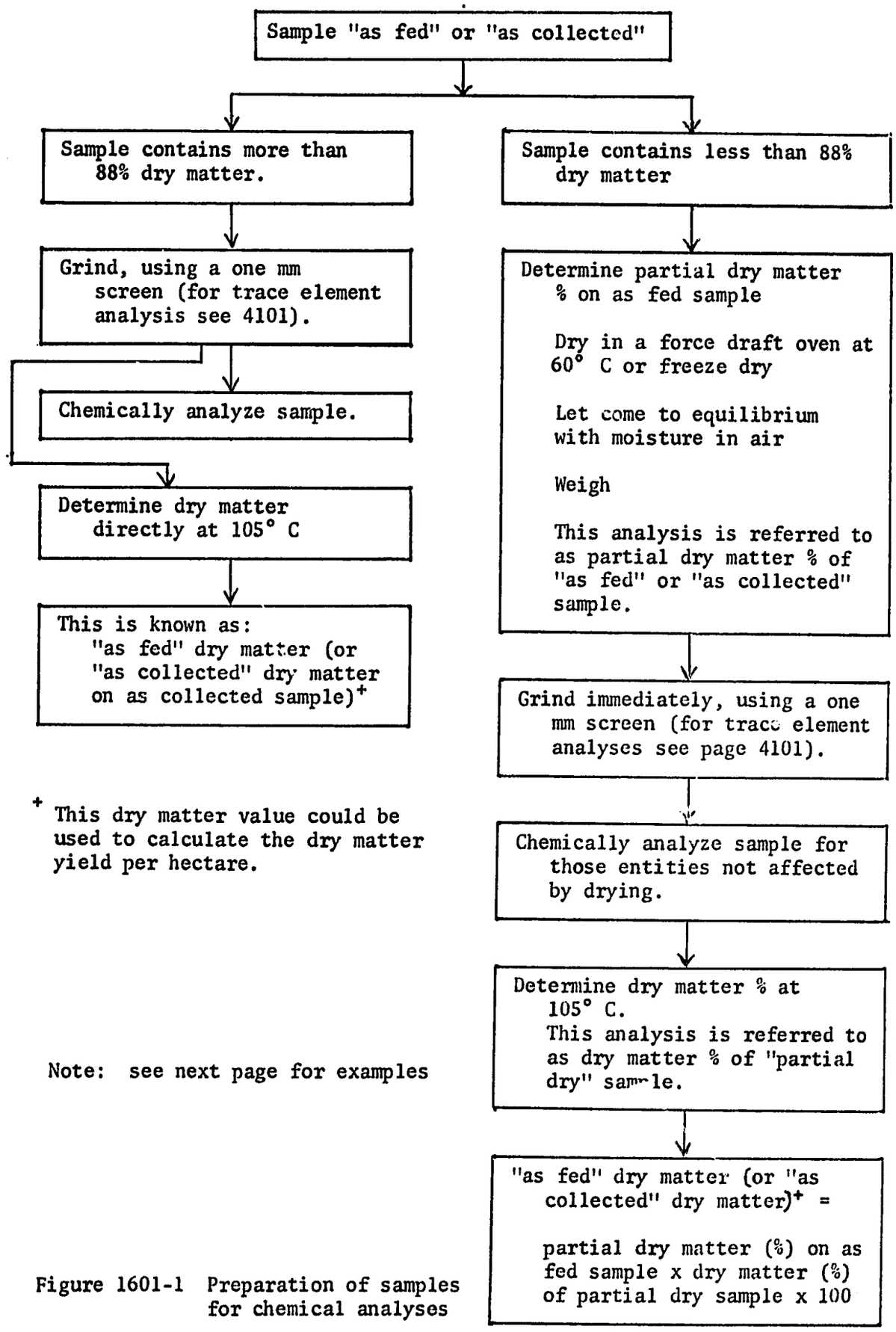
As explained in the definitions there are essentially two kinds of samples: (1) those dry enough to grind and analyze immediately (more than 88% dry matter) and (2) those which need to be partially dried or handled in a special way (less than 88% dry matter).

The flow chart outlined in figure 1601-1 shows how these two types of samples should be handled. (See next page)

The plan outlined in figure 1601-1 assumes that all samples with less than 88% dry matter are partially dried before analyzing. <u>However, the chemist should use his judgment and decide whether partial drying will affect the results of the analyses. If it does, then arrangements should be made to analyze the sample on the "as fed" or "as collected" basis.</u>

Recently it has been shown that the fiber fractions of esophageal samples are affected by drying samples at too high of a temperature. (Lesperance and Bohman, 1963). Therefore, care should be exercised in analyzing these samples.

Recently freeze drying equipment has become available. If this equipment is available this method should be substituted for partial drying in a forced draft oven at 60° C.



+ This dry matter value could be used to calculate the dry matter yield per hectare.

Note: see next page for examples

Figure 1601-1 Preparation of samples for chemical analyses

All analyses should be reported on a "dry," (100% dry matter or moisture free) basis on the source form. However, for certain samples there may be some advantage to reporting data on an "as fed" or as collected basis (blood or urine). Space has been provided on the source form for this.

To report data on the dry basis corrections need to be made as follows:

Adjusting to dry basis (the individual values of the sample, i.e., cellulose % or cobalt mg/kg):

(a) chemical or biological composition value in % or mg/kg (dry basis) =

$$\frac{\text{chemical or biological composition value in \% or mg/kg} \times 100}{\text{dry matter \% on "as fed" sample}}$$

or

(b) chemical or biological composition value in % or mg/kg (dry basis) =

$$\frac{\text{chemical or biological composition value in \% or mg/kg} \times 100}{\text{dry matter \% on "partial dry" sample}}$$

Example Calculations

(a) Dry matter % on as fed basis =

$$\frac{(\text{wt of dried sample and container - wt of drying container})}{\text{wt of sample in g on as fed basis}} \times 100$$

Example: If the as fed sample weighed 1.9444 g, the sample container weighed 12.3278 and the dried sample (after drying in 105° C oven) and container weighed 14.1011 g.

Dry matter % on as fed basis =

$$\frac{(14.1011 - 12.3278)}{1.9444} \times 100 = 91.2\%$$

(b) Partial dry matter % on as fed or as collected sample =

$$\frac{\text{wt of dried sample}}{\text{wt of sample in g on as fed or as collected basis}} \times 100$$

Example: This determination is made when as fed samples contain less than 88% dry matter such as silages and fresh forages, and a large sample is dried for further analysis. If the as fed or as collected sample weighed 468.2 g, the sample after drying at 60° C in a forced draft oven and equilibrating with moisture in the air weighed 155.4 g.

Partial dry matter % on as fed or as collected sample =

$$\frac{155.4}{468.2} \times 100 = 33.2\%$$

(c) Dry matter % on partial dry sample =

$$\frac{(\text{wt of dry sample and container - wt of container})}{\text{wt of sample on partial dry basis}} \times 100$$

Example: When the partial dry sample is equilibrated with the water content of the air, another dry matter determination is necessary to correct it to the dry matter basis. If the sample of partial dry material weighed 2.3146 g, the dried sample and container after drying at 105° C weighed 14.3957 g and the drying container weighed 12.2140 g.

dry matter % on partial dry sample =

$$\frac{(14.3957 - 12.2140)}{2.3146} \times 100 = 94.3\%$$

(d) To calculate as fed or as collected dry matter when partial dry matter was obtained on sample such as on silages or fresh forages and dry matter was determined on the partial dry sample.

Dry matter % of as fed or as collected sample =

$$\frac{\text{partial dry matter (\%) of as fed or as collected sample}}{100} \times \frac{\text{dry matter (\%) on partial dry sample}}{100} \times 100$$

Example: If partial dry matter of as fed or as collected sample was 33.2%, and the dry matter on the partial dry sample was 94.3%.

Dry matter % of as fed or as collected sample =

$$\frac{33.2\%}{100} \times \frac{94.3\%}{100} \times 100 = 0.332 \times 0.943 \times 100 = 31.3\%$$

(e) All other chemical analyses must also be converted to the dry basis. The following is an example of the calculation using cellulose analysis for the example.

Cellulose % on as fed basis =

$$\frac{(\text{wt of crucible and cellulose - wt of crucible and ash})}{\text{wt of sample in g on as fed basis}} \times 100$$

Example: If wt of crucible and cellulose after drying is 35.4375 g and wt of crucible and ash is 35.1131 g and the as fed sample used weighed 1.0341 g.

Cellulose % on as fed basis =

$$\frac{(35.4375 - 35.1131)}{1.0341} \times 100 = 31.4\%$$

To adjust as fed sample to dry basis:

$$\frac{\text{cellulose \% on as fed basis} \times 100}{\text{dry matter \% of as fed sample}}$$

Example:

$$\frac{31.4\% \text{ cellulose on as fed basis}}{91.2\% \text{ dry matter on as fed sample}} \times 100 = 34.4\% \text{ cellulose on dry basis}$$

or

Cellulose % on partial dry basis =

$$\frac{(\text{wt of crucible and cellulose} - \text{wt of crucible and ash})}{\text{wt of sample in g on partial dry basis}} \times 100$$

Example: If the sample weighed 1.0114 g, the crucible and dry cellulose 34.8393 g and the crucible and ash 34.5114 g.

Cellulose % on partial dry basis =

$$\frac{(34.8393 - 34.5114)}{1.0114} \times 100 = 32.4\%$$

To adjust partial dry sample to dry basis:

$$\frac{\text{cellulose \% on partial dry basis}}{\text{dry matter \% of partial dry sample}} \times 100$$

Example:

$$\frac{32.4\% \text{ cellulose on as fed basis}}{94.3\% \text{ dry matter on as fed sample}} \times 100 = 34.4\% \text{ cellulose on dry basis}$$

Reference

Harris, Lorin E. 1970. Nutrition Research Techniques for Domestic and Wild Animals. Published by L. E. Harris, 1408 Highland Drive, Logan, Utah, USA, 84321 (in press).

(Example heading for recording chemical analyses in bound books)

CELLULOSE

Date_____

Analyst_____

Lab No.	Beaker and crucible Nos.	Weights to obtain sample wt.	Crucible and cellulose	Crucible and ash	Cellulose	Cellulose of det.	Avg. cellulose of sample	Dry matter of sample (a.)	Cellulose on dry basis
		g	g	g	g	%	%	%	%
70	3	76.3223	34.8393			32.16	32.1	94.30	34.4
380	27	75.3028		34.5114					
2ECA		1.0195			.3279				
70	4		36.2591			32.68			
380	28	74.2923		35.7289					
260B		1.0105			.3302				

[a]This is dry matter of partial dry sample in this example.

DETERMINATION OF DRY MATTER

Principle

The moisture of the sample is lost by volatilization caused by heat. The amount of material left after the removal of the moisture is the dry matter.

Equipment

(a) Oven, 105° C.

(b) Covered aluminum dishes, 50 mm diameter with covers, or similar containers.

Determination

(a) Brush out the aluminum dishes between samples. If ether extract is to be determined on the sample rinse the dishes with 95% ethyl alcohol and finally with ethyl ether. Let the ether evaporate under a hood. Dry the dishes in 105° C oven for at least one hour. Place in desiccator, cool, and weigh. Handle dishes with metal tongs.

(b) Weigh by difference 1.5 to 2.0 g of sample into a weighed dish. Put top of dish on bottom of dish and place in 105° C oven over night. Remove dishes, put cover on top and place in desiccator and cool. Remove from desiccator and weigh as quickly as possible.

(c) The samples may be stored in a desiccator for use in ether extract determination.

Calculation

$$\% \text{ dry matter} = \frac{(\text{wt of dish} + \text{wt of dried sample}) - \text{wt of dish} \times 100}{\text{wt sample before drying}}$$

$$= \frac{\text{wt of dry sample} \times 100}{\text{wt sample before drying}}$$

Note: If the sample was analyzed on an as fed basis the dry matter is labeled "dry matter of as fed sample"

If the sample was analyzed on a partial dry basis the dry matter is labeled "dry matter of partial dry sample".

See page 1601 for definitions of "as fed", "partial dry" and "dry".

Reference

Official methods of analyses of the Association of Official Agricultural Chemists. 1960. 9th Edition. Published by the Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D.C.

DETERMINATION OF PARTIAL DRY MATTER

Principle

The sample is dried at a temperature of less than 65° C until about 95% of the water is driven off or it is freeze dried. The sample is then equilibrated with the moisture in the atmosphere.

Apparatus

(a) Containers. Use a container that does not absorb moisture and is large enough so that the sample can be spread out in a thin layer. Tin pans or pyrex glass dishes about 30 cm long, 14 cm wide and 4 cm deep may be used.

(b) Drier or freeze drier. Use a drier with a fan to circulate the air so part of the air goes away from the drier to remove the moisture or use a freeze drier.

(c) Balance. Use a balance which will weigh to an accuracy of 0.5 g.

Determination

(a) Preparation of pans. Wash the pans, and place in drier for five hours. Take out of drier, place on clean shelf, cool and weigh.

(b) Weighing samples. If the sample has been frozen bring it to the same temperature as the room. Remove the sample from its container, mix until homogeneous and weigh into one of the tared pans 200 to 500 g. The amount depends on the moisture content. Spread the sample over the surface of the pan in a thin layer.

(c) Drying. Place the pan and sample in the drier at a temperature of 60° C and leave at least 48 hours, or until about 95% of the moisture is removed. The temperature must not be higher, because it affects some determinations such as lignin; or use a freeze drier.

(d) Set the pan in a dust-proof, vermin-proof room or cabinet, where the moisture condition will be the same as when the sample is ground, for about 72 hours.

(e) Weigh the sample and grind immediately through a mill with a one millimeter screen (such as a Wiley mill). See page 4101 for directions on how to prepare samples for trace element analyses.

(f) Transfer the sample to an air tight container. An ideal container is a 250 ml plastic or glass bottle with an air tight lid.

(g) Label the sample with a tag as follows:

Laboratory No. _____
Project No. _____
Expt. No. _____
Date taken _____
Sample _____
_____
Project Leader _____
Initials of person doing work _____

Calculation

$$\text{Partial dry matter (\%)} = \frac{(\text{wt partial dry sample + pan}) - \text{wt pan} \times 100}{(\text{wt "as fed" or "as collected" sample + pan}) - \text{wt pan}}$$

or

$$\frac{\text{wt partial dry sample} \times 100}{\text{wt "as fed" or "as collected"}}$$

Reference

Harris, Lorin E. 1970. Nutrition Research Techniques for Domestic and Wild Animals. Published by L. E. Harris (in press).

DETERMINATION OF GROSS ENERGY

Principle

The amount of heat, measured in calories, that is released when a substance is completely oxidized in a bomb calorimeter, is called the gross energy (GE) of the substance. A sample of the material to be tested is weighed into a combustion capsule. The combustion capsule is placed in an oxygen bomb containing 25 to 30 atmospheres of oxygen. The oxygen bomb is covered with 2000 g of water in an adiabatic calorimeter. After the bomb and calorimeter have been adjusted to the same temperature, the sample is ignited with a fuse wire. The temperature rise is measured under adiabatic conditions. From the hydrothermal equivalent of the calorimeter times the temperature rise minus some small corrections for fuse wire oxidation and acid production, the caloric content of the sample is calculated.

See page 701 for definitions.

Equipment

(a) Parr oxygen bomb calorimeter and accessories or equivalent.

(b) The calorimeter may be equipped with an automatic temperature controller.[+] If the temperature controller is on the calorimeter, it will take less labor to run the analysis, but the controller is not necessary to obtain accurate results.

[+]Fisher Scientific Co., Catalog No. 4-364V2.

(c) Solution or trip balance with capacity to 3000 g and accurate to 0.1 g.

(d) For an illustration of the nomenclature of the Parr Bomb Calorimeter, refer to the illustration on page 1901-10.

Reagents

(a) Standard sodium carbonate solution, equivalent to 1 cal/ml (3.658 g Na_2Co_3 per liter).

(b) Methyl orange indicator.

(c) Bensoic acid combustion tablets or primary standard grade crystals.

Procedure

(a) Weigh by difference approximately 1.0 g of sample and place in a clean, empty combustion capsule. Samples may be pelleted, but this is usually not necessary.

(b) Attach a 10 cm length of fuse wire between the electrodes of the bomb (oxygen) and set the combustion capsule with sample in place in the loop electrode. Adjust the fuse wire so it is touching the sample.

(c) Place about 1 ml of water in the bomb cylinder and swirl it around to wet the sides. This is not necessary if the bomb is still wet from a preceding determination.

(d) Assemble the bomb, tighten the screw cap, close the pressure release valve and fill with oxygen to 25 atmospheres gauge pressure. Place the bucket (oval) in the calorimeter, set the bomb in the bucket, and attach the clip terminal.

(e) Weigh 2000 g distilled water on the solution or trip balance (use a 2000 ml volumetric flask to hold the water) and carefully pour into the calorimeter bucket. The water temperature must be within the range of the calorimeter thermometers.

(f) Close the cover (be sure thermometers are up), lower the thermometers and start the water circulating motor. Remove the cap from the calorimeter jacket cover and fill the cover with water until it runs out the drain hose (only necessary at the beginning of the day's run).

(g) Adjust the temperature of the water in the outer jacket to approximately equal that of the calorimeter by adding hot or cold water, and allow one minute to attain equilibrium. Then carefully adjust the temperatures to be exactly equal and check the calorimeter temperature at one-minute intervals for three minutes.

(h) Read and record the initial temperature to the nearest 0.0002° C and ignite the sample. Turn in hot or cold water to keep the jacket temperature equivalent to the calorimeter temperatures during the period of rise.

(i) Compare and adjust the temperature of the outer jacket to the inner bucket of the calorimeter temperature frequently and carefully to insure adiabatic condition or that the temperatures are equal. Read and record the final temperature after the same temperature is observed in three successive one-minute intervals.

(j) Raise the thermometers. Open the calorimeter, take the bomb from the calorimeter bucket, release the residual pressure of the bomb and open. Carefully remove the remaining pieces of fuse wire from the electrodes; straighten and measure the combined total length in centimeters. The calories of wire burned can be determined with the measuring

scale that is supplied with the wire.

(k) Rinse all inner bomb surfaces with a stream of neutral distilled water and collect all washings in a clean beaker. Titrate the washings with the standard sodium carbonate solution using methyl orange indicator to determine the amount of acid formed from the incidental oxidation of nitrogen and sulfur compounds. A correction is made to take care of the heat liberated in the formation of the acid.

(l) Correct the initial and final temperatures from the calibration curve supplied with the thermometer.

Calculations

$$\text{(a) GE (cal/g) on as fed basis} = \frac{\left[\text{final temp.} - \text{initial temp.}\right] \text{ hydrothermal equiv. of bomb} - \left[\text{length of fuse wire burned} \times \text{cal per cm}\right] - \text{ml } Na_2Co_3}{\text{wt. of sample}}$$

Example:

A 1.0214 g sample of feed (as fed basis) had an initial temperature of 23.13° C and a final temperature of 25.25° C. The hydrothermal equivalent of the bomb is 2412 cal per degree C. There was 7.0 cm of fuse wire burned with a correction of 2.3 cal per cm of wire and 6.0 ml of Na_2Co_3 titrated (equivalent of 6.0 cal).

$$\text{GE (cal/g)} = \frac{(25.25 - 23.13)\ 2412 - (7.0 \times 2.3) - 6.0}{1.0214 \text{ g sample on as fed basis}} = 3804 \text{ cal/g or } 3804 \text{ kcal/kg}$$

Note: Report the data on the source form as kcal/kg.

