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Animal feeding stuffs — Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES

National foreword

This British Standard is the UK implementation of EN 15621:2012. It supersedes DD CEN/TS 15621:2007, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Animal feeding stuffs - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES

Aliments pour animaux - Dosage du calcium, du sodium, du phosphore, du magnésium, du potassium, du soufre, du fer, du zinc, du cuivre, du manganèse et du cobalt après digestion sous pression par ICP-AES

Futtermittel - Bestimmung von Calcium, Natrium, Phosphor, Magnesium, Kalium, Schwefel, Eisen, Zink, Kupfer, Mangan und Kobalt nach Druckaufschluss mittels ICP-AES

This European Standard was approved by CEN on 30 December 2011.

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Contents

Foreword

This document (EN 15621:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2012, and conflicting national standards shall be withdrawn at the latest by August 2012.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 15621:2007.

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1 Scope

This European Standard specifies a method for the determination of the minerals calcium, sodium, phosphorus, magnesium, potassium and sulphur and the elements iron, zinc, copper, manganese, cobalt in animal feeding stuffs by inductively coupled plasma atomic emission spectrometry (ICP-AES) after pressure digestion.

The method was fully statistically tested and evaluated within 11 animal feeding stuff samples for the minerals calcium, sodium, phosphorus, magnesium, potassium and sulphur and the elements iron, zinc, copper, manganese and cobalt.

For potassium and sulphur the HORRAT values were mostly higher than 2. Therefore, for these elements the method is more applicable as a screening method and not for confirmatory purposes.

Other elements like molybdenum, lead, cadmium, arsenic were not fully statistically tested and evaluated within 11 animal feeding stuff samples because these elements did not occur in concentrations higher than the limit of quantification in most of these samples. A single laboratory validation is therefore necessary for the use of this multi element method for these elements.

The method limit of quantification for each element is dependent on the sample matrix as well as on the instrument. The method is not applicable for determination of low concentrations of elements. A limit of quantification of 1 mg/kg should normally be obtained.

NOTE This method can also be used for the determination of minerals in products with high mineral content (> 5 %), yet for this purpose other more precise analytical techniques are available.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

3 Principle

For the determination of calcium, sodium, phosphorus, magnesium, potassium and sulphur, iron, zinc, copper, manganese, cobalt, molybdenum, lead, cadmium and arsenic a test portion of the sample is digested under pressure.

The concentration of the elements is determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using external calibration or standard addition technique.

WARNING — The use of this European Standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

4 Reagents

Use only reagents of recognized analytical grade, and water conforming to grade 2 of EN ISO 3696.

4.1 Nitric acid concentrated, not less than 65 % (mass fraction), $c(HNO_3)$ = 14,4 mol/l, having a density of approximately ρ (HNO_3) 1,42 g/ml.

4.2 Nitric acid solution, with a volume fraction of 2 %.

To be prepared: pipette 20 ml nitric acid concentrated (4.1) in a 1 000 ml volumetric flask (5.4) and fill to the mark with water.

4.3 Hydrogen peroxide, not less than 30 % (mass fraction).

4.4 Element stock solutions.

Ca, Na, P, Mg, K, S, Fe, Zn, Cu, Mn, Co, Mo, Cd, Pb, As

c = 1 000 mg/l.

The user should choose a suitable stock solution. Both single-element stock solutions and multi-element stock solutions with adequate specification stating the acid used and the preparation technique are commercially available. It is advisable to use certified stock solutions.

NOTE Element stock solutions with concentrations different from 1 000 mg/l may be used as well.

4.5 Standard solutions.

Depending on the scope, different multi-element standard solutions may be necessary. In general, when combining multi-element standard solutions, their chemical compatibility and the possible hydrolysis of the components shall be regarded. Spectral interferences from other elements present in multi-element standards also need to be considered.

Various combinations of elements at different concentrations can be used, provided that the stock standard solutions (4.4) are diluted with the same acid and equal concentration as the acid in the test solution to a range of standards that covers the concentrations of the elements to be determined.

The multi-element standard solutions are considered to be stable for several months, if stored in the dark.

5 Apparatus

Usual laboratory apparatus and, in particular, the following:

NOTE For the determination of sodium in low concentrations it is advisable not to use glassware since glassware can be a source of sodium contamination.

5.1 Laboratory grinder.

5.1.1 Laboratory grinder, capable of grinding to a particle size of less than or equal to 1 mm, e.g. a knife mill or equivalent.

5.1.2 Laboratory grinder, capable of grinding to a particle size of less than or equal to 0,1 mm, e.g. a ball mill or equivalent.

NOTE It should be checked that the mill used does not influence the concentration of elements in the sample (e.g. adsorption, contamination) which have to be analysed.

5.2 Analytical balance, capable of weighing to an accuracy of 1 mg.

5.3 Pressure digestion apparatus, commercially available.

The apparatus shall be tested for safety pressure vessels made of acid-resistant materials and having holders for the sample of acid-resistant material with low level of contamination by elements to be determined. Apparatus is available which uses a high-pressure incinerator with or without ambient autoclave pressure.

Instead of polytetrafluoroethylene (PTFE) holders, it is better to use graduated quartz holders, fluorinated ethylene propylene (FEP) holders or perfluoroalkoxy (PFA) holders. Quartz is advisable to be used for decomposition temperatures above 230 °C.

5.4 One-mark volumetric flasks, of capacity 1 000 ml.

5.5 Inductively coupled plasma – Atomic Emission Spectrometer.

The instrument shall be equipped with radial plasma as a minimum requirement; axial plasma is equally acceptable. Background correction shall also be performed when necessary. Settings of the working conditions (e.g. viewing height, gas flows, RF or plasma power, sample uptake rate, integration time, and number of replicates) shall be optimised according the manufacturer’s instructions.

5.6 Freeze drying equipment, capable of freeze-drying liquid animal feeding stuffs.

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in EN ISO 6497 [2].

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage.

