STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Buffer soluble protein (sCP)
BIOVIT-nr: Arb 1171

1. Introduction

Buffer-soluble crude protein (ammonium nitrogen) is an easy and fast way to determine content of readily soluble degradable protein on. The extraction of protein can be affected by several factors:
Type of buffer, pH, temperature, extraction length, separation length and N analysis.
The method that this job description deals with is recommended by NorFor (dated 11/15/2006).

2. Principle of analysis, purpose

During the extraction, it is important to ensure a stable pH and temperature. This is done by using a borate-phosphate buffer with pH = 6.75 and keeping samples with a buffer of 39 °C below the incubation (1h). Ie. that one tries to imitate the physiological conditions as natural found in a rumen environment. After extraction, the samples are analyzed in the usual way at Kjeldahl-N- method.
For silo samples, the content of ammonium-N must be determined as a correction of lost Crude protein (such as ammonium-N) during drying. This loss is set at 60% ammonium.

3. Equipment

- 50ml centrifuge tubes or disposable tubes
- Funnel
- Filter (black)
- Water bath
- Centrifuge
- Kjeldahl equipment from Tecator

4. Reagents

- Distilled or ion-exchanged water
- Monosodium phosphate (NaH_2PO_4 x H_2O) 12.20g / l
- Sodium tetraborate ($Na_2B_4O_7$ x$10H_2O$) 8.91g / l

Borate phosphate buffer:

- Weigh 8.91 g $Na_2B_4O_7 \cdot 10H_2O$ in 950ml distilled water (dissolves slowly). Once resolved, add 12.20 g $NaH_2PO_4 \cdot H_2O$. Fill up with dest. water to 1 l. Adjust pH to 6.7-6.8.
- The buffer solution is made new just before use.

5. Sample material

1.5 g of dry, grinded (1 mm) sample material is needed.

6. Work procedure

1. Weigh about 1.5 g ± 1 mg (or 1.2 g and the tubes will not be so full) Sample in a centrifuge tube
2. Add 50 ± 0.5ml (or 40ml if it is 1.2g sample) borate-phosphate buffer (preheated to 39 °C) Include a blank with only borate-phosphate buffer on each series.
3. Put on the cork and shake the samples well.
4. Place the samples in a water bath at 39 ± 0.5 °C for incubation for 1 hour ± 5 minutes. Shake the samples every 15 minutes.
5. Centrifuge the samples at 3000 x g for 10min.
6. Filter the supernatant into a new tube
7. Pipette 20 ± 0.2 ml of the solution into a Kjeldahl tube
   Add 2 boiler drops and 15 ml sulfuric acid, put parafilm on the tubes and leave the block overnight above. To be placed on a cold block, use the program NMBU IHA RAMP on the block.
   From here, the sample is analyzed as a regular Kjeldahl-N sample

7. Calculation

$$\frac{\text{g/kg N (from kjeltec)}}{20} * 6{,}25 * 40$$

Where:
40 = volume (ml) of buffer added
20 = volume (ml) extract pipetted into Kjeldahl tubes

Optionally, the result can be stated as the proportion of buffer-soluble protein of total crude protein:

$$\frac{\text{sCP g / kg}}{\text{CP g / kg}} \times 1000 = \text{fraction sCP of total CP (g / kg)}$$

CP = crude protein and is obtained by multiplying the Kjeldahl-N value by 6.25