FORAGE

LABORATORY METHODS

FOR

FEED ANALYSIS

International Center for Agricultural Research in the Dry Areas
ICARDA

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INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH
IN THE DRY AREAS (ICARDA)

Contents

PREPARATION OF SAMPLES FOR LABORATORY ANALYSES

Experience has shown that good laboratory techniques and the use of sophisticated instruments will be little value if the actual sample being analysed does not truly represent the original specimen.

The preparation of samples for analysis is a very important procedure and deserves special attention. It may be necessary to determine a particular chemical constituent in the fresh sample as soon as it is harvested. Many changes which cause degradation of certain constituents occur in the living plant material which is freshly harvested. Some of these changes can be prevented or controlled by taking special precautions at the time of sampling. Although certain analysis must be done on the fresh material, the presence of the excessive moisture often makes the analyses of fresh material difficult. Fortunately, many analyses are done after the fresh sample has been sub-sampled, dried and ground.

The type of analyses to be done on a particular sample dictates the method to be followed in sample preparation.

Washing

Plants contaminated with soil may be washed under running tap water or in weak solution of non-ironic detergent. This washing should be carried out very quickly to minimize loss of soluble constituents, and should be followed by rinsing with distilled water and drying with a cloth or paper tissue. Roots can simply be washed under running tap water and dried with cloth. Silage and sugar beet pulp must not be washed because of the rapid loss of sugars and other soluble constituents.

Sub-sampling

With small samples, sub-sampling is often unnecessary; large samples however, may be sub-sampled as indicated in the following paragraphs. To avoid loss of moisture, this should be carried out as quickly as possible.

Compound feeds and grains

The sample should be mixed on a clean non-absorabent surface and subsampled by quartering. To do this, form a flattened heap, divide the heap into quarters and reject two diagonally opposite portions. Mix the remaining material and repeat the quartering until a sample of 200-300g is obtained. Samples weighing only a few hundred grammes should be prepared for analysis.

Fresh Herbage

Chaff should be cut into short lengths, throughly mixed and quartered down to approximately 200 g. If a laboratory chaff cutter is not available, mix the whole sample of a clean non-absorbent surface, sub-sample several hundred grammes and quarter this down to approximately 200 g. With small samples, take the whole of the sample for analysis.

Roughages

Roughages in long loose form cannot be satisfactorily sub-sampled. In the absence of a suitable laboratory corer, adopt the same sub-sampling procedure as for fresh herbage. Coring may be achieved in the laboratory by compressing the sample into a suitable box having a movable partition and using a corer, preferably of stainless steel, designed so that it can be operated with a variable speed electric hand drill.

When the roughages are received in the laboratory in the cored state, it should be mixed thoroughly before sub-sampling. Samples weighing less than 300 g should be prepared for analysis without subsampling.

Kale and other Green Brassicas

Three procedures can be used to sub-sample these materials:

a. chop the whole plants, with leaves attached, into short lengths and sub-sample the chopped material.
b. split large whole plants, with leaves attached, and divide into two four or six sections depending on stem thickness. Chop the split sections and sub-sample the chopped material.
c. remove the leaves from the stems of the plants. Weigh, chop and sub-sample the leaves and stem separately.

Take a sub-sample weighing approx. 200 g for analysis.

Roots (beet, potatoes, etc.)

Sub-sample by taking a 10 mm core or a diagonal slice approx. 5 mm thick through the root across the shoulder. Chop the slices or cores into 1 cm length and mix the pieces. Take a sub-sample of 200 - 400 for analysis.

Sliage

If the sample is cored, break up the cores and mix throughly. If the sample is in long form, treat as for fresh herbage. It is particularly important to sub-sample without delay.

Drying

Sub-sampling is followed by drying and this operation to a large extent can affect the results of subsequent determination, especially those involving sugars, vitamins and certain trace elements. It is not correct to simply place material in a hot oven until it feels dry. Different samples require different drying temperatures and drying times. Various ovens are used for such purposes and one with a forced-draught system is essnetial for fresh plant material.

As a general procedure, weigh 200 g. of samples into a suitable clean

metal tray, or cloth bag and dry as indicated in the following table.

Material	Drying time (hours) At 60°C	At 100 ± 2°C
Herbage, silage, hay		18
Brassicas		18
Sugar beet, carrots, turnips	48	
Potatoes	24 followed by	18
Cereal grains		40
Milled cereal and compound feeds		24

Samples for the determination of sugars and vitamin E should not be heat dried at all; air drying at 30°C in a forced-draught oven is often a reasonable compromise for these samples. At times there may be a small loss of dry matter due to respiration, but this is insignificant for most purposes.

