COMMISSION DIRECTIVE 2003/126/EC

of 23 December 2003

on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 70/373/EEC of 20 July 1970 on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs [1], and in particular Article 2 thereof,

Whereas:

(1) Pursuant to Directive 70/373/EEC, official controls of feedingstuffs, for the purpose of checking compliance with the requirements of the laws, regulations and administrative provisions governing their quality and composition, are to be carried out using Community sampling and analysis methods.

(2) Provisions on the labelling of feedingstuffs and requirements prohibiting the use of certain types of animal proteins in feedingstuffs for certain categories of animals imply the need to provide for reliable analytical methods to establish their presence and, if appropriate, their percentage.

(3) The method described in Commission Directive 98/88/EC of 13 November 1998 establishing guidelines for the microscopic identification and estimation of constituents of animal origin for the official control of feedingstuffs [2] is currently the only method validated to control the presence of animal proteins including these proteins treated at 133 °C/3 Bar/20', in feedingstuffs.

(4) An intercomparison study for the determination of processed animal proteins recently demonstrated that the variation in the application of the microscopic tests laid down in Directive 98/88/EC resulted in significant differences in the sensitivity, specificity and accuracy of the method. In order to harmonise and improve determination of processed animal proteins the provisions concerning the microscopic method should be further specified and made mandatory. It is necessary to ensure that analysts performing the method are adequately trained since the performance depends on the skills of the analyst.

(5) Directive 98/88/EC should therefore be replaced.

(6) The measures provided for in this Directive are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Member States shall provide that where official analysis of feedingstuffs is carried out with a view to officially controlling the presence, identification and/or estimation of the amount of constituents of animal origin in feedingstuffs, in the framework of the coordinated inspection programme in the field of animal nutrition in accordance with Council Directive 95/53/EC [3], it shall be carried out in accordance with the provisions of the Annex to this Directive.

Article 2

Member States shall ensure that laboratories carrying out official controls on the presence of animal constituents in feedingstuffs participate periodically in proficiency testing on the analytical methods, and that laboratory personnel carrying out analyses receive adequate training.

Article 3

Directive 98/88/EC is repealed.

References to the repealed Directive shall be construed as references to this Directive.

Article 4

1. Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 1 July 2004 at the latest. They shall forthwith communicate to the Commission the text of those provisions and a correlation table between those provisions and this Directive.

When Member States adopt those provisions, they shall contain a reference to this Directive or be accompanied by such a reference on the occasion of their official publication. Member States shall determine how such reference is to be made.

2. Member States shall communicate to the Commission the texts of the provisions of national law which they adopt in the field covered by this Directive.

[1] OJ L 170, 3.8.1970, p. 2 Directive as last amended by Regulation (EC) No 807/2003 (OJ L 122, 16.5.2003, p. 36).
[2] OJ L 318, 27.11.1998, p. 45.
[3] OJ L 265, 8.11.1995, p. 17. Directive as last amended by Directive 2001/46/EC of the European Parliament and of the Council (OJ L 234, 1.9.2001, p. 55).

Article 5

This Directive shall enter into force on the 20th day following that of its publication in the *Official Journal of the European Union*.

Article 6

This Directive is addressed to the Member States.

Done at Brussels, 23 December 2003.

For the Commission
David BYRNE
Member of the Commission

ANNEX

Conditions for the microscopic detection, identification or estimation of constituents of animal origin in feedingstuffs

1. **Objective and field of application**

These conditions shall be used when detection of constituents of animal origin (defined as products from processing bodies and body parts of mammals, poultry and fish) in feedingstuffs is carried out by means of microscopic examination in the framework of the coordinated inspection programme in the field of animal nutrition in accordance with Council Directive 95/53/EC. Provided that the methods in this Annex are used in all official tests, a second test may also be carried out using variant or alternative methods, in order to improve the detection of certain types of animal constituents or to specify further the origin of the animal constituents. Furthermore, a variant protocol may be used when examining certain specific animal constituents such as plasma or bones in tallow (see also point 9), provided that these analyses are made in addition to the analyses foreseen in the coordinated inspection programme.

