



VELP E-BOOK

BARLEY AND FEED

N/Protein Determination and
Fiber Determination

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INTRODUCTION

BARLEY AND FEED: A STRONG BOND

BARLEY AND FEED: A STRONG BOND

Barley ranks fourth in importance among cereals, after wheat, corn and rice. It represents two-thirds of the fodder grains demanded by the world and most of it is used to feed **livestock**, with industrial consumption remaining practically stable.

Barley is considered a medium-energy grain, low in starch and high in fiber. Regarding protein levels, barley is similar to wheat and superior to corn, the level of this nutrient can vary between 9% and 12-13%. It is an excellent source of some B vitamins (thiamine, riboflavin, pyridoxine, pantothenic acid) and niacin. Barley has a high fiber content, higher than that of corn and wheat, which results in a lower nutritional value for species sensitive to fiber content. Barley is one of the grains most commonly used in feed for dairy cows and fattening cattle. Due to its **high ruminal digestibility**, barley has high metabolizable energy values for ruminants.



BARLEY – OVERVIEW AND FIGURES

- High ruminal digestibility resulting in high metabolizable energy values for ruminants.
- Most commonly used in feed for dairy cows and fattening cattle.



Proteins

The level of proteins can vary between 9% and 12-13%.

Fiber

High fiber content, the level of Crude Fiber is around 4% and the level of Neutral Detergent Fiber is around 16%.

Vitamins

Excellent source of B vitamins and niacin.

Feed represents **70% of the costs of a livestock operation**, therefore, knowledge of different nutritional alternatives (alternative cereals) is an essential tool to maintain the profitability of the system. Feed is made up of **many components**, which are fats, oils, carbohydrates, protein, vitamins, minerals and product quality enhancement. In order to obtain a **nutritionally balanced diet** it is important to know in which amount these elements should be included in the diet, and in every single component of the feed, as for example, **barley**.



Within this scenario, **Proficiency Testing Programs** organized by institutions like **BIPEA** allow the comparison of several laboratories' results, in order to evaluate their analytical performance on the same homogeneous sample. By taking part in such programs on a regular basis, the performance of **VELP instruments** is constantly evaluated. Samples provided by BIPEA are analyzed in accordance with the official reference ISO and AOAC and the obtained results are compared with the given tolerance range.

MATERIALS, METHODS & RESULTS

PROTEIN AND FIBER
ANALYSIS: EVIDENCE
APPLYING DIFFERENT
METHODS

PROTEIN DETERMINATION

To determine the **quantity of proteins** it is possible to analyze the sample according to the **Dumas method** and the **Kjeldahl method**. Both are primary methods working in accordance to international standards such as AOAC, AACC, ASBC, ISO, IFFO, OIV.

The **Dumas method** for nitrogen determination, developed in 1831, is older than the **Kjeldahl**, 1883, but more convenient in many aspects such as speed, safety, cleanliness, productivity and cost per analysis.

The problem in the past was that it was not easy to reproduce the conditions required by the Dumas method and for this reason, the Kjeldahl technique took the lead and became considered as the classical method for nitrogen/protein determination.

Nowadays, thanks to steps forward in technology, the Dumas nitrogen determination is becoming more widespread.

Results obtained with the Dumas nitrogen determination are usually a little bit **higher than with Kjeldahl** since nitrogen compounds like nitrates, nitrites and heterocyclic compounds are detected.

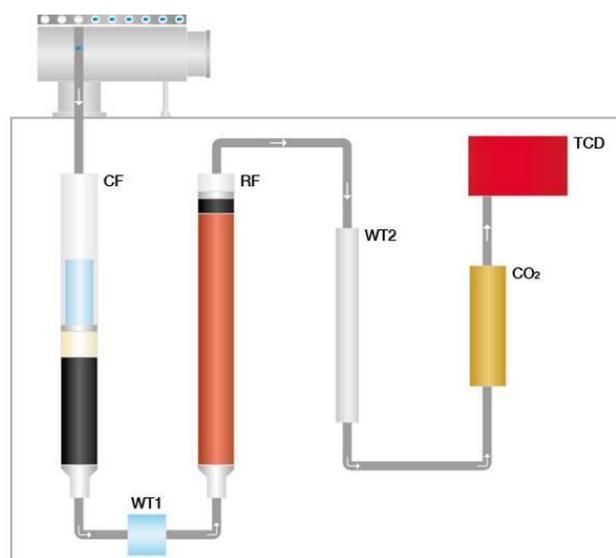
In the **Kjeldahl method**, such compounds are converted into the ammonium ion incompletely or not at all. The opposite could also happen (rarely) because in this kind of analysis there are lots of variables that could influence the final result.

