Application Note

Instrument: FP828

Determination of Nitrogen/Protein in Feeds, Grains, and Pet Food

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Introduction

Protein is one of the most significant nutrient components in animal feed products. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value of feed materials, but is also key to the economic value of these products. Protein in feed products is most commonly calculated using the measured nitrogen in the sample and a multiplier or conversion/protein factor (protein factors vary according to the sample matrix).

The LECO® FP828 is a combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a vertical quartz furnace. A thermoelectric cooler removes moisture from the combustion gases before they are collected in a ballast. The gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the combustion gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot gas is carried to a thermal conductivity cell (TC) for the detection of nitrogen (N_2).

Instrument Model and Configuration

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analyte gas compared to a reference/carrier gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater sensitivity of the detector. The FP828 supports either the use of helium or argon as the instrument's carrier gas. When used as a carrier gas, helium provides the highest sensitivity, and the best performance at the lower limit of the nitrogen range. The thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, therefore the detector is inherently less sensitive when using argon as a carrier gas.

Note: When changing carrier gas type, refer to the FP828 Operator's Instruction Manual for the procedure on setting the gas flow rate.

Method Reference

AOAC 990.03 - Protein (Crude) in Animal Feed, Combustion Method.

Sample Preparation

Samples must be of a uniform consistency to produce suitable results. Prior to analysis, feed samples can be ground to improve homogeneity and reduce bias from retained moisture. Reference materials should be prepared as directed by the certificate, prior to analysis.

Note: Nitrogen results for feed samples are typically reported on a dry basis in order to avoid a reporting bias due to fluctuations in moisture levels. Therefore, either the material can be dried prior to analysis, or the moisture content can be determined and entered into the software to correct for moisture. Feed samples should be dried in accordance with AOAC 930.15 - Loss on Drying (Moisture) for Feeds. The dried samples should be stored in a desiccator prior to use (within 24 hours).

Accessories

502-397 Large Tin Foil Cups, and 501-614 Spatula

Reference Materials

LCRM®, LRM®, NIST, or other suitable reference materials.

Method Parameters*

Gas Type	Helium or Argon
Furnace Temperature	950 °C
Afterburner Temperature	850 °C
Nominal Mass	1.0000 g
Purge Cycles	3
Ballast Equilibrate Time	10 s
Ballast Not Filled Timeout	300 s
Aliquot Loop Fill Pressure Drop	200 mm Hg
Aliquot Loop Equilibrate Time	6 s
Interleave Analysis	Yes
Sample Drop Detection	Disabled
Dose Loop Size**	3 cm^3

**Refer to FP828 Operator's Instruction Manual for Parameter definitions.*
*** The 3 cm^3 dose loop is recommended for this application.*

Element Parameters†

Parameter	*Helium*	*Argon*
Integration Delay	3 s	3 s
Starting Baseline	15 s	15 s
Post Baseline Delay	14 s	14 s
Use Comparator	No	No
Integration Time	35 s	60 s
Use Endline	Yes	Yes
Endline Delay	25 s	25 s
Ending Baseline	15 s	15 s
Use Profile Blank	---	Yes

†Refer to FP828 Operator's Instruction Manual for Parameter definitions.

Burn Profile

Base Model

Burn Step	Furnace Flow	Time
1	High	End

Performance Model

Burn Step	Furnace Flow	Time
1	5.0 L/min	End

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the System.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Initiate the analysis sequence.
   c. Set the blank following the procedure outlined in the operator's instruction manual.

   Note: The standard deviation of the last five blanks should be less than or equal to 0.001% (10 ppm) when utilizing Helium as a carrier gas, and less than or equal to 0.005% (50 ppm) when utilizing Argon as a carrier gas. Additional blanks beyond the recommended five may be required in order to achieve the recommended precision.
4. Calibrate/Drift Correct.
   a. Select the desired number of calibration/drift replicates in the Login screen (minimum of five).
   b. Weigh ~ 0.25 g of a suitable reference material into a 502-397 Large Tin Foil Cup and twist the top edges of the foil cup together to seal.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the tin foil cup containing the reference material to the appropriate position in the sample carousel.
   e. Perform steps 4b through 4d a minimum five times.
   f. Initiate the analysis sequence.
   g. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   h. Verify the calibration/drift correction by analyzing an appropriate mass of another/different suitable reference material and confirm that the results are within the acceptable tolerance range.

