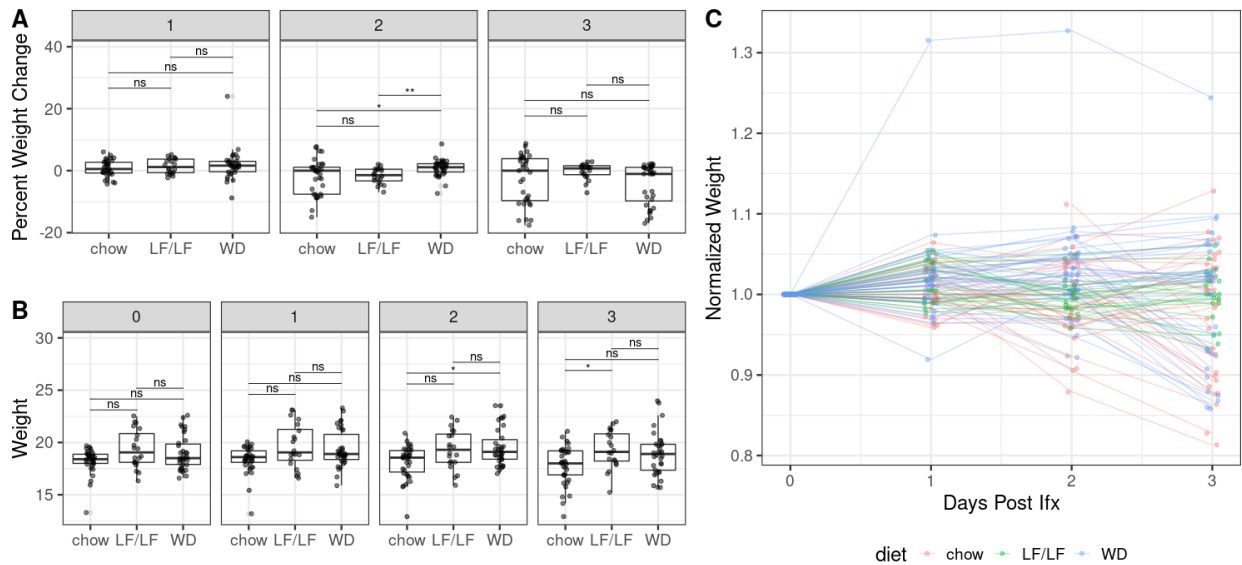**Supplementary Materials** for Hazleton K.Z., et al. *Dietary fat promotes antibiotic-induced*

