NorFor
Nordic Feed Evaluation System

Determination of Soluble Crude Protein (sCP)

1 Application / area of use

The procedure describes the determination of soluble crude protein (sCP) in all types of animal feeds. The procedure is intended for use in the Nordic Feed Evaluation System (NorFor).

2 Principle

The dried and milled sample is extracted with a borate-phosphate buffer pH 6.75 at 39^{o}C for 1 hour. After centrifugation the soluble crude protein in the supernatant is determined using Kjeldahl or other suitable methods for total nitrogen determination. For silage samples the content of ammonium nitrogen should also be determined as a correction for loss of crude protein as ammonium nitrogen during drying is needed in the calculation of sCP.

3 Sample preparation

The samples are dried as specified for the NorFor samples (< 60ºC) and ground on a hammer mill to pass a 1 mm sieve. Avoid over-grinding and heating during grinding. See note 7.1.

4 Reagents

Only use analytical grade reagents.

4.1 Water: Distilled or deionised water

4.2 Mono-sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) (CAS-No 10049-21-5)

4.3 Di-sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$) (CAS-No 1303-96-4)

4.4 Borate-phosphate buffer (modified from Licitra et al., 1996), pH 6.75±0.05. Dissolve 12.2 g of sodium dihydrogen phosphate and 8.91 g of sodium tetraborate in 900 ml of water. Check the pH with a pH-meter and if necessary adjust pH. Dilute with water in a 1000 ml volumetric flask. Prepare fresh buffer solution at least weekly.

4.5 Sulfuric acid, ρ_{20} 1.84 g ml^{-1}

4.6 Catalyst: Kjeltabs CF 5 g ($CuSO_4 \cdot 5H_2O$: Approximately 0.10 g Cu/tablet, Thompson & Capper Ltd.) or equivalent.

4.7 Titrate for the auto-burette in the Kjeltec apparatus, for example 0.1 mol L^{-1} HCl.

5 Equipment

5.1 Analytical balance capable of weighing to the nearest 1 mg

5.2 Centrifuge test tubes, 50 ml, with lids

5.3 Dispenser or pipette 50±0.5 ml

5.4 Water bath with continuous shaking, thermostated at 39±0.5°C (or incubating chamber with continuous shaking, 39±0.5°C)

5.5 Centrifuge suitable for the centrifuge tubes and capable of spinning at 3000 *x g* (given values of g are for the bottom of the test tubes)

5.6 Pipette 20±0.2 ml

5.7 Kjeldahl equipment or other equipment for total nitrogen determination in liquids

5.8 Heating block suitable for digestion of the samples

5.9 pH-meter, calibrated and capable of measuring pH to the nearest 0.01 unit

5.10 Nitrogen free filter paper e.g. AGF 607 Ø 90 mm, Munktell 00R

6 Procedure

6.1 Weigh approximately 1.2 g of the test sample to nearest 1 mg into a centrifuge tube (5.2).

6.2 Add 40±0.5 ml borate-phosphate buffer (4.4), pre-heated to 39 ºC (5.4), to the samples (See note 3).

6.3 A blank sample of 40 ml borate-phosphate buffer (4.4) should be included in each series of samples.

6.4 To hydrate the sample, swirl the tube gently. Then, put the lid on the tube and shake the sample thoroughly.

6.5 Incubate in a water bath or an incubating chamber with continuous shaking at 39±0.5°C for 1 h ± 5 minutes.

6.6 Centrifuge the tubes at 3000 × *g* for 15 min at 20ºC and filter the supernatant through a nitrogen free filter paper (5.11).

6.7 Pipette 20 ± 0.2 ml of the filtrate and transfer to Kjeldahl tubes.

6.8 Add salt/catalyst and the appropriate volume of sulphuric acid to the tubes according to the standard procedure in the lab. Some feed samples foam extensively when the acid is added. Foaming during digestion in the Kjeldahl analysis can be reduced if the acidified samples are allowed to stand at room temperature for 1-2 hours or overnight.

6.9 Increase the temperature of the digester stepwise, to prevent foaming. Do not include the time it takes to reach working temperature in the total digestion time.

6.10 Analyse the nitrogen content by Kjeldahl distillation.

6.11 Calculate the content of soluble crude protein.

7 Notes

7.1 The particle size of the ground material should be verified regularly according to EU regulation on animal feed analysis (EC No 152/2009). All the material should be able to pass through a sieve with a quadratic square mesh of 1 x 1 mm. Heating of samples during grinding should be avoided.

7.2 Depending on the facilities of the lab, other multiples of this sample:buffer ratio could be used, e.g. 3 g of sample and 100 ml buffer.

7.3 Analytical steps 6.2-6.8 (sulfuric acid addition) should be performed in sequence without interruption.

7.4 For samples containing measurable amounts of ammonia, it is necessary to correct the sCP for loss of sCP as ammonia during drying. This loss is at present set to 60%.

8 Calculations

The content of soluble crude protein per kg crude protein, for all samples in which the ammonia nitrogen content is zero, can be calculated according to:

$$g\ sCP/kgCP = \frac{(V_1 - V_0) * c * 14.007 * 6.25 * V_2 * 1000}{m * CP * V_3}$$

For silage samples, which have to be corrected for a 60% loss of ammonia nitrogen during drying, the equation is:

$$g\ sCP/kgCP = \frac{\frac{(V_1 - V_0) * c * 14.007 * 6.25 * V_2}{m * V_3} + \frac{0.60 * NH_3N * 1000 * 6.25}{DM1}}{CP_{uncorr} + \frac{0.6 * NH_3N * 1000 * 6.25}{DM1}} * 1000$$

Where:
V_0 = volume of HCl (4.7) used for titration of blank sample (ml)
V_1 = volume of HCl (4.7) used for titration of sample (ml)
V_2 = volume of buffer (4.4) added in step 6.2 (ml)
V_3 = volume of extract pipetted in step 6.7 (ml)
c = concentration of HCl used for titration (mol L^{-1})
m = sample size (g)
CP = crude protein in sample or pre-dried sample (g kg air dry $matter^{-1}$)
CP_{uncorr} = crude protein in pre-dried sample (g kg air dry $matter^{-1}$)
14.007 = molar weight for nitrogen (g mol^{-1})
6.25 = factor for conversion of nitrogen to crude protein

DM 1 = air dry matter in g kg^{-1} after drying at $60^{o}C$ and equilibration at room temperature for a minimum of four hours before grinding
NH_3N = ammonia nitrogen in fresh sample (g kg^{-1})
0.6 = estimated part of ammonia lost during drying (see note 7.4)

9 Precision

9.1 Repeatability

To be specified later.

9.2 Reproducibility

To be specified later.

10 References

Hansen-Møller, J. 2010. Ruggedness test of procedure for determination of soluble crude protein. Personal communication, 9 pp.

Licitra, G., T.M. Hernandez, P.J. Van Soest, 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 51: 347-358.

Udén, P. & T. Eriksson. 2012. Evaluation of some factors influencing the reliability of buffer soluble N recovery in feeds. Animal Feed Science and Technology 177: 218 – 224.

Åkerlind, M., M. Weisbjerg, T. Eriksson, R. Thøgersen, P. Udén, B.L. Ólafsson, O.M. Harstad and H. Volden, 2011. Feed analyses and digestion methods. In: NorFor – The Nordic feed evaluation system. EAAP publication No. 130, Wageningen Academic Publishers, 41-54.