Method for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff, IAG-Method A4

International Association of Feedingstuff Analysis
Section Feedingstuff Microscopy

1. Objective and field of application

The method is used for both qualitative and quantitative determination of ergot in animal feedingstuff. The method is suitable for the examination of feedingstuff of different particle sizes. In pelleted feedingstuff only qualitative determination is possible.

2. Principle

Ergot in feedingstuff is determined by the macroscopic and microscopic identification of the ergot sclerotia and fragments. Quantification is done by weighing the amount of identified sclerotia and fragments with a particle size > 0.5 mm.

3. Reagents

3.1 Chloral hydrate, ß = 60%

3.2 Sodium hydroxide (pelleted)

3.3 Potassium hydroxide (pelleted)

3.4 Ethanol, σ = 50%

3.5 Acetone

The reagents listed can be replaced by others which produce comparable results.

4. Equipment and accessories

4.1 Optical equipment

- 4.1.1 Stereo microscope (up to 70x magnification)
- 4.1.2 Magnifier (up to 10x magnification)

4.2 Mortar and pestle

4.3 Sieve fitted with square meshes of width of 0.5 mm

4.4 Analytical balance (accuracy 0.001 g)

4.5 Additional laboratory equipment is listed in supporting document (9)

4.6 Reference material

5. Procedure

The examination is performed in non-pelleted feedingstuffs. Pelleted feedingstuff have to be depelleted before examination (4.2; 8.1).

Qualitative determination of the sclerotia is performed macroscopically and microscopically considering ergot and its fragments in both the sieve fraction > 0.5mm and < 0.5mm.

Quantification is performed by selecting and weighing of ergot and its fragments with a particle size > 0.5mm out of the laboratory sample or an aliquot of it.

5.1 Preparation of the laboratory sample

5.1.1 Whole kernel feedingstuff (at least 250g) are weighed (4.4) and used directly for the investigation (5.2 and 5.3).

5.1.2 Non-pelleted feedingstuff (at least 10g) are weighed (4.4) and fractionated using supporting document (9., 5.3.1). The obtained fractions > 0.5mm and ≤ 0.5mm are weighed (4.4).

5.2 Identification of ergot

Ergot sclerotia are identified based on their characteristic features. The identification may be facilitated by comparison to reference material (4.6) and existing descriptions (10).

Morphology: Ergot Tul. sclerotia are elongated with a length up to several centimetres, coloured dark violet to black. The shape is similar to cereal kernels. They only consist of fungal hyphae.

Anatomy: Cross sections through the random parts of ergot sclerotia show very small, narrow interconnected hyphae which yield a dense pseudoparenchymatic tissue. The cells contain lots of fat oil. The outer layers of the hyphae are coloured dark violet to black, whereas the inner parts are coloured light pink to violet.

For the identification of ergot fragments in the sieve fractions <0.5mm the following colour reaction can be used. This staining procedure is only applicable to fresh sclerotia material.

A filter paper is soaked with a solution of 3ml ethanol (3.4) and 2 sodium hydroxide pellets (3.2) or 2 potassium hydroxide pellets (3.3). The sample is distributed on the filter paper.

After app. 5 min. a red-violet halo around the ergot fragments is observed.

The dark violet colouring of the outer hyphae layers is dissolved also in chloralhydrate (3.1) and colours it violet.

5.3 Quantification

The quantification of ergot is performed using the sieve fractions > 0.5 mm.

Material identified as ergot in each fraction is selected and weighed. An aliquot of the sieved fractions may be used if necessary. The ergot

content of the fractions >0.5mm is summarized and expressed in mg/kg feedingstuff (6.1).

6. Calculation and report

6.1 Calculation

The amount of ergot fragments in mg/kg (ppm) feedingstuff (original sample) is calculated using the following formula:

$$\mathbf{C} = \frac{\mathbf{BC \times 1000}}{\mathbf{E}} \text{ [mg/kg]}$$

C = amount of component in mg/kg feedingstuff (ppm)

BC = selected fragments of component in the laboratory sample or an aliquot of it [mg]

E = total weight of the laboratory sample or an examined aliquot of the laboratory sample [g]

6.2 Report

6.2.1 Negative result:

As far as was discernible using a microscope, ergot was not found in the submitted sample.

6.2.2 Positive result:

As far as was discernible using a microscope x mg ergot/kg feedingstuff were found in the submitted sample. For quantification ergot particles > 0.5 mm are considered.

6.2.3 Possible adding to the report:

In pelleted feedingstuff only qualitative determination of ergot is possible.

7. Validation

not yet validated

8. Remarks

8.1 For the identification of ergot in pelleted feedingstuffs the use of supporting document (9; 5.2.2.2 or 5.2.2.3) is recommended for depelleting the sample.

8.2 Ergot are the permanent forms or sclerotia of ergot which mainly occur in rye, more seldom in wheat, triticale and barley.

8.3 This method also is suitable for the examination of raw material and food.

8.4 This method has been developed by the International Association of Feedingstuff Analysis (IAG) – Section Feedingstuff Microscopy.

9. Supporting document

Sample Preparation for the Macroscopic and Microscopic Analysis, IAG-Method A1

10. Literature

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