Nitrogen/Protein determination in animal feed by Dumas Method using argon as carrier gas

Liliana Krotz, Francesco Leone and Guido Giazzi, Thermo Fisher Scientific, Strada Rivoltana, 20090 Rodano (MI), Italy

ABSTRACT

Protein determination is used for the evaluation of animal feed product quality. Elemental Analysis is a powerful analytical technique performing nitrogen determination for the protein content and it is an alternative to the classical Kjeldahl method. This poster shows the performance of the Thermo Scientific Flash*Smart* Elemental Analyzer for Nitrogen/Protein determination of raw and final animal feed products using argon as carrier gas. It shows also the reproducibility of the results in comparison with the values obtained using helium as carrier gas.

INTRODUCTION

One of the most important nutrients in animal nutrition is protein. Protein intake provides the building blocks needed by animals to build their own body proteins, which enable them to grow. The protein needs are very specie/phase specific: it is important to avoid excess and deficiency of amino acids.

The accurate quantification of proteins, through the determination of nitrogen, enables to characterize the nutritional quality of animal products. Protein content is the basis for trade of feed and official regulations establish protein content and labeling requirements. For these reasons, the Dumas method (combustion method) for the determination of nitrogen is a more reliable technique than the traditional Kjeldahl method.

Combustion Dumas method has been approved and adopted by the Association of Official Analytical Chemists (AOAC Official Method 990.03. Protein crude in Animal Feed 4.2.08). The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, meets the official regulations requirements alongside with the needs of laboratories, in terms of accuracy, day by day reproducibility and high sample throughput.

On the Flash*Smart* EA helium is usually run as carrier gas. It ensures high sensitivity. Considering the need for cost efficiencies and the likely increase in helium gas cost, due to its possible shortage, an alternative for the carrier gas, is needed. Argon which is readily available can be used as alternative to helium in the FlashSmart EA Flash*Smart* EA.

Figure 1. Thermo Scientific FlashSmart Elemental Analyzer.

METHOD

The Flash*Smart* EA operates according to the dynamic flash combustion of the sample. Samples are weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen. After combustion, the produced gases are carried by an argon flow to a second reactor filled with copper, then swept through CO_2 and H_2O traps, a GC column. Finally they are detected by a Thermal Conductivity Detector (TCD) (Figure 2).

A complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis. The dedicated software converts automatically the nitrogen content in protein content, by using a specific protein factor.

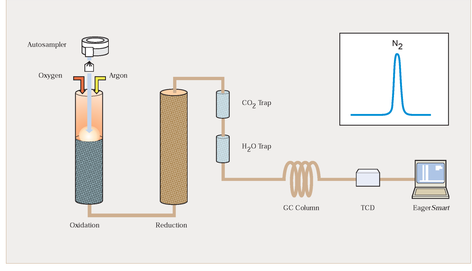

Figure 2. Nitrogen/Protein configuration.

RESULTS

To evaluate the performance of the system using argon as carrier gas, several reference materials as the Thermo Scientific Pasta Reference Material, were chosen. Instrument calibration was performed with about 50-70 mg of aspartic acid (10.52 N%) standard using K factor as calibration method. The protein factor 6.25 was used to calculate the protein content.

Table 1 shows the certified nitrogen values of the flours reference materials and Thermo Scientific Pasta Reference Material, the uncertainty declared by the supplier so as the accepted range according to the technical specification of the Flash*Smart* EA.

Table 2 shows the certified nitrogen values of BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, Paris, France) animal feed reference materials.

Table 3 shows the experimental data obtained using argon as carrier gas. Samples were analyzed in triplicate.

Table 1. Certified N% of Pasta and Flours Reference Materials.

Reference Material	N%	Uncertainty (±)
Thermo Scientific Pasta	2.227	0.097
Wheat Flour	1.36	0.25
Rice Flour	1.38	0.05
Barley Flour	1.90	0.04
Oatmeal	1.90	0.10
Soy Bean Meal	7.50	0.05

Table 2. Certified values of BIPEA Animal Feed Reference Materials.

