METHOD SPECIFICATION

Faculty of Biosciences, NMBU

Method name: Ammonium-N

BIOVIT-no. : Msp 1133

1. Method of analysis / Principle / Main instrument

Feed protein is normally divided into pure protein and non-protein nitrogen (NPN). Pure protein is what is normally called protein and it is made up of different amino acids that are put together in longer chains. NPN consists of simpler nitrogen compounds such as ammonia, nitrates, amides, nucleic acids, free amino acids and peptides of various sizes. Microbes in the rumen break down and hydrolyze the pure protein into peptides and amino acids, and these are further broken down into ammonia (NH_3), which is the most important starting material for microbial synthesis of proteins. 70% of the nitrogen that is bound up as NH_3 in the rumen comes from pure protein, while the remaining 30% comes from NPN.

In rumen juice, ammonia is not present as NH_3 but mainly as ammonium (NH_4^+), and it is the NH_4 + concentration that is determined in this analysis. The NH_4^+ concentration in rumen juice depends on the type of feed the animals receive and how long after feeding the sample is taken. The concentration normally varies between approx. 4-12 mmol / L (70-220 mg / L) and is highest approx. two hours after feeding.

In liquids, the NH_4^+ concentration can be determined in several ways, both photometrically and by distillation with a subsequent acid-base titration. In this method, the NH_4^+ concentration is determined using the last two steps in the Kjeldahl analysis, as shown below. The method is general and can be used to determine the concentration of NH_4^+ in most liquids.

Distillation

$NH_4^+ (aq) + OH^- (aq) \xrightarrow{\Delta} NH_3 (g) + H_2O (l)$

$B(OH)_3 (aq) + NH_3 (aq) + H_2O (l) \rightarrow NH_4^+ (aq) + B(OH)_4^- (aq)$

Titration

$B(OH)_4^- (aq) + HCl (aq) \leftrightarrows B(OH)_3 (aq) + H_2O (l) + Cl^- (aq)$

Equivalence point

$B(OH)_3 (aq) + 2\ H_2O (l) \leftrightarrows B(OH)_4^- (aq) + H_3O^+ (aq)$

Main instrument: Kjeltec 8400- automated distillation unit (Foss, Denmark)

2. Reference and any modification

AOAC Official method 2001.11 - Protein (crude) in animal feed, forage, grain and oilseeds. (Block Digestion with a Copper Catalyst and Steam Distillation into Boric Acid)

Modification: The support step is not performed.

3. Requirements for grinding and storage

Rumen juice must be preserved with concentrated formic acid in the ratio 5: 100 (0.5 mL formic acid per 9.5 mL rumen juice).

The sample must be stored cool and possibly frozen during prolonged storage.

Sample amount: minimum 4 mL canned rum juice

4. Contact persons

Lab manager: Hanne Kolsrud Hustoft
Responsible for analysis: Elin Kristoffersen / Heidi Askerud

5. Additional literature

1) Egli, H., Kjeldahl Guide, 1st edition, Büchi Labortechnik AG, Switzerland, 2008

2) Persson, J., Handbook for Kjeldahl Digestion, 4th edition, Sweden, 2008

3) Commission Regulation (EC) No 152/2009. 27 Jan 2009. Laying down the methods of sampling and analysis for the official control of feed. Annex III, P, Official Journal of the European Union L54 / 1 from 26/02/2009.