Method for the Identification and Estimation of Constituents in Animal Feedingstuff IAG-Method A2

International Association of Feedingstuff Analysis-Section
Feedingstuff Microscopy

1. Objective and field of application

The method is used for the identification and percentage estimation of the constituents present in animal feedingstuffs.

2. Principle

Constituents are identified using a representative sample prepared by a standardised method. The identification is based on typical macroscopic and microscopic characteristics. Different optical methods and staining reactions assist the identification process.

The percentage estimation of constituents is performed either by:

- the combined counting and weighing of the identified constituents from the coarse sieve fractions and the visual estimation of the fine sieve fractions or
- the visual estimation of the constituents in all sieve fractions.

3. Reagents

3.1 Embedding agents

3.1.1 Chloral hydrate, ß = 60%

3.1.2 Paraffin oil

3.1.3 Water

3.2 Staining Reagents

3.2.1 Bradford Reagent

3.2.2 Iodine/Potassium Iodide Solution (Lugol Solution)

3.2.3 Sudan-Glycerine Reagent

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

4. Equipment and Accessories

4.1 Stereo microscope (up to 70 X magnification).

4.2 Compound microscope (up to 400 X magnification) which may include: polarization, phase contrast, image support system.

4.3 Magnifier (up to 10 X magnification).

4.4 Analytical balance (accuracy 0,001g).

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

4.6 Reference material.

5. Procedure

5.1 Identification

A sample prepared according to the procedure detailed in (9.) is used. Fragments from the coarse sieve fractions (> 0,5 mm) are screened systematically on a plain support using a stereo microscope (4.1) and identified. Unidentified fragments are separated and examined using a compound microscope (4.2) or with staining reagents (3.2). If necessary, constituents may be thinly sliced to reveal diagnostic features.

Fragments from the fine sieve fractions (≤ 0,5 mm) are mounted in an embedding agent (3.1) on glass slides and identified with the aid of a compound microscope (4.2).

Constituents are identified by comparing them with visual and written descriptions (10.) and by using reference material (4.6). Different optical methods are used during microscopic examination, e.g. transmitted light, polarized light, phase contrast.

5.2 Estimation of constituents

5.2.1 Determination by weight

Fragments of individual constituents are selected from the coarse sieve fractions (> 0,5 mm) (9.) (or an aliquot thereof) using a stereo microscope (4.1) and weighed (4.4).

When an aliquot of each fraction is used, at least 0,01 g of each constituent should be represented.

With the fine sieve fractions (≤ 0,5 mm), a minimum of two slides are prepared. These are examined using a compound microscope and the proportion of similar fragments in the sample are estimated and their weight calculated (6.1).

5.2.2 Determination by visual estimation

Characteristic fragments belonging to individual constituents are estimated in each sieved fraction with the aid of both stereo- (4.1) and compound microscopes (4.2). A minimum of two slides are prepared from the fine sieve fractions (≤ 0,5 mm).

Constituent content can be estimated with the aid of reference material (4.6).

6. Calculation and Report

6.1 Calculation:

The percentage content of individual constituents obtained using the method detailed in 5.2.1 are calculated using the procedure given in

Example 1. The results are presented in increments of 5 percentage points.

Example 1 - Determination by weight:

(The table is an example and can be modified according to the number and size of sieve fractions).

Sample quantity: 10g = 100 %	Fraction 1 > 1 mm 4,550 g	Fraction 2 ≤ 1,0-0,5 mm 1,570 g	Fraction 3 ≤ 0,5 mm 3,880 g	Total amount of constituents 10,000 g		
Constituent:	Fraction 1*)	Fraction 2*)	Fraction 3 **)	Total amount of constituents	***) result	
Corn	2,550 g	0,630 g	5 % - 0,194 g	3,374 g - 33,74 %	*c.*35 %	30-35 %
Wheat	1,200 g	0,440 g	5 % - 0,194 g	1,843 g - 18,34 %	*c.*20 %	15-20 %
Soy bean	0,800 g	0,500 g	5 % - 0,194 g	1,494 g - 14,94 %	*c.*15 %	10-15 %
Rice flour	-	-	30% - 1,164 g	1,164 g - 11,64 %	*c.*10 %	10-15 %
Manioc flour	-	-	40% - 1,552 g	1,552 g - 15,52 %	*c.*15 %	15-20 %
Potato starch	-	-	15% - 0,582 g	0,582g - 5,82 %	*c.* 5 %	5-10 %
Total sum	4,550 g	1,570 g	100%-3,880g	10,000g 100,00 %	100 %	

*) selected (g/fraction)
**) estimated (%), calculated in g/fraction
***) estimated value rounded (%), indicated percentage may be documented as estimate span.

