APPLICATION NOTE 42497

Elemental Analysis: Combustion (Dumas) method for Nitrogen/ Protein determination of animal feed reference materials

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Goal

To assess the performance of the Flash*Smart* EA using helium and argon as carrier gas in alternative to the Kjeldahl method of animal feed reference materials analysis.

Introduction

One of the most important nutrients in animal nutrition is protein. The intake of protein provides animals with resource for energy, growth of muscles, production of hormones, milk, wool etc. The protein content can be monitored through the precise and accurate determination of nitrogen levels in the animal feed product.

Traditionally, the Kjeldahl method is used. However, it presents some challenges in terms of automation and reproducibility. For this reason, the use of an automated analytical techniques allowing fast nitrogen analysis with an excellent reproducibility is required.

The capabilities of the Dumas method (combustion method) for the determination of nitrogen have been greatly improved to make it faster, safer and more reliable 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, based on the dynamic flash combustion of the sample, meets a wide array of requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. The Flash*Smart* EA uses helium as carrier gas, which 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 giving excellent performance of the Flash*Smart* EA.

Figure 1. Thermo Scientific Flash*Smart* Elemental Analyzer

Method

The Flash*Smart* EA operates according to the dynamic flash combustion of the sample. The sample is 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 a helium or 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) (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 controls all analytical parameters of the instrument including the oxygen flow and the timing of oxygen injection. It calculates automatically the amount of oxygen, relative to the sample matrix and sample weight, through the dedicated Thermo Scientific™ OxyTune Function ensuring the complete combustion of the sample. Through this optimization also decreases the cost per analysis by not wasting oxygen or consuming the copper unnecessarily. Figure 3 shows the OxyTune Categories. From the nitrogen data obtained and a protein factor (6.25 as default), the software allows the automatic calculation of the protein content.

