



<b>Issuing Office(s)</b> _____ OACU _____
<b>Responsible University Officer(s)</b> _____ UNC IACUC _____

# UNIVERSITY STANDARD

---

## Title

---

### UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD ON FOOD AND/OR WATER RESTRICTION AND/OR DEPRIVATION IN RATS, MICE, RABBITS, AND FERRETS

---

## Introduction

---

### PURPOSE

The standards and procedures described below describe the requirements to all researchers and animal handlers for experimental procedures that utilize either dietary restriction or deprivation schedules. It does not, however, address fasting procedures used prior to anesthesia or surgery, veterinary recommendations for the care of an animal, or specialized diets which do not entail caloric restriction.

### SCOPE OF APPLICABILITY

Dietary (food and water) restriction and deprivation procedures are often necessary in physiological and neuroscience studies that focus on the relationship of caloric restriction to disease, obesity, life-span, and energy metabolism. Additionally, behavioral-oriented studies frequently utilize these procedures to motivate animals to learn or perform appetitive tasks. While some research objectives require varying periods of food or water deprivation, others require restricted access to limited amounts of food or water on a daily basis. As no single standard could cover the wide range of procedures and diverse species used in these types of studies, the IACUC considers each of these studies on a case-by-case basis.

The IACUC requires that all restriction/deprivation schedules be adequately described in the protocol and scientifically justified. The protocol also must describe the specific criteria to determine when animals should be returned to free or increased access to food or water. Records must be maintained that, when appropriate, include monitoring of body weight, food or water intake, and assessment of the animal's health status. Records must be readily available for review by veterinarians, IACUC, or external auditors.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DLAM Management.



Issuing Office(s)

\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)

\_\_\_\_ UNC IACUC \_\_\_\_\_

---

## Standard

---

### Information that must be included in the protocol:

1. A scientific rationale for the restriction or deprivation schedule
2. An indication that the investigator has consulted with an authorized institutional veterinarian as part of study design
3. Restriction or deprivation schedules described in terms of the duration of restriction/deprivation, time limited access, amount limited access, weekend scheduled access, and/or amount of food and water provided on a daily basis
4. Methods for assessing the health and well-being of the animals
5. Identification of measurable parameters used to assess the level of restriction/deprivation, such as percent ad libitum intake, percent body weight compared to baseline or an unrestricted control animal, or percent body weight compared to an established growth curve
6. If the animals are likely to experience distress as a result of the water or food restriction/deprivation procedures, a search for alternative and less stressful procedures must be conducted
7. Criteria to indicate when animals are to be returned to unlimited or increased access to food or water
8. Monitoring schedule for body weights and/or water intake

### General Guidelines:

1. The amount of restriction/deprivation used must be the minimum level that will achieve the studies objective.
2. Animals should not be both food and water restricted/deprived at the same time.
3. Many experiments involve restricting food or water to motivate animals to perform an appetitive task and are conducted 5 days per week. Therefore, planning for some ad libitum access to food or water over the weekend allows for the recovery of body weight and minimizes health risks associated with the restriction/deprivation schedule.
4. Restriction/deprivation schedules must be conducted with care and tailored to the feeding patterns of each species, its age, strain, and health status.
5. Before a restriction/deprivation study is initiated, ad libitum daily water and food intake should be determined for that species/strain/sex/age/health status.
6. Daily rations of food and water in an amount and quality to either maintain weight or maintain a suitable growth rate should be a priority.
7. Trained personnel should monitor body weight, body condition, hydration status and behavioral changes of the animal.



Issuing Office(s)

\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)

\_\_\_\_ UNC IACUC \_\_\_\_\_

### Documentation when food or water restriction/deprivation schedules are utilized:

1. During active restriction/deprivation periods, records should be available in the animal housing room. The [PI Husbandry Duties & Monitoring Check Sheet](#) (AKA PI Feed & Water Check Sheet) must be utilized for this purpose, and can be obtained from the DLAM Facility Manager. The [PI Food/Water Restriction Cards](#) are also available for this purpose, and should be used whenever animals are undergoing feed/water restriction/deprivation.
2. Documentation of health assessments must be maintained by the research staff and easily accessible for review either in the housing room or another location as designated by the veterinary staff.
3. Animals that have restricted access to food or water must be weighed at least 2 times per week. Up to date records of body weight should be maintained, and when appropriate daily water intake and amount of water obtained in the conditioning session.

### Criteria indicating the need for supportive care and interventional endpoints

1. Rapid loss of body weight
2. Dehydration >5% (indicated by the following clinical signs)
3. Loss of skin turgor or tenting of skin
4. Minimal or no urine output for >12 hours as evidenced by dry bedding, absence of feces or dry feces
5. Decrease food consumption and body weight loss of more than 10% over a 48 hour period
6. Sunken eyes, listlessness and inactivity
7. Dehydrated animals require immediate treatment. Supplemental oral fluids or parenteral hydration (such as subcutaneous Lactated Ringer's Solution) must be administered whenever the clinical signs of dehydration listed above are observed. Parenteral supplemental fluids must be administered gradually in order to prevent over-hydration.

