Determination of *Datura* spp. in Animal Feedingstuff, IAG-Method A3

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 *Datura* spp. in animal feedingstuffs.

2. Principle

Datura spp. content is determined by the visual identification of the seeds or seed husk fragments. Quantification is done by weighing the amount of identified seeds and seed husks in sieved fractions of the sample.

3. Reagents

3.1 Embedding agents

3.1.1 Chloral hydrate (ß = 60 %)

3.1.2 Water

The reagents listed may 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); recommended additional equipment: image support system

4.1.2 Compound microscope (up to 400x magnification); recommended additional equipment: polarization, phase contrast, image support system

4.1.3 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

5.1 Preparation of the laboratory sample

Analysis is done using unpelleted feedingstuffs. Therefore pelleted feedingstuffs must be depelleted before analysis using the procedure given in supporting document (9.). The unpelleted laboratory sample (approximately 10g) is weighed (4.4) and sieved as described in supporting document (9.). The weight of each fraction is recorded.

5.2 Identification of *Datura* spp.

Datura spp. are identified based on characteristic features of both the whole seeds and the fragments of the seed husks. The identification of seeds and seed husk fragments may be facilitated through comparison with reference material (4.6) and existing descriptions (10). The endosperm tissue and germ of *Datura* seeds do not possess diagnostic features.

Datura spp. seeds are 3-4 mm long, and are coloured yellow to blackish. They are flat and their surface shows indistinct cavities (pitted seed surface). The husk of mature seeds is dark whereas unmature husks are coloured light-brown. Internally, the seed husks possess typical epidermal cells. They are formed like palisades and comparatively large with irregular, waved and turned cellwalls in cross-section and light-yellow membranes. The cell content is dark. Outwards, the membrane loops end like small papillae.

5.3 Quantification of the *Datura* spp.

The quantification of *Datura* spp. seeds and seed husk fragments is performed using the sieve fractions (> 0,5 mm) only.

Material identified as *Datura* spp. seeds and seed husk fragments are each separated from the sample and weighed. An aliquot of the sieved fractions may be used if necessary.

The weight of the *Datura* spp.seed husk fragments determined in the different sieve fractions are added together and recorded as mg/kg feedingstuff (6.1). Whole *Datura* spp. seeds are similarily recorded (6.1)

6. Calculation and report

6.1 Calculation

The amount of *Datura* spp. seeds or *Datura* spp. seed husk fragments in mg/kg (ppm) feedingstuff (original sample) is calculated using the following formulas:

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

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]

The amount of *Datura* spp. seeds respectively *Datura* spp. seed husk fragments is specified separately.

6.2 Report

6.2.1 Negative result:

As far as was discernible using a microscope, seeds or seed husk fragments of *Datura* spp. were not found in the submitted sample.

6.2.2 Positive result in the case of seeds:

As far as was discernible using a microscope x mg seeds of *Datura* spp./kg feedingstuff were found in the submitted sample. For quantification *Datura* spp.seeds out of the sieve fractions > 0,5 mm have been determined.

6.2.3 Positive result in the case of seed husk fragments:

As far as was discernible using a microscope x mg seed husk fragments of *Datura* spp. /kg feedingstuff were found in the submitted sample. For quantification *Datura* spp. seed husk fragments out of the sieve fractions > 0,5 mm have been determined.

6.2.4 Possible supplement to the result:

The sieve fractions > 0,5 mm amount to x % of the laboratory sample.

7. Validation

Inapplicable

8. Remarks

8.1 In case of the determination of *Datura* spp. in pelleted feedingstuff, the sample preparation for depelleting according to document (9) is recommended.

8.2 The seeds of *Datura* spp. mainly occur as impurity in soybean seeds. Because of their toxic ingredients (alcaloids, e.g. scopolamin, hyoscyamin), they are undesirable substances in feedingstuffs.

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

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

- FREUND, H. (ED.), 1976: Handbuch der Mikroskopie in der Technik. Band VIII: Mikroskopie der Nahrungs- und Futtermittel, der Drogen und Genußmittel. Umschau- Verlag, Frankfurt/Main
- GASSNER, G.; HOHMANN, B. und DEUTSCHMANN, F., 1989: Mikroskopische Untersuchung pflanzlicher Lebensmittel. 5.Auflage, Gustav Fischer-Verlag, Stuttgart, New York, 66-70
- GILLET, H. 1987: Le *Datura* et les Tourteaux Argentins. In: Tagungsbericht der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Futtermittelmikroskopie und Futtermittelmikrobiologie, Speyer, 22-28
- HAHN. H. und MICHAELSEN, L., 1996: Mikroskopische Diagnostik pflanzlicher Nahrungs-, Genuß- und Futtermittel, einschließlich Gewürze. Springer-Verlag, Berlin, Heidelberg, New York
- MALKOMESIUS, E.; NEHRING, K.; CLAUS, G. und KUMMER, H., 1951: Die Untersuchung von Futtermitteln. In: Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch, Band III, 2. Auflage, Hrsg.R. Hermann), Neumann-Verlag, Radebeul und Berlin
- MESZAROS, L. 1987: Untersuchungen zur Morphologie und Anatomie von *Datura*-Arten. In: Tagungsbericht der internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Futtermittelmikroskopie und Futtermittel-Mikrobiologie, Speyer, 29-55
- PARADIES, I. 1987: Unterscheidung von *Datura*-Spezies mittels Elektrophorese. In: Tagungungsbericht der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Futtermittelmikroskopie und Futtermittelmikrobiologie, Speyer, 56-62
- RÖZSE, E., 2005: Atlas of Feed Microscopy. Part II: The Examination of non-desired Materials in Feed. National Institut for Agricultural Quality Control, Budapest
- RÖZSE, E. 1987: Zur Methodik der Mengenbestimmung von *Datura* in Sojaschrot. In: Tagungsbericht der Internationalen Arbeitsgemeinschaft für Futtermitteluntersuchung, Futtermittelmikroskopie und Futtermittelmikro-biologie, Speyer, 63-75
- VÖHRINGER, H., 1997: Leitfaden für die Futtermittel-Qualitätskontrolle durch Mikroskopie. Hoffmann-La Roche, 3. Fassung, Wien