Adjusting to dry basis:

(a) $\frac{\text{GE (kcal/kg on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$

or

(b) $\frac{\text{GE (kcal/kg) on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$

Determining the Hydrothermal Equivalent of the Bomb

(a) Determine the hydrothermal equivalent (calories per degree rise in temperature) of the bomb, bucket and water by determining the temperature rise using the same procedure as outlined above, but with a sample of known caloric content (benzoic acid combustion tablet). Make at least four determinations and use the average value. Once this value is determined it should not change unless some parts of the bomb are replaced.

(b) Dry the benzoic acid at 105° C overnight, cool in desiccator and weigh by difference from a covered weighing bottle one tablet or approximately a 1 g sample of dry calorific standard grade benzoic acid crystals. Determine the temperature rise from the benzoic acid in the bomb as with other samples.

(c) Examples of 4 determinations required to determine the hydrothermal equivalent and the calculations are shown below:

wt of sample and bottle	43.7622	42.7000	41.6695	40.6137
bottle tare wt	42.7000	41.6695	40.6137	39.5656
sample wt	1.0622	1.0305	1.0558	1.0481
final temp	23.045	24.17	25.12	25.76
initial temp	20.28	21.48	22.37	23.03
temp rise	2.765	2.69	2.75	2.73
acid (cal)	7.5	7.0	7.0	7.0
wire (cal)	11.0	16.5	15.0	10.0
heat of combustion of benzoic acid (cal)	6319	6319	6319	6319
cal from benzoic acid	6712	6512	6672	6623
total calories	6730	6535	6694	6640
hydrothermal cal/degree equivalent	2434	2434	2429	2432

$$\text{Hydrothermal equivalent per degree (cal)} = \frac{\text{wt of benzoic acid sample} \times \text{calories per g benzoic acid} + \left[\text{length of fuse wire burned} \times \text{cal/cm fuse wire}\right] + \text{ml } Na_2Co_3}{(\text{final temp.} - \text{initial temp.})}$$

Example:

A 1.0622 g sample of benzoic acid had a heat of combustion of 6319 cal per g. The corrected initial temperature of the bomb was 20.280° C and the final corrected temperature was 23.045° C. There were 4.8 cm of fuse wire burned with a caloric value of 2.3 cal per cm. There were 7.5 ml of Na_2Co_3 titrated (equivalent to 7.5 cal).

$$\text{Hydrothermal equivalent calories per degree} = \frac{(1.0622 \times 6319) + (4.8 \times 2.3) + 7.5}{(23.045 - 20.280)} \doteq 2434 \text{ small calories}$$

Determination of Gross Energy of Urine

(a) Urine energy (UE) can be estimated from the nitrogen content of the urine (UN) by the formula of Street et al. (1964).

UE (kcal per kg) = 117 + 26 (UN%)

This formula was developed with data from cattle and sheep. It may be used where the amount of urine energy represents a small part of the total energy such as in the determination of metabolizable energy of feeds for feed composition tables.

(b) If a more accurate determination of urine energy is needed, a sample of urine must be dried and the energy determined with an oxygen bomb calorimeter. Urine samples must be dried in contact with a known weight of a combustible solid. A small plastic beaker[+] serves as both a drying container and the solid fuel.

(c) A 5 ml urine sample may be weighed into a tared 5 ml polystyrene beaker. Urine samples are weighed as outlined on page 1501.

(d) Freeze drying is the preferred drying method when a freeze dryer is available.

(e) The next preferred method of drying the urine sample is in a vacuum oven at 40° C with over 700 mm of vacuum until the sample is almost dry.

(f) When neither a freeze dryer or vacuum oven are available, dry the urine sample in an oven at 45° C. The sample need not be completely dry to burn in the oxygen bomb. Overdrying will result in

[+]5 ml polystyrene beakers, catalog No. B2718, are available from Scientific Products, Evanston, Illinois, 60201, USA. These beakers weigh less than 1 g, can be heated to 135° C, and are relatively inexpensive.

unnecessary energy losses. After the urine sample in the plastic beaker is dried, gross energy is determined on the plastic beaker and urine according to the standard procedure for other solid samples.

(g) The average gross energy, cal/g, of polystyrene is determined by taking the average gross energy of four polystyrene beakers.

(h) The gross energy of urine is calculated as follows:

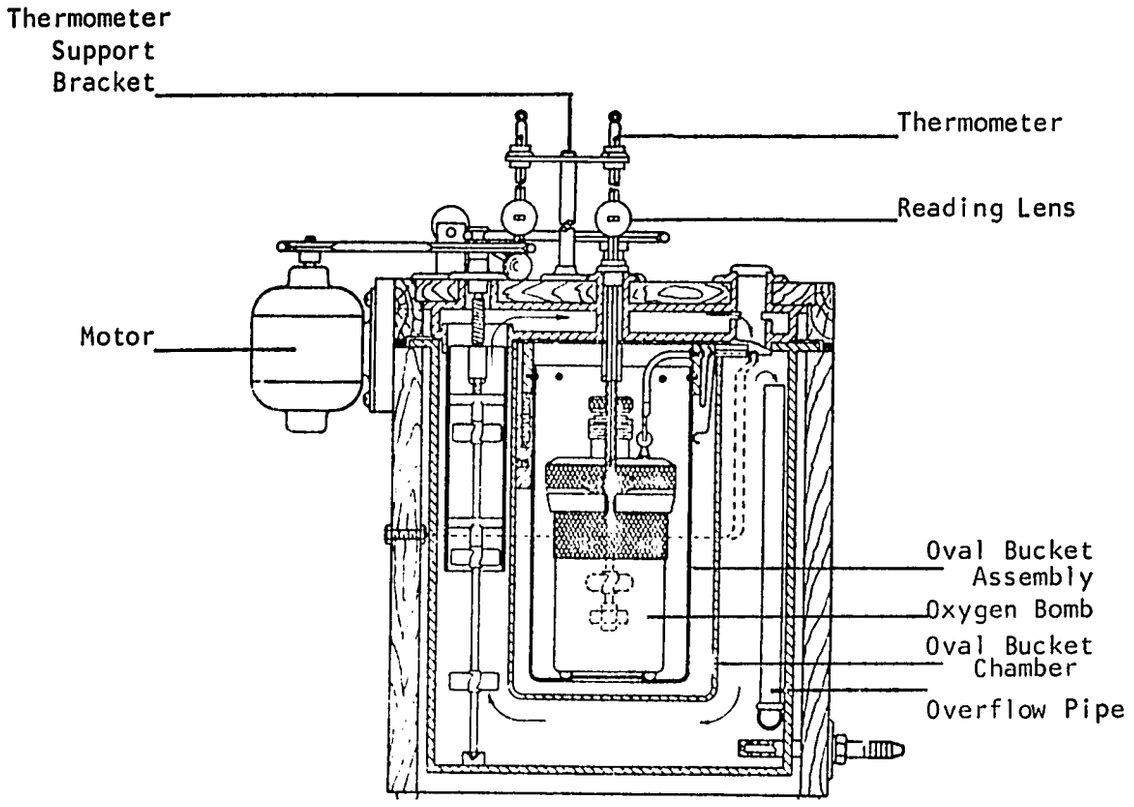
$$\text{GE of urine (cal/g)} = \frac{\left(\text{final temp.} - \text{initial temp.}\right) \text{ hydrothermal equiv. of bomb} - \left(\text{length fuse wire} \times \text{cal/cm wire}\right) - \text{ml } Na_2Co_3 - \left(\text{wt of polystyrene beaker} \times \text{cal/g polystyrene}\right)}{\text{wt urine sample in g}}$$

Note: Cal/g is the same as kcal/kg. Report data as kcal/kg.

References

Parr Instrument Company. 1966. Oxygen Bomb Calorimetry and Combustion Methods. Technical Manual No. 130. Parr Instrument Company, Molin, Illinois.

Street, J. C., J. E. Butcher and L. E. Harris. 1964. Estimating urine energy from urine nitrogen. J. Animal Sci. 23:1039.



Nomenclature of the Adiabatic Calorimeter

DETERMINATION OF ASH

Principle

The sample is ignited at 600° C to burn off all organic material. The inorganic material which does not volatilize at that temperature is called ash.

Equipment

(a) Muffle furnace

(b) Porcelain crucibles (for trace element analyses, see page 4101)

(c) Desiccator, with magnesium perchlorate desiccant.

Procedure

(a) Place new or clean crucibles in a muffle furnace at 600° C for one hour. Transfer crucibles from furnace to a desiccator and cool to room temperature. Weigh them as quickly as possible to prevent moisture absorption. Use metal tongs to move the crucibles after they are ashed or dried.

(b) Weigh by difference 1.5 to 2.0 g sample into a tared porcelain crucible. Place in a muffle furnace and hold the temperature at 600° C over night.

(c) Transfer the crucible to a desiccator and cool to room temperature . When cool, weigh the crucible as quickly as possible to prevent moisture absorption.

(d) Save the ash sample if mineral determinations are to be made.

Calculations

$$\text{Ash (\%) on partial dry or as fed basis} = \frac{\text{wt of ash} \times 100}{\text{wt of sample}}$$

Adjusting to dry basis:

(a) $$\frac{\text{ash \% on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{ash \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

Official Methods of Analysis of the Association of Official Agricultural Chemists. 1965. 10th Edition. Published by the Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D.C.

DETERMINATION OF CRUDE FIBER

Principle

A moisture-free and ether-extracted sample is digested first with a weak acid solution, then a weak base solution. The organic residue is collected in a filter crucible. The loss of weight on ignition is called crude fiber.

Equipment

(a) Extraction apparatus consisting of individually controlled heaters and water-cooled condensers designed to maintain constant volume of solution throughout digestion.

(b) Digestion containers. Use digestion containers of such size and shape that the solution is not less than 25 mm or more than 38 mm in depth. A 500 ml wide mouth Erlenmeyer flask or 600 ml tall form beaker is recommended, depending on the type of condenser used.

(c) Fit condensers into the top of two 5 liter round bottom flasks and put into a heating element that fits the flasks. Arrange to have a siphon made from rubber tubing. The purpose of this apparatus is to be able to preheat the sulfuric acid and sodium hydroxide to boiling before adding to the digestion containers.

(c) Linen cloth with about 20 threads per cm or No. 40 filtering cloth made by National Filter Media Corporation, New Haven 14, Connecticut.

Reagents

(a) Sulfuric acid solution. 0.255 N, 1.25 g H_2SO_4/100 ml. Check normality by titration and adjust if necessary to 0.255 N.

(b) Sodium hydroxide solution. 0.313 N, 1.25 g NaOH/100 ml, free from Na_2CO_3. Check normality by titration and adjust if necessary to 0.313 N.

(c) Asbestos. Gooch grade, medium fiber, acid-washed, and ignited is usually satisfactory. If it needs testing, digest on steam bath for eight hours with about 5% NaOH; wash thoroughly with hot water. Digest in similar manner eight hours with HCl (1 + 3) and again wash with hot water. Dry and ignite at bright red heat.

(d) Dow-Corning Antifoam A, spray or octyl alcohol.

(e) Ethyl alcohol.

Procedure

(a) The residue from the ether extract determination may be used (use original weight of sample before drying and extracting with ether). If this residue is not used, proceed as follows to obtain a dry ether extract free sample.

(b) Weigh by difference 1.5 to 2.0 g of sample into a clean alundum thimble. Dry in an oven at 105° C overnight (weigh 6 samples as outlined under e).

(c) Extract with ether (see ether extract determination page 2301) for 16 hours.

(d) Transfer the extracted residue, together with approximately 0.5 g asbestos to the digestion containers. Spray a small amount of Dow-Corning Antifoam A into the container, or add a drop or two of octyl alcohol. Siphon 200 ml of boiling sulfuric acid solution into the digestion container.

Place the beaker on preheated extraction heater and cover with condenser. The solution should start boiling within one minute. Rotate the container frequently until the sample is thoroughly wetted. Take care to keep material from remaining on the sides of the beaker out of contact with the solution. (A blast of air conducted into the flask serves to reduce frothing of liquid.) Repeat procedure on other samples at 5-minute intervals.

(e) When the first sample has boiled for exactly 30 minutes, remove it and filter onto a linen cloth in a fluted funnel, and wash with boiling water until washings are no longer acid. Wash the sample back into the container with the 200 ml of boiling sodium hydroxide solution. Place beaker back on hot plate. The solution should come to a boil within one minute. Allow a total of five minutes from time of removal of sulfuric acit treated sample from hot plate until the sodium hydroxide treated sample starts to boil. (With this timing sequence, six determinations can be run at a time by one operator.)

(f) When the first sodium hydroxide solution has boiled exactly 30 minutes, remove it and filter onto linen cloth as before. Wash the sample with boiling water. Filter with suction into a Gooch crucible with thin layer of asbestos in bottom. This is prepared by first having asbestos in water solution and pouring a small quantity into Gooch crucible. Finally wash sample in Gooch crucible with about 15 ml of 95% ethyl alcohol.

(g) Dry the sample in a 105° C oven overnight. Cool to room temperature in a desiccator and weigh.

(h) Ignite the contents of crucible in a muffle furnace at 600° C for an hour or longer. Cool in a desiccator to room temperature and weigh. Report the loss in weight as crude fiber.

If the crude fiber samples are difficult to filter through the Gooch crucibles, asbestos may be added to samples prior to addition of sulfuric

acid solution. Also, washing the sample while on the linen just prior to filtering through Gooch crucible with hot 10% K_2SO_4 solution may be helpful. The K_2SO_4 solution may be added during filtering whenever filtration becomes difficult.

Calculations

Crude fiber (%) on partial dry or as fed basis =

$$\frac{\text{loss of wt on ignition} \times 100}{\text{wt of sample before drying and extracting with ether}}$$

Adjusted to dry basis:

(a) $$\frac{\text{crude fiber \% on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{crude fiber \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

Official Methods of Analysis of the Association of Official Agricultural Chemists. 1965. 10th Edition. Published by the Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D.C.

DETERMINATION OF ETHER EXTRACT

Principle

Ether is continuously volatilized, then condensed and allowed to pass through the sample, extracting ether soluble materials. The extract is collected in a beaker. When the process is completed, the ether is distilled and collected in another container and the remaining crude fat is dried and weighed.

Equipment

(a) Goldfisch, fat extraction apparatus

(b) Solvent beakers

(c) Extraction thimbles, alundum

Reagents

(a) Diethyl ether, anhydrous

If anhydrous ether is not available, proceed as follows: Wash commercial ether with 2 or 3 portions of water, add solid sodium hydroxide or potassium hydroxide, and let stand until most of the water is abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic sodium and let stand until hydrogen evolution ceases. Keep ether, thus dehydrated, over metallic sodium in loosely stoppered bottles. Add zinc pellets to containers of both new and used ether to prevent formation of dangerous peroxides.

Procedure

(a) Weigh by difference a 1.5 to 2.0 g sample into a clean previously extracted alundum thimble.

(b) Dry the sample at 105° C over night (or use sample from dry matter determination).

(c) Clean and dry solvent beakers in 105° C oven for an hour. Cool them to room temperature in a desiccator, weigh and record the tare weight.

(d) Place thimble and sample in sample container and fix under the condenser of the Goldfisch extraction apparatus. Add 30 to 40 ml of diethyl ether to the solvent beaker and place on condenser with screw ring, which is tightened as much as possible by hand. Turn on the water that cools the condenser. Raise the hot plates until they are in contact with the beakers and turn on heaters. Check for ether leaks after the ether starts to boil and condense. When level of ether goes down in beaker to a constant level (because a portion is volatilizing and condensing) the apparatus may be left with occasional checks. Extract 16 hours.

(e) After the extraction is completed, lower the heater and allow the thimble to drain empty. Remove the samples and place glass ether reclaiming tubes under the condenser. Replace beaker, raise hot plate and distill ether into the reclaiming tubes. Just before ether in the beaker evaporates to dryness, lower the hot plate. Remove beakers. Pour ether from reclaiming tubes into a container for used ether. Complete drying of ether in beakers in open air and allow ether beakers to remain on bench.

Dry the ether extract in a 105° C explosion proof oven for 30 minutes. Cool in desiccator to room temperature and weigh.

Calculations

Ether extract (%) partial dry or as fed basis =

$$\frac{\text{wt ether extract} \times 100}{\text{wt of sample}}$$

Adjusting to dry basis:

(a) $$\frac{\text{ether extract \% on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{ether extract \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

USA Official Methods of Analysis of the Association of Official Agricultural Chemists. 1960. 9th Edition. Published by the Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D.C.

DETERMINATION OF NITROGEN FREE EXTRACT

Principle

Nitrogen free extract (NFE) of a feed is determined by difference after the analyses have been completed for ash, crude fiber, ether extract and crude protein. NFE is necessary for the calculation of TDN.

Calculation

NFE (%) on dry basis =

100% - [ash (%) on dry basis + crude fiber (%) on dry basis + ether extract (%) on dry basis + protein (%) on dry basis]

Example: If the ash on dry basis was 7.2%; crude fiber, 32.7%; ether extract, 3.3%; and the crude protein, 15.3%, then:

NFE (%) on dry basis =

100% - [7.2% + 32.7% + 3.3% + 15.3%] = 41.5%

Adjusting to as fed basis =

$$\frac{\text{constituent on dry basis (\%)}}{100} \times \frac{\text{as fed dry matter (\%)}}{100} \times 100$$

Example:

If as fed dry matter = 89.3%, then:

NFE (%) on as fed basis =

$$\frac{41.5}{100} \times \frac{89.3}{100} \times 100 = 0.415 \times 0.893 \times 100 = 37.1\%$$

Reference

Crampton, E. W., and L. E. Harris. 1969. Applied Animal Nutrition, The Use of Feedstuffs in the Formulation of Livestock Rations. Second Edition. W. H. Freeman and Co., San Francisco, California, USA.

DETERMINATION OF NITROGEN AND CRUDE PROTEIN

Principle

The nitrogen of protein and other compounds are transformed into ammonium sulfate by acid digestion with boiling sulfuric acid. The acid digest is cooled, diluted with water, and made strongly basic with sodium hydroxide. The ammonia is released and distilled into a boric acid solution. The ammonia in the boric acid solution is titrated with standardized sulfuric acid.

An alternate procedure is also given that uses standardized acid to collect the ammonia and standardized base to back-titrate the excess acid. Either method will give good results but there is a small saving in reagent costs with the alternate method. However, the boric acid procedure saves considerable time since the boric acid does not have to be standardized or measured accurately and there is no problem of absorbing all of the ammonia distilled over with high nitrogen samples.

Equipment

(a) Macro Kjeldahl nitrogen digestion and distillation apparatus.

(b) Kjeldahl flasks, 650 ml.

(c) Erlenmeyer flasks, 500 ml.

(d) Two burettes.

Reagents

(a) Indicator solution (0.1% methyl red and 0.2% bromocresol green in alcohol).

