

IACUC POLICY AND PROCEDURE STATEMENT**Policy/Procedure:** Food/Fluid Restriction**Policy/Procedure ID:** 08-02-001**Effective:** February 15, 2008**Revised:** March 26, 2014**A. Background**

A large body of published research exists demonstrating the utility to animal research of limiting access of research animals to food or fluids, or limiting the amount of food or fluids research animals consume on a daily basis. The Animal Welfare Act and Regulations (AWAR) §2.38 (f)(2)(ii) prohibit deprivation of food or fluids to train, work, or otherwise handle animals unless short-term withholding of food or water from animals is allowed in an Institutional Animal Care and Use Committee-approved activity that includes a description of monitoring procedures. The *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy) (II; IV (A)(1)) requires that institutions comply with the AWAR and the *Guide for the Care and Use of Laboratory Animals (the Guide)*.

It is the responsibility of the Bowling Green State University IACUC to ensure judicious and humane use of animals in its teaching and research programs, consistent with Federal requirements and BGSU's ethical standards. The *Guide* states that "Animals should be fed palatable, uncontaminated diets that meet their nutritional and behavioral needs at least daily, or according to their particular requirements, unless the protocol in which they are being used requires otherwise" (p. 65), and that "Animals should have access to potable, uncontaminated drinking water according to their particular requirements" (p. 67).

The United States Department of Agriculture Animal Care Policy #11 recognizes that restriction of food and/or fluid intake beyond that necessary for normal pre-surgical preparation has the potential to cause unrelieved pain and distress in animals. However, the *Guide* recognizes that "Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols" and "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. 30-31). The *Guide* also requires that "Written records should be maintained for each animal to document 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" (p. 31).

The 8th edition of the *Guide for the Care and Use of Laboratory Animals* (2011) defines procedures in which animals' access to food and/or fluids is restricted, but in which animals are allowed to consume food *ad libitum* during the access period as "Scheduled Access" procedures. In contrast, procedures in which the total weight of food or volume of fluid consumed is strictly controlled and monitored are defined as "Food/Fluid Restriction" procedures. In accordance with the 8th edition of the *Guide*, this Policy and Procedure Statement adheres to this differentiation between procedure types.

B. Definitions

1. Ad libitum food or fluid

Unrestricted food and/or fluid consumption, with the quantity and frequency of consumption being the free choice of the animal.

2. Scheduled access to food/fluid

In accordance with definitions found in the 8th edition of the *Guide for the Care and Use of Laboratory Animals*, "scheduled access" to food and/or fluid is defined as a regimen in which an animal is allowed to consume as much food or fluid as desired at scheduled intervals.

3. Food/fluid restriction

The 8th edition of the *Guide* defines "food or fluid restriction" as a regimen in which the total weight of food or volume of fluid consumed is controlled, limited, **and strictly monitored**.

4. Baseline weight

The stable weight of individual animals calculated before the animals are placed on any scheduled access or food/fluid restriction regime.

In adults with *ad libitum* access to food and fluid, baseline weight is calculated by averaging the body weight values recorded for at least three consecutive days immediately prior to the restriction period. These body weights must be within $\pm 2.5\%$ to indicate a stable weight[†].

In growing animals, baseline weights should be periodically recalculated based on standard growth curves for the species and strain of animal.

Procedures for recalculating the baseline weight of growing animals will be determined on a protocol-specific basis, with consultation between the PI and Attending Veterinarian.

5. Target weight

The weight at which researchers **should** attempt to maintain any animal placed on food restriction. This may be determined on a protocol-specific basis by the researcher, based on needs, but should not be less than 87% of the calculated baseline weight for each animal. For example, a rat with a calculated baseline weight of 400 grams should be food restricted to a target weight that is 87% of 400 grams, or 348 grams.

- a. See Appendix A for Pigeon Specific Target Weight

† For pigeon-specific procedures, see Appendix A.