## Rats and Mice

### General information regarding food and water requirements in mice and rats:

- **Recommendations for transporting mice/rats to lab setting:** Animals should be transported with feed/water (water bottle placed on cage) whenever possible.



Issuing Office(s)

\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)

\_\_\_\_ UNC IACUC \_\_\_\_\_

- Mice and rats consume 70-85% of their food and water during the night cycle<sup>10</sup>, typically in 10 small meals with drinking typically associated with meal consumption.
- An adult mouse consumes an average of 4-5 grams of food per day, whereas an adult rat will consume an average of 15-20 grams. The daily amount of food intake can vary by more than 2-fold across strains<sup>1</sup> and is highly dependent upon the palatability of the food as well as its fat and sugar content.
- The estimated daily water intake in an adult mouse is 5.8 mls and in an adult rat 25 mls. This amounts to 25% and 10% of the body weight of mice and rats, respectively. Water consumption in both mice and rats can vary by more than 2-fold across strains and is highly correlated with food consumption.<sup>1</sup>
- Food or water restriction/deprivation is not recommended in young, growing rodents (ex. < 6-8 weeks old).
- Restricting food intake can result in substantial increases in life span and health profiles<sup>8</sup> even under extreme conditions in which food amounts are limited to 45% of ad libitum control intake.<sup>3</sup>
- Moderate dietary restriction/deprivation schedules can initially induce levels of stress that are considered less than those typically observed with other stressors.<sup>2</sup>
- Healthy adult mice and rats that have been gradually reduced to 15% of their baseline body weight by restriction/deprivation schedules will rarely show clinical signs of distress.<sup>9</sup>
- Mice and rats routinely adapt to the types of deprivation/restriction schedules typically used in physiological, neuroscience and behavioral research with minimal or no observable signs of distress.<sup>9,11</sup>
- In young animals, growth is one of the main indicators of dietary adequacy.<sup>6</sup>

### **Food restriction/deprivation: Recommendations**

- Food restriction schedules should be gradually introduced over a minimum of 1 week.
- Providing limited access to food for ~1 hour per day or providing animals with a measured amount of food consistent with daily intake should be sufficient to maintain body weight.<sup>5</sup>
- Most rats and mice adapt well to once per day feeding schedules.
- Maintaining normal healthy adult animals at 15% weight loss from their initial body weight has minimal risk to the animal's health.<sup>9</sup>
- The maximum percent body weight loss for rats and mice on restricted diets should not exceed 20% relative to age- and sex-matched ad libitum fed controls.<sup>2,7</sup>



- Food or water deprivation periods in excess of 24 hours per day should not be utilized.
- In studies where restriction/deprivation schedules are used to motivate animals to perform appetitive tasks, consideration should be given to using highly palatable foods or liquids as a substitute for the restriction/deprivation schedule.

### **Water restriction/deprivation: Recommendations**

- For water restriction schedules that will last for more than a few weeks, the animal should be gradually weaned down over a minimum 3 days to the consumption goal.
- Daily access to water should be sufficient to maintain adequate food intake, as animals fed with limited access to water will eat less and lose weight.
- Water and food availability should coincide, thus promoting food consumption and the retention of fluids.
- For all types of water restriction schedules, rats and mice should be given free access to water when experimental testing is not scheduled.
- Immature animals are especially sensitive to water restriction and thus must be weighed, closely monitored and evaluated in relation to normal growth requirements.
- Hydration status should be assessed and sufficient amounts of water provided on a daily basis to keep the animal from becoming dehydrated.
- Levels of restriction/deprivation are critical in behavioral studies where water restriction is used to motivate animals to perform an appetitive task. In some cases, 14 hours of deprivation may not be sufficient for appetitive learning, whereas 21 hours is considered more optimal.<sup>4</sup> However, the degree of restriction/deprivation required for adequate learning or performance levels will depend on the type and parameters of the appetitive task.
- Restriction schedules must take into consideration the amount of time necessary for adequate daily water consumption, as even brief periods of water access (e.g., 30-60 minutes per day) can be sufficient to alleviate hydration deficits that can develop over a deprivation period.<sup>11</sup>

#### **References:**

1. Bachmanov AA, Beauchamp GK, Tordoff, MG (2002) Voluntary consumption of NaCl, KCl, CaCl<sub>2</sub>, and NH<sub>4</sub>Cl solutions. *Behav Genet* 32: 435-443.
2. Heiderstadt KM, McLaughlin RM, Wright DC, Walker SE, Gomez-Sanchez CE (2000) The effect of chronic food and water restriction on open-field behaviour and serum corticosterone levels in rats. *Lab Anim* 34:20-28.
3. Hubert MF, Laroque P, Gillet J-P, Keenan KP (2000) The effects of diet, ad libitum feeding, and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control Sprague-Dawley rats. *Tox Sci* 58:195-207
4. Hughes JE, Amyx H, Howard JL, Nanry KP, Pollard GT (1994) Health effects of water restriction to motivate lever-pressing in rats. *Lab Anim Sci* 44:135-140.
5. Hurwitz HMB, Davis H (1983) Depriving rats of food: a reappraisal of two techniques. *J Exp Anal Behav* 40:211-213.
6. Nutrient Requirements of Laboratory Animals, Fourth Revised Edition, 1995.