7 Preparation of the test sample

7.1 General

Prepare the test sample in accordance with prEN ISO 6498:

- The grinding must be done in conditions such that the substance is not appreciably heated and that no contamination takes place by the grinding tools;
- The operation is to be repeated as many times as is necessary and it must be effected as quickly as possible in order to prevent any gain or loss of constituents (water);
- The whole ground product is placed in a flask made of e.g. polypropylene, which can be stoppered and stored in such way to prevent any change in composition;
- Before any weighing is carried out for the analysis, the whole test sample must be thoroughly mixed for reasons of homogeneity. Since a maximum of 0,5 g of sample is used for the digestion it is of the utmost importance to have a homogeneous sample in order to take a representative sub sample.

7.2 Animal feeding stuffs which can be ground as such

Grind the laboratory sample (usually 500 g), using a laboratory grinder (0) or mortar, until a particle size of 1 mm or less has been reached.

7.3 Liquid animal feeding stuffs

7.3.1 General

Liquid feeding stuffs shall be pre-dried according to the procedure described in 7.3.2 or freeze-dried according to the procedure described in 7.3.3.

7.3.2 Pre-drying

Pre-dry the laboratory sample at a temperature of 70 °C ± 5 °C during at least 16 h to reduce the moisture content. The mass of the sample before and after the pre-drying is to be determined using an analytical balance (5.2). Grind the pre-dried sample in accordance with 7.2.

7.3.3 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (5.6). The mass of the sample before and after the freeze-drying is to be determined using an analytical balance (5.2). Grind the freeze-dried sample in accordance with 7.2.

7.4 Mineral animal feeding stuffs

Mineral compounds, except mineral products containing crystalline water, e.g. $MgCl_2.6H_2O$, shall be ground using a laboratory grinder (5.1.2) or mortar until a particle size of 0,1 mm or less has been reached. Mineral products containing crystalline water should not be ground.

8 Procedure

8.1 Pressure digestion – Preparation of the blank test solution and the test solution

8.1.1 General

Match the initial sample mass to the capacity of the digestion vessel, with the manufacturer's instructions being strictly observed for safety reasons. Determine the necessary digestion temperature and digestion time (see EN 13805 [1], see Annex B).

8.1.2 Example of microwave digestion

When using 100 ml vessels, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (4.1) and 0,5 ml of hydrogen peroxide (4.3), seal the digestion vessel and the pressure holders in the correct manner. Leave to pre-digest outside the microwave for about 30 min. Apply low microwave energy at the beginning of the digestion and slowly raise the energy to the maximum power, e.g. start with 100 W, raise up to 600 W within 5 min, hold for 5 min, raise to 1 000 W, hold for 10 min, cool down for minimum 20 min to 25 min. Treat a blank in the same way.

Dilute the digestion solution accordingly with water. The solution obtained after dilution is called the test solution. Proceed in accordance with 8.2.

8.1.3 Example of a high pressure digestion

When using a 100 ml vessel, weigh about 0,5 g of the prepared test sample to the nearest 1 mg. Add 3 ml of nitric acid (4.1), seal the digestion vessel and the pressure vessel in the correct manner and heat from room temperature to 150 °C in 60 min, then to 300 °C in 40 min and keep 300 °C for 90 min before cooling down. Treat a blank in the same way.

Dilute the digestion solution accordingly with water. The solution obtained after dilution is called the test solution. Proceed in accordance with 8.2.

8.2 Calibration

8.2.1 General

Calibration shall be performed by means of external calibration or standard addition technique. It is important that the measurements are made in the linear range of the instrument. Appropriate matrix matching of the calibration solutions shall be performed if an external calibration method is used (see Annex B).

8.2.2 External calibration

The calibration is performed with at least two calibration solutions, of which, one is a blank calibration solution. If the working range is not linear, the calibration should be performed with a blank calibration solution and at least three equidistant calibration solutions.

8.2.3 Standard addition technique

The standard addition curve should consist of at least two points, of which, one is an addition. For those elements whose concentration is near the limit of quantification, the standard addition curve should consist of at least four points, of which, three are additions. If three additions are used, the concentration of the highest standard should be 3 to 5 times the concentration in the sample solution.

8.3 Determination

8.3.1 General

Analytical lines, selectivity, limits of detection and quantification, precision, linear working area, and interferences have to be established before operating the ICP-AES system.

8.3.2 Determination by inductively coupled plasma – atomic emission spectrometry

8.3.2.1 General

Table 1 — Selected emission wavelengths and interferences for determination with ICP-AES

Element	Wavelength of emission (nm)	Interference
Ca	315,887	Co
	317,933	Fe, V
	393,366	
Co	228,616	Ti
Cu	324,754	Ti, Fe
	327,396	
Fe	238,200	Co
	259,940	
K	766,490	Mg, Ar
	769,900	
Mg	279,079	
	279,553	
	285,213	Fe
As	188,979	
	189,042	
	193,696	
	197,197	
Pb	216,999	
	220,353	
	261,418	Al, Co, Ti
Mn	257,610	Fe, Mo, Cr
	293,306	Al, Fe
Mo	202,030	Al, Fe
	204,598	
Na	330,237	
	588,995	
	589,592	Ar
P	178,287	I
	213,618	Cu, Fe, Mo, Zn
	214,914	Cu, Al, Mg
	177,428	Cu
S	181,972	
	182,036	
Zn	206,200	
	213,856	P
Cd	214,438	
	226,502	
	228,802	

Table 1 gives relevant analytical lines and possible interferences for the determination with ICP-AES. Other wavelengths than those specified in Table 1 can be used (see also Annex B).

8.3.2.2 External calibration method

Aspirate the blank test solution (8.1), the calibration solutions prepared from the standard solutions (4.5), and the test solution (8.1) in ascending order separately into the plasma and measure the emission of the element to be determined using an external calibration (8.2.2).

NOTE If the test solution contains residues, decant the solution into the test tube from which the test solution is aspirated into the ICP. Use the first decanted portion to rinse the test tube.

Perform at least two replicates. Average the values if the values fall within an accepted range. After each measurement, aspirate water or nitric acid solution (4.2).

8.3.2.3 Standard addition technique

Aspirate the blank test solution (8.1), the test solution (8.1), and the test solution prepared by standard addition technique (8.2.3) in ascending order separately into the plasma, and measure the emission of the element to be determined.

NOTE If the test solution (8.1) contains residues, decant the solution into the test tube from which the test solution is aspirated into the ICP. Use the first decanted portion to rinse the test tube.

Perform at least two replicates. Average the values if the values fall within an accepted range. After each measurement, aspirate water or nitric acid solution (4.2).