Crinding

It is often necessary to analyse powdered or finely ground samples and a variety of equipment is available for satisfactorily producing homogeneous powders from the dried samples. The most frequently used grinder or hammer mills are of the 8 in type. The feeding chute to the mill should be made of plastic. It is very important to clean the mill thoroughly between samples in order to avoid cross contamination. When a bag is used as the receiver of ground material it must be turned inside out, shaken and brushed with a nylon brush to remove adhering plant particles before being used for the next sample. The fineness of grinding can be important but, for general routine analyses samples milled to pass a 1 mm mesh sieve are

usually satisfactory. The entire dried sample must be ground, collected from the mill and thoroughly mixed.

The dried and ground samples should be stored away from direct sunlight in glass or plastic jars one with baskelite screw caps or in sealed polythene bags.

FEEDING-STUFF ANALYSIS

The analysis of a feeding-stuff usually involves what is known as a "proximate analysis". Such an analysis determines the precentages of water, ash, crude protein, ether-extract (oil) and crude fibre in the feed. The total of these percentages subtracted from 100 gives the percentage of nitrogen-free extract (N.F.E.), which is included in the proximate analysis. This scheme of analysis is usually supplemented by determinations which aim at a sub-division of the above fractions, e.g. the insoluble silica,calcium and phosphorus contents in the ash fraction, the true and digestible crude protein (D.C.P.) contents in the crude protein fraction etc.

Many of the methods used in feeding-stuff analysis are conventional and as they do not estimate definable chemical substances but conventionally defined fractions, accurate results depend on the strict adherence to the standardised procedures.

Sampling and preparation for Analysis

Before undertaking an analysis the results of which are to be used to represent the composition of a crop or a consignment of a feeding-stuff , you should assure yourself that the sample originally undertaken was sufficient in amount and that is was selected properly from the bulk so as to be fairly reprenentative of it. Sampling is, however, not a laboratory operation, and no more need be said about it here. The sample supplied has, if necessary, been dried sufficiently to enable it to be finely ground; it should always be well mixed before withdrawing any portion for analysis.

1. Moisture: Determine the water content by weighing out accurately about 2 g. of the sample into a silica or porcelain dish which has been previously ignited and weighed, and drying in the oven for 24 hours at 105^{o}C. Cool in a dessicator and weigh. Use this dried portion for the determination of ash and silica.

2. Ash: Char the residue over a flame and then ingite in the furnace at a dull red heat (about 550°C) until the ash is grey or nearly white. Cool and weigh.

3. Silica: Moisten the ash with 50% (by vol.) hydrochloric acid, and take to dryness on a water bath. Remoisten with conc. hydrochloric acid , again take to dryness and bake on a water bath for 2 hours. Add a few ml. 30% (by vol.) hydrochloric acid, and continue to heat on the water bath for 5-10 mins. Filter through a 12.5 cm. Whatman No. 1 paper, and wash well with hot water. Return filter paper and contents to dish, dry over a small flame, and ignite in the furnace at low red heat. Weigh and calculate per cent weight of silica, making allowance for the filter paper ash.

4. Ether-extract ('oil'): Fit up a Soxhlet extractor with reflux condenser and a small flask which has been previously dried in the oven and weighed. Accurately weigh about 3 g. of sample and transfer to a "fatfree extraction thimble", plug lightly with cotton wool, place the thimble in the extractor and add low boiling petroleum ether* (B.P. 40-60°C) until it siphons over once. Add more ether until the barrel of the extractor is half full, replace the condenser, see that the joints are tight and place on the heater. Adjust the source of heat so that the ether boils gently and leave it to siphon over. Leave overnight. Finally, watch until the ether is just short of siphoning over, then detach the flask and siphon the contents of the barrel of the extractor into the ether stock bottle. Drain well. Remove the thimble and dry it on a clock glass, away any flame.. Replace the condenser and flask and continue distilling the ether until the flask is practically dry. Detach the flask (which now contains all the 'oil') from the extractor and dry it in the oven to constant weight. Keep the extracted residue for the 'fibre' determination.

* Petroleum ether is a mixture of low boiling hydrocarbone and is not a true 'ether' in the proper chemical sense.

