# Designation of L-methionine as a feed additive

Ministry of Agriculture, Forestry and Fisheries (MAFF) will designate Muramidase as a feed additive and establish its standards and specifications in the ministerial ordinance. Outline of standards and specifications is as follows.

### Muramidase

### Specifications for method of manufacture of feed in general

Muramidase is allowed to be used as feed additives for broilers only.

### **Specifications for feed additives**

### Active Substance

## **Compositional specifications**

**Unit**  $\geq$  30,000 peptidoglycan degradation/g

### Physical and chemical properties

- (1) It comes in light-dark brown liquid.
- (2) pH of aqueous solution or aqueous suspension (1 part solute/100 parts solution) is 3.0 to 5.0.
- (3) It has the best enzyme activity when pH is between 4.0 and 7.5.

## **Purity test:**

- (1) Lead: 0.5 g (0.45~0.54 g) of this product is weighed. When lead is tested using this sample by the lead test method (Method No. 1 of atomic absorption spectrophotometry test method), concentration of lead in the solution shall not exceed 20  $\mu$ g/g.
- (2) Arsenic: 1.0 g (0.95~1.04 g) of this product is weighed. When arsenic is tested using this sample by Method No. 3 of arsenic test method (using the apparatus A), the concentration of arsenic in the solution shall not exceed  $2 \mu g/g$ .
- (3) Antibacterial activity: 1 g (0.5~1.4 g) of this product is weighed. When antibacterial activity is tested using this sample by the antibacterial activity test method, no antibacterial activity shall be exhibited for Micrococcus luteus ATCC 9341 and Escherichia coli ATCC 27166.

#### **Ignition residue:** $\leq 5.0\%$ (1 g)

Strength test of enzyme: Method of Strength test of decomposing peptidoglycan.

#### Standard for method of manufacture

Genetically modified *Trichoderma reesei* should be cultured. The cultured solution should be filtered or extracted with water and then filtered to remove bacteria. the filtrate must be concentrated to produce the product.

#### Standard for method of storage

The products must be stored in light-shielding and airtight containers.

### Standard for method of labelling

Containers or packaging of the products must be labeled that pH value (up to the first decimal place) showing the maximum enzyme activity.

### Product No. 1

### **Compositional specifications**

This product comes in the form of pieces, particles or powders and is produced by mixing with excipient substances to the Active Substance of Muramidase. If necessary, it is mixed with sodium sulfate.

**Unit** This product contains the amount of Muramidase corresponding to 85 to 170% of the peptidoglycan degradation unit of activity shown on the label.

Strength test of enzyme: Method of Strength test of decomposing peptidoglycan.

#### Standard for method of storage

Same as the standard for method of storage by Active Substance of Muramidase.

#### Standard for method of labelling

Same as the standard for method of labelling by Active Substance of Muramidase.

### Product No. 2

#### **Compositional specifications**

This product comes in the form of liquid and is produced by adding sorbitol to the Active Substance of Muramidase.

**Unit** This product contains the amount of Muramidase corresponding to 85 to 170% of the peptidoglycan degradation unit of activity shown on the label.

Strength test of enzyme: Method of Strength test of decomposing peptidoglycan.

**Preparation of Sample solution:** Strength test of enzyme for Active Substance of Muramidase. **Standard for method of storage** 

Same as the standard for method of storage by Active Substance of Muramidase.

#### **Standard for method of labelling**

Same as the standard for method of labelling by Active Substance of Muramidase.

#### Analytical method

#### Strength test of enzyme

## Method of Strength test of decomposing peptidoglycan

(See Japanese attachment.)