6. Critical weight

The minimum body weight of any animal placed on a food restriction protocol. This is 85% of calculated baseline weight of each animal. For example, for a rat with a calculated baseline weight of 400 grams, the critical weight will be 340 grams (85% of the baseline weight of 400 grams).

b. See Appendix A for Pigeon Specific Critical Weight

7. Dehydration

Excessive loss of water from the body, as from illness or fluid deprivation. Signs may include dry mucous membranes, decreased urination, decreased elasticity to the skin or sunken eyes, frequent blinking (birds). Severe dehydration, i.e. greater than 10% of body fluid, may lead to shock and death.

C. Policy

Placement of animals on Scheduled Access or Food/Fluid Restriction **must** be scientifically justified in an IACUC protocol and approved by the IACUC **prior to** initiation of the associated research. Because these procedures are carried out for many reasons, the IACUC will consider the maximum duration and type of restriction on a case-by-case basis with reference to the welfare of the animals (including consideration of the species and normal biology of the animal) and the goals of the particular study. The Animal Use Protocol proposal should include the minimum and maximum duration that the procedures will be imposed on the animals, type of procedure (Scheduled Access or Food/Fluid Restriction), and the effect on the animals that is believed to be necessary to achieve the stated study objectives.

In accordance with the *Guide*, in the case of conditioned-response protocols, a positive reinforcement such as a preferred food or fluid, is recommended instead of Scheduled Access or Food/Fluid Restriction procedures.

D. Procedures

Calculation of baseline weights

- 1) The Investigator or member of the research lab staff will weigh and record the *ad libitum* weight of each animal for three consecutive days at approximately the same time ± 2 hours each day.
- 2) After recording the weight on the 3rd day, the average weight will be calculated. The weights recorded each day will then be compared to the average. If the variation in the recorded weights is less than $\pm 2.5\%$ each day, the animal's *ad libitum*-fed weight is considered stable, and the average will be used as the baseline weight, from which target weights and critical weights will be calculated by the Investigator or member of the research lab staff.
 - a. See Appendix A for pigeon-specific baseline weight calculation procedures.

- b. Training on performing these calculations can be provided by the Investigator, or by the UAF Director upon request.

Scheduled Access or Food/Fluid Restriction procedures may not be initiated on any animal until it has been demonstrated, using the above method, that a stable *ad libitum*-fed weight has been achieved. If an animal has not reached a stable weight, as indicated by variation greater than $\pm 2.5\%$ on any of the three consecutive days, the procedure described in 3# will be continued each day until a stable weight is reached.

- 3) After the target weights and critical weights have been calculated, Scheduled Access or Food/Fluid Restriction procedures may begin. Cages housing animals on these regimes must bear an appropriate indicating tag, placed on the cage card holder. Tags are available from the UAF staff upon request.

On-going studies and record keeping

- 2) It is the responsibility of the Investigators to provide food/fluid to animals on Scheduled Access or Food/Fluid Restriction regimes. It is expected that animals will be weighed and fed at approximately the same time each day, ± 2 hours.
 - a. See Appendix A for Pigeon Specific feeding and weighing procedures
- 3) In procedures in which food intake is limited to achieve a target weight, animals must be weighed prior to providing the allotted amount of food for the day. **It is inappropriate to feed animals and weigh them immediately following a meal, as this will result in exaggerated weights.**
- 4) Animal weights and food or fluid intake of all animals used in Scheduled Access or Food/Fluid Restriction procedures must be measured and recorded **daily**, unless otherwise justified, and approved by the IACUC.

It is the responsibility of the Principal Investigator to ensure that all measurements are taken and all records are filled out daily.