5. Crude Protein: Weigh out 2 g. of the feeding stuff into a Kjeldahl flask. (For high protein cakes, etc. 1 g. will be sufficient). *Add 10 g. anhydrous potassium sulphate, 0.6 g. of copper sulphate and 0.1. g. of selenium, followed by 30 ml. conc. H_2SO_4. (The practice adopted in this department is to use 2 pellets, each weighing 5.0 g. and containing the appropriate amounts of K_2SO_4, $CuSO_4$ and Se). Place the flask over a small flame on the special Kjeldahl digestion rack and heat gently for 5 - 10 minutes. It is essential that the fume extraction fan should be running. During this period watch very carefully for signs of excessive frothing and if this occurs, turn out the burner and gently swirl the contents of the flask. When frothing has ceased, a stronger flame may be used and the contents of the flask digested until they are clear. Any black particles of carbon must be removed from the sides of the flask by occasionally shaking the flask with a swirling motion. Digest for a further one hour at full heat after the solution has cleared. Cool and before absolutely cold, dilute with 50 ml. water. Add the water carefully, keeping the neck of the flask pointed away from the face. Transfer to a 250 ml. graduated flask. Wash out the Kjeldahl flask several times; add all washings to the flask. before finally making to the mark, mix the contents of the flask so that contraction caused by the mixing of sulphuric acid and water takes place. Finally make to the mark and mix thoroughly. Determine $\%N_2$ in sample using the Micro-Kjeldahl apparatus.

* An alternative catalyst mixture, the one recommended in the Fertilisers and Feedingstuffs Regulations 1968, consists of 10g. anhydrous potassium suplphate and 0.5 g. of mercuric oxide.

THE USE OF THE MICRO-KJELDAHL DISTILLATION APPARATUS

The particular form of apparatus used in these laboratories is the Markham Distillation Apparatus which allows volatile substances (in the present case - ammonia) to be steam-distilled with complete collection of the

distillate. Provision is made for the introduction of strong alkali to bring about the release of ammonia from the previously acid solution.

Reagent 1% Boric acid solution containing 25 ml per 1. of the following mixed indicator:
2 parts of 0.2% Methyl Red + 3 parts of 0.2% Brom-cresol Green.

Procedure Steam out the apparatus for about 10 minutes before use. Remove the steam generator from the heat source and the developing vacuum will remove the condensed water. Place steam generator on heat source and pipette 5 ml. (or other known quantity of the nitrogen-containing digest) into the body of the apparatus via the small funnel aperture. If the mercury catalyst has been used 1 ml. of 1% solium thiosulphate should be added to prevent the formation of mercury-ammonia complexes.

At the receiving tip of the condenser place a 100 ml. conical flask containing 10 ml. of boric acid plus indicator solution. When all is ready, carefully lift the funnel plug and allow most of the caustic soda to run into the apparatus. To prevent any violent "Suck-back", hold the receiving flask just clear of the condenser tip while the caustic soda is running in, but quickly return it to its normal position as soon as possible. Continue the distillation for 2 minutes, at the end of which time, hold the flask clear of the condenser tip and allow 10 secs. for drainage from the condenser.

Titrate the contents of the receiving flask using 100 N (0.01 N) hydrochloric acid. The boric acid, while preventing loss of ammonia by the formation of ammonium borate, is a very weak acid and does not affect the indicator in any way . The titration may then be thought of as being essentially between ammonium hydroxide and hydrochloric acid.

$$1 \text{ ml. of } \frac{N}{100} \text{ (i.e. 0.01 N) HCl} = 0.00014 \text{ gm. } N_2$$

The % N_2 is multiplied by $\frac{100}{16}$ (i.e. 6.25) and the result called crude protein. This is based on the fact that protein is assumed to contain 16% N_2 .

6. Crude Fibre: In a 1000 ml. conical flask fitted with a 'cold finger' condenser place the residue from the ether extract determination, add 180 ml. of water and bring to the boil. Then add 20 ml. of 2.55 N. sulphuric acid (giving 200 ml. of 0.255 N. H_2SO_4), bring rapidly to the boil and note the time when boiling recommences. Adjust the flame so that the liquid boils gently and continue for 30 minutes. Keep rinsing back particles from the sides of the flask into the liquid by imparting a circular motion to the flask. Meanwhile, moisten a hardened filterpaper (Whatman No. 54 or equivalent) with distilled water and fit it into a Hartley-Buchner funnel. Pour in enough 'Celite' suspension to give approximately a 1/16 to $\frac{1}{8}$" layer of solid Celite in the funnel. Wash the layer with hot water, sufficient to heat up the Buchner funnel itself. At the completion of the 30 minutes boiling period, pour the digest into the funnel and filter by suction. The time of filtration should not exceed 10 minutes. Wash the residue with boiling water until the washings are free from acid. Remove the top part of the funnel and cut through the Celite layer with a spatula, transferring the feed residue + Celite back to the digestion flask. Pour 180 ml. of boiling water into the flask, using a little of it to wash down the spatula etc. Bring to the boil, add 20 ml. of 3.13 N sodium hydroxide (giving 200 ml. of 0.313 N. NaOH), bring rapidly back to the boil gently for 30 minutes. Repeat the filtration exactly as previously described, transferring the whole of the material to the filter. Wash with boiling water, then with 1% hydrochloric acid and again with boiling water until free from acid. Then wash the residue twice with 95% alcohol, and then three times with petroleum ether, using quite small quantities. Again, remove the top part of the funnel and cut through the Celite layer transferring the fibre residue + Celite to a silica dish and dry in an oven at 105°C to constant weight. Ignite to burn off all organic matter, cool and weigh. The loss on ignition is crude fibre.