2. **Sensitivity**

Dependent on the nature of the constituents of animal origin, very small amounts (< 0,1 %) in feedingstuffs can be detected.

3. **Principle**

A representative sample, taken in accordance with the provisions laid down in Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feedingstuffs [1] which has undergone suitable preparation is used for the identification. The following protocol is fit for handling feed with low moisture content. Feed with an amount of moisture higher than 14 % shall be dried (condensed) prior to handling. Special feed or feed materials (e.g. fats, oils) need dedicated treatment (see point 9). The constituents of animal origin are identified on the basis of typical, microscopically identifiable characteristics (i.e. muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers, egg shells, fish bones, scales). The identification has to be done both on the sieve fraction (6.1) and the concentrated sediment (6.2) of the sample.

4. **Reagents**

4.1. *Embedding agent*

4.1.1. Chloral hydrate (aqueous, 60 % w/v)

4.1.2. Lye (NaOH 2,5 % w/v or KOH 2,5 % w/v) for sieve fractions

4.1.3. Paraffin oil or glycerol (viscosity: 68-81) for microscopic observations in the sediment

4.2. *Rinsing agents*

4.2.1. Alcohol, 96 %

4.2.2. Acetone

4.3. *Concentrating agent*

4.3.1. Tetrachloroethylene (density 1,62)

4.4. *Staining reagents*

4.4.1. Iodine/potassium iodide solution (Dissolve 2 g potassium iodide in 100 ml water and add 1 g iodine while shaking frequently)

4.4.2. Alizarin Red (Dilute 2,5 ml 1M hydrocloric acid in 100 ml water and add 200 mg alizarine red to this solution)

4.4.3. Cystine reagent (2 g lead acetate, 10 g NaOH/100 ml H_2O)

4.4.4. Iodine/potassium iodide solution (dissolved in 70 % ethanol)

[1] OJ L 102, 15.4.1976, p. 1.

4.5. *Bleaching reagent*

4.5.1. Commercial sodium hypochlorite solution (9.6 % active chlorine)

5. **Equipment and accessories**

5.1. Analytical balance (accuracy of 0,01 g except for the concentrated sediment: 0,001 g)

5.2. Material for grinding (grinding mill or a mortar, especially for feed containing > 15 % fat on analysis)

5.3. Sieve fitted with sieve mesh with square meshes of width of 0,50 mm maximum

5.4. Separation funnel or conical bottomed settling beaker

5.5. Stereomicroscope (minimum 40' magnification)

5.6. Compound microscope (minimum 400' magnification), transmitted light or polarised light

5.7. Standard laboratory glassware

All equipment shall be thoroughly cleaned. Separation funnels and glassware need washing in a washing machine. Sieves need cleaning using a brush with stiff hairs.

6. **Procedure**

Pelleted feeds may be pre-sieved if both fractions are analysed as a separate sample.

At least 50 g of the sample shall be treated (ground with care using the suitable grinding equipment (5.2) if necessary in order to achieve an appropriate structure). From the ground material two representative portions shall be taken, one for the sieve fraction (at least 5 g) (6.1) and one for the concentrated sediment (at least 5 g) (6.2). Colouring with staining reagents (6.3) can additionally be applied for the identification.

In order to indicate the nature of the animal proteins and the origin of the particles, a decision support system such as Aries can be used and reference samples can be documented.

6.1. *Identification of constituents of animal origin in the sieve fractions*

At least 5 g of the sample is sieved through the sieve (5.3) in two fractions.

The sieve fraction(s) with the large particles (or a representative part of the fraction) is applied as a thin layer to a suitable support and screened systematically under the stereomicroscope (5.5) at various magnifications for constituents of animal origin.

Slides made with the sieve fraction(s) with the fine particles are screened systematically under the compound microscope (5.6) at various magnifications for constituents of animal origin.

