Texas Tech University

LECO

The procedures below are designed to determine the crude protein content of meat samples. **Warning: the sample boats are very hot, 1050°C.** Always wear gloves. LECO procedures can be used on either raw or cooked product.

LECO Procedure Set-up and Blanking

1. Turn Helium (He) and Oxygen (O_2) tank valves all of the way on. Do not move regulator valves.
2. Enter your first name on log-in menu and press **ENTER.** Machine conditions will appear.
3. Press **ESC** to exit this menu and enter the **MAIN MENU.**
4. Select **METHOD,** and choose the sample type (Meat) to run by highlighting your selection.
5. Press **ESC** to exit this menu and enter the **MAIN MENU.**
6. Press **MAINTENANCE** and then press **AMBIENT MONITOR.**
7. View the Ambient Monitor to assure all the parameters are checked. If any parameters are not in check then fix any problems before proceeding.
8. Press **ESC** to exit this menu and enter the **MAINTENANCE MENU.**
9. Then press **DIAGNOSTICS** and select **COMBUSTION LEAK CHECK** to run a Combustion Leak Check.
10. The difference between “system pressure” and “initial pressure” must be at least 80 mm. If the difference is less there may be a leak, and maintenance should be conducted.
11. Press **ESC** to exit this menu and enter the **MAIN MENU.**
12. You are about to “blank” the machine. This does not require any rack or boats but does require bottom container that catches the boats.
13. Select **ANALYZE** to begin the sampling process.
14. Select **ID code.** Using the arrow keys to move the highlighted bar, select “Blank” for ID and “Meat” as the method. Touch the **Exit** key.
15. Manually change the sample weight to 0.5g and then press **ENTER WEIGHT** 21 times. These samples are used to stabilize the machine, and calculate the blank.

16. Select **ANALYZE** to run the 21 blanks. Move the arrow to position 1 and select **ANALYZE.** The machine should start running blanks (this will take approximately 1hour).

17. Once the blanks are complete, check the standard deviation of the blanks. The standard deviation must be ~0.005.

18. Press **ESC** to exit this menu and enter the **MAIN MENU;** select **REPORTS** and then select **STATISTICS.**

19. When the **STATISTICS** page appears select at least the last five blanks by pressing **INCLUDE RESULTS,** then select **PROCESS RESULTS.** This page will display the standard deviation of the chosen results. If the standard deviation is > ~0.005 repeat steps 13 to 15 until the machine is stabilized.

20. Press **ESC** to exit this menu and enter the **MAIN MENU.**

21. Select **CALIBRATE.**

22. Select **BLANKS** and un-highlight **CARBON** and select **OK.**

23. Select **INCLUDE RESULTS** for the same samples that that standard deviation was determined by.

24. Select **PROCESS RESULTS** and select **OK** to save blank. Printer should print sheet with Nitrogen blank table. You are now done blanking and can calibrate (standardize) with the EDTA standard.

Calibration and Standardization

1. Press **ESC** to exit this menu and enter the **MAIN MENU** and select **ANALYZE.**

2. Perform scale validation. Select **ID code.** Using the arrow keys to move the highlighted bar, select “EDTA” for ID and “Meat” as the method. Touch the **EXIT** key.

3. Take the scale to zero. Run drift correction by weighing four (4) EDTA calibration standards (0.5 to 0.6g each) and placing each into a covered boat (lined with foil). Make sure to tare the covered boat on the scale. Add the EDTA and enter the weight. Select **PRINT** on the scale, and weight should be present on LECO screen. Make sure the sample is spread evenly in the boat, and load boat into rack (round end first). Do this process for each of the four standard samples (0.5 to 0.6 g each of EDTA in the ceramic boat, tared on the scale).

4. Load rack into the auto sampler.

5. Select **ANALYZE,** move the arrow to position 1, and select **ANALYZE.** Samples will run for approximately 15 to 20 minutes.

6. Press **ESC** from the **ANALYZE** menu to go back to the **MAIN MENU** and select **REPORTS** then select **STATISTICS.**

7. Highlight at least three (the 3 that are the closest) of the four EDTA samples by pressing **INCLUDE RESULTS,** and then press **PROCESS RESULTS.**

8. The standard deviation of the selected EDTA samples must be less ≤0.31. If it is >0.31 then steps 1-8 will need to be repeated until the standard deviation is ≤0.31.

9. Press **ESC** from the **STATISTICS** menu to go back to the **MAIN MENU** and select **CALIBRATE.**

10. Select **DRIFT CORRECTION**, un-highlight **CARBON** and select **OK,** then view the recently run 4 EDTA samples, which are the last three on the list.

11. Select **INCLUDE RESULT** three times to highlight the same three results that were used to determine the standard deviation.

12. Select **PROCESS RESULTS** and select **YES** to adjust factors, printer should print sheet with new factors.

13. Press **ESC** from **DRIFT CORRECTION** menu.

14. Make sure that the dump pan is free of used boats. Select **ANALYZE**. You are now ready to read samples.

Crude Protein Determinations in Meat

1. When weighing samples make sure samples that are out of the -80°C freezer do not begin to thaw. Samples may be held in the Large Lab Freezer while being used to reduce the chance of thaw.