This facilitates the clean removal of residues from the paper and may be used both for the acid and alkali filtrations.

7. Trichloroacetic Acid Method for Crude Fibre Determination

(Whitehouse, Zarrow and Shay J.A.O.A.C. 28(1) 1945)

This is an alternative procedure to the more conventional crude fibre method.

Reagent

Trichloroacetic Acid Digestion Reagent

Mix 500 ml. glacial acetic acid, 450 ml. water and 50 ml. conc.nitric acid. Dissolve 20 g. trichloroacetic acid in this mixture.

Method

Weigh out 1 g. milled material into a 500 ml. conical flask. Add 100 ml. digestion reagent, washing down the sides of the flask. Bring to the boil and reflux for exactly 40 minutes, counting from the time heating commenced. Use a 3 feet long air condenser or a water jacketed condenser to prevent loss of liqued. Remove the flask from the heater and cool under a cold tap. Filter through a prepared Gooch crucible. Wash 5 times with hot water and once with industrial spirit. Dry overnight at 105^{o}C.Transfer to a desiccator and weigh when cool. Ash at 600^{o}C to completion. Allow to cool and reweigh.

% Fibre = Difference in weighings x 100.

8. Nitrogen-free Extract : Add up the percentages of moisture ash, ether extract, crude protein and crude fibre, and subtract the total from 100. The difference is the percentage of nitrogen-free extract.

Calculate all results as percentages of the feed on an 'as received basis'. Recalculate the results as percentages of the feed on a 'dry matter basis'. Tabulate the results as follows:

Analysis of sample.

	% (as received basis)	% (dry matter basis)
Moisture	-	
*Ash	-	-
Crude Protein (C.P.)	-	-
Crude Fibre	-	-
Ether Extract	-	-
Nitrogen-free Extract	-	-
	100.0	100.0
* Includes Silica	-	-

Determination of True Protein

The nitrogenous compounds of feeding-stuff, apart from proteins, include amides, amino acids and perhaps ammonium salts and nitrates. In root crops particularly, a high proportion of nitrogen is present in these forms.

To determine the true protein, advantage is taken of the fact that the non-protein nitrogenous substances are soluble in water and that soluble proteins are precipitated by certain metallic salts e.g. Copper salts, in alkaline solution.

Reagents: (a) 6.0% Copper Sulphate Solution
(b) 5.0% Sodium Hydroxide Solution

Method

Weigh out 2 grams of sample into a 250 ml. beaker. Add 75 ml. hot water and 1 ml. of 50% hydrochloric acid and boil for 10 minutes. Stir vigorously and add 25 ml. of the 10.0% copper sulphate solution. Bring again to the boil, stir vigorously, and add 10 ml. of the 5.0% sodium hydroxide solution. Stir vigorously, remove from the flame and allow to settle.

Filter under reduced pressure, through a hardened filter paper supported in a Hartley-Buchner funnel. Clean the ppt. from the sides of the beaker with a "policeman". Wash the paper free from sulphate with very hot water (about 6 washes). Allow to drain well, and then transfer the residue and paper to a Kjeldahl flask and determine nitrogen as for ' Crude Protein'.

Determination of the Percentage Digestible Crude Protein

(Wedemeyer)

The percentage of digestible crude protein is determined chemically by simulating the conditions in the stomach, i.e. pepsin in acid solution maintained at body temperature. After a 48 hour digestion period the protein content in the undigested residue is determined. This value subtracted from the original crude protein content gives the amount of digestible crude protein in the feed.

Method

Weigh 2 grams of sample and place it in a 600 ml. Phillips conical beaker. Dissolve 1 gram of pure pepsin in 490 ml. distilled water and add it to the sample. Add 10 ml. 25% HCI (by weight) and place beaker and contents in an incubator (37-40^{o}C). At the end of exactly 24 hours add another 10 ml. of the acid, stir well, and return to the incubator. At the end of the second 24 hours filter under reduced pressure through a hardened filter paper supported in a Hartley-Buchner funnel and wash the residue until free from acid.

Transfer the filter paper and residue to a Kjeldahl flask and proceed to determine nitrogen by the usual method.

Digestible crude protein = % total crude protein - % crude protein present on the filter paper.

Determination of Calcium and Phosphorus in a feeding-stuff.

Calcium is most commonly determined by precipitating the element as calcium oxalate,

$$Ca^{++} + H_2C_2O_4 \longrightarrow CaC_2O_4 + 2H^+$$

Which being insoluble in hot water can be filtered and washed clear. The oxalate is then dissolved in acid, yielding oxalic acid, which may be titrated with potassium permanganate in the standard manner.