9 Calculation and expression of the result

9.1 General

Net signal is defined as the number of counts per second at the selected wavelength, corrected for background contributions.

9.2 External calibration

In the case of a linear calibration curve constructed with one blank calibration solution and one calibration solution, the calibration function can be described as follows:

$$S_{st} = c_{st} \times b + a \tag{1}$$

where

S_{st} is the net signal of the calibration solution;

c_{st} is the concentration, in mg/l, of the calibration solution;

a is the intersection;

b is the slope.

Calculate the element concentration c_f, in mg/l, in the test solution (8.1) using the slope *b* and the intersection *a* found in (1) as follows:

$$c_f = \frac{S_f - a}{b} \tag{2}$$

where

S_f is the net signal of the test solution.

9.3 Standard addition method with only one addition

In the most simple case of standard addition, where only one addition is made, the element concentration c_f, in mg per l, in the test solution (8.1) is determined as follows:

$$c_f = \frac{S_0 \times V_s \times c_s}{(S_1 - S_0) \times V_f} \tag{3}$$

where

c_s is the concentration, in mg/l, of the standard solution;

V_s is the volume, in l, of the standard solution added;

V_f is the volume, in l, of the test solution (8.1) used to prepare the solution without addition;

S_0 is the net signal of the solution without addition;

S_1 is the net signal after addition.

9.4 Standard addition method with several additions

In case of several additions, regression techniques on the linear model of variable y as a function of variable x, have to be used to determine the element concentration of the test solution (8.1). Generally, this model can be written as:

$$y_i = a + b \times x_i \qquad (4)$$

In this particular case of three standard additions,

$$y_i = S_i \qquad \text{(for i = 0, 1, 2, 3);} \qquad (5)$$

$$x_i = c_s \times V_i \qquad \text{(for i = 0, 1, 2, 3);} \qquad (6)$$

a is the intersection;

b is the slope;

where

c_s is the concentration, in mg/l, of the standard solution;

V_i are the various volumes, in litres, of the standard solution added;

S_i are the net signals after the various additions.

The values of *a* and *b* can then be calculated as follows:

$$b = \frac{n \times y_i - \sum x_i \sum y_i}{n \times \sum x_i^2 - \left(\sum x_i\right)^2} \qquad (7)$$

$$a = \frac{\sum y_i - b \times \sum x_i}{n} \qquad (8)$$

where

n is the number of solutions measured (*n* = 4 in case of three additions).

The element concentration c_f, in mg/l, of the test solution (8.1) can then be found using the following equation:

$$c_f = \frac{a/b}{V_f} \qquad (9)$$

where

V_f is the volume, in l, of the test solution (8.1) used to prepare the solution without addition.

9.5 Calculation of the element content in the sample

The element content for the minerals Ca, Na, P, Mg, K and S in the sample or mass fraction of element w_{elem}, expressed in percentage, is determined using the following equation:

$$w_{\text{elem}} = \frac{(c_f - c_{bl})}{m} \times V_t \times 0{,}0001 \quad (10)$$

The element content for the other elements Fe, Zn, Cu, Mn, Co, Mo, Cd, As, Pb in the sample or mass fraction of element w_{elem}, expressed in mg/kg, is determined using the following equation:

$$w_{\text{elem}} = \frac{(c_f - c_{bl})}{m} \times V_t \quad (11)$$

where

c_f is the concentration, in mg/l, of the test solution (8.1), as determined using equation (2) or (3) or (9);

c_{bl} is the concentration, in mg/l, of the blank solution;

m is the mass of sample, in kg, taken for the extraction by digestion, and corrected for water content;

V_t is the total volume, in l, of the test solution (8.1).

If the test solution (8.1) has been diluted further, take into account the dilution factor.

If the sample has been pre-dried or freeze-dried (7.3), recalculate the result to the fresh weight of the sample taking into account the loss of moisture during pre-drying or freeze-drying.

10 Precision

10.1 Inter-laboratory test

An inter-laboratory test has been carried out in 2007. Details of inter-laboratory tests on precision of the method are summarized in Annex A. The values derived from these tests may not be applicable to concentration ranges and matrices others than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of the cases be greater than the repeatability limit r given in Table 2 (minerals Ca, Na, Mg, P, K, S) and Table 3 (elements Fe, Mn, Cu, Zn, Co, Mo, Cd, As, Pb).

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in the same laboratory with different operators using different equipment, will in not more than 5% of the cases be greater than the reproducibility limit *R* given in Table 2 (minerals Ca, Na, Mg, P, K, S) and Table 3 (elements Fe, Mn, Cu, Zn, Co, Mo, Cd, As, Pb).

Table 2 — Precision data – Ca, K, Mg, Na, P, S

Ca			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	1,21	0,17	0,31
Sheep feed	1,12	0,10	0,17
MIN 1	24,1	0,8	2,9
MIN 2	18,1	0,8	2,2
MIN MIX	14,1	0,6	1,8
Com.Premix	4,59	0,53	1,60
CuSO4	< LOQ	--	--
Phosphate	21,7	0,7	1,9
MgO	1,36	0,11	0,31
CaCO3	35,4	1,3	3,5
Bentonite	0,75	0,03	0,10
K			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	0,73	0,06	0,17
Sheep feed	1,26	0,08	0,21
MIN 1	0,06	0,01	0,05
MIN 2	0,37	0,02	0,17
MIN MIX	0,05	0,01	0,04
Com.Premix	0,20	0,02	0,06
CuSO4	< LOQ	--	--
Phosphate	0,09	0,01	0,05
MgO	0,15	0,02	0,11
CaCO3	0,33	0,04	0,29
Bentonite	0,07	0,01	0,05
Mg			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	0,18	0,01	0,02
Sheep feed	0,35	0,02	0,05
MIN 1	3,75	0,13	0,85
MIN 2	2,85	0,14	0,40
MIN MIX	12,2	0,6	2,1
Com.Premix	0,13	0,01	0,05
CuSO4	< LOQ	--	--
Phosphate	0,70	0,03	0,11
MgO	48,9	2,2	6,2
CaCO3	0,30	0,02	0,09
Bentonite	0,70	0,06	0,91