6.2. *Identification of constituents of animal origin from the concentrated sediment*

At least 5 g (accurate to 0,01 g) of the sample shall be transferred into a separation funnel or conical bottomed settling beaker and treated with at least 50 ml of tetrachloroethylene (4.3.1). The mixture shall be shaken or stirred repeatedly.

— If a closed separation funnel is used the sediment shall be left to stand for a sufficient time (at least three minutes) before the sediment is separated off. Shaking shall be repeated and the sediment shall be left to stand again at least three minutes. The sediment shall be separated off again.

— If an open beaker is used, the sediment shall be left to stand for at least five minutes before the sediment is separated off.

The total sediment shall be dried and subsequently weighed (accurate to 0,001 g). The weighing is only necessary in case an estimation is required. If the sediment consists of many large particles it may be sieved through a sieve (5.3) in two fractions. The dried sediment shall be examined for bone constituents under the stereomicroscope (5.5) and the compound microscope (5.6).

6.3. *Use of embedding agents and staining reagents*

The microscopic identification of the constituents of animal origin can be supported by the use of special embedding agents and staining reagents.

Chloral hydrate (4.1.1):	By carefully heating, cell structures can be seen more clearly because starch grains gelatinise and unwanted cell contents are removed.
Lye (4.1.2):	Either sodiumhydroxide or potassiumhydroxide clears the material of the feed, assisting the detection of muscle fibres, hairs and other keratin structures.
Paraffin oil and glycerol (4.1.3):	Bone constituents can be well identified in this embedding agent because most lacunae remain filled with air and appear as black holes about 5-15 µm.
Iodine/potassium iodide solution (4.4.1):	Used for the detection of starch (blue-violet colour) and protein (yellow-orange colour). Solutions may be diluted if required.
Alizarin red solution (4.4.2):	Red/pink colouring of bones, fish bones and scales. Before drying the sediment (see section 6.2), the total sediment shall be transferred into a glass test tube and rinsed twice with approximately 5 ml alcohol (4.2.1) (each time a vortex shall be used, the solvent shall be let settle about one minute and poured off). Before using this staining reagent, the sediment shall be bleached by adding at least 1 ml sodium hypochlorite solution (4.5.1). The reaction shall be allowed to continue for 10 minutes. The tube shall be filled with water, the sediment shall be let settle two to three minutes, and the water and the suspended particles shall be poured off. The sediment shall be rinsed twice more with about 10 ml of water (a vortex shall be used, let settle, and the water poured off each time). Two to 10 or more drops (depending on the amount of residue) of the alizarine red solution shall be added. The mixture shall be shaken and the reaction shall be let occur a few seconds. The coloured sediment shall be rinsed twice with approximately 5 ml alcohol (4.2.1) followed by one rinse with acetone (4.2.2) (each time a vortex shall be used, the solvent shall be let settle about one minute and poured off). The sediment is then ready to be dried.
Cystin reagent (4.4.3):	By carefully heating, cystin-containing constituents (hair, feathers, etc.) become black-brown.

6.4. *Examination in feed possibly containing fishmeal*

At least one slide shall be examined from the fine sieve fraction and from the fine fraction of the sediment under the compound microscope (see sections 6.1 and 6.2).

Where the label indicates that the ingredients include fishmeal, or if the presence of fishmeal is suspected or detected in the initial examination, at least two additional slides of the fine sieve fraction from the original sample, and the total sediment fraction shall be examined.

7. **Calculation and evaluation**

Member States shall ensure that the procedures described in this point are used where an official analysis is carried out with a view to estimating the amount (and not simply the presence) of animal constituents.

The calculation can only be made if the constituents of animal origin contain bone fragments.

Bone fragments of terrestrial warm-blooded species (i.e. mammals and birds) can be distinguished from the different types of fish bone on the microscopic slide by means of the typical lacunae. The proportion of constituents of animal origin in the sample material is estimated taking into consideration:

— the estimated proportion (weight %) of bone fragments in the concentrated sediment and

— the proportion (weight %) of bone in the constituents of animal origin.