Phosphorus, as phosphate, may be determined by the formation of the yellow vanadomolybdophosphate or the blue molybdophospate, the concentration of either being estimated by spectrophotometry.

Spectrophotometry, which uses a narrow hand related to a definite wavelenght, and colorimetry, which uses a broader band selected by a filter, are based on Lambert's and Beer's Laws. These may be summarised by the statement that monochromatic light passing through a coloured medium will be absorbed in proportion to the thickness of the medium and the concentration of the coloured constitutuent. The relationship is logarithmic, so that

$$\log \frac{\text{(Intensity of incident ligh)}}{\text{(Intensity of transmitted light)}} = Kct$$

where c is concentration and t = thickness

Both the colorimeters and the spectrophotometers use the response of a photo-electric cell to measure density.

Preparation of Extract

Weigh out 5 gms. of feeding stuff into a silica or porcelain dish (2 g. if fish or meat and bone meal are present) and ash the sample over a bunsen burner. Complete the ashing in the furnace for 1 - 2 hours at about 550°C. Allow to cool, moisten with water. Add 1 ml. of conc. HCI and take to dryness in a water bath. Add a further 1 ml. of conc. HCI and dry again. Extract the residue with 30% HCI for 15 mins. over a small flame. Filter through a 12.5 cm. No. 1 filter paper into a 250 ml. graduated flask. Wash the residue on the paper with warm distilled water, allow the contents of the flask to cool, and make to volume.

Calcium

Pipette out two separate 100 ml. aliquots into two 400 ml. beakers (retaining the 50 ml. of extract for the phosphorus determination) . Add a few drops of methyl red as indicator. Make just alkaline with 10% ammonia and just acidify with 15% acetic acid. Bring to the boil and and add about powdered ammonium oxalate. Boil for 1 minute, transfer to a water bath and leave for 30 mins. Filter through a No 1 paper (12.5 cm) and wash 6-8 times with hot water. Transfer the paper back to the beaker used for the precipitation and dissolve the calcium oxalate in 15-20 ml. of bench (5N) H_2SO_4 . Add 100 ml. of hot water, heat to 70°C and titrate to pink with $\frac{N}{10}$ $KMnO_4$.

$$1 \text{ ml } \frac{N}{10} \ KMnO_4 \ \begin{matrix} - \\ - \\ - \\ - \\ - \end{matrix} \ 0.0028 \text{ g CaO}$$

$$\text{or} \ \begin{matrix} - \\ - \\ - \\ - \\ - \end{matrix} \ 0.0020 \text{ g Ca}$$

N.B. The most frequent error in this determination is inadequate washing of the calcium oxalate precipitate and its separation from the ammonium oxalate reagent. As a result the reagent oxalate is also titrated by permanganate giving a result which is too high.

Phosphorus (vanadomolybdophosphate procedure)

(Note: As it is important that the volume of extract used in this determination should produce a colour density within a restricted range, it is not possible to give an aliquot volume which will be suitable for all samples - discuss this point with the demonstrator).

Transfer two 2 ml. aliquots of the acid extract to 100 ml volumetric flasks. Add 10 ml. of ammonia vanadomolybdate reagent and make up to volume with distilled water. Shake the flasks and leave on the bench for about 30 minutes.

Using an SP 600 spectrophotometer, read the optical density at 400 mu and subtract the density of a blank prepared as above, using distilled water instead of the ash extract. Determine the concentration of phosphate by comparison with the provided standard phosphate solution which must be processed exactly as for the sample.

Determination of Manganese in a feeding-stuff

Manganese occurs in plant material in very variable quantity, usually to the extent of between 50-250 p.p.m. In general, plants growing in acid soils contain more manganese than those in neutral or alkaline soils.

The chemical determination of manganese in plant material is based on its oxidation to potassium permanganate, the colour of which is proportional to the amount of manganese present. The intensity of colour is measured on a photo-electric colorimeter and compared with a standard calibration graph, or with prepared standards.

Method

Weigh 5 g. of material into a silica or porcelain dish, char over a bunsen flame and ash completely in a furnace maintained at 550°C. Carefully moisten the ash with 50% HNO_2 plus a few ml. of water. Cover with a

watch-glass and allow to digest for a few minutes on a water bath or over a very small bunsen flame. Filter though a small filter paper collecting the filtrate in a graduated 100 ml. flask. Wash with small amounts of hot water, the final volume of filtrate being about 30 ml. Add 5 ml. of conc. phosphoric acid to prevent interference by the ferric ion and about 0.3 g. of potassium periodate (the oxidasing agent). Heat in a beaker of boiling water until the colour of potassium permanganate forms. At this stage, add a further quantity of water to make the volume up to about 90 ml. and re-heat for 5 mins. Finally cool and make to volume, shaking the flask well. Measure the colour intensity on the colorimeter and determine the manganese content in p.p.m. (mg per kg) by comparison with the colour intensity of the standard manganese solution provided which must be processed exactly as the sample.

