Short and long-term effects of a synbiotic on clinical signs, the fecal microbiome, and metabolomic profiles in healthy research cats receiving clindamycin: A randomized, controlled trial

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Background. Antibiotic-associated gastrointestinal signs (AAGS) occur commonly in cats. Co-administration of synbiotics is associated with decreased AAGS in people, potentially due to stabilization of the fecal microbiome and metabolome. The purpose of this double-blinded randomized-controlled trial was to compare AAGS and the fecal microbiome and metabolome between healthy cats that received clindamycin with a placebo or synbiotic.

Methods. 16 healthy domestic shorthair cats from a research colony were randomized to receive 150 mg clindamycin with either a placebo (8 cats) or commercially-available synbiotic (8 cats) once daily for 21 days with reevaluation 603 days thereafter. All cats ate the same diet. Food consumption, vomiting, and fecal score were recorded. Fecal samples were collected daily on the last 3 days of baseline (days 5-7), treatment (26-28), and recovery (631-633). Sequencing of 16S rRNA genes and gas chromatography time-of-flight mass spectrometry was performed. Clinical signs, alpha and beta diversity metrics, dysbiosis indices, proportions of bacteria groups, and metabolite profiles were compared between treatment groups using repeated measures ANOVAs. Fecal metabolite pathway analysis was performed. $P<0.05$ was considered significant. The Benjamini & Hochberg's False Discovery Rate was used to adjust for multiple comparisons.

Results. Median age was 6 and 5 years, respectively, for cats in the placebo and synbiotic groups. Hyporexia, vomiting, diarrhea, or some combination therein were induced in all cats. Though vomiting was less in cats receiving a synbiotic, the difference was not statistically significant. Bacterial diversity decreased significantly on days 26-28 in both treatment groups. Decreases in *Actinobacteria* (*Bifidobacterium*, *Collinsella*, *Slackia*), *Bacteriodetes* (*Bacteroides*), *Lachnospiraceae* (*Blautia*, *Coprococcus*, *Roseburia*), *Ruminococcaceae* (*Faecilobacterium*, *Ruminococcus*), and *Erysipelotrichaceae* (*Bulleidia*, [*Eubacterium*]) and increases in *Clostridiaceae* (*Clostridium*) and *Proteobacteria* (*Aeromonadales*, *Enterobacteriaceae*) occurred in both treatment groups, with incomplete normalization by days 631-633. Derangements in short-chain fatty acid, bile acid, indole, sphingolipid, benzoic acid, cinnaminic acid, and polyamine profiles also occurred, some of which persisted through the terminal sampling timepoint and differed between treatment groups.

Discussion. Cats administered clindamycin commonly develop AAGS, as well as short- and long-term dysbiosis and alterations in fecal metabolites. Despite a lack of differences in clinical signs between treatment groups, significant differences in their fecal metabolomic profiles were identified. Further

investigation is warranted to determine whether antibiotic-induced dysbiosis is associated with an increased risk of future AAGS or metabolic diseases in cats and whether synbiotic administration ameliorates this risk.

1   **Short and long-term effects of a synbiotic on clinical signs, the fecal microbiome, and**

2   **metabolomic profiles in healthy research cats receiving clindamycin: a randomized,**

3   **controlled trial**

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22   **Background.** Antibiotic-associated gastrointestinal signs (AAGS) occur commonly in cats. Co-

23   administration of synbiotics is associated with decreased AAGS in people, potentially due to

24   stabilization of the fecal microbiome and metabolome. The purpose of this double-blinded

25   randomized-controlled trial was to compare AAGS and the fecal microbiome and metabolome

26   between healthy cats that received clindamycin with a placebo or synbiotic.

27   **Methods.** 16 healthy domestic shorthair cats from a research colony were randomized to receive

28   150 mg clindamycin with either a placebo (8 cats) or commercially-available synbiotic (8 cats)

29   once daily for 21 days with reevaluation 603 days thereafter. All cats ate the same diet. Food

30   consumption, vomiting, and fecal score were recorded. Fecal samples were collected daily on the

31   last 3 days of baseline (days 5-7), treatment (26-28), and recovery (631-633). Sequencing of 16S

32   rRNA genes and gas chromatography time-of-flight mass spectrometry was performed. Clinical

33   signs, alpha and beta diversity metrics, dysbiosis indices, proportions of bacteria groups, and

34   metabolite profiles were compared between treatment groups using repeated measures

35   ANOVAs. Fecal metabolite pathway analysis was performed. $P<0.05$ was considered significant.

36   The Benjamini & Hochberg's False Discovery Rate was used to adjust for multiple comparisons.

37   **Results.** Median age was 6 and 5 years, respectively, for cats in the placebo and synbiotic

38   groups. Hyporexia, vomiting, diarrhea, or some combination therein were induced in all cats.

39   Though vomiting was less in cats receiving a synbiotic, the difference was not statistically

40   significant. Bacterial diversity decreased significantly on days 26-28 in both treatment groups.

41   Decreases in *Actinobacteria* (*Bifidobacterium*, *Collinsella, Slackia*), *Bacteriodetes* (*Bacteroides*),

42   *Lachnospiraceae* (*Blautia, Coprococcus, Roseburia*), *Ruminococcaceae* (*Faecilobacterium*,

43   *Ruminococcus*), and *Erysipelotrichaceae* (*Bulleidia*, [*Eubacterium*]) and increases in

44   *Clostridiaceae* (*Clostridium*) and *Proteobacteria* (*Aeromonadales, Enterobacteriaceae*) occurred

45   in both treatment groups, with incomplete normalization by days 631-633. Derangements in

46   short-chain fatty acid, bile acid, indole, sphingolipid, benzoic acid, cinnaminic acid, and

47   polyamine profiles also occurred, some of which persisted through the terminal sampling

48   timepoint and differed between treatment groups.

49   **Discussion.** Cats administered clindamycin commonly develop AAGS, as well as short- and

50   long-term dysbiosis and alterations in fecal metabolites. Despite a lack of differences in clinical

51   signs between treatment groups, significant differences in their fecal metabolomic profiles were

52   identified. Further investigation is warranted to determine whether antibiotic-induced dysbiosis

53   is associated with an increased risk of future AAGS or metabolic diseases in cats and whether

54   synbiotic administration ameliorates this risk.

56   **Introduction**

57   Antibiotic administration is associated with profound, and sometimes prolonged,

58   derangements of the fecal microbiome and metabolome of people and animals (de La Cochetière

59   et al. 2010; Dethlefsen et al. 2008; Jakobsson et al. 2010; Suchodolski 2016b; Suchodolski et al.

60   2009). This dysbiosis is believed to be a primary contributor to the development of antibiotic-

61   associated gastrointestinal signs (AAGS), such as hyporexia, vomiting, and diarrhea (Hempel et

62   al. 2012; McFarland 2008; Videlock & Cremonini 2012). Susceptibility to AAGS and

63   derangements in the microbiome both increase with repeated antibiotic exposure (Ouwehand et

64   al. 2016).

65   Antibiotic-associated gastrointestinal signs occur commonly in cats (Albarellos &

66   Landoni 2009; 1995; Hunter et al. 1995) and people (Hempel et al. 2012; Lenoir-Wijnkoop et al.

67   2014; McFarland 2008), and they are an important cause of antibiotic non-compliance (Chan et

68   al. 2012; Jefferds et al. 2002; Kardas et al. 2005; Llor et al. 2013; Muñoz et al. 2014; Pechere et

69   al. 2007). Co-administration of probiotics or synbiotics (commercial mixtures of probiotics and

70   prebiotics) with antibiotics is associated with up to a 3-fold decrease in AAGS in people

71   (Hempel et al. 2012; Johnston et al. 2011; Lenoir-Wijnkoop et al. 2014; Narayan et al. 2010;

72   Selinger et al. 2013). Positive effects of probiotics on AAGS in people are postulated to result

73   from stabilization of the fecal microbiome and metabolome (Hempel et al. 2012; Ouwehand et

74   al. 2016).

75   The impact of synbiotics on AAGS and antibiotic-induced dysbiosis in cats is currently

76   unknown. However, the administration of 1 commercially-available multi-strain synbiotic[b]

77   resulted in significantly improved fecal scores for 72% of cats with idiopathic chronic diarrhea

78   (Hart et al. 2012), suggesting potential efficacy for reducing gastrointestinal signs due to other

79   causes. Prevention or mitigation of AAGS could decrease noncompliance by clients and, thus,

80   patient morbidity. Even if direct clinical effects are not observed, synbiotic administration might

81   be warranted if it is found to decrease antibiotic-induced changes in the microbiome and

82   metabolome and, thus, potentially reduce cumulative long-term sensitivity to AAGS and/or

83   metabolic disorders associated with antibiotic exposure, such as inflammatory bowel disease and

84   obesity (Aniwan et al. 2018; Kronman et al. 2012; Ouwehand et al. 2016).

85   The purpose of this study was to compare changes in food intake, vomiting, fecal scores,

86   the fecal microbiome, and the fecal metabolome of healthy cats receiving either a placebo or a

87   commercially-available synbiotic[b] with 150 mg clindamycin orally once daily for 21 days. A

88   secondary objective of the study was to assess the long-term impacts of antibiotic and synbiotic

89   administration on the fecal microbiome and untargeted metabolomic profiles of healthy cats.