BIPEA Ref.Mat.	Moisture	Fat	Carbo-hydrate	Kjeldahl Protein		Combustion Protein	
	%	%	%	Av.%	Tol.	Av.%	Tol.
Feed for Sow	9.8	2.8	48.7	16.0	0.6	16.2	0.6
Dehydrated Alfalfa	7.7	-	29.3	14.8	0.6	15.1	0.6
Hyperproteic Powder	-	0.8	-	85.4	3.4	86.4	3.5

Table 3. Experimental data of Reference Materials using argon carrier gas.

Reference Material	Weight (mg)	N%	RSD%	Protein %	RSD%
Thermo Scientific Pasta	150-160	2.22 2.21 2.22	0.26	13.87 13.81 13.87	0.26
Wheat Flour	140-150	1.37 1.36 1.35	0.74	8.57 8.51 8.41	0.95
Rice Flour	140-150	1.40 1.38 1.39	0.72	8.73 8.65 8.69	0.46
Barley Flour	140-150	1.90 1.92 1.92	0.60	11.90 11.97 12.01	0.47
Oatmeal	140-150	1.88 1.88 1.86	0.62	11.76 11.73 11.61	0.68
Soy Bean Meal	140-150	7.52 7.51 7.51	0.07	46.98 46.93 46.93	0.06
Feed for Sow	100-120	2.60 2.63 2.61	0.58	16.22 16.41 16.32	0.58
Dehydrated Alfalfa	100-140	2.37 2.38 2.37	0.24	14.81 14.87 14.79	0.28
Hyperproteic Powder	100-120	13.69 13.69 13.67	0.08	85.57 85.55 85.43	0.09

Table 4 shows the Nitrogen/Protein data of different raw and final animal feed samples analyzed in triplicate using argon and helium carrier gas. The protein factor used to calculate the protein content was 6.25. No noteworthy difference was observed.

Table 4. Reproducibility of N/Protein data using argon and helium as carrier gas.

Sample Name	Argon carrier gas					Helium carrier gas				
	W (mg)	N %	RSD %	Prot. %	RSD %	W (mg)	N %	RSD %	Prot. %	RSD %
Wheat Flour	130-140	1.91 1.93 1.94	0.79	11.93 12.05 12.14	0.87	160-200	1.92 1.93 1.90	0.79	11.98 12.06 11.88	0.75
Mais 1	130-140	1.25 1.25 1.26	0.46	7.84 7.83 7.87	0.27	150-200	1.26 1.26 1.27	0.46	7.88 7.93 7.91	0.32
Mais 2	130-140	1.48 1.49 1.49	0.39	9.24 9.29 9.29	0.31	150-200	1.50 1.50 1.49	0.38	9.41 9.39 9.34	0.38
Soy	130-140	8.11 8.08 8.00	0.71	50.66 50.51 49.99	0.70	170-180	7.98 8.03 8.01	0.31	49.87 50.21 50.06	0.34
Soy Meal 1	130-140	7.14 7.15 7.14	0.08	44.63 44.70 44.62	0.10	230-240	7.14 7.11 7.10	0.29	44.61 44.44 44.39	0.26
Soy Meal 2	130-140	8.18 8.19 8.17	0.12	51.11 51.17 51.03	0.14	230-280	8.16 8.19 8.18	0.19	50.99 51.19 51.11	0.20
Corn Distiller	138-139	4.89 4.89 4.90	0.12	30.55 30.55 30.63	0.15	210-215	4.87 4.87 4.86	0.12	30.44 30.46 30.38	0.14
Mix Distiller	137-140	4.98 4.98 4.96	0.23	31.11 31.14 31.02	0.20	240-245	4.98 5.00 4.99	0.20	31.15 31.27 31.19	0.20
DDGS*	120-130	4.79 4.78 4.83	0.55	29.92 29.85 30.21	0.64	150-160	4.79 4.80 4.77	0.31	29.92 30.03 29.80	0.38
Sunflower 1	100-120	6.02 6.08 6.09	0.62	37.62 38.01 38.08	0.65	170-200	6.10 6.07 6.09	0.25	38.15 37.91 38.07	0.31
Sunflower 2	130-140	5.69 5.75 5.71	0.53	35.54 35.95 35.68	0.58	170-200	5.65 5.61 5.64	0.37	35.31 35.05 35.22	0.37
Gluten 1	130-140	9.31 9.27 9.28	0.22	58.20 57.92 58.02	0.24	170-180	9.34 9.33 9.31	0.16	58.36 58.33 58.21	0.14
Gluten 2	130-140	9.31 9.35 9.28	0.38	58.18 58.44 57.98	0.40	170-180	9.32 9.37 9.36	0.28	58.28 58.55 58.50	0.25
Poultry Feed 1	120-140	2.91 2.98 2.96	1.22	18.18 18.62 18.48	1.22	150-160	2.95 2.96 2.90	1.09	18.43 18.53 18.13	1.13
Poultry Feed 2	130-140	4.31 4.39 4.30	1.14	26.96 27.43 26.87	1.11	150-160	4.37 4.35 4.42	0.82	27.30 27.17 27.65	0.91
Forage	130-132	1.10 1.11 1.09	0.91	6.85 6.91 6.79	0.88	190-200	1.09 1.08 1.09	0.53	6.80 6.77 6.81	0.31

