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Dosing Animals Via Diet

This can be a simple and efficient method for delivering compounds to lab animals when done correctly.

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Often, experimental protocols call for dosing animals with a compound. Traditional dosing methods such as oral gavage or injections have significant drawbacks. One important consideration is the stress associated with handling the animal. The animal's stress response can affect their behaviour and metabolism, potentially confounding the parameters you are measuring. Many animal handlers will admit that such dosing can also be stressful for them as well. The other key issue is the time involved in these methods of dosing. Even an experienced person would likely take about 2 hours to gavage 100 mice—time which can be used for other purposes.

The idea of dosing animals by adding a compound to their diet has been around for quite some time and is successfully used with everything from experimental drugs to approved pharmaceuticals to food/plant extracts. When dosing via diet, the concerns of both handling-related stress and the time involved are removed.

So what should be taken into consideration when deciding how to dose animals via diet?

The researcher must first realise that oral administration (whether via diet or gavage) will almost surely produce different pharmacokinetics (what the body does to a drug) compared to an injection. This is because when taken orally, the liver and intestine have a chance to metabolise the drug before it reaches the general circulation. Injections bypass the liver and intestine and so would be expected to result in different pharmacokinetics. Furthermore, a single daily dose given via oral gavage and the same dose consumed in the diet over the course of the animal's nocturnal feeding are likely to produce different plasma levels of the compound. This may result in different effects on the biological systems being measured. Also, the researcher should determine whether any dietary ingredients have the potential to interfere with the absorption of the compound. This is likely to be more of a potential issue when using grain-based diets as compared to purified ingredient diets (see below).

Once the researcher decides to dose the compound via diet, there are other decisions to make.

WHAT TYPE OF DIET SHOULD I USE?

Lab animal diets fall into two main categories: purified ingredient diets or grain-based 'chow' diets. In a previous issue of *ALN World*'s sister publication, *ALN* (September/October 2005), we discussed in detail the differences between these types of diets. Briefly, chow diets, while completely nutritionally adequate, often contain non-nutritive substances that can affect the phenotype of the animal. Examples include phytoestrogens (which can have pro- or anti-estrogenic activity) and heavy metals, such as arsenic. Some researchers have had eye-opening experiences when they discovered that the estrogen or arsenic they were giving to the animal (either orally or via injection) had little effect on the animal precisely because they had already and unknowingly 'dosed' the animal via diet. Because the content of the relatively unrefined plant material used in chow diets can change over time and across harvest locations, there can be variation from batch to batch of chow diets. And, because each ingredient in chow diets contains multiple nutrient (and non-nutrient) materials, it is difficult to fine-tune the content of a chow diet. An example would be removing some or all of a specific vitamin from the diet—this is really not possible using a chow diet.

Purified ingredient diets, as the name implies, use highly refined materials, with the goal that each ingredient essentially adds only one nutrient. The advantages of using purified ingredients include minimal batch to batch variability, absence of extraneous materials such as phytoestrogens or heavy metals and ease of formula revision.

If a researcher decides to add a compound to a purified ingredient diet for animals previously fed a chow, they should first switch the animals to the new diet (without added compound) for at least a few weeks for acclimation and to 'wash out' any effects of the prior diet. This period of time will depend on the parameters being measured (plasma cholesterol, gene expression, etc.). Since chows and purified diets are different in many ways, feeding a new diet can change the phenotype. Therefore, it would not be recommended to simultaneously change their diet and dose them with a compound—the effects of the compound could be clouded by the diet change itself.

IS THE COMPOUND STABLE TO DIET PRODUCTION CONDITIONS?

If the researcher is concerned that their compound will be destroyed during diet production, they should ask the diet manufacturer about their methods. If the researcher wants powdered diet, compound stability should be less of a concern, since the ingredients plus the compound are simply mixed together without further manipulation (see later comments on using powdered vs. pelleted diet). If pelleted diet is desired, the water added (required for pelleting) and heat generated during pelleting are conditions that the researcher should know about, as they can potentially affect the stability of the compound. To enter some animal facilities, the diet needs to be irradiated and so the potential effects of gamma irradiation on the compound should be discussed.

CAN THE COMPOUND BE HOMOGENOUSLY INCORPORATED INTO DIET?

Most of the time, the answer is yes and the diet manufacturer should be able to tell you this based on the physical characteristics of your compound. What physical form is the compound? The best type to work with is a free-flowing powder, since it will easily mix well with the other powdered diet ingredients. If the compound is a hard crystal, it would first need to be ground into smaller, finer particles and this can be done either by the researcher or the diet manufacturer. Sometimes, the compound has a syrup-like consistency. If it is soluble in ethanol, the resulting solution can be poured into the diet, since the ethanol evaporates fairly quickly, leaving the compound evenly distributed throughout the diet. Problematic compounds include those with very high water content, such as slurries—especially if the researcher wants the compound added at a relatively high dose. High water content in lab animal diets can lead to microbial growth and inability to form a pellet, and so in these cases it would be best if the compound were first dried down to a powder. This is most often done by the researcher or a commercial freeze-drying facility.

SHOULD I FEED THE DIET AS A PELLET OR A POWDER?

Most researchers prefer pelleted diet for several reasons—it is easier to feed, easier to measure food intake, less diet is wasted and there is no chance that ingredients will 'settle out' and no longer be uniformly distributed throughout the diet. There is even the chance than animals could avoid compound rich areas in powdered diet. One reason for using powdered diets is that the researcher has the freedom to mix in the compound themselves (perhaps trying many different doses in a pilot study). In these situations, the researcher will need to adequately mix the diet plus compound. This is more difficult for the researcher to do in the case of high fat diets. High fat diet 'powders' have a consistency of something like sticky, wet sand and so the researcher should be aware that it may not be possible to homogenously mix in their compound at this stage. (Ideally, the compound would be mixed with the dry ingredients and a small amount of the fat earlier in the mixing process). Finally, if the compound is not stable to pelleting conditions (addition of water and mild heating), using powdered diet would be necessary.