2. Perform a scale check unless the same scale that was used to weight the EDTA samples is used and tare the scale to zero. Up to 49 different boats can be analyzed in one run. **Place the boat on the scale, tare the scale to zero, add 0.5 to 1.0 g of homogenized tissue and place the boat back on the scale.** Select **ID code.** Using the arrow keys to move the highlighted bar, select the last sample ID code (example 2-c), this will be the only thing besides EDTA, Halt, or Blank under the Meat method.

3. Touch **LOG-IN SAMPLE** to highlight box. Enter the sample ID# with the replicate designation on the LECO touch pad and press **ENTER.** Select **PRINT** on the scale, weight should be present on screen. Make sure the sample is spread evenly in the boat and load boat into rack (round end first). The RACK DOES NOT HAVE TO BE FULL TO RUN!

4. Each tissue sample should be run in quadruplicate. For each run, three validation samples (the validation samples can be EDTA or other samples with a known amount of crude protein or nitrogen) should be run at the beginning, middle and end of the run.

5. Once all boats are loaded, load rack into auto sampler. Make sure samples are loaded into the rack according to the proper order (from top to bottom, left to right).

6. Select all boats are loaded, load rack into auto sampler. Make sure samples are loaded into the rack according to the proper order (from top to bottom, left to right).

7. DO NOT REMOVE BOATS FROM THE DUMP PAN for at least 10 minutes after completion (they are over 1,000°C)!

8. After all samples run, press **ESC** to main menu and log-off.

9. Remove print-out from the printer; write the study number (if not printed on the top of the first page) on the top of the first sheet. Date and sign the last page. Place the print-out in the Intervet study notebook.

10. Turn off He and O2 bottles. The AIR SUPPLY MUST BE LEFT ON AT ALL TIMES.

Protein Data Entry and Calculations

1. Once raw data for crude protein determination is collected, date and sign the printouts. Verify the three validation (internal standard) samples are within ±2.5% of there respective nominal values. Document all three of the validation samples are acceptable. If one or more of the validation samples are found to be unacceptable (greater than ±2.5% of the approximate known value) all samples will have to be reanalyzed. Reasons for reanalyzing samples should be documented in the study notebook with the unaccepted data.

2. If all of the validations samples are acceptable, enter the data, including sample identification, into an Excel spreadsheet.

3. In Excel, determine the coefficient of variation (CV) for each set of quadruplicate samples:
    a. CV = [(standard deviation)/mean] X 100

4. Print, sign and date the Excel spreadsheet. Label it with the study number.

5. If a CV of ≥10% can be reduced to less than 10% be deleting one **(ONLY ONE)** data point per sample, then this new mean will be acceptable. In a new Excel spread sheet, delete one data point from replicates that have a CV of 10% or greater. If the CV of the three closest results is >10%, repeat the protein analysis procedure for this sample.

6. Print, sign and date this second spreadsheet. Label it with the study number.

7. Place Excel spreadsheets in the study notebook.

8. Once analyses are complete and the CV has been found to be acceptable for each sample, the quadruplicates (or triplicates) shall be averaged to form an average percent moisture for each individual carcass in a third spreadsheet.

9. Print, sign and date this third spreadsheet. Label it with the study number. Place with other study documentation.

Printer Malfunctions

1. If the printer malfunctions and the data can not be printed out, the data should be recorded on a Note to the Study File Form. Make sure to list why the data is being recorded on the Note to the Study File Form (e.g. Printer Malfunction), the date of the recording, the samples being recorded and signature of the person making the recording.

Routine Maintenance

1. Maintenance will be conducted according toe the Periodic Maintenance Schedule. Leak checks (helium, combustion chamber and ballast) should be conducted as indicated in the schedule. All maintenance should be documented and maintained with the study documentation in the study notebook.

Periodic Maintenance Schedule for Leco

Equipment and Material	Replacement and Cleaning Schedule	Inspection Schedule
Aliquot Dosing Valve	Cleaning as necessary depending on type of samples analyzed	Every 1000 samples
Ballast Test	Clean every 6 months	Every 1000 samples
Catalyst Heater Tube	Approximately every 500 samples or every 6 months, which ever comes first	
Boston Filter (Disposable Particle Filters)	Replace once per year or when the filter becomes discolored (not white)	Every 1000 samples
Fan Filters	Clean as necessary depending on condition of filter	Every Month
Helium Flow Reagent	Invert tube at approximately 50 samples, replace packing at approximately 100 samples (reset counters)	Every Day
Measure Flow Reagent	Invert tube at approximately 50 samples, replace packing at approximately 100 samples (reset counters)	Every Day
Precooler Block Cleaning	Clean every time the primary filter tube is repacked	
Primary Furnace Filter Tube	Replace as necessary; Pack with 1 ½ inches of glass wool, 8.5 g steal wool, and 1 ½ in glass wool	Every Day
Secondary Furnace Filter Tube	Replace as necessary; Pack with 5 inches (10 g) glass wool	Every Day
Screen Filters	Clean when reagents are replaced	
Touch-Screen	Clean as necessary depending on condition of	Every Day

	Touch-screen	
Helium Tank	Suggest to order new tank at 1000 psi and replace tank at approximately 200 psi	Every Day
Oxygen Tank	Suggest to order new tank at 1000 psi and replace tank at approximately 200 psi	Every Day
Leak Checks	Perform helium (run weekly), combustion chamber (run daily), and ballast (as needed) leak checks	Every Day