Lab Diet Management

a look at feed and diet handling, affecting research, and the concerns of the lab animal manager

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first, some thoughts

- back in the old days
  - animals were fed the scientist’s or family’s leftovers
- today’s terminology, what is food
  - call it feed, if it comes packaged
  - call it diet, if it’s specially prepared
  - call it slop?, only for pigs
- nutrition is relatively easy today
  - we take from the bag and provide it to the animal
  - but, potential issues could be overlooked through a complacent approach to nutriture
now, some new thoughts

- in these new days, we have
  - zebra fish
  - exotics
  - dietary catalysts
  - nutri-ceuticals
  - nutri-phenomics

- moreover, an improved understanding of nutrition that says, what if ... ?
Feed and Nutrition

- how do we know what is healthy
- what is nutritious
- what about dietetics
- what about food allergies
- what do we feed animals in general

lab animal concerns:
- off feed, anorexia, bulimia
- vomiting and diarrhea
- post-op care
- hydration
- nutrient intake
- increasing palatability
- etc.
What’s in food/feed

**The nutrients**
- protein
- carbohydrate
- fat
- minerals
- vitamins
- water

**Other stuff**
- preservatives (anti-oxidants)
- non-nutritive items (drugs)
- contaminants (glass, plastic, insects, etc.)
- heavy metals (arsenic, lead, mercury)
- flavorings
- colorings
- pesticides
- and more
<table>
<thead>
<tr>
<th>Feed or diet? different terms</th>
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<td><strong>Standard feed</strong></td>
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<td><strong>Test or medicated diet</strong></td>
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<td><strong>Semi-purified, purified, or chemically-defined diet</strong></td>
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<td><strong>Therapeutic or dietetic diet</strong></td>
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- **Other terms**
  - fixed formula, ingredients used remain constant
  - variable formula, Inclusion rate and ingredients can be changed batch to batch
  - open and closed formulae
different digestive systems

The process of decomposing organic matter (as in sewage) by bacteria.

The organic process by which food is converted into substances that can be absorbed into the body.
different players in animal nutrition
(USA, others in Europe and Asia)
facility design
incorporating feed processing

- receiving dock
- location of feed storage room
- diet preparation kitchen
- pest management
- containment
  - HVAC, dust, waste
- storage of specialty or test diets for PIs
receipt of feed products

- receiving area
  - dedicated
  - does it serve as the waste path, too?
- ease of cleaning the area and path to storage
  - bird nests and fecal material present
- condition of the delivery truck
  - vendor-owned, UPS, common carrier
SOP:
Sanitation of the delivery truck

PURPOSE:
- To carry out the cleaning and sanitizing of company owned, leased or rental vehicles. The cargo areas of feed and bedding delivery vehicles shall be cleaned and sanitized at the end of each day to insure that appropriate sanitary conditions are maintained during the shipping of feed/bedding products from Harlan facilities to customers or other Harlan facilities.

SAFETY:
- Follow instructions provided by the manufacturer and on the Material Safety Data Sheets for safe handling and use of detergents and chlorine dioxide (i.e. MB-10). Always wear gloves when preparing or using chlorine dioxide (MB-10) sanitizing solution or handling materials, which are still damp from the chlorine dioxide solution. Material Safety Data Sheets for products used to clean and/or sanitize animal holding rooms shall be maintained by local Harlan Transportation Services Departments and by the Administrative Department. Only persons trained in the safe and effective use of cleaning and sanitizing products shall be permitted to do so. Unless otherwise directed by the U.S. Fleet Manager and/or Vice President of Teklad Operations, the Transportation Services Hub Manager is responsible for insuring that employees have been trained in the safe handling and use of any/all chemicals employed in the cleaning and sanitizing of company delivery vehicles.