The Estimation of Carotene in Feeds

Reference : NELSON, W.A.G. (1947) . Analyst. 72, 200
(This method is only suitable for use on dried samples)

Reagents : (1) Bone meal A satisfactory bone meal, standardised for carotene estimation, is prepared by B.D.H.

(2) Petroleum Ether 80-100^{o}C boiling fraction.

Apparatus: Chromatographic Column Loosely plug a Buchner adaptor tube with glass wool and poor into the tube sufficient bone meal, mixed into a slurry with a little petroleum ether, to fill it to a depth of about 2 inches after draining off the ether. The settling of the bone meal may be assisted by gentle suction. A very thin layer of cotton wool is applied to the top of the column.

Stock Carotene Solution : 20 mg. of purified carotene crystals are placed in a litre flask with 20 ml. of pure di-ethyl. Petroleum ether is added and the crystals allowed to dissolve. The solution is made to volume with petroleum ether (1 ml. = 20 ug B - carotene).

Preparation of Transmittancy and Standard Curve

Prepare a series of diluted standards ranging from 0 - 100 ug carotene/100 ml. and construct a transmittancy curve and standard curve using the Unicam Spectrophotometer. In preparing both curves, as well as in subsequent readings, use petroleum ether to zero the instrument in place of water.

Method: 2 g. of dried milled material are boiled with 50-60 ml. of petroleum ether (80-100^{o}C) in a small flask under a reflux condenser for 1 hour. The flask and its contents are cooled and then decanted on to the surface of the column. Suction is applied to the column which is further eluted with petroleum ether. The eluted petroleum ether is made up to a suitable volume (preferably 100 ml.) and the optical density measured in the spectrophotometer.

Note

The chromatographic column may be used for a number of determinations if it is washed, between experiments, with a solution containing 3 volumes of petroleum ether (40-60^{o}B.pt): 1 volume of acetone: 1 volume of di - ethyl ether.

Determination of Detergent Fibre and Detergent Lignin Fractions in Forages.

(Van Soest. U.S.D.A.)

Despite the very considerable use which has been made of the conventionally determined crude fibre content of feeds in predicting their digestibility, there has always been concern that the fiber fraction on determined has no chmical identity. In addition, the conventional crude fibre

procedure is a somewhat tedious one. Many alternative methods have been proposed by which definite fractions such as cellulose or lignin are estimated but many of these are fairly involved.

However the following two methods which show considerable promise and in which a quaternary ammonium detergent, hexa-decyltrimethylammonium bromide (CTAB), is used as reagent are reasonably suitable for class-work .

It should be pointed out that although the 'fibre' and 'lignin' fractions obtained by these methods are more chemically justifiable than that obtained by the conventional crude fibre method, nevertheless, they are still to some extent empirical and should be always prefaced by the term 'detergent'.

Note: While the lignin method follows on form the fibre method, both fractions cannot be obtained from the one sample. Two initial samples should therefore be taken, one will stop at the fibre stage while the other will continue through to lignin.

Detergent Fibre Procedure

Reagent: 2 percent detergent (hexadecyltrimethylammonium bromide) in 1.O.N. H_2SO_4 .

Method : Weigh a 1 g. sample into a 250 ml. wide-mouthed flask.Add 50 ml. of the acid-detergent reagent and heat on a hot-plate or over a small flame so that boiling commences in 10-12 minuts. Continue boiling at a higher heat for 60 minutes. Filter through a sinterd glass crucible, or through a gooch crucible prepared with an asbestos pad, using gentle suction. Wash with hot water followed by several washings with acetone. Dry and weigh; determine the weight of fibre as the loss in weight on ignition at $500^{o}C$.

Detergent Lignin Procedure

Additional Reagent : 72 percent H_2SO_4 by weight.

Method : Proceed as for fibre method up to the point of filtering through a sinterd glass crucible including the washing stages. Allow the fibre to air-dry (DO NOT ASH). To the crucible add a small quantity of asbestos and sufficient 72 percent H_2SO_4, previously cooled to about 15^oC to cover the contents. Stir with a 3-inch glass rod which is allowed to remain in the crucible during the determination. Make sure that all particles come in contact with the acid by breaking up any lamps. place the crucible in a 50 ml. beaker for support and for receiving the acid which slowly percolates through. Maintain the temperature as close to 20^oC as possible; stir occasionally and add fresh acid as necessary. At the end of about 3 hours, return the crucible to a suction flask and remove the acid without washing. Wash the residue thoroughly with hot water to remove all traces of acid. Dry overnight at 105^oC and weigh. The lignin is determined as the loss in weight on ignition at 500^oC .