Clostridioides difficile mortality in mice

Figure S1: Weight of mice before and after infection with *C. difficile* in Cohort 1. (A) Percent weight change of mice after 1,2, and 3 days of infection across diets (baseline was day of infection); (B) weight in grams of mice across diets pre and 1,2, and 3 days post infection and (C) scatter plot of fractional weight change of each mouse, colored by diet. p-values were determined using a Kruskal-Wallis with Dunn's post hoc test. Median and IQR indicated. (* : p < 0.05, ** : p < 0.01).
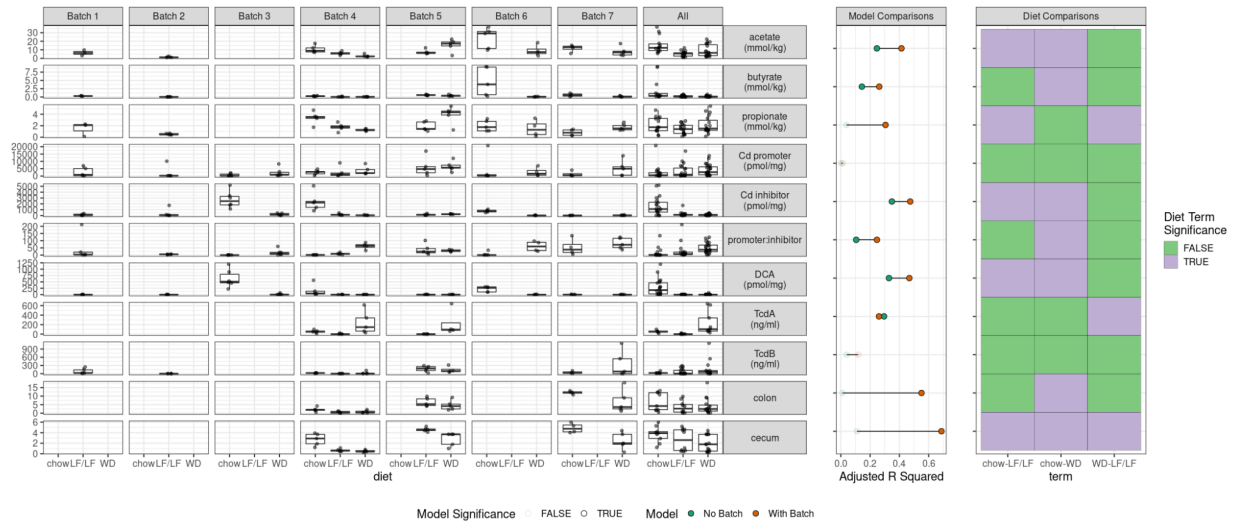
Figure S2: Analyte concentrations and inflammatory scores across different batches. The middle panel compares the adjusted R squared of linear models without considering batch (readout ~ diet) to linear models while considering batch (readout ~ diet + batch). Models are opaque if the corrected p values < 0.05 and translucent if not. The right panel shows significant differences between diet groups for models that consider batches.
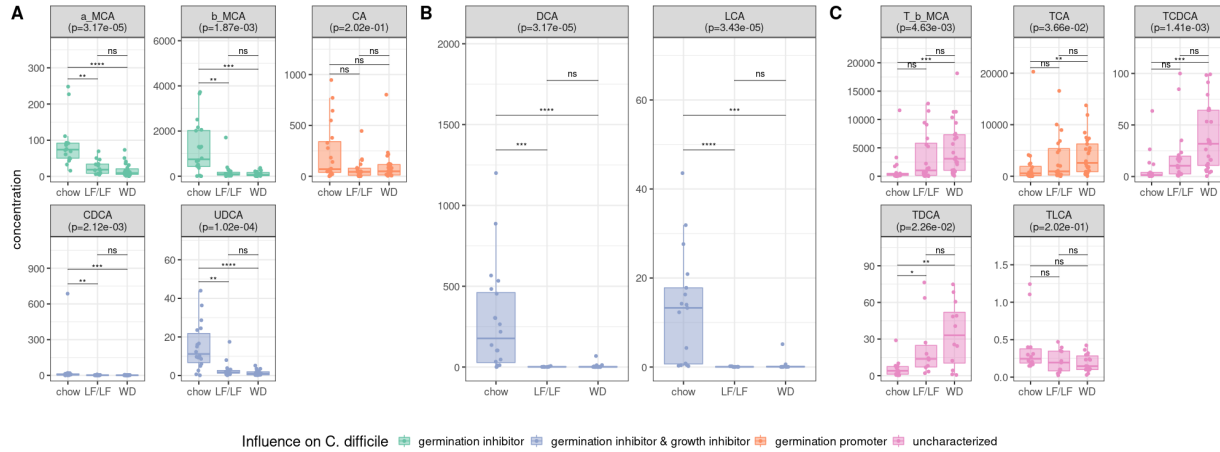
Figure S3. Cecal bile acid concentrations (pmol/kg) by diet. Unconjugated primary bile acids (A), secondary bile acid (B) and taurine-conjugated bile acids (C). a_MCA (alpha muricholic acid); b_MCA (beta muricholic acid); CA (cholic acid); CDCA (chenodeoxycholic acid); UDCA (ursodeoxycholic acid); DCA (deoxycholic acid); LCA (lithocholic acid); T_b_MCA (tauro-beta muricholic acid); TCA (taurocholic acid); TCA_3_SO4 (taurocholic acid 3-sulfate); TCDCA (taurochenodeoxycholic acid); TDCA (taurodeoxycholic acid); TLCA (taurolithocholic acid). P-values for the Kruskal-Wallis test with Dunn's post hoc are noted. Median and IQR are indicated. Plots are colored based on the previously described influence of each bile acid on *C. difficile*.
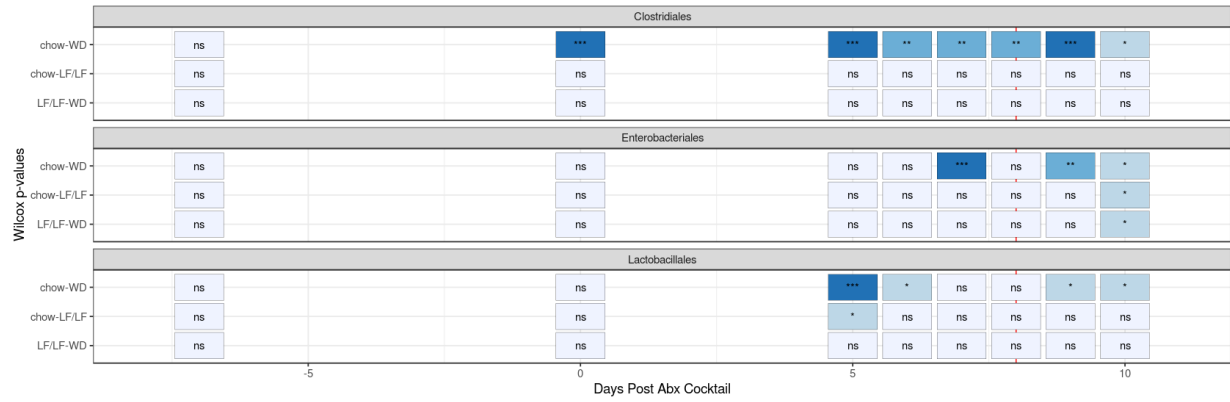
Figure S4: Statistical summary of relative abundances of key taxa from Figure 7a. Significant differences between groups are noted as calculated with the Wilcoxon rank-sum test. *******: p<0.001. ******: p<0.01, *****: p<0.05, ns= non significant.

