



IACUC Guidance:	<b>TAMU-G-031</b>	Title:	<b>Guidelines on Experimental Food or Water Restriction or Manipulation in Research Animals</b>
-----------------	-------------------	--------	---

	Location	Effective Date	Review By
	<b>College Station/Dallas/Galveston/Kingsville</b>	10/20/2022	7/31/2025
	<b>Houston</b>	10/01/2022	7/31/2025

**1. PURPOSE**

- 1.1. Food and water restriction can be an important and commonly used research tool in many experimental studies. This restriction has the potential to adversely affect an animal’s well-being. In keeping within federal and university Guidelines to minimize pain, distress and discomfort of animals used in research, the following document has been developed for implementing food or water restriction or manipulation in experimental studies involving animals at Texas A&M University.

**2. SCOPE**

- 2.1. Does not apply to pre-anesthetic fasting with access to water. See the AV (or designee) for species-specific anesthesia guidelines, as well as TAMU-G-002, TAMU-G-013, TAMU-G-018, and TAMU-G-035 as applicable for additional information on pre-anesthetic fasting.
- 2.2. Does not apply to standard production methods such as controlled feeding of broiler breeder pullets during rearing to prevent skeletal and other abnormalities that occur with excessive weight.
- 2.3. Does not apply to animals whose clinical care includes directives from the AV (or designee) to decrease body condition.
- 2.4. Does not apply to shipping or transportation.

**3. RESPONSIBILITY**

- 3.1. The **IACUC** is responsible for:
  - 3.1.1. Evaluating the level of restriction and potential adverse consequences of regulating food or fluid.
  - 3.1.2. Evaluating the methods for assessing the health and well-being of animals involved in activities that regulate food or fluid consumption.
  - 3.1.3. Approving scientific justifications for departures from the recommendations in the *Guide for the Care and Use of Laboratory Animals*.
- 3.2. The **AV** is required to approve restriction of water for Non-Human Primates
- 3.3. The **PI** is responsible for:
  - 3.3.1. Labeling and discarding food and/or water additives appropriately
  - 3.3.2. Utilizing the least restriction necessary to achieve scientific objectives while maintaining animal well-being
  - 3.3.3. Providing a complete description of food/fluid manipulation/restriction in the AUP
  - 3.3.4. Maintaining written records, when applicable, for each animal to document body weight, daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol

**4. DEFINITIONS AND/OR ACRONYMS**

- 4.1. **AUP:** Animal Use Protocol. Document submitted by the PI indicating the housing and research procedures involving animals.
- 4.2. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.3. **BCS:** Body Condition Score. Visual assessment of the amount of fat/muscle covering the bones of an animal.
- 4.4. **BW:** Body weight
- 4.5. Centrally administered support service for animal research and teaching programs at Texas A&M University:

- 4.5.1. **CMP:** Comparative Medicine Program supports the Texas A&M College Station and Dallas campuses
- 4.5.2. **PAR:** Program for Animal Resources supports the Institute of Biosciences and Technology vivarium
- 4.5.3. **PRF:** Pharmaceutical Research Facility supports the Kingsville Pharmaceutical Science Facility vivarium
- 4.5.4. **Sea Life:** The Sea Life Facility supports the Galveston campus
- 4.6. **Conspecifics:** Animals of the same species
- 4.7. **Guidance:** Guidance documents are developed by the IACUC to provide procedural standards to the research community on the topics identified. Animal care and use program participants are expected to adhere to the standards described unless an exception has been requested and approved by the IACUC.
- 4.8. **(Food/Fluid) Manipulation:** A change in the composition of the normally offered food or water. This can include addition or deletion of a nutrient in an animal diet and/or addition of a compound, medication or other substance to the food or drinking water provided to the animal.
- 4.9. **NHP:** Nonhuman primate
- 4.10. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
- 4.11. **(Food/Fluid) Restriction:** A limitation placed on an animal's access to food or water when compared to normal standards for the species or age of the animal. It can be described in terms of either the amount of food or water provided on a daily basis (volume or weight) or the amount of time daily that an animal is given access to food and/or water.
- 4.12. **USDA:** United States Department of Agriculture. USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations (AWAR).