   Note: Typically, the LECO FP828 can be calibrated using several replicates of a single mass range (0.25 g) of EDTA or other suitable reference material utilizing a single standard, forced through origin calibration. This is a cost-effective and simple process. The calibration can be verified by analyzing a different compound such as Phenylalanine (~ 0.10 g). A multi-point calibration (fractional weight or multiple calibration samples) may be used to calibrate if desired.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Weigh ~ 0.25 g of the feed sample into a 502-397 Large Tin Foil Cup and twist the top edges of the foil cup together to seal.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the tin foil cup containing the sample to the appropriate position in the sample carousel.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.

TYPICAL RESULTS

Data was generated utilizing a single standard, forced through origin calibration using ~ 0.25 g of 502-896 EDTA LCRM Lot #1002 (9.57% N). The calibration was verified using ~ 0.10 g of 502-642 Phenylalanine LCRM Lot #1018 (8.47% N). Samples were analyzed as received and the nitrogen values were then corrected for moisture using the instrument's software. A protein factor of 6.25[††] was used for all samples to calculate the protein content.

	Helium			**Argon**		
	Mass (g)	*Nitrogen (%)*	*Protein (%)*	*Mass (g)*	*Nitrogen (%)*	*Protein (%)*
NIST 3290	0.2794	5.30	33.1	0.2483	5.30	33.1
Dry Cat Food	0.2603	5.26	32.9	0.2603	5.29	33.0
32.77 ± 0.30% Protein	0.2524	5.30	33.1	0.2458	5.26	32.9
	0.2577	5.29	33.0	0.2447	5.31	33.2
	0.2546	5.28	33.0	0.2468	5.28	33.0
	Avg =	***5.29***	***33.0***	***Avg =***	***5.29***	***33.0***
	s =	***0.02***	***0.1***	***s =***	***0.02***	***0.1***
Hay	0.2451	3.43	21.4	0.2529	3.41	21.3
	0.2529	3.43	21.5	0.2560	3.45	21.6
	0.2519	3.44	21.5	0.2534	3.43	21.4
	0.2528	3.43	21.5	0.2549	3.40	21.2
	0.2556	3.44	21.5	0.2524	3.42	21.4
	Avg =	***3.43***	***21.5***	***Avg =***	***3.42***	***21.4***
	s =	***0.01***	***0.03***	***s =***	***0.02***	***0.1***
Corn Silage	0.2522	1.10	6.90	0.2497	1.15	7.17
	0.2518	1.11	6.92	0.2492	1.14	7.11
	0.2494	1.10	6.87	0.2492	1.10	6.89
	0.2512	1.10	6.89	0.2475	1.16	7.23
	0.2503	1.09	6.83	0.2527	1.13	7.07
	Avg =	***1.10***	***6.88***	***Avg =***	***1.14***	***7.09***
	s =	***0.01***	***0.03***	***s =***	***0.02***	***0.13***
Distillers Grain	0.2596	5.24	32.8	0.2495	5.19	32.4
	0.2503	5.22	32.6	0.2511	5.17	32.3
	0.2544	5.22	32.6	0.2504	5.22	32.6
	0.2477	5.23	32.7	0.2506	5.23	32.7
	0.2564	5.20	32.5	0.2524	5.23	32.7
	Avg =	***5.22***	***32.6***	***Avg =***	***5.21***	***32.6***
	s =	***0.02***	***0.1***	***s =***	***0.03***	***0.2***
Feed	0.2569	3.87	24.2	0.2567	3.85	24.0
	0.2531	3.76	23.5	0.2479	3.93	24.6
	0.2551	3.89	24.3	0.2531	3.85	24.0
	0.2506	3.88	24.3	0.2470	3.78	23.6
	0.2507	3.84	24.0	0.2606	3.99	24.9
	Avg =	***3.85***	***24.0***	***Avg =***	***3.88***	***24.2***
	s =	***0.05***	***0.3***	***s =***	***0.08***	***0.5***
Corn Grain	0.2540	1.27	7.95	0.2545	1.31	8.16
	0.2557	1.27	7.95	0.2597	1.29	8.03
	0.2554	1.28	8.01	0.2530	1.27	7.92
	0.2479	1.27	7.94	0.2525	1.25	7.83
	0.2542	1.27	7.95	0.2502	1.28	7.99
	Avg =	***1.27***	***7.96***	***Avg =***	***1.28***	***7.99***
	s =	***< 0.01***	***0.03***	***s =***	***0.02***	***0.12***

[††]Protein factor was obtained from the United States Department of Agriculture, Circular No. 183. The choice of protein factor to be used for determining protein content in different materials is the subject of some debate. As a result, if being used for commerce, the value of this conversion factor should be part of the contractual agreement between buyer and seller.

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