Effects of Dietary Supportive Care on Bioluminescent and Fluorescent Imaging in Mus musculus

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Introduction

Optical imaging is widely used as preclinical imaging modality due to ease of use and cost effectiveness. However, tissue autofluorescence/luminescence (mainly due to NADH, FAD, lipo-pigments and porphyrins), food signal (due to high chlorophyll content), light scattering, and signal absorption due to blood limit its ability to detect low intensity signals. When implementing optical imaging at the onset of metastasis, background signal must be reduced as low as possible to accurately detect low signal lesions and to avoid false positives. It is well-established that chlorophyll containing diets have significant auto-fluorescence and -luminescence that interferes with detectability of these small lesions (see Figures 1 and 2 below). Current literature recommends feeding a complete purified alfalfa-free control diet (AIN93G, BioServ, Flemington, NJ) for at least 3 days prior to imaging for background reduction.[1] This is particularly important when the fluorophore of interest has an emission wavelength longer than 630 nm, as a standard diet typically yields higher background signal compared to the control feed in the visible range.

Efficacy studies may also require nonpharmaceutical dietary supportive care to improve quality-of-life of rodent models and many of these feeds may show higher background levels of fluorescence and luminescence, limiting supportive care options. We evaluated 5 commercially available complete diets and 5 supplemental feeds (test feeds) and compared to a control diet (AIN93G) to characterize the background signal. As fluorescence signal intensity varies as a function of wavelength, all diets were imaged at 4 different filter pair settings corresponding to commonly used fluorophores (GFP, mCherry, AF680 and AF750) covering visible to near infrared range (NIR).

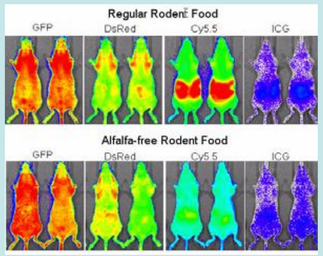

Figure 1: Representative images at various wavelengths (Image courtesy: IVIS Spectrum manual)

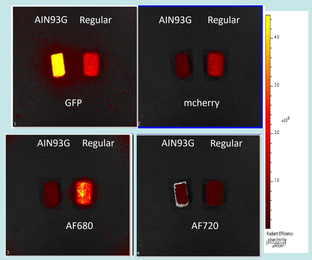

Figure 2: Regular Vs. AIN93G diet fluorescence signal comparison at various wavelengths

Objectives

- Determine background signal of commonly used supportive care test feeds.
- Provide appropriate supportive care for animal models of cancer with minimal interference to optical in vivo imaging.

Materials and Methods

Imaging parameters:

	Bioluminescence	Fluorescence
Scanner	IVIS spectrum imager (PerkinElmer Inc., Waltham, MA).	
Software	LivingImage (version 4.3.1)	
Exposure	Auto (1-120 seconds)	
F-stop	1	
Binning	Medium (8X8)	
Excitation filter	Block	465 (GFP), 570 (mCherry), 675 (AF680), 745 (AF750)
Emission filter	Open	520 (GFP), 620 (mCherry), 720 (AF680), 800 (AF750)

10 test diets were compared to a control diet:

Complete	Supplement
DietGel® 76A*	DietGel® Boost*
DietGel® 31M*	DietGel® Recovery*
Nutra-Gel™, Purified Formula, Bacon flavored†	DietGel® Prenatal*
Nutra-Gel™, NCI†	MediGel® Sucralose*
DietGel 5020*	HydroGel™*

* Clear H2O, Portland, ME

† BioServ, Flemington, NJ

Samples from 3 different diet batches were imaged. Region of interest (ROI) was drawn over the samples and total flux (bioluminescence) and total radiance efficiency (fluorescence) was measured. These values were normalized by the ROI area. Percentage change in signal for each diet with respect to the control diet was calculated.

Results

Fluorescence: MediGel Sucralose and HydroGel (supplemental feeds) showed lower fluorescent signal for all four fluorophores. The GFP fluorophore showed the broadest range of complete diets with lower signal compared to control diet (DietGel 76A, DietGel 31M, Dietgel 5020, and NutraGel NCI).

