🌐 U Cinn - Meal Pattern Analysis Food Intake Procedure
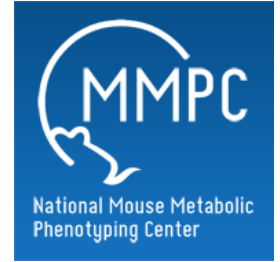
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Abstract

Summary:

Food Intake Monitoring - Oxymax/CLAMS monitors the cumulative amount of food eaten as well as the amount eaten in each bout with the use of a Mettler Toledo balance with a resolution of 0.01g. Feeders are designed to account for spillage of food and to prevent foraging, a combination which provides the most accurate food intake monitor on the market.

Food Access Control - Access to food can be placed under automatic computerized control. A pneumatic driven shield prevents access to the food, and can be controlled by times intervals and/or by mass of food. Additionally, this option also includes paradigms for Yolked or Paired feeding.

Center Feeder Cages: The Center Feeder Cage maintains the same sealed environment but adds a feeder to the center of the cage to allow food intake measurements. The Center Feeder is the most popular and universal in application, supporting all subject body types from lean to obese. An adjustable food guard allows mechanical fine tuning of food access to minimize foraging.

Materials

MATERIALS

⊗ Clorox Wipes **Office Depot Catalog** #821-808

⊗ Clean-up Clorox Spray **Office Depot Catalog** #115-581

⊗ Drierite 5lb 8 Mesh **Drierite Catalog** #23005

⊗ Cal Gas Mixed Gas 20.5% O2 5000ppm CO2 bal N2 Primary Standard **Praxair Catalog** #NI CDOXP27-Q

⊗ Electro Chemical Cell for O2 Analyzer **Columbus Instruments Catalog** #7260-D54

⊗ Oxymax Sample Max Channels 1-8 **Columbus Instruments Catalog** #090678-7-CH1-8

⊗ Oxymax Sample Max Channels 9-16 & reference **Columbus Instruments Catalog** #090678-8-CH9-17

⊗ Oxymax Flow Max Channels 1-8 **Columbus Instruments Catalog** #090678-5-CH1-8

⊗ Oxymax Flow Max Channels 9-16 **Columbus Instruments Catalog** #090678-6-CH9-16

⊗ Oxygen Sensor **Columbus Instruments Catalog** #090678-3-NHV1-WO

⊗ Carbon Dioxide Sensor **Columbus Instruments Catalog** #090678-4-PPS-WO

⊗ Sample Pump **Columbus Instruments Catalog** #090678-2-WO

⊗ Gas Sample Drier **Columbus Instruments Catalog** #090678-SD-WO

⊗ Fresh Air Pump **Columbus Instruments Catalog** #1108000802C

⊗ Tso's High Fat Butter (20%) w/green dye **Open Source Diets Catalog** #D03082706

⊗ 7012 Teklad LM-485 Mouse/Rat Sterilizable Diet **Envigo (Harlan) Catalog** #LM-485

1   **Meal Pattern Analysis Protocol** (Columbus Instruments)

1. Take Body Composition via QMR before you put animals in this experiment.

2. Set up cages (this can be done at any time before starting an experiment)
   a. Weigh animals and place into Food Intake chambers
   b. Supply food and water
     i. To fill food hoppers, turn the feeding tube upside down so that the metal mesh is at the bottom.

    ii. Fill feeding tube 90% full with diet.
    iii. Slide metal washer onto the post running down the center of the feed tube.

    iv. Using the red handled tool (Supplied with the Columbus Inst system), slide the larger "catch bin" onto the tool, then followed by the spring.

Red Handle Tool

Catch Bin

Spring

v. Position the tool over top of the center post of the feeding tube.

vi. Leaving the tool attached to the feeding tube, carefully slide the spring and catch bin over the feeding tube.

vii. Line up the slot holes in the catch bin with the slots in the feeding tubes. Push down and twist so that the feeding tube and catch bin are locked snuggly together.

viii. Place full feeding tube on top of each scale and place chamber over top.

