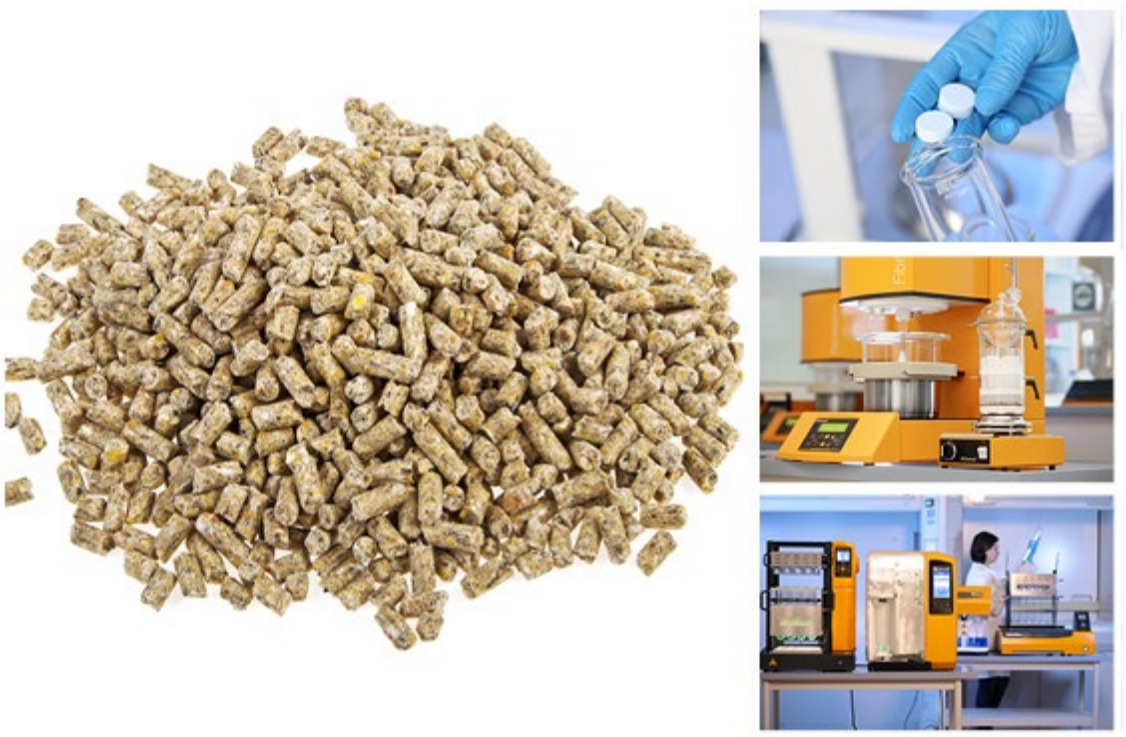
Application Kjeldahl

A.1.2.1 NDLXP- Determination in Animal Feed



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1 Principle and Introduction

The sample is boiled for one hour with a neutral detergent solution (NDF solution). A thermally stable α-Amylase is added in the process. The undissolved residue is washed and dried and then subjected to N-determination using the Kjeldahl method. The N quantity multiplied by the factor 6.25 in relation to the sample weight results in the content of crude protein bound to the NDF. The difference between the crude protein content of the sample and the content of crude protein bound to the NDF results in the content of neutral-detergent-soluble crude protein (NDLXP).

FibreBags make it easier to handle the dissolving and filtering of the components. The treatment process in the FIBRETHERM is a preliminary treatment for subsequent protein determination.

2 Method

The method is based on the specification:

- Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Association of German Agricultural Analytic and Research Institutes) (VDLUFA), 2017: Method 4.13.1 Determination of neutral-detergent-soluble crude protein (NDLXP). In: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA- Methodenbuch), Volume III. Die chemische Untersuchung von Futtermitteln, 3rd edition, 8th amendment, VDLUFA-Verlag, Darmstadt

This application document is intended to be a guide to assist users in the initial use of C. Gerhardt analytical equipment. It is not a definitive method. Users may have to adapt this method to the ambient conditions and to suit their own analytical requirements.

