
**Animal feeding stuffs — Determination of
phytase activity**

Aliments des animaux — Détermination de l'activité phytasique



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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.

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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 30024 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 327, *Animal feeding stuffs — Methods of sampling and analysis*, in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

This International Standard has been developed to quantify phytase products in feed samples to enable the European Commission to control the phytase content of animal feed products. However, the method cannot be used to evaluate the *in vivo* efficacy of the phytase products.

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Animal feeding stuffs — Determination of phytase activity

1 Scope

This International Standard specifies the determination of phytase activity in feed samples.

The method does not distinguish between phytase added as a feed additive and endogenous phytase already present in the feed materials.

The method cannot be used to evaluate or compare the *in vivo* efficacy of the phytase product. It is not a predictive method of the *in vivo* efficacy of phytases present on the market as they can develop different *in vivo* efficacy per unit of activity.

The method is suitable and validated exclusively for the determination of phytase activity and exclusively in complete feeds.

NOTE The harmonized method was developed on the basis of the presently existing phytase products [E1600 (EC 3.1.3.8, 3-phytase), E1614 (EC 3.1.3.26, 4-phytase), and E1640 (EC 3.1.3.26, 4-phytase)]. Therefore, it might not necessarily be suitable as such for phytase products that are developed in the future. The harmonized method is thus a tool which is useful only to evaluate the total phytase activity in feed samples.

2 Terms and definitions

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

2.1

phytase unit

U

amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute under the reaction conditions specified in this International Standard

3 Principle

Phytase releases phosphate from the substrate *myo*-inositol hexakisphosphate (phytate). The released inorganic phosphate is determined by forming a yellow complex with an acidic molybdate/vanadate reagent. The optical density (OD) of the yellow complex is measured at a wavelength of 415 nm and the inorganic phosphate released is quantified from a phosphate standard calibration curve.