Total Fat Determination in Feed

according to the Randall method

Reference:

UNI EN ISO 11085:2015 Cereals, cereals-based products and animal feeding stuffs -- Determination of crude fat and total fat content by the Randall extraction method

AOAC 2003.06 Crude Fat in Feeds, Cereal Grains, and Forages

Tested with **VELP Scientifica SER 158/6 Solvent AutoExtractor** (Code S303A0380) and **HU6 Hydrolysis Unit** (Code F30300110)

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TOTAL FAT DETERMINATION IN FEED
RANDALL METHOD

Introduction

Fat is an important nutrient in feed rations for cattle, pig, poultry, sheep, horse and pet foods, as a high energy feed ingredient. Fats and oils, contain about 2.25 times as much digestible energy as the carbohydrates in grain. They are very concentrated sources of energy when added to animal feeds to increase the energy density of the ration. Adding fats and oils will reduce the dustiness of feeds, and reduce 'fines' in pelleted diets, adding desirable characteristics which have value. Fats and oils can also improve a ration by improving palatability.
In particular, cow nutrition is dependent upon adequate energy, protein, vitamins and minerals in a balanced diet, but research is showing that fat content in a cow's diet can enhance rebreeding success.

Fat Determination in Feed

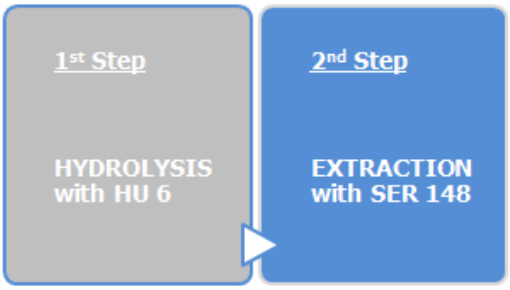
Hot solvent extraction process with SER 158 Series can be summed up in 5 steps, for a fully unattended operation:

During IMMERSION the sample is immersed in boiling solvent. Then the REMOVING step automatically lowers the level of the solvent to below the extraction thimble. During WASHING the condensed solvent flows over the sample and through the thimble to complete the extraction process. The fourth step involves solvent RECOVERY. Approximately 90% of the solvent used is collected in the internal recovery tank. The final step is the COOLING of the extraction cups containing the extracted matter. The cups are raised to prevent burning. The extraction cups containing the extract are placed in a drying oven, cooled in a desiccator and weighed for the extract percentage calculation.

Sample

Feed standard AAFCO Fat labeled range: 5.939 – 6.814 %

In order to determine the total fat in feed a direct extraction with solvents is not efficient because a part of lipids is chemically bonded with other components. The determination of the total fat content of these samples, requires a *preliminary hydrolysis* using hydrochloric acid 4 N, followed by filtration and washing, in order to free completely the fat molecules. Then the hydrolyzed sample is ready for the extraction and it can be easily transferred to SER 158 Solvent AutoExtractor avoiding any possible sample loss and ensuring improved accuracy of results (see the following picture).



Chemicals and Equipment Required

- Grinder
- Glass sand, 0.4-0.8 mm (Code A00000089)
- Celite 545 (Code A00000097)
- Glass crucibles P1 (Code A00000086)
- Hydrochloric acid 4N
- Test tube 250 ml (Code A000000144)
- Glass bottle for waste collector (Code A0000088)
- Glass extraction cups (Code A00000290)
- Vaflon seals (Code A00000288)
- Diethyl ether as solvent

All the accessories required for the hydrolysis are included in the kit code A00000085.

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Sample Preparation

- Hydrolysis Crucibles Preparation

Put 25 g of glass sand in glass crucibles P1 and 3g of Celite 545: the two layers resulting have not be mixed together otherwise the phase in Celite may bypass the crucible negatively affecting the end result.

- Sample Preparation in Test Tube

Grind the sample, reducing particle size to 0.75 -1 mm. Weigh 7 g of the crushed sample (M_{sample}) and 2 g of Celite 545 in the test tube. For each test tube add 50 ml of 4N hydrochloric acid, shake gently and carefully, and finally add a further 50 ml hydrochloric acid in order to rinse the sample residue that may remain on the walls of the test tube.