The estimate has to be based on at least three (if possible) slides and at least five fields per slide. In compound feedingstuffs, the concentrated sediment as a rule contains not only terrestrial animal bone and fish bone fragments, but also other particles of high specific weight, e.g. minerals, sand, lignified plant fragments and the like.

7.1. *Estimated value of the percentage of bone fragments*

% terrestrial bone fragments = (S × c)/W

% fish bone and scale fragments = (S × d)/W

(S = sediment weight (mg), c = correction factor (%) for the estimated portion of terrestrial animal bones in the sediment, d = correction factor (%) for the estimated portion of fish bones and scale fragments in the sediment, W = weight of the sample material for the sedimentation (mg)).

7.2. *Estimated value of constituents of animal origin*

The proportion of bone in animal products can vary greatly. (The percentage of bone in the case of bonemeals is of the order of 50 to 60 % and in the case of meat meals of the order of 20 to 30 %; in the case of fish meals bone and scale contents vary according to the category and origin of the fishmeal, normally in the order of 10 to 20 %).

If the type of animal meal present in the sample is known, it is possible to estimate the content:

Estimated content of constituents of terrestrial animal products (%) = (S × c)/(W × f) × 100

Estimated content of constituents of fish products (%) = (S × d)/(W × f) × 100

(S = sediment weight (mg), c = correction factor (%) for the estimated portion of terrestrial animal bone constituents in the sediment, d = correction factor (%) for the estimated portion of fish bones and scale fragments in the sediment, f = correction factor for the proportion of bone in the constituents of animal origin in the sample examined, W = weight of the sample material for the sedimentation (mg)).

8. **Expression of the result of the examination**

The report shall at least contain information on the presence of constituents derived from terrestrial animals and from fishmeal. The different cases shall be reported in the following way:

8.1. *With regard to the presence of constituents derived from terrestrial animals:*

— As far as was discernible using a microscope, no constituents derived from terrestrial animals were found in the submitted sample,

or:

— As far as was discernible using a microscope, constituents derived from terrestrial animals were found in the submitted sample.

8.2. *With regard to the presence of fishmeal:*

— As far as was discernible using a microscope, no constituents derived from fish were found in the submitted sample,

or:

— As far as was discernible using a microscope, constituents derived from fish were found in the submitted sample.

In case constituents derived from fish or terrestrial animals are found, the report of the examination result, if required, can further indicate an estimation of the amount of constituents detected (x %, < 0,1 %, 0,1-0,5 %, 0,5-5 % or > 5 %), further specification of the type of terrestrial animal if possible and the animal constituents identified (muscle fibres, cartilage, bones, horn, hair, bristles, feathers, blood, egg shells, fish bones, scales).

For the case where the amount of animal ingredients is estimated the correction factor f used shall be mentioned.

For the cases where bone constituents from terrestrial animals are identified, the report shall contain the additional clause:

'The possibility that the above constituents are derived from mammals cannot be excluded.'

This additional clause is not necessary in cases where the bone fragments from terrestrial animals have been specified as bone fragments from poultry or mammals.

9. **Optional protocol for analysing fat or oil**

The following protocol may be used for the analysis of fat or oil:

— If the fat is solid, it is warmed for example in a microwave oven until it is liquid.

— Using a pipette, 40 ml of fat is transferred from the bottom of the sample to a centrifugation tube.

— Centrifuge for 10 minutes at 4 000 rpm.

— If the fat is solid after centrifugation, it is warmed once more in an oven until it is liquid. Repeat the centrifugation for five minutes at 4 000 rpm.

— Using a small spoon or a spatula, one half of the decanted impurities is transferred to a small petri dish or a microscopic slide for microscopic identification of a possible content of animal constituents (meat fibres, feathers, bone fragments). As an embedding agent for microscopy, paraffin oil or glycerol is recommended.

— The remaining impurities are used for sedimentation as described in point 6.2.