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Replacing GB/T 13093-1991

Examination of bacterial count in feeds

饲料中细菌总数的测定

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Examination of bacterial count in feeds

1 Scope

This standard specifies the method for the determination of the bacterial count in feed.

This standard is applicable to the determination of the bacterial count in compound feed, concentrated feed, feed raw materials (fishmeal, etc.), supplementary cattle concentrate.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard; however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

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

GB/T 14699.1 Feeding stuffs - Sampling

GB/T 20195-2006 Animal feeding stuffs - Preparation of test samples

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1

Bacterial count

After the feed specimen is processed and cultured under certain conditions (such as culture ingredients, culture temperature and time, etc.), the bacterial count in 1 g (mL) of the specimen obtained.

Note: It is mainly used as a sign to determine the degree of contamination of feeds. This method can also be used to observe the dynamics of bacterial reproduction in feed, so as to provide a basis for hygienic evaluation of the tested samples.

4 Principles

After the specimen is processed, it is diluted to an appropriate concentration, cultured under certain conditions (such as using a specific medium, culturing at a temperature of 30 °C ± 1 °C for 72 h ± 3 h, etc.), the bacterial count in the resulting 1 g (mL) of specimen is obtained.

5 Equipment and materials

5.1 Analytical balance: Sensitivity is 0.1 g.

5.2 Oscillator: Reciprocating.

5.3 Pulverizer: It is not a whirlwind mill, with good airtightness.

5.4 Autoclave: Sterilization pressure 0 ~ 3 kg/cm^2.

5.5 Refrigerator: Ordinary refrigerator.

5.6 Constant temperature water bath: 46 °C ± 1 °C.

5.7 Constant temperature incubator: 30 °C ± 1 °C.

5.8 Micromixer.

5.9 Sterile conical flasks of 100 mL, 250 mL, 500 mL.

5.10 Sterile pipette: 1 mL, 10 mL.

5.11 Sterile test tube: 16 mm x 160 mm.

5.12 Sterile glass beads: 5 mm in diameter.

5.13 Sterile petri dish: 90 mm in diameter.

5.14 Sterilize metal spoons, knives, etc.

6 Media and reagents

6.1 Nutrient agar medium: See Appendix A.

6.2 Phosphate buffer solution (diluent): See Appendix A.

6.3 0.85% normal saline: See Appendix A.

6.4 Water agar medium: See Appendix A.

9 Measurement steps

9.1 Specimen dilution and incubation

9.1.1 Weigh 25 g (or 10 g) of the specimen (Chapter 7) by aseptic operation. Put it in a sterilized conical flask, which contains 225 mL (or 90 mL) of diluent or normal saline (the bottle is pre-filled with appropriate number of glass beads). Place it on an oscillator, to oscillate it for 30 minutes. After fully shaking, prepare a uniform dilution of 1:10. It is best to process in a homogenizer, at a speed of 8000 r/min ~ 10000 r/min for 1 min.

9.1.2 Use a 1 mL sterilized pipette, to absorb 1 mL of the 1:10 diluent. Slowly pour it into a test tube, which contains 9 mL of the sterilized diluent or normal saline, along the tube wall (note that the tip of the pipette shall not touch the diluent in the tube). Shake the test tube. OR put it on a micromixer, to mix for 30 s. Mix well. Make a 1:100 diluent.

9.1.3 Take another 1 mL sterilized straw. Make a 10-fold incremental dilution, according to the above operation method. Every time the incremental dilution is performed, replace a sterilized straw.

9.1.4 According to the requirements of the feed hygiene standards or the estimation of the contamination degree of the specimen, select 2 ~ 3 appropriate dilutions, respectively. While making 10-fold incremental dilutions, pipette 1 mL of the dilution into the sterilized petri dish, to make two petri dishes for each dilution.

9.1.5 After the dilution liquid is transferred into the petri dish, about 15 mL of the medium, which is cooled to 46 °C ± 1 °C (it can be kept in a water bath at 46 °C ± 1 °C), shall be poured into the petri dish in time. Rotate the culture dish carefully, to make the specimen mix well with medium. The time -- between diluting the specimen and pouring the culture medium -- shall not exceed 30 min.