(b) Standardized sulfuric acid solutions, 0.1 N. Standardize acid against tris(hydroxymethyl) aminomethane, also known as 2-amino-2-(hydroxymethyl)-1,3-propanediol, which has an equivalent wt of 121.14 g. A 0.3029 g sample dissolved in water is equivalent to 25 ml of 0.10 N H_2SO_4. Use the indicator solution under (a). Make at least 3 determinations and use the average normality of the three.

(c) Concentrated sulfuric acid, 93-98%, reagent grade.

(d) Catalyst mixture. Mix 7% reagent grade fine crystalline copper sulfate with reagent grade potassium sulfate (premeasured catalyst packages are also available).

(e) Zinc, mossey.

(f) Sodium hydroxide solution, nitrogen-free (dissolve 450 g sodium hydroxide flakes per liter of solution). Usually 18 to 25 liters are mixed at a time. Mix in a large metal container by alternately adding small amounts of water and sodium hydroxide. Stir with metal or glass rod until completely dissolved after each addition of sodium hydroxide. Let stand over night to cool then transfer by using a porcelain pitcher to a polyethylene storage bottle.

(g) Boric acid solution, 4%. Dissolve 40 g boric acid reagent per liter and add 5 ml indicator solution.

Alternate Procedure Reagents

(a) All reagents as described in (b) through (f) above.

(b) Sodium hydroxide solution, 0.1 N. Add 4 g of NaOH (reagent grade) per liter of distilled water (free from CO_2). Standardize the sodium hydroxide solution against potassium biphthalate which has an equivalent wt of 204.228 g. A 0.5106 g sample dissolved in water is equivalent to 25 ml of 0.10 N NaOH.

(c) Methyl red indicator (0.2% methyl red in 95% ethyl alcohol).

Procedure

(a) Weigh by difference a sample to contain approximately 25 to 50 mg N. For feed samples this is 1.5 to 2.0 g. For fresh feces this is 4 to 6 g. For fresh urine use 5.0 ml, but weigh the material because urines vary considerably in specific gravity. Place a folded filter paper in a small funnel set in a small bottle. Place solid samples in the filter paper. Twist the top of the filter paper around the sample and introduce into a Kjeldahl flask.

(b) Run two reagent blanks (with filter paper) through all the steps of the procedure and subtract the blank titration from the sample titrations. Usually the average of two blanks are sufficient for a day's run.

(c) Add 10 g of the catalyst mixture with a small measuring scoop followed by 25 ml of concentrated sulfuric acid. Increase the amount of sulfuric acid used by 10 ml for each gram of organic matter in sample above 2 g (for samples very low in nitrogen).

(d) Place the flask on the digestion heaters of the Kjeldahl apparatus and turn on heat and exhaust fan. Watch the digestion process until frothing ceases. If the froth of a sample starts up the neck of the flask remove it from the heat to allow froth to subside and return

to heater. Frothing is common with liquid samples. Continue digestion, turning flasks occasionally for 30 minutes after the solution clears and all carbon has been oxidized. Turn off the heat and allow the flasks to cool on the heaters until fuming ceases; then remove the flasks and continue cooling on a rack.

(e) Before the digest solidifies, carefully add 250 ml cold tap water while cooling the flask under running cold water. If the digest cooled to a solid state dissolve the solids by swirling before continuing the procedure.

(f) Add 50 ml of the boric acid solution to the 500 ml Erlenmeyer flasks and set them under the condensers with the tips beneath the surface of the solution (see alternate procedure below).

(g) Turn on the water for the condenser. Turn on the heaters on the distillation rack so that they are hot when the distillation begins. This prevents the boric acid from being drawn up into the distillation flasks.

(h) Carefully add 110 ml of NaOH with the flask tilted so that the reagent runs down the side to the bottom of the solution. (If additional H_2SO_4 was used add 35 ml of NaOH for each 10 ml of H_2SO_4.) Add a few pieces of mossy zinc (1-2 g) and quickly attach the flask to the distillation condenser. With the stopper in place, gently mix the contents of the flask with a rotary motion. The copper ions will form a dark blue copper ammonium complex that indicates that enough NaOH was added to neutralize the excess sulfuric acid and release the ammonia.

(i) Distill about two-thirds of the liquid in the flask or until about 200 ml of liquid are contained in the receiving flask. Lower the

receiving flasks and allow the condensers to drain for five minutes while the Kjeldahl flasks are cooling on the distillation heaters. Rinse the bottom of the condensers with neutral water (titrated with indicator and sulfuric acid).

(j) Titrate the ammonia with standardized 0.1 N sulfuric acid to a faint purple or colorless endpoint.

(k) Empty the Erlenmeyer flasks and let drain upside down on a rack. They are then ready for the next run without washing.

(l) Drain Kjeldahl flasks through a screen to collect the zinc. (The undissolved pieces of zinc can be reused.) Rinse in tap water and drain upside down on a rack. They are then ready for the next run without washing.

Alternate Procedure

(a) Weigh sample, digest and dilute digest as in (a) through (c) above.

(b) Add from a burette or volumetric pipet exactly 50 ml of standardized 0.1 N sulfuric acid to the 500 ml Erlenmeyer flasks (rather than the boric acid) and set them under the condensers as in (f) above. Continue the procedure through the distillation as in (g), (h) and (i) above.

(c) Add 5 drops of the methyl red indicator solution. Back-titrate the sulfuric acid that has not been neutralized by ammonia with the standardized 0.1 N sodium hydroxide solution. (If the back-titration is zero then the sample contained too much ammonia for 50 ml of 0.1 N H_2SO_4 to absorb. Repeat the determination with a smaller sample or larger volume of standardized sulfuric acid.)

(d) Continue the procedure as in (k) and (l) above.

Calculations

(a) Calculate the % nitrogen of sample when using boric acid as follows:

$$\text{Nitrogen \% of sample} = \frac{\left(\left(\begin{matrix}\text{ml acid}\\ \text{titrated}\end{matrix} - \begin{matrix}\text{ml blank}\\ \text{titration}\end{matrix}\right)(\text{acid N})\right) \times 0.014 \times 100}{\text{wt of sample in g}}$$

(b) Calculate the % nitrogen of sample when using sulfuric acid to collect the ammonia as follows:

$$\text{Nitrogen \% of sample} = \frac{(\text{ml acid} \times \text{acid N}) - \left(\begin{matrix}\text{ml base}\\ \text{titrated}\end{matrix} \times \text{base N}\right) \times 0.014 \times 100}{\text{wt of sample in g}}$$

(c) To convert % nitrogen to % crude protein:

Crude protein = N % x 6.25

(d) Adjust % crude protein to dry basis:

$$\frac{\text{Protein \% on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

$$\frac{\text{Protein \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

Scales, F. M., and A. P. Harrison. 1920. Boric acid modification of the Kjeldahl method for crop and soil analysis. J. Ind. Eng. Chem. 12:350-352.

ORGANIC MATTER

Organic matter is the material which burns in the presence of oxygen. The ash or mineral matter remains.

If the percent ash is determined on the sample, the organic matter may be calculated by difference.

$$\text{\% organic matter on a dry basis} = \text{\% dry matter} - \text{\% ash on dry basis}$$

DETERMINATION OF CELL CONTENTS

Principle

The more readily utilizable nutrients are enclosed by the cell wall and can be grouped as cell contents. These include the protein, soluble carbohydrate, soluble minerals and lipids. A high percentage of cell contents is a good index of high nutritive value of a feed. The cell content is determined as the difference between the % cell walls and 100%.

Calculation

Cell contents (%) on dry basis = 100% - cell walls (%) on dry basis.

Example:

If the % cell walls on the dry basis is 59.3% then:

cell contents (%) on dry basis = 100% - 59.3% = 40.7%.

Adjusting to as fed basis:

$$\frac{\text{constituent on dry basis (\%)}}{100} \times \frac{\text{as fed dry matter \%}}{100} \times 100$$

Example:

If as fed dry matter = 89.3%, then:

cell contents (%) on as fed basis = $\frac{40.7\%}{100} \times \frac{89.3\%}{100} \times 100 =$

$0.407 \times 0.893 \times 100 = 36.3\%$.

Reference

Van Soest, P. J. 1967. Development of a comprehensive system of feed analysis and its application to forages. J. Animal Sci. 26:119.

DETERMINATION OF CELL WALLS (NEUTRAL DETERGENT FIBER) AND CELL CONTENTS[a]

Principle

The neutral-detergent procedure for cell walls (CW) is a rapid method for the total fiber in fibrous plant feedstuffs. It appears to divide the dry matter of feeds very near the point which separates the nutritively available and soluble constituents from those which are incompletely available or dependent on a microbial fermentation. This method cannot be used with feeds that have a high protein and low fiber content.

Equipment

(a) Refluxing apparatus. Use any conventional apparatus suitable for crude fiber determinations. Berzelius beakers (600 ml) and condensers made from 500 ml round flasks are preferred.

(b) Scintered glass crucibles. Use tall form, coarse porosity with plate 40 mm in diameter, and large enough to contain 40-50 ml of liquid.

(c) Cleaning of crucibles. After much use, crucibles tend to become clogged with residual matter, resistant to the commonly used chromic acid filtration. A reliable cleaning procedure is to ash at 500° C (if not already ashed) and force water in reverse flow through the filter plate. When crucibles become clogged with mineral particles after several uses a hot solution of 20% KOH, 5% Na_3PO_4, and 0.5% disodium ethylenediaminetetra-

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[a]Also see page 2701.

acetate forced through the scintered glass plate in reverse flow will usually be effective. Overuse of the alkaline cleaning solution should be avoided, as it tends to erode the glass.

Reagents

(a) Neutral detergent solution. Add 30 g sodium lauryl sulfate, USP grade, 18.61 g disodium dihydrogen ethylenediaminetetraacetate dihydrate, reagent, 4.56 g disodium hydrogen phosphate reagent, 6.81 g sodium borate decahydrate reagent, and 10 ml 2-ethoxyethanol (ethylene glycol, monoethyl ether), purified grade, per liter of distilled water and agitate to dissolve. Check pH of solution to be within range of 6.9 to 7.0 and adjust as necessary.

(b) Decalin, reagent grade decahydronaphthalene.

(c) Acetone, reagent grade.

(d) Sodium sulfite, reagent grade.

Procedure

(a) Weigh by difference approximately a 1.0 g sample that has been ground to pass through a 1 mm screen and place the sample in a Berzelius beaker for refluxing.

(b) Add, in order, 100 ml cold (room temperature) neutral-detergent solution, 2 ml decahydronaphthalene, and 0.5 g sodium sulfite with a calibrated scoop. Heat to boiling in 5-10 minutes. Reduce heat as boiling begins, to avoid foaming. Adjust boiling to an even level and reflux for 60 minutes, timed from onset of boiling.

(c) Place previously tared crucibles on filtering apparatus. Swirl beaker to suspend solids and fill crucible. Do not admit vacuum until

after crucible has been filled. Use low vacuum at first, increasing it only as more force is needed. Rinse sample into crucible with a minimum of hot (80° C) water. Remove vacuum, break up mat, and fill crucible with hot water. Filter liquid and repeat washing procedure.

(d) Wash twice with acetone in same manner and suck dry. Dry crucibles at 105° C overnight and weigh.

(e) Report yield of recovered neutral-detergent fiber as cell walls.

(f) Estimate cell content (soluble material) by subtracting this value from 100 (see page 2701).

Calculations

Cell walls % on as fed or partial dry basis =

$$\frac{\text{wt of crucible and cell walls - wt of crucible}}{\text{wt of sample}} \times 100$$

Adjusting to dry basis:

(a) $$\frac{\text{cell walls \% on as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$$

or

(b) $$\frac{\text{cell walls \% on partial dry sample}}{\text{dry matter \% of partial dry sample}} \times 100$$

<u>Note</u>: See page 2701 for calculations of cell contents.

Reference

Van Soest, P. J., and R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. The determination of plant cell wall constituents. J. Assn. Official Anal. Chem. 50:50.

DETERMINATION OF ACID DETERGENT FIBER

Principle

The acid-detergent fiber procedure provides a rapid method for lignocellulose in feedstuffs. The residue also includes silica, however. The difference between the cell walls and acid-detergent fiber is an estimate of hemicellulose, although this difference does include some protein attached to cell walls. The acid-detergent fiber is used as a preparatory step for lignin determination. Hemicellulose can be estimated from the difference between cell-wall constituents and acid detergent fiber.

Equipment

(a) Same as used with cell wall analysis, neutral detergent fiber procedure (see page 2801).

Reagents

(a) Acid-detergent solution. Sulfuric acid, reagent grade standardized to 1 N. This is achieved by adding 49.04 g (correct for assay) H_2SO_4 within each liter of distilled water. For 18 liter of solution it requires 882.72 g of H_2SO_4. Add 20 g of cetyl trimethylammonium bromide (CTAB), technical grade to one liter of 1 N H_2SO_4 solution, or 360 g per 18 liters. Stir to facilitate solution.

(b) Decalin, technical grade dicahydronaphthalene.

(c) Acetone, reagent grade.

(d) Hexane, reagent grade.

Procedure

(a) Weigh by difference approximately 1 g of sample into a beaker or container suitable for refluxing.

(b) Add 100 ml cold (room temperature) acid-detergent solution and 2 ml decahydronaphthalene. Heat to boiling in 5 to 10 min. Reduce heat to avoid foaming as boiling begins. Reflux 60 min. from onset of boiling, adjusting boiling to a slow, even level.

(c) Filter on a previously tared crucible, which is set on the fiber manifold, using light suction. Break up the filtered mat with a rod and wash twice with hot water (90-100° C). Rinse sides of the crucible in the same manner.

(d) Repeat wash with acetone until it removes no more color, breaking up all lumps so that the solvent comes into contact with all particles of fiber.

(e) Wash with hexane. Hexane should be added while crucible still contains some acetone. (Hexane can be omitted if lumping is not a problem.) Suck the acid-detergent fiber free of hexane and dry at 105°C 8 hr. or overnight, cool in a desiccator to room temperature and weigh.

Calculations

Acid detergent fiber % on partial dry or as fed basis =

$$\frac{(\text{wt of crucible + fiber - tare wt of crucible}) \times 100}{\text{wt of sample}}$$

Adjusted to dry basis =

(a) $$\frac{\text{Acid detergent fiber \% on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{Acid detergent fiber \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46(5):829.

DETERMINATION OF ACID DETERGENT LIGNIN

Principle

The acid-detergent lignin procedure utilizes the acid-detergent fiber procedure as a preparatory step. The detergent removes the protein and other acid-soluble material which would interfere with the lignin determination. The principle of the procedure is that the acid detergent fiber residue is primarily lignocellulose of which the cellulose is dissolved by the 72% H_2SO_4 solution. The remaining residue consists of lignin and acid-insoluble ash; however, with samples containing large amounts of cutin this also is measured as part of the lignin.

Equipment

(a) All equipment required for determination of cell walls (page 2801).

(b) Glass tray.

(c) Muffle furnace, temperature controlled at 500° C.

Reagents

(a) Sulfuric acid, 72%. Calculation of g acid and water needed in 1 liter of solution:

$$\frac{100 \times 98.08 \text{ molecular weight} \times 12 \text{ moles}}{H_2SO_4 \text{ assay } (\%)} = \text{g } H_2SO_4 \text{ needed.}$$

$1000 \times 1.634^{+} - g\ H_2SO_4 = g\ H_2O$ needed

$^{+}$specific gravity of 72% H_2SO_4.

Weigh the water needed for 1 liter in a 2000 ml beaker. Weigh the acid needed in a separate container. Gradually add the acid to the water with occasional stirring while the solution is being cooled in a cold water bath (sink). Check the specific gravity by measuring a 5 ml aliquot (at 20° C) with a volumetric pipette, placing it in a covered weighing bottle and weighing it on an analytical balance. Adjust the specific gravity to 1.634 at 20° C by adding small measured amounts of water or acid and recheck the specific gravity as before.

Procedure

(a) The first step is to prepare acid-detergent fiber as described on page 3201.

(b) Place the crucibles in the glass tray. Have one end of the tray 2 cm higher so acid will drain away from the crucibles.

(c) Cover the contents of the crucible with cooled (5° C) 72% H_2SO_4 and stir with a glass rod to a smooth paste, breaking all lumps. Fill crucible about halfway with acid and stir. Let glass rod remain in crucible. Refill with 72% H_2SO_4 and stir at hourly intervals as acid drains away. Crucibles do not need to be kept full at all times. Three additions suffice.

(d) Keep crucible 20-23° C.

(e) After 3 hours, filter off as much acid as possible with vacuum. Rinse the residue once with 72% acid and filter it off.

(f) Wash contents with hot water (85-95° C) until free from acid.

Rinse and remove stirring rod.

(g) Dry crucible overnight at 100° C and weigh.

(h) Ignite crucible in a muffle furnace at 500° C for 3 hours. Cool to 250° C and transfer to a desiccator. Cool to room temperature and weigh.

Calculations

Lignin (%) on as fed or partial dry basis =

$$\frac{(\text{wt of crucible and lignin - wt of crucible and ash})}{\text{wt of sample}} \times 100$$

Adjusting to dry basis:

(a) $\frac{\text{lignin \% on as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$

(b) $\frac{\text{lignin \% on partial dry sample}}{\text{dry matter \% of partial dry sample}} \times 100$

Reference

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46:829.

DETERMINATION OF PERMANGANATE LIGNIN, CELLULOSE AND SILICA (INSOLUBLE ASH)

Principle

An indirect method for lignin utilizing permanganate allows the determination of cellulose and insoluble ash in the same sample. The insoluble ash is an estimate of silica content, which in many grasses is a primary factor in reducing digestibility. The permanganate lignin method is an alternative procedure to the 72% sulfuric acid method; each has its own advantages. Choice of methods will depend on materials analyzed and on the purpose for which the values are to be used. Advantages of the permanganate method over the 72% acid method include (1) a shorter procedure, (2) the permanganate reagents are much less corrosive and require no standardization, and (3) values are less affected by heat damage artifacts and are closer to a true lignin figure.

However, cutin, which is important in many seed hulls, is not measured. A variation for the analysis of seed hulls is to prepare the permanganate cellulose and treat with 72% sulfuric acid and asbestos for 3 hours. This will result in the partitioning of crude lignin into two fractions as described below.

One disadvantage of the permanganate method is that large particles are poorly penetrated by the reagents and yield low values. Consequently materials high in water content must be partially dried and

ground through a 1 mm screen, and the method is not applicable to fresh feces and forages which have been ground in a meat grinder. Because of higher sensitivity to heat damage, 72% acid is preferred for assaying artifact lignin.

Theory of the method: Interfering matter is removed by preparing acid-detergent fiber, which is chiefly composed of lignin, cellulose, and insoluble minerals. Lignin is oxidized with an excess of acetic acid-buffered potassium permanganate solution, containing trivalent iron and monovalent silver as catalysts. Deposited manganese and iron oxides are dissolved with an alcoholic solution of oxalic and hydrochloric acids leaving cellulose and insoluble minerals. Lignin is measured as the weight lost by these treatments, while cellulose is determined as the weight loss upon ashing. The ash residue is mainly silica and much of the non-silica matter can be removed by leaching with concentrated hydrobromic acid.