Issuing Office(s)

\_\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)

\_\_\_\_\_ UNC IACUC \_\_\_\_\_

7. Peck JW (1978) Rats defend different body weights depending on palatability and accessibility of their food. J Comp Physiol Psychol 92:555–570.
8. Roe FJC (1994) Historical histopathological control data for laboratory rodents: valuable treasure or worthless trash? Lab Anim 28:148–154.
9. Rowland NE (2007) Food or fluid restriction in common laboratory animals: Balancing welfare considerations with scientific inquiry. Comp Med 57:149–160.
10. Siegel PS (1961) Food intake in the rat in relation to the dark-light cycle. J comp physiol Psychol, 1961, 54, 294-301.
11. Toth LA, Gardiner TW (2000) Food and water restriction protocols: Physiological and behavioral considerations. Contemp Top Lab Anim Sci 39:9-17.

## Rabbits

### General information and guidelines regarding food and water requirements in rabbits:

- Rabbits consume 50-150 mls/kg of water daily, an amount that varies across breeds, age, sex, health and type of food available.<sup>2</sup>
- Rabbits typically eat small meals throughout the day.
- If water deprived, you may also inadvertently food restrict them as well, since rabbits will decrease eating and after three days without water they will stop eating.
- When a rabbit does not have access to food, it will drink excessive amounts of water.
- Because of disturbances associated with fasting, rabbits should not undergo a fast (pre-surgical or otherwise) without a specific justification.<sup>1</sup>
- Calorie restriction is an accepted practice for long-term housing of rabbits.

#### References:

1. Manning PJ, Ringler DH, Newcomer DC (1994) The Biology of the Laboratory Rabbit.
2. Suckow, MA (1997) The Laboratory Rabbit.

## Ferrets

### General information and guidelines regarding food and water requirements in ferrets:

- Food deprivation that exceeds 4 hours can result in hypoglycemia.
- Average water consumption is generally considered to be 75-100 mls/kg/24 hours, with bouts of drinking occurring 20 times per day.
- Fasting in ferrets should be avoided.



Issuing Office(s)

\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)

\_\_\_\_ UNC IACUC \_\_\_\_\_

- Daily feed consumption averages 42 g/kg body weight for males and 49 g/kg for females.<sup>1</sup>
- When fluid restrictions are imposed, the ferrets should receive a minimum of 60 ml/kg of water daily.
- Ferrets on water restriction/deprivation procedures should be weighed at least 3 times per week to ensure absence of weight loss and tested for dehydration by skin pinch.
- Under no conditions should ferrets be allowed to weigh less than 85% of the baseline body weights.
- When the research objectives of the study require water restriction for experimental sessions conducted Monday through Friday, free access to water should be available on the weekends.

**References:**

1. Bleavins MR, Aulerich RJ (1981) Food consumption and food passage time in mink (*Mustela vison*) and European ferrets (*Mustela putorius furo*). *Lab Anim Sci* 31:268-269.

## EXCEPTIONS

Requests for exceptions to this Standard must be reviewed and approved by the IACUC and/or DLAM Management.

---

## Definitions

---

**IACUC:** Institutional Animal Care and Use Committee

**DLAM:** Division of Laboratory Animal Medicine

**University Standard:** The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

**Ad libitum:** Animals have continuous access to food and water. This is standard animal husbandry at most research institutions.

**Deprivation:** Absence of ad libitum availability of food or water, typically described in terms of the time interval during which food or water is withheld (e.g., water is withheld for >12 hours, food is withheld for >24 hours).

**Restriction:** Limiting access to food or water, typically described in terms of providing limited amounts or time-limited periods of access to food or water on a daily basis (e.g., the diet is restricted in total calories to maintain 80-90 percent of ad libitum body weight).



Issuing Office(s)  
\_\_\_\_ OACU \_\_\_\_\_

Responsible University Officer(s)  
\_\_\_\_ UNC IACUC \_\_\_\_\_

---

### Related Requirements

---

#### UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

Please refer to the [University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes](#).

Public Health Service Policy on Humane Care and Use of Laboratory Animals  
Animal Welfare Act Regulations. 9 CFR, Chapter 1, Subchapter A.

The Guide for the Care and Use of Laboratory Animals, Eighth Edition. 2011.

---

### Contact Information

---

Subject	Contact	Telephone	Email
IACUC Protocol	OACU	919-966-5569	<a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a>
Husbandry	DLAM	919-962-5335	
Veterinary Services	DLAM	919-966-2609	

---

### Important Dates

---

- Effective Date and title of Approver: March, 2016; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: March, 2017, Nov 2017.

Approved by: UNC IACUC

---

Dr. Mitchell Picker  
UNC IACUC Chair

11/2017