

Institutional Animal Care & Use Program - UTEP	
Title: Food and Water Restriction, Regulation, or Diet Manipulation	
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Introduction

This guideline describes the recommendations for protocols that include the regulation or restriction of an animal’s food and/or water. The *Guide for the Care and Use of Laboratory Animals* (the Guide, NRC 2011) states: “The objective when these studies are being planned and executed should be to use the least restriction necessary to achieve the scientific objective while maintaining animal well-being” (p. 31). The restriction or regulation of food/water must be described in detail in the protocol application and approved by the IACUC.

Definitions

Regulation of food/water is defined as the scheduled access to food and/or water sources, so an animal consumes as much as desired at regular intervals. For example, rats may have regulated access to food where the food is withheld for several hours in order for them to work for food as a reward in a behavioral test.

Restriction of food/water is where the quantity or total volume consumed is strictly monitored and controlled (NRC 2003).

Fasting is the removal of food (but not water) for a certain period of time prior to experimental manipulation such as surgery, glucose tolerance testing, etc.

Special Diet/Water is any experimental or non-standard diet or water type for the species.

Treats/Food Rewards are a highly palatable food and/or fluid.

Policy

Protocols should propose minimal restrictions or food regulation limits necessary to achieve the scientific objective while maintaining animal well-being. Protocol applications must address the

necessary level of regulation/restriction, the methods for assessing the health and well-being of the animals, the potential adverse consequences of regulation/restriction, and the steps to be taken to address the adverse health effects.

Animals must always have access to water unless justified and approved in the IACUC protocol.

Even on food/water restriction or in the case of special diets, minimum requirements for adequate nutrition and hydration should be met and can be assured by monitoring body weight, as well as behavioral and clinical changes. In the case of these conditioned-response research protocols, the use of a highly preferred food or fluid as positive reinforcement, instead of restriction/regulation, is recommended. If an acclimation period for food regulation or restriction is needed this should be described in the IACUC protocol. Animals experiencing adverse effects due to food/water regulation/restriction must be evaluated by a veterinarian.

Special considerations

- Life stage (e.g., young, growing, pregnant, and lactating) and health status may affect the caloric requirement for maintaining animals. When determining food restriction or regulation protocols these situations should be considered and appropriate monitoring implemented (e.g., weight assessment, comparison with expected growth curve).

Experimental/Special Diet and/or Water or Treats/Rewards

- Diets should be acquired from reputable vendors and must be kept, after opening, in labeled, clean, easily sanitized, vermin-proof containers. Exceptions must be listed in the animal protocol and approved by the IACUC.
- Diets must be irradiated at the vendor.
 - This is consistent with Laboratory Animal Resources Center (LARC) practices to reduce the potential for adventitious disease.
 - If unable to be irradiated, LARC veterinarians must be notified prior to use to discuss options.
- Diet must be used within six (6) months of the mill date, unless otherwise specified by the manufacturer.

- Special water bottles must be replaced monthly with fresh bottles and fresh water to prevent bacterial overgrowth and biofilm creation, or as needed.
- All treats and food rewards, not already approved as standard LARC enrichment, must be listed and approved in the IACUC protocol.

Restriction/Fasting

- Pre-surgical fasting is the veterinary standard for larger species (cats, dogs, pigs) and does not need to be detailed in the IACUC protocol. Small research animals are not typically fasted before surgery. Therefore, pre-surgical fasting of rodents and rabbits must be justified in the IACUC protocol.
- Fasting may be necessary in preparation for non-surgical experimental manipulations.
 - The length of the fasting period must be specifically defined in the IACUC protocol.
 - Any mammal without access to food for more than 24 hours must be listed in Category E. Non-mammalian species will be addressed on a case-by-case basis. Additionally, if scientific needs require a weight loss of >20%, these animals must be listed in Category E.
- Animals without water for longer than 12 hours must be listed in Category E.

Recordkeeping

The following parameters should be measured to ensure that the nutritional needs of the animals are met. Animals must be assessed daily, and written records must be maintained for each animal to document these parameters, see Recordkeeping Policy.

- Mammalian animals must be weighed before experiments begin to establish a baseline weight. Weights must be recorded (at least) three times weekly during the periods of food/water regulation and time periods must be specified in the IACUC approved protocol. Non-mammalian animals will be assessed on a case-by-case basis.
 - An adult mouse consumes an average of 5 g/100 g body weight of food per day, whereas an adult rat will consume an average of 12g/100g body weight. The

maximum percent body weight loss for animals on restricted diets should not exceed 20% relative to age- and sex-matched *ad libitum* fed controls.

- Due to variation in food requirements and nutritive status, use of average guidelines for food intake is not appropriate. Mature or obese animals can tolerate greater food restriction than their young or thin counterparts.
- If weight loss is expected, this will need to be outlined in the IACUC approved protocol. Determination of weight loss should be specified in the protocol and can be based on baseline weights, *ad libitum* controls, and/or a standardized growth curve. Adjustments may need to be made as animals age.
- Body condition scores can also be used to assess health status (Appendix 1)
 - Ideal score is 3/5; scores of 2/5 or less are considered under-conditioned to emaciated.
 - Scores lower than 2/5 must be justified in IACUC protocol

For Water Restriction Only

- The estimated daily water intake in an adult mouse is 8-10 ml /100g body weight and in an adult rat is 15 ml/100g body weight. This amounts to 25% and 10% of the body weight of mice and rats, respectively.
- These values may be used as a starting point to determine regulation or restriction parameters. However, individual variation in water consumption based on factors such as strain, sex, age, and health status must be taken into account when establishing fluid regulation or restriction paradigms.
- Mice, being one logarithmic unit smaller in body weight than rats, have correspondingly higher Basal Metabolic Rates (BMRs) and correspondingly faster water turnover. Mice are therefore less tolerant of water restriction and PIs must be aware of these differences if extending observations made on rats to mice.
- Skin elasticity should be evaluated to assess the animal's hydration status when water is restricted.

- Solid and liquid waste can be evaluated to assess the animal's hydration status and physiological compensation for fluid regulation if necessary.
- Rough hair coat and sunken eyes are indicators of dehydration.

Appendix 1

Table 1. Rodent Body Condition Score

	BC 1 Mouse is emaciated. ◦ <i>Skeletal structure extremely prominent; little or no flesh cover.</i> ◦ <i>Vertebrae distinctly segmented.</i>
	BC 2 Mouse is underconditioned. ◦ <i>Segmentation of vertebral column evident.</i> ◦ <i>Dorsal pelvic bones are readily palpable.</i>
	BC 3 Mouse is well-conditioned. ◦ <i>Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.</i>
	BC 4 Mouse is overconditioned. ◦ <i>Spine is a continuous column.</i> ◦ <i>Vertebrae palpable only with firm pressure.</i>
	BC 5 Mouse is obese. ◦ <i>Mouse is smooth and bulky.</i> ◦ <i>Bone structure disappears under flesh and subcutaneous fat.</i>

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Ullman-Cullere MH, Foltz CJ. 1999. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Laboratory animal science* 49:319-323.

References

1. NRC 2011. Guide for the care and use of laboratory animals, 8th edition. Washington: National Academies Press.
2. NRC 2003. Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington: National Academies Press.
3. Bekkevold CM, Robertson KL, Reinhard MK, Battles AH, Rowland NE. 2013. Dehydration Parameters and Standards for Laboratory Mice. *J Am Assoc Lab Anim Sci* 52: 233-239.