Equipment

(a) Same as for acid detergent lignin (page 3301).

Reagents

(a) Saturated potassium permanganate. Dissolve 50 g reagent-grade $KMnO_4$ per liter of distilled water, 900 g $KMnO_4$ per 18 liters of distilled water. Keep out of direct sunlight.

(b) Lignin buffer solution. For one liter dissolve 6.0 g ferric nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$) and 0.15 g silver nitrate in 100 ml distilled water. Combine with 500 ml glacial acetic acid and 5.0 g potassium acetate (reagent grades). Add 400 ml tertiary butyl alcohol

and mix (reagent grade). For 12 liters use the following amounts of chemicals:

ferric nitrate nonahydrate	72 g
silver nitrate	1.8 g
acetic acid	6.0 liter
potassium acetate	60 g
t-butyl alcohol	4.8 liter
distilled water	1.2 liter

(c) Combined permanganate solution. Combine and mix saturated potassium permanganate and the lignin buffer solution in the ratio of 2:1 by volume before use. Unused mixed solution may be kept about a week in a refrigerator in the absence of light. Solution is usable if it is purple in color and contains no precipitate.

(d) Demineralizing solution. For one liter, dissolve 50 g oxalic acid dihydrate in 700 ml 95% ethyl alcohol. Add 50 ml concentrated (approximately 12 N) hydrochloric acid and 250 ml distilled water and mix. For 18 liters use the following amounts of chemicals:

oxalic acid	900 g
95% ethanol	12.6 liter
concentrated hydrochloric acid	900 ml
distilled water	4.5 liter

(e) Ethyl alcohol 80%. For one liter mix 155 ml distilled water and 845 ml 95% ethyl alcohol. For 18 liters, mix 2.8 liters distilled water and 15.2 liters 95% ethyl alcohol.

(f) Hydrobromic acid, reagent grade.

Procedure

(a) The residue from the acid-detergent fiber determination may be used (use original weight of sample). If this residue is not used, proceed as outlined under (b), (c), and (d).

(b) Weigh approximately a 1.0 g sample that has been ground through a 1 mm screen. On sample containing over 15% lignin, use 0.5 g.

(c) Place crucible in a muffle furnace at 500° C for an hour or longer. Cool in a desiccator to room temperature and weigh.

(d) Prepare acid detergent fiber according to the procedure outlined on page 3201.

(e) Place crucibles containing the acid detergent fiber in a shallow glass tray containing approximately 1 cm cold water. Fiber in crucibles should not be wet.

(f) Combine and mix saturated potassium permanganate and buffer solution 2:1 by volume, and add approximately 25 ml to the crucibles. Do not overfill. Adjust level of water in pan to reduce flow of solution out of crucibles. Place a short glass rod in each crucible to stir contents, to break lumps, and to draw permanganate solution up on sides of crucibles to wet all particles.

(g) Allow crucibles to stand 90 ± 10 minutes at a temperature of 20 to 25° C, adding more mixed permanganate solution if necessary. Purple color must be present at all times.

(h) Remove crucibles to filtering apparatus and filter off visible liquid. Do not wash. Place in a clean enamel or glass pan, and fill crucibles no more than half full with demineralizing solution. After about 5 minutes, filter off visible liquid and refill halfway with demineralizing solution. Care must be taken to avoid spillage by foaming. Repeat the demineralizing solution treatment a third time if the filtrate from the second treatment is very brown. Rinse down the sides of the crucibles with a fine stream of demineralizing solution from a squeeze bottle. Treat until fiber is white. Total time required is 20 to 30 minutes.

(i) Fill and thoroughly wash crucible and contents with 90% ethyl alcohol, filter off liquid and repeat wash two times. Wash and filter twice in similar manner using acetone.

(j) Lignin content. Dry at 105° [illegible] overnight, cool in a desiccator and weigh. Calculate lignin content as loss in weight from acid-detergent fiber.

(k) Cellulose content. Ash at 600° [illegible] for 3 hours, cool in a desiccator and weigh. [illegible]

[illegible]

obtained [illegible] hydrogen [illegible] with acetone [illegible] cool in a desiccator [illegible] ash [illegible] less than [illegible] of the original [illegible]

Irregularity in crucible, combustion tube or [illegible] will require [illegible] more solution than is necessary. Appearance of a yellow or brown color indicates exhaustion of permanganate. If crucible is full, [illegible] solution off on a vacuum and add more reagent. A yellow color persisting after treatment of fiber with decolorizing [illegible] incomplete removal of lignin. This will occur only on material of a very high lignin content. Cutin material present in seed coats or other plant parts is not oxidized by permanganate and thus [illegible] determined as lignin nor [illegible] with the treatment. [illegible] will appear as colored flecks among white cellulose particles [illegible] should not be confused with incomplete oxidation.

Excessive flow of permanganate solution through the [illegible] fiber

[illegible]

should be avoided with samples of low lignin content, particularly in the case of immature grasses. Here a single addition of permanganate solution suffices. Fiber from immature grasses is very rapidly delignified and there is danger in these materials of loss of cellulosic carbohydrates if there is too much flow. Reduction in flow is accomplished by adjusting pan water level to near that in the crucibles. These precautions are not needed with the demineralizing solution.

Calculations

Lignin % on partial dry or as fed basis =

$$\frac{(\text{wt acid detergent fiber} - \text{wt permanganate fiber residue})}{\text{wt of sample before determination of acid detergent fiber}} \times 100$$

Cellulose % on partial dry or as fed basis =

$$\frac{(\text{wt crucible and permanganate fiber residue} - \text{wt crucible and ash})}{\text{wt of sample before determination of acid detergent fiber}} \times 100$$

Silica % on partial dry or as fed basis =

$$\frac{(\text{wt of ash after hydrogen bromide washing})}{\text{wt of sample before determination of acid detergent fiber}} \times 100$$

Adjusting to dry basis:

(a) $\frac{\text{analysis \% on as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$

or

(b) $\frac{\text{analysis \% on partial dry sample}}{\text{dry matter \% of partial dry sample}} \times 100$

Reference

Van Soest, P. J., and R. H. Wine. 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. J. Assoc. Official Anal. Chem. 51:780.

PREPARATION OF ASH SOLUTION FOR THE DETERMINATION OF CALCIUM, MAGNESIUM AND PHOSPHORUS

Principle

The ash which is soluble is put into solution in an acid medium. The insoluble part is filtered off.

Equipment

(a) Hot plate

Reagents

(a) Concentrated HCl

Procedure

(a) Add 5 ml of concentrated HCl acid to the porcelain crucibles containing the ashed sample which was saved from the determination of ash procedure.

(b) Add about 20 ml of distilled water and place on 100° C hot plate. Evaporate the samples to about 10 ml.

(c) Add 10 ml distilled water and heat to around 90° C. Cool and filter the samples through Sharkskin, S & S, filter paper (filter paper of low ash content) in long stem funnels into 100 ml volumetric flasks. Rinse crucible and filter paper with distilled water.

(d) Make the solutions to volume with distilled water.

(e) Save for determinations of calcium, magnesium, and phosphorus.

References

Easley, J. F., McCall, J. T., Davis, G. K. and Shirley, R. L. 1965. Analytical methods for feeds and tissues. Nutrition Laboratory, Department of Animal Science, University of Florida.

Snell, Foster Dee and Cornelia T. Snell. 1949. Colorimetric Methods of Analysis. 3rd Edition, Vol. II. D. Van Nostrand Company, Inc., Princeton, New Jersey.

DETERMINATION OF CALCIUM

Principle

Ethylenediaminetetraacetic acid (EDTA) forms, in basic solution a soluble and slightly ionized complex with calcium and magnesium as well as many other metals. When the magnesium ion is present, a good end point is observed with Eriochrome Black T indicator. Calcium is complexed by EDTA before magnesium. So, an unknown calcium solution containing a known amount of magnesium may be titrated with EDTA to determine the calcium present. Interference by other ions must be eliminated.

Equipment

(a) Micro burettes

Reagents

(a) Buffer solution. Mix 67.5 g of ammonium chloride with 570 ml of concentrated ammonium hydroxide and dilute to 1 liter.

(b) Indicator. Dissolve 0.25 g of Eriochrome Black T in 50 ml of diethanolamine.

(c) Magnesium sulfate solution. Dissolve about 2.0 g of $MgSO_4 \cdot 7H_2O$ in a liter of water. An exact EDTA titer must be determined by titrating the magnesium sulfate solution with the EDTA solution. (1 ml of magnesium sulfate solution needs to be evaluated in regard to how many ml of the EDTA solution it is equivalent to.)

(d) EDTA solution. Dissolve 6.65 g of reagent grade disodium ethylene-diaminetetraacetic (EDTA) in sufficient water to make 1 liter of solution. (1.0 ml of EDTA needs to be evaluated in regard to how many mg of calcium it is equivalent to.)

(e) Saturated ammonium exalate.

(f) Concentrated hydrochloric acid.

(g) Methyl red indicator. 0.1% alcoholic solution. Dissolve 0.1 g in 95% ethyl alcohol and dilute to 100 ml.

(h) Calcium standard (one mg Ca per ml). Dissolve 2.4973 g pure calcium carbonate in about 100 ml of dilute HCl. Dilute to exactly 1 liter with water.

(i) Ammonium hydroxide, 1%. Dilute 10 ml of reagent NH_4OH to 1 liter with water.

Procedure+

(a) Pipette an aliquot of the ash solution containing from 0.05 mg to 1.5 mg of calcium into a 15 ml conical-tipped centrifuge tube containing 3 ml of saturated ammonium oxalate.

(b) Add 1 drop of methyl red indicator and adjust the pH to 5.0-5.5 (faint pink color of indicator) using a dilute solution of hydrochloric acid or ammonium hydroxide. Mix the contents thoroughly, let stand for one hour and centrifuge for five minutes at 3,000 rpm.

(c) Carefully pour off the supernatant liquid, resuspend the precipitate and wash the sides of the tube with about 3 ml of 1% ammonium hydroxide, centrifuge, again pour off the supernatant liquid.

+See page 3651 for preparation of ash solution.

(d) Dissolve the precipitate in 0.5 ml of concentrated hydrochloric acid. Wash the sample with distilled water into a 100-ml beaker. Dilute to about 30 ml, add 5.0 ml of the buffer and several drops of the Eriochrome Black T indicator.

(e) Add 0.5 ml of the magnesium sulfate solution from a burette. The color should be wine red.

(f) Add EDTA solution from a burette until a true blue color is obtained, then add 0.1-0.5 ml extra. Back titrate with the magnesium sulfate solution until the very first wine red tinge appears in the blue solution.

(g) If a clear end point is not obtained, add about 0.25 g of sodium cyanide to the sample after the buffer has been added.

Calculations

Calcium (%) on either partial dry or as fed basis =

$$\frac{\text{calcium titer of EDTA [ml EDTA - (Mg titer of EDTA x ml } MgSO_4\text{)]}}{\text{ml aliquot of ash solution x wt of sample before ashing in g}} \times \frac{100}{100} \times 1000$$

or

$$\frac{\text{calcium titer of EDTA [ml EDTA - (Mg titer of EDTA x ml } MgSO_4\text{)]}}{\text{ml aliquot of ash solution x wt of sample before ashing in g}} \times 10$$

Adjusting to dry basis:

(a) $\frac{\text{calcium \% on as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$

or

(b) $\frac{\text{calcium \% on partial dry sample}}{\text{dry matter \% of partial-dry sample}} \times 100$

Reference

Easley, J. F., J. T. McCall, G. K. Davis and R. L. Shirley. 1965. Analytical methods for feeds and tissues. Nutrition Laboratory, Department of Aniaml Science, University of Florida.

Welcher, Frank J. 1957. The analytical uses of ethylenediaminetetraacetic acid. D. Van Nostrand Company, Inc., Princeton, New Jersey.

DETERMINATION OF MAGNESIUM

Principle

Ethylenediaminetetraacetic acid (EDTA) forms, in basic solution, a soluble and slightly ionized complex with calcium and magnesium as well as many other metals. All calcium ions are complexed before the magnesium ions. When using Eriochrome Black T indicator, a good end point is observed in the presence of magnesium ions. The calcium is determined as in the calcium procedure, and the equivalent amount of EDTA is subtracted leaving the amount due to magnesium ions. Using the magnesium equivalent of the EDTA solution titer, the milligrams of magnesium in sample may be calculated.

Equipment

(a) Micro burettes

Reagents

(a) Buffer solution. Mix 67.5 g of ammonium chloride with 570 ml of concentrated ammonium hydroxide and dilute to 1 liter.

(b) Indicator. Dissolve 0.25 g of Eriochrome Black T in 50 ml of diethanolamine.

(c) Magnesium sulfate solution. Dissolve about 2 g of $MgSO \cdot 7H_2O$ in a liter of water. The EDTA equivalent of this solution must be determined by titration with the EDTA solution. (1 ml of magnesium

sulfate solution needs to be evaluated in regard to how many milliliters of EDTA solution it is equivalent to.)

(c) Magnesium standard. One milligram per ml. Dissolve 8.3606 g of reagent grade magnesium chloride 6-hydrate in a small volume of water and dilute to 1 liter with water.

Procedure+

(a) Pipette an aliquot of the ash solution containing 0.02-1.5 mg of magnesium into a 100 ml beaker and dilute to about 30 ml with distilled water.

(b) Add 5 ml of the buffer solution and a few drops of the indicator solution.

(c) Add 0.2-0.5 ml of magnesium sulfate solution and titrate to a true blue color with the EDTA solution.

(d) Add 0.2-0.5 ml extra EDTA solution to the first faint wine-colored tinge in the blue solution.

(e) If a clear end point is not obtained, add about 0.25 g of sodium cyanide to the sample after the buffer has been added. Clean equipment with care so that it does not contaminate other equipment or yourself. If interference persists, see reference below.

+ See page 3651 for preparation of ash solution.

Calculations

Magnesium (%) partial dry or as fed basis =

$$\frac{\text{magnesium equivalent of EDTA solution}\left(\text{ml EDTA titrated} - \text{ml EDTA equivalent of calcium in aliquot} - \text{ml EDTA equivalent of } MgSO_4 \text{ added}\right) \times 10}{\text{ml aliquot ash solution} \times \text{wt ashed sample in g}}$$

Adjusting to dry basis:

(a) $\frac{\text{magnesium \% on as fed basis} \times 100}{\text{dry matter \% of as fed sample}}$

or

(b) $\frac{\text{magnesium \% on partial dry basis} \times 100}{\text{dry matter \% of partial dry sample}}$

Reference

Welcher, Frank J. 1957. The analytical uses of ethylenediaminetetraacetic acid. D. Van Nostrand Company, Inc., Princeton, New Jersey.

DETERMINATION OF PHOSPHORUS

Principle

The orthophosphate ion reacts with ammonium molybdate to form a phosphomolybdate compound. The phosphomolybdate compound is reduced to molybdenum blue with 1-amino-2-naphthol-4-sulfonic acid. The blue color formed is in direct proportion to the orthophosphate present.

Equipment

(a) Spectronic "20" spectrophotometer or equivalent.

Reagents

(a) Standard phosphorus solution, 0.1 mg phosphorus per ml. Dissolve 0.4394 g of pure, dry KH_2PO_4 in 300 ml of distilled water and 200 ml of 1 N sulfuric acid. Add a few drops of 2% potassium permanganate as a preservative and dilute with water to 1 liter. Dilute as needed for standards.

(b) Sulfuric acid solution, 10 N. Carefully pour 280 ml of concentrated sulfuric acid into 720 ml water.

(c) Ammonium molybdate reagent. Dissolve 12.5 g of ammonium molybdate in about 100 ml of water. Add the molybdate solution to 150 ml of 10 N sulfuric acid in a 500 ml volumetric flask and dilute to volume with water. Store in refrigerator.

(d) Sodium bisulfite solution, 15%. Dissolve 15 g of sodium bisulfite in water and dilute to 100 ml.

(e) Sodium sulfite, 20%. Dissolve 10 g of anhydrous sodium sulfite in water and dilute to 50 ml.

(f) Amino-naphthol sulfonic acid reagent (ANSA). Weigh 250 mg of reagent grade 1-amino-2-naphthol-4-sulfonic acid. Make a paste with a drop or two of the 15% sodium bisulfite solution. Then add 97.5 ml of the 15% sodium bisulfite solution and 2.5 ml of the 20% sodium sulfite solution. If all the aminonaphthol sulfonic acid does not readily dissolve, add more of the 20% sodium sulfite solution, 0.5 ml at a time, until solution is complete. Do not add an excess. Let stand overnight, then filter through No. 41 Whatman filter paper or equivalent. Store in a brown glass bottle.

Procedure+

(a) Pipette an aliquot of the ash solution containing 0.01 mg to 0.2 mg of phosphorus into a 50 ml volumetric flask. Add about 25 ml of distilled water. Add 5 ml of the ammonium molybdate reagent. Swirl the flask to mix the solution. Add 2 ml of ANSA reagent. Fill to volume with water. Stopper and mix well.

(b) Record the absorbancy of the solution at exactly 20 minutes after addition of the ANSA reagent. Compare the absorbancy to a reagent blank using distilled water in place of sample aliquot. Read the phosphorus concentration by comparing the absorbancy to a previously prepared standard phosphorus curve ranging from 0.01 to 0.25 mg phosphorus per 50 ml.

+ See page 3651 for preparation of ash solution

Calculations

Phosphorus % partial dry or as fed basis =

$$\frac{\text{mg phosphorus in aliquot} \times 10}{\text{ml aliquot ash solution} \times \text{wt ashed sample}}$$

Adjusting to dry basis =

(a) $$\frac{\text{phosphorus \% on as fed sample} \times 100}{\text{dry matter \% on as fed basis}}$$

or

(b) $$\frac{\text{phosphorus \% on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

References

Bolts, D. F., and M. G. Mellon. 1948. Spectrophotometric determination of phosphorus as molybdiphosphoric acid. Anal. Chem. 20:749

Fiske, C. A., and I. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

TRACE ELEMENT ANALYSIS

In the determination of trace elements (usually considered less than 0.01%) certain precautions should be taken. Glassware as free as possible from traces of the element desired should be used. In the case of ashing crucibles, this means using Vycor brand, which is 96% silica glass, the remainder being boric oxide, aluminum, sodium, iron, and arsenic. This is comparable to fused quartz in thermal and chemical resistance. It is very stable with acids and mild alkalies. All other glassware used should be of Pyrex brand quality. This is a low-alkali-content, boro-silicate glass. It is free from magnesia-lime-zinc group elements, heavy metals, arsenic, and antimony. For boron determinations, use alkali-resistant glass which is free from boron.

All glassware used must be washed well with a detergent, rinsed with tap water, rinsed with 10% hydrochloric acid solution and then rinsed with triple distilled water.

Special care should be taken in preparation of samples if the trace metals are to be determined. Samples prepared for proximate analyses are frequently ground in Wiley mills, generally equipped with bronze sieves. The bronze sieves have been shown to contribute significant quantities of trace metals to the ground material. Stainless steel sieves are available for the Wiley mill which should reduce contamination and should be used when trace metals are to be determined on the ground sample. If stainless steel sieves for the grinding mill are not available,

stainless steel scissors can be used with certain types of forages for cutting or chopping the sample in lieu of grinding. In this case, subsamples of greater weight than those usually taken can be used to aid in obtaining representative material if the sample is cut rather than ground. If at all possible, it should be established that the preparation procedure being utilized does not contaminate the samples with metals to be determined.

