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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

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GB/T 13089-2020

Replacing GB/T 13089-1991

**Method for Determination of Oxazolidinethione in
Feeds**

饲料噁唑烷硫酮的测定方法

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Foreword

This Standard was drafted as per the rules specified in GB/T 1.1-2009.

This Standard replaced GB/T 13089-1991 *Method for Determination of Oxazolidinethione in Feeds*.

Compared with GB/T 13089-1991, this Standard has the major differences in technical contents as follows besides the editorial modifications:

- Modify the scope of application of the method and increase the limit of quantification (see Clause 1 of this Edition; Clause 1 of 1991 Edition);
- Modify the principle of the method (see Clause 3 of this Edition; Clause 2 of 1991 Edition);
- Modify the preparation method of white myrosinase (see 4.10 of this Edition; 3.4 of 1991 Edition);
- Modify the dosage and test procedures of white myrosinase (see Clause 7 of this Edition; Clause 6 of 1991 Edition);
- Modify test data processing (see Clause 8 of this Edition; Clause 7 of 1991 Edition);
- Modify the precision (see Clause 9 of this Edition; Clause 7 of 1991 Edition).

Please note some contents of this document may involve patents. The issuing agency of this document shall not assume the responsibility to identify these patents.

This Standard was proposed by and under the jurisdiction of National Technical Committee for Standardization of Feed Industry (SAC/TC 76).

Drafting organizations of this Standard: Sichuan Will Test Technology Co., Ltd.; Institute of Animal Sciences of Chinese Academy of Agricultural Sciences; Public Monitoring Center for Agro-Product of Guangdong Academy of Agricultural Sciences; Tongwei Co., Ltd.; and China Feed Industry Association.

Chief drafting staffs of this Standard: Zhang Fengping, Tong Jianming, Wang Weili, Lei Baoliang, Yang Fashu, Wang Liwen, Song Tao, and Lu Jiawen.

The historical edition replaced by this Standard is as follows:

- GB/T 13089-1991.

Method for Determination of Oxazolidinethione in Feeds

1 Scope

This Standard specifies the UV spectrophotometric method for the determination of oxazolidinethione in feeds.

This Standard is applicable to the determination of oxazolidinethione in rapeseed and its processed products, as well as compound feeds, concentrated feeds and concentrate supplements containing rapeseed and its processed products.

The limit of quantification of this Standard method is 150mg/kg.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 5520 Inspection of Grain and Oils - Germination Test of Seeds

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

GB/T 20195 Animal Feeding Stuffs – Preparation of Test Samples

3 Principle

The glucosinolate in the specimen is hydrolyzed into isothiocyanate under the action of white myrosinase in the buffer solution with pH 7.0. After extraction with dichloromethane, the isothiocyanate with hydroxyl reacts with ethanol. The oxazolidinethione is cyclized and formed, which is determined by ultraviolet spectrophotometry.

4 Reagents and Materials

Unless otherwise specified, only use analytically-pure reagents.

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

4.2 Petroleum ether (boiling range 30°C ~60°C).

4.3 Dichloromethane.

4.4 Absolute ethanol.

4.5 Citric acid solution (0.1mol/L): accurately take 4.20g of citric acid ($C_6H_8O_7 \cdot H_2O$) and dissolve and make constant volume by water to 200mL; and mix evenly. Prepare for current use.

4.6 Disodium hydrogen phosphate (0.2mol/L): accurately take 28.39g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and dissolve and make constant volume by water to 1L; and mix evenly. Prepare for current use.

4.7 Hydrochloric acid (0.01mol/L): accurately pipette 0.9mL of concentrated hydrochloric acid; dilute and make constant volume by water to 1L; and mix evenly.

4.8 Sodium hydroxide solution (0.01mol/L): accurately take 0.40g of sodium hydroxide and dissolve and make constant volume by water to 1L; and mix evenly.

4.9 Buffer solution with pH 7.0: pipette 176.5 mL of citric acid solution (4.5) in a 1000 mL volumetric flask; dilute and make constant volume by disodium hydrogen phosphate solution (4.6) to 1000 mL; mix evenly. Use hydrochloric acid solution (4.7) or sodium hydroxide solution (4.8) to adjust the pH to 7.0. Prepare for current use.

4.10 White myrosinase: take 50g of white mustard (*Sinapis alba* L.) seeds (make the germination rate test in accordance with GB/T 5520; the germination rate must be greater than 85% within 72h; and the storage period shall not exceed 2 years); after crushing, place them in a 500mL beaker; add 100mL of petroleum ether (4.2); stir for 2min; let stand; discard the supernatant liquid. Repeat degreasing for 10 times, make the fat content less than 2%. Put the solvent in a fume hood to evaporate. And then crush it again, 80% passed through the 0.28mm test sieve; put in an enclosed container and store at -18°C below. The validity period is 6 months. In order to ensure the activity of white myrosinase, the environment temperature shall be kept at 30°C below during the preparation process. Pay attention to a small amount and multiple times during the two crushing to prevent the crusher from overheating.

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