APPLICATION NOTE

F&F-D-002-2021/A1

N/Protein Determination in Fishmeal

Dumas and Kjeldahl method comparison

Kjeldahl reference: **AOAC 2001.11** Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds; **REG CE 152/2009; EN ISO 5983-2:2009** Animal feeding stuffs

Dumas reference: **AOAC 990.03** Protein (Crude) in Animal Feed — Combustion Method, **ISO 16634-1:2008** Oilseeds and animal feeding stuffs

Tested with **VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit** (Code S30100210) **UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler** (Code S30200160) and **VELP Scientifica NDA 702 Dual Carrier Gas Dumas Nitrogen Analyzer** (Code F30800080)

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N/PROTEIN DETERMINATION IN FISHMEAL
DUMAS AND KJELDAHL METHOD COMPARISON

Introduction

Fishmeal is obtained from small fish (used whole, including the entrails) or from the carcasses of large fish (salmon, trout, sturgeon, tuna), waste of the choices for baby food and for gastronomy.
The mass is then crushed to extract any fish oil, then the product is cooled, dehydrated and ground to obtain a powder.
Fishmeal is an important food used in animal husbandry and in aquaculture. It is also the main ingredient of the commonly marketed feed for aquarium fish.
Human nutrition also involves the consumption of fishmeal, but only top-quality parts of the fish are used and the finished product is free of additives and preservatives that prevent rancidity of fats (allowed only in feed for dogs and cats).
Both Kjeldahl and Dumas techniques are officially approved for the determination of the protein content in fishmeal.
Performances of VELP Kjeldahl system and Dumas unit were evaluated by participating in the **Proficiency Testing Program** organized by **BIPEA** (Bureau Interprofessionnel d'Etudes Analytiques).
The obtained results (as % Protein) were compared with the BIPEA assigned values.

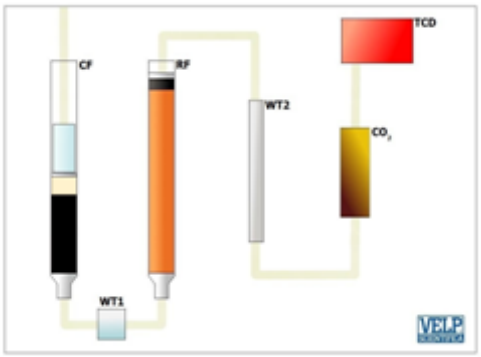
Protein Determination in BIPEA sample Fishmeal

This application note compares the nitrogen/protein determination in fishmeal by using **NDA 702 Dumas Nitrogen Analyzer** and **UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler**.

The specific methods used in this study are summarized briefly here.

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 - **DriStep™**), placed after the combustion, and a second chemical one (WT2). Between the two, the elemental substances passed through a reduction furnace (RF).
The auto-regenerative CO_2 absorbers (CO_2) let pass only the elemental nitrogen that is detected by the **LoGas™** innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.
The NDA 702 is controlled via PC through the intuitive **DUMASoft™**.



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.

Sample

BIPEA Fishmeal	ID: 11-0313-0217	
Dumas protein:	assigned value: 70.1	acceptability range: 68.0 -72.2
Kjeldahl protein:	assigned value: 69.0	acceptability range: 66.9- 71.1

Sample has been grinded by using a laboratory grinder (particle size 1 mm).

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Dumas analysis

NDA 702 Preliminary Operations (daily)

Follow the operating manual to start the NDA 702 and check that the following parameters are set:
Temperature Combustion reactor (Code A00000158): 1030 °C
Temperature Reduction reactor (Code A00000226): 650 °C
Flow rate MFC1 He: 190 ml/min
Flow rate MFC2 He: 220 ml/min
Condition the system by testing 2 EDTA standard (Code A00000149) and 3 to 5 empty tin foils (Code A00000153) as Check up. Verify the calibration curve with one or more tests as Standard by testing EDTA, used for the curve creation.