- 5) In addition to records maintained for use by the investigator's lab, weight measurements and daily food/fluid intake of each animal must also be recorded using a Food/Fluid Intake Log. This record is used to monitor food/fluid intake and weight of animals on Scheduled Access or Food/Fluid Restriction regimes. UAF staff will monitor this record daily to ensure that all animals are given proper access to food/water, and have not dropped below critical weight. This record must remain in, or immediately outside, the animal housing room and must be readily available for inspection at any time by the Attending Veterinarian, UAF staff, IACUC, and any appropriate regulatory agencies or accrediting organizations. If the records are not maintained in the same location in which the animals are housed, they must be maintained as close as possible to the housing area and notation regarding their location is required (contact the UAF staff for assistance in this regard.).

Weight/feeding records must indicate that Scheduled Access or Food/Fluid Restriction has been approved by the IACUC, and must include the **current** IACUC protocol number, the target weight of the animal, the baseline weight

upon which the target weight is calculated, time(s) of feeding/access to fluids, weight/volume of food/fluid consumed, and the initials of the animal handler.

- 6) If an animal is discovered without access to food in the course of daily room checks, but no tag indicating scheduled access or food restriction is indicated, reasonable attempts will be made to determine the status of the animal. If the UAF staff member is unable to determine positively whether an animal's access to food/fluid should be restricted and the animal is without food or fluid, **the UAF staff will be obligated to provide food or fluid for the animal in question.**
- 7) In the event that animals used in Scheduled Access or Food/Fluid Restriction Procedures will not be used for testing or collection of data for more than one week, or if no member of the Investigator's lab staff will be available to provide food/fluid to the animals (no matter the duration of time), they must be removed from the Scheduled Access or Food/Fluid Restriction regime and allowed *ad libitum* access to food and fluids.

Unforeseen Circumstances

- 1) If unforeseen circumstances (e.g., illness or injury to a bird participating in the experiment or to the person conducting the experiment) cause a bird having to be placed temporarily (few hours to 1 week) back on free-feeding even though the current experimental series has not yet been completed, then the most recent baseline weight used for this birds to calculate target and critical weights will be reused upon resumption of food restriction.
- 2) If unforeseen circumstances (e.g., equipment malfunction) causes a bird on food restriction not to be worked with in the experimental apparatus within the seven-day time frame normally required whilst on food restriction, then the PI has 48 hours to resolve the issue before having to place the bird back on *ad libitum* food.

Adverse events

- 1) Any animal with limited access to fluid must be observed carefully at least daily by trained animal care staff. If the animal shows signs of dehydration, the staff will notify the Principal Investigator, and will contact the Attending Veterinarian immediately. If the AV determines that the animal's health is compromised, an appropriate course of action will be recommended and initiated immediately. This course of action may or may not include removal of the animal from the study.

- 2) If the weight of an animal under food and/or fluid restriction falls below its critical weight, food and fluid intake will be increased immediately by the research team until the animal regains at least its target weight. If the animal falls below its critical weight for two or more consecutive days, then the animal user(s) must also inform the UAF Director, or a member of the UAF staff, immediately. The UAF staff will notify the Attending Veterinarian. If the AV determines that the animal's health is compromised, an appropriate course of action will be recommended and initiated immediately. This course of action may or may not include removal of the animal from the study.
- 3) In the event that animals are removed from study due to dehydration or an underweight condition, the decision of whether and when the animal will be allowed to return to the study rests solely with the Attending Veterinarian.

Appendix A: Pigeon-specific food restriction procedures

All procedures described in section D of this policy apply equally to food restriction procedures used in pigeons, with the following exception:

- 1) During calculation of baseline weights, variation in the recorded daily weights must be less than $\pm 5\%$ each day (versus $\pm 2.5\%$ used for rats or mice). This larger variability is permissible to take into account egg laying and variation in crop fill at the time of weighing.
- 2) For pigeons the target and critical weights will be calculated as 82% and 80% of the baseline weight for each bird.
- 3) It is expected that animals will be weighed at the approximately the same time each day, ± 2 hours. It is expected that animals will be fed at least once every 24 hours.