Table 2 — *(continued)*

Na			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	0,24	0,02	0,05
Sheep feed	0,30	0,02	0,05
MIN 1	6,90	0,29	1,25
MIN 2	8,88	0,26	1,68
MIN MIX	11,9	0,7	1,6
Com.Premix	0,96	0,05	0,15
CuSO4	< LOQ	--	--
Phosphate	0,12	0,01	0,03
MgO	< LOQ	--	--
CaCO3	< LOQ	--	--
Bentonite	1,20	0,06	0,19
P			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	0,51	0,04	0,71
Sheep feed	0,69	0,04	0,10
MIN 1	3,37	0,27	0,63
MIN 2	2,16	0,14	0,30
MIN MIX	3,83	0,13	0,76
Com.Premix	0,15	0,02	0,07
CuSO4	< LOQ	--	--
Phosphate	21,9	1,2	2,3
MgO	0,047	0,004	0,014
CaCO3	0,023	0,003	0,007
Bentonite	0,020	0,003	0,012
S			
Samples	Mean, $\bar{x}$ (%)	*r* (%)	*R* (%)
Pig feed	0,19	0,02	0,04
Sheep feed	0,29	0,02	0,06
MIN 1	0,78	0,06	0,37
MIN 2	0,17	0,01	0,15
MIN MIX	0,35	0,04	0,10
Com.Premix	2,64	0,26	0,67
CuSO4	12,1	0,4	2,6
Phosphate	0,98	0,08	0,44
MgO	< LOQ	--	--
CaCO3	0,39	0,02	0,32
Bentonite	0,22	0,02	0,06

Table 3 — Precision data – Co, Cu, Fe, Mn, Mo, Zn, Cd, As, Pb

Co			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	< LOQ	--	--
Sheep feed	1,85	0,17	1,05
MIN 1	24,0	1,3	15,0
MIN 2	13,6	1,0	6,2
MIN MIX	47,4	9,2	36,4
Com.Premix	21 540	1 988	3 252
CuSO4	< LOQ	--	--
Phosphate	< LOQ	--	--
MgO	2,65	0,78	2,29
CaCO3	5,75	0,77	3,44
Bentonite	1,46	0,36	1,66
Cu			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	22,6	10,1	11,6
Sheep feed	11,6	1,9	5,4
MIN 1	947	59	209
MIN 2	574	30	156
MIN MIX	850	77	335
Com.Premix	2024	123	397
CuSO4	264 900	13 920	60 750
Phosphate	11,6	1,5	6,1
MgO	28,4	6,6	14,1
CaCO3	6,26	1,35	7,80
Bentonite	6,17	2,10	7,80
Fe			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	386	67	115
Sheep feed	277	47	96
MIN 1	2 512	202	669
MIN 2	3 205	287	860
MIN MIX	3 240	431	895
Com.Premix	15 940	1 938	3 942
CuSO4	24,2	6,1	12,7
Phosphate	2 074	63	380
MgO	21 280	1 018	4 067
CaCO3	6 939	423	1 565
Bentonite	11 390	1 093	13 790

Table 3 — *(continued)*

Mn

Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	89,6	13,1	36,9
Sheep feed	95,8	8,4	21,5
MIN 1	2 444	163	663
MIN 2	2 542	150	662
MIN MIX	2 143	177	491
Com.Premix	15 590	1 940	6 526
CuSO4	< LOQ	--	--
Phosphate	118	2	39
MgO	718	38	206
CaCO3	1 509	56	402
Bentonite	88,0	8,7	12,0

Mo

Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	< LOQ	--	--
Sheep feed	< LOQ	--	--
MIN 1	< LOQ	--	--
MIN 2	< LOQ	--	--
MIN MIX	< LOQ	--	--
Com.Premix	17 920	2 351	6 483
CuSO4	< LOQ	--	--
Phosphate	< LOQ	--	--
MgO	< LOQ	--	--
CaCO3	< LOQ	--	--
Bentonite	< LOQ	--	--

Zn

Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	127	12	47
Sheep feed	155	14	46
MIN 1	6 844	525	1 595
MIN 2	5 440	348	1 144
MIN MIX	3 789	247	940
Com.Premix	10 310	611	1 559
CuSO4	< LOQ	--	--
Phosphate	220	12	86
MgO	< LOQ	--	--
CaCO3	11,5	2,7	10,8
Bentonite	83,5	19,5	33,0

Table 3 — *(continued*

Cd			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	< LOQ	--	--
Sheep feed	< LOQ	--	--
MIN 1	37,9	1,7	19,9
MIN 2	< LOQ	--	--
MIN MIX	< LOQ	--	--
Com.Premix	< LOQ	--	--
CuSO4	< LOQ	--	--
Phosphate	4,90	0,40	1,96
MgO	< LOQ	--	--
CaCO3	< LOQ	--	--
Bentonite	< LOQ	--	--
As			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	< LOQ	--	--
Sheep feed	< LOQ	--	--
MIN 1	< LOQ	--	--
MIN 2	< LOQ	--	--
MIN MIX	< LOQ	--	--
Com.Premix	< LOQ	--	--
CuSO4	< LOQ	--	--
Phosphate	< LOQ	--	--
MgO	< LOQ	--	--
CaCO3	9,42	0,99	4,82
Bentonite	< LOQ	--	--
Pb			
Samples	Mean, $\bar{x}$ (mg/kg)	*r* (mg/kg)	*R* (mg/kg)
Pig feed	< LOQ	--	--
Sheep feed	< LOQ	--	--
MIN 1	< LOQ	--	--
MIN 2	< LOQ	--	--
MIN MIX	< LOQ	--	--
Com.Premix	< LOQ	--	--
CuSO4	< LOQ	--	--
Phosphate	3,97	0,84	3,32
MgO	< LOQ	--	--
CaCO3	< LOQ	--	--
Bentonite	35,6	3,8	15,3

11 Test report

The test report shall contain at least the following information:

a) the test method used, with reference to this European Standard;

b) all information necessary for the complete identification of the sample;

c) any particular points observed in the course of the test;

d) all operation details not specified in this document, or regarded as optional, together with details of any incidents which might have affected the results;

e) the results obtained of the determination, expressed as mass fraction of animal feeding stuff w_{elem}, in percentage for the minerals or in mg/kg for the trace elements and heavy metals.