## 5. GUIDELINES OR PROCEDURE

### Manipulation

- 5.1. When manipulation of a diet occurs:
  - 5.1.1. Deviations from the standard diet must be described in the AUP
  - 5.1.2. The investigator is responsible for assuring that specially formulated diets are nutritionally adequate and palatable unless the scientific needs of the research protocol necessitate otherwise. If this is the case, the investigator must describe the adverse effects of the diet.
  - 5.1.3. The PI should consider acclimating animals to novel food items before adding drugs or other manipulations.
- 5.2. Special considerations for manipulation of drinking water:
  - 5.2.1. To encourage voluntary consumption, substances might be mixed with flavored water to promote palatability.
  - 5.2.2. The addition of analgesics in the drinking water may be problematic. See TAMU-G-002 for more information.
  - 5.2.3. Care should be taken to ensure animals consume the appropriate amount of the agent offered.

### Restriction

- 5.3. In a natural environment, animals have restricted access to food and/or water throughout the day. Hunger and thirst influence animal behavior in the wild. In captive animals, food is often used to manage behavior by manipulating amount, timing and location of meals and rewards.
- 5.4. The food/fluid regulation process may entail scheduled access to food or fluid sources, so an animal consumes as much as desired at regular intervals, or restriction, in which the total volume of food or fluid consumed is strictly monitored and controlled.
  - 5.4.1. When using food or fluid restriction to provide motivation, consider whether the use of highly preferred food or fluid as positive reinforcement can be used instead of restriction
  - 5.4.2. If restriction is necessary, use the least restriction that will achieve the scientific objective while maintaining animal well-being, understanding that multiple factors including species, strain or stock, age

and reproductive status of the animals, thermoregulatory demand and type of housing, time of feeding, nutritive value and fiber content of the diet can all influence the amount of food or fluid restriction that can be safely used in a given protocol

5.4.3. In order to determine the appropriate level of food/fluid restriction, it is necessary to know the normal quantities of food that are required for the maintenance of the species in question. Life stage (growth, pregnancy, lactation, geriatric, etc.) and state of health must also be taken in consideration when determining maintenance requirements.

5.5. Common experimental purposes for using food or water deprivation or restriction:

5.5.1. Studies pertaining to metabolism, energy regulation, fluid balance, etc.

5.5.2. Studies pertaining to mechanisms and motivations of hunger and/or thirst

5.5.3. Studies using hunger or thirst as a motivating force to perform behavioral tasks

5.5.4. Studies analyzing the effects of restriction or manipulation on aging or cancer

5.6. Animals that do not have access to food or water for at least the periods outlined below are defined as restricted.

5.6.1. Table 1: Food

Frequency	Species
(Once) Daily	Dogs weaned Cats (weaned) Rabbits (weaned) Non-human primate (weaned) Guinea Pigs Hamsters Exotic species Agricultural species including poultry
Access > 18 hrs/day	Rodents (weaned)
Ad libitum	Birds (all ages)
As per recommendation of veterinarian	All other animals

5.6.2. Table 2: Water

Frequency	Species
Ad libitum or unlimited amount for more than 1 hour twice daily	Non-human primate (>1 yr old)
Access > 18 hrs/day	Rodents (weaned)
(Once) Daily	Guinea Pigs Hamsters Rabbits
Ad libitum or as often as necessary for the health and comfort of the animal	Exotic species Agricultural species
Ad libitum	Dogs (weaned) Cats (weaned) Birds (all ages)
As per recommendation of veterinarian	All other animals

5.7. Food and water provisions to animals outside of the above tables (e.g., a fast of 6 hours or longer in rodents) constitute restriction and must adhere to the following:

5.7.1. Description within the animal use protocol including:

5.7.1.1. The method and duration of restriction (complete restriction for a set period, vs. a percentage of the animal's daily ration over a period of time)

