WHITEPAPER

Crude protein analysis: New VDLUFA method for crude protein fractionation

Based on
VDLUFA 2023 METHOD BOOK III 4.13.2

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Content

Whitepaper by CG

We have been a developer and producer of analytical systems and basic products for laboratory work since 1846. The efficient use and analytical accuracy of our systems in your laboratory are our top priority. That is why we accompany you in your daily laboratory routine even after you have purchased a Gerhardt apparatus. Because the transfer of our specialist knowledge and the constant expansion of this knowledge are of the utmost importance to us.

Focus on feed efficiency

Our world population has been steadily increasing for decades, and in parallel, the consumption behaviour of society is changing. In this context, the so-called 'basic necessities', such as drinking water or food, are of particular importance for ensuring people's nutrition.

Looking at consumption in the food sector alone, the annual global meat consumption per person increased by almost 1 kg between 2010 and 2020. This increased consumption in turn led to an increased demand for livestock and thus also for animal feed. The accurate assessment and efficient use of animal feed has therefore become more important.

Figure 1: Feed for cattle

On the one hand, animal feed plays a crucial role in the composition and quality of animal products such as meat, milk and eggs. On the other hand, the absorption and utilisation of animal feed varies among different types of farm animals and can therefore be specifically adapted.

When adapting animal feed, it is important to ensure that it is well processed by the animal. On the one hand, this is important for the animal welfare, because only healthy animals produce high-quality food. On the other hand, it also reduces environmentally harmful emissions. Specifically composed animal feed can also be used more effectively, which in turn saves resources and reduces costs.

A precise animal feed analysis for a feeding strategy based on needs is therefore desirable. That is why members of the Association of German Agricultural Research and Testing Institutes (VDLUFA) are constantly researching the composition of various animal feeds and the determination and effect of various parameters.

Feed rationing acc. to CNCPS

The composition and processing of animal feed are crucial criteria for efficient use. However, the key lies in evaluating the quality of the ingredients of animal feed. In this context, the aim is to adapt the nutrient supply to the nutrient requirements.

This includes a corresponding feed analysis with standard methods, such as the Weende analysis or, in addition, the detergent fibre analysis according to Van Soest. In the Weende analytics, the organic substances contained in feed are categorised into the components: crude protein, crude fat, crude fibre and nitrogen-free extract substances. However, in order to be able to classify the analytical results, it is also of considerable importance to collect data on the digestion, metabolism and excretion processes in livestock.

„The key to the efficient use of animal feed lies in the most accurate prediction possible of the nutrient requirements and the optimal supply of nutrients to the animals.“

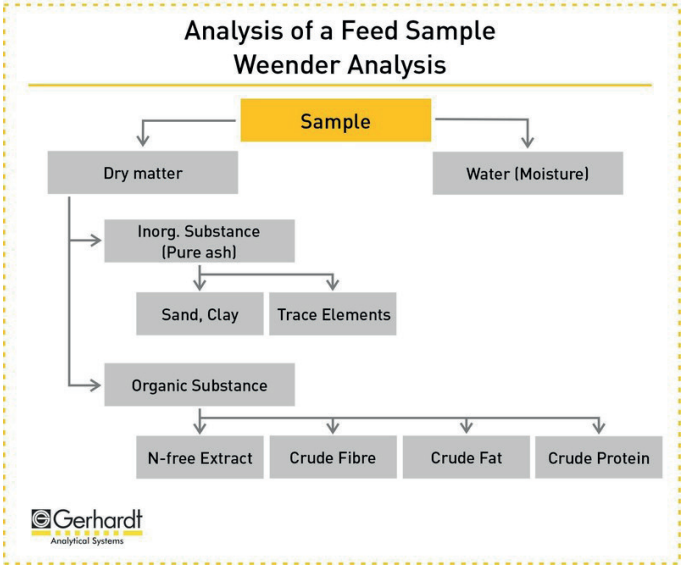

Figure 2: Weender Analysis

In the past, feed rations were optimised using empirical prediction equations developed under controlled research conditions.

However, these controlled conditions come with a 'safety margin'. This means that additional nutrients are included to ensure that the animals' nutrient requirements are always met. This safety margin often results in increased nutrient excretion on the part of the animals. This not only affects animal welfare, but also has a negative impact on groundwater and air quality. In addition, resources are wasted because the excess nutrients are excreted by the animal again.