Annex A
(informative)

Results of the inter-laboratory test

An inter-laboratory comparison has been organized by Technische Universität München, Research Center for Nutrition and Food Sciences, Bioanalytic Weihenstephan in 2007 with 20 participating laboratories and 11 different animal feeding stuffs, including a complete feed for pigs, a complete feed for sheep, three mineral mixture feeds, a mineral premixture and five minerals (rock phosphate, copper sulphate, magnesium oxide, calcium carbonate, bentonite). The samples were homogenized centrally and distributed to the participants. The tests yielded the data given in Tables A.1 and A.15. Repeatability and reproducibility were calculated according to ISO 5725-1 [3].

Details are given only in the final report [4].

Table A.1 — Statistical results of an inter-laboratory test – Calcium (Ca)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Calcium (Ca)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	19	19	20	20	19	20
No. of outlier labs	2	0	2	1	2	0		2	2	1	2
No. of non-compliant labs	0	0	0	0	0	0		0	0	0	0
No. of valid labs	18	20	18	19	18	19		18	18	18	18
Mean value, %	1,21	1,12	24,1	18,1	14,1	4,59	<LOQ	21,7	1,36	35,4	0,75
s_r, %	0,058	0,036	0,288	0,297	0,208	0,190		0,254	0,039	0,464	0,011
r, %	0,17	0,10	0,80	0,84	0,58	0,53		0,70	0,11	1,3	0,03
RSD(r), %	4,8	3,2	1,2	1,6	1,5	4,2		1,2	2,8	1,3	1,5
s_R, %	0,11	0,063	1,060	0,801	0,662	0,578		0,688	0,113	1,26	0,038
R, %	0,31	0,17	2,9	2,2	1,8	1,6		1,9	0,31	3,5	0,10
RSD(R), %	9,2	5,6	4,4	4,4	4,7	12,9		3,2	8,3	3,6	5,0
HORRAT value	2,4	1,4	1,8	1,7	1,8	4,0	- - -	1,3	2,2	1,5	1,2

Table A.2 — Statistical results of an inter-laboratory test – Potassium (K)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Potassium (K)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	19	16	20	20	20	20
No. of outlier labs	1	0	0	1	3	0		2	0	1	1
No. of non-compliant labs	0	0	2	0	0	0		0	1	0	0
No. of valid labs	19	20	18	19	17	19		18	19	19	19
Mean value, %	0,73	1,26	0,055	0,37	0,053	0,20	<LOQ	0,087	0,15	0,33	0,074
s_r, %	0,021	0,029	0,004	0,009	0,0042	0,006		0,0028	0,007	0,015	0,003
r, %	0,06	0,08	0,01	0,02	0,012	0,02		0,008	0,02	0,04	0,01
RSD(r), %	2,8	2,3	6,4	2,4	7,9	2,8		3,3	4,3	4,6	3,4
s_R, %	0,059	0,074	0,018	0,061	0,0155	0,023		0,0182	0,038	0,103	0,019
R, %	0,17	0,21	0,05	0,17	0,043	0,06		0,05	0,11	0,29	0,053
RSD(R), %	8,1	5,9	32,5	16,3	29,0	11,8		21,0	25,2	31,4	25,8
HORRAT value	1,9	1,5	5,3	3,5	4,7	2,3	- - -	3,6	4,8	6,6	4,4

Table A.3 — Statistical results of an inter-laboratory test – Magnesium (Mg)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Magnesium (Mg)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	19	17	20	19	20	20
No. of outlier labs	2	2	4	0	1	0		2	2	0	2
No. of non-compliant labs	0	0	0	0	0	0		0	0	0	0
No. of valid labs	18	18	16	20	19	19		18	17	20	18
Mean value, %	0,18	0,35	3,75	2,85	12,2	0,13	<LOQ	0,70	48,9	0,30	0,70
s_r, %	0,003	0,007	0,046	0,067	0,23	0,0029		0,010	0,79	0,009	0,022
r, %	0,01	0,02	0,13	0,19	0,6	0,008		0,03	2,2	0,02	0,06
RSD(r), %	2,0	2,1	1,2	2,4	1,9	2,3		1,4	1,6	2,9	3,1
s_R, %	0,007	0,019	0,308	0,143	0,76	0,017		0,039	2,23	0,034	0,33
R, %	0,02	0,05	0,85	0,40	2,1	0,05		0,11	6,2	0,09	0,91
RSD(R), %	3,9	5,5	8,2	5,0	6,2	13,6		5,4	4,6	11,2	46,7
HORRAT value	0,7	1,2	2,5	1,5	2,3	2,5	- - -	1,3	2,0	2,3	11,1

Table A.4 — Statistical results of an inter-laboratory test – Sodium (Na)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Sodium (Na)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	19	19	20	20	20	20
No. of outlier labs	2	2	3	3	2	3		19			3
No. of non-compliant labs	0	0	0	0	0	0		0			0
No. of valid labs	18	18	17	17	18	16		1			17
Mean value, %	0,24	0,30	6,90	8,88	11,87	0,96	<LOQ	0,12	<LOQ	<LOQ	1,20
s_r, %	0,006	0,009	0,106	0,093	0,257	0,017		0,003			0,021
r, %	0,02	0,02	0,29	0,26	0,71	0,05		0,01			0,06
RSD(r), %	2,5	3,0	1,5	1,0	2,2	1,8		2,4			1,7
s_R, %	0,016	0,020	0,452	0,607	0,572	0,054		0,012			0,070
R, %	0,05	0,05	1,25	1,68	1,59	0,15		0,03			0,19
RSD(R), %	6,8	6,6	6,6	6,8	4,8	5,6		10,1			5,8
HORRAT value	1,4	1,4	2,2	2,4	1,7	1,4	- - -	1,8	- - -	- - -	1,5