THE VISUAL ASSESSMENT AND CHEMICAL EVALUATION OF SILAGE

While chemical analysis remains the best available method of assessing the probable nutritive value of silage, short of carrying out a feeding trial, visual assessment can be extremely useful. The present schedule aims at comparing these two approaches when applied to one or more samples of silage of varying types.

Visual Assessment (acknowledgements to N. Trinder, N.A.A.S. Nutrition Chemist, Northern Region, U.K).

Examine the silages provided for colour, smell and taste; note the physical from and texture. Using pH test papers (range - about 3.6 to 5.1pH) attain an approximate pH of the silage. The test paper should be pressed between two handfuls of silage and the colour compared with the chart provided.

On the basis of these observations decide to which of the following four categories the sample belongs.

(a) Well-made silage (type I)

Colour : Olive-yellow; yellowish-brown; or khaki
Smell : Pleasant, fruity (ester-like), vinegary, raisiny
Texture : Firm
Taste : Sharp acid - like vinegar
pH : 3.6 - 4.3 unless showing signs of mould.

(b) Butyric Silage (type II)

Colour : Olive-green; greenish-yellow; or greenish-brown; dark green or bright green in severe cases.
Smell : Unpleasant, strong and clinging, often cheesy,
Texture : Wet very firm, Slimy in severe cases
Structure going in severe cases
Taste : Insipid, little if any acid taste, May be unpleasant.
pH : 4.4 - 4.7 if slightly butyric; 4.8 and over if butyric or very butyric .

(c) Overheated Silage (type III)

Colour : Golden-brown ranging through to brown, dark-brown and black.
Smell : Caramel, tobacco, charred, Occasionally of beer.
Texture : Very firm, even harsh in severe cases.
Taste : Usually acid, but not sharply acid.
pH : 3.6 - 4.3 unless moulding. 4.4 - 5.1 if beery.
Over 5.1 if moulding heavily.

(d) Composted Silage (type IV)

Colour : Very dark greenish-brown or black.
Smell : Ammoniacal or compost-like.
Texture : Slimy, structure almost gone.
Taste : Insipid
pH : Over 5.1

Visual Estimattion of Dry-matter content

The dry-matter content may be estimated by the ease with which juice can be expressed. Rubber or plastic gloves should be worn and a handful is squesszed with increasing pressure. From the following table estimate the dry-matter content.

Chemical Evaluation

One of the major problems associated with the chemical analysis of silage is in the obtaining of a representative sample from the whole silo.

Drying of Silage Sample

Most analyses are performed on dry, milled samples; however, if the pH of a silage is more than 4.4, it is usually to determine the nitrogen on a fresh sample. This is because such a silgae will have a considerable amount of volatile non-protein nitrogen which will be lost during the drying process. While lower drying temperatures than the usual 105^{o}C will reduce the loss of volatile materials these conditions usually lead to increased enzymatic activity in the sample. Ideally, a silage sample should be heated for a time at 80^{o}C to destroy enzymes and then at 65^{o}C for some hours.

Determination of pH

(a) Fresh silage

Take 100 g. of fresh, mixed, chopped sample in a beaker and add 100 ml. of distilled water. Boil for 5 minutes, decant off the supernatant liquid, and cool to room temperature. Determine the pH of the supernate using a glass electrode and pH meter.

(b) Milled dried silage

To 10 g. of dried sample in a beaker add 40 ml. of water. Warm the mixture at 60°C for 5 minutes stirring constantly. Filter and cool the filtrate. Determine the pH as in the previous section.

Compare your results with the following equation (Batnett 1957)

pH (dried silage) = -0.880 + 1.305 pH (fresh silage)

Total Nitrogen Content (Crude Protein)

(a) Fresh sample

Weigh out 10 g. of freshly minced or chopped silage and determine the total nitrogen by the Kjeldahl procedure.

(b) Dried sample

Weigh out 2 g. of dried silage sample and determine the total nitrogen by the Kjeldahl procedure.

(c) Determine the dry matter contents of the fresh and airdried samples, and compare the nitrogen contents.

Digestible Crude Protein (D.C.P.)

Determine the % D.C.P. by the Wedemeyer procedure as given previously under general feedingstuff analysis (page 8) .

Crude Fibre

Determine the crude fibre content of the silage using the Trichloroacetic Acid Method as given previously under general feedingstuff analysis (page 6).

Estimation of the Starch Equivalent (S.E)

It is usual to estimate the S.E. on the basis of regression equations taking S.E. with some other characteristics (s). The following table is based on such equations and has been devised by N. Trinder, N.A.A.S, Nutrition Chemist, Northern Region, U.K.