90

91 **Materials and Methods**

92 Study population: This study was performed at the University of Tennessee's Veterinary Medical

93 Center and was approved by the Institutional Animal Care and Use Committee at the University

94 of Tennessee, Knoxville (protocol number 2169). Twenty purpose-bred, domestic short hair

95 research cats from the University of Tennessee, College of Veterinary Medicine teaching and

96 research colony determined to be healthy based on unremarkable physical examinations at the

97 time of study initiation and lack of pertinent abnormalities on CBC or biochemistry profiles

98 performed within the previous 6 months were enrolled. Prior antibiotic use was not noted in any

99 of the medical records. All cats ate the same commercial adult dry cat food for the duration of

100 the study.[c] Cats were transitioned to individual housing the week prior to the start of the study

101 and randomized into 1 of 2 treatment groups (placebo vs. synbiotic) at the start of the study using

102 a random number sequence.

103 Study periods: The study consisted of a baseline period (days 1-7), a treatment period (days 8-

104 28), and a long-term follow-up period (days 631-633) (Figure 1). During the baseline period, no

105 treatments were administered. During the treatment period, all cats received 150 mg (34

106 mg/kg/d, range 25.9-44.1 mg/kg/d) of clindamycin[d] PO once daily with food for 21 days. The

107 dose was chosen based on the manufacturer drug insert,[a] which noted changes in fecal

108 consistency and emesis in cats receiving higher doses of clindamycin. At the time of antibiotic

109 administration, cats received either 1 capsule of placebo or a commercially-available synbiotic[b]

110 PO once daily at the time of antibiotic administration. The synbiotic contains 5 billion *cfu* of a

111 proprietary mixture of *Bifidobacterium bifidum, Enterococcus faecium* and *thermophilus,* and

112 *Lactobacillus acidophilus, bulgaricus, casei,* and *lantarum* per capsule, as well a proprietary

113 blend of fructooligosaccharide and arabinogalactan. The time period for treatment was chosen

114 based on previous veterinary publications (Garcia-Mazcorro et al. 2011; Hart et al. 2012;

115 Koeppel et al. 2006), which showed significant changes in the fecal microbiome or fecal score

116 by 21 days of treatment. After completion of treatment, cats were returned to group housing in

117 the colony. On day 631, cats remaining in the teaching and research pool were reevaluated.

118 Based on review of their medical records, none of the cats had received antibiotics, probiotics,

119 other medications known to affect the microbiome, or dietary change in the intervening time.

120 Data collection: All cats underwent weekly physical examinations, including determination of

121 body weight, throughout the study. Daily observations, including food intake and vomiting

122 (present vs. absent), were collected throughout the study by an observer blinded to the treatment

123 groups. Photographs of defecated feces were taken daily by the same observer. Fecal consistency

124 was scored (Greco 2011) using still photographs at the completion of data collection by 2

125 investigators (JCW and JES), who were blinded to the cat, treatment group, and day for each

126 fecal sample.

127 Fecal samples: To limit the impact of daily variation in bacterial populations and fecal

128 metabolites, as well as differential distribution of bacterial groups and metabolites within

129 individual fecal samples, first morning naturally-voided fecal samples were collected daily for 3

130 days for each cat at each of 3 timepoints: the conclusion of baseline (days 5-7), the conclusion of

131 treatment (days 26-28), and after long-term recovery (days 631-633). 2 grams from the center

132 portion of each fecal sample was subdivided into 2 aliquots, with each aliquot placed into a 2 mL

133 cryovial. Samples were immediately frozen and remained in storage at -80ºC pending completion

134 of data collection. Samples for each cat from each timepoint were combined directly prior to

135 sample analysis to generate pooled samples for microbiome and metabolomic analysis.

136 Microbiome analysis: Genomic DNA was extracted from 100 mg of feces from each pooled

137   sample using a commercially available kit according to manufacturer's protocol.[e]

138   Amplification and sequencing of the V4 variable region (primers 515F/806R) of the 16S

139   rRNA gene was performed on a MiSeq (Illumina)[f] as previously described (Bell et al. 2014). The

140   software Quantitative Insights Into Microbial Ecology (QIIME, v. 1.8)[g] was used for processing

141   and analyzing the sequences. The raw sequence data were de-multiplexed, and low quality reads

142   were filtered using default parameters for QIIME. Chimeric sequences were filtered from the

143   reads using USEARCH against the 97% clustered representative sequences from the Greengenes

144   database (v. 13.8) and removed. The remaining sequences were assigned to operational

145   taxonomic units (OTUs) using an open-reference OTU picking protocol using the default QIIME

146   parameters, uclust consensus taxonomy assigner, and Greengenes database. The OTU table was

147   rarefied to 35,000 sequences per sample. Alpha rarefaction plots, alpha diversity metrics (Chao1,

148   Shannon, Goods Coverage, and Observed Species), and beta diversity metrics (weighted and

149   unweighted UniFrac distance matrices) were created using QIIME scripts.

150   Quantitative PCR was performed for selected bacterial groups (total bacterial,

151   *Faecalibacterium* spp.*, Turicibacter* spp.*, Streptococcus* spp.*, Escherichia coli, Blautia* spp.*,

152   *Fusobacterium* spp.*, Clostridium hiranonis*) using extracted DNA as has been previously

153   described (AlShawaqfeh et al. 2017). Briefly, 2 µl of normalized DNA (final concentration: 5

154   ng/µl) was combined with 5 µl of a DNA-binding dye,[h] 0.4 µl each of a forward and reverse

155   primer (final concentration: 400 nM), and 2.6 µl of PCR water to achieve a total reaction volume

156   of 10 µl. Oligonucleotide primers and probes, as well as respective annealing temperatures, are

157   summarized in Table 1. Data were expressed as log amount of DNA (fg) for each particular

158   bacterial group per 10 ng of isolated total DNA.

159

160 Metabolomics analysis: 10 mg of lyophilized feces from each pooled sample were analyzed at a

161 metabolomics facility[h] using gas chromatography time-of-flight mass spectrometry (GC-TOF-

162 MS) and standardized protocols as described in detail elsewhere (Fiehn et al. 2008; Minamoto et

163 al. 2015). Raw data files were processed using ChromaTOF v. 2.32. BinBase algorithm was

164 applied to match spectra to database compounds[i] or to characterize as an unknown compound as

165 reported previously (Honneffer et al. 2017). Quantification was reported by peak height of an ion

166 at the specific retention index characteristic of the compound across all samples.

167 Statistical analyses: Descriptive statistics were generated for each parameter. Samples were

168 analyzed for normality using the Shapiro-Wilk test and, for the presence of outliers, using box-

169 and-whisker plots. Age and weight for the 2 treatment groups were compared using an

170 independent two-sample Student's t-test. Mean percent food intake, percent days of vomiting,

171 and mean fecal scores were determined for each week within each study period (baseline and

172 treatment weeks 1, 2, and 3). Mean food intake for each week in each study period was

173 calculated as a percentage of food intake of each cat during the week prior to the start of the

174 study. Inter-rater correlation coefficients were calculated for fecal scores. The mean of fecal

175 scores assigned by the 2 investigators were used for all further statistical analyses. Mean food

176 intake, percent days vomiting per week, and mean fecal score were compared between treatment

177 groups using a multivariate repeated measures ANOVA. Treatment group (placebo or synbiotic),

178 week, and cat were included as categorical variables. Treatment, week, and the treatment-by-

179 week interaction were included as fixed effects. Week was included as a repeated measure with

180 subject as cat. Age, sex, and weight were initially included as covariates but were not retained in

181 the final models. Model assumptions regarding normally distributed residuals were verified with

182 the Shapiro-Wilk test for normality. Model assumptions regarding equality of variances were

183   verified with Levene's Test for Equality of Variances.

184   Global changes in microbiota communities (beta diversity) between individuals were

185   determined using unweighted Unifrac distance metrics; principal coordinates analysis (PCoA)

186   plots and rarefaction curves were plotted using QIIME software. The ANOSIM function in

187   PRIMER 6[j] was used to compare beta diversity metrics across time and between treatment

188   groups (Suchodolski et al. 2012). A dysbiosis index was calculated through methods previously

189   described (AlShawaqfeh et al. 2017). The dysbiosis index is a quantitative PCR-based assay that

190   summarizes the quantitative abundances of 8 major bacterial groups in feces in 1 number. The

191   higher the dysbiosis index, the more deviation of the microbiota from normobiosis. Global

192   changes in untargeted metabolomic profiles were determined using principal component analysis

193   (PCA) plots developed using an online metabolomics software analysis suite[k] as per standard

194   protocols (Xia et al. 2015; Xia & Wishart 2011). Pathway analysis was performed in the same

195   software suite using the *Homo sapiens* pathway library, interquantile range data filtering, log

196   transformation, and Pareto scaling.