Indeed, there are many minor variants of the Kjeldahl method, involving the use of different catalysts, heating times, volumes and distribution of sulfuric acid and masses of test portion: this shows that the Kjeldahl

procedure may be influenced by experimental errors. Recovery is the same for both the methods ($\geq 99.5\%$), while the detection limit is lower for Dumas than for Kjeldahl (0.001 mg N absolute vs. ≥ 0.1 mg N absolute).

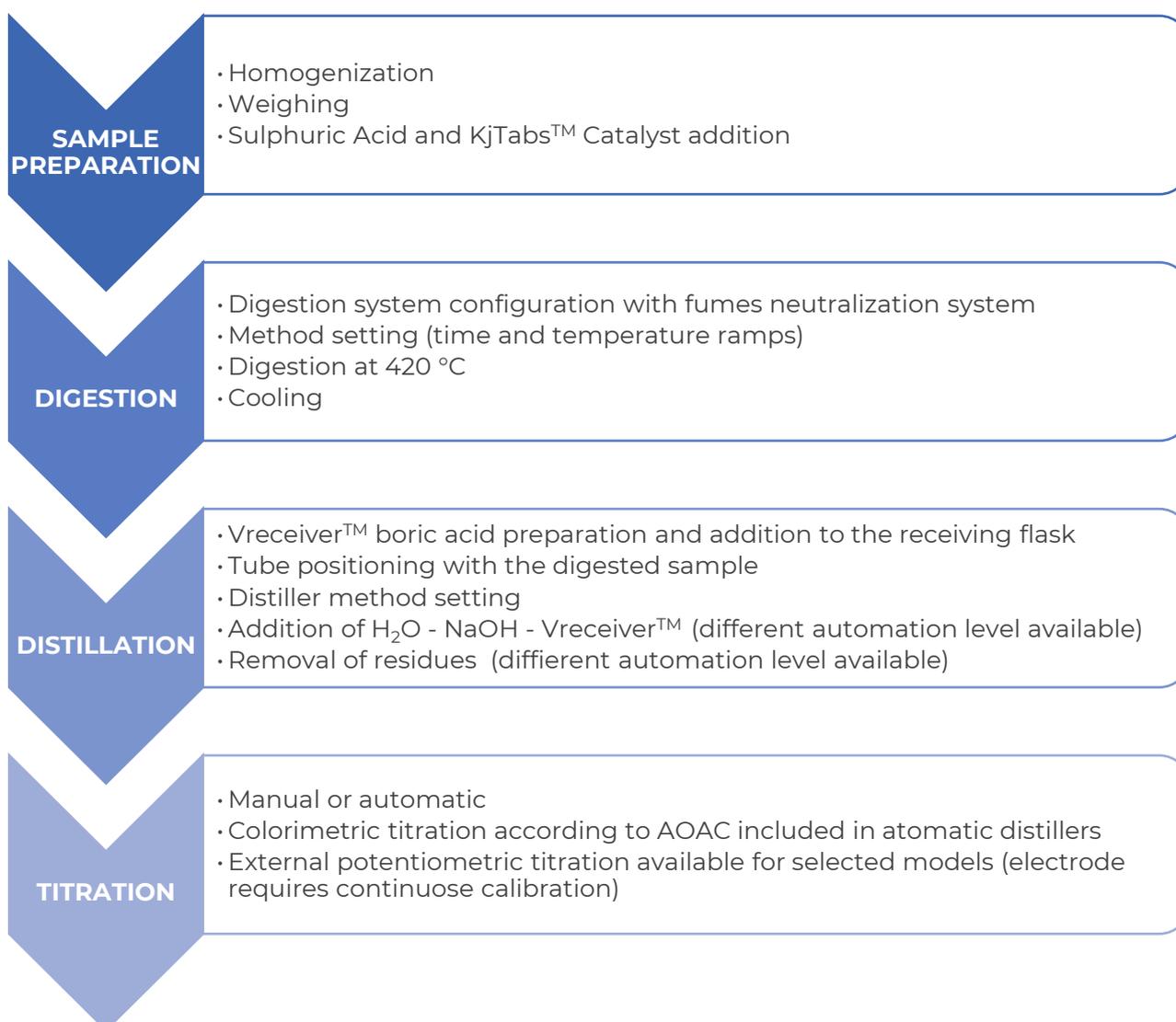
DUMAS METHOD

The **Dumas method** starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds. Water is removed by a first physical trap (WT1) and a second chemical one (WT2). Between the two, the elemental substances pass through a reduction furnace (RF). The auto-regenerative CO₂ absorbers let only the elemental nitrogen pass, which is detected by Thermal Conductivity Detector (TCD).



KJELDAHL METHOD

The **Kjeldahl method** consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperatures higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.



N/PROTEIN RESULTS

Materials:

- NDA 702 – N/Protein Elemental Analyzer
- DKL 20 – Digestion Unit
- JP Pump and SMS Scrubber for fumes neutralization
- UDK 159 – Distillation Unit

Table 1 – Kjeldahl and Dumas Nitrogen and Protein results

	Sample quantity	Nitrogen %	Protein %
Dumas	199.97	1.319	8.244
	199.84	1.327	8.294
	199.89	1.315	8.219
	Average ± SD%	1.320 ± 0.006	8.252 ± 0.038
Kjeldahl	1.0030 g	1.264%	7.900%
	1.0011 g	1.287%	8.044%
	1.0048 g	1.282%	8.012%
	Average ± SD%	1.278 ± 0.010	7.985 ± 0.075

BIPEA Protein Assigned Value - Dumas: 8.2 ± 0.2 % P

BIPEA Protein Assigned Value - Kjeldahl: 8.1 ± 0.2 % P

FIBER DETERMINATION

Fiber is a component of the vegetable cell walls and as a primary function of plant support. From a nutritional perspective we can define it as the hydrolytically indigestible partially fermentable components of feed. A chemical perspective defines fiber as a variable mixture of predominantly cellulose, hemicelluloses, lignin, and soluble dietary fibers (e.g., pectins).

