

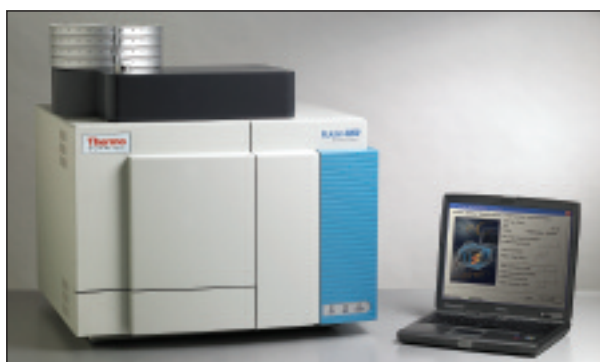
# Nitrogen/Protein determination in Animal Feed by the Thermo Scientific FLASH 4000 Series

Liliana Krotz and Guido Giazzi, Thermo Fisher Scientific, Milan, Italy

## Key Words

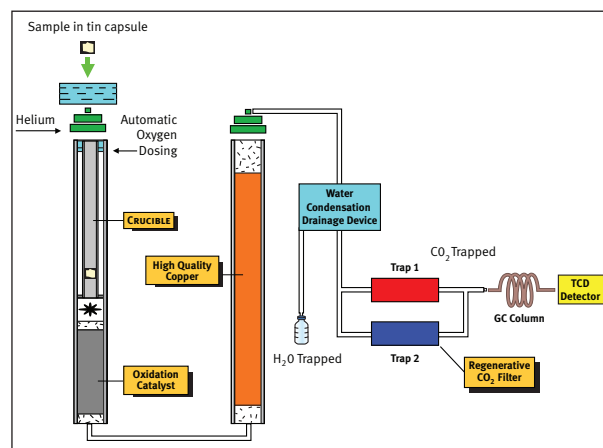
- Food Safety
- DDG
- Flash combustion
- Nitrogen/Protein

## Introduction



One of the most important nutrients in animal food is protein. Protein intake provides the building blocks needed by the animal to produce its own proteins in order to grow, maintain or produce muscles, enzymes, hormones, milk, wool, etc. The quantity of Protein required by animals is very precise as excess amounts of lead to amino acid deficiency and generates unnecessary amounts of energy. The precise and accurate determination of its amount, through the determination of Nitrogen, is fundamental to achieving high nutritional quality of finished animal products.

## Analytical Configuration



The capabilities of the combustion method for the determination of Nitrogen have been greatly improved to make analyses faster, safer and more reliable than the traditional Kjeldahl method. For this reason, the Dumas Combustion method has been approved and adopted by the Association of Official Analytical Chemists for this application (AOAC Official Method 990.03. Protein crude in Animal Feed 4.2.08).

The FLASH 4000 Nitrogen/Protein Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and high sample throughput.



## Analytical conditions

T left tube: 950°C  
T right tube: 840°C  
T oven: 50°C

Carrier Flow: 300 ml/min  
Reference Flow: 300 ml/min

Standard: 500 mg EDTA 9.59 %N  
EDTA: EthyleneDiamineTetraAcetic acid

Sample weight: 1 gram

*Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.*

## Official method requirements for Protein determination by Combustion

AOAC (Method 990.03) indicates that the suitable fineness of grind must be determined (for each different material analyzed) to achieve precision shown by an RSD of  $\leq 2\%$  for 10 successive determinations of Nitrogen. The fineness of 0.5 mm required to achieve this precision must be used for all mixed feeds and other non-homogenous materials.

## Results

Different animal feed products were chosen to demonstrate the suitability of the method for all ranges of Protein content without matrix effect and with reduced sample preparation. The Protein content is calculated automatically by the dedicated Thermo Scientific Eager Xperience software using a Protein factor of 6.25.

Table 1 shows the Nitrogen/Protein determination in **maize flour**, **wheat flour** and **wheat midd** samples. Samples were homogenized to particle size 2 mm.

