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GB/T 18246-2019

Replacing GB/T 18246-2000

Determination of amino acids in feeds

饲料中氨基酸的测定

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1 Scope

GB/T 18246-2019 is the Chinese national standard on determination of amino acids in feeds, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 10 December 2019 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 July 2020. Classification: ICS 65.120, CCS B46. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This Standard specifies the determination methods for total amino acids (peptide bonded and free) and free amino acids (natural and added) in feeds, including conventional acid hydrolysis method, oxidative acid hydrolysis method, alkaline hydrolysis method and acid extraction method. The conventional acid hydrolysis method applies to the determination of the content of sulfur-containing amino acids (cystine, cysteine, methionine) and other amino

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acids other than tryptophan (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine) in feed raw materials, compound feeds, concentrated feeds, concentrate supplements and additive premixes. The oxidative acid hydrolysis method applies to the determination of sulfur-containing amino acids (cystine, cysteine and methionine) in feed raw materials, compound feeds, concentrated feeds and concentrate supplements. When sodium metabisulfite is used as the oxidation terminator, other proteolytic amino acids except tyrosine and tryptophan can be determined; when hydrobromic acid is used as the terminator, other proteolytic amino acids except tyrosine, phenylalanine, histidine and tryptophan can be determined. The alkaline hydrolysis method applies to the determination of tryptophan in feed raw materials, compound feeds, concentrated feeds and concentrate supplements. The acid extraction method applies to the determination of free amino acids in compound feeds, concentrated feeds, concentrate supplements and additive premixes. The quantitative limits of each amino acid are shown in Table A.1 in Appendix A.

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2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, Water for analytical laboratory use - Specification and test methods

GB/T 15399, Determination of sulfur amino acids in feeds - Ion exchange chromatography

GB/T 15400, Determination of tryptophan in feeds

GB/T 20195, Animal feeding stuffs. Preparation of test samples JJG 1064, Automatic amino acid analyzer

3 Conventional acid hydrolysis method

3.1 Principle The protein in the feed is hydrolyzed into amino acids at 110 °C and 6 mol/L hydrochloric acid solution, separated by ion exchange chromatography and derivatized on a ninhydrin column. Proline is determined at a wavelength of 440 nm, and other amino acids are determined at a wavelength of 570 nm.

3.2 Reagents or materials Unless otherwise specified, all reagents used in this Standard are analytical reagents.

3.2.1 Water: Shall comply with the requirements of Grade 1 water in GB/T 6682.

3.2.2 Hydrochloric acid: guaranteed reagent.

3.2.3 Hydrochloric acid hydrating solution (6 mol/L): Mix 500 mL of hydrochloric acid (3.2.2) with 500 mL of water; add 1 g of phenol; mix well.

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3.2.4 Sodium citrate buffer solution [pH = 2.2, c(Na+) =

0.2 mol/L]: Weigh

19.6 g of trisodium citrate into a 1 000 mL volumetric flask; add water to dissolve; then, add

16.5 mL of hydrochloric acid (3.2.2),

5.0 mL of thiodiglycol, and 1 g of phenol; use water to fix the volume and filter.

3.2.5 Amino acid standard stock solution: Containing 17 kinds of amino acids for routine protein hydrolysate analysis (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine). The concentration of each component is 2.50 μmol/mL. Certified standard solutions shall be used.

3.2.6 Amino acid standard working solution: Accurately transfer 2 mL of amino acid standard stock solution (3.2.5) into a 50 mL volumetric flask; use sodium citrate buffer solution (3.2.4) to fix the volume, with a concentration of 100 nmol/mL for each amino acid component; package and seal in ampoules, approximately 1 mL each; store at 2 °C ~ 8 °C, valid for 3 months.

3.2.7 Sodium citrate buffer solutions and ninhydrin solutions of different pH values and ionic strengths: Prepare or purchase according to the instrument manual.

3.2.8 Liquid nitrogen.

3.3 Instruments and equipment

3.3.1 Automatic amino acid analyzer: equipped with cation exchange column, ninhydrin post-column derivatization device and 570 nm and 440 nm photometric detectors.

3.3.2 Balance: sensitivity

0.1 mg and

0.01 mg.

3.3.3 Vacuum pump.

3.3.4 Blowtorch.

3.3.5 Constant temperature drying oven: The temperature can reach 110 °C ± 2 °C.

3.3.6 Centrifuge: speed not less than 4 000 r/min.

3.3.7 Rotary evaporator or concentrator: The temperature can be adjusted between room temperature and 65 °C, with a temperature control accuracy of ±1 °C and a vacuum degree as low as 3.3x103 Pa (25 mmHg).

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3.4 Test sample Prepare the sample according to GB/T 20195; crush it through a

0.25 mm sieve; mix it evenly; store it in a sealed container for later use. For samples with crude fat content greater than or equal to 5%, the defatted samples need to be air-dried, mixed, and placed in a sealed container for later use. For samples with crude fat content less than 5%, they can be weighed directly.

3.5 Test procedure

3.5.1 Sample pretreatment Perform two tests in parallel. Weigh 50 mg ~ 100 mg of the sample (containing about

7.5 mg ~ 25 mg of protein, accurate to

0.1 mg) into a 20 mL ampoule or hydrolysis tube; accurately add 10 mL of hydrochloric acid hydrolysis solution (3.2.3); freeze in liquid nitrogen (3.2.8); use a vacuum pump to evacuate to

7 Pa (<=5x10-2 mmHg); then use a blowtorch to seal the mouth or fill with nitrogen for 1 min and tighten the tube cap. Place the ampoule or hydrolysis tube in a constant temperature drying oven at 110 °C ± 2 °C and hydrolyze for 22 h ~ 24 h. Cool; mix well; open the tube; filter; use a pipette to accurately draw an appropriate amount of filtrate; place it in a rotary evaporator or concentrator; evaporate to dryness under vacuum at 60 °C. If necessary, add a little water and repeat the evaporation 1 ~ 2 times. Add 3 mL ~ 5 mL of sodium citrate buffer solution (3.2.4) to re-dissolve, so that the amino acid concentration in the sample solution reaches 50 nmol/mL ~ 250 nmol/mL; shake well; filter or centrifuge; take the supernatant for determination.

3.5.2 Determination Vi - injection volume of sample solution, in microliters (μL); F - crude fat content, %. Report the result as the arithmetic mean of the results of two parallel samples, retaining two decimal places.

3.7 Precision Under repeatability conditions, the absolute difference between two independent measurement results and their arithmetic mean shall not exceed 5% of the arithmetic mean of the two measurement values when the content is not greater than 0.5%; and shall not exceed 4% when the content is greater than 0.5%.

4 Oxidative acid hydrolysis method

4.1 Determination of sulfur-containing amino acids Carry out according to the provisions of GB/T 15399.

4.2 Determination of other amino acids

4.2.1 Principle When sodium metabisulfite is used as the oxidation terminator, the content

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