Care must also be taken to prevent gross contamination of the sample through accidental introduction of metal from common articles of the laboratory -- orin from ring stands, nickel from crucible tongs, copper and zinc from burners, zinc from rubber, etc.

Water used in trace mineral analysis should be glass distilled.

A blank should be carried through all the steps of the determination proper. This takes account of the amounts of constituent contributed by the vessels and by the reagents. A zero blank is not necessarily an indication that all is well. It may mean that a foreign substance in the reagent is combining with the desired constituent and preventing its reaction with the color reagent in colorimetric analysis.

Dilute solutions of metal ions may undergo a serious loss in strength on standing as a result of absorption on, or cation exchange with, the glass container. The loss is greatest in neutral or slightly basic medium, but may occur as well in slightly acidic solution. The weaker the solution, the greater will be the relative decrease in concentration. Therefore, all standards and solutions should be stored in polyethylene containers. Solutions in organic solvents should never be stored in polyethylene bottles.

Fungus growth may remove metals from solution.

Standard solutions may be prepared from pure metals or from pure salts of definite composition. If a good grade of "reagent-quality" salt is available, it may, as a rule, be used without further purification for the purposes of colorimetry. Whenever possible, anhydrous salts are used. These should be powdered and dried at 100° C or higher to remove most of the vacuole water. Hydrated salts may, in many instances, be used with satisfaction if they are not deliquescent or markedly efflorescent. The crystals should be large enough to permit the picking out of clear uneffloresced individuals. Such air-dry crystals may contain a few parts per thousand of foreign water but this will rarely matter in trace analysis.

In practically all cases the organic material must be destroyed before trace analysis. This may be done by dry ashing in a muffle furnace at 500-600° C or by wet digestion with acids.

References

Easley, J. R., J. T. McCall, G. K. Davis and R. L. Shirley. 1965. Analytical methods for feeds and tissues. Nutrition Laboratory, Department of Animal Science, University of Florida, Gainesville, Florida.

Sandell, E. B. 1959. Colorimetric Determination of Traces of Metals. 3rd Edition. Interscience Publishers, Inc., New York, New York.

DETERMINATION OF COBALT

Principle

Cobalt is extracted from ash solution with dithizone. Cobalt forms a red complex with sodium 1-nitroso-2-naphthol-3, 6-disulfonate, nitroso-R salt, which is stable in boiling nitric acid. This last property makes possible a determination that is nearly specific for cobalt. Three moles of reagent combine with one of cobalt. This reaction furnishes a most sensitive method for the determination of traces of cobalt such as occur in soils, plants, and animal organs.

Equipment

(a) Spectronic "20" or equivalent spectrophotometer.

(b) 5 liter separatory funnel

Reagents

(a) Nitroso-R salt (sodium 1-nitroso-2-hydroxynaphthalene-3, 6-disulfonate), 0.2% aqueous solution. This solution undergoes no change for months if kept in the dark.

(b) Standard cobalt stock solution, 0.0100% cobalt. Dissolve 0.040 grams of $CoCl_2 \cdot 6H_2O$ in water, add 1 ml of concentrated HCl and dilute to 100 ml. From this solution prepare a more dilute one (e.g., 10 micrograms of cobalt per milliliter).

(c) Citric acid, 0.20 M. Dissolve 4.2 g of the monohydrate in water and dilute to 100 ml.

(d) Buffer solution. Use 6.2 g of boric acid, 35.6 g of disodium

phosphate dihydrate, and 500 ml of 1.00 N sodium hydroxide in a total volume of 1 liter.

(e) Dithizone. Dissolve 0.5 g dithizone in 600-700 ml CCl_4. Filter into 5 liter separatory funnel containing 2.5-3.0 liters of 0.02 N NH_4OH, shake well, and discard CCl_4 layer. Shake with 50 ml portions of high grade CCl_4 until CCl_4 phase, as it separates, has pure green color. Add 1 liter of CCl_4 and acidify slightly with HCl (1 + 1). Shake the dithizone into CCl_4 layer and discard aqueous layer. Store in cool, dark place, preferably in refrigerator.

(f) Ammonium citrate solution, 40%. Dissolve 800 g of citric acid in 600 ml of water and, while stirring, add slowly 900 ml NH_4OH. Adjust pH to 8.5, if necessary. Dilute to 2 liters and extract with 25 ml portions of dithizone solution until aqueous phase stays orange and CCl_4 remains predominantly green. Then extract solution with CCl_4 until all orange color is removed.

(g) Hydrogen Peroxide, 30%.

(h) Concentrated sulfuric acid.

(i) Concentrated nitric acid.

(j) Concentrated hydrochloric acid.

(k) Phenolphthalein, 1% alcoholic solution. Dissolve 1 gram in 95% ethyl alcohol and dilute to 100 ml.

(l) Ammonium hydroxide (1 + 1). Distill concentrated NH_4OH into equal volume of water.

(m) HNO_3 + H_2SO_4 solution. Combine 45 ml concentrated nitric acid and 30 ml concentrated sulfuric acid.

Procedure

See instructions on how to prepare samples (page 4101). Weigh about 6 g sample (or larger amount) into a Vycor crucible. Ash at 500° C overnight. Cool. Dissolve ash with 3 ml concentrated HCl and add 25 ml water. Evaporate two-thirds of solution. Add 15 ml water. Evaporate two-thirds of solution. Add 15 ml water. Evaporate one-half of solution. Filter into 25 ml volumetric flask and make to volume. Transfer all or part of this solution to a 150 ml separatory funnel. Add 5 ml of the ammonium citrate solution and one drop phenolphthalein solution; adjust to pH 8.5 with NH_4OH (1 + 1). If precipitate forms, add additional ammonium citrate. Add 10 ml of the dithizone in CCl_4 and shake the solution five minutes. Drain CCl_4 phase into 100 ml beaker. Repeat as many times as necessary, using 5 ml quantities of dithizone solution and shaking five minutes each time. Extraction is complete when aqueous phase remains orange and CCl_4 phase remains predominantly green. Then add 10 ml CCl_4, shake five minutes, and combine with CCl_4 extract. Final 10 ml CCl_4 should be pure green. If not, extraction was incomplete and must be repeated. Add 2 ml of HNO_3-H_2SO_4 solution and evaporate just to dryness. Cool. Add 20 ml water. Add 2 ml 30% H_2O_2 until solution is clear. Evaporate solution to 5 ml volume. Add 1 ml of citric acid solution and 1.2 ml of phosphate-boric acid buffer. The pH should be 8.0; if not, add more buffer until pH 8.0 is reached. Add exactly 0.5 ml of nitroso-R salt solution while stirring. Boil for one minute, add 1 ml of concentrated nitric acid, and again boil for exactly one minute. Cool immediately in the dark and make up to 10 ml after transferring to volumetric flask. Observe the optical density of the

solution at 420 millimicrons. Compare the observed optical density to a standard cobalt curve ranging from 0 to 10 microgram cobalt per 10 ml final volume.

Calculations

Cobalt (milligrams per kg) on partial dry or as fed basis =

$$\frac{\text{micrograms cobalt in sample}}{\text{wt of sample}}$$

Adjusting to dry basis

(a) $$\frac{\text{cobalt (milligrams per kg) on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{cobalt (milligrams per kg) on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

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DETERMINATION OF MANGANESE

Principle

The colorimetric determination of manganese by oxidation to permanganate in acid solution is both sensitive and specific. Periodate oxidizes manganous salts smoothly to permanganate. The reaction is as follows:

$$2Mn^{++}+5IO_4^{-}+3H_2O \rightarrow 2MnO_4^{-}+5IO_3^{-}+6H$$

Equipment

(a) Spectronic "20" or other spectrophotometer

(b) Vycor ashing crucibles or equivalent

Reagents

(a) Concentrated H_2SO_4

(b) Concentrated HNO_3.

(c) Phosphoric acid (85%)

(d) Sodium or potassium periodate (meta).

(e) Hydrofluoric acid (48%)

(f) Standard manganese solution. This is prepared by dissolving pure electrolytic manganese metal in dilute nitric acid, boiling out oxides of nitrogen, and diluting to volume. A standard solution may also be obtained by weighing reagent grade, crystalline potassium permanganate, acidifying with sulfuric acid, and reducing with a little sulfite.

Procedure

(a) See instructions on how to prepare samples (page 4100).

(b) Ash sample of plant tissue or biological material of suitable size in a muffle furnace at 500° C., cool.

(c) Add 10 ml of 48% hydrofluoric acid. This will prevent loss of manganese as insoluble silicate. Warm to dissolve, cool, add 10 ml concentrated sulfuric acid and evaporate under a fume hood until fumes of sulfur trioxide come off. Transfer quantitatively to a 100 ml volumetric flask and dilute to volume. The ash may also be taken up directly in 10 ml concentrated nitric acid and diluted to volume.

(d) The solution, ready for analysis, (0.1 to 1.0 mg manganese), should contain 10 ml concentrated sulfuric or 15 to 20 ml nitric acid; and, in addition, 5 to 10 ml of 85% phosphoric acid in a volume of 100 ml. However, if the amount of manganese present is of the order of a few micrograms, make the solution 2 N in sulfuric acid and add 20 mg of silver nitrate, in addition to phosphoric acid.

(e) Add 0.3 grams of potassium meta-periodate, or equivalent amount of sodium meta-periodate for each 100 ml of solution. Heat to boiling with stirring, and keep at or slightly below the boiling point for 10 minutes (or at least one hour for any small amounts of manganese); cool.

(f) Dilute to volume and observe the optical density at 525 millimicrons in a spectrophotometer. Compare the observed optical density to a standard manganese curve ranging from 0.1 to 1.0 mg of manganese per 100 ml.

Calculations

Manganese (milligrams per kg) partial dry or as fed basis =

$$\frac{\text{Milligrams manganese in sample} \times 1000}{\text{wt of sample}}$$

Adjusting to dry basis:

(a) $$\frac{\text{manganese (milligrams per kg) on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{manganese (milligrams per kg) on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$$

References

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DETERMINATION OF CAROTENE IN FEEDS AND FORAGES

Principle

Carotene and other pigments are extracted from plant material by an organic solvent. The carotene is eluted on a chromatographic column which retains other pigments. The carotene concentration is determined by comparing to a standard curve on spectrophotometer.

Equipment

(a) Solvent extraction apparatus. Goldfisch or equivalent.

(b) Spectronic "20" Spectrophotometer or equivalent.

(c) Chromatographic tubes. 20 x 400 mm, with fritted glass filter.

Reagents

(a) Acetone. Dry, alcohol-free.

(b) Hexane. B. P. 60-70° C.

(c) Activated Magnesia. SeaSorb 43, Fisher Scientific Company or equivalent.

(d) Diatomaceous earth. Hyflo Super-cel, Johns-Manville or Fisher Scientific Company.

Procedure

(a) For hays and dried plants, grind sample through a 1 mm screen.

Weigh by difference 1.000 to 4.000 g depending on amount of carotene in sample in relation to the standard curves and place in flask of extractor (Goldfisch). Add 30 ml acetone-hexane mixture (3 + 7) to flask and reflux one hour on high heat setting. Cool to room temperature.

(b) Filter extract into 100 ml volumetric flask, wash residue, and dilute to volume with hexane (this solution now contains 9% acetone).

(c) For fresh plant materials and silages, cut material finely with scissors or knife. If analysis cannot be performed immediately, blanch in boiling water for 5 to 10 minutes and store in frozen condition. Place weighed sample, 2 to 5 g, in high speed blender; add 40 ml acetone, 60 ml hexane, and 0.1 g $MgCO_3$, and blend for five minutes. Let residue settle and decant into separatory funnel, wash residue with two 25 ml portions of acetone, then with 25 ml of hexane, and combine extracts. Wash acetone from extract with five 100 ml portions of water, transfer upper layer to 100 ml volumetric flask containing 9 ml acetone, and dilute to volume with hexane.

(d) Prepare chromatographic column with 1 + 1 mixture of activated magnesia and diatomaceous earth. To prepare column, add loose adsorbend to 15 cm depth, attach tube to suction flask, and apply full vacuum of water. Use flat instrument (such as inverted cork mounted on a rod) to gently press adsorbent and flatten surface (packed column should be about 10 cm deep). Place 1 cm layer of anhydrous Na_2SO_4 above adsorbent. All materials must be completely dry. If necessary, dry them in 105° C oven.

(e) With vacuum continuously applied to flask, pour extract into column. Use 50 ml acetone-hexane (1 + 9), or slightly more if necessary, to develop chromatogram and wash visible carotenes through adsorbent. Keep top of column covered with layer of solvent during entire operation. Collect entire eluate. (Carotenes pass rapidly through column; bands of

xanthophylls, carotene oxidation products, and chlorophylls should be present in column when operation is complete.)

(f) Transfer eluate, which has been reduced in volume by loss of vapor through water vacuum, to 100 ml volumetric flask, and dilute to volume with acetone-hexane (1 + 9). Determine carotene content of this solution at 436 millimicrons on the spectrophotometer.

(g) A standard curve may be run with a range of 0.0001 to 0.005 mg beta-carotene per ml of acetone-hexane mixture (1 + 9); or 0.01 to 0.5 mg beta-carotene per 100 ml of solution.

Calculations

Carotene (milligrams per kg) on partial dry or as fed basis =

$$\frac{\text{mg carotene in sample} \times 1000}{\text{wt of sample in g}}$$

Adjusting to dry basis:

(a) $$\frac{\text{carotene (milligrams per kg) on as fed basis} \times 100}{\text{dry matter \% of as fed sample}}$$

or

(b) $$\frac{\text{carotene (milligrams per kg) on partial dry basis} \times 100}{\text{dry matter \% of partial dry sample}}$$

Reference

Official Methods of Analysis of the Association of Official Agricultural Chemists. 1960. 9th Edition. Published by the Association of Official Agricultural Chemists, P. O. Box 540, Benjamin Franklin Station, Washington 4, D. C.

COLORIMETRIC DETERMINATION OF CAROTENE AND VITAMIN A IN BLOOD

Principle

Carotene and vitamin A are extracted from the blood with petroleum ether. The vitamin A is determined colorimetrically by the antimony trichloride reaction which produces a vivid blue. The carotene content is determined directly with the aid of a colorimeter.

Equipment

(a) Spectronic "20" spectrophotometer or similar equipment

(b) 50 ml glass-stoppered centrifuge tubes

(c) Supply of nitrogen gas and regulator

Reagents

(a) Antimony trichloride. Dissolve 25 g antimony trichloride in 75 ml chloroform, add acetic anhydride to clear cloudiness, then make to 100 ml with chloroform. Grind antimony trichloride with mortar and pestle to hasten speed of solution. Store in brown glass-stoppered bottle in a desiccator.

(b) Petroleum ether. Use best grade of petroleum ether and redistill if necessary.

(c) Chloroform. Use best grade of chloroform and redistill if necessary.

(d) Ethyl alcohol, 95%.

Procedure

Approximately 25 ml blood is drawn into test tube containing 1 ml lithium citrate (20%) and mix thoroughly by inverting 20 times. Centrifuge for 30 minutes at approximately 2,800 rpm. Pipette 10 ml plasma into 50 ml glass-stoppered centrifuge tube, add 10 ml 95% ethyl alcohol and 15 ml petroleum ether. Shake mixture vigorously for 10 minutes. Pipette 5 ml of the petroleum ether to each of two Spectronic "20" test tubes. Read the optical density at 440 millimicrons using petroleum ether as blank. Evaporate the petroleum ether by placing the tubes in hot water and allowing a stream of nitrogen to blow into the tubes. Prepare a blank containing 1 ml of chloroform with 4 ml of 25% antimony trichloride and adjust the spectrophotometer to 100 optical density at 620 millimicrons. Add 1 ml chloroform to the tube with the vitamin A and position the tube in the spectrophotometer. Add 4 ml 25% antimony trichloride to the tube quickly by inverting 4 ml volumetric pipette. Read optical density as soon as possible after adding reagent (2-3 seconds) as the color will rapidly fade away.

Establish carotene curve by determining optical density at 440 millimicrons of 0.1-1.0 micrograms pure beta-carotene per ml of petroleum ether. Use petroleum ether as blank.

Establish vitamin A curve by determining optical density of 1-25 micrograms pure vitamin A alcohol per ml of the 5 ml solution (1 ml chloroform + 4 ml 25% antimony trichloride). Set spectrophotometer at 620 millimicrons. Use 1 ml chloroform + 4 ml 25% antimony trichloride solution as blank as above.

For correction factor of vitamin A color due to carotene in sample, evaporate carotene curve solutions and read as in vitamin A curve. Calculate the amount of vitamin A color developed from amounts of beta-carotene used (micrograms per ml from the vitamin A curve).

Calculation

(a) The carotene and vitamin A of 10 ml plasma are extracted into 15 ml of petroleum ether. The carotene concentration is determined on this petroleum ether solution.

$$\text{Beta-carotene, micrograms per ml plasma} = \frac{\text{15 ml petroleum ether solution} \times \text{carotene curve factor of micrograms per ml}}{\text{10 ml plasma}}$$

$$= 1.5 \times \text{carotene curve factor of petroleum ether solution}$$

(b) Only 5 ml of the [illegible] vitamin A petroleum ether solution are used [illegible] this represents the vitamin A in 10 ml of plasma. The vitamin A concentration per ml of plasma is therefore:

$$\text{Vitamin A alcohol, micrograms per ml plasma} = \left[\frac{\text{15 petroleum ether solution} \times \text{micrograms vitamin A per ml from curve}}{\text{5 ml plasma sample}}\right] - \text{Vitamin A color due to carotene, micrograms per ml}$$

$$= 0.5 \times \text{micrograms vitamin A per ml from curve} - \text{vitamin A color due to carotene, micrograms per ml}$$

(c) Report data on the [illegible] in terms of micrograms per 100 ml (micrograms per [illegible]). This data would be reported under other analyses on the [illegible]

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VITAMIN A DETERMINATION IN LIVER

Principle

Vitamin A forms a vivid blue when combined with antimony trichloride in chloroform. The intensity of this color is easily measured in a spectrophotometer. Any interference from carotene, which also forms a blue color with antimony trichloride is corrected for by determining carotene content of sample. The amount of vitamin A color developed from carotene is subtracted from sample reading.

Equipment

(a) Spectrophotometer

(b) 50 ml glass-stoppered test tubes

(c) Glass homogenizing tubes

(d) Supply of hidrogen gas and regulator

Reagents

(a) Antimony trichloride, 25%. Dissolve 25 g antimony trichloride in 75 ml dry choroform, add acetic anhydride to clear the solution. Make to 100 ml with chloroform. Grind antimony trichloride with mortar and pestle to hasten speed of solution. Store in brown, glass stoppered bottle in a desiccator.

(b) Petroleum ether, reagent grade, A.C.S. B.P. 30-60° C.

(c) Chloroform, reagent grade, A.C.S.