INSTRUCTIONS:
- Remove trash and personal items from the cab. Remove dirt and debris from the seats and floor. Wipe the dash and accessory equipment with a sponge dampened with clean water and a cleanser as necessary. Clean the windows and windshield as necessary.
- Clean all surfaces of the cargo area by applying detergent solution (e.g. Breakthrough, Hotsy Corporation Detergent Division, Inglewood, Colorado; Esterville, Iowa) and rinsing with clean water. Utilize a pressure washer for straight trucks and trailers or a sponge or sponge mop and a water hose for vans if a pressure washer is not available. Scour surfaces of the cargo area as required.
- After cleaning the cargo area, spray or fog with fresh (i.e. no more than 10 days after preparation) chlorine dioxide sanitizing solution. Apply the solution to sufficiently wet all surfaces of the cargo area. Take care to minimize the amount of solution entering the driver's compartment, dash and under-dash area, as the solution is corrosive. Indicate that the vehicle has been cleaned and sanitized by checking the appropriate box on your Trip Report. Allow vehicle cargo area to dry prior to loading.
transport of feed products

just how far is it from ...
... the receiving area ...
... to the storage area ...
... to the usage areas

- ergonomic issues
  - adoption of the 15 kg bag standard
  - how many times handled
- carts and trollies
- transport dollies
- ease of cleaning the vehicle
  - can it go through the washer?
inspection of feed products

- what else was in the delivery truck that could be a contaminant?
  - just plain dirty, grimy, smelly, etc.
  - spider webs, mold, feed

- examine packaging
  - peculiar odors (rancid)
  - torn bags, dented or expanded or rusted cans, stained cartons
  - missing identification or labels
storage of feed products

- in the vivarium or PI lab?
- a room, a shelf, a refrigerator, a freezer
- signage: no food for human consumption to be stored herein
- get rid of the cardboard and paper!
- rotation: FIFO – first in, first out
- labeling, identification, and ownership
  - set the expiration date
  - whose is it, how old is it (IACUC and AAALAC)
- Need a sign
what’s wrong here?
- not a lab animal facility -
storage of feed products

- dunnage racks
- disposable, cleanable plastic pallets
- “taping” clear area in the storage room for sanitation purposes
protecting feed/diet

- effects of air ($O_2$, $N_2$, $CO_2$, $H_2O$)
  - rancidity through oxidation
  - adding nitrogen gas to bags
  - gas tight or permeable containers
- effects of light
  - destruction of vitamins
- effects of temperature
  - frozen (-80°C, -20°C), refrigerated (4°C), room (20°C)
  - ice crystallization
- effects of moisture
  - low, drying/desiccating out the product
  - high, causing microbial growth
- interaction with the container
  - leaching of minerals in metal or BPA or plasticizers in plastics
- cross-contamination from fines or dust
storage room environment

- easily cleaned surfaces
  - metal or cinder block walls
  - epoxy or monolithic flooring
  - floor drain?
- controlled and monitored temperature
  - cool/refrigerated or freezer
- controlled and monitored humidity
  - low but not drying
- controlled and monitored lighting
  - automatic timers and sensors
- controlled and monitored air flow
  - to draw away odors
what does certified diet mean to you?

Certified diets meet the requirements of the FDA's GLP (Good Laboratory Practices) program. Certified diets are assayed prior to shipment, and each bag is certified not to exceed maximum concentrations of key contaminants (heavy metals, aflatoxins, chlorinated hydrocarbons, organophosphates, and specified nutrients).
what does certified diet mean to you?

- pesticides?
  - malathion and others have declined in analyses
  - newer pesticides aren’t in typical profiles
- PCBs, poly-chlorinated biphenyls?
  - have declined to less than detectable levels
- heavy metals?
  - Hg, As, Cd, Pb, Se
- microbial contamination (actually the toxin)?
- GLPs?
  - The regs (EPA/FDA) require PIs to analyze the diet for contaminants that might be expected to influence studies in tox and drug safety testing
  - CFR 21 58.90 (g): “Feed and water used for animals shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed and water are not present above those specified in the protocol. Documentation of such analyses shall be maintained as raw data.”
- Review your own list for what is appropriate per study and acceptable for maximum levels
components of sanitation