Feeding Tube

c. Make sure chamber lids are clasped firmly and evenly

Ob/Ob Center Feeder Cage
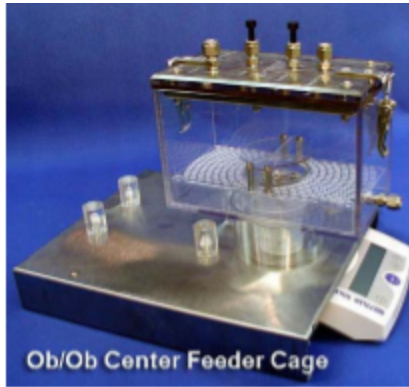
3. Check to see if Drierite and Lime Soda needs to be replaced (if Drierite is pink, replace with new; if Soda Lime is **not** white, replace with new.)

   a. To change Drierite or Soda Lime, remove canister from holder and unscrew the lid (you do not have to remove hoses from container).

   b. Remove spring, metal lid and cotton filter.

   c. Empty old Drierite or Soda Lime and replace with new (DO NOT FILL PAST THE TOP HOLE IN THE CANISTER).

   d. Tap bottom of canister to insure Drierite/Soda Lime is tamped down.

   e. Insert cotton filter, metal disc, spring, and then screw on the top (in that order).

   f. Push the canister back onto the holder

2   Running an Experiment

1. Turn on all of the surge protectors and make sure that the power lights indicate that all of the equipment is on. Make sure the PC is powered on and logged in.

2. Run "Oxymax for Windows" and select the appropriate hardware configuration.

   ♦ After selecting the appropriate hardware configuration, the Oxymax will initialize a system scan to determine if there are any errors with the equipment/hardware. If all is detected properly, the status window will automatically minimize itself. If error messages occur, check the hardware to make sure all is on and it is set up properly, then select "**Refresh**."

3. Allow approximately **2 hours** of warm up time before calibrating the system or starting an experiment. (pg.29 in User Manual)

3   Gas Sensor Calibration

Calibration must be performed before the start of EVERY new experiment. This can be done before or after creating a new experiment configuration. (Before: click "**Tools**>>**Calibration**"/ After: click "**Experiment**>>**Calibration**")

   *Calibration Gas Concentrations should consist of: **(Primary Standard Grade or better)**
   20.50% Oxygen
  0.50% Carbon Dioxide
  Balanced with Nitrogen

Before calibrating, make sure that the proper calibration gas concentrations are registered for the **ACTUAL** gases that will be used for calibrating. (The concentrations will be found next to the "Span" gases, the "Zero" gases should be left at 0)
♦ Click on the "**Cal Gas**" button next to the sensor group label. After making any changes select "**Apply**" before selecting "**Close**".
♦ Before calibrating any sensor, set the sample flow meter (on the front of the System Sample Pump) to about **400 mL/min** (the middle of the indicator ball should float on top of the line marked **.4 L/min)**
* ***DO NOT*** *re-adjust the sample flow meter before starting a new experiment. If flow sample flow meter is adjusted, recalibrate.*

When turning on the tank of calibration gas, set the outlet pressure on the tank regulator to read between **5-10 PSI**. Locate the calibration sample tube on the back of the sample pump. The tube should have a Tfitting with one end open. Insert the tube from the cal gas tank and insert it into the open T-fitting end. There should be a noticeable flow of gas from the exhaust tube. (It is better to have the gas pressure too high than too low for proper calibration)

After the system has warmed up, click the **"Calibrate"** buttons under either the "O2" or "CO2" to initiate the calibration procedure.

4   **Oxygen Sensor Calibration**
1. Click the "**Calibrate**" button under the "O2" header to open the oxygen sensor calibration.

2. Make sure that the sample flow is at about **400 mL/min**.

3. Turn on the cal gas and adjust pressure so that excess gas is apparent at the exhaust tube (5-10psi)

4. Click "**NEXT**" to begin purging the sensor with the cal gas.

5. When purging is complete, the prompt will display the target O2 concentration for the cal gas.
   ♦ Use the "Course Gain" and "Fine Gain" knobs on the O2 sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target concentration. The values will turn **_green_** when the target value is reached. (_There will be a lag time when adjusting the knobs so be sure to wait a few minutes before proceeding_)

6. After the measured value matches the target concentration, click "**Next**" to display the finalization instructions.

7. Click "**Finish**" and immediately proceed to calibrating the CO2 sensor.

5   **Carbon Dioxide Sensor Calibration**
1. Click the "**Calibrate**" button under the "CO2" header to open the carbon dioxide sensor calibration.

2. Make sure cal gas is turned on and check to see that the column of Soda Lime is **WHITE**. (if Soda Lime is not white, exchange old Soda Lime with new) The Soda Lime is used for the "CO2 Zero" calibration

3. Click "**NEXT**" to begin purging the sensor with the cal gas.
   ♦ The system will measure the "Zero" point of the CO2 sensor (the "Ready" light will turn off for a few seconds which is normal)

4. After the "Zero" has been established, the system will begin purging the "Span" gas.

5. When purging is complete, the system will measure the gain necessary to calibrate the sensor.
   ♦ Oxymax will display a prompt listing the calculated offset and gain for the CO2 sensor.