5.7.1.2. Scientific justification of the food and/or fluid restriction

- 5.7.1.2.1. Using scheduled access to food or fluid sources may be justified by describing procedures based on performance standards that assure adequate maintenance of hydration, body weight, and behavioral and clinical health
- 5.7.1.2.2. The degree of food or fluid restriction necessary for consistent behavioral performance is influenced by the difficulty of the task, the individual animal, the motivation required of the animal, and the effectiveness of animal training for a specific protocol-related task
- 5.7.1.3. Procedures for selection and training of animals, as needed
- 5.7.1.4. The collection of baseline physiological data, as needed
- 5.7.1.5. Any research specific endpoints related to the restriction and the course of action (euthanasia, intervention including return to unrestricted access to food/water, removal from study) for animals that reach those endpoints
- 5.7.1.6. If animals are receiving their daily ration of food/water as part of the experimental procedure, the protocol must clearly define when and how food and water will be provided on days when no experiments are conducted
- 5.7.1.7. Any additional enrichment planned for the period of restriction
- 5.7.1.8. The method(s) and frequency of monitoring in order to assure animal health for:
  - 5.7.1.8.1. ongoing restriction resulting in planned weight loss compared to ad libitum weight or conspecifics
  - 5.7.1.8.2. repeated short-term food restriction lasting less than 24 hours (48 hours for ruminants), or water deprivation of less than 24 hours (any species), without a return to normal eating and starting body weight between events
  - 5.7.1.8.3. Animals deprived of food for longer than 24 hours (48 hours for ruminants), or of water for 24 hours for any species, when repeated for more than a single event
  - 5.7.1.8.4. Diligent record keeping on daily food or fluid consumed, hydration status, appearance, general affect, behavioral changes, plasma osmolality (if determined), experimental performance, and routine body weights (as applicable) should be readily available for review by the veterinary staff and IACUC. (See Monitoring: methods and frequency section below)
- 5.7.2. Acclimate animals to new restriction paradigms gradually. Ideally, during acclimation, the animal should not lose more than 10% body weight in a week
  - 5.7.2.1. For studies that require body weight loss greater than 10%, restrict food gradually to allow for physiological and psychological adaptation
- 5.8. Animals deprived of food for longer than 24 hours (48 hours for ruminants), or of water for 24 hours for any species, when repeated for more than a single event may be considered prolonged restriction and placed in Category E.
- 5.9. Animals experiencing food/fluid regulation resulting in more than 20% loss of body weight compared to ad lib fed age- and sex- matched conspecifics may be placed in Category E.
- 5.10. Special considerations for water restriction
  - 5.10.1. To ensure that the total amount of water an animal receives is adequate to meet daily needs, it is common to either allow the animal to work to satiation during the experiment or to provide a period of free access to fluid after the experiment.
    - 5.10.1.1. Animals often drink to satiation within the first 30 minutes of access to a water source, even if it is available for longer.
    - 5.10.1.2. When water is restricted, food consumption will also often decrease. Therefore, food should be available during periods when water is provided or shortly after, to encourage eating. This will also allow animals to consume solutes to help maintain fluids in circulation.
- 5.11. Monitoring: methods and frequency
  - 5.11.1. It may be necessary to monitor both food and fluid intake if regulation of one influences consumption of the other.

- 5.11.2. Percent (%) Body Weight (BW) either as a % from when animal was fed ad libitum prior to restriction or as compared to a matched conspecific (as applicable to the study design and/or age or growth stage of the animals)
  - 5.11.2.1. For food regulation studies, an animal's BW should not decrease by more than the percentage approved in the animal use protocol (e.g. 15-20% of their pre-restricted body weight. If an animal reaches the percentage weight loss indicated (early removal criterion), it should be euthanized or placed back onto the normal diet for that species.
  - 5.11.2.2. Body weights should be recorded as outlined in the AUP
    - 5.11.2.2.1. Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (the *Guide*, 8 ed.).
    - 5.11.2.2.2. Measure body weights at physiologically appropriate times (i.e., before and after periods of water deprivation may be useful)
    - 5.11.2.2.3. Scales used to weigh animals must be properly calibrated and should be checked periodically throughout the study (e.g., monthly)
  - 5.11.2.3. Calculation of % loss of BW
    - 5.11.2.3.1. Obese animals: Should be calculated from ideal BW, not starting obese weight. If an animal is obese and on calorie restriction but is intended to be used in a restriction protocol, the weight should be initially recorded when the animal reaches an "optimum" healthy weight. Subsequent weight loss calculations should be derived from the initial optimum healthy weight not the starting obese weight.
    - 5.11.2.3.2. Growing Animals: Young or growing animals are especially sensitive to fluid restriction and malnutrition. Special concern for their health and minimum growth requirements must be met.
      - 5.11.2.3.2.1. Body weight for growing animals should not be compared with the animal's baseline body weight. Instead, animals should be maintained within a specific percentage (i.e., 85%) of an age/sex/strain-matched control littermate with ad libitum food and water. If no control conspecifics are present, vendor growth charts can also be used as comparison.
      - 5.11.2.3.2.2. An animal should not be more than 20% below normal weight for that age group, unless otherwise approved in the AUP.
- 5.11.3. Measuring amount of food/fluid consumed
  - 5.11.3.1. The amount earned during experimentation plus any other "free" food or water provided must total to an amount sufficient to maintain the animal in a healthy state
  - 5.11.3.2. Fluids must at least meet daily maintenance amounts, which vary widely by species, strain, environment, and efficiency of fluid-saving mechanisms. The animal must be provided with enough fluid to replace calculated daily losses, which may vary depending on the physiology of the animal (diabetes, etc.).
  - 5.11.3.3. Daily recording of food or fluid intake amounts is recommended
  - 5.11.3.4. The diet should still be complete and balanced to meet the species' need (e.g. still providing a source of vitamin C to NHPs and guinea pigs)
- 5.11.4. Solid and fluid waste output and moistness of feces
  - 5.11.4.1. Animals adapting to food or fluid restriction may produce concentrated urine or have diminished quantities of excrement that are less moist than normal. Absence of urine or feces (e.g., few or no fecal pellets for > 24 hours) indicates inadequate intake and should be reported to the veterinary staff.