This approach of empirical prediction equations was replaced in 1992 – at least for dairy and beef cattle – by a mathematical calculation model. This model takes a holistic approach and has been continuously developed since its publication: the **Cornell Net Carbohydrate and Protein System (CNCPS)**.

Here, the digestibility and the passage rate for the nutrient supply of ruminants are included. Certain nutrients are therefore considered independently of each other, so that the digestibility of the individual substances can be determined on the basis of their solubility and availability in the rumen. This makes it possible to determine which of the nutrients are available to the animal and can therefore be utilised.

„Farmers can use the CNCPS to incorporate individual data and thereby determine the performance, feed efficiency, sustainability and profitability of their operations."

In addition to feed composition and the digestion and metabolism process, the CNCPS also includes individual characteristics such as husbandry, performance and environmental factors or physiological conditions of the animals in the calculation. This means that operators can supplement this model with individual data and thereby determine the performance, feed efficiency, sustainability and economic efficiency for their own farm.

Crude protein fractionation acc. to VDLUFA

When breaking down the ingredients into crude protein, crude fat, crude fibre and nitrogen-free extract substances, there is a need for more specific information. This is because more in-depth analytical methods improve the assessment of protein quality and availability, which in turn increases the informative value of the animal feed's digestibility and usability.

That is why the CNCPS goes one step further and divides the various parameters into usability, digestibility and energy concentration. In doing so, qualitative characteristics such as amino acid patterns, crude protein fractions, fibre components or fatty acid spectra are determined.

In this white paper, we focus in particular on crude protein fractionation, specifically on the fractionation of crude protein as the basis for crude protein evaluation in ruminants.

Crude protein fractionation is necessary because, among other things, the crude protein content of a sample is determined by the analysed nitrogen content. For example, the crude protein content in an animal feed is calculated by multiplying the measured nitrogen content by the protein factor 6.25. However, there are qualitative differences in the usability of the crude protein that should not be ignored. In the analysis of crude protein fractionation, proteins are therefore subdivided into different fractions or groups based on Licitra et al 1996, according to their degree of solubility and the resulting degradability and/or usability.

According to the new VDLUFA method, the following parameters are analysed first:

Table 1: Overview of the parameters to be determined, with corresponding abbreviations and simplified descriptions of how the parameters are determined.

Parameter	Abbreviation	Simplified description of the analytical procedure
Crude protein	XP	Direct crude protein determination
Tungstate-insoluble crude protein	WUXP	1. Incubation at pH 2 overnight in a tungstate solution. 2. Filtration with a suitable filter paper or bag. 3. Crude protein determination of the insoluble residue.
Borate-phosphate buffer-insoluble crude protein	BUXP	1. Incubation for 3 hours at room temperature in a borate-phosphate buffer 2. Filtration with a suitable filter paper or bag. 3. Crude protein determination of the insoluble residue.
Crude protein insoluble in neutral detergent	NDUXP	1. Boiling for 1 hour in a neutral detergent solution 2. Filtration with a suitable filter paper or bag. 3. Crude protein determination of the insoluble residue.
Acid detergent-insoluble crude protein	ADUXP	1. Boiling for 1 hour in acid detergent solution 2. Filtration with a suitable filter paper or bag. 3. Crude protein determination of the insoluble residue.

„Fibre determination is the preparatory analytical step in the determination and classification of the various crude protein fractions prior to protein determination.“

The column ‘Simplified description of the analytical procedure’ shows that both fibre and protein determination are used for the determination of the individual parameters. This shows the relationship between how crude protein is present and how it is available.

This is partly due to the fact that the so-called fibre proteins or structural proteins serve as a scaffold for cells of plants or living organisms. This means that the scaffold contains protein. However, in order to determine this protein content, the sample must first be isolated by fibre analysis. This is important because many proteins that are bound in the cell wall are only partially or not at all usable by animals.

The following crude protein fractions can be calculated from the analysed parameters listed in Table 1:

Table 2: Overview of crude protein fractions with information on utilisation and calculation of the individual fractions.