6. Turn off the flow of cal gas and click "**Finish**" to commit the calibration to memory.

<center>**＊DO NOT RECALIBRATE BETWEEN FED AND FASTING CYCLES**</center>

6   **Starting an Experiment**
It is recommended that all hardware errors have been resolved before starting an experiment.

Configuring Experiment
1. Select an experiment configuration template; "**File>>Open Experiment Configuration**" (Select a mouse configuration for mice and a rat configuration for rats)

2. Fill out the subject roster; "**Experiment>>Setup**"

   ♦ Right click the chamber ID to enable/disable the chambers in the system when disabled, the Group/Cage ID should look like
~~0109~~

   ♦ Enter in the animals body weights

   ♦ Set the fresh air flow; animals sharing a flow controller (chambers1-8 and chambers 9-16) will have identical flows

     o Mice: 0.50 LPM for ~20g animals; 0.60 LPM for ~30g animals

     o Rats; 2.0 LPM for ~200g animals; 3.5 LPM for ~600g animals

   ♦ Enter in any other information in the "Label=Information" field o Gender, color, genotype, age, treatment, etc

   ♦ Choose a location on the hard disk to store the experiment data "Data Filename"

3. Verify experiment settings; "**Experiment>>Properties**"

     o Intervals- The number of intervals to be recorded. Set to zero (0) if you want experiment to run indefinitely.

     o Cage/Reference Settle- The amount of time the system waits before taking another sample

      (both should be set at **90**)

     o Cage/Reference Measure: The amount of time the system measures the sample (both should be set at **30**)

   ♦ Choose reference method based on the number of cages. o If room air is not stable, more references are required.

   ♦ The Volume Rate Units should be set at **mL/kg/min**

   ♦ Select the "Feeding" tab at the top of the page and select the amount of time you would like the program to analyze the feeding bouts. (ex: If you select 1 minute feeding bouts, the program will generate data every minute while the experiment is in progress. Select 5 Minutes and the program will generate readings every 5 minutes.)

Run Experiment

1. Select "**Experiment>>Run**"

2. Monitor first few readings to verify system is working properly

   ♦ "O2in" should be around 20.94%

   ♦ "CO2in" should be around 0.04%

   ♦ RER should be between **0.7** and **1.0**

     o　0.7 = fat being burned as energy

     o　1.0 = carbohydrates being burned as energy

3. Right click on the tabs at the top of the screen (i.e.: VO2, VCO2, RER, Heat, etc...) and set the furthest decimal place (0.00000)

Stop Experiment

1. Select "**Experiment>>Stop**"

2. Fresh air will continued to be delivered to the chambers while software is running.

3. When exiting out of the Oxymax program, it will ask if you would like to turn off the fresh air. If all animals are out of the chambers, select "**Yes**," otherwise keep fresh air flowing so that the animals do not suffocate.

Exporting/Generating Data

1. Open the desired experiment data file

♦ The data generated from a just-finished experiment is still open after stopping it.

♦To open a previously saved experiment, click "**File>>Open Experiment Data**" and select desired .CDTA file.

2. Click "**File>>Generate Subject CSVs**" to automatically generate CSV files for all subjects in data file.

Cleaning Chambers

1. DO **NOT** USE SANI-CLOTH PLUS WIPES OR ANY OTHER CLEANERS THAT WILL RUIN ACRYLIC TO CLEAN OUT CHAMBERS!!!!! Only use a mild bleach solution or Clorox Wipes to clean out the chambers. If you have questions about what to use, contact someone before cleaning.

2. Wipe/rinse out the chamber thoroughly, including plastic mesh grates at bottom of chambers.

3. Remove water bottles from lids

♦ Loosen the nut where the water bottle is fastened to the lid and pull the water bottle up.

♦ Empty water bottles and wash out. Make sure bottles are rinsed well (Do NOT use Sani-Cloths on the plastic bottles).

