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Determination of nitrite in feeds - Colorimetry method

饲料中亚硝酸盐的测定 比色法

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Determination of nitrite in feeds - Colorimetry method

1 Scope

This Standard specifies the colorimetry method for the determination of nitrite in feeds.

This Standard applies to the determination of nitrite in feed raw materials, compound feeds, concentrated feeds and concentrate supplements.

The detection limit of this method is 0.70 mg/kg, and the quantitation limit is 2.0 mg/kg.

2 Normative references

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

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

GB/T 14699.1, Feeding stuffs - Sampling

GB/T 20195, Animal feeding stuffs - Preparation of test samples

3 Principle

The sample removes protein under weakly alkaline conditions, and the nitrite in the sample reacts with p-aminobenzenesulfonic acid under weakly acidic conditions to form diazo compounds, which are then coupled with N-1-naphthylethylenediamine to form purple compounds. Under the condition of wavelength 538 nm, carry out the colorimetric determination.

4 Reagents or materials

Unless otherwise specified, only use reagents confirmed to be of analytical grade in the analysis.

4.1 Water: grade-II water as specified in GB/T 6682.

4.2 Dilute hydrochloric acid solution: Take 5 mL of hydrochloric acid; add it to a 100 mL volumetric flask; use water to dilute to a constant volume; mix well.

4.3 Dilute ammonia water: Take 5 mL of ammonia water; add it to a 100 mL volumetric flask; use water to dilute to a constant volume; mix well.

4.4 Ammonium chloride buffer solution: Measure 500 mL of water into a 1 000 mL volumetric flask; add 20 mL of hydrochloric acid; mix well; add 50 mL of ammonia water; use water to dilute to the mark. Use dilute hydrochloric acid (4.2) and dilute ammonia water (4.3) to adjust the pH to 9.6 ~ 9.7.

4.5 Zinc sulfate solution: Weigh 120 g of zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$); use water to dissolve, and dilute to a constant volume of 1 000 mL; mix well.

4.6 Sodium hydroxide solution: Weigh 20 g of sodium hydroxide; use water to dissolve, and dilute to a constant volume of 1 000 mL; mix well.

4.7 Acetic acid solution: Measure 600 mL of glacial acetic acid in a 1 000 mL volumetric flask; use water to dilute to the mark; mix well.

4.8 P-aminobenzenesulfonic acid solution: Weigh 5.0 g of p-aminobenzenesulfonic acid; dissolve it in 700 mL of water and 300 mL of glacial acetic acid; mix well; store in a brown bottle. The validity period is 7 days.

4.9 N-1-Naphthylethylenediamine solution: Weigh 0.1 g of N-1-naphthylethylenediamine; add acetic acid solution (4.7) to dissolve and dilute to a constant volume of 100 mL; mix well; put it in a brown bottle; store it in a refrigerator at 4 °C ~ 10 °C. The validity period is 7 days.

4.10 Color-developing agent: Mix equal volumes of p-aminobenzenesulfonic acid solution (4.8) and N-1-naphthylethylenediamine solution (4.9) before use.

4.11 Standard stock solution of sodium nitrite (500 µg/mL): Accurately weigh 250.0 mg of sodium nitrite which has been baked at $115\text{ °C} \pm 5\text{ °C}$ to constant mass; add water to dissolve; transfer to a 500 mL volumetric flask; add 100 mL of ammonium chloride buffer solution (4.4); add water to dilute to the volume; mix well; store at $2\text{ °C} \sim 8\text{ °C}$ in the dark. The validity period is 6 months. Alternatively, use certified sodium nitrite standard solution for preparation.

4.12 Sodium nitrite standard working solution (5.0 µg/mL): Accurately pipette 1.00 mL of sodium nitrite standard stock solution (4.11) into a 100 mL volumetric flask; add water to dilute to a constant volume to the mark; mix well. Prepare when necessary.

5 Instruments and equipment

5.1 Spectrophotometry.

5.2 Water bath: The temperature control range is $25\text{ °C} \sim 100\text{ °C}$; the temperature control accuracy is $\pm 1\text{ °C}$.

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