**Table 1 – N/Protein determination in flours samples**

Maize flour			Wheat flour			Wheat midd		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
1006.1	1.274	7.963	1020.3	1.836	11.475	1001.3	2.737	17.109
1005.0	1.257	7.858	1016.3	1.856	11.600	1008.3	2.763	17.271
1005.8	1.288	8.053	1017.5	1.832	11.450	1002.1	2.759	17.242
1007.2	1.259	7.867	1008.4	1.841	11.506	999.3	2.776	17.347
1002.9	1.268	7.928	1009.8	1.844	11.525	1006.7	2.789	17.434
1004.1	1.287	8.043	1004.4	1.848	11.550	1004.5	2.769	17.309
996.4	1.285	8.032	1002.6	1.859	11.619	1015.3	2.776	17.349
994.8	1.267	7.918	1012.4	1.849	11.556	1003.5	2.722	17.015
1003.1	1.257	7.856	1000.6	1.857	11.606	1000.7	2.729	17.054
996.1	1.275	7.967	1007.3	1.848	11.550	1015.3	2.730	17.063
<b>Average</b>	<b>1.272</b>	<b>7.949</b>	<b>Average</b>	<b>1.844</b>	<b>11.525</b>	<b>Average</b>	<b>2.757</b>	<b>17.232</b>
<b>RSD %</b>	<b>0.959</b>	<b>0.959</b>	<b>RSD %</b>	<b>0.459</b>	<b>0.459</b>	<b>RSD %</b>	<b>0.858</b>	<b>0.858</b>

Table 2 shows the analysis of 10 consecutive determinations of soya and sunflower samples homogenized to 2 mm particle size.

**Table 2 – N/Protein determination in soya and sunflower**

Soya			Sunflower		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
995.8	7.452	46.574	998.1	3.088	19.302
1000.4	7.464	46.647	694.9	3.092	19.328
999.5	7.451	46.569	991.5	3.041	19.008
1010.3	7.454	46.587	795.1	3.029	18.9300
1007.2	7.453	46.582	692.2	3.074	19.216
999.6	7.448	46.550	994.9	3.052	19.077
1005.7	7.461	46.631	802.4	3.066	19.161
108.8	7.443	46.518	996.5	3.077	19.233
1008.2	7.447	46.544	897.6	3.061	19.129
1002.1	7.469	46.681	1002.1	3.058	19.115
<b>Average</b>	<b>7.454</b>	<b>46.55</b>	<b>Average</b>	<b>3.064</b>	<b>19.150</b>
<b>RSD %</b>	<b>0.161</b>	<b>0.161</b>	<b>RSD %</b>	<b>0.655</b>	<b>0.655</b>

Table 3 shows the N/Protein data obtained from DDG, gluten and fish meal samples. DDG is the dried distilled grain fraction after removing ethyl alcohol from the yeast fermentation.

**Table 3 – N/Protein determination in gluten, DDG and fish meal**

DDG			Gluten			Fish meal		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
1010.5	5.298	33.112	1001.5	9.538	59.614	999.8	11.404	71.275
1002.4	5.324	33.277	1001.7	9.542	59.635	1007.3	11.396	71.226
1008.5	5.367	33.545	1009.7	9.621	60.134	1003.6	11.369	71.055
1009.2	5.345	33.407	1010.2	9.623	60.146	1010.2	11.416	71.350
1012.3	5.377	33.604	1012.3	9.544	59.651	998.9	11.357	70.978
1008.6	5.350	33.438	1011.8	9.549	59.683	1004.6	11.418	71.365
1001.4	5.326	33.290	1029.8	9.600	59.998	1000.3	11.418	71.362
1008.3	5.363	33.519	1015.7	9.560	59.748	1008.4	11.339	70.868
1012.2	5.336	33.353	1006.4	9.559	59.745	1008.2	11.277	70.483
1015.7	5.309	33.185	1008.8	9.587	59.916	997.5	11.321	70.755
<b>Average</b>	<b>5.340</b>	<b>33.373</b>	<b>Average</b>	<b>9.572</b>	<b>59.827</b>	<b>Average</b>	<b>11.372</b>	<b>71.072</b>
<b>RSD %</b>	<b>0.479</b>	<b>0.479</b>	<b>RSD %</b>	<b>0.343</b>	<b>0.343</b>	<b>RSD %</b>	<b>0.421</b>	<b>0.422</b>

## Conclusion

The data obtained of 10 consecutive determinations show an excellent reproducibility. In all cases the relative standard deviation was less than 2 %, according to the official method indicating that it is not necessary a fine homogenization and no memory effect was observed when changing the type of sample, indicating the complete detection of the Nitrogen present in the sample. This demonstrates that the Thermo Scientific FLASH 4000 Analyzer is able to analyze Nitrogen in a wide range from low to high content without matrix effect.

Note: Thanks to Cargill Animal Feed Division, Spessa(Pv), Italy, for the collaborative studies. Part of this application data was presented at PittCon 2009 as an oral lecture.

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