Crude protein fraction	Description	Enzymatic degradation	Calculation
A	Non-protein-nitrogen	Not applicable	XP - WUXP
B1	Pure protein soluble in buffer solution	fast	WUXP - BUXP
B2	Buffer-insoluble pure protein (ND soluble)	variable	BUXP - NDUXP
B3	Cell-wall bound, soluble pure protein	Variable to slow	NDUXP - ADUXP
C	Cell-wall-bound, insoluble pure protein	Indigestible	ADUXP

„The information obtained through crude protein fractionation is used to supply ruminants according to their needs and to make optimal use of available resources.“

The information obtained through crude protein fractionation is used to supply ruminants with nitrogen as needed and to make optimal use of available resources. The differentiated analysis of the protein content of animal feeds brings several advantages for agricultural businesses:

- Promoting animal health by avoiding inappropriate feeding
- Minimising negative environmental impacts, e.g. by reducing the excretion of nitrogen and phosphorus
- Effective use of feed and the associated cost savings

Analytical background

The analytics behind crude protein fractionation are quite complex, since XP, WUXP, BUXP, NDUXP and ADUXP must first be determined in order to calculate the crude protein fractions. Although the analytical processes all conclude with a crude protein determination, they differ in the preparatory steps.

All parameters are based on a crude protein determination. This can be carried out, for example, using the Kjeldahl method, which can be roughly divided into the following four steps:

1. Sample weighing
2. Digestion of samples with sulphuric acid
3. Distillation of the digestion solution with steam
4. Titration of the distillate and calculation of the result

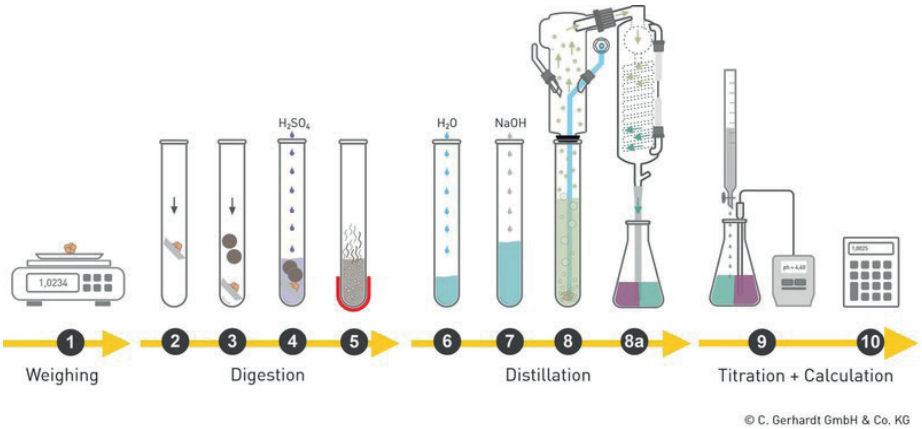

Figure 3: Kjeldahl analytical process

„Thanks to the simplified and standardised filtration conditions with FibreBag, analytical results are more reliable and reproducible.“

While nitrogen determination according to Kjeldahl is sufficient for calculating the crude protein parameter, the other four parameters require additional sample preparation steps.

For example, in the determination of WUXP and BUXP, the sample is incubated and then filtered. The filter residue is then analysed for its crude protein content. The filtration can be carried out either with the aid of a conventional filter paper or C. Gerhardt's FibreBag technology.

Thanks to the simplified and precisely reproducible filtration conditions when using the FibreBags, the analytical results are highly reliable and highly reproducible. To go with its FibreBag technology, C. Gerhardt also offers a filter insert that simplifies the handling of samples during the filtration process.

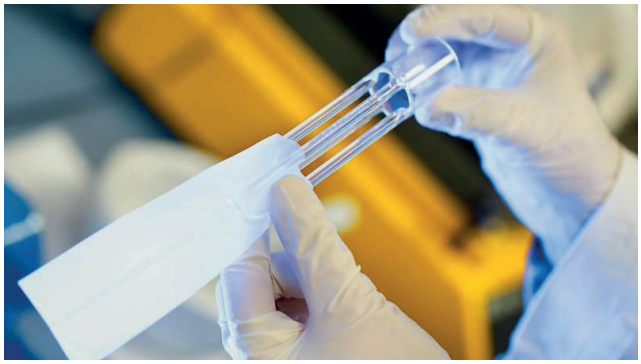

Figure 4: FibreBag technology by C. Gerhardt

The determination of NDUXP first requires sample preparation in the form of isolating the neutral detergent fibres after amylase treatment (aNDF).

