The University of Hong Kong CULATR Policy on Food and Fluid Restriction in Research Animals

1. Purpose

The purpose of this policy is to provide guidance to principal investigators and their staff with regard to withholding of feed and/or fluid from laboratory animals.

The goals of these guidelines are to ensure that (a) dietary/fluid restrictions are well justified and necessary for study objectives and (b) that animals subjected to dietary /fluid restriction are appropriately monitored.

2. Scope

This policy is intended as guidelines for research staff who intend to withhold feed and/or fluid from laboratory animals in their approved CULATR protocol.

Common experimental protocols that require food/fluid restriction include:

- 1. Behavior study protocols in which withholding food and/or fluid is required to train animals to perform a task, while providing food or fluid as a reward for the correct behavior.
- 2. Nutrition studies may require altering levels of specific nutrients in the daily diet.
- 3. Limit-feeding, which is common for some sedentary laboratory animals in order to control obesity or maximize life-span (such as maintaining 85% of body weight).

3. Definition

Fluid deprivation/ restriction:

- Fluid deprivation is defined as complete withdrawal of fluids. Fluid restriction is defined as any restriction of fluids below the normal ad-libitum fluid consumption
- Temporary fluid deprivation for very short durations does not require CULATR approval, such as:
 - During transportation. If transportation is expected to exceed one hour however, providing liquid in the form of a gel pack is required.
 - o Routine husbandry procedures such as water bottle or cage changing.
 - During experimental procedures of short duration where it is not physically feasible to provide water (such as during irradiation or imaging). Whilst animals are in the cage waiting to undergo such procedures however the expectation is that water will still always be available.

Food deprivation/restriction:

- Food deprivation is defined as withdrawal of diet in animals for more than 16-hours in any 24-hour period
- Food restriction is defined as any caloric restriction and/or limited access to ad-libitum diet to animals that would result in reduction of their age matched body weight or body condition score.

- Food deprivation or restriction of less than 16 hours does not require CULATR approval.
- Pre-surgical fast of up to 24-hours in large animals such as pigs and goats is permitted without CULATR approval.

Skin tenting demonstrates the degree of elasticity in the skin. Skin that has lost elasticity, i.e., doesn't 'snap' back after being pinched, is caused by dehydration.

Body Condition Score: a visual assessment of an animal's weight relative to age, strain and stock based on the amount of tissue cover between the points of the hip, over the transverse processes of the lumbar vertebrae, the cover over the ribs, and the pin bones below the tail. Scoring is done on a scale of 1 to 5, with 1 being emaciated, 3 being optimal and 5 being obese.

4. Procedure

- 1. Food/fluid restriction/deprivation beyond the defined parameters in section 3, must be approved by the CULATR and justified based on the scientific objectives of the study. The least amount of restriction that will achieve the scientific objectives must be used.
- 2. Baseline body weight of animal(s) must be recorded before food or fluid restriction/deprivation occurs. In the case of young growing animals, body weight loss must be adjusted to normal body weight gain for age matched animals.
- 3. Restriction must be based on a measurable parameter, i.e. percentage of *ad libitum* intake or duration of fluid restriction.
- 4. In the case of a conditioned-response behavioral research protocol, use of a highly preferred food or fluid as positive reinforcement instead of restriction is recommended.
- 5. Experimental endpoints, clinical symptoms, and conditions for temporary or permanent removal of an animal from a study must be described in the CULATR application. Examples include:
- a. Body weight loss of greater than 15%
- b. Appearance (sunken eyes, body condition score of 2 or 1 out of 5)
- c. Behavior (listless, lethargic)
- d. Other health issues
- e. Failure for growing animals to gain weight
- 6. It is important to note that restriction/deprivation of fluid will concurrently cause a rodent to eat less and vice versa.
- 7. Animals that are co- or group-housed must be observed when feed/fluid is given to ensure that all animals are receiving adequate intake.

Fluid Restriction/Deprivation

- 1. When using fluid rewards as motivation for task performance, it is imperative for the investigator to ensure that the daily requirements to maintain a healthy state are met by the sum of earned rewards and supplemental fluid offered.
- 2. Skin tenting must be used to assess hydration status. When the skin is 'tented,' it should snap back quickly. If the tent of skin does not snap back, this indicates at least a 5% dehydration status.
- 3. Animals that have weight loss of 10% from baseline, or age matched, weight, are considered clinically dehydrated and should be allowed to freely drink fluid without interruption. In addition, 0.5-1ml (mouse) or 2-3ml (rat) of subcutaneous warmed isotonic saline (0.9% NaCl) must be administered and the veterinary staff consulted.

Food Restriction / Deprivation

- 1. An animal must not lose more than 15% of its baseline or age matched weight during a food restriction/deprivation study. Animals who lose more than 15% of its baseline body weight must be immediately provided with *ad libitum diet*, or provided with sufficient food to consistently maintain >85% age matched body weight for those study protocols involving lifetime feed restrictions.
- 2. Average feed consumption amounts are determined based on age, sex, stock, and strain of animal and should be established before beginning any restriction study.

5. Quality Control & Assurance

Monitoring and Documentation

- 1. Each cage must be marked with a "food/fluid restriction." card
- 2. A log sheet must be maintained for each cage of animals on restricted food / fluid protocols and kept in the animal holding room for the duration of the study.
- 3. The log sheet/cage card should include:
- a. Current approved CULATR protocol number
- b. Investigator name
- c. Contact name, email, and phone number (including after hours)
- d. Animal identification
- e. Animals' initial body weight
- f. Type of restriction
- g. Length of restriction
- h. Daily/weekly body weights
- i. Daily/weekly body condition score
- j. Presence/Absence of urine and feces (for fluid restriction only)
- k. Skin tenting (for fluid restriction only)
- I. Amount of food/fluid given, including amount of time allowed to eat/drink, if applicable
- m. Amount of food/fluid consumed

- 4. Body weight and skin tenting must be recorded at least daily for any animal that has fluid restriction/deprivation of 4-24 hours.
- 5. Body weight and skin tenting must be recorded at least twice daily for any animal that has fluid restriction/deprivation for >24 hours.
- 6. Body weight must be measured with or without a body condition score and recorded at least twice weekly for any animal begins any food restriction.
- 7. Body weight must be measured and body condition score recorded at least once daily for any animal on food restriction that has lost >10% body weight.
- 8. For animals on prolonged food restriction, such as maintaining 85% body weight based on growth curve for the strain and species, animals must be weighed at least 2-3 times each week.
- 9. Research staff are responsible for monitoring all animals on food/fluid restriction/deprivation studies.
- 10. Research staff should be trained and competent to evaluate animal's condition.
- 11. Veterinary staff and/or CULATR have the right to request these documents for review at any time and to stop/amend food or fluid restriction durations at any time to prevent animal suffering.