

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

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 13904:2016 sisaldab Euroopa standardi EN ISO 13904:2016 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 13904:2016 consists of the English text of the European standard EN ISO 13904:2016.
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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)

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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of an identical text or by endorsement, at the latest by September 2016, and conflicting national standards shall be withdrawn at the latest by September 2016.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.

This document supersedes EN ISO 13904:2005.

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Endorsement notice

The text of ISO 13904:2016 has been approved by CEN as EN ISO 13904:2016 without any modification.

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Foreword

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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.

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 $(C/C_0) \times 100$ where C_0 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 %.