5.11.5. Clinical signs of distress

5.11.5.1. An animal with a Body Condition Score (BCS) of 2/5 or less is considered under conditioned and should be reported to CMP/PAR/PRF veterinary staff.

5.11.5.2. The texture and elasticity of the skin are important indicators of an animal's hydration status.

5.11.5.2.1. The skin should return to normal position rapidly after being pinched. If the skin does not return to its normal position the animal is considered dehydrated and should be reported to veterinary staff.

5.11.5.2.2. Other clinical signs of illness, including lethargy, a rough coat, sunken eyes, dry mucous membranes, and poor performance on behavioral tests should be reported to the veterinary staff.

5.11.5.3. Chemistry values

5.11.5.3.1. Serum protein levels, albumin, osmolality, BUN/Creatinine, electrolyte values, ketone levels or other acid-base imbalances indicating metabolic acidosis may be helpful in monitoring the health status of a fasted or water deprived animal. Urine specific gravity may also help establish the kidney health of water-deprived animals.

5.12. Other considerations

5.12.1. For animals with specific conditions that alter food and water homeostasis (e.g., diabetes, genetic obesity), this condition should be taken into account when reviewing a restriction protocol and the above recommendations may not apply.

5.12.2. Animals on a restricted diet should be allowed a short-term period of unrestricted feeding/drinking prior to a surgical fast to help prevent hypoglycemia or dehydration.

5.12.3. Between experiments, it may be advisable to allow the animal an unrestricted period of feeding/drinking to reestablish baseline weights and hydration status. The intake should still be gradually introduced and carefully monitored to prevent adverse effects from rapid consumption of large amounts of food (e.g., bloat).

5.13. Species-Specific Considerations for Food/Water Restriction

5.13.1. Mice & Rats: Meal-eaters rather than nibblers; most mice and rats have a circadian rhythm of feeding and are likely to eat during dark cycle; some stocks/strains which tend to eat continuously (Sprague-Dawley, Long-Evans hooded rats), may never stabilize at an ad libitum fed weight, and so at 80% of this (overweight) weight, they may not work reliably for food. Note: mice are more tolerant of food restriction than are rats.

5.13.1.1. Since rodents are more active during the dark phase, it may be more appropriate to provide the limited item (food or water) during that phase to encourage maximum intake while it is available

5.13.1.2. Some studies have indicated that for long-term restriction protocols, rodents may adapt better to water restriction than food restriction, with less overall weight loss and lower cortisol levels.

5.13.2. Hamsters: Have limited flexibility in their meal size and do not compensate for infrequent availability of food by increasing meal size.

5.13.3. Guinea Pigs: Can be more difficult and delicate than other rodents to alter food type or access; may need more highly preferred foods, because use of regular food/water reinforcers have been found to be problematic by some investigators.

5.13.4. Guinea Pigs and Rabbits: May benefit by providing hay or other roughage ad libitum to support gut health; especially when feeding pelleted diet once daily.

5.13.5. Non-Human Primates: May require more individually tailored protocols as metabolic requirements can vary greatly between individuals. Reduction to a percentage of free-feeding weight may be necessary in initial training but may be able to be more relaxed once the task is learned. Use of positive reinforcement is highly preferred e.g., administration of fruit juice.

