I Detection method for animal bones by microscopy

[Method listed in the guidelines]

1 Sampling method

Sample 20-30 scoops (about 5-10 kg in weight) of a product during transport at a frequency around one scoop per minute using a shovel etc. at the site of carrying out the final product (at shipping in bulk or immediately before packing in paper bags or container bags) in the manufacturing series of formula feed for cattle. Obtain about 300-500 g as the final sample by increment reduction or using a riffle sampler. Also, an autosampler can be used if the plant is equipped with one.

2 Detection method

(1) Sample preparation
Grind the sample until it passes a mesh sieve of 1 mm.

(2) Detection of meat and bone meal etc.
Put 1 g of the ground sample in a funnel for separation by specific gravity that contain the specific gravity solution (chloroform),[1] stir with a thin glass bar, leave at rest for about 20 minutes to separate materials in the sample by specific gravity. Filter feed materials separated in the lower layer (meat and bone meal, etc. are separated in this fraction.) and chloroform with filter paper (5A), and wash feed materials adhered to the wall with ethanol to collect on filter paper. Dry the filter paper, and then transfer the residue on the filter paper to a 100-mL tall beaker with a brush, etc., add 20 mL of 5 % sodium hydroxide solution, and boil for 30 minutes. Fill the beaker with water, leave at rest, aspirate the supernatant, and repeat the washing procedure until the water layer becomes transparent.

Transfer all the precipitate in the beaker to a small dish, and observe with a light microscope (x50~100 magnification) or a stereomicroscope (x20~30) to identify the presence or absence of tissue fragments derived from meat and bone meal in the sample. [2]

(3) Estimation of the amount of meat and bone meal etc.
Prepare control samples for identification by adding 0.1 %, 0.2 %, 0.3 %, 0.4 %, 0.5 % and 1.0 %, respectively, of meat and bone meal to a ground formula feed for cattle without contamination with meat and bone meal.

Treat the respective control samples for identification and the sample to be estimated for the amount of meat and bone meal, etc. by the detection method in 1, and estimate the amount of meat and bone meal, etc in the feed based on the amount of tissue fragments derived from meat and bone meal, etc. in the control samples for identification and in the sample.

<<Summary of analysis method>>

This method was defined in the attachment of “Guidelines concerning the prevention of contamination of ruminant feeds with proteins derived from ruminants (Notification from the Director-General of Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, 13 Seichiku No. 1366 dated June 1, 2001, which was abolished in parallel with the new release of “Guidelines concerning the prevention of contamination of ruminant feeds with animal derived proteins” dated on September 16, 2003.), and indicates basics of detection methods etc. concerning the implementation of the test for contamination with meat and bone meal, etc. according to the stipulation in 3-3-(1) in the Guidelines.

<<Notes and precautions>>

[1] Bone tissues can be more selectively separated by using the zinc chloride solution shown in Section 19, Identification 1 Screening by specific gravity, <<Notes and precautions>> [1]

[2] As microscopy requires experience, conduct identification with reference to photos in such as “Illustrated Guide to Feed Ingredients” (issued by Japan Scientific Feeds Association).