197   Shannon indices, goods coverage, the Chao 1 metric, the dysbiosis index, proportions of

198   bacteria groups, and fecal metabolite profiles were compared between treatment groups using a

199   mixed model, split-plot repeated measures ANOVA that included fixed effects of treatment

200   (placebo or synbiotic), time period, and treatment-by-time period interaction. Cat nested within

201   group was included as a random effect. Age and sex were included as covariates in the initial

202   analysis, but they were not retained in the final model due to lack of significant effects. The

203   repeated measure of time period was accounted for in a repeated statement. A compound

204   symmetry variance/covariance structure was incorporated into each model to account for the

205   potential inclusion of constant covariates over time. The Shapiro-Wilk test of normality of the

206   residuals was evaluated for each marker to confirm the assumption of normally distributed

207   residuals had been met. Model assumptions regarding equality of variances were verified with

208   Levene's Test for Equality of Variances. Differences in least squares means were determined for

209   markers with significant main effect or interaction terms. Due to a marked lack of variability in

210   microbiome results for cats on days 26-28 (see Results below), rank transformation had to be

211   employed to allow convergence of the model for analysis of the proportions of bacteria groups

212   and ensure the statistical assumptions regarding normally distributed residuals and equality of

213   variances were met. Only bacteria taxa that were present in at least 50% of cats in $\geq$ 1 group at $\geq$

214   1 time point were included in statistical analyses.

215         $P < 0.05$ was considered statistically significant. P-values were corrected for multiple

216   comparisons on each phylogenetic level and for untargeted metabolomics using the Benjamini &

217   Hochberg's False Discovery Rate (fdr). Publicly-accessible software packages[g,k-m] were used for

218   all analyses.

219

220   **Results**

221   Study population: There were 4 female spayed and 6 male castrated cats in the placebo group

222   and 5 female spayed cats and 5 male castrated cats in the synbiotic group. Median age was 6

223   years (range 3-8 years) for cats in the placebo group and 5 years (range 3-8 years) for cats in the

224   synbiotic group. Median weight was 4.2 kg (range 3.4-5.4 kg) for cats in the placebo group and

225   4.3 kg (3.4-5.8 kg) for cats in the synbiotic group. No cat vomited any of the administered

226   capsules during the study. Four cats were removed from the study for marked hyporexia (2

227   placebo, 1 synbiotic) or high baseline fecal scoring (1 synbiotic).

228   After completion of treatment, cats were returned to the colony. On day 631, 15/16 of the

229   cats that completed the trial were available for re-examination. One cat (from the synbiotic

230   group) had been retired from the colony with subsequent private adoption. None of the cats had

231   received antibiotics, probiotics, or other medications known to affect the microbiome in the

232   intervening time, and none had undergone diet change. None of the cats were experiencing

233   vomiting, abnormal fecal scores, or inadequate body condition scores.

234   Antibiotic-associated gastrointestinal signs: Food intake, percent days vomiting, and fecal scores

235   over time by treatment group are summarized in Table 2. Food intake was decreased during

236   treatment in 3/8 cats in the placebo group and 4/8 cats treated with the synbiotic. Food intake

237   was significantly associated with week of treatment (F-value 5.2, $P < 0.01$) but did not differ

238   between treatment groups ($P = 0.5$). Percent days vomiting per week was significantly associated

239   with week of treatment (F-value 8.6, $P < 0.01$) but not treatment group ($P = 0.4$). It was highest

240   in the first week of treatment, during which 14/16 cats had at least 1 episode of vomiting, 7 in

241   each group. Fecal scores increased significantly during treatment (F-value 9.4, $P < 0.01$) but did

242   not differ between treatment groups ($P = 0.2$). All cats in each treatment group had fecal scores $\geq$

243   5 at some point during treatment, although high daily variability was noted. There was no

244   significant effect of age, weight, or sex on any of the clinical parameters evaluated.

245   Fecal microbiome: Cats in both treatment groups had marked changes in their fecal microbiome

246   over time (Figures 2-3). Alpha diversity was significantly lower ($P < 0.01$) on days 26-28, based

247   on the Shannon index, goods coverage, and Chao1 metric (Table 3). There was no significant

248   association between treatment group and changes in Shannon index, goods coverage, or the

249   Chao1 metric. Similarly, beta diversity was significantly altered on days 26-28 compared to

250   baseline and days 631-633 in each treatment group ($P < 0.01$; R = 0.73-0.76) with no significant

251   differences between treatment groups (Figure 2). The dysbiosis index was significantly higher on

252   days 26-28 than at baseline or on days 631-633 in each treatment group (Table 3), and a

253   significant association was found between treatment group and dysbiosis index (Table 3, F-value

254   13.6, $P < 0.01$).

255   Five phyla were identified based on sequencing analysis (mean baseline prevalences):

256   *Actinobacteria* (67.95%), *Firmicutes* (30.39%), *Proteobacteria* (1.03%), *Bacteroidetes* (0.73%),

257   and *Fusobacteria* (0.02%), with marked and significant differences over time in relative

258   abundances of all phyla except *Fusobacteria* (Figure 3, Table 4). There was no significant effect

259   of treatment group on changes in relative bacterial abundances at any timepoint, and there was

260   no significant association between age or sex and changes in any of the analyzed fecal

261   microbiome parameters.

262   Metabolome: Untargeted fecal metabolomics profiles differed markedly over time for cats in

263   both treatment groups (Figures 4-5), with a variety of patterns of changes found. Fecal profiles

264   for 178 of 252 assayed compounds (71%) differed significantly over time (see Additional file 1).

265   Of those that differed over time, 47 of 178 (26.4%) metabolites had equivalent profiles at

266   baseline and days 631-633. Partial return to baseline profiles were identified for an additional 14

267   metabolites (7.9%), and 21 metabolites (11.8%) had altered profiles on days 26-28 compared to

268   baseline with no significant changes between days 26-28 and days 631-633. Changes in profiles

269   for the remaining 96 metabolites (53.9%) were more complex. Profiles for 33 compounds

270   (18.5%) differed significantly on days 631-633 from both baseline and days 26-28 with no

271   difference between baseline and days 26-28, and profiles for 14 metabolites (7.9%) were

272   significantly higher or lower at days 26-28 compared to baseline with further significant changes

273   in the same direction by days 631-633. In contrast, profiles for 30 metabolites (16.9%) were

274   significantly higher at days 26-28 than baseline but also significantly lower at days 631-633 than

275   baseline; profiles for another 14 metabolites (7.9%) were decreased on days 26-28 with much

276   higher profiles on days 631-633 than baseline. Finally, 5 metabolites had individual patterns of

277   change.

278   Significant differences (fdr adjusted $P < 0.05$) were noted between treatment groups for 7

279   metabolite profiles (Figure 6). Significant group by time interactions were found for fecal

280   profiles of putrescine, isopentadecanoic acid, cellobiose, ethanolamine, lactose, and D-erythro-

281   sphingosine, while fecal profiles for N-acetylglutamate differed significantly by treatment group

282   alone.

283   With regard to metabolites of known biological importance, there were significant

284   changes in profiles of some short-chain fatty acid (SCFA) synthesis metabolites, bile acids,

285   tryptophan degradation pathway metabolites, sphingolipids, polyamines, and benzoic and

286   cinnaminic acids over time (Table 5). Pathway analysis revealed significant changes (fdr

287   adjusted $P < 0.05$) over time for 55 metabolic pathways; 47 of which had impact factors > 0

288   (Table 6). The majority of pathways affected were related to SCFA, amino acid, sugar, and

289   nucleotide synthesis and degradation. Amino acid pathways that were affected included those

290   related to tryptophan degradation. Other pathways significantly affected included those related to

291   linoleic acid, sphingolipid, and glycerolipid metabolism.

292

293   **Discussion**

294   The incidence of AAGS varies in people based on individual antibiotic agent, prior

295   antibiotic exposure, subject age, and other factors (Hempel et al. 2012; Lenoir-Wijnkoop et al.

296   2014; McFarland 2008; Ouwehand et al. 2016). Antibiotics, such as clindamycin, can cause

297   AAGS directly by stimulating the intestinal motilin receptor (Bartlett 2002). However,

298   antibiotic-induced dysbiosis, with secondary alterations in fecal metabolites and overgrowth of

299   opportunistic pathogens, is believed to be the primary contributor to AAGS (Gustafsson et al.

300   1998; McFarland 2008; Varughese et al. 2013; Videlock & Cremonini 2012). Dysbiosis is

301   greatest for broad-spectrum and poorly-absorbed antibiotics, including macrolides,

302   cephalosporins, and fluoroquinolones (Jakobsson et al. 2010; Löfmark et al. 2006; McFarland

303   2008; Ouwehand et al. 2016), and alterations to the microbiome can persist at least 4 years after

304   short-term antibiotic therapy (Jakobsson et al. 2010; Löfmark et al. 2006).

305   Multiple metanalyses support the efficacy of probiotics in preventing AAGS in people

306   (Goldenberg et al. 2015; Lau & Chamberlain 2016; McFarland 2016; Ouwehand et al. 2016;

307   Szajewska et al. 2016; Videlock & Cremonini 2012). Although some gastrointestinal effects

308   appear to be dose-related (Ouwehand et al. 2016; Pagnini et al. 2010; Szajewska et al. 2016),

309   effects do not correlate directly with the relative abundances of probiotic bacteria or measurable

310   shifts in OTUs in the fecal microbiome (Biagi et al. 2013; Garcia-Mazcorro et al. 2011;

311   Marshall-Jones et al. 2006; Rossi et al. 2014). The ideal microbial strain(s), dose, and timing

312   relative to antibiotic administration likely vary by antibiotic, underlying disease processes,

313   patient (age, inpatient status, historical antibiotic exposure), and other factors (Lau &

314   Chamberlain 2016; McFarland 2009; Szajewska et al. 2016). Positive effects of probiotics

315   include stimulation of the host's innate system and epithelial secretion of antimicrobial

316   substances, restoration of intestinal barrier function, alteration of the gastrointestinal

317   metabolome, disruption of biofilms through production of bacteriocins, generation of other

318   antimicrobial agents, and competitive exclusion of pathogens (Ohland & MacNaughton 2010;

319   Ouwehand et al. 2016; Pagnini et al. 2010; Quigley 2012; Rossi et al. 2014; Strompfová et al.

320   2004).