(d) 15% KOH. Dissolve 15 g KOH in water and make to 100 ml with water.

(e) 1:1 HCl. 50 ml concentrated HCl plus 50 ml water.

(f) 95% ethyl alcohol.

Procedure

Weigh 3 g (or other appropriate amount) of tissue into a homogenizing tube. (Protect samples and solutions containing vitamin A from light throughout the procedure.) Add 1 ml water and 2 ml of 15% KOH. Put the test tube in a beaker of boiling water for 15 minutes with frequent stirring so that the liver is completely dissolved. Cool the tube with running tap water. Add an amount of 1:1 HCl to neutralize the alkali. (The amount is previously determined). Insert glass pestle and thoroughly homogenize. Remove pestle and dilute with water to 15 ml. Pipet 5 ml of the mixture into each of two 50 ml glass-stoppered test tubes. Add 5 ml of 95% alcohol to each, then add 15 ml of petroleum ether and shake gently for 10 minutes. Allow the ether layer to separate pipet 3 ml (more or less depending on amount of vitamin A in sample) of petroleum ether into colorimeter tube. Read the optical density at 440 millimicrons using petroleum ether as blank. Determine amount of carotene by comparison with carotene calibration curve. Evaporate the petroleum ether by placing tube in hot water and allowing a stream of nitrogen to blow into tubes. Prepare a blank containing 1 ml of chloroform with 4 ml of antimony trichloride and adjust the spectrophotometer to zero optical density at 620 millimicrons. Add 1 ml chloroform to tube with the vitamin A and position the tube in the spectrophotometer. Add 4 ml 25% antimony trichloride to the tube quickly by inventing 4 ml volumetric pipette. Read optical density as soon as possible after adding reagent (2-3 seconds) as the color will rapidly fade away.

Establish carotene curve by determining optical density at 440 millimicrons of 0.1-1.0 micrograms pure beta-carotene per milliliter of petroleum ether. Use petroleum ether as blank.

Establish vitamin A curve by determining optical density of 1-25 micrograms pure vitamin A alcohol per 5 ml solution (1 ml chloroform + 4 ml 25% antimony trichloride). Appropriate amount of vitamin A is first dissolved in petroleum ether then aliquot evaporated as above. Read optical density at 620 millimicrons on the spectrophotometer using 1 ml chlor[illegible] + 4 ml 25% antimony trichloride as blank as above.

For correction factor of vitamin A color due to carotene in sample, evaporate carotene curve solutions and read as in vitamin A curve. Calculate the amount of vitamin A color developed from amounts of beta-carotene used.

Calculations

Vitamin A alcohol (micrograms per g) of fresh liver tissue =

$$\frac{\text{micrograms vitamin A in sample - adjustment for carotene content}}{\text{wt of sample in g}}$$

Adjusting to dry basis

$$\frac{\text{vitamin A (micrograms per g) on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$$

Note: Run a dry matter in an oven at 105° C to correct the data to a dry basis.

References

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DETERMINATION OF CYANIDE IN PLANTS

Principle

Cyanide is distilled from a chloroform and water solution into KOH solution, forming KCN. KCN is titrated with silver nitrate; $2KCN+AgNO_3 = KCN \cdot AgCN+KNO_3$. An excess of $AgNO_3$ produces insoluble AgCN, which is the end point of the titration: $Ag(CN)_2K+AgNO_3 = 2AgCN+KNO_3$.

Equipment

(a) Kjeldahl distillation apparatus (macro)

(b) 5 ml burette (micro)

Reagents

(a) 2% KOH solution. Dissolve 2 g KOH in 100 ml H_2O.

(b) Chloroform. Spectranalyzed, Fisher Certified, A.C.S. or equivalent.

(c) Potassium iodide.

(d) Potassium chloride. Recrystallized three times with H_2O, dry at 105° C, and then heat at about 500° C to constant weight.

(e) Potassium chromate solution. 5% solution of K_2CrO_4 in H_2O.

(f) Silver nitrate solution - 0.1 N. Dissolve slightly more than theoretical quantity of $AgNO_3$ (equivalent weight - 169.89) in halogen-free H_2O and dilute to volume. Thoroughly clean glassware, avoid contact

with dust, and keep prepared solution in amber glass-stoppered bottles away from light. Standardize as follows: weigh accurately sufficient quantity of KCl to yield titration of about 40 ml (about 0.3 g for 0.1 N solution) and transfer to 250 ml glass-stoppered Erlenmeyer with 40 ml H_2O. Add 1 ml 5% K_2CrO_4 solution and titrate with the $AgNO_3$ solution until appearance of first perceptible pale red-brown color. Subtract from titration milliliters of the $AgNO_3$ solution required to produce end point color in 75 ml H_2O containing 1 ml of the K_2CrO_4 solution. Calculate normality of $AgNO_3$ solution.

Procedure

Cut sample of forage into one-fourth inch lengths. Mix well. Place an 8 g sample in an 800 ml Kjeldahl flask. Add about 200 ml distilled water and 5 ml chloroform. Steam distill into a 100 ml test tube containing 5 ml 2% KOH, with the adaptor end of the condenser below the surface of the KOH solution. Distill over 60-70 ml and transfer to 600 ml beaker. Add enough H_2O to make 300 ml. Add a few crystals of KI and titrate to faint opalescence with the $AgNO_3$ solution. A drop of the silver salt in excess will produce a permanent turbidity, owing to the following reaction:

$Ag(CN)_2K+AgNO_3 = 2AgCN+KNO_3$, the insoluble AgCN being formed. (In making this titration, it is advantageous to have beaker over black surface.) From milliliters 0.1 N $AgNO_3$ used to calculate percent CN. Reaction is represented by equation:

$2NaCN+AgNO_3 = NaCN \cdot AgCN+NaNO_3$; hence 1 ml 0.1 N $AgNO_3$ = 0.005204 g CN.

Calculations

Cyanide (milligrams per kg) on partial dry or as fed basis =

$$\frac{52 \text{ ml } AgNO_3 \times N\ AgNO_3 \times 1000}{\text{wt sample in g}}$$

Adjusted to dry basis:

(a) $\frac{\text{cyanide (mg per kg) on as fed sample} \times 100}{\text{dry matter \% of as fed sample}}$

or

(b) $\frac{\text{cyanide (mg per kg) on partial dry sample} \times 100}{\text{dry matter \% of partial dry sample}}$

References

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DETERMINATION OF FINENESS OF FEED MATERIALS (GRAINS AND OTHER CONCENTRATES, BUT NOT FORAGES) BY SIEVING†

Purpose and Scope

The purpose of this standard is to define a test procedure to determine the fineness of feed ingredients and to define a method of expressing the particle size of the material. The particle size determined can be used to calculate surface area and number of particles per unit weight.

This standard shall be used to determine the fineness of feed ingredients where the reduction process yields particles which are essentially spherical or cubical. It is not adequate to define the particle size of materials such as steamed and rolled grains which are a flaked product, or products, such as chopped hay, where a substantial fraction consists of elongated particles.

Test Equipment

(a) A set of woven-wire cloth sieves having a diameter of 233 millimeters shall be used. With the most common shaking

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†Proposed by a subcommittee of the USA American Feed Manufacturers Association; approved by the American Society Agricultural Engineers (ASAE) Electric Power and Processing Division Technical Committee; adopted by the ASAE December 1968; approved by the following: American Society of Agronomy, American Society of Animal Science, American Society of Dairy Science, American Society of Poultry Science.

equipment, sieves having a height of 25 millimeters (1 inch) or half-height sieves are most suitable to avoid the necessity of resieving the finer fraction. A set of sieves as specified in American Society for Testing and Materials E11, Standard Specifications for Sieves for Testing Purposes (also designated United States of America Standard Z23.1) shall consist of the following sizes:

USA standard sieve No.	Normal sieve opening mm
3.5	5.66
5	4.00
7	2.83
10	2.00
14	1.41
18	1.00
25	.707
35	.500
45	.354
60	.250
80	.177
120	.125
170	.088
230	.063

The above sieve sizes have been proposed as International standard sizes. The sieve openings listed are for guidance only and any similar group of sieves can be substituted. The calculations use the actual sieve opening diameter and hence the results obtained will be correct regardless of which sieve set is used.

(b) A suitable sieve shaker, such as Ro-tap†, is required.

(c) A balance having an accuracy of at least ± 0.1 grams shall be used.

(d) Sieve agitators† such as plastic or leather rings, or small

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†Registered trade name (W. S. Tyler Co., Mentor, Ohio).

rubber balls may be required to break up agglomerates on finer sieves, usually those smaller than U.S. No. 50.

(e) A dispersion agent[+] should be available to facilitate sieving of high fat or similar materials.

(f) Sieve openings must be kept free of feed particles so that normal sieving can be accomplished. A stiff bristle sieve cleaning brush, or compressed air, is useful for cleaning sieves which have become clogged with feed particles. Sieves must be cleaned periodically to remove oil. Oil can be removed by washing with water containing a detergent. Sieves must be dried before use.

Method of Sieving

(a) A sample size of 100 g should be used although smaller samples may be used if extra care is taken to recover all material from the sieves.

(b) Place the sample on the top sieve of the set of sieves and shake until the weight of material, on the smallest sieve which contains any material, reaches equilibrium. Equilibrium shall be determined by inspecting and weighing at 5 minute intervals after an initial sieving time of 10 minutes. If the weight on the smallest sieve containing any material changes by 0.2 percent or less of the total sample weight during a 5 minute period, the sieving shall be considered complete at the onset of the previous period.

(c) Material on all sieves shall be weighed and recorded.

[+]Dispersion agents include Cab-O-Sil M5 available from the Cabot Corp., Boston; Zoilex 23A and Zeofree 80 available from the J.M. Huber Corp., New York; and Flo-Gard available from the Pittsburgh Plate Glass Co., St. Louis.

(d) If a dispersing agent is required, it should be added at a level of 0.5 percent, and its effect on particle size need not be recorded.

(e) If 20 percent or more of the material by weight passes the smallest sieve, the fine material shall be subjected to a nonsieving particle size analysis, such as microscopic measurement or sedimentation testing, and such analysis shall be reported separately.

Data Analysis

(a) Analysis of weight distribution data of all ground feeds and feed ingredients are based on the assumption that these distributions are logarithmic normally distributed.

(b) Calculation of particle size. The size of particles shall be reported in terms of geometric mean diameter and geometric standard deviation by weight.

(c) Calculated values are obtained as follows:

$$d_{gw} = \log^{-1}\left[\frac{\Sigma(W_i \log \bar{d}_i)}{\Sigma W_i}\right]$$

$$S_{gw} = \log^{-1}\left[\frac{\Sigma W_i (\log \bar{d}_i - \log d_{gw})^2}{\Sigma W_i}\right]^{1/2}$$

where

d_i = diameter of sieve openings of the i'th sieve

d_{i+1} = diameter of openings in next larger than i'th sieve (just above in a set)

d_{gw} = geometric mean diameter

d_i = geometric mean diameter of particles on i'th sieve $(d_i \times d_{i+1})^{1/2}$

S_{gw} = geometric standard deviation

W_i = weight fraction on i'th sieve

(d) Material passing U.S. Sieve No. 230 shall be considered to have a mean diameter of 44 microns.

(e) Graphical solutions for geometric mean diameter and log-normal geometric standard deviation may be obtained by plotting results on logarithmic probability graph paper. Figure 1 shows an example where:

$d_{gw} = d_{50}$ = particle diameter at 50 percent probability

$$S_{gw} = \frac{d_{84}}{d_{50}} = \frac{d_{50}}{d_{16}} =$$

particle size at 84 percent probability/d_{gw} = d_{gw}/ particle diameter at 16 percent probability

or d_{gw} = 350 microns

and $$S_{gw} = \frac{640}{350} = \frac{350}{191} = 1.83.$$

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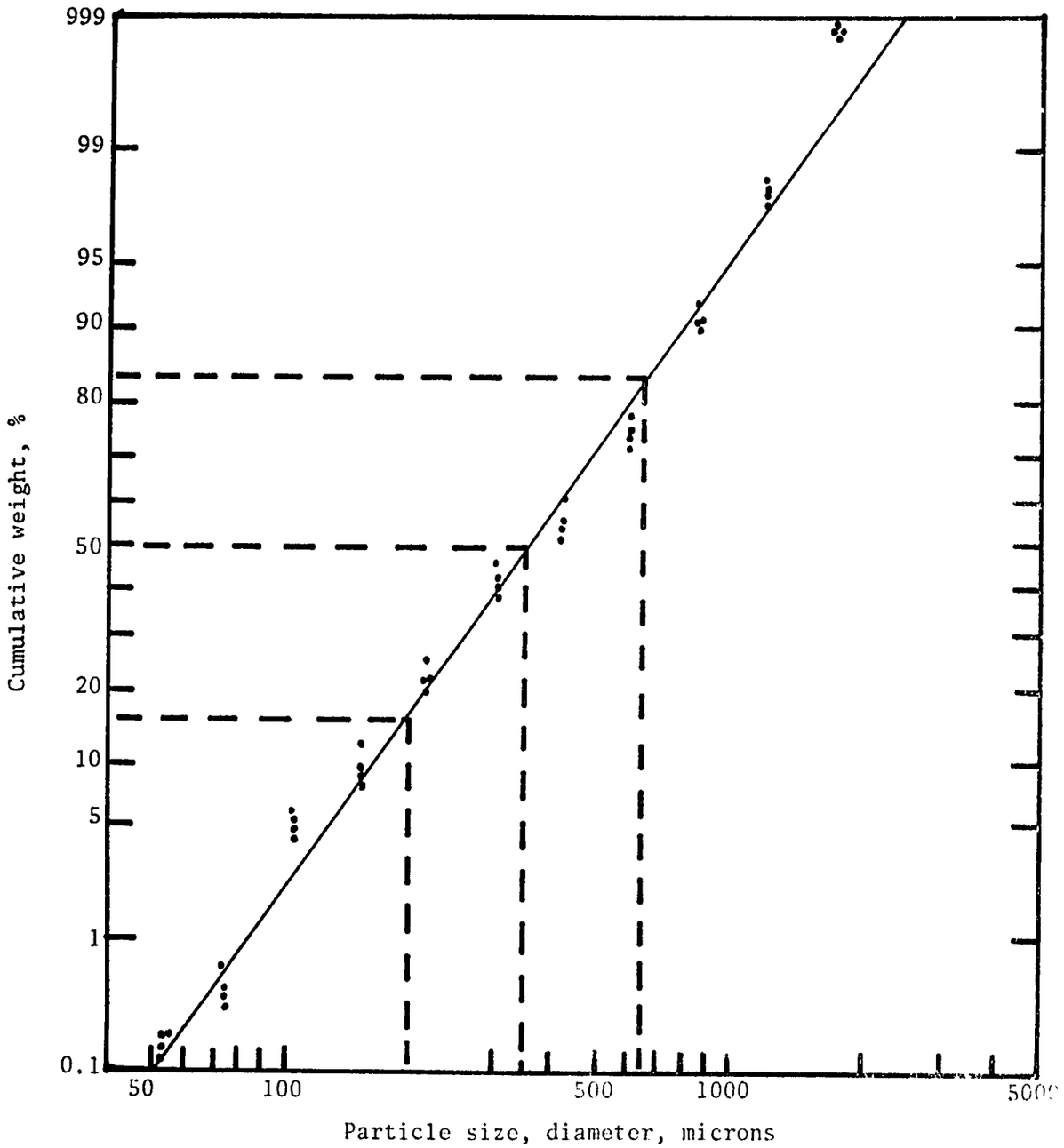

Figure 1. Lognormal distribution for sorghum grain ground through a 32 mm screen.

DETERMINATION OF NITRATES AND NITRITES IN FORAGES

Nitrates and nitrites are known to have injurious effects on animals when excess amounts are ingested. Nitrites are much more toxic but nitrates are reduced to nitrites by bacterial action in the digestive tract.

Nitrites (NO_2^-) are rarely found in plants in appreciable amounts except under very unusual conditions such as severe disease infections; therefore, the results are usually reported in terms of nitrate (NO_3^-). The usual method is to determine the total nitrate and nitrite in terms of nitrate. However, the method can also be used to determine the nitrite content of a feed separately if a nitrite problem is suspected.

Principle

Nitrate is reduced to nitrite by zinc and manganese (II) sulfate. The reaction then consists of diazotization of sulfanilic acid by the nitrite ion and subsequent coupling with 1-naphthylamine to form a red dye. The reaction is optimum in the pH range of 1.7 - 3.0. Interfering iron is complexed with citrate. Copper from 0.2 to 1.0 ppm aids the reaction but over 1.2 ppm interferes.

Equipment

(a) Spectronic "20" spectrophotometer or equivalent.

(b) Centrifuge.

Reagents

(a) Standard nitrate solution. Dissolve 1.37 g of sodium nitrate in 1 liter of water for a stock solution of 0.1% nitrate (1000 micrograms per ml).

(b) To 200 ml of acetic acid, add 5.0 ml of $CuSO_4$ solution (0.1572 g of $CuSO_4.5H_2O$ per liter) and dilute to 1000 ml. This solution is then 20% acetic acid with 0.2 ppm Cu.

(c) Powder mixture (Bray's indicator). One hundred g of barium sulfate (dried at 105° C), 10 g of manganese (II) sulfate monohydrate, 2 g of finely powdered zinc, 75 g of powdered citric acid, 4 g of sulfanilic acid, and 2 g of 1-naphthylamine. Grind any coarse material to a fine powder. Mix the manganese (II) sulfate, powdered zinc, sulfanilic acid, and 1-naphthylamine separately with portions of barium sulfate. Then mix thoroughly all ingredients including the barium sulfate and citric acid. Use extreme care to have room, table top, and equipment free of nitrate and nitrite. Store the powder in a blackened bottle, since light affects the 1-napthylamine. The reagent is stable for many months in a bottle painted on the outside with black paint. If the determination of nitrite in the presence of nitrate is desired, make up a separate powder mixture that does not contain the powdered zinc and manganese (II) sulfate.

(d) 0.1 N HCl. 8.18 ml of 37-38% HCl per 1.0 liter of water. Exact standardization is not necessary.

(e) Activated charcoal. Merck number 18351 activated charcoal N.F. powder.

Sampling of Feeds

The nitrate content of plants or feeds from different parts of a field, stack or silo often varies extremely. For example, the nitrate content of plants in a field vary between: different plant species, different stages of plant growth, different parts of a plant, different areas in a field, different times of day, and different days as climatic conditions vary. Nitrate contents of feeds in stacks and silos vary because of the above factors and because of [illegible] hay or silage is handled in the field [illegible] leaching from rain); and losses and [illegible] during fermentation in the silo.

Therefore, at any one sampling [illegible] obtain separate samples from different places [illegible] or silo and analyze each sample separately, for [illegible] the field, samples should be collected and [illegible].

Nitrates may be lost from moist plant or [illegible] between the time the sample is collected and the time it is analyzed if the samples are not handled properly. Such losses may be due to enzymatic or microbiological reduction of nitrates in the moist sample or to volatilization of nitrogen gases or acid [illegible] drying. Therefore, either the moist plant or feed samples should be frozen as soon as possible after they are collected and kept frozen until they are analyzed, or plant samples (but not silage samples) may be dried immediately in an oven with adequate ventilation at 60° C.