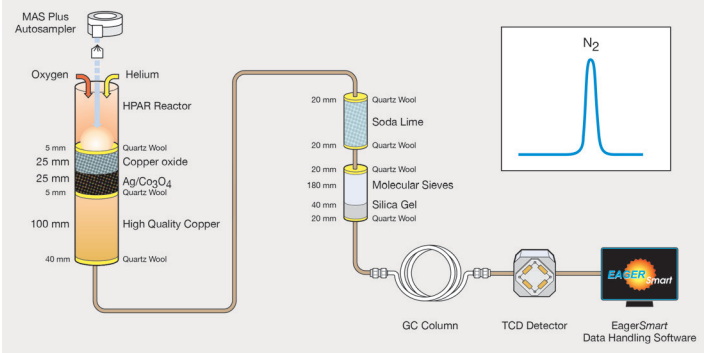

Figure 2. Nitrogen configuration

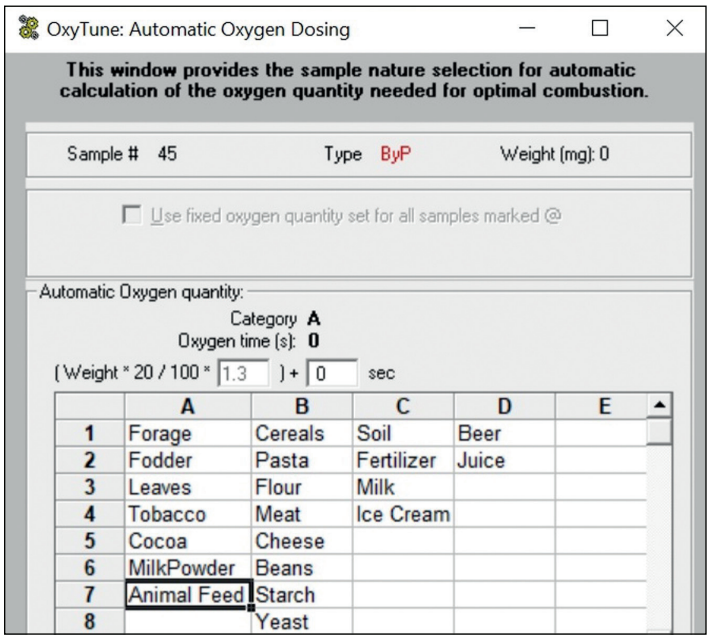

Figure 3. OxyTune Eager*Smart* Data Handling Software window

Results

The accuracy and precision of the Flash*Smart* EA was evaluated through the analysis of BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, France) Reference Materials. The materials were characterized through an intercomparison laboratory test using Kjeldahl and combustion methods. The results obtained were compared with the accepted range indicated in the relative reports of BIPEA.

For the intercomparison laboratory test, BIPEA considered the following Official Methods:

- **For Kjeldahl method:** NF EN ISO 5983-1: Determination of nitrogen content and calculation of crude protein content -Part 1: Kjeldahl method; NF EN ISO 5983-2: Determination of nitrogen content and calculation of crude protein content -Part 2: Block digestion and steam distillation method; REGULATION (EC) n° 152/2009 – Methods of sampling and analysis for the official control of feed.
- **For Dumas method:** NF EN ISO 16634-1: Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content -Part 1: Oilseeds and animal feeding stuffs.

Different reference materials were chosen to demonstrate the availability of the method for a large range of protein content without matrix effect. Samples were homogenized by a ball mill. Table 1 shows the BIPEA accepted range for both Kjeldahl and combustion method, and the protein average content of 10 consecutive analyses of BIPEA Reference Materials obtained with the Analyzer using helium as carrier gas.

Table 1. Protein data of BIPEA Reference Materials

Sample name	Protein % – BIPEA accepted range		Protein %
	Kjeldahl method	Combustion method	Flash*Smart* EA
Feed for sow	15.4–16.6	15.6–16.8	16.32
Hyperproteic powder	82.0–88.8	82.9–89.9	85.84
Laying hen feed 1	16.0–17.0	16.3–17.3	16.90
Laying hen feed 2	16.2–17.2	16.5–17.5	16.76
Fish meal	67.7–71.9	68.8–73.0	71.72
Feed for chicken 1	17.6–18.6	17.8–19.0	18.82
Feed for rabbit	14.7–15.7	15.1–16.1	15.71
Molasse	5.1–5.9	5.1–5.9	5.29
Cat food	33.0–35.0	33.3–35.3	35.07
Dehydrated alfalfa 1	14.4–15.2	14.6–15.6	14.90
Dehydrated alfalfa 2	14.2–15.4	14.5–15.7	15.17
Growing finishing pig meal	15.0–16.0	15.2–16.2	15.73
Horse beans	24.9–26.5	25.2–26.8	26.52
Triticale	9.7–10.5	9.7–10.5	10.29
Bran	15.5–16.5	15.8–16.8	16.37
Common wheat	12.2–13.0	12.3–13.1	12.59
Feed for piglet	18.0–19.2	18.2–19.4	19.12
Corn	6.1–6.9	6.1–6.9	6.75
Milk replacer for cow	20.7–21.9	21.1–22.5	21.91
Poultry meal	63.4–67.4	64.4–68.4	66.44

To demonstrate the performance of the Flash*Smart* Elemental Analyzer using argon as carrier gas, several BIPEA Reference Materials were analyzed for N/Protein determination. The instrument calibration was performed with aspartic acid or nicotinamide standard using K factor as calibration method. Table 2 shows the sample weight and the standard when helium or argon is used as carrier gas. Table 3 shows the N/Protein data obtained using helium and argon as carrier gas. Each sample was analyzed ten times. The data are comparable and the repeatability is more than acceptable giving in both cases a RSD% less than 2% as Official Methods requirements. Figure 4 shows the relationship of the protein content using helium and argon carrier gas, the good correlation obtained confirm the possibility to use argon as alternative carrier gas for N/Protein analysis.