Table S1: Composition of low-fat/low-fiber/high-sucrose diet

	Low-fat/Low-fiber/High-Sucrose
Fat (% kcal)	17.0
(% SFA)	*(19.5)*
(% MUFA)	*(41.7)*
(% PUFA)	*(38.8)*
Carbohydrates (% kcal)	64.5
(Sucrose)	*(26.7)*
Protein (%kcal)	18.6
Fiber (g/kg)	50 (cellulose)

Table S2: Description of samples sizes across different assays in cohorts 1 and 2.

		Batches	Chow	LF/LF	WD
Cohort 1 * (stool collection)		Total	20	20	20
		Batch1	10	0	10
		Batch2	10	10	0
		Batch3	0	5	5
		Batch4	0	5	5
	16S rRNA	Batches 1,2,3	13	5	13
Cohort 2 ** (tissue collection)		Total	20	20	26
		Batch1	0	5	0
		Batch2	0	5	0
		Batch3	6	0	6
		Batch4	5	5	5
		Batch5	0	5	5
		Batch6	5	0	5
		Batch7	4	0	5
	histology	Batches 4,5,7	9	10	14
	TcdA	4,5	5	10	9
	TcdB	1,2,4,5,7	8	20	15
	Bile acids	All batches	20	20	25
	SCFAs	1,2,4-7	14	18	19

*Cohort 1 (longitudinal mortality analysis with serial fecal collection and microbiome sequencing) was conducted in 4 different batches of mice with per batch counts across diets indicated.

**Cohort 2 (tissue collection – intestines for histopathology and cecal aspirates for bile acids, SCFAs, and toxins) was conducted in 7 different batches of mice with per batch counts across diets indicated. 16S rRNA, histology, toxins TcdA and TcdB, bile acids and SCFAs were all measured for particular sets of batches as indicated.

Table S3

Complete results of linear modeling statistical analyses performed. **[Table in separate Excel spreadsheet file]**

Table S4

Ingredient (g/KG)	WD	LF/LF
Anhydrous Milkfat	36.3	50
Beef Tallow	24.8	0
Casein	190	0
Cellulose	30	35
Cholesterol	0.4	0
Choline Bitartrate	2.1	0.01
Corn Oil	16.5	6.5
Corn Starch	230	392.2
L-Cystine	2.85	3
Lard	28	0
Maltodextrin	70	100
Mineral Mix, nTWD (110422)	35	15
Olive Oil	28	16.5
Sodium Chloride	4	0
Soybean Oil	31.4	23
Sucrose	255.6	24
TBHQ, antioxidant	0.028	0.014
Thiamin (81%)	0.015	0.002
Vitamin K1, phylloquinone	0.003	0.014
Vitamin Mix*	15	2.75

* WD used the Vitamin Mix nTWD (110423) and LF/LF diet used AIN-93-VX (94047)