321   Although AAGS are a recognized complication of antibiotic therapy in cats (Albarellos &

322   Landoni 2009; Bureau of Veterinary Drugs 1995; Hunter et al. 1995), the incidence of AAGS,

323   short- and long-term effects on the microbiome and metabolome, and impact of concurrent

324   synbiotic administration had not been previously characterized. In this study, AAGS developed

325   in 100% of cats treated with high dose clindamycin once daily for 21 days. Vomiting was most

326   severe in the first week of therapy, although it persisted for the duration of treatment in some

327   cats. A temporal association between antibiotic administration and vomiting was not noted,

328   suggesting stimulation of the motilin receptor by clindamycin had minimal impact on the

329   incidence of vomiting. The incidence of vomiting per week was less in cats receiving the

330   synbiotic, but the difference between treatment groups was not statistically significant. Fecal

331   consistency and food intake declined progressively throughout the course of treatment in both

332   treatment groups.

333   There are a number of potential explanations for the lack of statistically significant

334   differences in AAGS between treatment groups in this study. A high antibiotic dose was used to

335   increase the likelihood of detecting a difference between groups because the incidence of AAGS

336   due to clindamycin was unknown. Ironically, this might have obscured positive results due to the

337   severity of AAGS induced. The synbiotic dose chosen for the study was based on a prior report

338   describing clinical improvement in cats with chronic enteropathy treated with the same synbiotic

339   (Hart et al. 2012), but use of a higher dose might be required to prevent AAGS. We elected to

340   administer the synbiotic concomitantly with clindamycin because compliance with medication

341   regimens is inversely correlated with the number or frequency of treatments (Adams et al. 2005;

342   van den Bemt et al. 2016), primarily due to forgetfulness and/or logistical difficulties of

343   balancing treatment schedules against other daily responsibilities (Col et al. 1990). Although

344 macrolide resistance occurs in a relatively high percentage of *Enterococcus faecium* and

345 *Lactobacillus* strains, as well as a *Bifidobacterium animalis* probiotic (European Food Safety

346 Authority 2012; Ouwehand et al. 2016; Strompfová et al. 2004), antibiotic sensitivity of the

347 bacterial strains in the study drug were not determined so neutralization due to concomitant

348 antibiotic administration cannot be ruled out.

349   To further assess the potential efficacy of synbiotics in preventing AAGS in cats, our

350 group has since performed a follow-up study (Stokes et al. 2017) using a crossover design with a

351 6-week washout period, a more clinically relevant clindamycin dose (mean 18 mg/kg/d), a higher

352 synbiotic dose (10 billion *cfu*/d), and delaying administration of the synbiotic for 1 hour after

353 antibiotic administration to limit potential synbiotic neutralization. Cats receiving the synbiotic

354 were significantly more likely to complete the initial treatment course, vomited less, and had

355 higher food intake than cats receiving the placebo. Interesting, strong period effects were noted –

356 suggesting ameliorative effects of synbiotics on AAGS may persist for at least 6 weeks after

357 administration. Because the antibiotic and synbiotic doses, as well as timing of synbiotic

358 administration, were all altered in that study, it is not possible to ascertain which factor had the

359 greatest impact on clinical signs.

360   In addition to causing AAGS, clindamycin administration was associated with marked

361 changes in the fecal microbiome and metabolome in this study. Alterations in alpha diversity

362 (Shannon index, Goods coverage, Chao1 metric), beta diversity, and the dysbiosis index returned

363 to baseline levels by the end of the washout period. The return of diversity at a global level was

364 not due to return to baseline abundances for individual OTUs. Instead, changes in relative

365 abundances of many individual OTUs persisted through the terminal sampling timepoint (> 600

366 days after discontinuation of antibiotic therapy) in both treatment groups. Changes included

367   reductions in the abundances of *Actinobacteria*, including *Bifidobacterium*, *Collinsella,* and

368   *Slackia*; *Bacteroidetes*, including *Bacteroides*; many members of the *Clostridium* clusters IV and

369   XIV, including *Lachnospiraceae* (*Blautia*, *Coprococcus*, and *Roseburia*), *Ruminococcaceae*

370   (*Faecalibacterium* and *Ruminococcus*), and *Erysipelotrichaceae* (*Bulleidia* and [*Eubacterium*]);

371   and *Veillonellaceae*, including *Megamonas*. Marked increases in abundance were identified for

372   *Clostridiaceae*, including *Clostridium*, and *Proteobacteria*, including *Aeromonadales* but

373   primarily due to increases in *Enterobacteriaceae*. Alterations were generally consistent with

374   those found in cats (Ramadan et al. 2014; Suchodolski et al. 2015) and people (Schubert et al.

375   2014) with dysbiosis due to naturally-occurring diarrhea. Interestingly, decreased relative

376   abundances of *Lachnospiraceae* and *Ruminococcaceae* also have been found in cats infected

377   with *Giardia* and feline immunodeficiency virus (Šlapeta et al. 2015; Weese et al. 2015).

378   Antibiotic-induced dysbiosis and gastrointestinal signs have been associated with

379   alterations in a variety of metabolite profiles. Consistent with prior work in people (Gustafsson et

380   al. 1998; McFarland 2008; Woodmansey et al. 2004), we found marked derangements in SCFA

381   profiles and pathways during antibiotic administration, some of which persisted at days 631-633.

382   No difference was found in SCFA profiles between treatment groups. In addition to being an

383   important energy source for colonocytes, SCFAs have anti-inflammatory and

384   immunomodulatory effects, regulate intestinal motility, and maintain intestinal barrier function

385   (Quigley 2012; Suchodolski 2016a). Persistent alterations in SCFA profiles could perpetuate

386   ongoing dysbiosis after resolution of the initial insult.

387   Decreased proportions of secondary bile acids, important for maintaining eubiosis and

388   modulating the immune system, have been found in people and dogs with gastrointestinal disease

389   (Duboc et al. 2013; Honneffer et al. 2015). Similarly, we identified a marked reduction in

390   secondary versus primary bile acids during antibiotic administration, followed by an increase at

391   days 631-633 above baseline. It is possible, though speculative, that the inversion in the ratio of

392   primary to secondary bile acids on days 631-633 reflects a compensatory response to persistent,

393   occult dysbiosis and inflammation. Temporal alterations in bile acid biosynthesis were also noted

394   on pathway analysis but did not achieve significance (fdr adjusted $P = 0.06$).

395   Indole profiles, the product of tryptophan degradation by commensal *E. coli*, have

396   previously been found to be decreased in people and animals with gastrointestinal disease

397   (Suchodolski 2016a). In this study, indole profiles decreased significantly during antibiotic

398   administration in both treatment groups. Indole profiles failed to return to baseline values by

399   days 631-633 for cats in the placebo group, while they were mixed for cats in the synbiotic

400   group. Direct effects of indole within the gut lumen include decreased production of adherence

401   and virulence factors, motility, biofilm formation, and adherence of pathogenic *E. coli* (Bansal et

402   al. 2007), while effects on the intestine include strengthening of intestinal tight junctions,

403   increased production of mucin to shield the epithelium from bacterial adherence, and modulation

404   of inflammation (Bansal et al. 2010). Persistent alterations in indole profiles in cats not

405   administered a synbiotic with antibiotics could contribute to a pro-inflammatory state, as well as

406   chronically increased intestinal permeability and risk of bacterial colonization and translocation.

407   Significant temporal changes also were found in profiles of D-erythro-

408   sphingosine, isopentadecanoic acid, and cellobiose. D-erythro-sphingosine is a sphingolipid,

409   while isopentadecanoic acid and cellobiose are components of sphingolipids and

410   sphingophospholipids (Chao et al. 1992; Minamino et al. 2003; Vesper et al. 1999).

411   Sphingolipids and sphingophospholipids have antineoplastic properties, stimulate the innate

412   immune system, and increase bacterial clearance (Chao et al. 1992; Fujiwara et al. 2013;

413   Minamino et al. 2003). Persistent reductions in putrescine profiles in cats in the placebo, but not

414   the synbiotic, group after antibiotic exposure also were noted. Putrescine, along with spermine

415   and spermidine, are polyamines. Polyamine supplementation increases resistance to oxidative

416   stress in mice, while depletion is associated with development of pancreatitis (Minois et al.

417   2011). Further study will be necessary to determine the clinical relevance of differing

418   sphingolipid and putrescine profiles between treatment groups over time.