- 5.13.6. Fish & Other Aquatics/Reptiles/Amphibians: Individual species have different needs and requirements. Please make sure that you are familiar with your individual model and consult CMP/PAR/PRF veterinarians with any questions.
- 5.13.7. Avian species: As applicable to the species, birds may experience crop compaction post fasting due to gorging behavior. Consideration should be given to volume of feed provided immediately post fasting.
- 5.13.8. Waterfowl: Under feed restriction, breeders may show stereotypic pecking on non-nutritive objects and excessive drinking of water and reduce fertility. Adequate feeder space may benefit subordinate animals and environmental enrichment devices are useful to satisfy feed-seeking behaviors.

## 6. EXCEPTIONS

- 6.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

## 7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- 7.1. References:
  - 7.1.1. OLAW:
    - 7.1.1.1. [PHS Policy on Humane Care and Use of Laboratory Animals](#)
    - 7.1.1.2. <https://olaw.nih.gov/faqs#/search/442/restriction>
    - 7.1.1.3. [Guide for the Care and Use of Laboratory Animals](#)
  - 7.1.2. USDA:
    - 7.1.2.1. [Animal Welfare Regulations 9 CFR Ch.1 Subpart C § 2.31](#)
    - 7.1.2.2. [Animal Welfare Inspection Guide](#)
  - 7.1.3. American Society of Animal Science: [The Guide for the Care and Use of Agricultural Animals in Research and Teaching](#)
- 7.2. [IACUC/AWO Referenced Documents](#): (requires TAMU NetID authentication)
  - 7.2.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
  - 7.2.2. TAMU-G-002 IACUC Guidelines on the use of Anesthesia and Analgesia
  - 7.2.3. TAMU-G-013 Guidelines for Surgical Procedures in Rodents
  - 7.2.4. TAMU-G-018 IACUC Guidelines for Performing Surgical Procedures in Non-Rodent Mammals
  - 7.2.5. TAMU-G-035 Guidelines for Surgery in Fish
  - 7.2.6. Body Condition Score Charts
    - 7.2.6.1. AWO-O-058 – Mice & Rats
    - 7.2.6.2. AWO-O-059 – Rabbits
    - 7.2.6.3. AWO-O-060 – Dogs
    - 7.2.6.4. AWO-O-061 – Cats
    - 7.2.6.5. AWO-O-062 – Guinea Pigs
    - 7.2.6.6. AWO-O-063 – Pigs
- 7.3. Resources:
  - 7.3.1. Heiderstadt K.M., McLaughlin R.M., Wright D.C., Walker S.E., Gomez-Sanchez C.E. The effect of chronic food and water restriction on open-field behavior and serum corticosterone levels in rats. *Laboratory Animals* 2000; 34 (p.20-28)
  - 7.3.2. Hughes J.E., Amyx H., Howard J.L., Nanry K.P., Pollard G.T. Health effects of water restriction to motivate lever-pressing in rats. *Lab Animal Sci* 1994; 44 (2) p. 135-140
  - 7.3.3. Morrison, Evan, Ator, Nakamura. 2002. Report of a NIH workshop: Methods and Welfare Considerations in Behavioral Research with Animals. Chapter 4: Manipulation of Food and Fluid Access.
  - 7.3.4. NRC 2003, [Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research](#), Washington: National Academies Press)



- 7.3.5. Rowland NE. Food or Fluid Restriction in Common Laboratory Animals: Balancing Welfare Considerations with Scientific Inquiry. Comparative Medicine, April 2007; 57(2): 149-160.
- 7.3.6. Toth, L.A., Gardiner, T.W. Food and Water Restriction Protocols: Physiological and Behavioral Considerations. Contemp top lab animal sci, 2000; 39(6) p. 9-17.
- 7.3.7. Tucci V., Hardy A., Nolan P.M. A comparison of physiological and behavioral parameters in C57BL/6J mice undergoing food or water restriction regimes. Behavioral Brain Research 2006; 173 (p.22-29)

7.4. For more information on food and water restriction, please contact:

- 7.4.1.1. [CMP](#): at (979) 845-7433
- 7.4.1.2. [PAR](#): at (713) 677-7471
- 7.4.1.3. [PRF](#): at (361) 221-0770
- 7.4.1.4. Sea Life Center: at (409) 740-4574

7.5. Acknowledgement

- 7.5.1. This document was partially prepared using materials obtained from McGill and the University of Michigan.

**8. HISTORY**

Effective Date	Version #	Description
08/01/2022	000	College Station/Dallas/Galveston: New document; Reviewed and approved via email.
10/01/2022	001	Houston/Kingsville: New document; Reviewed and approved via email.
10/20/2022	002	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.