- inspecting the packaging containers
- decontaminating the container
  - spray off, misting chambers
- cleaning floors
  - sweep, mop, vacuum
- ease of wiping down racks
- dip or dunk tanks (bleach)
- feed as a source of contamination
  - dust from diet preparation
- feeding implements, cutting boards
  - micro-organisms (salmonella)
  - cross-contamination

courtesy of Richard Hitzelberg
ViraTek, Inc.
feed/diet QC/QA

- assessing the manufacturer’s qualifications
- dating
  - mill date, expiry date
- contamination control
  - rust/Fe, faucets/Cr, other metals
  - air filtration – spores, particles, etc.
feed selection
- what kind of nutriture -

- appropriate to the species
  - National Resource Council books
  - providing nutrients that are essential
  - balanced diet for growth, maintenance, and repair of the body of any living organism

- appropriate to the stage of life
  - pregnant, lactating, sick, growing, adult, elderly
  - colostrum, milk, solid/liquid diet

- experimental requirements
  - special diet
  - restricted consumption vs. ad libitum
feed types

- extruded
- precision pellets
- soft, mashed, or dough diet
- purified, semi-purified
- natural ingredient
  - hay, corn, fish, insect, meat, etc.
- constant formula
- sterile: autoclaved, pasteurized, or irradiated
- canned, fresh, frozen
- dry, semi-moist, moistened
- perishable vs. stable/prepared
the variety of diets
thinking about phytoestrogens

- can confound results in studies with estrogens or indicating a sex-linked pathology
- present in most diets
  - isoflavones (genistin and diadzin) in soya
  - coumestans (coumestrol) in alfalfa
  - lignans in plants
- being soy-free does not mean estrogen free
- consider the potential effects on your in-house breeding program
pelleted diets

- 5/8” oval pellets have traditionally been fed to mice and rats
- 3/16” round pellets are fed to guinea pigs, rabbits, and swine
- ingredients are combined in a mixer
- steam is applied which gelatinizes a portion of the starches
- pellets are made by forcing the partially gelatinized diet mixture through a die

extruded diets

- diets for dogs, cats, and non-human primates are extruded
- the extruder is used to produce a wide variety of diets in various shapes and sizes
- extruded diets are processed at a higher moisture, temperature, and pressure conditions
- greater destruction of microorganisms during manufacture
autoclaving versus irradiation

- vendors offer Co$^{60}$ gamma irradiation services

- Yes, you can autoclave ‘regular’ diet, but in doing so the nutritional value will change. So, don’t.

- Yes, you can feed un-autoclaved diet that is meant to be autoclaved, but the nutritional value won’t be what it is supposed to be. Nutrients destroyed by high heat may be present at excessive levels. So, don’t.

- autoclaving can result in “brick” diet, a possible issue for weakened incisors
making your own diet?

Considerations

- not always so simple
  - recipes, “kitchen,” equipment
- downfalls
  - over-mixing, under-mixing
  - over heating
  - nutrient changes
  - homogeneity
- dosed diets
  - safe mixing of potentially hazardous compounds
  - radioisotopes, carcinogens,
  - DOT issues
- helpful companies
mixing your own diet

- P-K or Patterson Kelley machines
- Hobart machines
- blenders
QC sampling

- analyses, examples
  - nutrients
    - proximate analysis
    - story of melamine
      - nitrogen?
  - microbes
    - bacterial, mold
  - sensory
    - odor
  - consistency
    - fines, dust, powdery
    - granular, solid
    - homogeneous
  - non-food items
- saving a reserve sample

Bag Sampling Technique

Insert, Slot Down

Turn and withdraw, slot up
determining shelf life

- accepting the manufacturer’s recommendation
- based on the most short-lived ingredient
- based upon storage conditions, too
  - temperature
  - humidity
  - light
- varies whether in storage or in the cage
- what about specialty diets in cups and standard feed in hoppers?
some scenarios

- It’s winter time in the northeast. A new study with guinea pigs is to start next week and your feed order with 5321 is scheduled for Thursday. A 3’ snow storm (or hurricane or earthquake) hits and trucks can’t make deliveries. What are you going to do?

- The same truck was going to deliver NHP feed. You have some old stock, but the expiry date is next week. What are you going to do?

