Application Note

LEEMPOWERING RESULTS

## Instrument: FP928

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

*LECO Corporation; Saint Joseph, Michigan USA*

## 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 content in feed products is most commonly calculated using the measured nitrogen content in the sample and a multiplier or conversion/protein factor (protein factors vary according to the sample matrix).

## Instrument Model and Configuration

The LECO FP928 is a macro combustion Nitrogen/Protein determinator that utilizes a pure Oxygen environment in a high-temperature horizontal ceramic combustion furnace, using ceramic boats designed to handle macro sample masses (~1 g). 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 gas is extracted and introduced into a flowing stream of inert carrier gas (Helium or Argon) for analysis. The aliquot of gas is carried through a heated reduction tube, filled with copper, to convert Nitrogen Oxide combustion gas species ($NO_x$) to Nitrogen ($N_2$). The aliquot gas is then carried to a thermal conductivity cell (TC) for the detection of Nitrogen ($N_2$).

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 the sensitivity of the detector. The FP928 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.

The FP928 offers the additional advantage of utilizing either a 10 $cm^3$ aliquot loop or a 3 $cm^3$ aliquot loop within the instrument's gas collection and handling system. The 10 $cm^3$ aliquot loop optimizes the system for the lowest Nitrogen range and provides the best precision. The 3 $cm^3$ aliquot loop extends reagent life expectancy by approximately three-fold when compared to the 10 $cm^3$ aliquot loop, while providing the lowest cost-per-analysis with minimal impact on practical application performance for the determination of Nitrogen/Protein in feeds, grains and pet foods (see Typical Results section).

*Note: When changing carrier gas type, the flow needs to be adjusted following instructions provided in the 928 Series Operator's Instruction Manual. The aliquot loop size is changed by selecting the desired aliquot loop size in the software's Method Parameters.*

## Method Reference

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

## Sample Preparation

Samples must be of uniform consistency to produce suitable results. Prior to analysis, samples should be ground to improve homogeneity and reduce bias from retained moisture. Typically, samples should be ground to a fineness of <0.5 mm. Refer to AOAC 990.03 or AOCS Ba 4f for additional information regarding sample preparation. Reference materials should be prepared as directed by the certificate, prior to analysis.

*Note: Nitrogen results for feed, grain and pet food 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 material can be analyzed as received and the moisture content can be determined and entered into the software to correct for moisture. 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

528-203 Ceramic Combustion Boats, 611-844 Spatula Flat Spoon

## Reference Materials

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

## Method Parameters*

| | |
|---|---|
| Gas Type | Helium or Argon |
| Furnace Temperature | 1100 °C |
| Dehydration Time | 0 s |
| 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 | 4 s |
| Dose Loop Size | Large (10 $cm^3$)or Small (3 $cm^3$) |

## *Element Parameters**

| Parameter | Helium 10 $cm^3$ and 3 $cm^3$ | Argon 10 $cm^3$ and 3 $cm^3$ |
|---|---|---|
| Integration Delay | 0 s | 9 s |
| Starting Baseline | 10 s | 10 s |
| Post Baseline Delay | 20 s | 20 s |
| Use Comparator | No | No |
| Integration Time | 50 s | 61 s |
| Use Endline | Yes | Yes |
| Endline Delay | 30 s | 30 s |
| Ending Baseline | 5 s | 5 s |

**Refer to 928 Series Operator's Instruction Manual for Parameter definitions.*

## *Burn Profile*

| *Burn Step* | *Lance Flow* | *Furnace Flow* | *Time* |
|---|---|---|---|
| 1 | No | Yes | 5 s |
| 2 | Yes | Yes | 35 s |
| 3 | Yes | No | 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 (ceramic combustion boat is not required).
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Place 528-203 Ceramic Combustion Boats in the appropriate positions in the autoloader.
   c. Initiate the analysis sequence.
   d. 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 an appropriate mass of a suitable reference material into a 528-203 Ceramic Combustion Boat.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the ceramic combustion boat containing the reference material to the appropriate position in the autoloader.
   e. Perform steps 4b through 4d a minimum five times for each calibration/drift material used.
   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 by analyzing an appropriate mass of another suitable reference material and confirm that the results are within the acceptable tolerance range.

   *Note: Typically, the LECO FP928 can be calibrated utilizing several replicates of a single mass range (nominal 0.75 g) of EDTA utilizing a single standard calibration (linear, force through origin calibration). This is a cost effective and simple process. The calibration can be verified by analyzing different compounds such as nicotinic acid (0.15 to 0.35 g), or phenylalanine (0.15 to 0.5 g). A multi-point calibration (fractional masses 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 approximately 1.0 g of the sample material into a 528-203 Ceramic Combustion Boat.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the ceramic combustion boat containing the sample to the appropriate position in the autoloader.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.

