





# The Impact of Laboratory Animal Diets on Autofluorescence Imaging in Animals

Fluorescence optical imaging is a powerful technique used to monitor biological processes, and allows for longitudinal studies in individual animals. Diets containing alfalfa (chlorophyll) fluoresces naturally, causes the imaging quality to be compromised.

### **OBJECTIVE:**

To determine whether a grain based diet, such as 5V75 is a suitable option to purified diets in fluorescence imaging studies in order to reduce per diem costs.

### **EXPERIMENTAL DESIGN:**

Adult male C57BL/6 mice (5 mice/diet group) were assigned to one of three diet groups that contained a pre-imaging diet and imaging diet (Table 1). Each group were fed a pre-imaging diet for 10 days (day -9 to 0) then transitioned to an imaging diet for an additional six days (day 1-7). The mice were imaged at day 0 to day 7. The in vivo images were taken using the autoexposure setting for Alexa Fluor 680 (675 nm excitation – 720 nm emission, 640 nm excitation - 700 nm emission) and plum fluorescent protein (570 nm excitation – 640 nm emission, 605 nm excitation - 660 nm emission). The fluorescence efficiency (fluorescence emission proportional to incident excitation light intensity) was measured over the abdominal area of each animal. All images were taken using the IVIS Spectrum optical imaging system (Perkin Elmer, Waltham, MA).

#### Pre-Imaging Vs. Imaging Diet (Table 1)

GROUP#	PRE-IMAGING DIET	IMAGING DIET
1	LabDiet 5001	AIN-93M
2	LabDiet 5001	LabDiet 5V75
3	LabDiet 5V75	AIN-93M

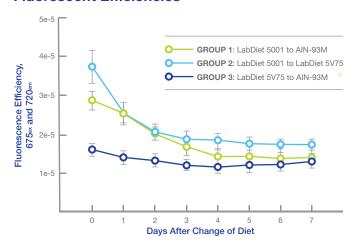
**5001**: Ground corn, dehulled SBM, dried beet pulp, fish meal, ground oats **5V75**: Ground corn, ground wheat, wheat middlings, corn gluten meal, cane molasses

AIN-93M: Cornstarch, dextrin, casein, sucrose

### **RESULTS:**

Autofluorescence was higher with the animals fed the pre-imaging diet 5001 diet than 5V75 at both Alexa Fluor 680 wavelengths and one plum fluorescent protein (605 nm excitation – 660 nm emission) wavelength on day 0. Three to four days after changing to the imaging diets, group 1 and 2 autofluorescence decreased to similar levels seen with the mice originally on the 5V75 (Group 3) at the Alexa Fluor 680 wavelengths. For plum fluorescent protein wavelength, 605 nm excitation – 660 nm emission, a similar decrease in autofluorescence was seen after 4 days for group 1 and group 2. However, this was not true for wavelength at 570 nm excitation – 640 nm emission, in which there was no gradual decrease in autofluorescence for any dietary treatment.

#### **Fluorescent Efficiencies**



## **CONCLUSION:**

5V75 is able to decrease autofluorescence levels within 4 days when fed as an imaging diet; and elicits minimal autofluorescence levels similar to AIN-93M that are needed for *in vivo* bio-imaging.

# Grain based and purified diets reduce autofluorescence *in vivo* at wavelengths detecting Alexa 680

Each group (5 mice/group) were fed a pre-imaging diet for 10 days (day -9 to 0) then transitioned to an imaging diet for an additional seven days (day 1-6). The graph (to the left) shows the Fluorescence Efficiency for the 675 excitation /720 emission wavelength at 0 to 7 days after diet change (Mean  $\pm$  SE). Fluorescent images are from the ventral side of C57B6 mouse fed a pre-imaging diet for 10 days, then transitioned to AIN-93M or 5V75 for 7 days. The images shown are at 0, 3, and 6 days after mice were transition to imaging diet.