- Vitamin C is the issue, the determinant of shelf-life.
types of feeding equipment

- “J,” slotted hoppers
- bowls
- timed feeders
- calibrated feeders
- devices for liquid diets
powdered diet feeders
feeders for enrichment
metabolism cage

- about $1000
- can be gas tight
  - oxygen in
  - carbon dioxide out
- measures input
  - water
  - food (powdered diet)
- measures output
  - feces
  - urine
  - separation of same
- urine can be kept cold to avoid evaporation and degradation of contents
feed room pest management

- pheromone strips for insects
  - cockroaches, beetles, flying insects
- booklouse problem in paper products
- flour beetles, Indianmeal moths, weevils, etc.
- using insecticides
  - no spraying (pesticides are toxic and carcinogenic)
  - boric acid and amorphous silica
  - pyrethrins are preferred if something must be used
- my grain beetle story with ferret feed

Sawtooth grain beetle (Oryzaephilus surinamensis) is a major pest of stored grains and other stored products.
feed room pest management

- vermin traps (Victor® TIN CAT® Mouse Trap )
  - rodents, snakes, etc.
  - inside and/or outside of the building
  - sticky traps and the welfare issue of a stuck mouse
- using rodenticides (Warfarin®) or other pesticides?
- build pests out by caulking/sealing cracks
- door sweeps (fixed and falling-into-place styles)
- best is to practice good sanitation
PLAN A "PROACTIVE APPROACH TO FACILITY PEST CONTROL MANAGEMENT TODAY"

Summer months are here again. Let's be mindful of how rising temperatures impact your feed, bedding and work areas. Here are some things to remember:

- Inspect your incoming products from all vendors including paper and boxed items:
- Rotate stock regularly (first in, first out) - Track Date Codes
- Don't overstock during the summer months
- Do not store any seed or grain items. Consider Refrigeration
- Implement/ Monitor Internal Pest Control Program
- Monitor Air Conditioning, Humidity and Airflow in Feed Storage and Animal Rooms

**Internal Audits**

- Lunch or Coffee Rooms ~ watch for food products left out. Be mindful of your trash receptacles.
- Autoclave/Cage Wash Areas ~ Moisture is a breeding place for insects. Include regular inspections of drains and dirty bedding dispenser area
- Automatic Bedding Dispenser ~ Have a plan for cleaning on a regular basis
- Spills and Fines ~ Be sure to clean up spills immediately and clean the fines out of Bins and Containers
- Locker Rooms and Storage Areas
- Hallways and Offices
- Washroom and Shower Areas ~ inspect drains regularly, beware of standing water

**MOST IMPORTANTLY ~ UTILIZE AND RELY ON YOUR PEST CONTROL EXPERTS**

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT JODI BARB AT (800) 289-2469 ext 215 OR DANI CARTWRIGHT AT ext 212.
Pest Control Guideline List

Weekly Inspection /Monthly Inspection
(Circle One)

- Inspected incoming products from all vendors including paper and boxed items
- Stock Rotated (first in, first out)
- Date codes tracked
- Reviewed Internal Pest Control Program
- Inspected Air Conditioning, Humidity and Airflow in Feed Storage
- Washroom and Shower Areas inspected for standing water
- Automatic Bedding Dispenser Cleaned
- Autoclave/Cage Wash Areas inspected
- Lunch, Locker, and Storage Rooms Checked for spillage or left out food
- Storage Areas inspected for over stocked items
- Seed and Grain Items clear of Storage

UTILIZE AND RELY ON YOUR PEST CONTROL EXPERTS

___________ Date ___________  __________ Inspected By

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT US AT (800) 289-2469 ext 215 OR ext 212.
Subject: [TECHLINK] Bullfrogs

Thank you for the information on bullfrogs. I was telling the investigator the results and he wanted some further info. He has been trying to feed his frogs crickets without success. I told him about you guys saying you feed goldfish now he wants to know about force feeding. Anybody? To me this seems like a lousy idea.

Thanks, Bill McAllister LVT, VAWNY Healthcare System

A diet of only goldfish would not be nutritionally advisable, they are very fattening. If you're going to consider fish in the diet, consider minnows or guppies. However, I don't think it's a great idea. Bringing fish in would be a great way to expose your frogs to diseases. Also, depending on what your IACUC decides, you may well need an IACUC protocol to cover the goldfish (they are vertebrates, after all, and being fed to a hungry frog is not an approved method of euthanasia). I would do force-feeding as a last resort only. First I would try to find out why the frogs aren't eating on their own. Make sure water quality and housing conditions are reasonable. They need both wet and dry areas. Also, make sure your crickets are nutritious by feeding them ("gut-loading").

