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UC Davis - Meal Pattern Analysis

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We use this protocol and it's working

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Abstract

Summary:

Behaviorally, meals are defined as periods of intense feeding and drinking separated by periods of activity, grooming, and rest. A “meal” is the primary data unit for characterizing food intake behavior. Changes in the patterning of meals (number, size or duration) may affect food intake and energy balance indirectly. "Meal", in our SOP, is defined by a minimum food intake of 0.02g and at least 10 min between food bout events. Detailed analysis of food intake behavior includes an assessment of average meal duration, average meal size, number of meals, average inter-meal interval (IMI), as well as calculation of satiety ratio. In addition, the diurnal (light cycle/dark cycle) patterns of food intake behavior are also calculated to examine temporal shifts in feeding behavior. Meal pattern and food intake behavior is measured in the Columbus Instruments Oxymax system within a temperature and light controlled cabinet. Animals are acclimated to the facility for at least 1 week. Animals are then acclimated to the CLAMS cages and powdered diet for 48 hours and to the light and temperature-controlled chamber for 24 hours prior to testing. Analyzed data constitutes data collected from 48 hours of continuous measurement (2 light/2 dark cycles).

Materials

MATERIALS

- Acclimation cages with lids and fans **Columbus Instruments**
- Water bottles for acclimation cages **Columbus Instruments**
- Cylindrical feeder tube with wire mesh top **Columbus Instruments**
- Test cages with tight lids for use inside CLAMS incubator **Columbus Instruments**
- Mineral drops **VER1REV Enterprise Catalog #**ICMD (8 oz)
- Conductivity meter **Columbus Instruments**
- O2/CO2 gas tank (20.5% Oxygen/0.5% CO2 Balance Nitrogen) **Airgas**
- OxyVal to validate system (Columbus Instruments) **Columbus Instruments**
- CO2 20%/Nitrogen balance gas tank for OxyVal **Airgas**
- Drierite (Desiccant- Anhydrous) **W.A. Hammond Drierite Company**
- Soda Lime (Airgas) **Airgas**
- Ammonia Filter **Columbus Instruments**

Vendor contact Number

Columbus Instruments (1-800-669-5011)
Airgas (1-510-812-9710)
W.A. Hammond Drierite Company (1-937376-2927)
Ionique Concentrated Mineral Drops (1-888464-6642)

Reagents and Materials:

- T-shaped rod with blunt end (used for pushing down mesh to load food in feeder tube)
- Two 1 Liter bottles with attached lids
- De-ionized water
- Sifted powdered mouse chow or defined diet
- Gloves
- 10 % Nolvasan
- Coverage Plus
- Lab Coat
- Paper Towels
- Animal Room Monitoring Log sheets (door)
- Weight/Food/Water Intake Log sheet and pens
- Animal and System Remote Monitoring Log sheet access thru computer
- Laboratory journal for CLAMS equipment, calibrations, repairs, update and software

1 Recommend acclimation to MBP Vivarium Room 115 for one week prior to study.

a. Animals will be placed in single housing (duplex cages: one cage with clear divider) after transfer. While acclimating in the MBAL animal room the mice will be introduced to the sifted powdered version of their normal accustomed rodent chow via specialized plastic feeders placed in their home cages in addition to their normal pelleted chow 2 days before transferring to the specialized acclimation cages in the CLAMS room.

b. Mice will also be given de-ionized water with a mineral content of approximately 400 PPM. Mineral drops (6-7 drops) will be added to 1L of de-ionized water and measured on the conductivity meter to approximately 400-425 PPM. This water will be given during the study in order to monitor water intake via conductivity in the CLAMS unit.

c. The powdered food and water will be grossly observed as eaten or spilled and documented on the Weight/Food/Water Intake Log sheet.

2 Acclimation in the CLAMS acclimation cages in the CLAMS room for 24 hours followed by 24 hours acclimation in the incubator.

a. In the AM (before 12:00, if possible) feeders will be filled to capacity with the powdered feed to be used in the calorimetry study. Food guard size should be matched to animal size to prevent foraging.

b. Mice will be weighed and their weights recorded on the Weight/Food/Water intake log sheet before being placed into the CLAMS room acclimation cages.

c. Lixit bottles will be filled with de-ionized water, checked for a mineral content of approximately 400-425 PPM and placed in the appropriate slot on the top of the acclimation cages.

d. Power will be supplied to the fans to circulate air in the cages.

e. Lids will be tightened and cage cards placed on the front of each cage via the front hinge.

f. The time at which animals were placed in the acclimation cages will be recorded in the Animal and System Monitoring Log sheet.

g. After all animals are housed in the acclimation cages, the door to the room will be closed and a “Testing in Progress” sign will be placed on the door while the animals are acclimating. Room temperature will be maintained at 24°C.

h. The acclimation room will be maintained on a 12 hour light/dark cycle, with lights off at 18:00 and on at 06:00. Lux as measured inside the cages is 100-200 lux from the adjusted room light and the incubator lights (CLAMS equipment is turned on, no door panels).

i. Animal Monitoring Log Sheet on the door will be filled out daily as per campus requirements.

j. At the end of 24 hours, animals will be weighed and the food and water grossly observed and documented on the Weight/Food/Water Intake Log sheet.