Note that some visual estimate of the approximate clover/grass percentages are required.

Use this table to estimate the S.E. of the sample and compare all the chemical data with that from the visual examination.

CALCULATION OF THE STRACH EQUIVALENT OF A FEED FROM ITS CHEMICAL ANALYSIS

If the chemical analysis of a feed is known along with the digestibilities of the chemical fractions, the S.E. may be calculated.

The digestibilities will either have to come from animal data or from tables of digestibilities such as published in " feeds of the world" (Schneider).

If 100 kg. of barley contains:

	Factors from Kellner
7.6 kg dig. Crude Protein	.94
1.2 kg dig. Ether Extract	2.10
60.9 kg dig. N.F.E.	1.00
2.5 kg dig. Crude Fibre	1.00

Then the S.E. equals

7.6	x	.94	= 7.2
1.2	x	2.10	= 2.5
60.9	x	1.00	= 60.9
2.5	x	1.00	= 2.5
			73.1

Therefore, the Starch Equivalent (theoretical) of this sample of barley is 73.1. In other words, 100 kg of this barley would have the same fattening potential as 73.1 kg of pure starch.

Corrections to Covert S.E. (theoretical) into S.E. (practical)

Roughages:

Fodder

	Deduct the following units of S.E. for each 1% Fibre. (N.B. Crude fibre not Digest C. Fibre).
Hay and straw - chopped	0.29
- long	0.58

Green fodders:

4.0 - 5.9 C. Fibre %	0.29
6.0 - 7.9 " " "	0.34
8.0 - 9.9 " " "	0.38
10.0 - 11.9 " " "	0.43
12.0 - 13.9 " " "	0.48
14.0 - 15.9 " " "	0.53
16.0 and over	0.58

Concentrates:

For concentrates a different system of correction is used. This involves the use of published 'V' values which range from about 80 - 95% .

$$\text{Calculated S.F.} \times \frac{V}{100} = \text{Corrected S.E.}$$

STARCH EQUIVALENT TABLE FOR SILAGE SAMPLES (% OF DRY MATTER)

Composition (% Grass=G ; Clover=C																	
%G	100	80	60	40	20	0	% Fibre in Dry Matter										
%C	0	20	40	60	80	100	21 22 23	24 25 26	27 28 29	30 31 32	33 34 35	36 37 38	39 40 41	42 43 44
% Crude protein in dry matter	18	19	20	21	22	23	61 60 60	59 59 58	57 57 56	56 55 54	54 53 53	52 51 51	50 50 49	48 48 47
	17	18	19	20	21	22	61 60 59	58 57 56	56 55 55	54 53 53	52 52 51	50 50 49	49 48 47	47 46 46
	16	17	18	19	20	21	61 60 58	57 55 54	54 53 53	52 51 51	50 50 49	48 48 47	47 46 45	45 44 44
	15	16	17	18	19	20	- 59 58	57 55 54	53 51 51	50 49 49	48 48 47	46 46 45	45 44 43	43 42 42
	14	15	16	17	18	19	- - 57	56 55 53	51 49 48	48 47 47	46 45 45	44 44 43	42 42 41	41 40 39
	13	14	15	16	17	18	- - -	56 54 53	51 49 48	47 46 46	45 44 44	43 43 42	41 41 40	40 39 38
	12	13	14	15	16	17	- - -	- 54 53	51 49 48	46 45 44	44 43 43	42 41 41	40 40 39	38 38 37
	11	12	13	14	15	16	- - -	- - 53	51 49 48	46 45 44	43 42 41	41 40 40	39 38 38	37 37 36
	10	11	12	13	14	15	- - -	- - -	51 49 48	46 45 43	42 40 39	39 38 38	37 36 36	35 35 34
	9	10	11	12	13	14	- - -	- - -	- 49 48	46 45 43	42 40 39	38 37 37	36 35 35	34 34 33
	8	9	10	11	12	13	- - -	- - -	- - 48	46 45 43	42 40 38	36 35 35	34 34 33	32 32 31
	7	8	9	10	11	12	- - -	- - -	- - -	46 45 43	42 40 38	36 35 34	33 33 32	31 31 30
	6	7	8	9	10	11	- - -	- - -	- - -	- 45 43	41 40 38	36 35 33	32 31 31	30 30 29

Less deduction for loss of disgestible crude protein

Example Laboratory Analysis 13% crude protein in dry matter
36% fibre in dry matter
Estimated clover content 20%

Then looking horizontally along the line containing the figure 13 under the 20 clover box and vertically down the line headed 36% fibre, the figure for stach equivalent = 42% in dry matter.

Note This table applied to grass and clover mixtures it also applies to arable silage to some extent.