

STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: AIA (Acid Insoluble Ash)

BIOVIT No.: Arb1034

1. Introduction

AIA indicates the proportion of silica and silicates (silica is the main constituent of sand) in a sample and can be used as a marker for digestibility of different types of feed. The analysis is based on the combustion of organic material, boiling in HCl and re-ashing of the sample, before gravimetric measurement (1).

2. Reagents

- Concentrated HCl (37%)
- RO water

3M HCl

- Have about 700 mL RO water in 1 L volumetric flask
- Add 250 mL HCl (concentrated 12 M)
- Top up to the mark with RO water

3. Risk assessment

HCl: Harmful in contact with skin (corrosive/irritating), eye contact (corrosive/irritating) and if swallowed.

- Wear gloves and goggles and work in the exhaust.
- In case of skin contact: Wash with water and remove contaminated clothing/shoes. Call a doctor if necessary.
- In case of eye contact: Rinse with water, call a doctor.

The furnace must **not** be opened at 550 °C. If there is still organic material left, a flame will go out when the door is opened!

- Wait until the temperature is around 200 °C or lower.
- Use pliers and gloves when taking the samples from the furnace.
- Should you burn yourself; use running cold water for the first few minutes. Then use temperate running water so that frost damage does not occur.

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4. Equipment

- Crucible (telleglass)
- Weight
- Steel tray
- Drying cabinet
- Desiccators
- Hotplate/sand bath
- Ash-free filter (Folding filter black band 589-1 ½ 150mm).
- Muffle furnace (550 °C)

5. Sample material

The method can be used for feed, feed ingredients and feces. For mineral samples/mixtures, an alternative procedure must be used (see procedure B in ISO 5985 (2)). About 2 grams of sample are weighed.

6. Special remarks

At the first incineration only one tray with crucibles should be inserted (because of the large amount of organic matter in the furnace) (step 6 in the section below).

7. Work procedure

1. Weigh the crucible and register the weight (**W₀**).
2. Tare the weight and weigh in about 2 g of sample (**W₁**).
3. The samples are placed in a drying cabinet at 103 °C ± 2 °C for a minimum of 4 hours or overnight.
4. Dried samples are placed in a desiccator to cool.
5. When the temperature of the samples has become stable (room temperature), the samples are weighed (**W₂**).
6. The samples are then placed in the muffle furnace (550 °C) and incinerated for 16 hours.
7. The ashed samples are placed in a desiccator to cool.
8. When the temperature of the samples has become stable (room temperature), the samples are weighed (**W₃**).
9. Mark the samples and put on the screw cap.

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10. Transfer sample to 100 mL beaker and add 30 mL of 3M HCl.
11. Boil the samples on a hot plate for 7 min.
12. Rinse the crucible with boiling water (to remove acid residues).
13. Filter solution through an ash-free folding filter.
14. Clean the filter with boiling RO water (3-4 times).
15. Carefully remove the filter with the acid-insoluble ash and return it to the rinsed crucible.
16. Ash the filter for 8 hours (overnight) at 550 ° C.
17. Hold the crucible with "acid-insoluble ash" in the desiccator for cooling and weigh the crucible (**W₄**).

8. Calculation

$$\frac{(W_4 - W_0)}{W_1} \times 100 = \text{amount of AIA in the sample (\%)}$$

Reference

- (1) Sales, J, and G. Janssens. 2003. "Acid-insoluble Ash as a Marker in Digestibility Studies: a Review." *Journal of Animal and Feed Sciences* 12 (3): 383–401.
- (2) ISO 5985: Animal feeding stuff- Determination of ash insoluble in hydrochloric acid.

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