419   Finally, significant associations were found in profiles for cinnaminic acid and benzoic

420   acid compounds. Compounds in both treatment groups had lower results at days 26-28 than

421   baseline. Benzoic acid profiles were higher at days 631-633 than at baseline, while cinnaminic

422   profiles did not return to baseline levels. Both cinnaminic and benzoic acids have been found to

423   have antimicrobial effects, and cinnaminic acids have additional anti-inflammatory properties

424   (Alam et al. 2016; Manuja et al. 2013). The persistent decrease in cinnaminic acid profiles after

425   antibiotic administration is particularly intriguing given work in research models suggesting

426   potential benefits of cinnaminic acids in management of obesity and diabetes mellitus (Alam et

427   al. 2016). Repeated exposure to antibiotics has been associated with increased risks of childhood

428   obesity and diabetes mellitus (Mikkelsen et al. 2016; Scott et al. 2016). It is possible, though

429   certainly speculative at this juncture, that associations between antibiotic exposure and increased

430   risk of obesity and diabetes are at least partially due to cumulative derangements in cinnaminic

431   acid profiles.

432   One factor that could impact clinical application of these results is the use of healthy

433   research cats. Creation of AAGS in healthy privately-owned animals for antibiotic-related

434   investigations poses concerns regarding compliance with the study protocol, while evaluations in

435   clinically ill animals can be confounded by heterogeneity in underlying disease, the antibiotic

436   required for the primary disease, prior antibiotic exposure, and husbandry factors. The

437   predominant phylum identified at baseline in this study, *Actinobacteria*, conflicted with that

438   found in recent reports in privately-owned healthy cats, *Firmicutes* (Bell et al. 2014; Suchodolski

439   et al. 2015; Weese et al. 2015), though they agree with findings of other studies, particularly

440   those using research colony cats (Abecia et al. 2010; Desai et al. 2009; Jia et al. 2011a; Jia et al.

441   2011b). Although *Firmicutes* was the predominant phyla in the first 3 studies (Bell et al. 2014;

442   Suchodolski et al. 2015; Weese et al. 2015), relative abundance ranged from 50–80% with

443   similar variability in *Actinobacteria* (0.11–9.9%), *Bacteroidetes* (0.15–33%), *Fusobacteria* (0–

444   1.15%) and *Proteobacteria* (0–30%). Differences among studies also could reflect geographical

445   variation, heterogeneity in diet, or variable exposure to other treatments known to impact the

446   microbiome. Although cats in this study had not previously received antibiotics, probiotics or

447   proton-pump inhibitors, data on potential exposure to them were not included in the other

448   reports.

449   Because there was not a control group that received neither antibiotics nor probiotics, it is

450   not possible to definitively ascribe long-term changes in the fecal microbiome and metabolome

451   to antibiotic administration. Although the fecal microbiome of cats evolves with age,

452   predominant bacterial groups and relative abundances appear relatively stable in colony cats

453   based on evaluation of cats from 1 to 9 months of age and 8 to 14 years from the same colony

454   (Jia et al. 2011a; Jia et al. 2011b). Further, we did not identify an association between the age of

455   cats and the composition of the microbiome at baseline. As such, exposure to antibiotics is the

456   most likely cause for the marked changes in fecal microbiome and metabolomic profiles over

457   time. Interestingly, we also found no association between age and AAGS, in contrast to work in

458   people (McFarland 2008).

459

Conclusions

461 High-dose clindamycin therapy induces AAGS in 100% of cats, and severity of signs is

462 often treatment-limiting. The incidence of vomiting was lower in cats concurrently administered

463 a synbiotic, although the difference was not statistically significant. Antibiotic-induced dysbiosis

464 and alterations in fecal metabolite profiles mirror those found in people. Changes in fecal

465 metabolomic profiles included derangements in SCFA, bile acid, indole, sphingolipid,

466 polyamine, benzoic acid, and cinnaminic acid metabolism. Changes in relative abundances of

467 many OTUs and metabolite profiles persisted for > 600 days after treatment discontinuation.

468 Significant differences between synbiotic and placebo groups were noted for metabolites that

469 affect immunomodulation, intestinal permeability and barrier function, colonization resistance,

470 and oxidative stress. Further investigation is warranted to determine whether synbiotics blunt

471 AAGS or the risk of antibiotic-associated metabolic derangements, such as obesity, in cats

472 undergoing repeated antibiotic therapy.

473

Footnotes

475 [a]Antirobe®/Antirobe® Aquadrops manufacturer insert, Pfizer Pharmacia, March 2010.

476 [b]Proviable-*DC*®, Nutramax Laboratories Veterinary Sciences, Inc., Lancaster, SC USA.

477 [c]Hill's® Science Diet® Light, Hill's Pet Nutrition, Inc., Topeka, KS USA.

478 [d]Antirobe®/Antirobe® Aquadrops, Pfizer, Pharmacia & Upjohn Company, New York, NY USA.

479 [e]PowerSoil®, Mo Bio, Carlsbad, CA USA.

480 [f]MR DNA (Molecular Research LP), Shallowater, TX USA.

481 [g]QIIME, v. 1.8. Available at: http://www.qiime.org.

482 [h]SsoFast EvaGreen® supermix, Bio-Rad Laboratories, CA USA.

483 [i]West Coast Metabolomics Core, University of California, Davis, CA USA.

484 [j]PRIMER 6, PRIMER-E Ltd, Ivybridge, UK.

485 [k]MetaboAnalyst 3.0. Available at: http://www.metaboanalyst.ca.

486 [l]MedCalc 15.8 MedCalc Software, Ostend, Belgium.

487 [m]SAS 9.4 release TS1M3, SAS Institute Inc., Cary, NC USA.

488

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Flowchart illustrating study design, duration, observations, and sampling.

The study spanned 633 days (D1-633) and was broken into 3 study periods: baseline (D1-D7), treatment (D8-D28), and washout. Cats were randomized to receive either a placebo or synbiotic with 150 mg clindamycin PO once daily during treatment. Food intake, vomiting, and fecal score were recorded daily (•) and weight (W) weekly (*) by an individual blinded to treatment group. 2 grams were collected from the center portion of each first morning naturally-voided fecal samples for each cat once daily for the last 3 days of each study period: baseline (open circles), treatment (open squares), and recovery (open diamonds). Each sample was subdivided into 2 aliquots, with each aliquot placed into a 2 mL cryovial and immediately frozen at -80ºC pending completion of data collection.

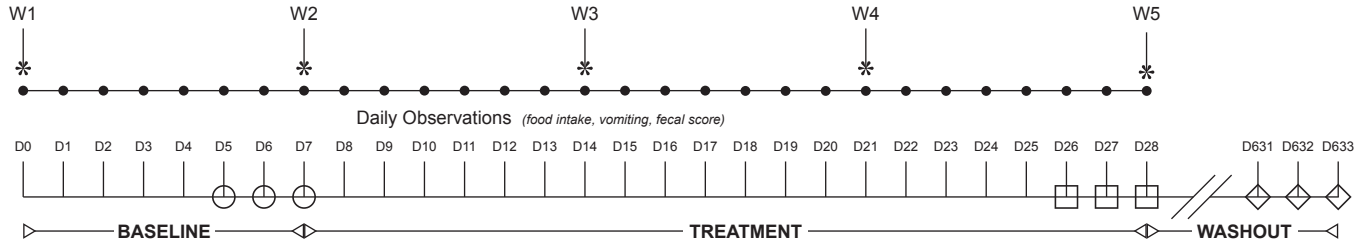



Figure 2

Principal Coordinate Analysis (PCoA) of unweighted UniFrac distances of 16S rRNA genes.

Gene sequences were determined using fecal samples collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable on days 631-633.