Fluorescent signal between the test diets varied significantly between the four fluorophore channels with higher signal observed for the GFP (visible) channel and lowest for the AF750 (NIR) channel.

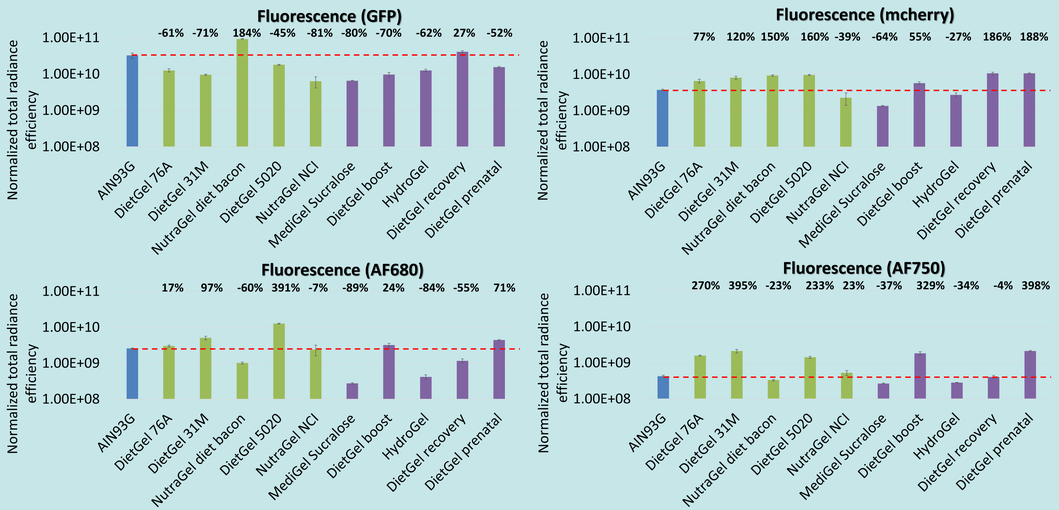

Figure 3: Plots show normalized total radiance efficiency for GFP, mCherry, AF680 and AF750 channels. % indicate change in signal intensity w.r.t. the control diet. Red dashed line indicates signal level for the control diet.

Results

Bioluminescence:

One complete (DietGel® 76A) and four supplemental (MediGel® Sucralose, DietGel® Boost, HydroGel™, and DietGel® Recovery) showed equal or lower bioluminescence signal compared to the control diet.

Bioluminescence signal for the complete diets was generally higher than the supplemental diets.

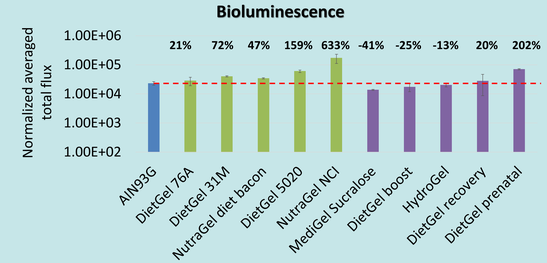

Figure 4: Plot shows normalized total flux values. % indicate change in signal intensity w.r.t. the control diet. Red dashed line indicates signal level for the control diet.

Conclusion

Evaluation of commercially available complete and supplemental feeds identified multiple options for supportive care for rodents undergoing in vivo optical imaging resulting in equal or superior reduction in background signal when compared to the standard control diet (AIN93G). Institutions wishing to recommend nutritional supportive care should be aware of the potential variability, especially between fluorophore channels and choose supportive care diets based on background signal levels. Additional consideration should also be given to the effect of the diet on the animal and any experimental manipulations. Future studies may encompass the effects of the change in diet on various tumor cell lines.

References

1. Bhaumik S, DePuy J, Klimash J. 2007. Strategies to minimize background autofluorescence in live mice during noninvasive fluorescence optical imaging. Lab Animal 36:40-43.

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.