3 Chemicals and Accessories

Quality p. a.

1. Water distilled or demineralised
2. NDF-Solution:
   While being heated gently, 93 g EDTA ethylenediamine tetra acetic acid-disodium salt and 34 g disodium tetra borate-decahydrate are dissolved in a beaker in 2 l water. After cooling off, 150 g dodecylsulphate-sodium salt and 50 ml 2-ethoxyethanol are added. Due to the toxicity of 2-ethoxyethanole triethylene glycol can be used as a substitute instead.
   In another beaker, 22.8 g sodium dihydrogenphosphate are dissolved in 2 l water being gently heated up and after cooling off, are added to the first batch. The solution is filled up with distilled water to make about 5 l; the pH value has to be between 6.9 and 7.1 (if necessary, standardize with phosphoric acid, acetic acid or sodium hydroxide solution).
3. Heat-stable α-amylase, e.g. Termamyl 2X (Novozymes) – 1 ml α-amylase per sample (no amylase to be added to blank value sample
4. Petroleum Ether, Boiling Range 40 to 60 °C
5. Acetone p.a
6. Antifoam Solution for Fibre determination, Order No.: 10-0143; 5 - 10 drops for 1.3 l of detergent solution
7. Concentrated sulphuric acid H_2SO_4
8. Catalyst tablets KJELCAT Cu 5.0 g K_2SO_4 + 0.5 g $CuSO_4 + 5H_2O$ (Art. 12-0328) or comparable
9. Caustic soda NaOH 32 %
10. Boric acid H_3BO_3 2 %
11. Indicator solution M5 (Merck) or comparable

12. Standard solution: Hydrochloric acid c(HCl)= 0.1 mol/l or sulphuric acid c(H2SO4) = 0.05 mol/l
13. Acetanilid
14. Saccharose, nitrogen free
15. Ammonium sulphate, to be dried for at least 2 hours at a temperature of 102° C ± 2°C immediately before usage and subsequently to be kept in a desiccator for cooling to room temperature
16. Weighing paper WP250 (Art. 1004939) or paper weighing boats
17. Glass Spacer for FibreBags, to open and fix the bags, Package with 6 pieces, Order No.: 10-0124
18. FibreBags for ADF/NDF determination, bag with 100 pcs, Order No.: 10-0127
19. Incineration Module for FibreBags, 12-place, complete with handle and 12 quartz glass crucibles, Order No.: 13-0092
20. Degreasing module/ ADL- module made of PTFE, for the simultaneous degreasing of up to 6 samples, incl. handle and beaker 1000 ml, Order No.: 13-0045

4 Instruments

- FIBRETHERM FT 12, cat.no. 13-0026, with accessory
- External dosing unit 230 VAC with housing for the automatic addition of amylase, complete with teflon tubings and mains cable, Order No.: 1004430
- FRITSCH Pulverisette P14, sieve ring 1mm
- Desiccator with a drying agent as silica gel
- Drying chamber, electric driven, temperature 100°C +/- 5°C
- Timer or alarm clock
- Fume cabinet
- Kjeldahl digestion system KJELDATHERM, TURBOTHERM or flask heater for Kjeldahl flasks with enlarged neck
- Fume Scrubber TURBOSOG, alternatively VACUSOG or water jet pump
- VAPODEST Steam distillation system, models 200 to 450 without titrator, titration has to be performed by means of a manual burette (class A, according to ISO 385), 50 ml nominal volume, with volume scale in 0.05 ml steps or a Titrator, or instead of indicator solution with a pH meter with a combination electrode. The titration is performed automatically in case of VAPODEST 450 with external titrator or VAPODEST 500/500c with integrated titrator.