Procedure

(a) Weigh by difference approximately 1.0 g of air dry plant material or 3.0 to 5.0 g of moist sample. The sample should contain 1 to 8 milligrams nitrate to be within the range of the standard curve. Place the sample in a 125 ml glass stoppered Erlenmeyer flask.

(b) Add 100 ml of 0.1 N HCl. Stopper the flask and shake the contents until all parts of the sample are wetted. Let the sample stand with occasional shaking for 1 hour to extract nitrate. If the extract is strongly colored, decolorize it by adding about 1 g of activated charcoal to the extract in the Erlenmeyer flask and shake well. Filter the extract through filter paper.

(c) Pipet 1 ml of the extract and 9 ml of 50% acetic acid solution to a glass stoppered centrifuge tube. Add about 0.5 g of the Brayts indicator powder with a small measuring scoop. Stopper and shake each tube 1 minute. Shade the solution from strong light.

(d) Place the tubes in the centrifuge tube holder and centrifuge for 5 minutes at 3,000 revolutions per minute or until the supernatant liquid is clear. Remove any film on top and pour the clear red solution into a spectrophotometer cuvet.

(e) Observe the optical density at 520 millimicrons. Set the instrument at 100% transmittancy with a blank solution that has been treated exactly as the sample solutions.

(f) Compare the observations to a standard nitrate curve ranging from 0 to 10 micrograms nitrate per ml of solution in the cuvet. (Make the standard curve by diluting 0, 2, 5 and 10 ml aliquots of the standard nitrate solution to 100 ml in volumetric flasks with water. Transfer 1 ml

of the diluted solution to centrifuge tubes and continue the procedure from (c) on. The solution in the cuvets will contain 0, 1, 2, 5 and 10 micrograms nitrate per ml. The remaining solution in the 100 ml volumetric flasks may be transferred to polyethylene bottles and stored for several months and reused as needed.)

(g) When nitrites are to be determined in the presence of nitrates follow the same procedure except use the powder mixture that does not contain powdered zinc or manganese (II) sulfate.

Calculations

(a) To determine the nitrate plus nitrite content, the powder mixture containing zinc and manganese sulfate is used and all nitrates are reduced to nitrites. The result is the total of nitrate and nitrite in terms of nitrate when the standard curve under (f) is used.

Total nitrate (%) on partial dry or as fed basis =

$$\frac{\text{concentration of nitrate in final solution in micrograms per ml}}{\text{wt of sample in g} \times 1{,}000{,}000} \times 1000 \times 100 = \text{from curve}$$

(b) To determine only nitrite in the presence of nitrate, the zinc and manganese sulfate are omitted from the powder mixture.

The nitrite % on partial dry or as fed basis =

$$\frac{\text{concentration of nitrogen in final solution in micrograms per ml}}{\text{wt of sample in g} \times 1{,}000{,}000} \times 1000 \times 100 = \text{from curve}$$

The nitrite determination is used only to add information when nitrite poisoning is suspected in local areas. The amount will be included in the analysis of total nitrate and therefore need not be reported separately on the source form.

(c) Adjusting to dry basis for total nitrate (nitrate plus nitrite):

$$\frac{\text{\% nitrate in as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$$

or

$$\frac{\text{\% nitrate in partial dry sample}}{\text{dry matter \% of partial dry sample}} \times 100$$

Note: report % of nitrates (nitrate plus nitrite) on the source form under other analyses.

Reference

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in vitro DRY MATTER AND ORGANIC MATTER DIGESTION

Principle

This procedure follows the "Tilley and Terry" two-stage technique and involves, first, a 48-hr incubation with rumen microorganisms and buffer and, second, a hydrochloric acid-pepsin digestion. The amount of dry matter or organic matter disappearing after both stages is considered to have been "digested."

Supplies and Equipment

(a) 100 ml centrifuge tubes, plastic (Nalgene) or glass without pourout (if centrifugation is used, see alternate procedure p and q below, tubes must fit centrifuge carriers).

(b) Rubber stoppers for item (a) fitted with Bunsen valve for gas release (rubber policemen with longitudinal slit about 6 mm long).

(c) Test tube racks for item (a).

(d) Scintered glass crucibles, 50 ml, coarse porosity, low form (Corning # 32940-50C, 40-60 micron).

(e) Vacuum filtration device for crucibles, item (d): Crucible holder, (Fisher 8-238B or equivalent), rubber adapter (Fisher 8-239B or equivalent), and vacuum source (pump or aspirator).

(f) Round bottom flask, 5 liter, with short heavy neck, Pyrex (Corning 4260).

(g) Dispensing burette, 500 ml.

(h) Water bath which will accept 5 liter flask, (f) above.

(i) Incubator or water bath which will accept centrifuge tubes, (a) above, with bunsen valve stoppers, (b) above, in place.

(j) pH meter with combination electrode usable in centrifuge tube.

(k) Other permanent equipment: analytical balance, drying oven, muffle furnace, CO_2 tank and reduction valve (also a centrifuge if alternate procedure p and q, below, are used).

(l) Other expendable supplies: beakers, thermometers, desiccators, graduated cylinders, cheesecloth, glass wool, tongs, porcelain crucibles, asbestos gloves, pipettes, etc.

Forage Preparation

Forages should be air dry (88-92% dry matter) and ground through a 1 mm screen (such as in a Wiley mill). Representative samples should be placed in air-tight containers which facilitate easy removal of aliquots (glass or plastic bottles).

Reagents

(a) McDougals' Artificial Saliva. (40 ml required per tube)
Since insoluble calcium phosphate will precipitate at the high pH of the buffer, the saliva must be prepared in two parts if it is to be stored more than 2 hours.

1. Buffer (quantities per liter, w/v)

   9.80 g $NaHCO_3$
   7.00 g $Na_2HPO_4 \cdot 7H_2O$ (3.71 g anhydrous)
   0.57 g KCl
   0.47 g NaCl
   0.12 g $MgSO_4 \cdot 7H_2O$

2. 4% (w/v) calcium chloride solution

4.0 g $CaCl_2$(5.3 g $CaCl_2 \cdot 2H_2O$) per 100 ml water

Just before use, add 1 ml 4% $CaCl_2$ per liter of buffer to provide 0.04 g $CaCl_2$ per liter of artificial saliva.

(b) 20% (v/v) HCl: Dilute 200 ml concentrated HCl to one liter (approximately 2.4 N HCl) (6 ml required per tube).

(c) 5% (w/v) pepsin: Add 5 g of pepsin to 100 ml distilled water (2 ml required per tube).

(d) Strained rumen fluid (10 ml required per tube): Great variation will exist among laboratories in the source of rumen fluid and the manner in which it is obtained. Cattle, sheep or goats may be used and it will generally be most convenient to use an animal with a permanent rumen fistula. The type of diet will also affect results. It is important that donor animals be fed a diet composed predominately of forage, generally hay, and that it be similar to the forages to be subjected to the *in vitro* digestion. When grass hay is the diet, it may be desirable to provide supplemental protein, minerals, and vitamins, for example for a steer: 900 g soybean meal, 75 g trace mineralized salt, 50 g dicalcium phosphate, 20,000 IU of Vitamin A per 500 kg body weight. In no instance where the test materials are forages, should any concentrate be fed, except the protein source. Whatever the diet chosen, it should be maintained as constant as possible.

Many variations exist in methods of obtaining and processing rumen fluid. The following general recommendations should be followed:

1. The donor animal should not have access to feed or water during one hour prior to collecting rumen fluid; always

collect rumen fluid at about the same time each day.

2. Rumen fluid should not be obtained from the bottom of the rumen since this will have many small feed particles which will interfere with the results.
3. Strain the rumen fluid through glasswool (place four layers of cheesecloth in a funnel and cover this with a double layer of glass wool).
4. If rumen fluid must be transported some distance to the laboratory, care must be taken to maintain anaerobiosis. This can be achieved by completely filling a bottle and stoppering so that all air is excluded. Using an insulated flask to keep the sample warm may also be necessary in some instances.

Procedure

(a) Determine sample dry matter and organic matter content: place about 0.5 g sample in a porcelain crucible (in duplicate) and record weight to at least 3 decimals (the difference method may be used); dry overnight at 105° C, cool in desiccator and weigh; ash in muffle furnace at 500° C, cool in desiccator and weigh; calculate % dry matter and % organic matter (dry matter basis).

(b) Weigh out samples for <u>in vitro</u> digestion: place about 0.5 g sample in a numbered centrifuge tube (in duplicate) and record weight to at least 3 decimals (the difference method may be used). (Note: the weighings should be completed at least by the day prior to the start of the digestion.)

(c) Set aside four empty tubes for blanks.

(d) Turn on incubation baths or incubators (39° C).

(e) Place appropriate quantity of buffer (40 ml per tube plus about 200 ml extra) in a 5 liter round bottom flask (maximum amount = 4 liters), add 1 ml 4%$CaCl_2$ per liter, place flask in water bath at 39° C, and bubble CO_2 through the solution at a gentle rate.

(f) Add 2 ml distilled water to all centrifuge tubes, wetting forage particles.

(g) Check pH of artificial saliva; it should be approximately 6.9-7.0; the CO_2 bubbling through the solution lowers the pH; when solution is saturated with CO_2 and the bicarbonate buffer system is established, the pH should be 6.9-7.0; about 30 min is required to adjust 4 liters of artificial saliva to pH 6.9-7.0 and 39°C. (Note: 4 liters buffer is sufficient for 96 tubes. (46 samples in duplicate, plus 4 blank tubes and some excess.) If more than this is used in a run, either use a larger flask or use 2 flasks; if 2 flasks are used, prepare 4 blank tubes for each and keep the tubes in sets.)

(h) Prepare strained rumen fluid and add one (1) part rumen fluid to four (4) parts artificial saliva in the round bottom flask.

(i) Allow the media (rumen fluid plus saliva) to mix for 10 minutes through the action of the bubbling CO_2.

(j) Flow CO_2 into the dispensing burette and fill the burette with media. (The effluent CO_2 from the round bottom flask may be directed into the burette and the media may be transferred anaerobically to the burette through tygon tubing; flow can be achieved by momentarily closing the effluent CO_2 tube thus increasing the pressure in the flask.)

(k) Add 50 ml of media to each centrifuge tube, including blanks

(add media to the tubes at random and disperse the blanks randomly among the set of tubes).

(l) Immediately after adding media, sweep the remaining air out of the tubes by passing CO_2 into the tube for 15 seconds (do not bubble CO_2 into the media in the tubes); quickly stopper the tubes with the bunsen valve.

(m) Place tubes in incubator or water bath at 39° C.

(n) Swirl contents in tubes with a gentle lateral rotating motion to insure that all forage particles are wet with media; repeat twice the first day and three times the second day.

(o) After 48 hours incubation, swirl the tubes and remove the stoppers; wash any forage particles adhering to the stoppers into the tube with a minimum of distilled water (check pH of a few tubes at random; should be less than 7).

(p) Add 1 ml 20% HCl, after a few minutes swirl tube; add another 1 ml 20% HCl, swirl and finally add 4 ml 20% HCl and swirl (total HCl = 6 ml/tube).

(q) Add 2 ml 5% pepsin, swirl thoroughly and place tubes in water bath or incubator at 39° C; repeat swirling twice the first day and three times the second. (Note: alternative for steps p and q: centrifuge the tubes for 15 minutes at 1000 relative centrifugal force, e. g. 2000 rpm in International Electric Centrifuge, size 2; decant supernatant and add 50 ml of 0.2% pepsin in 0.1 N HCl).

(r) Wash, dry (2 hours) and weigh scintered glass crucibles (this should be completed the day before the final day of digestion).

(s) After 46 hours pepsin digestion, transfer the contents of the centrifuge tubes to the dried and tared scintered glass crucibles.

Wash the tubes and residue in the crucibles with distilled water (if a hot water dispenser is available, this can be used).

(t) Place crucibles in drying oven at 105° C and dry to constant weight (overnite), cool in desiccator and weigh.

(u) Place crucibles in muffle furnace at 500° C for 3 hours, cool in desiccator and weigh.

(v) Record the following values:

weight of air dry sample placed in tube	(A)
% dry matter of sample	(B)
% organic matter of sample, (DM basis)	(C)
tare weight of empty crucible	(D)
dry weight of crucible with residue	(E)
ashed weight of crucible with residue	(F)

Calculations

Initial dry matter, $g = \frac{A \cdot B}{100}$ (G)

Residual dry matter, sample, $g = E - D$ (H)

Residual dry matter, blank, $g = E - D$ (average 4 tubes) (I)

in vitro dry matter digestion, $\% = \frac{G - (H-1)}{G} \times 100$

Initial organic matter, $g = \frac{G \cdot C}{100}$ (J)

Residual organic matter, sample, $g = E - F$ (K)

Residual organic matter, blank, $g = E - F$ (average 4 tubes) (L)

in vitro organic matter digestion, $\% = \frac{J - (K-L)}{J} \times 100$

Suggested Time Sequence for One *in vitro* Digestion Trial

Wednesday	pm	start weighing samples into tubes
Thursday	--	complete weighing samples into tubes
Monday	am	inoculate
Wednesday	am	acidify and add pepsin
Wednesday	pm	wash crucibles and place in drying oven
Thursday	--	transfer crucibles to desiccator, cool and weigh
Friday	am	transfer residues to tared crucibles and place in drying oven
Monday	am	transfer crucibles from oven to desiccator
Monday	pm	weigh dry crucibles
Tuesday	am	place crucibles in muffle furnace (3 hrs) and transfer crucibles from furnace to desiccator
Tuesday	pm	weigh ashed crucibles and calculate data

(The drying, ashing and weighings for initial dry matter and organic matter determinations may be done at any convenient times)

References

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DETERMINATION OF VOLUNTARY INTAKE AND DIGESTION COEFFICIENTS OF CURED FORAGES

Principle

Animals are fed the forage to be studied long enough for them to become accustomed to it. When they reach a point where their intake is relatively the same from day to day the amount of forage consumed is tabulated. During the last seven days of the trial, feces are collected to determine digestion coefficients. The higher the intake of forage and the higher the digestible dry matter, the better the quality of the forage.

General

(a) A 12 to 16 day supply of sun-cured or fan air dried forages are chopped through a forage harvester into a bin; forage is not chopped during a measurement or collection period.

(b) Sheep are fed in individual pens or cages, about 0.6 meter x 1.2 meter, preferably with a slotted floor. Water is provided continuously through an automatic system, or supplied twice a day by hand. Dicalcium phosphate or the equivalent such as bone meal and trace mineralized salt are kept in separate boxes before each animal.

(c) Feces are collected in canvas bags attached to the animal by means of a harness; urine is not collected.

(d) A randomized block experimental design is used; sheep are as-

signed to a block according to weight and randomly assigned to treatments within the blocks. Sheep are randomly assigned to pens. At least six sheep receive each treatment. Four sheep may be fed each forage in two periods and the sheep are randomly reassigned to the treatments at the start of the second period.

(c) One week prior to an experiment, the sheep are confined, in a barn, preferably in a slotted floor pen and all are fed the same hay plus salt and dicalcium phosphate; the sheep are wormed on the first day of this confinement period.

Intake Measurement and Fecal Collections

(a) On the first day of the experiment the sheep are weighed and placed in individual metabolism cages; the feces bags are attached at this time and the experimental feeds are offered according to the design of the experiment and the weights of the animal.

(b) Voluntary intake measurement is achieved by offering 15 percent more than is consumed (adjustments are made each day). Orts or weigh-backs are never refed. This procedure is followed through the experiment.

(c) The first 14 days of the experiment are considered as a preliminary period during which the amount of forage offered is adjusted to achieve voluntary intake, as defined above.

(d) The 15th through 21st days (7 days) are considered as the intake measurement period; on each of these days a sample of forage is taken representing a constant percentage of the total amount offered to all sheep receiving that forage; the percentage used is selected such that the total composite cured forage sample after 7 days will be between 1 and 5 kg. It is collected and saved in a large tin can with air-tight lid or plastic

bottle, or heavy plastic bag.

(e) The total quantity of orts from each sheep is collected and saved each day during the intake measurement period. The feeders are cleaned thoroughly before feeding on the 15th day (and the orts collected on the 16th through 22nd days are saved; this corresponds to the forage offered on the 15th through 21st days (7 days).) After weighing to calculate the amount to feed, the orts are transferred to a paper sack: one sack is used for each sheep throughout the collection and the orts for each sheep for the 7 days are composited.

(f) Feces bags are emptied daily throughout the experiment; this is done immediately after removing and weighing orts and prior to feeding. On the 16th day, the feces bags are emptied completely. On the 17th through 23rd days (7 days) the total quantity of feces voided during the past 24 hours is collected in a bucket, weighed, mixed by hand, and a representative 20% aliquot is placed in a plastic bag and frozen.

(g) After fecal collection and before feeding on the 23rd day, the sheep are weighed.

(h) The following summarizes the experimental calendar discussed above:

Day	Action
1	Weigh sheep, place in metabolism cages, attach feces bags, start experimental diets, determine voluntary intake.
2 to 14	Adjust feeding to ad libitum intake, remove orts each day.
15	Collect forage sample
16	" " " collect orts
17	" " " " " collect feces
18	" " " " " " "
19	" " " " " " "
20	" " " " " " "
21	" " " " " " "
22	" " " "
23	" " , weigh sheep

Sample Preparation

(a) The daily forage samples (if sun-cured or fan air dried) are composited and the entire amount is ground through a small hammermill (about 40 cm diameter) with a 6 mm screen, mixed, and about 2 kg are placed in a sealed plastic bag to prevent changes in moisture content. As soon as possible, the 2 kg aliquot is reground through a Wiley mill with a 4 mm screen and a 1 gallon plastic jar is filled with approximately 800 g and sealed for long-term storage in a freezer. Another portion (about 200 g) is ground through a 1 mm screen and stored in an air tight container, such as a 250 ml glass or plastic bottle.

(b) The total orts composited from each sheep for 7 days are reweighed and immediately ground through a small hammermill, as above, and approximately 300 g are placed in a sealed plastic bag to prevent changes in moisture content. This sample is reground through the Wiley mill, both 4 mm and 1 mm screens, as above, and stored in an air-tight container.

(c) The 7 daily feces samples from each sheep, representing 20% of the total voided, are dried under forced air at 45° C. After equilibration with atmospheric moisture for at least 48 hours, the total sample is weighed and immediately ground through the 4 mm screen of the Wiley mill. A representative sub-sample (approximately 200 g) is immediately reground through the 1 mm screen of the Wiley mill and stored in an air-tight container. If nitrogen digestibility is a critical measurement, fresh feces should be analyzed as described in "Digestibility and Balance Trial for Sheep" (page 5301).

(d) Dry matter analyses are conducted as soon as possible on the forage, orts and feces samples ground through the 1 mm screen. Separate

analyses are made on each orts and feces sample.