The **demand for fiber content determination in feed** is constantly growing because it is a fundamental parameter for the assessment of Feed's Quality.

The fiber amount of feed has impact on livestock productivity and the cost of the feed. For example, the calculation of the correct amount of fiber in the feedstuff influences milk production, milk quality and fat content of milk.



*“Because there is no guarantee of direct correspondence between chemical solubility and nutritional availability, in reality, fiber is defined by the method used to isolate it. **The actual definition of fiber becomes method-dependent**”.* (AAFCO - Critical Factors in Determining Fiber in Feeds and Forages)

This passage of an AAFCO document suggests that is important to follow official method exactly in order to obtain reproducible results and that any modification leads to incomparability of tests.

FIBER DETERMINATION METHODS

Crude Fiber, CF

The method is based on the solubilization (digestion) of non-cellulosic compounds by sulfuric acid and potassium hydroxide solutions. Crude fiber is the loss on ignition of the dried residue remaining after digestion of the sample and is determined by weight difference.

This method is applicable to grains, meals, flours, feeds, and fiber-bearing material from which fat can be extracted to leave workable residue.

Neutral Detergent Fiber, NDF

The sample is digested in the Neutral Detergent Solution NDS with heat-stable α -amylase-treated enzyme to separate the neutral detergent soluble fraction (sugars, starches and pectin soluble, filtered) from the neutral detergent insoluble fraction (cell walls substances, hemicellulose, cellulose and lignin, residues). The cell contents are highly digestible (about 98 %) and include various sugars, starches, pectins and other soluble carbohydrates, proteins, non-protein nitrogenous compounds, lipids, water-soluble minerals and vitamins.