6.2 The percentage content of individual constituents obtained using the method detailed in 5.2.2 are calculated using the procedure given in Example 2. The results are presented in increments of 5 percentage points.

Example 2 - Determination by visual estimation:

(The table is an example and can be modified according to the number and size of sieve fractions).

Sample quantity: 10g = 100 %	Fraction 1 > 1 mm 4,550 g	Fraction 2 ≤ 1,0-0,5 mm 1,570 g	Fraction 3 ≤ 0,5 mm 3,880 g	Total amount of constituents 10,000 g	
Constituents:	Fraction 1*)	Fraction 2*)	Fraction 3 **)	Total amount	results **
	% * 45,5	% * 15,7	% * 38,8	%	%
Corn	50 22,75	50 7,85	5 1,94	32,54	30-35
Wheat	30 13,65	25 3,93	5 1,94	19,52	15-20
Soy bean	20 9,10	25 3,93	5 1,94	14,97	10-15
Rice flour	-	-	30 11,64	11,64	10-15
Manioc flour	-	-	40 15,52	15,52	15-20
Potato starch	-	-	15 - 5,82	5,82	5-10
Total sum	100 %	100 %	100 %	100 %	

*) estimated (%), calculated on fraction amount
**) estimated value rounded in %, may be documented as estimate span.

6.3 Report

6.3.1 Without declaration:

As far as was discernible using a microscope the following constituents were found in the submitted sample.

(Depending on the experience of the analyst additional remarks on the constituents and their amount in the sample are possible).

6.3.2 Partly open declaration (descending sequence):

As far as was discernible using a microscope the constituents in the submitted sample were found in the declared sequence.

6.3.3 Open declaration (percentile declaration):

As far as was discernible using a microscope, the declared constituents were found in the submitted sample in the declared amount.

(Depending on the experience of the analyst additional remarks on the constituents and their amount in the sample are possible).

6.3.4 Negative result:

As far as was discernible using a microscope, the declared constituent [*name*] was not found in the submitted sample.

6.3.5 Additional result:

In addition to the declared constituents of the submitted sample constituent [*name*] was found by microscopic investigation. (As far as was discernible using a microscope, an amount of [*number*] % was estimated in the submitted sample. Depending on the identified constituent, amounts lower than 2% are reported as traces).

6.3.6 Deficiency of the declared amount of a constituent:

The constituent [*name*] was found by microscopic investigation in the submitted sample with an amount deviating from the declared value.

(As far as was discernible using a microscope an amount of [*number*] % was estimated.

Depending on the identified constituent, amounts lower than 2% are reported as traces).

6.3.7 Additional remarks:

Quantification done by microscopy may be subject to significant variation especially in the case of pelleted feed.

Constituents, such as fat and oil, molasses, fish solubles and other constituents, which lack characteristic morphological structures cannot be determined microscopically. Therefore variations in the estimated results are possible.

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

7. Validation

The accuracy of percentage estimation of feedingstuff constituents by microscopy is strongly influenced by a range of factors which are outside the control of the feed microscopist. These factors include structure of individual components, methods used in feed manufacture and choice of raw material in compound feedingstuffs.

Following extensive ring-trials conducted by IAG to address these issues, the following uncertainty intervals have been developed:

>	2 – 5	%	+/-	100 r
>	5 – 10	%	+/-	5 a
>	10 – 20	%	+/-	50 r
>	20 – 50	%	+/-	10 a
>	50	%	+/-	20 r

8. Remarks

Macroscopic and microscopic identification of constituents may be confirmed by staining, e.g.

	Reagent	Stained Component	Colour
8.1	Bradford Reagent (3.2.1)	protein containing animal and plant constituents	blue colouring
8.2	Iodine / Potassium Iodide Solution (Lugol Solution) (3.2.2)	protein containing animal and plant constituents, yeast, bacteria	brown colouring
		horn containing animal parts, connective tissue, feathers	brown-yellow colouring
		starch	blue-violet colouring

		hydrolysed starch	blue colouring
8.3	Sudan-Glycerine Reagent (3.2.3)	oils and fats	orange-red colouring

8.4 Chloral hydrate (3.1.1) applied to fine sieved fragments on a glass slide and gently heated in a fume cupboard can be used to dissipate the obscuring effect of starch during microscopic observation. Additional to a chloral hydrate preparation, slides with fragments embedded in water can be used especially to examine starch products.

8.5 If the amount of only one constituent is to be determined in a compound feedingstuff, it is recommended to determine all constituents for confirmation of the result.

8.6 Constituents that are difficult or cannot be determined by microscopy e.g. molasses, fish solubles, fat and oil can be considered by taking the data from the chemical analysis or the declaration into account.

9. Supporting document

Sample Preparation for the Macroscopic and Microscopic Analysis, IAG-Method A 1

10. Literature

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