Important checkpoints during this stage:

After 24 hours in the room acclimation cages, the animals should be around the same weight as before they were placed in the acclimation cages. Any mouse that loses between 10-15% of its original body weight over the acclimation period will be closely monitored and the reason for the weight loss inspected. If no hardware issues (will check water and food intake) are found and the cause of the weight loss cannot be determined, this animal will be assessed more closely to see if being placed in the CLAMS apparatus is feasible; this will be conducted on a case-by-case basis. If it is decided that the animal is to be taken out of the study, it will be placed back in its home cage. Weight and health of this animal will be monitored and checked daily to ensure it returns to its normal weight. If the animal continues in poor health, the campus vet will be notified and further action will be taken upon the recommendations of the campus vet. If a large amount of powdered diet has been spilled during this acclimation period, the food guard size should be checked to ensure that the animal is not able to forage or a put pressure on the scale to avoid errors in food intake measurements.

3 Running animals in CLAMS incubator (24 hours incubator acclimation) to be initiated in the AM (before 12:00).

a. Computer set-up (see step-by-step procedure, (**Appendix 1**)

b. Checking of hardware (Drierite, soda lime, ammonia filter)

c. Fill container with 2 L of de-ionized water and using conductivity meter add mineral drops (67 drops/L) until you achieve a mineral content of approximately 400-425 PPM. Fill two 1 Liter bottles with the measured water and note the mineral content of the water on the Animal and System Monitoring Log sheet.

d. Fill water lines through the ‘pump status’ option in the Oxymax software

e. Calibrate the gases using 20% CO_2, Nitrogen balance (**Appendix 1**).

f. Put mesh food holders with food on balances in CLAMS apparatus.

g. Turn on and tare the balances (should read 0 grams).

h. Weigh animals and note on data sheet.

i. Put acclimation cage containing each animal in the incubator and secure the lid. These are the same cages that the animals have been acclimating in the room.

j. Incubator temperature is set at 22°C and lights set on at 06:00 and off at 18:00. Light intensity as measured inside the cage is 100-200 lux. Light is from the lights inside the incubator and adjusted room lights. Door panels are not used on the incubator. This enables us to monitor the animals, food and equipment without opening the doors (glass doors) thus not disturbing the animals, the animals acclimate to the incubator better by seeing out and the room lights contributes to a more even light intensity within the incubator.

k. In Oxymax, click Experiment→ Run

l. Begin recording and check RER measurements each hour to ensure proper function (RER below 0.7 or > 1.3 may signal cage lid or gas line leakage, e.g.).

m. Recording to continue for 24 hours incubator acclimation followed by 48 hours of test or longer as determined by the study.

n. To end study: in Oxymax click Experiment → Stop.

o. After the study is finished, the animals are weighed and returned to their home cage. Body composition values of the mice are to be determined by DEXA immediately following CLAMS analysis.

p. The CLAMS cages are washed and disinfected, water lines flushed with 10% bleach and rinsed with de-ionized water. Data are collected and analyzed.

Important checkpoints during this stage:

Mice are to be monitored via the CLAX program every two hours starting when they are first put into the CLAMS between MBALs' normal business hours of 08:00-17:00 Monday-Friday. Animals will be visually checked in the morning before 10:00 and again in the afternoon before 16:00. Monitoring will continue remotely via personal computers between 21:00 and 22:00. Upon checking the mice, food and water intake will be monitored as well as activity, RER, environmental conditions and electrical power and

documented by MBAL personnel and recorded in the Animal and System Remote Monitoring Log sheet located in that specific experiment's CLAMS folder on the M-drive. Environmental conditions will also be checked. Any problems will be evaluated with the proper action.

Other events that might occur:

Temperature in CLAMS goes out of range:

The CLAMS apparatus has a fail-safe range (15-30 degrees Celsius) where the apparatus will automatically shut itself off if the temperature within the apparatus surpasses the range. If an event such as this occurs, the temperature and animals within the CLAMS will be closely monitored and checked via the 'temperature' measurement in CLAX which can be assessed through the M-drive → CLAMS DATA → Studies folder name → MDTA file → CLAX →Time graph→Temperature. If the temperature inside the CLAMS fails to correct itself, the animals will be taken out immediately and returned to their home cages.

Power outage:

The CLAMS has a monitoring system in case of power outages. In the event the power goes out, MBAL personnel will automatically be notified via the monitoring system; MBAL personal will also be notified when power is restored. If the power is restored within 30 minutes, the back-up battery will generate enough power to sustain the CLAMS and keep the experiment going. If this occurs, the animals will be closely monitored and the system verified to still be running remotely. If, however, the power does not return within thirty minutes and/or the CLAMS shuts off prematurely within the 30 minutes, the animals will be taken out of the CLAMS and returned to their home cages within the hour.

4 Data collection.

a. Food intake (amount, time of eating and accumulated amount)- food bout file for each animal is scanned for irregularities- such as "overload".

b. The food bout "B" .csv files for each subject are generated from the .CDTA file using the Oxymax software.

c. The .csv files are combined into 1 data folder including the meal pattern analysis template and a custom MS Excel-based (Visual basic) macro is run to calculate and collate the data. Data parameters include: Avg Meal Duration-Active Eating Time (sec), Avg Meal Size (g), # of Meals, Avg Inter-Meal Interval (IMI) (sec), Total Meal Duration, Satiety Ratio, Avg Eating Rate, Avg Light-Meal Duration, Avg Dark-Meal Duration, Avg

Light-Meal Size, Avg Dark-Meal Size, Avg Light-IMI, Avg Dark-IMI, Light- # Meals, Dark- # Meals, Light-Satiety Ratio, Dark-Satiety Ratio, Light-Eating Rate (g/min), Dark-Eating Rate (g/min), Food Intake/BW).

5 Data QC

a. Feed Accumulated (Feed Acc. 1) has no “underload,” “overload” or “No response” messages.