



SLOVENSKI STANDARD SIST EN ISO 13904:2016

01-maj-2016

Nadomešča:
SIST EN ISO 13904:2005

Krma - Določevanje triptofana (ISO 13904:2016)

Animal feeding stuffs - Determination of tryptophan content (ISO 13904:2016)

Futtermittel - Bestimmung des Tryptophangehalts (ISO 13904:2016)

Aliments des animaux - Détermination de la teneur en tryptophane (ISO 13904:2016)

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EUROPEAN STANDARD
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EUROPÄISCHE NORM

EN ISO 13904

March 2016

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English Version

**Animal feeding stuffs - Determination of tryptophan
content (ISO 13904:2016)**

Aliments des animaux - Dosage du tryptophane (ISO
13904:2016)

Futtermittel - Bestimmung des Tryptophangehalts (ISO
13904:2016)

This European Standard was approved by CEN on 30 January 2016.

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COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN ISO 13904:2016) has been prepared by Technical Committee ISO/TC 34 "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of an identical text or by endorsement, at the latest by September 2016, and conflicting national standards shall be withdrawn at the latest by September 2016.

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.

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According to the CEN-CENELEC Internal Regulations, the national standards organizations 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, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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INTERNATIONAL STANDARD

**ISO
13904**

Second edition
2016-02-15

Animal feeding stuffs — Determination of tryptophan content

Aliments des animaux — Dosage du tryptophane

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](http://www.iso.org/foreword)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition cancels and replaces the first edition (ISO 13904:2005), which has been technically revised.

Animal feeding stuffs — Determination of tryptophan content

1 Scope

This International Standard specifies a method for determination of the total and free tryptophan (Trp) content in feeding stuffs (e.g. complete and complementary feeds, supplementary feeds, raw materials, ingredients, and concentrates) and determination of free tryptophan in commercial pure substances and premixtures containing more than 2 % of tryptophan.

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. For commercial pure substances and premixtures containing more than 2 % of tryptophan, it is possible to add the internal standard after the extraction.

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

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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 and control substance: tryptophan (purity ≥99 %) dried under vacuum over phosphorus pentoxide.

The two products are considered as 100 % pure. Control substance shall come from another manufacturer than the standard substance (see [3.17.2](#)).

NOTE The control of the purity of the standard substance can be performed by measuring the absorbance of a solution of tryptophan at 280 nm. Prepare a solution of about 5 mg/l in HCl 10⁻³ N from a stock solution and measure the Optical Density (OD) at 280 nm versus HCl 10⁻³ N. Then, the concentration of tryptophan is:

$$C = OD/5\,630 \times 10^{+06}$$

where

5 630 is the molar extinction coefficient of tryptophan in water at 280 nm;

C is expressed in µmole/l.

The standard substance purity is then (C/C₀)*100 where C₀ is the theoretical concentration of the diluted solution, expressed in µmole/l (about 25 µmole/l).

The control of the purity is performed every 6 months of use; it shall be ≥99 %.