*DDGS: Dried Distillers Grains with Solubles

CONCLUSIONS

For the N/Protein determination, the application showed that the Dumas Method meets manufacturers and laboratories requirements, including the compliance to official methods.
The Thermo Scientific Flash*Smart* Elemental Analyzer, based on the combustion method (Dumas), offers advantages over the Kjeldahl Method for the N/Protein determination in terms of automation, ease of use and cost per sample.
The Flash*Smart* Elemental Analyzer, using argon as carrier gas, enables to analyze nitrogen in a wide range from low to high content without matrix effect. The nitrogen data obtained fall within the tolerance declared in the certificates and the RSD% obtained was less than 2% of the performance requirements of the Official Methods.

The Dumas Combustion method has been approved and adopted by Official Organizations such as ASBC, AOAC, AACC, AOCS, IDF, IFFO and ISO (Table 5).

Table 5. Most relevant Official Methods (partial list).

Official Association	Official Method
AACC (American Association of Cereal Chemists)	Crude Protein in Cereal, 46-30, 1999.
AOAC (Association of Official Analytical Chemists)	Official Method 990.03. Protein (crude) in Animal Feed 4.2.08
AOAC (Association of Official Analytical Chemists)	Official Method 992.15. Crude Protein in Meat and Meat Products including Pet Foods 39.1.16
AOAC (Association of Official Analytical Chemists)	Official Method 992.23. Crude Protein in Cereal Grains and Oilseeds 32.2.02
AOCS (American Oil Chemists Society)	Official Method Ba 4e-93 (revised 1995). Combustion method for Crude Protein determination.
ASBC (American Society of Brewing Chemists)	Official Method 1996. Nitrogen determination in Barley.
ASBC (American Society of Brewing Chemists)	Total Nitrogen in Wort and Beer by combustion method. Report of subcommittee, 1994
Office International de la Vigne et du Vin	Resolution OENO 13/2002 Quantification de l'Azote Total selon la methode de Dumas (Mouts et Vins (Quantification of Total Nitrogen by Dumas Method (must and wines)
IFFO (International Fishmeal and Fish Oil Organization Ltd.)	Nitrogen determination in fish meal by combustion method.
ISO 14891 (International Organization for Standarization) FIL 185 (International Dairy Federation)	Nitrogen determination in dairy products by combustion method.

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