Calculations

(a) Voluntary dry matter intake and an apparent dry matter digestion coefficient are calculated for each sheep as follows:

average daily dry matter intake (g) = A - B

apparent dry matter digestibility = $\frac{A-B-C}{A-B} \times 100$

where:

A = average daily dry matter offered
B = average daily dry matter refused
C = average daily dry matter voided in feces

A, B, and C are calculated as follows:

$$A = \frac{(\text{\% dry matter of forage}) \times (\text{average daily forage offered}^{1/})}{100}$$

$$B = \frac{(\text{\% dry matter of orts}) \times (\text{average daily orts}^{2/})}{100}$$

$$C = \frac{(\text{\% dry matter of partial dry feces}) \times 5 \times (\text{weight of feces aliquot}^{3/})}{100}$$

1/ Average weight of hay offered according to daily barn records, for days 15 through 21.

2/ Weight of orts just prior to grinding through hammermill, divided by the number of days in collection period.

3/ Weight of feces just prior to grinding through the 4 mm screen of the Wiley mill (this represents 20% of the total feces voided), divided by the number of days in collection period.

(b) Digestion coefficients for all other nutrients are calculated using the dry matter values A, B and C, above, and the % of nutrients in

hay, orts and feces expressed on the dry matter basis.

Reference

Moore, J. E. 1969. Procedure for determining voluntary intake and nutrient digestibility of hay with sheep. University of Florida Animal Science Department. Unpublished.

A DIGESTION AND BALANCE TRIAL FOR CATTLE

Where possible use animals for which you wish to have the data apply. If cattle are raised in your country and you wish to have the data apply to cattle, use them for collecting *in vivo* data. The procedure should be modified to fit local conditions and to obtain the desired data from the experiment. If working with trace elements, see trace element analysis, page 4101.

Principle

The animal is fed a quantitative amount of feed for a definite period of time and the excreta resulting therefrom are collected quantitatively for chemical analyses. The excreta are preserved by refrigeration or by freezing to prevent decomposition until they are analyzed. By analyzing the feed and feces, digestion coefficients and digestible nutrients may be calculated. By further analyzing the urine and products such as milk, the amount of some of the nutrients absorbed and retained in the body or stored as a product may be calculated by difference.

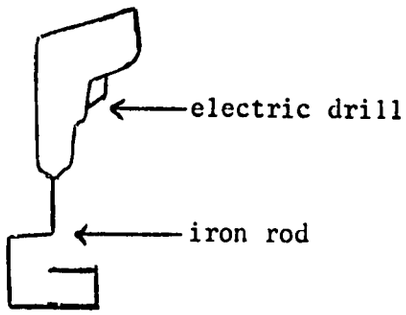
Apparatus

(a) Metabolism cages or stalls. Use metabolism cages or stalls which permit the giving of a quantitative amount of feed and water and the quantitative collection of feces and urine.

(b) Urine containers. Use glass or plastic containers to collect urine under the cage or stall. Use 20 liter containers (plastic) with large lids to store the urine aliquots during the collection period.

(c) Feces containers. Use plastic or tin tubs to collect feces under the cage or stall. Use 4 liter containers (plastic) with large lids to store the feces during the collection period.

(d) Feces stirrer. Make a feces stirrer with an iron rod attached to an electric drill as follows:



(e) Scales. To weigh animals, feed, feces and urine.

(f) Containers. Use bottles with air tight lids or heavy plastic bags for collecting samples of grain, and for holding partially dried feces samples before grinding them in the Wiley mill. Use 250 ml glass or plastic bottles fitted with air tight lids to hold samples after they have been ground through the Wiley mill. Use glass or plastic 100 ml bottles for urine samples.

(g) Use air tight, large plastic bags, plastic containers or 20 liter cans for forage samples.

(h) Deep freeze unit. A small chest type or a walk in unit.

Feeding and Watering

(a) Weigh an amount of feed (forage or grain or mixed ration), the afternoon before it is to be fed, which will last the animal for one day and put it in a container in front of the animal. This makes it possible

to feed all animals quickly the next morning.

(b) Arrange to feed the animals at the same time each day (preferable between 7:00 and 8:00 a.m.).

(c) If a balance trial is to be conducted and the water contains materials which are to be studied, it is necessary to weigh and analyze the water for these materials (this is especially true for trace minerals). Weigh the water the animal drinks. Fill buckets with water and set them on the scales and obtain the gross weight. Let the animal drink twice daily at the same time each day. After the animal has drunk its water, again weigh the buckets with the remaining water and record the weight (or if the metabolism cage or stall has a movable water container, it may be filled and weighed in the morning and then weighed again the next morning).

Preliminary Period

(a) The purpose of the preliminary period is to acquaint the animal with the metabolism cage or stall, to make the necessary adjustments in order that the feces and urine are collected properly, and to adjust the animal to its intake of feed in relation to the excretion of feces and urine. It is difficult to set a definite length of time for this preliminary period since it depends upon when the animal adjusts itself to eating a definite amount of feed. If the animals are not trained, it may take 3 to 6 weeks to acquaint them with the procedures before they begin to eat a definite amount of feed or until the conditions of the experiment are met. If the animals are trained, they should be on the preliminary period for at least 10 days prior to the beginning of the collection period. This refers to the time they are receiving a definite amount of feed.

(b) Treat the animals for external parasites if necessary, and worm them at least 10 days before the preliminary period starts.

(c) It is preferable to keep the animals in a slotted floor pen during the first part of this period.

(d) During the preliminary period the animals should be cleaned externally to avoid the possibility of any foreign material contaminating the feces and urine collections.

Collection Period

(a) Transfer the animal to the collection cage or stall 7 days before the collection period begins.

(b) Weigh the animals 3 days before the collection period starts and the morning after the feces and urine are collected on the last day of the collection period. The weighing should take place at the same time each day, preferably before feeding at about 7:00 a.m.

(c) The collection period usually runs for 7 consecutive days, but it may be varied depending on how the animal eats and the information wanted in the experiment. Keep track of the exact time the period starts and ends.

(d) The afternoon before the collection period is initiated, the backs of the cages or stalls and the collection area should be cleaned thoroughly.

(e) The collection period should begin on the morning after the animal has been eating a constant amount of feed for at least 10 days.

(f) During the collection period, save a certain percentage of the feed sample (at the time it is weighed out for feeding). Start saving this sample two days before the collection of feces begins and end the collection 2 days before the collection of feces stops.

Collection and Sampling of Feces and Urine

(g) The collection of feces, urine and milk (if the animal is lactating) should be started 48 hours after the collection period begins. In this procedure it is assumed that all the feces are collected. Collect consecutive samples. A good plan would be to remove the feces and urine containers beginning at 8:00 a.m. each morning and replace them with clean containers. Collect feces and urine for 7 consecutive days, providing feed intake and collections have [illegible] period.

(h) Weigh the feces by difference. Place the [illegible] container (4 liter plastic container with large lid) on the balance and obtain a tare weight and then add sufficient feces to give the correct aliquot (2.5 to 5.0%) to the container for each day [illegible] record the tare and gross weight on a record form. Do not use [illegible] paper. [illegible] the samples frozen in a deep freeze. Repeat this procedure for each day during the collection period.

(i) Weigh the total urine excreted [illegible] and record the gross and tare weight. Mix the urine by [illegible] and take an aliquot (2.5 to 5.0%) and weigh it into the plastic containers with large [illegible] as described for the feces. Keep the urine samples frozen in a deep freeze. Repeat this procedure for each day during the collection period.

(j) If the animal is lactating, weigh the total amount of milk each day. Sample by saving an aliquot in a glass or plastic bottle (follow same procedure as described for feces). Keep samples in refrigerator at 1° C.

Sampling Feces for Chemical Analyses

(a) Thaw the feces sample, but do not let it remain for long periods

at room temperature. Remove the feces quantitatively from the container and mix on a piece of stainless steel or tin with a small trowel (if trace minerals are to be run, use a piece of plastic).

(b) Be prompt in obtaining two samples in 50 ml weighing bottles to prevent loss of moisture. Take these to the laboratory immediately and determine dry matter, nitrogen and energy on the fresh feces without partial drying (see "Weighing Samples by Difference" page 1501).

Analyze each sample once. If the analyses check, a good job has been done in sampling the feces. If the analyses do not check, take the stored fresh feces sample out of the deep freeze (see c below) and analyze samples from it. Bring it to room temperature before sampling.

(c) Save a 500 ml bottle of feces and label and store in deep freeze. This sample should be saved until the chemical analyses are complete.

(d) Weigh out a sample (about 700 g) of feces for partial dry matter analysis. Analyze this sample for those materials that are not affected by drying.

Sampling Urine for Chemical Analyses

(a) Thaw the urine sample, but do not let it remain for long periods at room temperature. Mix thoroughly and place a sample in a 250 ml plastic bottle. Analyze immediately (see "Weighing Samples by Difference", page 1501).

(b) Save a 250 ml sample in the deep freeze until all chemical analyses are finished.

Sampling Feeds, Orts or Weighback

(a) If the animal refuses to eat all its food during the collection period or if any is wasted by being thrown out of the manger, it should be saved.

(b) If the weighback is dry enough to grind, a weight should be taken prior to grinding. However, if water was spilled on the weighback or if it is wet from saliva, it should be dried in a forced draft oven (temperature 60° C) then placed in a room for 48 hours to come to moisture equilibrium with the air. Just prior to grinding it should be weighed.

(c) Grind the feed sample, orts or weighback and partial dry feces through a mill with a one millimeter screen (if trace minerals are to be run see "Trace Element Analyses" page 4101). Save samples in 250 ml (about 3/4 full) glass or plastic bottles.

Calculations

(a) Apparent digestion.

Apparent digestion coefficient =

$$\frac{\text{nutrient in feed} - \text{nutrient in feces} \times 100}{\text{nutrient in feed}}$$

Example:

$$\text{GE}^{+}\text{ apparent digestion coefficient} = \frac{(\text{GE of feed per unit dry wt} \times \text{dry wt of feed}) - (\text{GE of feces per unit dry wt} \times \text{dry wt of feces})}{\text{GE of feed per unit dry wt} \times \text{dry wt of feed}} \times 100$$

(b) A balance is the relation of the material in the feed to the output of the material. For most nutrition work the feed, feces and urine are considered:

a balance = material in feed - material in feces and urine

Example:

Nitrogen balance (NB) =

$$(\text{N of feed per unit dry wt} \times \text{dry wt of feed}) - (\text{N of feces per unit dry wt} \times \text{dry wt of feces}) - \text{urine N}$$

+ Gross Energy

or

Nitrogen balance = N in feed - N in feces - N in urine

However, for extremely precise work, the material lost through perspiration and cutaneous material should be taken into account (cutaneous nitrogen). For some types of research, the nitrogen in the products synthesized -- such as milk, eggs (for poultry), or wool (for sheep) -- should be considered. For a carbon and energy balance the gases need to be considered.

Reference

Harris, Lorin E. 1970. Nutrition research techniques for domestic and wild animals. Published by L. E. Harris (in press).

A DIGESTION AND BALANCE TRIAL FOR SHEEP

Where possible use animals for which you wish to have the data apply. If sheep are raised in your country and you wish to have the data apply to sheep, use them for collecting in vivo data. The procedure should be modified to fit local conditions and to obtain the desired data from the experiment.

Principle

The animal is fed a quantitative amount of feed for a definite period of time and the excreta resulting therefrom are collected quantitatively for chemical analyses. The excreta are preserved by refrigeration or by freezing to prevent decomposition until they are analyzed. By analyzing the feed and feces, digestion coefficients and digestible nutrients can be calculated. By further analyzing the urine and products such as milk or wool the amount of some of the nutrients absorbed and retained in the body or stored as a product may be calculated by difference.

Apparatus

(a) Metabolism cages. Use metabolism cages which permit the giving of a quantitative amount of feed and water and the quantitative collection of feces and urine.

(b) Urine containers. Use glass or plastic containers to collect urine under the metabolism cage. Use 20 liter glass or plastic bottles to

store the urine during the collection period.

(c) Feces containers. Use glass or plastic containers to collect feces under the metabolism cage. Use 4 liter cans or plastic buckets to store the feces during the collection period.

(d) Feces stirrer. Use a vertical dough mixer (large Hobart type mixer or equivalent). The action of this mixer breaks up the feces pellets so they do not need to be ground. If such a mixer is not available use a large electric meat grinder or a hand food grinder.

(e) Scales. To weigh animals, feed, feces, and urine.

(f) Containers. Use bottles with air tight lids or heavy plastic bags for collecting samples of grain, and for holding partial dried feces samples before grinding them in the Wiley mill. Use 250 ml glass or plastic bottles fitted with air tight lids to hold samples after they have been ground through the Wiley mill. Use glass or plastic bottles for urine samples.

(g) Use air tight, large plastic bags, plastic containers or 20 liter cans for forage samples.

Feeding and Watering

(a) Weigh an amount of feed (forage or grain or mixed ration), the afternoon before it is to be fed, which will last the animal for one day and put it in a container in front of the animal. This makes it possible to feed all animals quickly the next morning.

(b) Arrange to feed the animals at the same time each day (preferably between 7:00 and 8:00 a.m.).

(c) If a balance trial is to be conducted and the water contains materials which are to be studied, it is necessary to weigh and analyze

the water for these materials (this is especially true for trace minerals). Weigh the water the animal drinks. Fill buckets with water and set these on the scales and obtain the gross weight. Let the animal drink twice daily at the same time each day. After the animal has drunk its water, again weigh the buckets with the remaining water and record the weight. If the metabolism cage or stall has a movable water container, it may be filled and weighed in the morning and then weighed again the next morning.

Preliminary Period

(a) The purpose of the preliminary period is to acquaint the animal with the metabolism cage or stall, to make the necessary adjustments in order that the feces and urine are collected properly, and to adjust the animal to its intake of feed in relation to the excretion of feces and urine. It is difficult to set a definite length of time for this preliminary period since it depends upon when the animal adjusts itself to eating a definite amount of feed. If the animals are not trained, it may take 3 to 6 weeks to acquaint them with the procedures before they begin to eat a definite amount of feed or until the conditions of the experiment are met. If the animals are trained, they should be on the preliminary period for at least 10 days prior to the beginning of the collection period. This refers to the time they are receiving a definite amount of feed.

(b) Treat the animals for external parasites if necessary, and worm them at least 10 days before the preliminary period starts.

(c) It is preferable to keep the animals in slotted floor pens during the first part of this period.

(d) During the preliminary period the animals should be cleaned

externally to avoid the possibility of any foreign material contaminating the feces and urine collections.

Collection Period

(a) Transfer the animal to the collection cage 7 days before the collection period begins.

(b) Weigh the animal 3 days before the collection starts and the morning after the feces and urine are collected to end the period. The weighing should take place at the same time each day, preferably before feeding at about 7:00 a.m.

(c) The collection period usually runs for 7 consecutive days, but it may be varied depending on how the animal eats and the information wanted in the experiment. Keep track of the exact time the period starts and ends. Arrange to end the experiment so there are 24 hours per day.

(d) The afternoon before the collection is initiated the backs of the cages and the collection area should be cleaned thoroughly.

(e) The collection period should begin on the morning after the animal has been eating a constant amount of feed for at least 10 days.

(f) During the collection period, save a certain percentage of feed sample at the time it is weighed out for feeding. Start saving this sample two days before the collection of feces begins and end the collection two days before the collection of feces stops.

Collection and Sampling of Feces, Urine and Milk

(g) The collection of feces, urine and milk (if the animal is lactating) should be started 48 hours after the collection period begins. In this procedure it is assumed that all the feces are collected. Collect consecutive

samples. A good plan would be to remove the feces and urine containers beginning at 8:00 a.m. each morning and replace them with clean containers. Collect feces and urine for 7 consecutive days, providing feed intake and collections have been normal.

(h) Save all feces for the entire collection period. Keep in refrigerator at 1° C. If space is a problem, weigh and save 20%.

(i) Add 150 ml of 1 to 4 HCl to each urine collection container each day. Save all the urine for the entire collection period in the glass or plastic bottles. Keep in a refrigerator at 1° C. If space is a problem, weigh and save 20%.

(j) If the animal is lactating and a balance trial is being conducted, weigh the total amount of milk each day. Sample by saving an aliquot in a glass or plastic bottle. Keep samples in refrigerator at 1° C.

(k) Weigh the animal 3 days before the collection period starts and the morning after the feces and urine are collected to end the period. The weighing should take place the same time each day, preferably before feeding at about 7:00 a.m.

Sampling Feces

(a) Remove all 7 days' collection of feces from container by transferring to the bowl of the vertical (Hobart) dough mixer. Mix for three minutes (or until the pellets are broken up). Weigh to obtain total weight. Divide by the number of days on collection to obtain the daily weight.

If a Hobart type mixer is not available, weigh the feces, empty into a tin-lined box with sloping ends about [illegible] x [illegible]. Mix the feces with a hoe or with your hands (with rubber gloves on). Select a random sample of about 2 liters. Grind this sample through an electric meat grinder or use a hand food chopper. Mix the sample after grinding with your hands. Work fast to prevent loss of moisture.

(b) Be prompt in obtaining two samples in 500 ml sampling bottles to prevent loss of moisture. Take them to the laboratory immediately and determine dry matter, nitrogen and energy on the fresh feces without partial drying (see "Handling samples for differences", page [illegible]).

Analyze each sample [illegible] has been done [illegible] the stored feces [illegible] samples from [illegible]

(c) [illegible] freeze. This [illegible] complete.

(d) Weigh [illegible] Analyze this sample for [illegible]

[illegible]

(a) Weigh [illegible] a sample in [illegible] fill completely [illegible] immediately [illegible]

(b) Sa[illegible] analyses are [illegible]

Sampling Orts or Weighback

(a) If the animal refuses to eat all its food during the collection period or if any is wasted by being thrown out of the manger, it should be saved.

(b) If the weighback is dry enough to grind, a weight should be taken prior to grinding. However, if water was spilled on the weighback or if it is wet from saliva, it should be dried in a forced draft oven (temperature 65° C), then placed in a room for 48 hours to come to moisture equilibrium with the air. Just prior to grinding it should be weighed.

(c) Grind the feed sample, orts or weighback and partially dry feces through a mill with a one millimeter screen (if trace minerals are to be run see "Trace Mineral Analyses," page 4101).

Calculations

(a) Apparent digestion.

Apparent digestion coefficient =

$$\frac{\text{nutrient in feed - nutrient in feces x 100}}{\text{nutrient in feed}}$$

Example:

$$\text{N}^{+}\text{ apparent digestion coefficient} = \frac{\text{(N of feed per unit dry wt x dry wt of feed) - (N of feces per unit dry wt x dry wt of feces) x 100}}{\text{N of feed per unit dry wt x dry wt of feed}}$$

(b) A balance is the relation of the material in the feed to the output of the same material. For most nutrition work, the feed, feces, and urine are considered:

A balance = material in feed-material in feces and urine.

Example:

Nitrogen balance (NB) =

(N in feed per unit dry wt x dry wt of feed) - (N of feces per unit dry wt x dry wt of feces) - Urine N

However, for extremely precise work, the material lost through perspiration and cutaneous material should be taken into account (cutaneous nitrogen). For some types of research, the nitrogen in the products synthesized--such as milk, eggs (for poultry), or wool--should be considered. For a carbon and energy balance the gases need to be considered.

Reference

Harris, Lorin E. 1970. Nutrition research techniques for domestic and wild animals. Published by L. E. Harris (in press).

+Nitrogen