The remaining dry matter is estimated and the proportion gives the neutral detergent fiber (NDF).

Acid Detergent Fiber, ADF

The Acid detergent solution (ADS) solubilizes the hemicellulose while lignin and cellulose remain insoluble. The residue is weighed for the determination of ADF. It includes cellulose and lignin.

Acid Detergent Lignin, ADL

The remaining residue from the ADF analysis, is solubilized by 72% sulfuric acid, leaving the lignin (ADL) which is determined gravimetrically.



FIBER RESULTS

Materials:

- FIWE Advance – Fully Automatic Fiber Analyzer
- COEX – Cold Extractor

Crude Fiber calculation

To calculate this parameter, it is necessary to subtract the weight of the crucible after the dry process minus the weight of the crucible after the incineration in the muffle.

$$\% \text{ Crude Fiber} = (M_{dry} - M_{ash}) * 100 / M_{sample}$$

M_{dry} = sample weight after drying

M_{ash} = sample weight after ashing

M_{sample} = sample weight

Table 2 – Crude Fiber results

M_{sample} (g)	M_{dry} (g)	M_{ash} (g)	CF %
1.0349	30.4385	30.394	4.1067
1.0511	31.221	31.1732	4.3573
1.0043	30.5511	30.5073	4.1621
		Average ± SD%	4.21 ± 0.13

BIPEA Crude Fiber Assigned Value: 4.2 ± 0.4 %

Neutral Detergent Fiber calculation

To calculate this parameter, it is necessary to subtract the weight of the crucible after the time in the oven minus the weight of the crucible after the incineration process in the muffle.

$$\text{aNDF \%} = (M_{dry} - M_{ash} - (B_{dry} - B_{ash})) * 100 / M_{sample}$$

M_{dry} = sample weight after drying

M_{ash} = sample weight after ashing

M_{sample} = sample weight

B_{dry} = blank weight after drying

B_{ash} = blank weight after ashing

Table 3 – Neutral Detergent Fiber results

M_{sample} (g)	M_{dry} (g)	M_{ash} (g)	aNDF %
0.5009	30.101	30.0205	16.15%
0.5026	30.7516	30.669	16.43%
0.5068	31.0118	30.9294	16.26%
		Average ± SD%	16.28 ± 0.39

BIPEA NDF Assigned Value: 16.7% ± 0.8%

Acid Detergent Fiber calculation

To calculate this parameter, it is necessary to subtract the weight of the crucible before the analysis minus the weight of the crucible after the digestion end the dry process.

$$\text{ADF \%} = (M_{\text{dry}} - M_{\text{tare}} - (B_{\text{dry}} - B_{\text{tare}})) * 100 / M_{\text{sample}}$$

M_{dry} = sample weight after drying

M_{tare} = tare of the sample

M_{sample} = sample weight

B_{dry} = blank weight after drying

B_{tare} = tare of the blank

Table 4 – Acid Detergent Fiber results

M_{tare} (g)	M_{sample} (g)	M_{dry} (g)	ADF %
30.4512	1.0342	30.459	4.844%
30.992	0.9972	30.9987	4.8335
31.1974	1.0085	31.2067	4.9380
		Average ± SD%	4.87 ± 0.06

BIPEA ADF Assigned Value: 5.0% ± 0.8%

Acid Detergent Lignin calculation

To calculate this parameter, it is necessary to subtract the weight of the crucible after the dry process minus the weight of the crucible after the incineration process in the muffle.

$$\text{ADL \%} = (M_{\text{dry}} - M_{\text{ash}} - (B_{\text{dry}} - B_{\text{ash}})) * 100 / M_{\text{sample}}$$

M_{dry} = sample weight after drying

M_{ash} = sample weight after ashing

M_{sample} = sample weight

B_{dry} = blank weight after drying

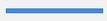
B_{ash} = blank weight after ashing

Table 5 – Acid Detergent Lignin results

M_{sample} (g)	M_{dry} (g)	M_{ash} (g)	ADL %
1.0342	30.459	30.4441	1.440
0.9972	30.9987	30.9836	1.5142
1.0085	31.2067	31.1924	1.4179
		Average ± SD%	1.46 ± 0.05

