

STANDARD OPERATION PROCEDURE

Faculty of Biosciences, NMBU

Method name: Ammonium-N

BIOVIT-no. : Arb1133

1. Introduction

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 sources

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. The method is general and can be used to determine the concentration of NH_4^+ in most liquids.

2. Reagents

- Boric acid with methyl red and bromocresol green (indicators)
- Sodium hydroxide, NaOH (35%).
- Hydrochloric acid (0.2 M)
- RO water

3. Risk assessment

Sodium hydroxide and hydrochloric acid are corrosive. In case of spillage on clothes, these must be removed immediately and the skin rinsed with large amounts of water. In case of spillage on bare skin, rinse with large amounts of water. The boric acid solution is very dilute and has no danger label.

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4. Equipment

- Kjelttec Auto TM 8400
- Cooking block 2520 Auto
- Kjeldahl blocks w / Kjeldahl pipes.
- Pipette, 5 mL

5. Sample material

Pipette out 4 mL of rumen juice preserved with concentrated formic acid (5: 100).

6. Special remarks

7. Work procedure

- 1) Centrifuge the samples at 3000 rpm for 20 min
- 2) Fill an entire stand with Kjeldahl tubes
- 3) Note the number on the stand and leave the surface the number is on at row no. 1, where sample no. 1 starts from the left side
- 4) Make a blank sample (No. 1)
- 5) Make a standard - pipette out 4 mL of the standard solution (150 mg NH₄⁺ / L)
- 6) Pipette out 4 mL of rumen juice and transfer this to a Kjeldahl tube
- 7) The samples are now ready for analysis on the Kjelttec 8400
- 8) Check that the scale has the correct program for weighing in Ammonium-N.
 - Touch application> system> peripherals
 - Printer should be set to "OFF", Host should be set to "RS232", press "OK" and "exit"
 - see if necessary Own manual attached to the scale

Enter sequence in Compass (PC at the far right of the weighing room)

- 9) Open "launch Compass" on the desktop, notification appears, press "YES".
- 10) Login: Username: Admin
- 11) Pin code: 1234 OK.
- 12) Touch the new batch icon (top left).
- 13) Enter block number on «batch name».
- 14) It should say kjeldahl on Analysis type.
- 15) Press 20rack 250 ml on «Rack name».
- 16) Click on "samples" at the top.

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- 17) Tube 1 is always listed as mL blank, press OK for the number of blanks we want. If we are going to have 3 blanks, press OK three times
- 18) Make the last blank a sample (tube 4).
- a) Press «Show details», «sample type»: convert blank to sample,
 - b) "Result type": convert ml to mg N / L.
- 19) Enter ID on the test: ID ex: Requisition number 36-1 etc.
- 20) Place the pointer in the field next to "Get", enter 4.0, press OK.
- a) Check that the weight has entered.
- 21) The program automatically goes to the next pipe, enter the next ID.
- 22) If it is not a full block, remember to press "delete sample" on the last sample that appears.
- 23) Press «Save»,
- 24) Press «Send batches»

8. Calculation

Kjeltec 8400 calculates the results and publishes them in **mg N / L samples**.

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