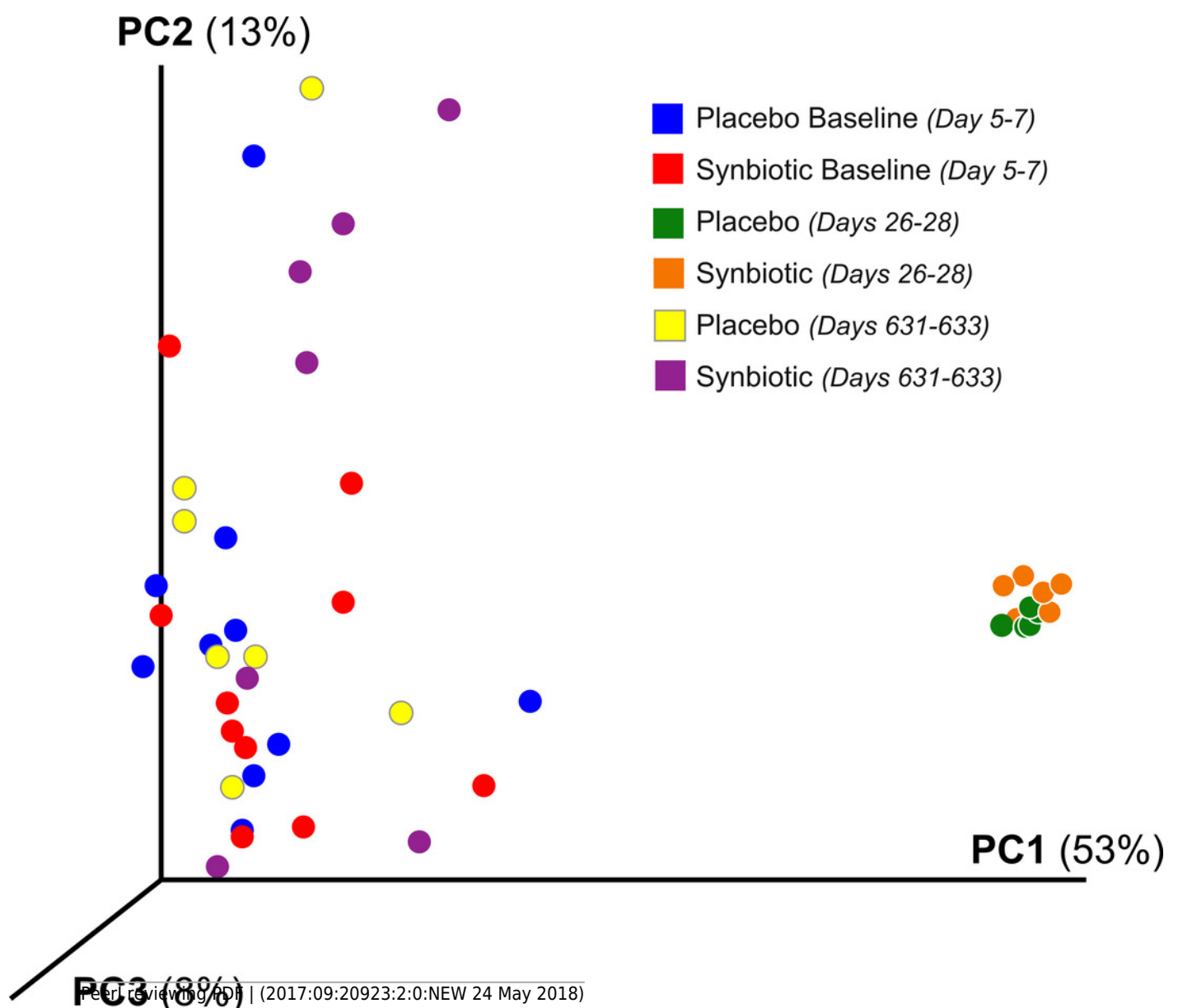



Figure 3

Phylum- and class-level composition of fecal microbiota obtained from feline fecal samples.

(A) Placebo. (B) Synbiotic. Samples were collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable on days 631-633. Legend for all detected classes is shown, grouped by phylum.

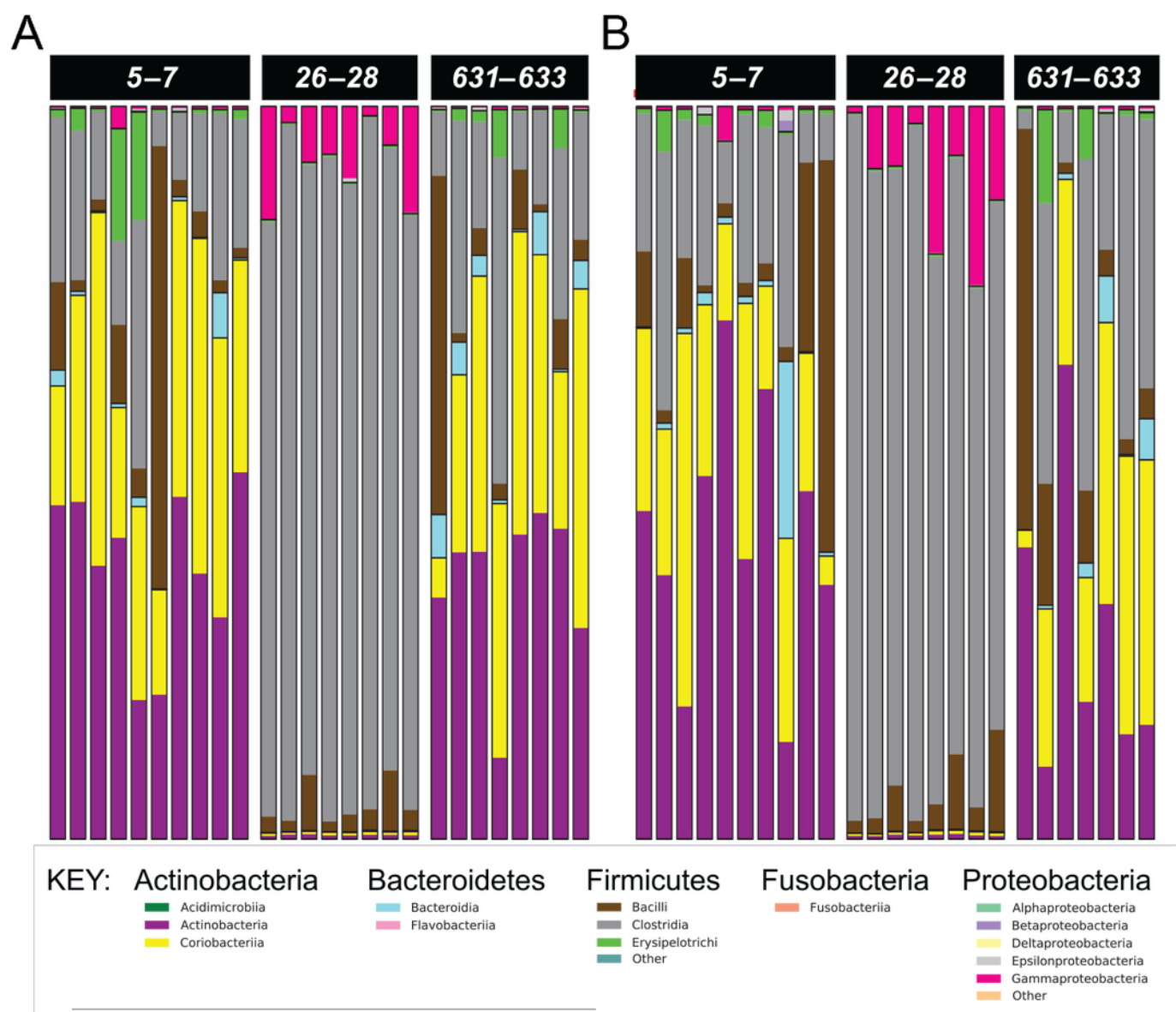



Figure 4

Dual hierarchical dendrogram of metabolites, clustered by pathway, that differed significantly over time in feline fecal samples.

Samples were collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable on days 631-633.

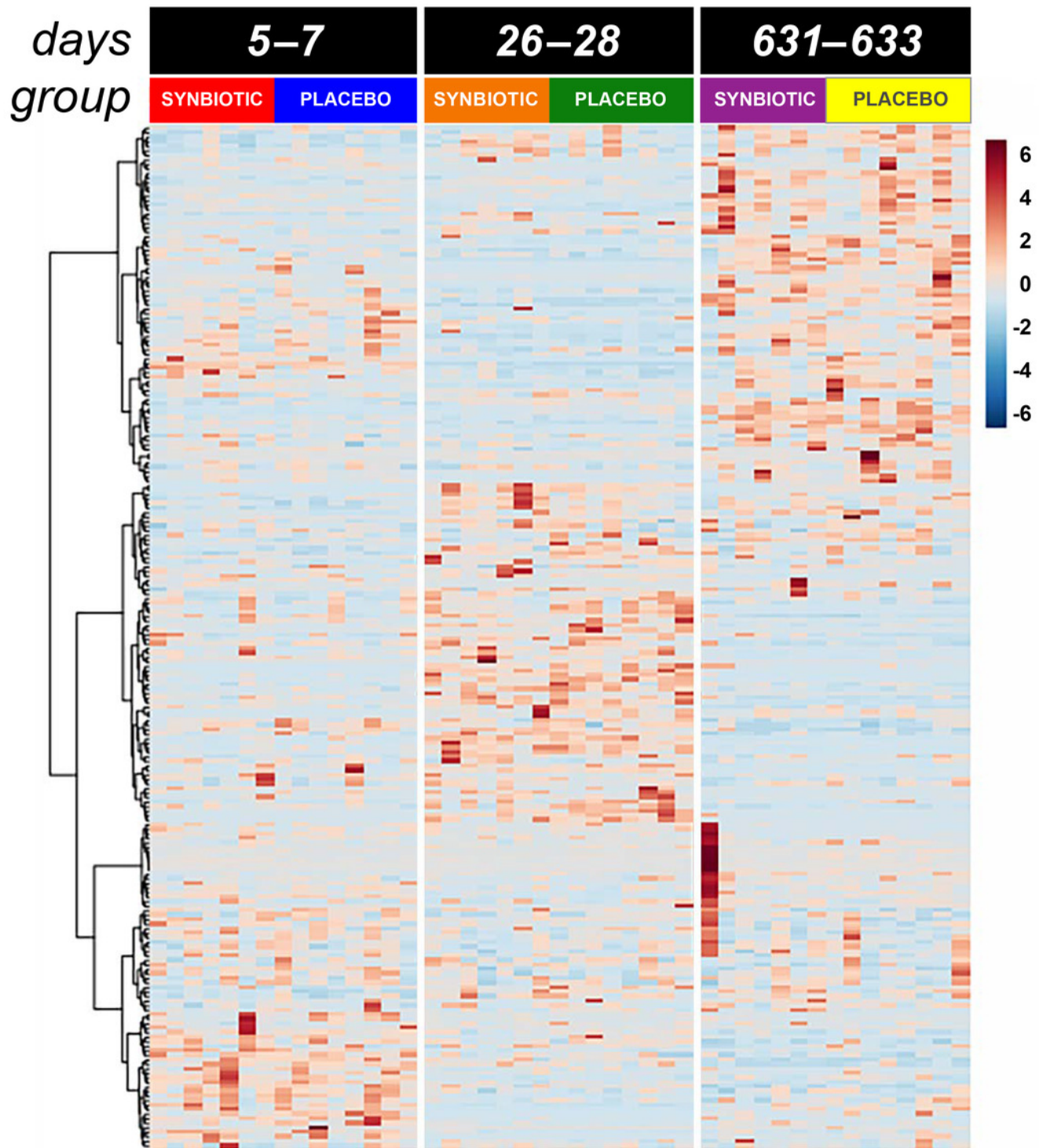



Figure 5

Principal Component Analysis (PCA) of metabolic pathway analyses from feline fecal samples

Samples were collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable on days 631-633.