   *Note: If soot (carbon black) is noticed in the primary filter (steel wool filter), reduce the sample mass to prevent soot build-up in this filter. Soot can be produced when larger masses of high fat samples are analyzed.*

## *TYPICAL RESULTS*

*Data was generated utilizing a linear, force through origin calibration using ~0.75 g of 502-896 (Lot 1003) EDTA LCRM (9.58% N). The calibration was verified using ~0.20 g of 502-642 (Lot 1019) Phenylalanine LCRM (8.46% N). Samples were analyzed as received and the Nitrogen values were corrected for moisture using the instrument's software. A protein factor of 6.25** was used for all samples to calculate the protein content.*

| | 10 $cm^3$ Helium | | | 3 $cm^3$ Helium | | | 10 $cm^3$ Argon | | | 3 $cm^3$ Argon | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mass (g) | % N | % Protein | Mass (g) | % N | % Protein | Mass (g) | % N | % Protein | Mass (g) | % N | % Protein |
| **Dry Cat Food** | 1.0143 | 5.29 | 33.0 | 1.0305 | 5.27 | 33.1 | 1.0449 | 5.29 | 33.1 | 1.0156 | 5.28 | 33.0 |
| **NIST 3290** | 1.0205 | 5.28 | 33.0 | 1.0330 | 5.29 | 33.1 | 1.0359 | 5.29 | 33.1 | 1.0279 | 5.29 | 33.1 |
| **32.77 ± 0.30 %** | 1.0307 | 5.29 | 33.1 | 1.0742 | 5.29 | 33.1 | 1.0079 | 5.29 | 33.0 | 1.0728 | 5.29 | 33.1 |
| **Protein** | 1.0447 | 5.27 | 32.9 | 1.0769 | 5.29 | 33.1 | 1.0781 | 5.29 | 33.1 | 1.0149 | 5.27 | 32.9 |
| | 1.0227 | 5.28 | 33.0 | 1.0559 | 5.29 | 33.1 | 1.0198 | 5.30 | 33.1 | 1.0845 | 5.28 | 33.0 |
| | ***Avg =*** | ***5.28*** | ***33.0*** | ***Avg =*** | ***5.29*** | ***33.1*** | ***Avg =*** | ***5.29*** | ***33.1*** | ***Avg =*** | ***5.28*** | ***33.0*** |
| | ***s =*** | ***0.01*** | ***< 0.1*** | ***s =*** | ***0.01*** | ***< 0.1*** | ***s =*** | ***< 0.01*** | ***< 0.1*** | ***s =*** | ***0.01*** | ***0.1*** |
| **Hay** | 1.0116 | 3.41 | 21.3 | 1.1800 | 3.42 | 21.4 | 1.0341 | 3.37 | 21.0 | 1.0039 | 3.41 | 21.3 |
| | 1.0090 | 3.43 | 21.4 | 1.0254 | 3.42 | 21.4 | 0.9959 | 3.39 | 21.2 | 1.0410 | 3.40 | 21.3 |
| | 1.0138 | 3.38 | 21.1 | 1.0211 | 3.43 | 21.4 | 1.0206 | 3.41 | 21.3 | 1.0251 | 3.41 | 21.3 |
| | 1.0319 | 3.42 | 21.4 | 1.0392 | 3.41 | 21.3 | 1.0179 | 3.39 | 21.2 | 1.0222 | 3.41 | 21.3 |
| | 1.0533 | 3.39 | 21.2 | 1.0321 | 3.43 | 21.4 | 1.0322 | 3.40 | 21.3 | 1.0531 | 3.41 | 21.3 |
| | ***Avg =*** | ***3.41*** | ***21.3*** | ***Avg =*** | ***3.42*** | ***21.4*** | ***Avg =*** | ***3.39*** | ***21.2*** | ***Avg =*** | ***3.41*** | ***21.3*** |
| | ***s =*** | ***0.02*** | ***0.1*** | ***s =*** | ***0.01*** | ***< 0.1*** | ***s =*** | ***0.02*** | ***0.1*** | ***s =*** | ***< 0.01*** | ***< 0.1*** |
| **Corn Silage** | 1.0058 | 1.13 | 7.06 | 1.0118 | 1.12 | 7.03 | 1.0125 | 1.12 | 6.98 | 1.0206 | 1.07 | 6.71 |
| | 1.0511 | 1.11 | 6.91 | 1.0229 | 1.12 | 7.00 | 1.0011 | 1.12 | 7.02 | 1.0100 | 1.11 | 6.94 |
| | 1.0196 | 1.12 | 7.00 | 1.0007 | 1.12 | 7.02 | 1.0434 | 1.10 | 6.88 | 1.0100 | 1.11 | 6.95 |
| | 1.0662 | 1.11 | 6.95 | 0.9983 | 1.13 | 7.04 | 1.0209 | 1.11 | 6.91 | 1.0856 | 1.10 | 6.84 |
