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

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to the 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], as last amended by the Act of Accession of Austria, Finland and Sweden, and in particular Article 2 thereof,

Whereas Directive 70/373/EEC stipulates that official controls of feedingstuffs for the purpose of checking compliance with the requirements arising under the laws, regulations and administrative provisions governing their quality and composition must be carried out using Community sampling and analysis methods;

Whereas Commission Decision 94/381/EC of 27 June 1994 concerning certain protection measures with regard to bovine spongiform encephalopathy and the feeding of mammalian derived protein [2], as amended by Decision 95/60/EC [3] prohibits the feeding of protein derived from all mammalian tissues to ruminants, with the exception of certain animal products and by-products;

Whereas Commission Decision 91/516/EEC of 9 September 1991 establishing a list of ingredients whose use is prohibited in compound feedingstuffs [4], as last amended by Decision 97/582/EC [5], prohibits the use of protein derived from mammalian tissue in compound feedingstuffs for ruminants;

Whereas Council Directive 79/373/EEC of 2 April 1979 on the marketing of compound feedingstuffs [6], as last amended by Commission Directive 97/47/EC [7], provides in Article 5c that all ingredients must be mentioned where a declaration of the ingredients is provided and that the listing of ingredients is subject to several rules, *inter alia*, the listing of ingredients in descending order by weight for compound feedingstuffs intended for animals other than pets;

Whereas Directive 97/47/EC amending the Annexes to Council Directives 77/101/EEC [8], 79/373/EEC and 91/357/EEC [9] introduces appropriate labelling provisions with regard to the prohibition of these products on their use in ruminant feed;

Whereas Member States may have adopted more stringent provisions, in accordance with Article 1(2) of Council Directive 90/667/EEC of 27 November 1990 laying down the veterinary rules for the disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedstuffs of animal or fish origin and amending Directive 90/425/EEC [10], as last amended by the Act of Accession of Austria, Finland and Sweden;

Whereas by microscopic examination the presence of constituents of animal origin can be established; whereas bones of terrestrial animals and bones of fish can be distinguished by microscopic examination; whereas the possibility of distinguishing, by microscopic examination, the bones of mammalian origin from bones of poultry origin depends on the experience of the analyst; whereas the possibility of estimation of the quantity of constituents of animal origin depends also largely on the experience of the analyst; whereas it may be appropriate according to the progress of scientific and technological knowledge, to combine microscopic examination with other methods of analysis; whereas the fixing of these guidelines for the microscopic examination do not exclude the use, instead or in addition, of methods of analysis, other than microscopic examination, which have been proved to be scientifically valid;

Whereas it is therefore advisable to lay down the provisions concerning microscopic examination as guidelines;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Feedingstuffs,

[1] OJ L 170, 3. 8. 1970, p. 2.
[2] OJ L 172, 7. 7. 1994, p. 23.
[3] OJ L 55, 11. 3. 1995, p. 43.
[4] OJ L 281, 9. 10. 1991, p. 23.
[5] OJ L 237, 28. 8. 1997, p. 39.
[6] OJ L 86, 6. 4. 1979, p. 30.
[7] OJ L 211, 5. 8. 1997, p. 45.
[8] OJ L 32, 3. 2. 1977, p. 1.
[9] OJ L 193, 17. 7. 1991, p. 34.
[10] OJ L 363, 27. 12. 1990, p. 51.

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall provide that where, with a view to officially controlling the identification and/or estimation of the amount of constituents of animal origin in feedingstuffs, microscopic examination is carried out, it shall be carried out using the guidelines set out in the Annex hereto.

In accordance with the requirements posed by the competent authorities to the analysis, point 7 'Calculation and evaluation' of these guidelines are to be considered as optional, provided that in the case where the estimation of the quantity is carried out, the provisions laid down in point 7 have to be followed.

The fixing of these guidelines, in respect of the procedure for microscopic examination does not exclude the use, instead or in addition, of methods of analysis, other than microscopic examination, which have been scientifically proved to be valid for the identification and/or estimation of the amount of constituents of animal origin.

Article 2

The Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with the provisions of this Directive, not later than 1 September 1999. They shall forthwith notify the Commission thereof.

When Member States adopt these provisions, the provisions shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by the Member States.

Article 3

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

This Directive is addressed to the Member States.

Done at Brussels, 13 November 1998.

For the Commission

Franz FISCHLER

Member of the Commission

ANNEX

Guidelines for the microscopic identification and estimation of constituents of animal origin in feedstuffs

1. *Objective and field of application*

These guidelines should be used where 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 case where the estimation of the quantity of animal constituents is carried out, the provisions under point 7 of these guidelines have to be followed.