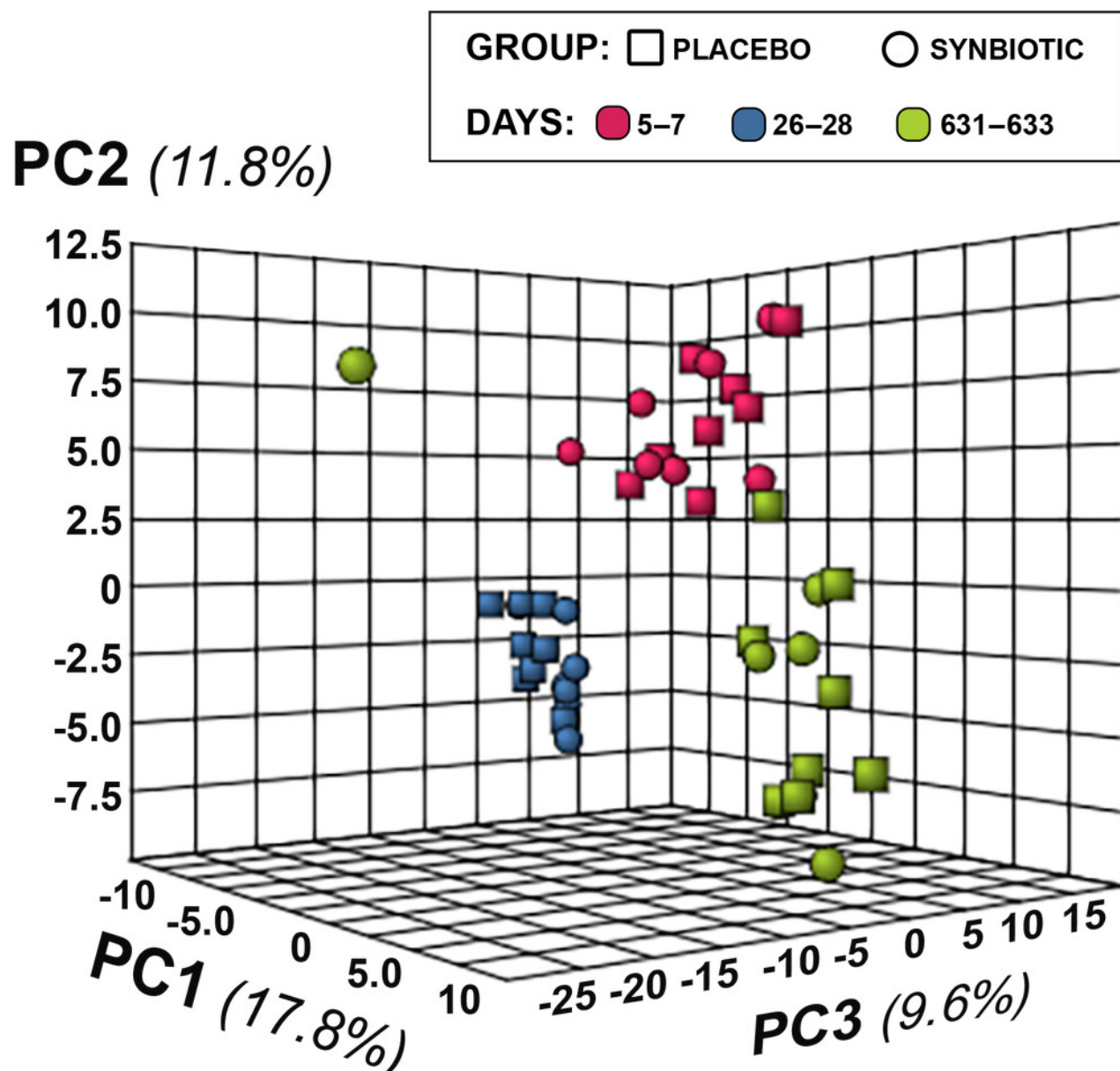
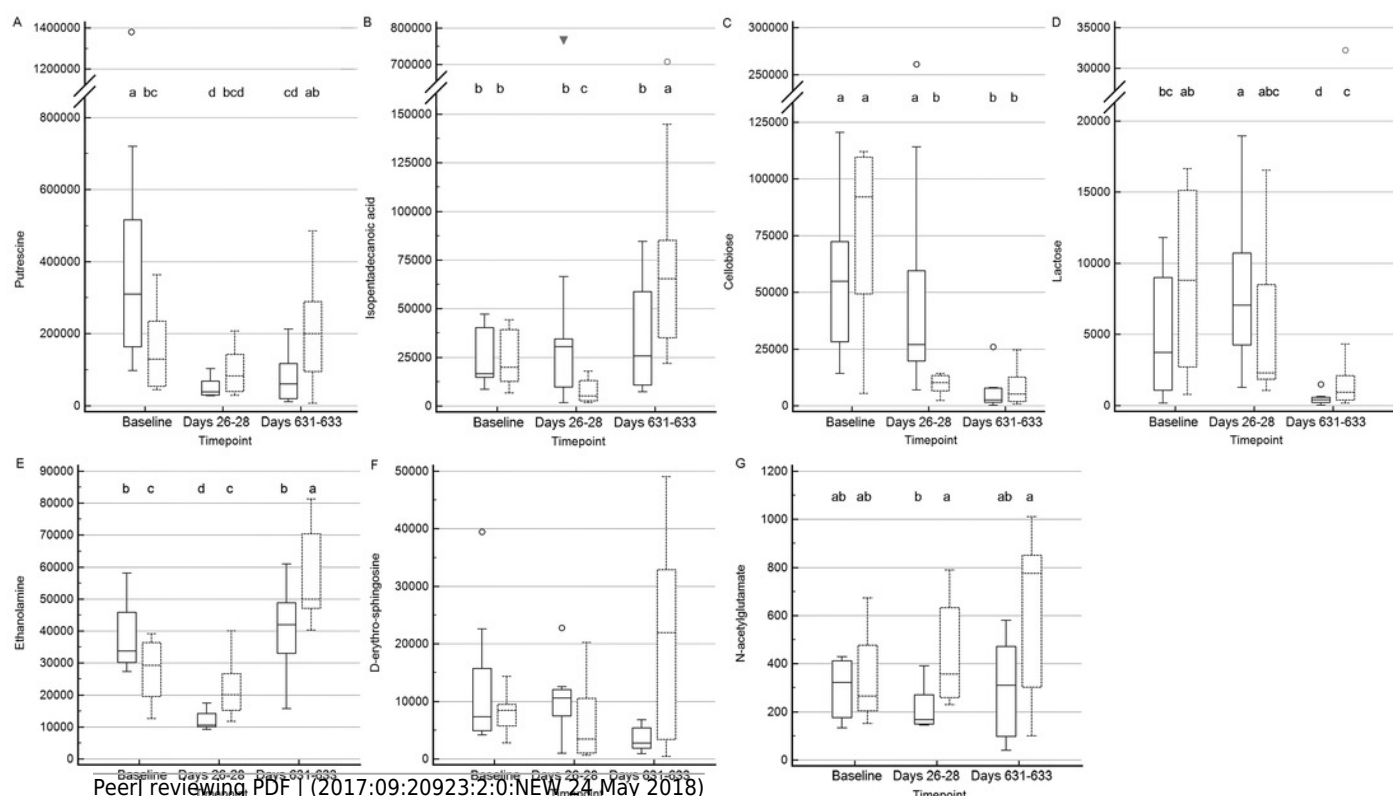



Figure 6

Box and whisker plots of fecal metabolite profiles for 7 fecal metabolites that differed significantly (fdr adjusted *P* < 0.05) between treatment groups and over time.

(A) Putrescine. (B) Isopentadecanoic acid. (C) Cellobiose. (D) Lactose. (E) Ethanolamine. (F) D-erythro-sphingosine. (G) N-acetylglutamate. Medians, interquartile ranges, and minimum and maximum values are presented for cats in the placebo (boxes with solid borders) and synbiotic (boxes with dashed borders) treatment groups. Open circles and closed triangles denote outlier values. Boxes that do not share a letter differed significantly (fdr-adjusted *P* < 0.05) based on post-hoc analysis. Fecal samples were collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable on days 631-633. Significance was set as *P* < 0.05, with *P*-values adjusted based on the Benjamini and Hochberg False discovery rate (fdr).

Target bacteria, oligonucleotide primers/probes, and annealing temperatures used for quantitative PCR analysis.