If it is estimated that the microorganisms, which are contained in the specimen, may grow on the surface of the medium plate, after the medium is completely set, pour 4 mL of water agar medium, which is cooled to 46 °C ± 1 °C, on the surface of the medium.

9.1.6 After the agar has solidified, invert the plate and incubate it in a constant temperature incubator at 30 °C ± 1 °C, for 72 h ± 3 h. Take it out. Calculate the bacterial count in the plate. Multiply the bacterial count by the dilution factor, to obtain the bacterial count per gram of specimen.

9.2 Calculation method of bacterial count

When doing plate colony counting, it can be observed with the naked eye. If the colony shape is small, it can be checked with the help of a magnifying glass, to prevent omissions. After calculating the bacterial count on each plate, calculate the average value of the two plate colonies, which have the same dilution.

9.3 Reporting of bacterial counts

9.3.1 Selection of the bacterial count on the plate

Select a plate, which has a bacterial count between 30 and 300, as the standard for the determination of the bacterial count. For each dilution, use the average number of colonies on two plates. If one of the two plates has larger flake colonies, it should not be used. Instead, the average number of plate colonies without flake colonies shall be used as the bacterial count of this dilution. If the flake colony is less than half of the plate, whilst the other half of the colony is evenly distributed, THEN, the half plate can be calculated and multiplied by 2, to represent the bacterial count on the plate.

9.3.2 Selection of dilution

9.3.2.1 The dilution, which has an average bacterial count between 30 and 300, shall be selected, multiplied by the dilution factor and reported (see case 1 in Table 1).

9.3.2.2 If there are two dilutions and the bacterial count grown is within 30 ~ 300, it shall be decided based on the ratio of the two. If the ratio is less than or equal to 2, the average value shall be reported; if it is greater than 2, the smaller number shall be reported (see case 2 and case 3 in Table 1).

9.3.2.3 If the average bacterial count in all dilutions is greater than 300, it shall be reported as the average bacterial count in the highest dilution multiplied by the dilution factor (see case 4 in Table 1).

9.3.2.4 If the average bacterial count in all dilutions is less than 30, it shall be reported as the average bacterial count in the lowest dilution multiplied by the dilution factor (see case 5 in Table 1).

9.3.2.5 If there is no colony growth at all dilutions, report it as less than 1 multiplied by the lowest dilution (see case 6 in Table 1).

9.3.2.6 If the average bacterial count in all dilutions is not between 30 and 300, meanwhile some of them are greater than 300 or less than 30, then it is reported as the average bacterial count closest to 30 or 300 multiplied by the dilution factor (see case 7 of Table 1).

9.3.3 Report of total bacterial count

When the bacterial count is less than 100, it is reported according to the actual number; when it is greater than 100, it takes two significant figures, meanwhile the value after the two significant figures is rounded off. In order to shorten the number of zeros after the number, it can also be represented by an exponent of 10 (see Table 1).

Appendix A

(Normative)

Media and reagents

Unless otherwise specified, all reagents used are of analytical grade; water complies with the requirements of grade-3 water in GB/T 6682.

A.1 Nutrient agar medium

A.1.1 Composition

Peptone: 10 g

Beef extract: 3 g

Sodium chloride: 5 g

Agar: 15 g ~ 20 g

Distilled water: 1000 mL

A.1.2 Preparation method

Dissolve all components, except agar, in distilled water. Add about 2 mL of 15% sodium hydroxide solution, to correct the pH to 7.2 ~ 7.4. Add agar and heat to boil, to dissolve the agar. Divide into conical flasks and autoclave at 121 °C for 20 min.

Note: This medium is used for general bacterial culture. It can be poured into a flat plate or made into a slope. For colony counting, the agar shall be 1.5%, and if it is made into a plate or slope, it shall be 2%.

A.2 Phosphate buffer (diluent)

A.2.1 Storage solution

Potassium dihydrogen phosphate: 34 g

1 mol/L sodium hydroxide solution: 175 mL

Distilled water: 1000 mL

A.2.2 Preparation method

Dissolve phosphate in 500 mL of distilled water. Use 1 mol/L sodium hydroxide solution, to correct the pH to 7.0 ~ 7.2. Then use distilled water to dilute it to 1000 mL.

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