5 Sample Type and Preparation

Approx. 5 - 100 g of the feed are grinded down to a particle size of approx. 1 mm.
The glass spacers are marked with a heat- proofed marker.

- The glass spacers are put into the FibreBags.
- The weight of the sample to be put into the FibreBag should be around 1 g and should be weighed with 1 mg preciseness.
- The glass spacers with the samples are inserted into the FIBRETHERM.
- The blank value is determined in parallel to the regular analysis.
- The dry matter of the sample should be determined separately and is important for the calculation of NDLXP content (result related to the dry matter).

De-fatting of the sample is important especially for samples with a fat content higher than 10 %:
The carousel is immersed three times in a row into 40/60 petroleum ether. By turning it as well as moving it up and down the sample is defatted. This facilitates the washing and filtration process, which will follow. Furthermore, no fibre content is lost. The first petroleum ether fraction is thrown away but the following can be re-used. After a short drying process in the fume cupboard (about 2 minutes) the samples are inserted into the FIBRETHERM. 5 – 10 drops of the Antifoam Solution (3.6.) is added and the method is started.

Table 1: Analysis Parameter FIBRETHERM for aNDF

Settings Rinsing

1.	Water filling	1.3	Liter	
2.	Circulation Time	9	Seconds	
3.	Circulation Pause	4	Seconds	
4.	Suction	2 to 3 min	min / s	The boiling vessel shall be empty.
5.	Wash out time	1 min	min / s	

Settings Preheating

1.	Circulation Time	10	Seconds	
2.	Circulation Pause	30	Seconds	
3.	Heating delay after start	30	Seconds	
4.	ADF / H_2SO_4 heat up to	80 to 85	°C	
5.	NDF / KOH heat up to	75 - 78	°C	The sample solution shall boil.
6.	H_2O-Wash heat up to	80 to 85	°C	The sample solution shall boil.
7.	ADF / H_2SO_4 cool down to	85	°C	
8.	NDF / KOH cool down to	85	°C	
9.	H_2O-Wash cool down to	85	°C	

Method Settings

1.	Method Definition	X	No.	
2.	<A> Detergent	NDF-Solution	Type	
3.	<A> Add	1.3	Liter	
4.	<A> Add Amylase	12	ml	amount for 12 samples
4.	<A> Heating Power	30 to 35	%	The sample solution shall gently boil.
5.	<A> Circulation Time	10	s	Strong foaming: 5s
6.	<A> Circulation Pause	25	s	Strong foaming: 5s
7.	<A> Boiling Time	1 h 0min	h / min	
8.	<A> Suction	2 to 3 min	min / s	
9.	<A> Rinsing	2	Cycles	
10.	<B> Detergent	H_2O-Wash	Type	
11.	<B> Add	1.3	Liter	
12.	<A> Add Amylase	0	ml	Only if amount under 4. was not sufficient
13.	<B> Heating Power	55	%	
14.	<B> Circulation Time	10	s	
15.	<B> Circulation Pause	15	s	
16.	<B> Boiling Time	0 h 5 min	h / min	
17.	<B> Suction	2 to 3 min	min / s	
18.	<B> Rinsing	0	Cycles	
19.	<C> Detergent	H_2O-Wash	Type	
20.	<C> Add	1.3	Liter	
21.	<C> Add Amylase	0	ml	
22.	<C> Heating Power	55	%	
23.	<C> Circulation Time	10	s	
24.	<C> Circulation Pause	15	s	
25.	<C> Boiling Time	0 h 5 min	h / min	
26.	<C> Suction	2 to 3 min	min / s	
27.	<C> Rinsing	0	Cycles	
28.	<D> Detergent	H2O-Wash	Type	

29.	<D> Add	1.3	Liter	
30.	<D> Add Amylase	0	ml	
31.	<D> Heating Power	55	%	
32.	<D> Circulation Time	10	s	
33.	<D> Circulation Pause	15	s	
34.	<D> Boiling Time	0 h 5 min	h / min	
35.	<D> Suction	2 to 3 min	min / s	
36.	<D> Rinsing	1	Cycles	
37.	Meth. X defined Time:		h / min	

Drying of the FibreBags

The FibreBags are taken out of the carousel. The glass spacers are removed from the FibreBags rinsing them carefully with water.
The drained FibreBags are put into crucibles and are dried in the drying chamber at 105 °C for minimum 2 hours. The FibreBags should be dry before starting the Kjeldahl digestion (danger of overfoaming with the concentrated sulfuric acid).

