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APPLICATION NOTE 42200

Nitrogen/Protein determination in animal feed by the Thermo Scientific Flash*Smart* Elemental Analyzer using argon as carrier gas

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Keywords

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Goal

This application note presents data on Nitrogen/Protein determination in reference materials, raw and final animal products with different nitrogen concentrations to show the performance of the system using argon as an alternative carrier gas and show the reproducibility of the results obtained.

Introduction

One of the most important nutrients in animal nutrition is protein. The intake provides with resource for energy, growth and development of muscles, hormones, milk, wool etc.

Protein is a compound of amino acids. The body cannot create essential amino acids and must therefore be taken through food. Essential amino acids differ between species, and as such the level of proteins/amino acids in animal food must exist in specific and correct levels to achieve the nutritional quality of the finished product. They must provide a balanced level of protein to prevent excess or avoid a deficiency in amino acids. This can be monitored through the precise and accurate determination of the levels of nitrogen in the product.

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For this reason, modern advances in instrumentation for the determination of nitrogen have greatly improved the capabilities of the combustion method, making it faster, safer and more reliable than the traditional Kjeldahl method. The Dumas (combustion) 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 supply of helium over the last few years has been beset with problems, namely world-wide shortages and subsequent price increases. It has therefore been necessary to test an alternative gas, argon, which is readily available.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with a wide array of laboratory requirements, such as accuracy, day by day reproducibility and high sample throughput.

Figure 1. Thermo Scientific Flash*Smart* Elemental Analyzer.

Methods

Samples are weighed in tin capsules and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen. After combustion, the resultant 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 and finally detected by a Thermal Conductivity Detector (TCD). The analytical configuration as well as the TCD Detector are the same as those used with helium as the carrier gas (see 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 Eager*Smart* Data Handling Software provides a new option AGO (Argon Gas Option) through which modifies the argon carrier flow during the run to optimize the analysis.

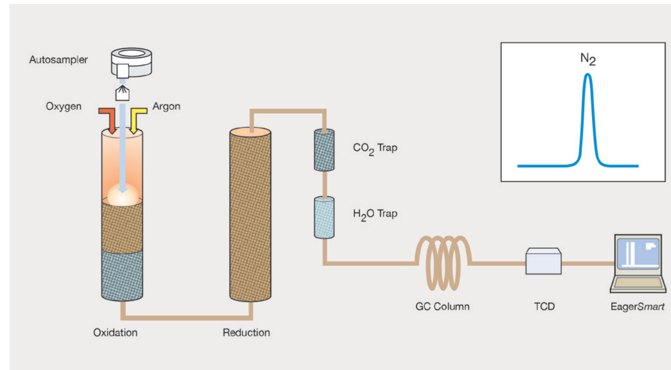

Figure 2. Nitrogen configuration.

Analytical Conditions	
Combustion Furnace Temperature	950 °C
Reduction Furnace Temperature	840 °C
Oven Temperature	50 °C (GC column inside the oven)
Argon Carrier Flow	60 ml/min
Argon Reference Flow	60 ml/min
Oxygen Flow	300 ml/min
Sample Delay	10 sec
Run Time	10 mins

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune function of the Eager*Smart* Data Handling Software.

Official methods requirements for Protein determination by combustion

AOAC (Method 990.03) indicates that a suitable fineness of grind must be determined (for each different material analyzed) to achieve precision that gives RSD of ≤ 2% for 10 successive determinations of nitrogen.

Results

Different reference materials, raw and final animal feed products were chosen to demonstrate the availability of the method for all range of protein content without matrix effect.

Tables 1 and 2 show the sample information and the reproducibility of 10 consecutive analyses of BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, France) Reference Materials using a sample weight of approximately 100–120 mg for feed for sow and hyperproteic powder, and 100–140 mg for alfalfa. The materials were characterized through a laboratory intercomparison using Kjeldahl and Combustion methods.

The protein factor used to calculate the protein content was the default value 6.25 present in the Eager*Smart* Data Handling Software. Instrument calibration was performed with aspartic acid (10.52 %N) standard using K factor as calibration method. The data obtained are inside the tolerance declared in the relative certificates.

Table 1. BIPEA sample information.

Sample	Moisture	Fat	Carbohydrate	Kjeldahl protein		Combustion protein	
	%	%	%	Av. %	Tolerance	Av. %	Tolerance
BIPEA – feed for sow 3/2009	9.8	2.8	48.7	16.0	0.6	16.2	0.6
BIPEA – dehydrated alfalfa 3/2009	7.7	-	29.3	14.8	0.6	15.1	0.6
BIPEA – hyperproteic powder 1/2008	-	0.8	-	85.4	3.4	86.4	3.5

Table 2. Reproducibility of Nitrogen/Protein determination in BIPEA reference materials.

Sample	BIPEA – feed for sow		BIPEA – dehydrated alfalfa		BIPEA – hyperproteic powder	
%	N%	Protein %	N%	Protein %	N%	Protein %
	2.67	16.71	2.47	15.46	13.53	84.59
	2.67	16.67	2.49	15.57	13.56	84.74
	2.66	16.63	2.45	15.34	13.67	85.42
	2.60	16.22	2.41	15.04	13.62	85.10
	2.63	16.41	2.47	15.41	13.69	85.57
	2.67	14.72	2.48	15.49	13.66	85.36
	2.67	16.66	2.37	14.81	13.60	85.02
	2.67	16.71	2.44	15.27	13.63	85.21
	2.61	16.32	2.38	14.87	13.71	85.72
	2.65	16.55	2.37	14.79	13.67	85.43
Average	2.65	16.56	2.43	15.21	13.63	85.22
RSD%	2.65	1.09	1.93	1.97	0.42	0.42

Table 3 shows the Nitrogen/Protein data of different animal feed samples using argon and helium carrier gas including the range of weight used for analysis. The protein factor used was 6.25. No significative difference was observed.

Table 3. N/Protein data of animal feed samples.

Sample	Flash*Smart* EA using argon carrier gas					Flash*Smart* EA using helium carrier gas				
Name	**Weight (mg)**	**N%**	**RSD%**	**Protein %**	**RSD%**	**Weight (mg)**	**N%**	**RSD%**	**Protein %**	**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
Maize 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
Maize 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
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
Soya	130-150	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
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	130-140	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	120-140	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

Conclusions

Good repeatability, accuracy and precision was obtained with the Flash*Smart* EA using argon as carrier gas to analyze nitrogen in a wide range from low to high content without matrix effect.

The Nitrogen/Protein data obtained of the BIPEA Reference Materials fall within the tolerance declared in relative certificates and the RSD% obtained was less than 2% according to the Official Methods Performance Requirements.

No memory effect was observed when changing the type of sample, indicating complete combustion and detection of the element. Samples used were chosen for their high content of protein.

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