Table A.5 — Statistical results of an inter-laboratory test – Phosphorus (P)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Phosphorus (P)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	18	15	20	19	17	17
No. of outlier labs	0	0	0	0	3	1		2	3	0	1
No. of non-compliant labs	0	0	0	0	0	0		0	4	2	2
No. of valid labs	20	20	20	20	17	17		18	12	15	14
Mean value, %	0,51	0,69	3,37	2,16	3,83	0,15	<LOQ	21,9	0,047	0,023	0,020
s_r, %	0,015	0,015	0,099	0,051	0,046	0,007		0,42	0,0015	0,0010	0,0012
r, %	0,04	0,04	0,27	0,14	0,13	0,02		1,2	0,004	0,003	0,003
RSD(r), %	2,9	2,2	2,9	2,3	1,2	4,9		1,9	3,3	4,6	5,7
s_R, %	0,026	0,035	0,229	0,108	0,273	0,024		0,84	0,0052	0,0026	0,004
R, %	0,71	0,10	0,63	0,30	0,76	0,07		2,3	0,014	0,007	0,012
RSD(R), %	5,0	5,0	6,8	5,0	7,1	16,7		3,8	11,2	11,2	20,5
HORRAT value	1,1	1,2	2,0	1,4	2,2	3,1	- - -	1,5	1,8	1,6	2,9

Table A.6 — Statistical results of an inter-laboratory test – Sulphor (S)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Sulphor (S)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	19	19	19	19	19	19	18	19	19	19	19
No. of outlier labs	2	2	2	3	2	0	1	0		3	1
No. of non-compliant labs	0	0	0	0	0	0	0	0		0	0
No. of valid labs	17	17	17	16	17	19	17	19		16	18
Mean value, %	0,19	0,29	0,78	0,17	0,35	2,64	12,1	0,98	<LOQ	0,39	0,22
s_r, %	0,006	0,008	0,023	0,004	0,013	0,093	0,14	0,029		0,006	0,006
r, %	0,02	0,02	0,06	0,01	0,04	0,26	0,4	0,08		0,02	0,02
RSD(r), %	3,0	2,9	2,9	2,5	3,6	3,5	1,1	3,0		1,4	2,8
s_R, %	0,015	0,023	0,134	0,056	0,037	0,242	0,93	0,158		0,117	0,020
R, %	0,04	0,06	0,37	0,15	0,10	0,67	2,6	0,44		0,32	0,06
RSD(R), %	7,9	8,0	17,1	32,7	10,6	9,2	7,7	16,2		29,8	9,3
HORRAT value	1,5	1,7	4,1	6,3	2,3	2,7	2,8	4,0	- - -	6,5	1,9

Table A.7 — Statistical results of an inter-laboratory test – Cobalt (Co)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Cobalt (Co)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	17	17	18	18	18	17	15	17	16	17	16
No. of outlier labs		3	2	1	1	2			0	1	1
No. of non-compliant labs		3	1	1	1	0			5	1	4
No. of valid labs	17	11	15	16	16	15			11	15	11
Mean value, mg/kg	<LOQ	1,85	24,0	13,6	47,4	21 540	<LOQ	<LOQ	2,65	5,75	1,46
s_r, mg/kg		0,060	0,47	0,38	3,31	717,1			0,280	0,276	0,129
r, mg/kg		0,17	1,3	1,0	9,2	1 988			0,78	0,77	0,36
RSD(r), %		3,2	2,0	2,8	7,0	3,3			10,6	4,8	8,9
s_R, mg/kg		0,379	5,41	2,23	13,14	1 173			0,825	1,242	0,599
R, mg/kg		1,05	15,0	6,2	36,4	3 252			2,29	3,44	1,66
RSD(R), %		20,5	23,5	16,3	27,7	5,4			31,1	21,6	41,0
HORRAT value	- - -	1,4	2,4	1,5	3,1	1,5	- - -	- - -	2,3	1,8	2,7

Table A.8 — Statistical results of an inter-laboratory test – Copper (Cu)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Copper (Cu)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	18	18	19	20	18	18	19	18	18	16	18
No. of outlier labs	3	0	6	2	4	2	2	7	2	3	1
No. of non-compliant labs	0	0	0	0	0	0	0	0	0	3	2
No. of valid labs	15	18	13	18	14	16	17	11	16	10	15
Mean value, mg/kg	22,6	11,6	947	574	850	2 024	264 900	11,6	28,4	6,26	6,17
s_r, mg/kg	3,63	0,68	21,3	11,0	27,6	44,4	5 022	0,55	2,39	0,487	0,746
r, mg/kg	10,1	1,9	59	30	77	123	13 920	1,5	6,6	1,35	2,1
RSD(r), %	16,1	5,9	2,3	1,9	3,3	2,2	1,9	4,8	8,4	7,8	12,1
s_R, mg/kg	4,19	1,960	75,4	56,3	120,8	143,3	21 920	2,19	5,07	2,80	2,80
R, mg/kg	11,6	5,4	209	156	335	397	60 750	6,1	14,1	7,8	7,8
RSD(R), %	18,5	16,9	8,0	9,8	14,2	7,1	8,3	18,9	17,8	44,8	45,4
HORRAT value	1,8	1,5	1,4	1,6	2,5	1,4	3,4	2,1	1,8	3,7	3,7

Table A.9 — Statistical results of an inter-laboratory test – Iron (Fe)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Iron (Fe)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	19	20	20	18	19	20	19	20	19
No. of outlier labs	1	0	3	0	2	0	1	2	3	1	2
No. of non-compliant labs	0	0	0	0	0	0	0	0	0	0	0
No. of valid labs	19	20	16	20	18	18	18	18	16	19	17
Mean value, mg/kg	386	277	2 512	3 205	3 240	15 940	24,2	2 074	21 280	6 939	11 390
s_r, mg/kg	24,2	17,1	72,8	103,5	155,6	699	2,18	22,6	367,20	152,7	394,3
r, mg/kg	67	47	202	287	431	1 938	6,1	63	1 018	423	1 093
RSD(r), %	6,3	6,2	2,9	3,2	4,8	4,4	9,0	1,1	1,7	2,2	3,5
s_R, mg/kg	41,6	34,5	241,2	310,2	322,8	1 422	4,58	137,1	1 467	564,50	4 974
R, mg/kg	115	96	669	860	895	3 942	12,7	380	4 067	1 565	13 790
RSD(R), %	10,8	12,4	9,6	9,7	10,0	8,9	18,9	6,6	6,9	8,1	43,7
HORRAT value	1,7	1,8	1,9	2,0	2,1	2,4	1,9	1,3	1,9	1,9	11,1