Determination of the nitrogen content

The dried FibreBags with the dry residue are transferred to the Kjeldahl digestion tubes and the chemicals (see below: 3. Chemicals) are added.
In parallel, the nitrogen is determined from the original sample.

Digestion

Chemicals	Amount per sample
Sulphuric acid	20 ml
KJELCAT	2

Digestions with KJELDATHERM, time-optimised (differs from reference digestion time, however leads to comparable results in nearly all cases)

For digestions with a KJELDATHERM system with 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

Phase	Step	hh:mm	Temp. [°C]	Power [%]	Lift	Suc	Cool Vent	Cool Water
Digestion	1/2	01:30	410			☑	☐	☐
Cooling	2/2	00:30	-	-		☑	☑	☐
Done		-	-	-		☐	☐	☐

If your digester does not have an automatic lift system, take out the insert rack after digestion manually and leave the samples for cooling.

Note: Shorten the digestion time by placing the samples in a pre-heated digester.

For digestions with a TURBOTHERM system with 12 x 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

Note	Step	hh:mm	Power [%]	Suc
Heat-up of the system until boiling of the digestion solution	1/3	00:15	100	☑
After 20 – 30 minutes the digestion solution should be clear	2/3	01:15	75	☑
Sample cooling	3/3	00:30	0	☑
Digestion done		-	-	☐

Digestions with TURBOTHERM TTs and foaming samples

For digestions with a TURBOTHERM system with 12 x 250 ml KJELDATHERM digestions tubes, we recommend the following program parameters:

Note	Step	hh:mm	Power [%]	Suc
Heat-Up of the system until boiling of the digestion solution	1/7	00:05	100	☑
	2/7	00:05	0	☑
	3/7	00:05	100	☑
	4/7	00:05	0	☑
	5/7	00:05	100	☑
After 20 – 30 minutes the digestion solution should be clear	6/7	01:30	75	☑
Sample cooling	7/7	00:30	-	☑
Digestion done		-	-	-

Choose the method from the method library or program an older TURBOTHERM unit following the method „Foaming Samples“.

Digestions with a classic flask heater

For digestions with classic flask heaters in Kjeldahl flasks of 500 ml or 750 ml volume with enlarged neck, we recommend the following program parameters:

Time [min]	Power level	Note
20	3	Heating and evaporation until the digestion solution boils and white foams occur
60	1,5	Boiling of digestion solution
30	-	Cooling down samples

Suction of the digestion fumes

During the digestion, a fume scrubber (TURBOSOG or VACUSOG) must be activated. For the washing bottle we recommend to fill approx. 1.200 ml of caustic soda (concentration approx. 15 %). The suction power is adjusted correctly when no fumes come out of the tubes.
You can check if the caustic soda is still usable by adding an indicator and checking the pH value.
Allow 30 minutes for cooling down after taking out the insert rack or after deactivating the heating. Leave the fume scrubber activated during this time.

Note: You can reduce cooling down time of your samples by half with a KJELDATHERM ECO KIT.