♦ Use bleach or Sani-Cloths to wipe off metal spigots and rinse with H2O

♦ Set bottles up so they can dry

4. Wipe down all lids thoroughly (if you use Sani-Cloths, do NOT let them hit the acrylic/plastic parts of the lids)

5. Allow the chambers to dry completely before placing the lids back onto them.

7 **CLAX**

1. Select the **CLAX** icon or select Start and find the CLAX program in the Program files. If CLAX is not installed on your computer, contact Dana for the installation disk

2. On the "Manage Databench" screen, select **Add Experiment**

3. Locate the experiment you want to view and select **Open**. Multiple files CAN be added to the same databench for comparison

4. When the file has been added to the databench, there will be three (3) tabs labeled "Experiments," "Subjects," and "Measurements"

    a. **Experiments** – Under the "Experiments" tab, you can view the   Hardware Configuration, Experiment Configuration and Oxymax settings for that particular experiment. This tab also allows you to set the light/dark cycle times for your experiment (light data will be in yellow/ dark data will be in pink when looking at the raw data and the graphs).

        i. **Hardware Configuration** – Selecting the Hardware Configuration will allow you to view the specifications of the Oxymax system hardware.

        ii. **Experiment Configuration** – Selecting the Experiment Configuration will allow you to view the technical information and measurement parameters for that particular experiment.

        iii. **Oxymax** – Selecting the Oxymax icon in the Manage Databench screen will also allow you to view your experiments parameters (i.e.: date of experiment, number of animals, reference time, measurement times, etc)

    b. **Subjects** – Under the "Subjects" tab, you can view the body weights and other notes for each particular chamber/animal. This is also where you can add "Tags" for grouping animals together when looking at graphs and data. Tags can be the genotype of the animal, color, gender, treatment, etc.

        i. To add tags, go to the bottom, right hand side of the "Manage Databench" page and type in the necessary tags you want to add to the animals in the experiment. After typing in the tag, select Create Tag

        ii. After you have added all of the tags you wish to add to the animal IDs, select an animal ID and select which tag(s) are appropriate for that animal. Do this for all animals in the experiment.

    c. **Measurements** – Under the "Measurements" tab, you can make new equations for different measurements.

        *See "Feed Measurements" below for definitions and equations.

5. Once you have added the experiment(s) to the databench; locate the **Data** icon at the top of the screen and select it. This will open the raw data produced be the Oxymax system.

    a. You can click on a specific parameter to view the results of all animals for that particular parameter.

        i. **High Resolution** feeding data will give you the amount of food consumed at the time interval that was selected when starting the experiment.

        ii. **Feeding Bouts** data will give you the duration of each time the animal eats and the consumption of each meal.

    b. You can also click on an animal number to view all of the raw data for each parameter for that particular animal.

    c. You can select the ALL box to view all data for all animals.

6. To copy the data to add to another program (Excel, Prism...) select **Export** and **Copy to Clipboard**. Open the program that you want to insert the data into, right click on the page, and select **Paste** (as if you would copy and paste like anything else).

7. Next to the "Data" icon at the top of the screen, there are other ways to look at the data including Statistics, Time Graphs, Box Plot, Histogram, and Correlation. Use these tools to help visualize, understand the data, and troubleshoot if necessary.
* NOTE: you can not use the Time Graph to compare two different experiments as of May 1, 2010. Columbus Instrument personnel are working on updating the CLAX program to allow for this in the future

8   **Feed Measurements**
Oxymax for Windows makes feeding measurements in several methods to suit a variety of research needs. Feeding data is recorded in three different methods:
» Synchronously with the metabolic data. Consumption data for a chamber is measured and logged at the same time as the metabolic data for that chamber. This data appears under the "Feeding" tab and is stored in the .CDTA file.
» Asynchronously as the animal interacts with its food. Each time the balance is disturbed the software keeps track of how long the animal disturbs the feeder and how much food it removes. This data appears under the "Feeding Events" tab and is stored in the .BDTA file.
Periodically at a higher rate than the metabolic sampling frequency. This rate is adjustable in the experiment settings. This data is only viewable when it has been exported and is stored in the .FDTA file.

Both the synchronous and high-frequency measurements consist of the same basic measurements:
Date – The date and time the measurement was taken.
Status – If the balance is being disturbed at the time of the measurement it cannot be read and Oxymax for Windows flags the measurement as "Unstable". The software will then record the difference between the last measurement and the last "Stable" reading from the balance. If the balance is "Stable", the value can be read and recorded immediately.
Feed – The amount of food that has been consumed since the last measurement.
Feed Acc – The total amount of food that has been consumed since the beginning of the experiment.

The "Feeding Events" measurements contain additional information about the subjects feeding behavior:
Count – The number of feeding events a balance has recorded.
Interval – The metabolic sampling interval in which the event took place.
Date – The date of the feeding event.
Start – The time at which the feeding event started.

End – The time at which the feeding event ended.

Duration – The length of time the event lasted.

Weight – The amount of food consumed during the feeding event.

Accum – The total amount of food consumed during the experiment.