Table 2. Sample weight and standard information

Sample name	Helium carrier gas		Argon carrier gas	
	Weight (mg)	Calibration standard	Weight (mg)	Calibration standard
Feed for sow	200–300	50–90 mg aspartic acid	125–135	50–70 mg aspartic acid
Hyperproteic powder	200–250	90–100 mg nicotinamide	100–120	50–60 mg nicotinamide
Laying hen feed 1	200–300	50–90 mg aspartic acid	125–135	50–70 mg aspartic acid
Laying hen feed 2	200–300	50–90 mg aspartic acid	125–135	50–70 mg aspartic acid
Fish meal	200–250	90–100 mg nicotinamide	120–140	50–60 mg nicotinamide
Feed for rabbit	200–210	60–70 mg aspartic acid	125–145	50–60 mg aspartic acid
Molasse	90–100	50–60 mg aspartic acid	90–100	50–60 mg aspartic acid
Dehydrated alfalfa 1	190–200	50–70 mg aspartic acid	120–130	50–70 mg aspartic acid
Dehydrated alfalfa 2	190–200	50–70 mg aspartic acid	120–130	50–70 mg aspartic acid
Growing finishing pig meal	190–200	50–70 mg aspartic acid	100–110	50–70 mg aspartic acid
Milk replacer for cow	120–140	50–70 mg aspartic acid	120–140	50–70 mg aspartic acid

Table 3. N/Protein data of BIPEA Reference Materials using helium and argon carrier gas

Sample name	Helium carrier gas				Argon carrier gas			
	N%	RSD%	Protein %	RSD%	N%	RSD%	Protein %	RSD%
Molasse	0.85	0.83	5.29	0.94	0.84	0.99	5.28	0.94
Dehydrated alfalfa 1	2.38	0.79	14.90	0.75	2.40	0.87	15.01	0.90
Dehydrated alfalfa 2	2.42	0.29	15.17	0.37	2.43	0.55	15.21	0.55
Feed for rabbit	2.51	0.80	15.71	0.84	2.51	1.14	15.71	1.16
Growing finishing pig meal	2.52	0.73	15.73	0.66	2.51	1.15	15.69	1.17
Feed for sow	2.61	0.33	16.32	0.30	2.65	0.50	16.56	0.50
Laying hen feed 2	2.68	0.86	16.76	0.81	2.68	0.88	16.72	0.82
Laying hen feed 1	2.70	0.43	16.90	0.40	2.71	0.92	16.93	0.91
Milk replacer for cow	3.50	0.79	21.91	0.76	3.51	0.71	21.95	0.76
Fish meal	11.48	0.16	71.72	0.15	11.50	0.40	71.89	0.40
Hyperproteic powder	13.73	0.20	85.84	0.20	13.63	0.42	85.22	0.42

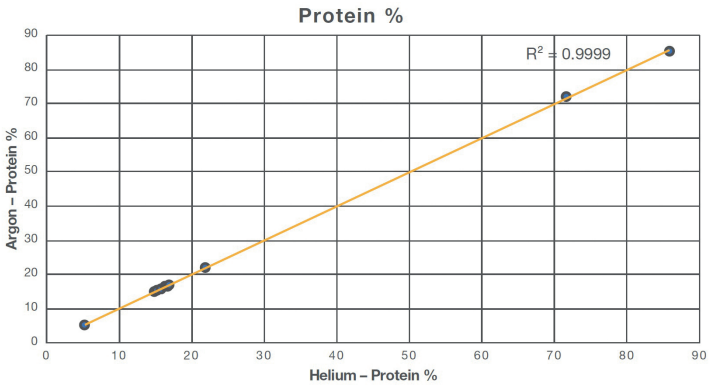

Figure 4. Correlation of protein content

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, easy to use and cost per sample.

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

Good repeatability, accuracy and precision was obtained with the Flash*Smart* EA using helium and argon carrier gas to analyze nitrogen in a wide range from low to high content.

No memory effect was observed when changing the type of sample, indicating complete combustion and detection of the element.

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

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