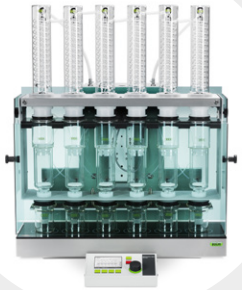
Application Note
No. 189/2015

Fat determination in Animal feed samples

Extraction Unit E-816 ECE:
Fat Determination in Animal Feed samples using Twisselmann extraction



1. Introduction

An effective procedure for fat determination in animal feed samples according to COMMISSION REGULATION (EC) No 152/2009 is presented [1]. The sample is hydrolyzed prior the extraction. The Twisselmann extraction is performed with the Extraction Unit E-816 ECE (Economic Continuous Extraction).This allows the sample to be constantly kept in hot solvent vapor whilst efficiently rinsed with freshly distilled solvent. After the extract has been dried to a constant weight the total fat content is determined gravimetrically.
The reference value was determined using Soxhlet extraction.

2. Equipment

- Extraction Unit E-816 ECE
- Analytical balance (accuracy ± 0.1 mg)
- Drying oven

3. Chemicals and Materials

Chemicals:

- Hexane, Carlo Erba (528949)
- Petroleum ether 40 °C – 60 °C, analytical grade, Carlo Erba (447831)
- 5 L of 3 M Hydrochloric acid (HCl) are prepared by dilution of 1.25 L HCl 37 % (VWR, 20252324) to 5 L with deionised water

Materials:

- Pleated filter paper MN 615 ff, Macherey-Nagel (591024)
- Paper thimble 33 x 94 mm, BUCHI (41883)

For a safe handling please pay attention to all corresponding MSDS!

Samples:

- Dry dog feed, specified fat content: 20.4 ± 1 g/100g
- Turkey feed, specified fat content: 9.3 ± 1 g/100g

The samples were purchased at a local supermarket.

4. Procedure

The determination of fat includes the following steps:

- Homogenization of the sample by crushing
- Acid hydrolysis of the sample
- Fat extraction of the sample following Twisselmann extraction, using the Extraction Unit E-816 ECE
- Calculation of the fat content

4.1. Homogenization of the sample

- The animal feed samples were crushed and then sieved through a 1 mm sieve [1]

4.2. Acid hydrolysis of the sample

1. Add the appropriate amount of sample1 to the digestion vessel and record the accurate weight of the sample
2. Add 100 mL hydrochloric acid (3 M)
3. Hydrolyze the sample for 1 hour
4. Collect the hydrolyzate in a filter and rinse with hot water until a neutral pH is reached
5. Put the filter paper into a cellulose thimble
6. Dry the filter in a drying oven (45 min at 100 °C)
7. Allow the filter to cool down to room temperature in a desiccator

4.3. Fat extraction of the sample following Twisselmann extraction, using the Extraction Unit E-816 ECE

4.3.1.Preparation of the beakers

Always use dry and clean beakers for the Twisselmann extraction. Add a boiling aid (e.g. boiling stones) to each beaker and dry them for at least 30 min at 102 °C. Let them cool down to ambient temperature in a desiccator for at least 1 h. Record the exact weight prior to extraction.

4.3.2.Twisselmann extraction

Put the sample tubes into the extraction chamber using the pliers. See Figure 1.

Figure 1: Twisselmann extraction chamber with sample before start

Fill the solvent into the beakers and place them on their corresponding heating plate. Close the safety shield and lower the rack. Activate the occupied positions, open the cooling water or switch on the connected chiller and start the extraction according to the parameters listed in Table 1.

Table 1: Parameters for the extraction with the Extraction Unit E-816 ECE

Method parameters Extraction Unit E-816 ECE	
Solvent	Hexane, Petroleum ether[2]
Extraction step	50 min (Heater 100 %)[3]
Drying step	10 min (Heater 100 %)[4]
Solvent volume	70 mL

[1] The sample weight has to be chosen according to the approximate fat content of the sample.
80-100 %: 0.7-1 g 20-50 % 1.5-3.5 g <10 %: 7- 10 g
50-80 %: 1-1.5 g 10-20 % 3.5-7 g
[2] Please select the solvent, which is the default in the menu.
[3] Choose the heater between 100 – 120 % so the boiling is sufficient.
[4] Choose the same parameter for the heater that was selected for the extraction step.

4.3.3. Drying of the extract

Dry the beakers containing the extract in a drying oven at 102 °C until a constant weight is reached. Let the beakers cool down to ambient temperature for at least 1 h in a desiccator and record the weight.

Make sure that the cooling down time of the beakers in the dessicator is the same before and after extraction. Differences in beakers temperature falsify the results.

4.4. Calculation

The results are calculated as percentage of the fat according to equation (1).

$$\%\,\text{Fat} = \frac{(m_{\text{Total}} - m_{\text{Beaker}})}{m_{\text{Sample}}} \bullet 100\% \qquad (1)$$

% Fat : Percentage of fat in the sample
m_{Total} : Beaker + extract [g]
m_{Beaker} : Empty beaker weight with boiling aid [g]
m_{Sample} : Sample weight [g]

5. Results

The fat contents of the animal feed samples obtained using the Twisselmann extraction method are comparable with the reference value determined by Soxhlet extraction. The results are summarized in Tables 2 and 3.

Table 2: Dry dog feed (Reference 20.4 +/- 1 g/100g)

	Hexane
Sample 1	20.98
Sample 2	19.65
Mean value [g/100g]	**20.31**
rsd [%]	**4.63**

Table 3: Turkey feed (Reference 9.3 +/- 1 g/100g)

	Hexane	Petroleum ether
Sample 1	9.61	9.66
Sample 2	9.61	9.66
Mean value [g/100g]	**9.61**	**9.66**
rsd [%]	**0.02**	**0.05**

6. Conclusion

The fat content in different animal feed samples, which was determined by a well-known analytical laboratory, using the Extraction Unit E-816 ECE, is comparable to the results obtained with the Soxhlet extraction method following Weibull-Stoldt. The results show low relative standard deviations (rsd).

7. References

[1] COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

Operation Manual of Extraction Unit E-816 ECE