**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.

**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.
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**

$$\frac{\left(W\_{4}-W\_{0}\right)}{W\_{1}}×100=amount of AIA in the sample \left(\%\right)$$

*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.