BIPEA ADL Assigned Value: 1.2% ± 0.5%

CONCLUSIONS



RESULTS DISCUSSION

The results obtained are reliable, reproducible in accordance with the expected values of **the proficiency testing program organized by BIPEA.**

It means that **VELP instruments are able** to determine accurate and precise parameters for feed analysis in barley, in order to obtain a **complete diet for animals.**

PROTEIN ANALYSIS

The obtained results for barley fell within the expected value range indicated by BIPEA, demonstrating the high performances of **VELP Kjeldahl analytical instruments** and **VELP N/Protein Elemental Analyzers**, for **protein determination.** Both techniques are efficient and capable of analyzing barley samples with **high accuracy and repeatability.**

VELP N/Protein Elemental Analyzers with high productivity and non-stop performances, are indeed ideal for high throughput, being fully automated and requiring just 3-4 minutes per analysis.

VELP Kjeldahl system, is still a robust solution for protein determination in food and feed field.

FIBER ANALYSIS

The fiber results obtained are reliable and in accordance with the expected values. The use of an **extraction apparatus** purposely devised for this method as **VELP Fiber Analyzer**, makes the standardization of analytical conditions very easy.

- Fast analysis (2 hours with VELP Fiber Analyzers vs. 6 hours manually)
- Ease of use with convenient filtration, with pump and air pressure
- High reproducibility of the results, $\pm 1\%$ relative or better
- Results in accordance with official procedures

It is a great advantage that a single company as **VELP Scientifica**, is able to design, produce and support these three series of instruments to determine the crucial feed parameters in barley.

Furthermore, **VELP Scientifica internally produces the main consumables** to offer the most suitable **turnkey solution** for your analyses guaranteeing a considerable advantage over the competition, since all the instruments and consumables to optimize the performance of the laboratory come from a single source.

VELP SOLUTIONS

ELEMENTAL ANALYZERS

KJELDAHL ANALYZERS

FIBER ANALYZERS

VELP ELEMENTAL ANALYZERS



ermes enabled

NDA 701 – Dumas Elemental Analyzer

- Nitrogen and Protein determination in 3-4 minutes
- Accurate and precise
- Versatile and cloud-enabled
- Helium as carrier gas



ermes enabled

NDA 702 – Dumas Elemental Analyzer

- Nitrogen and Protein determination in 3-4 minutes
- Accurate and precise
- Unmatched LOD of 0.001 mgN (with He as carrier gas)
- Versatile and cloud-enabled
- Helium and Argon as carrier gas



ermes enabled

CN 802 – Elemental Analyzer

- Carbon, Nitrogen, C:N ratio, TOC and TIC determination
- Fast analysis in 3-5 minutes
- Versatile and cloud-enabled
- Helium and Argon as carrier gas

VELP KJELDAHL ANALYZERS



UDK Series – Distillation Units

- Full range of distillers with different automation to match any lab requirement
- Exclusive patented titanium condenser and steam generator to maximize accuracy and efficiency
- Robust and chemical resistant



DKL Series – Automatic Kjeldahl Digesters

- Fully automatic digesters in 8, 12, 20 and 42 positions
- Stable and homogeneous temperature (± 0.5 °C)
- Fast, flexible and safe
- Robust and compact



DK Series – Kjeldahl Digesters

- Semi-automatic digester in 6, 8, 18, 20 and 42 positions
- Stable and homogeneous temperature (± 0.5 °C)
- Intuitive and easy-to-use

VELP FIBER ANALYZERS



ermes enabled

FIWE Advance – Automatic Fiber Analyzer

- Crude and detergent fiber determination to official standards (ISO and AOAC)
- Fully automatic solution with reagent loading and pre-set methods
- Safe, fast and cloud-enabled



FIWE – Fiber Analyzer

- Crude and detergent fiber determination to official standards (ISO and AOAC)
- Semi-automatic solution with timer and acoustic signal
- High reproducibility



COEX – Cold Extractor

- Preliminary defatting of the sample prior to fiber determination
- Reliable and easy-to-use



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