The sample is boiled with a neutral solution (NDF solution). During the boiling process, a heat-stable amylase is added. The sample is then filtered. At the end, the insoluble residue remains, which is dried and then analysed for crude protein using the Kjeldahl method.

The determination of ADUXP requires a sample preparation in the form of determinating the ADF (acid detergent fibre) content. The analytical process is similar to that for aNDF determination. However, instead of the NDF solution, the sample is boiled in an acidic solution, i.e. an ADF solution.

Automation of analytics

A total of five parameters must therefore be determined: crude protein, WUXP, BUXP, NDXUP and ADUXP.

„The automation of all analytical steps within closed systems ensures that the analysis is not only rapid but also subject to constant conditions.“

While the steps in the classic Kjeldahl protein determination are carried out manually, automated laboratory systems are available today.

C. Gerhardt offers two different digestion units: a block digestion system in which the heat transfer takes place by means of cast heaters and an infrared digestion system in which the heat transfer takes place by means of contact-free infrared radiation. For the subsequent distillation and titration that take place after digestion, C. Gerhardt offers the VAPODEST series. These are various steam distillation systems that are either partially or fully automated.

Figure 5: Kjeldahl series by C. Gerhardt

The automation of all analytical steps within closed systems makes the analysis faster than the manual Kjeldahl analysis and ensures that it is carried out under constant analytical conditions. This makes the analytical results highly precise and highly reproducible. Another advantage is that the time spent at the apparatus is reduced. This allows for more effective use of working hours.

As with the classic Kjeldahl method, the boiling process, the addition of amylase and the filtration in the determination of ADF and aNDF are originally steps of a manual analysis. This is because fibre analysis is also based on classic apparatus. However, analytics have also developed in this area, with the result that automated systems are now available.

One example is FIBRETHERM, which can control all cooking, washing and filtration processes for up to 12 samples. In addition, all detergents are automatically dosed and added by means of calibrated pumps, without interrupting the analytical process. The fact that the work steps are carried out within a single system means that the analytical results are also highly precise and reproducible. In addition, the automation of all analytical steps reduces the time spent at the apparatus, which leads to increased efficiency and occupational safety.

The FibreBag technology can therefore be used for both manual and automated sample treatment and optimises the washing and filtration process.

„The more specifically the components of animal feed can be identified and analysed, the better the nutrient supply can be matched to the animals‘ nutrient requirements.“

Figure 6: FIBRETHERM and manual FibreBag system from C. Gerhardt

Against this background, the efficiency of determinig crude protein fractions can be significantly increased.

Conclusion

In summary, it can be said that crude protein fractionation is very valuable for evaluating animal feed in terms of the usability of crude protein in ruminants. The more specifically the components of an animal feed can be identified and analysed, the better the nutrient supply can be tailored to the animals‘ nutrient requirements. This optimises animal health and, as a result, animal welfare.

In addition, it avoids wasting resources. What‘s more, the available resources are used more efficiently. This is not only sustainable for the environment, but also economical for the company.

Automated laboratory systems can significantly reduce the manual effort required for the complex analytical process of determining crude protein fractions. This makes the process more rapid, reliable and standardised. As a result, not only is efficiency increased in the laboratory, but the quality of the analytical results is also improved.

The use of innovative consumables such as FibreBag can further optimise the process: after all, the performance of an analytical system is only as good as its consumables!

As a result, even lengthy analytical processes such as crude protein fractionation can be carried out as rapidly and easily as possible.

Literature

(1) VDLUFA 2023 METHOD BOOK III 4.13.2

(2) Publication of LICITRA, G., HERNANDEZ, T., VAN SOEST, P. J., 1996: Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358

(3) Federal Statistical Office, https://www.destatis.de/DE/Themen/Laender-Regionen/Internationales/Thema/landwirtschaft-fischerei/tierhaltung-fleischkonsum/_inhalt.html, Stand: 28.09.2023