Table A.10 — Statistical results of an inter-laboratory test – Manganese (Mn)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Manganese (Mn)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	19	19	19	18	16	20	19	19	20
No. of outlier labs	0	1	4	0	0	0		2	2	2	0
No. of non-compliant labs	0	0	0	0	0	0		0	0	0	0
No. of valid labs	20	19	15	19	19	18		18	17	17	20
Mean value, mg/kg	89,6	95,8	2 444	2 542	2 143	15 590	<LOQ	118	718	1 509	88,0
s_r, mg/kg	4,72	3,0	58,6	54,2	42,7	700		1,79	13,7	20,1	3,13
r, mg/kg	13,1	8,4	163	150	118	1 940		5,0	38	56	8,7
RSD(r), %	5,3	3,2	2,4	2,1	2,0	4,5		1,5	1,9	1,3	3,6
s_R, mg/kg	13,32	7,8	239,2	238,8	177	2 354		14,2	74,2	145	11
R, mg/kg	36,9	21,5	663	662	491	6 526		39	206	402	29,2
RSD(R), %	14,9	8,1	9,8	9,4	8,3	15,1		12,1	10,3	9,6	12,0
HORRAT value	1,8	1,0	2,0	1,9	1,6	4,0	- - -	1,6	1,7	1,8	1,5

Table A.11 — Statistical results of an inter-laboratory test – Molybdenum (Mo)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Molybdenum (Mo)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	15	16	15	15	14	17	12	14	14	15	15
No. of outlier labs						0					
No. of non-compliant labs						0					
No. of valid labs						17					
Mean value, mg/kg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	17 920	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
s_r, mg/kg						848,3					
r, mg/kg						2 351					
RSD(r), %						4,7					
s_R, mg/kg						2 339					
R, mg/kg						6 483					
RSD(R), %						13,1					
HORRAT value	- - -	- - -	- - -	- - -	- - -	3,6	- - -	- - -	- - -	- - -	- - -

Table A.12 — Statistical results of an inter-laboratory test – Zinc (Zn)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Zinc (Zn)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	20	20	20	20	20	19	19	20	17	19	20
No. of outlier labs	2	1	4	1	0	1		1		1	1
No. of non-compliant labs	0	0	0	0	0	0		0		1	0
No. of valid labs	18	19	16	19	20	18		19		17	19
Mean value, mg/kg	127	155	6 844	5 440	3 789	10 310	<LOQ	220	<LOQ	11,5	83,50
s_r, mg/kg	4,5	4,9	189,3	125,5	89,0	220,5		4,3		0,98	7,02
r, mg/kg	12	14	525	348	247	611		12		2,7	19,5
RSD(r), %	3,5	3,2	2,8	2,3	2,3	2,1		2,0		8,5	8,4
s_R, mg/kg	16,8	16,7	575,4	412,7	339,1	562,5		31,1		3,88	11,9
R, mg/kg	47	46	1 595	1 144	940	1559		86		10,8	33
RSD(R), %	13,2	10,8	8,4	7,6	8,9	5,5		14,2		33,7	14,3
HORRAT value	1,7	1,4	2,0	1,7	1,9	1,4	- - -	2,0	- - -	3,0	1,7

Table A.13 — Statistical results of an inter-laboratory test – Cadmium (Cd)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Cadmium (Cd)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	12	12	12	11	12	12	12	12	12	12	12
No. of outlier labs			2					1			
No. of non-compliant labs			0					0			
No. of valid labs			10					11			
Mean value, mg/kg	<LOQ	<LOQ	37,9	<LOQ	<LOQ	<LOQ	<LOQ	4,90	<LOQ	<LOQ	<LOQ
s_r, mg/kg			0,62					0,145			
r, mg/kg			1,7					0,40			
RSD(r), %			1,6					3,0			
s_R, mg/kg			7,19					0,707			
R, mg/kg			19,9					2,0			
RSD(R), %			19,0					14,4			
HORRAT value	- - -	- - -	2,0	- - -	- - -	- - -	- - -	1,1	- - -	- - -	- - -

Table A.14 — Statistical results of an inter-laboratory test – Arsenic (As)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Arsenic (As)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	9	9	9	9	9	9	8	9	9	9	9
No. of outlier labs										0	
No. of non-compliant labs										1	
No. of valid labs										8	
Mean value, mg/kg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9,42	<LOQ
s_r, mg/kg										0,355	
r, mg/kg										0,99	
RSD(r), %										3,8	
s_R, mg/kg										1,740	
R, mg/kg										4,82	
RSD(R), %										18,5	
HORRAT value	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	1,6	- - -

Table A.15 — Statistical results of an inter-laboratory test – Lead (Pb)

Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Element Lead (Pb)	Pig feed	Sheep feed	MIN 1	MIN 2	MIN MIX	Com.Premix	CuSO4	Phosphate	MgO	CaCO3	Bentonite
No. of labs	11	11	11	12	12	11	11	11	11	11	11
No. of outlier labs								0			0
No. of non-compliant labs								3			0
No. of valid labs								8			11
Mean value, mg/kg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3,97	<LOQ	<LOQ	35,6
s_r, mg/kg								0,303			1,39
r, mg/kg								0,84			3,8
RSD(r), %								7,6			3,9
s_R, mg/kg								1,20			5,54
R, mg/kg								3,3			15,3
RSD(R), %								30,2			15,6
HORRAT value	- - -	- - -	- - -	- - -	- - -	- - -	- - -	2,3	- - -	- - -	1,7

Annex B
(informative)

Notes on the detection technique, interferences and quantification, pressure digestion

B.1 General

Atomic emission spectroscopic techniques are widely used for qualitative and quantitative analysis. This Annex describes some phenomena that can be of importance for the interpretation of the procedures of this standard. Although some theoretical considerations will be made, this annex has not the intention of being a handbook of spectroscopic techniques.

B.2 Interferences

B.2.1 General

For the determination of a specific analyte in a sample, usually the most sensitive lines are preferred. In case of interferences, especially spectral interferences, another line has to be selected, even when it is a less sensitive one. It is known that the ICP-AES technique suffers from a variety of interferences that are shortly described hereafter.

B.2.2 Spectral line interference

Spectral line interference, where atomic lines overlap or are unresolved, is often encountered in atomic emission where light is emitted not only by the element of interest but also from all other elements present in the sample. Very often, this kind of interference can be eliminated by the proper choice of emission line.

