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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 17811-2025

Replacing GB/T 17811-2008

**Determination of pepsin digestibility for animal-derived protein
feeds - Filtration method**

动物源性蛋白质饲料胃蛋白酶消化率的测定 过滤法

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Table of Contents

Foreword	3
1 Scope	5
2 Normative References	5
3 Terms and Definitions	5
4 Principle	6
5 Reagents and Materials	6
6 Instruments and Apparatuses	6
7 Samples	7
8 Test Procedures	7
9 Test Data Processing	8
10 Precision	8
Appendix A (Normative) Determination of Pepsin Activity	10

1 Scope

This Document describes a method for determining the digestibility of pepsin in animal-derived protein feeds.

This Document applies to the determination of pepsin digestibility in animal-derived protein feeds.

2 Normative References

GB/T 6432

GB/T 6433

GB/T 6682

GB/T 20195

3 Terms and Definitions

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

3.1 Pepsin digestibility for animal-derived protein feeds

Under conditions of 45 °C and pH 1~2, the ratio of digested crude protein to total crude protein in animal-derived protein feed after digestion with 20 U/mL pepsin solution for 16 h

is given.

NOTE. The pepsin digestibility of animal-derived protein feed is not directly related to its in vivo digestibility.

4 Principle

The specimen was digested with pepsin under specified conditions; the insoluble residue was separated; the undigested crude protein content in the residue and the crude protein content of the specimen were determined; and the pepsin digestibility was calculated.

5 Reagents and Materials

Unless otherwise specified, use only analytically pure reagents.

5.1 Water. GB/T 6682, Grade III.

5.2 Petroleum ether. Boiling range 30°C~60°C. 5.3 Anhydrous ethanol.

5.4 Pepsin. Derived from porcine stomach, CAS No. 9001-75-6, enzyme activity determined

according to Appendix A.

5.5 Hydrochloric acid solution. Transfer 6.1 mL of hydrochloric acid; dilute with water to 1000 mL (pH 1~2); and mix well.

5.6 Pepsin solution (20 U/mL). Take an appropriate amount of pepsin (5.4) and dissolve it in hydrochloric acid solution (5.5) heated to 42 °C ~ 45 °C in a water bath (6.5) to achieve a pepsin enzyme activity concentration of 20 U/mL. Prepare fresh before use. 5.7 Filter Paper. Quick.

6 Instruments and Apparatuses

6.1 Analytical balance. Accuracy 0.1 mg.

6.2 Thermostatic shaker. Temperature control accuracy ± 1 °C, rotation speed 15 r/min ~ 300 r/min.

6.3 Electric heating drying oven. Temperature control accuracy ± 2 °C.

6.4 Buchner Funnel. Diameter 100 mm.

6.5 Water Bath. Temperature control accuracy ± 1 °C.

7 Samples

Prepare samples according to GB/T 20195, at least 200 g. Crush the solid sample until it

passes entirely through a 0.84 mm sieve; mix thoroughly; and store in a sealed container for later use.

8.1 Defatting

Take 5 g of the specimen; and defat it with petroleum ether according to the extraction method in GB/T 6433. After defatting, air-dry the specimen at room temperature.

8.2 Digestion

Perform two parallel tests. Take 1 g (accurate to 0.1 mg) of the defatted specimen (8.1) and place it in a 250 mL stoppered ground glass bottle. Add 150 mL of pepsin solution preheated to 45 °C (5.6) to completely immerse the specimen. Tighten the stopper and digest the specimen for 16 h on a constant temperature shaker (6.2) at 45 °C ± 1 °C.

8.3 Treatment of digestion residue

Remove the ground glass bottle from the constant temperature shaker and let it stand at an angle for at least 15 min. Filter using a Buchner funnel (6.4) lined with rapid filter paper. First, wash the residue on the stopper with a small amount of water onto the Buchner funnel. Then, keeping the bottle mouth at the same angle as when the sediment was set, move it onto the Buchner funnel and slowly pour out the contents; avoiding stirring the residue. After the upper liquid has passed through the filter paper; wash the ground glass bottle with 15 mL of anhydrous ethanol (5.3); and transfer the residue to the Buchner funnel. Repeat this process for 3 times to ensure complete transfer of the residue. After all the liquid has passed through the filter paper, wash the residue twice with a small amount of anhydrous ethanol. Carefully remove the filter paper containing the residue from the Buchner funnel; wrap it; transfer it to a tray; and dry it in an electric hot air drying oven at 105 °C. Simultaneously, perform blank tests according to 8.2 and 8.3 to obtain an enzyme blank.

8.4 Determination of undigested crude protein content

Determine the crude protein content (w₁) in the residue (8.3) according to GB/T 6432. Use the enzyme blank (8.3) as the titration blank to calculate the undigested crude protein content.

8.5 Determination of crude protein content in the specimen

Determine the crude protein content (w₂) in the defatted specimen (8.1) according to the provisions of GB/T 6432.