HOW CAN I CALCULATE THE DIET DOSE?

In order to determine how much compound needs to be added to the diet, you will need to know 1) the daily dose you want to achieve (per kg body weight), such as 3 mg/kg, 2) the animal's body weight and 3) the animal's daily food intake. In the equation below, DD is the diet dose (in mg/kg diet), SD is single, daily dose you want to deliver (in mg/kg), BW is body weight (in g) and FI is daily food intake (in g).

$$DD = (SD \times BW)/FI$$

This obviously works best if you have an accurate idea of your animals' weight and how much they eat. Of course, growing animals will weigh more over time, but they will also eat more, and the researcher should continue to monitor these parameters in order to continuously calculate the dose over time.

A brief word here about dosing multiple diets that have different caloric densities. A good example of this is high and low fat diets, which contain a different number of calories per gram of food given that fat is more calorically dense than carbohydrate and protein. When a researcher wants to add a compound to both a high and low fat diet, they will need to decide whether to add the compound to the diets on a weight or calorie basis. A weight basis means that the compound would be added at say, 100 mg/kg in each diet. A calorie basis means that the compound would be added at say, 100 mg/4,000 calories in diet. This decision is important because very often animals will 'eat for calories', not for weight. For example, rats and mice will often eat fewer grams of a high fat diet when switched from a low fat diet because high fat diets contain more calories per gram of food than low fat diets. There is usually a period of adjustment (a week or two) after which the low and high fat fed groups eat the same number of calories of food (but not the same number of grams). If the diets were dosed with compound based on weight, the high and low fat groups would be receiving different amounts of compound each day. If they are dosed based on calories, the assumption is made that the groups will eat the same number of calories, on average, over time and therefore eat the same dose of compound.

DO I FEED THE DIET AD LIBITUM?

The above discussions (calculating the dose, dosing by weight or by calories) assumes that the animals will be given the diet ad libitum, which is the most common practice. That is, they will dose themselves in the normal course of eating food when they choose to eat it. This method clearly reduces the involvement of humans, since we only need to offer the animals plenty of food and let them eat normally.

Another, less common method would be to deliver the compound in a discrete amount of diet, something much less than their normal average food intake (metered feeding). If for example, a researcher wanted the animal to eat all of the compound in a relatively short amount of time, they could offer only one pellet (or a fraction of a pellet) containing the full day's dose of compound. Since rodents are mainly nocturnal eaters, if they were offered this small amount of diet toward the end of the light cycle, they would likely consume it as they should be preparing to become more active and eat. Another way to improve the chances that the animal will eat all of the pellet would be to first fast them for a time, increasing their drive to eat when presented with food. The drawback to these methods is that unless you watch them consume this small amount of food, you cannot be sure that they have eaten all of it, when you want them to eat it. Also, a fasted animal will have a different hormonal state and metabolism, and this itself could potentially change the effects of the compound. So while metered feeding does still have advantages over more stressful procedures like oral gavage or injection, it does require more work than pure ad libitum feeding.

WILL THE COMPOUND AFFECT THE PALATABILITY OF THE DIET?

This is often a difficult question to answer with certainty since defining palatability in lab animals is another area of study altogether. If the compound itself gives the diet a flavor or taste that changes food intake, this will change the dose the animals receive. However, if the dose in the diet is relatively small, it is unlikely to have any measurable effect on food intake from a palatability perspective. In addition, unless the compound is toxic, the animals will typically eat the diet since it is their only source of food. Of course, it may be a good idea to do a pilot study to test this, though more often than not, this is not an area of great concern.

In summary, dosing via diet is an established and simple method for delivering a compound to lab animals. It greatly reduces stress and saves time over other procedures such as oral gavage and injections. Finally, as long as you have accurate body weight and food intake data, you can easily calculate the dose they are receiving over the course of an experiment.

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Simple, Safe Dosing

Research Diets, Inc. will incorporate your compounds into any experimental diet. Feeding test compounds eliminates dosing related stress to the animal, eliminates vehicle effects, and saves time and labor. Consult with one of our scientists on the formula, determine the dosage required and the diet will be produced and shipped in 5 to 7 business days.

Consistent OpenSource Diet® formulation provides a clean, repeatable control diet for your research. Precise, graded addition of test compounds to your specified control diet allows evaluation of dose response effects in your animal model. We can blend your compound homogeneously into any diet, typically to ppm and even as low as parts per billion.

Typical Formula

Product #	D10001	
	gm%	*kcal%*
Protein	20	*21*
Carbohydrate	66	*68*
Fat	5	*12*
Total		*100.0*
kcal/gm	3.90	
Ingredient	**gm**	***kcal***
Casein	200	*800*
DL-Methionine	3	*12*
Corn Starch	150	*600*
Sucrose	500	*2000*
Cellulose, BW200	50	*0*
Corn Oil	50	*450*
Mineral Mix S10001	35	*0*
Vitamin Mix V10001	10	*40*
Choline Bitartrate	2	*0*
Total	**1000**	***3902***

Examples of Compounds Added

- Chemotherapeutics
- COX-2 Inhibitors
- Antioxidants
- Nutraceuticals
- Statins
- Insulin sensitizers
- NSAIDS

YOUR COMPOUND

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