A kind of spectral interference encountered in emission techniques is the occurrence of band-emission spectra due to the presence of molecular species.

B.2.3 Ionisation interferences

Ionisation interferences are caused by the presence of easily ionisable elements in the matrix of the sample, resulting in a change of the ionisation equilibrium of the analyte due to an increase in the electron number density.

Adding larger amounts of an easily ionisable element to sample and calibration solutions can be used to overcome this kind of interference.

B.2.4 Physical interferences

Physical interferences are caused by differences in some physical properties of the solutions (sample and calibration standards) such as viscosity, surface tension, and vapour pressure. These differences can then cause changes in aspiration, nebulization, or atomisation efficiency.

They can be overcome to some extent by applying matrix matching of the calibration solutions, by dilution or adding relatively high acid concentrations, or by means of the standard addition technique.

B.3 Quantification and matrix matching

B.3.1 General

As spectroscopic techniques are not able to measure concentrations directly, but by means of a conversion of the emission signal into concentration, calibration is inevitable. Calibration can be performed by means of a calibration curve or by means of standard addition.

Given the various kinds of interferences, and the fact that most animal feeding stuffs have complex matrices, some kind of matrix matching between calibration solutions and sample solution has to be performed, in order to eliminate matrix effects.

If an unknown sample is to be handled, it seems more appropriate to determine the concentration of the analyte by means of standard addition.

B.3.2 Calibration curve

A calibration curve is constructed by adding increasing amounts of the substance to be studied to a solution of a supporting matrix. The most difficult condition to meet is making the solutions used for the calibration curve exactly identical to those for the sample analysis. However, calibration curves are frequently recorded in solutions containing only the studied compound, whereas the sample itself introduces various other substances.

Insufficient knowledge about the sample composition may create serious difficulties for matrix matching. However, in case the composition of the samples in very well known, and does not vary too much from sample to sample, matrix matching is preferred over standard addition, especially in case of multi-element determinations.

Often the ratio of the analyte intensity to the intensity of a second element added to the sample (internal standard) is used to improve the precision of the analysis. In this way, also some of the variables in the excitation and processing of spectra can be minimized or eliminated by adopting the internal standard technique.

If the linearity is guaranteed, two calibration solutions should be enough to set up the calibration curve. Nevertheless, three to five calibration solutions are recommended. In all cases, the linearity should be checked on a regular basis. The working area should be chosen in such a way that the concentration of the sample solution is situated in the middle of the calibration curve.

B.3.3 Matrix matching

In case of known matrices the technique of matrix matching between calibration solutions and sample solutions is done by adding the appropriate amounts of analytical grade reagents to the calibration solutions in order to imitate the matrix of the sample solution.

B.3.4 Standard addition

Standard addition is a way of measuring concentrations suited particularly well for samples with high but unknown total ionic strength (matrix) or for samples with highly variable solution components. This approach does not require the preparation of a calibration curve. The standard addition method is also used in this way to compensate for chemical and other matrix interferences and effects.

Usually the standard addition is a small volume of a concentrated solution so that the total solution volume and ionic strength are not changed appreciably. The most accurate determinations are made when the change in concentration of the element in study is such that the total concentration is approximately doubled.

Standard addition is particularly affected by non-linearity's, and a minimum number of additions of three to five is therefore recommended. The best precision can be achieved by adding several small increments, rather than one single standard addition measurement.

When this method is used, the condition of identical compositions of compared solutions is most closely fulfilled.

B.4 Pressure digestion conditions

B.4.1 General

As an alternative for dry ashing or wet digestion, pressure digestion can be used. Before the pressure digestion apparatus is used, read operating manual and observe safety instructions. Pay particular attention to the risk posed to the laboratory staff by nitrous gases (see EN 13805 [1]).

B.4.2 Initial sample mass and acid volumes

Match the initial sample mass to the capacity of the digestion vessel, with the manufacturer's instructions being strictly observed for safety reasons. When digesting unknown samples, observe caution since a too large amount of sample may lead to explosions.

If the capacity is e.g. 100 ml, up to 500 mg (to the nearest milligram) of dry matter equivalent to a carbon content of 250 mg, can as a rule be digested. If the carbon content is lower, the test portion may be increased.

The volume of acid necessary for the digestion will depend on the nature of the sample material. Usually 3 ml of concentrated nitric acid will be sufficient to digest the amounts mentioned above.

B.4.3 Digestion temperature

Determine the necessary digestion temperature and consequently the completeness of the digestion.

A smooth rise in temperature at the beginning of the digestion is advantageous. In general, it applies that the quality of the digestion will become better with increasing digestion temperature.

B.4.4 Digestion time

A suggested digestion time for homogenised sample material is about 3 h. In case of microwave systems digestion time is typically 15 min to 30 min. For some samples, the digestion will be gentler if a preliminary reaction is allowed to take place at room temperature, e.g. overnight, after adding the acid.

B.4.5 Digestion solution

To reduce the pressure inside the digestion vessel, cool the still sealed pressure vessel to near the ambient temperature.

After the digestion vessel has been cooled and opened, initially place it under a fume hood until fumes are no longer visible. It is highly recommended to degas the digestion solution in an ultrasonic bath. The digestion solution shall be clear and its volume roughly the same as before digestion. A marked reduction in volume indicates that the pressure vessel was not leak-tight and in such cases, the digestion shall be repeated.

Transfer the digestion solutions to vessels made of quartz, FEP or PFA and fill up to a specified volume with water (diluted digestion solution).

B.4.6 Blank solution

To check for contamination, prepare a reagent blank containing the same amount of acids as in the sample and up to 4 ml of water (depending on the initial sample weight), then carry out all the steps described in the method.

Bibliography

[1] EN 13805, *Foodstuffs — Determination of trace elements — Pressure digestion*

[2] EN ISO 6497, *Animal feeding stuffs — Sampling (ISO 6497:2002)*

[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] Validation of an analytical method to determine calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES – Final report of the collaborative study – Animal feeding stuffs - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES – Jürgen Danier (project leader) c/o Bioanalytic Weihenstephan, Research Centre for Nutrition and Food Science (ZIEL) of TUM, 85350 Freising, Germany: http://www.wzw-bioanalytik.de/download_e.php

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