



BSI Standards Publication

**Animal feeding stuffs - Methods of sampling
and analysis - Determination of calcium,
sodium, phosphorus, magnesium, potassium,
iron, zinc, copper, manganese, cobalt,
molybdenum and lead by ICP-AES**

National foreword

This British Standard is the UK implementation of EN 15510:2017. It supersedes BS EN 15510:2007, which is withdrawn.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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English Version

Animal feeding stuffs: Methods of sampling and analysis -
Determination of calcium, sodium, phosphorus,
magnesium, potassium, iron, zinc, copper, manganese,
cobalt, molybdenum and lead by ICP-AES

Aliments des animaux - Méthodes d'échantillonnage et
d'analyse - Détermination des teneurs en calcium,
sodium, phosphore, magnésium, potassium, fer, zinc,
cuivre, manganèse, cobalt, molybdène et plomb par
ICP-AES

Futtermittel - Probenahme- und
Untersuchungsverfahren - Bestimmung von Calcium,
Natrium, Phosphor, Magnesium, Kalium, Eisen, Zink,
Kupfer, Mangan, Cobalt, Molybdän und Blei mittels ICP-
AES

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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European foreword

This document (EN 15510:2017) 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of an identical text or by endorsement, at the latest by February 2018, and conflicting national standards shall be withdrawn at the latest by February 2018.

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

This document supersedes EN 15510:2007.

WARNING — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard specifies the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method for the determination of the elements calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead.

The elements calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead are extracted either in feeds mainly consisting of organic matter after dry ashing and dissolving in hydrochloric acid or in feeds mainly consisting of inorganic matter after wet digestion with hydrochloric acid.

For the determination of extractable lead in minerals and feeds containing phyllosilicates (e.g. kaolinite clay) wet digestion with diluted nitric acid should be used.

The method was successfully tested for:

- calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt and molybdenum in the following animal feeding stuffs: 2 complete feeds (pig feed, sheep feed), 1 feed material (phosphate), 1 mineral premixture and 2 complementary feeds (2 mineral feeds),
- lead in 2 feed materials (phosphate, CaCO_3), 2 feed additives (Bentonite, CuSO_4), 1 complementary feed (mineral feed)

For elements with a HORRAT value higher than 2 (see Annex A) the method is more applicable as a screening method and not for confirmatory purposes.

The method detection limit for each element is dependent on the sample matrix and the instrument. The method is not applicable for the determination of a low concentration of elements. The limit of quantification should be 3 mg/kg or lower.

This method also applies for the determination in products with high element content (>5 %). For this purpose the accuracy of the method has to be checked individually.

NOTE 1 Results using EN 15550 may be lower than those obtained when applying EN 15621 as pressure digestion is used in EN 15621.

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)*

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply:

3.1

limit of detection (LOD)

smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

Note 1 to entry: The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations ($n \geq 10$, where n = number of measures) performed under reproducibility conditions.

3.2

limit of quantification (LOQ)

lowest content of the analyte that can be measured with reasonable statistical certainty

Note 1 to entry: If both trueness and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to ten times the standard deviation of the mean of blank determinations ($n \geq 10$, where n = number of measures) performed under reproducibility conditions.

3.3

feed additives

substances that comply with the definition of feed additives given in the Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition [3]

3.4

animal feeding stuffs

substances that comply with the definition of animal feeding stuffs given in the Regulation (EC) No 178/2002 [4]

4 Principle

For the determination of the elements calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, lead, a test portion of the sample is

- dry ashed and dissolved in hydrochloric acid for feeds mainly consisting of organic matter or
- wet digested with hydrochloric acid for feeds mainly consisting of inorganic matter.

For the determination of extractable lead in minerals and feeds, containing phyllosilicates (e.g. kaolinite clay) wet digestion with nitric acid should be used.

The concentration of the elements calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead is determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using external calibration or standard addition technique.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

WARNING —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.

5.1 Water, complying with grade 2 as defined in EN ISO 3696.

5.2 Nitric acid, concentrated, not less than 65 % (m/m), having a density of approximately (HNO₃) 1,42 g/ml.

5.3 Dilute nitric acid, to be prepared by mixing 1 volume of nitric acid (5.2) with 1 volume of water.

5.4 Nitric acid solution of 5 % (m/v), to be prepared: pipette 160 ml of dilute nitric acid (5.3) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

5.5 Nitric acid solution of 2 % (v/v), to be prepared: pipette 20 ml of nitric acid (5.2) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

5.6 Hydrochloric acid, concentrated, not less than 30 % (mass fraction), having a density of approximately (HCl) 1,15 g/ml.

5.7 Dilute hydrochloric acid, to be prepared by mixing 1 volume of hydrochloric acid (5.6) with 1 volume of water.

5.8 Hydrochloric acid solution of 1 % (m/v), to be prepared: pipette 60 ml of dilute hydrochloric acid (5.7) into a 1 000 ml volumetric flask (6.7) and fill to the mark with water.

5.9 Element stock solutions

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

$c = 1\,000\text{ mg/l}$.