Distillation with VAPODEST
After cooling down the samples, a steam distillation is performed with the following program:

	Method Food / Feed TKN	VAP 200	VAP 300	VAP 400	VAP 450	VAP 500 / 500c
H_2O Addition	100 ml	•	✓	✓	✓	✓
NaOH Addition	80 ml	✓	✓	✓	✓	✓
Reaction time	0 s	✓	✓	✓	✓	✓
Distillation time	240 s	✓	✓	✓	✓	✓
Steam power	100 %	✓	✓	✓	✓	✓
Sample suction	30 s	-	✓	✓	✓	✓
H_3BO_3 Addition	80 ml	•	•	✓	✓	✓
Suction receiver solution	30 s	-	-	-	✓	✓
Titration	-	•	•	•	✓	✓
Calculation	-	•	•	•	•	✓
Reading pH value, fixed endpoint or automatic endpoint	-	-	-	-	✓	✓
Titration online	-	-	-	-	-	✓

✓ = automatic • = manual - = not applicable

Choose the method from the method library or program an older VAPODEST unit following the method „Food / Feed TKN“.
Note: If you use a different amount of sulfuric acid for digestion, also the addition of water and caustic soda during distillation has to be adjusted accordingly.
A guideline for the proportions is: „1 part acid : 5 parts water : 4 parts caustic soda“ .

Titration
Add 3-4 drops of mixed indicator M5 to the receiver solution (**Fehler! Verweisquelle konnte nicht gefunden werden.**) and titrate with standard solution (**Fehler! Verweisquelle konnte nicht gefunden werden.**) until the colour changes from green to violet.
If you determine the endpoint with a pH meter or titrator, you do not have to add the mixed indicator M5.

Blank value
For blank value determination, perform the analysis (digestion + distillation + titration) just with the indicated chemicals and 1 g saccharose (3.14) instead of the sample. The chemical consumption has to be taken into account for the calculation.

Performance check
To check the analytical performance of your water steam distillation system, perform a distillation and titration of 0.12 g ammonium sulphate (3.15). The percentage of the recovered nitrogen must be between 99.0 and 100.0 % taking the purity of the standard solution into account a recovery rate up to 101 % is still acceptable in sporadic cases.

To verify the method, determine the nitrogen content of 0.18 g tryptophane or 0.16 g lysine-hydrochloride following this procedure. The recovery rate of nitrogen must be at least 99 %.

6 Calculation

The NDLXP content (neutral-detergent-soluble crude protein) w_{NDLXP} as % of the dry mass of the sample is calculated using the following formula:

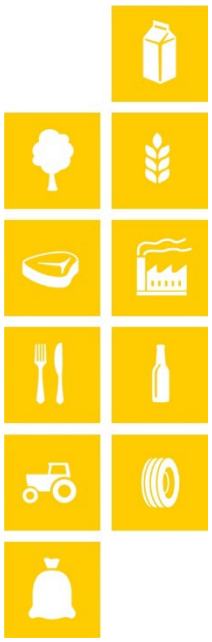
$$w_{NDLXP}\ \% = XP_P - \frac{(N_F - N_B) \times 6.25 \times 10}{E \times TM}$$

XP_P = Crude protein content of sample as % in relation to the dry mass
N_F = Nitrogen quantity of filtration residue in mg
N_B = Mean nitrogen quantity of blank value in mg
E = Sample weight in g
TM = Dry mass of sample as %

For the **VAP 500** (specifying the crude protein content as %), the formula is simplified as follows:

$$w_{NDLXP}\ \% = XP_P - XP_F$$

XP_P = Crude protein content of sample as % in relation to the dry mass
XP_F = Crude protein content of FibreBag residue as % in relation to the dry mass



COMPREHENSIVE APPLICATION DATA BASE

C. Gerhardt offers a wide range of application notes for many methods and procedures. Please contact our application lab team via application@gerhardt.de for deeper information on:

- Nitrogen in food and feed samples according to Kjeldahl and Dumas
- Crude fibre, ADF and NDF in feed
- Fat in food and feed
- Alcohol determination
- Total cyanide in water
- Trace metal in soil and sludge
- COD determination in water
- Total nitrogen determination in water, soil and plants
- Many more application notes on request.

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