Sample Preparation

Weigh around 50 mg of sample in a tin foil directly on the analytical balance.
Close the tin foil, obtaining a capsule, and load it into the autosampler.

Analysis Procedure

Fill the following fields in the database of the software Dumasoft™: **Sample name, Weight, Method, Sample type, Calibration number**
The "FEED FOR ANIMALS, DRY" method shows the following parameters:
Protein factor: 6.25
O_2 flow rate: 400 ml/min **O_2 factor**: 1.6 ml/mg
Press to start the analysis.
Analysis time: from 3 minutes for one run.
Results have been obtained with the following calibration curve: in a range of 0 - 7 mg N with 7 measurements of EDTA standard (%N = 9.58) (Code A00000149). The data obtained are included in the tolerance admitted by the EDTA certificate.

Kjeldahl analysis

1. Sample Digestion

Weight about 1.0 grams in a nitrogen-free weighing boat (Code CM0486000) and transfer in a test tube.
In each test tube add:

- 2 catalyst tablets VCM (code A00000274; 3.5 g K_2SO_4, 0.1 g $CuSO_4$ $5H_20$ Missouri)
- 2 antifoam tablets VS (Code A00000283)
- 20 ml concentrated sulfuric acid (96-98%)
- 5 ml hydrogen peroxyde (H_2O_2)

Prepare some blanks with all chemicals and without sample.

Connect the Digestion Unit to a proper Aspiration Pump (JP code F30620198) and a Fume Neutralization System (SMS Scrubber code F307C0199) to neutralize the acid fumes created during digestion phase.
Digest the sample for 40 minutes at 300 °C plus 90 minutes at 420 °C, according to the method "animal feed" (n°7 on DKL 20).

2. Distillation and Titration

Let the test tubes cool down to 50-60 °C.
Condition the UDK 169 unit by performing the Automatic Check up in Menu-System and a Wash down.
Distill the samples selecting the predefined methods n°7.

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- H_2O (dilution water): 50 ml
- H_2SO_4 (0.2 N) as titrant solution
- NaOH (32%): 70 ml
- Protein factor: 6.25
- Vreceiver™ (A00000316): 30 ml

Distillation &Titration analysis time: from 4 minutes for one test.

Results on BIPEA sample Fishmeal

The table below shows the nitrogen/protein results, obtained by NDA702 Dumas unit and VELP Kjeldahl system.

Technique	Sample quantity (mg)	Nitrogen %	Protein %
Dumas	50.07	11.41	71.31
	50.04	11.36	70.97
	49.86	11.45	71.56
	Average ± SD%	**11.41 ± 0.05**	**71.28 ± 0.29**
	RSD% *	**0.41**	**0.41**
Technique	**Sample quantity (g)**	**Nitrogen %**	**Protein %**
Kjeldahl	1.0021	10.93	68.30
	1.0007	10.97	68.56
	1.0003	11.09	69.34
	Average ± SD%	**11.00 ± 0.08**	**68.73 ± 0.54**
	RSD% *	**0.79**	**0.79**
acceptability Dumas range: **68.0 - 72.2** % P			
acceptability Kjeldahl range: **66.9 - 71.1** % P			

Conclusions

The obtained values fell within the expected protein range indicated by **BIPEA**, demonstrating the high performance of both VELP Analytical Instruments, **Kjeldahl system** and **Dumas unit NDA 702**. Excellent repeatability is ensured with both techniques, as demonstrated by low RSD values.

NDA 702 Dumas combustion Elemental Analyzer with high productivity and non-stop performance, is indeed ideal for high throughput, being fully automated and requiring just 3-4 minutes per analysis.

VELP Kjeldahl system, using genuine **catalyst tablets KjTabs™**, is still a robust solution for protein determination in food and feed field.

The complete procedure was verified by using 5 ml of glycine standard solution (3%) containing 28 mg of nitrogen, as reference substance. The obtained recovery falls into the expected range: between 98% and 102%.

In conclusion both techniques are efficient and capable of analyzing the fishmeal sample with high accuracy and repeatability.

Discover all the VELP solutions for N/Protein Determination >

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