Nirah H Shomer, DVM, PhD, DACLAM  
Director, Laboratory Animal Resources  
Merck Research Labs  
Boston, MA 02115
Subject: [CompMed] Powdered diet feeders

We would appreciate nominations for feeders to place into mice boxes for feeding medicated feed for a drug trial. The boxes are on ventilated racks and the diet base will be ground rodent pellets into which the drug will be mixed. We are concerned about reliable access to the food, while at the same time reducing urine/fecal contamination and the wasteful dispersal of the diet by rodent parlour games. Thanks for your suggestions.

Thanks, Phillip T. Robinson, MS, DVM, Dipl., ACZM, University of Toledo

- I ran into a similar situation with a client, and found that the only available powdered feed holders were the ones from UniFab. They worked pretty well, but there was some waste.
- Have you considered pressing your own pellets? We do that in our department here and there if we are in need of a more customized diet.
- It might work for you too and you could probably use the normal feeders.
- For mice, I have seen a trough made out from 50 ml conical tube split vertically down the middle and positioned in the bottom of the cage so that it wouldn't spill over....they supported it some kind of way that I don't recall exactly, but it worked for a short term session.
- In a previous incarnation with a pharmaceutical company, I can recall using stainless steel rodent feeders for powdered feed. I remember the rat feeders more clearly than the mouse feeders - they were about the same size as a "low ball" cocktail glass. The powdered feed was placed in the cup, and then a stainless steel disc was placed on top of the feed. This disc was slightly smaller than the cup, and had maybe 8-10 ~1cm holes in it. This allowed the rats to access the feed but kept it relatively clean, dry, and contained. A stainless steel "rim"
- was then fitted on top which went around the outside of the cup and covered ~1cm into the center, thus keeping the disc in the cup. I don't remember our mouse feeders as clearly...they were smaller and rectangular in shape, but worked on the same principle (had a perforated insert between the animals and the feed). We did have occasional problems with the rat cups bending out of shape as they went through cagewash, but we would generally just repurpose these as primate enrichment.
mice grinding diet

Why?, many theories:
• boredom
• “psycho” mice
• “inherent” in the strain
• noise (perhaps inaudible to us)
• vibrations (e.g., HVAC)
• construction or other disruptions
• the wrong feed
malocclusion

- is heritable
- can be a problem with soft diets

courtesy of Dr. Nirah Shomer
dietary interference with imaging studies

Diet selection can affect mouse *in vivo* imaging

- Red signal is fluorescence in the 700-channel
- Prior to day 1, mouse is fed 7012 (standard diet; contains alfalfa meal)
- Day 1, mouse switched to purified diet (TD.97184) and imaged daily
- 4 days needed to clear residual from intestinal tract.

rodents have a rapid GI transit time

there are reports of PIs fasting animals 2 days to clear the “agents” like chlorophyll – an IACUC issue
dietary interference with imaging studies?

- one diet does not fit all
- that’s not to say that feeds and diets are imperfect
- know the research need and document what the animal is fed
final comments

- Feed or diet is what goes straight into the animal.
- As lab managers, we don’t necessarily need to know nutritional science. But we do need to manage the process and be aware of the concerns for our PIs.
- Components in what we and non-human animals eat can have profound effects on metabolism and can be used therapeutically.
- There are many companies that can assist us with this aspect of our jobs in support of bio-medical research.
I am indebted to:

- LAMA and the program committee
- Leo Yanas, Novartis
- Karena Thek and Jamie Lecker, Bio-Serv
- Terry Burns-Heffner, Harlan
- Dani Cartwright, Newco/PMI
- the many web sites from which I “stole” images
have you thought about ....

Why isn't there mouse-flavored cat food?

When dog food is advertised as new and improved, do you wonder who did the taste testing?

Nutrition engineering a la Dagwood Bumstead ...