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 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* [2]

4.1. Embedding agent

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

4.1.2. Paraffin oil

4.2. Concentrating agent

4.2.1. Tetrachloroethylene (density 1,62)

4.3. Staining reagents

4.3.1. Bradford reagent

4.3.2. Iodine/potassium iodide solution

4.3.3. Millon reagent

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

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

5. *Equipment and accessories*

5.1. Analytical balance (accuracy of 0,001 g)

5.2. Material for grinding (rasp, mill, etc.)

5.3. Sieve fitted with sieve mesh with square meshes of width 0,1 to 2 mm

5.4. Stereomicroscope (up to 50′ magnification)

5.5. Compound microscope (up to 400′ magnification), transmitted light/polarised light

5.6. Standard laboratory glassware

[1] OJ L 102, 15. 4. 1976, p. 1.
[2] The reagents listed are commercially available, if no other indication is given.

6. *Procedure*

At least 10 g of the sample should, if necessary, depending on the nature of the material be treated (depelletarised or ground with care using the suitable grinding equipment) and then divided into two representative parts, one of at least 5 g for the sieve fraction (6.1) and one of at least 2 g for the concentrated sediment (6.2). Colouring with staining reagents (6.3) is recommended for the identification.

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

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

The sieve fraction(s) $>0{,}5$ mm (or a representative part of the fraction) is applied as a thin layer to a suitable support and screened systematically under the stereomicroscope (5.4) at various magnifications for constituents of animal origin.

Slides made with the sieve fraction(s) $<$ 0,5 mm are screened systematically under the compound microscope (5.5) at various magnifications for constituents of animal origin.

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

At least 2 g (accurate to 0,001 g) of the sample are weighed into a test tube or a separating funnel and treated with at least 15 ml of tetrachloroethylene (4.2.1). After the mixture has been stirred/shaken repeatedly and left to stand for a sufficient time (at least one minute and no more than two to three minutes), the sediment is separated off.

The sediment is dried in a fume cupboard and subsequently weighed (accurate to 0,001 g). The weighing is only necessary in case an estimation is required. Examine the entire dried sediment or part thereof for bone constituents under the stereomicroscope (5.4) and the compound microscope (5.5).

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.
Paraffin oil (4.1.2):	Bone constituents can be well identified in this embedding agent because most lacunae remain filled with air and appear as black holes about 5 to 15 μm.
Bradford reagent (4.3.1):	Is used for the detection of protein (typical blue colour). Dilute with water approx. 1:4.
Iodine/potassium iodide solution (4.3.2):	Is used for the detection of starch (blue-violet colour) and protein (yellow-orange colour). Dilution can be made if required.
Millon reagent (4.3.3):	By carefully heating, the bone constituents become pink.
Cystin reagent (4.3.4):	By carefully heating, cystin-containing constituents (hair, feathers, etc.) become black-brown.

7. *Calculation and evaluation*

In the case where the estimation of the quantity of animal constituents is carried out the provisions under this point have to be followed.

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 in 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

$$\%\ \text{terrestrial bone fragments} = \frac{S \times c}{W}$$

$$\%\ \text{fish bone and scale fragments} = \frac{S \times d}{W}$$

(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 bone meals 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 the category and origin of fish meal, 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:

$$\text{Estimated content of constituents of terrestrial animal products (\%)} = \frac{S \times c}{W \times f} \times 100$$

$$\text{Estimated content of constituents of fish products (\%)} = \frac{S \times d}{W \times f} \times 100$$

(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 different cases could be reported in the following way:

8.1. As far as was discernible under the microscope, no constituents of animal origin (as defined in point 1) were found in the submitted sample.

8.2. As far as was discernible under the microscope constituents of animal origin [1] were found in the submitted sample.

In this case, the reporting of the result of the examination, if required, can be further specified as:

8.2.1. As far as was discernible under the microscope, small amounts of constituents of animal origin [1] were found in the submitted sample.

8.2.2. Dependent on the experience of the analyst:

— either, as far as was discernible under the microscope, constituents of animal origin [1] were found in the submitted sample. The content of bone fragments (fish/terrestrial animals — in the case of bone fragments of terrestrial animals, eventually specified as bone fragments from poultry or mammalians, see remark 9.3) is estimated in an order of magnitude of ...%, equal to ...% of animal constituents when calculated on the basis of ...% bone in the animal constituents' product (= correction factor f used),

— or, as far as was discernible under the microscope, constituents of animal origin [1] were found in the submitted sample in measurable quantities.

[1] The type of constituents found, for example bones (fish or terrestrial animals), meat constituents, etc. should be indicated here.

For the cases under point 8.2, 8.2.1 and 8.2.2, when 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 clause is not necessary in cases where the bone fragments from terrestrial animals have been specified as bone fragments from poultry or mammalians (see remark 9.3).

9. *Remarks*

9.1. It is recommended in the case of many and big constituents in the concentrated sediment to sieve the sediment into two fractions (i.e. use of a 320 μm sieve). The fraction with the big constituents can be examined as a paraffin oil preparation under a stereomicroscope with transmitted light. The fraction with the fine constituents must be examined under the compound microscope.

9.2. The concentrated sediment obtained (6.2), can, if necessary, be divided further using a concentrating agent with a greater density.

9.3. Dependent on the experience of the analyst, the distinction between constituents of mammalian or poultry origin can be made, making use of specific histological features, by which this distinction can be made.