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.

Stock solutions are not to be used after the expiry date. Element stock solutions with concentrations different from 1 000 mg/l may also be used.

5.10 Standard solutions

5.10.1 General

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 in multi-element standard solutions also need to be considered (Annex B, B.2.2). The examples given below also consider the measuring range of various inductively coupled plasma atomic emission spectrometers and the expected concentration of the element in animal feeding stuffs.

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

Other combinations of elements at different concentrations can be used, provided that the element stock solutions (5.9) 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.

5.10.2 Multi-element standard solution – Ca, Na, P, Mg, K in 1 % HCl

$c(\text{Ca, Na, P, Mg, K}) = 40\text{ mg/l}$

Pipette 40,0 ml of each element stock solution (Ca, Na, P, Mg, K) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml of dilute hydrochloric acid (5.7). Fill to the mark with water and transfer to a suitable storage bottle.

5.10.3 Multi-element standard solution - Fe, Zn, Cu, Mn, Co, Mo in 1 % HCl

$c(\text{Fe, Zn, Cu, Mn, Co, Mo}) = 50\text{ mg/l}$

Pipette 50,0 ml of each element stock solution (Fe, Zn, Cu, Mn, Co, Mo) (5.9) into a 1 000 ml volumetric flask (6.7). Add 60 ml of dilute hydrochloric acid (5.7). Fill to the mark with water and transfer to a suitable storage bottle.

5.10.4 Multi-element standard solution – Pb in 5 % HNO₃

$c(\text{Pb}) = 100 \text{ mg/l}$

Pipette 100,0 ml of each element stock solution (Pb) (5.9) into a 1 000 ml volumetric flask (6.7). Add 160 ml dilute nitric acid (5.3). Fill to the mark with water and transfer to a suitable storage bottle.

6 Apparatus

6.1 Laboratory grinder

6.1.1 Use laboratory grinders that are equipped such that they do not lead to contamination of the samples.

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

6.1.3 Laboratory grinder for mineral matrices capable of grinding to a particle size of less than or equal to 0,5 mm, e.g. a ball mill, centrifuge mill or equivalent.

6.1.4 Mortar with pestle, free of contamination.

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

6.3 Electric hot plate, with temperature control.

6.4 Ashing crucibles, of platinum, quartz or porcelain.

6.5 Electric muffle-furnace, capable of being maintained at a temperature of $450 \text{ }^{\circ}\text{C} \pm 20 \text{ }^{\circ}\text{C}$.

The real temperature in the furnace has to be checked, because this temperature may be substantially different from the adjust temperature.

6.6 Beaker, of capacity 250 ml.

6.7 One-mark volumetric flasks, of capacities 100 ml, 500 ml, 1 000 ml.

6.8 Inductively coupled plasma – Atomic Emission Spectrometer

The instrument shall be equipped with a radial plasma as a minimum requirement; an 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, number of measurements) shall be optimized according the manufacturer's instructions.

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

6.10 Oven for pre-drying, capable to hold a temperature of $(70 \pm 5) \text{ }^{\circ}\text{C}$.

7 Sampling

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

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

8 Sample preparation

8.1 General

Prepare the test sample in accordance with EN ISO 6498.

- Grinding must be carried out in conditions such that the substance is not appreciably heated.
- Operation is to be repeated as many times as is necessary and it must be affected as quickly as possible in order to prevent any gain or loss of constituents (water).
- 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.

8.2 Animal feeding stuffs, which can be ground as such

Grind the laboratory sample (usually 500 g), using a grinder (6.1.2) or mortar (6.1.4), until a particle size of 1 mm or less has been reached.

8.3 Liquid animal feeding stuffs

8.3.1 General

Liquid feeding stuffs shall be pre-dried according to the procedure described in 8.3.2 or freeze-dried according to the procedure described in 8.3.3.

8.3.2 Pre-drying

Pre-dry the laboratory sample at $70\text{ °C} \pm 5\text{ °C}$ over at least 16 h to reduce the moisture content using an oven (6.10). The mass of the sample before and after the pre-drying is determined using an analytical balance (6.2). Grind the pre-dried sample in accordance with 8.2.

8.3.3 Freeze-drying

Freeze-dry the laboratory sample following the instructions of the freeze-drying equipment (6.9). The mass of the sample before and after the freeze-drying is determined using an analytical balance (6.2). Grind the freeze-dried sample in accordance with 8.2.

8.4 Mineral matrices

Mineral matrices, except mineral products containing crystalline water, e.g. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, shall be ground using a grinder (6.1.3) or mortar (6.1.4) until a particle size 0,5 mm or less has been reached. Mineral products containing crystalline water should not be ground.

9 Procedure

9.1 Digestion

9.1.1 Selection of the procedure

9.1.1.1 Determination of Ca, Na, P, Mg, K, Fe, Zn, Cu, Mn, Co, Mo, Pb

If the test sample is from feeds mainly consisting of inorganic matter or a product potentially containing phosphates, proceed in accordance with 9.1.2.