



SLOVENSKI STANDARD
SIST EN ISO 13904:2005

01-september-2005

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Animal feeding stuffs - Determination of tryptophan content (ISO 13904:2005)

Futtermittel - Bestimmung des Tryptophangehalts (ISO 13904:2005)

Aliments des animaux - Détermination de la teneur en tryptophane (ISO 13904:2005)

Ta slovenski standard je istoveten z: EN ISO 13904:2005

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Animal feeding stuffs

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 13904

May 2005

ICS 65.120

English version

**Animal feeding stuffs - Determination of tryptophan content (ISO
13904:2005)**

Aliments des animaux - Détermination de la teneur en
tryptophane (ISO 13904:2005)

Futtermittel - Bestimmung des Tryptophangehalts (ISO
13904:2005)

This European Standard was approved by CEN on 4 April 2005.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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

Management Centre: rue de Stassart, 36 B-1050 Brussels

EN ISO 13904:2005 (E)**Foreword**

This document (EN ISO 13904:2005) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis", 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of an identical text or by endorsement, at the latest by November 2005, and conflicting national standards shall be withdrawn at the latest by November 2005.

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

Endorsement notice

The text of ISO 13904:2005 has been approved by CEN as EN ISO 13904:2005 without any modifications.

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INTERNATIONAL
STANDARD

ISO
13904

First edition
2005-05-01

**Animal feeding stuffs — Determination
of tryptophan content**

Aliments des animaux — Détermination de la teneur en tryptophane

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ISO 13904:2005(E)**Foreword**

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 13904 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

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Animal feeding stuffs — Determination of tryptophan content

1 Scope

This International Standard describes determination of the total and free tryptophan content in feeding stuffs (e.g. complete and complementary feeds, supplementary feeds, raw materials, ingredients, premixtures and concentrates). It does not distinguish between D- and L-forms.

2 Principle

For the determination of the total tryptophan, the sample is hydrolysed under alkaline conditions with saturated barium hydroxide solution and heated to 110 °C for 20 h. After hydrolysis, an internal standard is added.

For the determination of free tryptophan, the sample is extracted under mild acidic conditions in the presence of an internal standard.

The tryptophan and the internal standard in the hydrolysate or in the extract are determined by reversed phase C₁₈ HPLC with fluorescence detection.

3 Reagents and materials

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

3.1 Double-distilled water, or water of equivalent purity (conductivity < 10 µS/cm).

3.2 Standard substance: tryptophan (purity/content ≥ 99 %) dried under vacuum over phosphorus pentoxide.

3.3 Internal standard substance: α-methyltryptophan (purity/content ≥ 99 %), dried under vacuum over phosphorus pentoxide.

3.4 Barium hydroxide octahydrate.

Care should be taken not to expose the Ba(OH)₂·8H₂O excessively to air in order to avoid formation of BaCO₃, which could disturb the determination (see observation in B.3).

3.5 Sodium hydroxide.

3.6 Orthophosphoric acid, *w* = 85 %.

3.7 Concentrated hydrochloric acid, $\rho_{20} = 1,19$ g/ml.

3.8 Methanol, HPLC grade.

3.9 Light petroleum, boiling range 40 °C to 60 °C.

3.10 Sodium hydroxide solution, *c* = 1 mol/l.

Dissolve 40,0 g of NaOH (3.5) in water (3.1) and make up to 1 l with water (3.1).