| | 0.9932 | 1.09 | 6.79 | 1.0078 | 1.12 | 6.99 | 1.0148 | 1.10 | 6.88 | 1.0242 | 1.11 | 6.92 |
| | ***Avg =*** | ***1.11*** | ***6.94*** | ***Avg =*** | ***1.12*** | ***7.01*** | ***Avg =*** | ***1.11*** | ***6.93*** | ***Avg =*** | ***1.10*** | ***6.87*** |
| | ***s =*** | ***0.02*** | ***0.10*** | ***s =*** | ***< 0.01*** | ***0.02*** | ***s =*** | ***0.01*** | ***0.06*** | ***s =*** | ***0.02*** | ***0.10*** |
| **Distillers Grain** | 1.0305 | 5.20 | 32.5 | 1.0743 | 5.19 | 32.4 | 1.0481 | 5.21 | 32.6 | 1.0847 | 5.19 | 32.4 |
| | 1.0629 | 5.20 | 32.5 | 1.0381 | 5.20 | 32.5 | 1.0058 | 5.22 | 32.6 | 1.0833 | 5.06 | 31.6 |
| | 1.0225 | 5.21 | 32.6 | 1.0182 | 5.18 | 32.4 | 1.0089 | 5.21 | 32.6 | 1.0115 | 5.16 | 32.3 |
| | 1.0461 | 5.21 | 32.6 | 1.0723 | 5.18 | 32.4 | 1.0530 | 5.23 | 32.7 | 1.0168 | 5.18 | 32.4 |
| | 1.0499 | 5.20 | 32.5 | 1.0169 | 5.18 | 32.4 | 1.0206 | 5.22 | 32.6 | 1.0692 | 5.17 | 32.3 |
| | ***Avg =*** | ***5.20*** | ***32.5*** | ***Avg =*** | ***5.18*** | ***32.4*** | ***Avg =*** | ***5.22*** | ***32.6*** | ***Avg =*** | ***5.15*** | ***32.2*** |
| | ***s =*** | ***0.01*** | ***0.1*** | ***s =*** | ***0.01*** | ***0.1*** | ***s =*** | ***0.01*** | ***< 0.1*** | ***s =*** | ***0.05*** | ***0.3*** |
| **Feed** | 1.0205 | 3.86 | 24.1 | 1.0820 | 3.89 | 24.3 | 1.0793 | 3.92 | 24.5 | 1.0405 | 3.93 | 24.5 |
| | 1.0028 | 3.85 | 24.0 | 1.0489 | 3.75 | 23.4 | 1.0085 | 3.82 | 23.9 | 1.0816 | 3.88 | 24.3 |
| | 1.0523 | 3.89 | 24.3 | 1.0129 | 3.89 | 24.3 | 1.0519 | 3.88 | 24.3 | 1.0305 | 3.86 | 24.1 |
| | 1.0095 | 3.81 | 23.8 | 1.0178 | 3.89 | 24.3 | 1.0040 | 3.73 | 23.3 | 1.0009 | 3.70 | 23.1 |
| | 1.0580 | 3.87 | 24.2 | 1.0710 | 3.90 | 24.4 | 1.0304 | 3.87 | 24.2 | 1.0086 | 3.86 | 24.1 |
| | ***Avg =*** | ***3.85*** | ***24.1*** | ***Avg =*** | ***3.86*** | ***24.1*** | ***Avg =*** | ***3.84*** | ***24.0*** | ***Avg =*** | ***3.84*** | ***24.0*** |
| | ***s =*** | ***0.03*** | ***0.2*** | ***s =*** | ***0.07*** | ***0.4*** | ***s =*** | ***0.07*** | ***0.5*** | ***s =*** | ***0.09*** | ***0.5*** |
| **Corn Grain** | 1.0546 | 1.29 | 8.08 | 1.0460 | 1.31 | 8.20 | 1.0211 | 1.28 | 8.02 | 1.0223 | 1.30 | 8.10 |
| | 1.0974 | 1.29 | 8.09 | 1.0566 | 1.31 | 8.16 | 1.0329 | 1.29 | 8.09 | 1.0010 | 1.29 | 8.08 |
| | 1.0832 | 1.29 | 8.09 | 1.0254 | 1.30 | 8.13 | 1.0360 | 1.29 | 8.04 | 1.0201 | 1.26 | 7.89 |
| | 1.0504 | 1.30 | 8.10 | 1.0254 | 1.31 | 8.18 | 1.0819 | 1.30 | 8.11 | 1.0504 | 1.26 | 7.86 |
| | 1.0666 | 1.29 | 8.09 | 1.0744 | 1.30 | 8.14 | 1.0747 | 1.25 | 7.81 | 1.0820 | 1.27 | 7.95 |
| | ***Avg =*** | ***1.29*** | ***8.09*** | ***Avg =*** | ***1.31*** | ***8.16*** | ***Avg =*** | ***1.28*** | ***8.01*** | ***Avg =*** | ***1.28*** | ***7.98*** |
| | ***s =*** | ***< 0.01*** | ***0.01*** | ***s =*** | ***< 0.01*** | ***0.03*** | ***s =*** | ***0.02*** | ***0.12*** | ***s =*** | ***0.02*** | ***0.11*** |

***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.*


**LECO Corporation** | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
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