- Glass Extraction Cups Preparation

Keep the empty glass extraction cups in oven at 105 °C for 1 hour. Cool them in a dessicator and record the accurate weight of the tare (M_{tare}).

Hydrolysis Procedure with HU 6

Place 6 crucibles P1 on the HU6 and connect the aspirating tubes: one side needs to be placed on the sealing ring of the crucible and the other one has to be in contact with the corresponding test tube.
Set 170 °C for 60 minutes.
Place the 6 test tubes in the heating block, lower the glassware and activate the vacuum pump.

➢ *In case of foam, add 4 N hydrochloric acid drop by drop inside the test tubes.*

At the end of the procedure, switch off and allow aspiration of the content of the test tubes in the crucible.
Raise the glassware to its maximum point and secure it, by tightening the knob.
Then, add hot water (40-50 °C, about 250 ml) slowly, in order to aspirate all the residues of hydrolysis in the test tubes.
Remove the aspirating tubes and the crucibles containing the sample after the washing: mix the layer of hydrolyzed sample with the layer of Celite by using a spatula in order to break any clot.

➢ *Take care to not damage the layer of glass sand.*

This operation helps the sample drying, which must take place in an oven at 105 °C for a time between 1.5 to 6 hours.

If dried test portions will not be immediately extracted, put them in a desiccator, or let the crucibles cool to room temperature and then, mix the layer of hydrolyzed sample and Celite with a spatula to obtain a powdery hydrolyzed.

➢ *Take care not damage the layer of glass sand.*

Extraction Procedure with SER 158

Fix the crucibles with the crucible holders (Code A00000293). The extraction cups containing the crucibles can now be placed on the ultra-fast heating plate of SER 158.

On the ControlPad select “Analysis”, and then method “Total fat in feed” including the following parameters:

- Immersion Time: 55 minutes
- Removing Time: 10 minutes
- Washing Time: 55 minutes
- Recovery Time 30 minutes
- Cooling Time: 5 minutes
- Diethyl Ether, 100 ml

Close the safety guard and add the solvent using the automatic solvent dispensing system SolventXpress™ to minimize exposure to the solvent ensuring operator safety.
Press START to begin the extraction process. At the end of analysis position the extraction cups containing the extract in a drying oven (1 hour at 105 °C), cool them in a desiccator to room temperature and record the accurate weight (M_{tot}).

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Typical Results on Feed

Analysis results are calculated automatically and stored in the ControlPad when entering the weights into the software (manually or automatically through a balance). The extract percentage calculation is performed by using the following formulas:

Extract (g) = (Total - Tare)
Extract (%) = Extract x 100 / (Sample)

Where:

Sample = sample weight (g)
Tare= weight of the empty extraction cup (g)
Total = weight of the extraction cup + extract (g)

Tare (g)	Sample (g)	Total (g)	Extract (g)	Extract (%)
129.668	6.997	130.085	0.417	5.96
130.592	7.008	131.010	0.418	5.96
129.932	7.033	130.358	0.427	6.06
130.353	7.037	130.779	0.426	6.06
130.281	6.992	130.700	0.419	6.00
127.371	7.004	127.787	0.416	5.94
			Average ± SD%	**6.00 ± 0.06**
			RSD% **	**0.97**
Fat Labeled range: 5.939 – 6.814 %				

****** RSD% = (Standard Deviation x 100) / Average

Conclusion

The results obtained are reliable and reproducible in accordance with the expected values, with a low relative standard deviation (RSD < 1%), that means high repeatability of the results.
Therefore, SER 158 Solvent AutoExtractor is ideal for the fat content determination in feed.

Benefits of HU 6 Hydrolysis Unit are:
- safety with performance, reducing manual handling to the minimum
- no sample transfer required when passing from HU 6 to SER 158
- suitable for both acid and basic hydrolysis

Benefits of hot solvent extraction (Randall) by using 158 Solvent AutoExtractor:
- up to 5 times faster than Soxhlet (hot solvent vs. cold solvent)
- low solvent consumption (high solvent recovery, approximately 90%) - limited cost per analysis
- no exposure to solvent
- worldwide official method
- full traceability with automatic result calculation and on-board archive

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