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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 NorFor feed evaluation system.

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 (< 60º C) and ground on a hammer mill to pass a 1 mm sieve**.**

4 Reagents

Only use reagents of recognized analytical grade

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 in 900 ml of water 12.2 g of sodium dihydrogen phosphate and 8.91 g of sodium tetraborate. Check the pH with a pH-meter and if necessary adjust pH. Dilute in a 1000 ml volumetric flask to the mark with water. Prepare fresh buffer solution daily

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 pr tablet, Thompson & Capper Ltd.) or equivalent.

4.7 Titrant for the auto-burette in the Kjeltech 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 lid

5.3 Dispenser or pipette 50 ± 0.5 ml

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

5.5 Glass rods

5.6 Centrifuge suitable for the centrifuge tubes and capable of spinning at 3000 g (values of g is given for the bottom of the test tubes)

5.7 Pipette 20 ± 0.2 ml

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

5.9 Heating block suitable for digestion of the samples

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

6 Procedure

6.1 Weigh approximately 1.5 g of the test sample to nearest 1 mg in a centrifuge tube (5.2) (See note 7.1).

6.2 Add 50 ± 0.5 ml borate-phosphate buffer (4.4), pre-heated to 39 ºC (5.4), to the samples (See note 7.2)

6.3 A blank sample of 50 mL borate-phosphate buffer (4.4) should be included in each series of samples

6.4 To hydrate the sample, mix the sample gently with e.g. a glass rod (5.5). Then put the lid on the tube and shake the sample thoroughly.

6.5 Incubate in a water bath or an incubating chamber at 39 ± 0.5 °C for 1 h ± 5 minutes. Shake the tubes manually every 15th minute.

6.6 Centrifuge the tubes at 3000 × g for 10 min (see note7.3).

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

6.8 Add salt/catalyst and the 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 over night.

6.9 Increase the temperature of the digestor stepwise, to prevent foaming of the samples. 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 In the procedure 1.5 g sample and 50 ml buffer is recommended. If using the common 50 ml centrifuge tubes from Falcon, NUNC, Greiner etc the tube will be very full when using 50 ml of buffer and the shaking might be a problem. The recommendation is then to use 1.2 g sample and 40 ml of buffer. Depending on the facilities in the lab other multiples of this sample:buffer ratio could be used, e.g. 3 g of sample and 100 ml buffer.

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

7.3 Some insoluble particles (containing trapped air), particularly from forages may float on the surface after centrifugation, but if the supernatant is carefully pipetted the insoluble matter will not cause contamination. The particles may be removed with a spoon or a paper tissue. If still a problem the supernatant can carefully be poured into a beaker through a tea-strainer and then pipetted.

7.4 For samples containing measurable amounts of ammonium 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 pr kg crude protein is calculated according to:

$$g\ sCP/kg\ CP = \frac{\frac{(V_1 - V_0) * c * 14.007 * 6.25 * V_2}{m * V_3} + Ammonium\_CP\_Loss}{CPuncorr + Ammonium\_CP\_Loss} * 1000$$

Where:
V_0 = volume (ml) of HCl (4.7) used for titration of blank sample
V_1 = volume (ml) of HCl (4.7) used for titration of sample
V_2 = volume (ml) of buffer (4.4) added in step 6.2
V_3 = volume (ml)of extract pipetted in step 6.7
c = concentration of titrant (mol L^{-1})
m = sample size (g)
CPuncorr = crude protein (g kg $DM1^{-1}$) in predried sample
14.007 = Mw for nitrogen (g)
6.25 = Factor for conversion of nitrogen content to crude protein
Ammonium_CP_Loss = correction for loss of CP as volatile ammonia during drying. This is set to 60% of ammonia.

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

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

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

$$g\ sCP/kg\,CP = \frac{\frac{(V_1 - V_0) * c * 14.007 * 6.25 * V_2}{m * V_3} + \frac{0.60 * Am\_N * 1000 * 6{,}25}{DM1}}{CPuncorr + \frac{0.6 * Am\_N * 1000 * 6{,}25}{DM1}} * 1000$$

Where:
DM 1 = Dry matter in g kg^{-1} after drying at < 60 ^{o}C and equilibrating with air before grinding
Am_N = g ammonia N in fresh sample
60 = percentage of ammonia lost during the drying (see note 7.4)

9 Precision

9.1 Repeatability

The absolute difference between two independent single tests results, obtained on the identical material in the same laboratory by the same operator using the same equipment within a short interval of time, will not more than 5% of the cases be greater than the repeatability limit of XXXX (to be calculated later)

9.2 Reproducibility

The absolute difference between two independent single tests results, obtained with the same method on identical material in different laboratories with different operators using different equipment, will not more than 5% of the cases be greater than the reproducibility limit: XXXX (to be calculated later)

10 References

10.1 Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 51, 347-358