Target	Primer type	Sequence (5'-3')	Annealing (ºC)
Universal bacteria	Forward	CCTACGGGAGGCAGCAGT	59
	Reverse	ATTACCGCGGCTGCTGG	
Blautia spp.	Forward	TCTGATGTGAAAGGCTGGGGCTTA	56
	Reverse	GGCTTAGCCACCCGACACCTA	
Clostridium hiranonis	Forward	AGTAAGCTCCTGATACTGTCT	50
	Reverse	AGGGAAAGAGGAGATTAGTCC	
Escherichia coli	Forward	GTTAATACCTTTGCTCATTGA	55
	Reverse	ACCAGGGTATCTAATCCTGTT	
Faecalibacterium spp.	Forward	GAAGGCGGCCTACTGGGCAC	60
	Reverse	GTGCAGGCGAGTTGCAGCCT	
Fusobacteria spp.	Forward	KGGGCTCAACMCMGTATTGCGT	51
	Reverse	TCGCGTTAGCTTGGGCGCTG	
Streptococcus spp.	Forward	TTATTTGAAAGGGGCAATTGCT	54
	Reverse	GTGAACTTTCCACTCTCACAC	
Turicibacter spp.	Forward	CAGACGGGGACAACGATTGGA	63
	Reverse	TACGCATCGTCGCCTTGGTA	

Antibiotic-associated gastrointestinal signs for cats that received clindamycin and a placebo or synbiotic.

Mean (± standard deviation) percent food intake compared to the week prior to initiation of the study, percent days vomiting per week, and fecal score for 16 healthy cats, 8 per group, that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days.

	Baseline	Treatment		
		Week 1	Week 2	Week 3
Food intake (%)				
– Placebo	97.8 ± 4.1	89.9 ± 5.5	89.7 ± 11.3	88.1 ± 13.2
– Synbiotic	98.3 ± 4.1	92.7 ± 8.2	94.1 ± 8.4	92.7 ± 11.1
Vomiting (%)				
– Placebo	0 ± 0	26.8 ± 22.2	14.3 ± 20.2	9.0 ± 17.0
– Synbiotic	1.8 ± 5.1	21.5 ± 15.3	9.0 ± 15.2	1.8 ± 5.1
Fecal score				
– Placebo	2.6 ± 0.8	3.2 ± 0.8	3.5 ± 0.8	3.7 ± 1.3
– Synbiotic	2.7 ± 0.8	3.7 ± 0.9	3.8 ± 0.7	4.2 ± 0.4

Alpha diversity and dysbiosis index results for cats that received clindamycin and placebo or synbiotic.

Median (range) results for feces collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable at the days 631-633 timepoint. Cells that do not share a common superscript letter differed significantly ($P < 0.05$) based on post-hoc analysis.

	Baseline		Days 26-28		Days 631-633		P-value
	Placebo	**Synbiotic**	**Placebo**	**Synbiotic**	**Placebo**	**Synbiotic**	
Shannon index	3.9[a] (3.3-5.3)	4.2[a] (3.5-4.8)	2.5[b] (1.8-3.0)	2.8[b] (2.1-3.1)	4.4[a] (3.6-5.2)	4.9[a] (3.0-5.6)	<0.001
Goods coverage	0.9859[a] (0.9794-0.9919)	0.9857[a] (0.9819-0.9920)	0.9906[b] (0.9870-0.9942)	0.9907[b] (0.9875-0.9927)	0.9858[a] (0.9809-0.9909)	0.9866[a] (0.9815-0.9894)	<0.001
Chao1 metric	2,085[a] (1,126-3,158)	1,960[a] (1,044-2,930)	1,419[b] (830-2,026)	1,408[b] (1,043-2,062)	2,119[a] (1,272-2,945)	1,976[a] (1,349-2,622)	<0.001
Dysbiosis index	-2.9[c] (-5.5 to -1.6)	-3.0[c] (-5.2 to -0.2)	1.6[b] (0.4 to 3.3)	4.7[a] (1.1 to 5.4)	-2.6[c] (-5.1 to -1.0)	-4.4[c] (-5.2 to -0.4)	<0.001

Median percent abundance (range) for taxa with significantly different relative abundances over time.

Median (range) percent relative abundances of different taxa in feces were collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable at the days 631-633 timepoint. *P*-values adjusted based on the Benjamini and Hochberg False discovery rate (fdr). Relative abundances that do not share a common superscript letter differed significantly (fdr-adjusted $P < 0.05$) based on post-hoc analysis.

	Baseline		Days 26-28		Days 631-633		Fdr-adjusted P-value*
	Placebo	Synbiotic	Placebo	Synbiotic	Placebo	Synbiotic	
Actinobacteria	68.02[a] (34.02-87.12)	71.32[a] (55.95-83.97)	0.96[b] (0.82-1.07)	0.92[b] (0.69-1.20)	69.43[a] (38.38-82.91)	51.75[a] (31.39-90.03)	<0.001
• *Actinobacteria*	68.02[a] (34.02-87.12)	71.32[a] (55.95-83.97)	0.96[b] (0.82-1.07)	0.92[b] (0.69-1.20)	69.43[a] (38.38-82.91)	51.75[a] (31.39-90.03)	<0.001
− *Actinomycetales*	0.04[a] (0.00-0.07)	0.04[a] (0.02-0.06)	0.00[b] (0.00-0.01)	0.00[b] (0.00-0.01)	0.03[a] (0.01-0.13)	0.03[a] (0.02-0.16)	<0.001
o *Actinomycetaceae*	0.03[a] (0.00-0.06)	0.04[a] (0.01-0.06)	0.00[b] (0.00-0.01)	0.00[b] (0.00-0.01)	0.03[a] (0.01-0.13)	0.03[a] (0.01-0.14)	<0.001
▪ *Actinomyces*	0.033[a] (0.000-0.060)	0.037[a] (0.009-0.060)	0.001[b] (0.000-0.006)	0.003[b] (0.000-0.006)	0.026[a] (0.006-0.126)	0.034[a] (0.014-0.143)	<0.001
− *Bifidobacteriales*	39.16[a] (18.91-46.70)	46.10[a] (18.05-70.73)	0.54[b] (0.45-0.65)	0.49[b] (0.42-0.71)	39.16[c] (10.98-44.47)	18.72[c] (9.85-64.69)	<0.001
o *Bifidobacteriaceae*	39.16[a] (18.91-46.70)	46.10[a] (18.05-70.73)	0.54[b] (0.45-0.65)	0.49[b] (0.42-0.71)	39.16[c] (10.98-44.47)	18.72[c] (9.85-64.69)	<0.001
▪ *Bifidobacterium*	39.12[a] (18.91-46.70)	46.08[a] (18.01-70.72)	0.54[b] (0.45-0.65)	0.49[b] (0.42-0.71)	39.12[c] (10.97-44.46)	18.70[c] (9.82-64.66)	<0.001
• *Coriobacteriia*	27.29[a] (14.31-48.21)	21.64[a] (13.19-50.92)	0.42[b] (0.32-0.48)	0.40[b] (0.26-0.53)	34.95[a] (5.43-46.25)	25.28[a] (2.33-38.40)	<0.001
− *Coriobacteriales*	27.29[a] (14.31-48.21)	21.64[a] (13.19-50.92)	0.42[b] (0.32-0.48)	0.40[b] (0.26-0.53)	34.95[a] (5.43-46.25)	25.28[a] (2.33-38.40)	<0.001
o *Coriobacteriaceae*	27.29[a] (14.31-48.21)	21.64[a] (13.19-50.92)	0.42[b] (0.32-0.48)	0.40[b] (0.26-0.53)	34.95[a] (5.43-46.25)	25.28[a] (2.33-38.40)	<0.001
▪ *Adlercreutzia*	0.024[a] (0.003-0.051)	0.014[a] (0.006-0.037)	0.003[b] (0.000-0.003)	0.000[b] (0.000-0.003)	0.059[c] (0.006-0.094)	0.031[c] (0.009-0.217)	<0.001
▪ *Collinsella*	9.39[a] (1.39-25.36)	8.91[a] (3.19-21.39)	0.21[b] (0.17-0.24)	0.21[b] (0.16-0.25)	8.69[a] (4.17-33.50)	16.18[a] (1.88-20.90)	<0.001
▪ *Slackia*	0.13[a] (0.04-0.21)	0.11[a] (0.05-0.27)	0.01[b] (0.00-0.02)	0.01[b] (0.00-0.01)	0.17[a] (0.14-0.30)	0.19[a] (0.01-0.35)	<0.001
Bacteroidetes	0.54[a] (0.11-2.17)	0.80[a] (0.17-1.67)	0.09[b] (0.05-0.15)	0.10[b] (0.05-0.13)	3.35[a] (0.30-5.91)	0.82[a] (0.08-6.37)	<0.001
• *Bacteroidia*	0.54[a] (0.11-2.17)	0.80[a] (0.17-1.67)	0.09[b] (0.05-0.15)	0.10[b] (0.05-0.13)	3.35[a] (0.30-5.91)	0.81[a] (0.08-6.37)	<0.001
− *Bacteroidales*	0.54[a]	0.80[a]	0.09[b]	0.10[b]	3.35[a]	0.81[a]	<0.001

		(0.11-2.17)	(0.17-1.67)	(0.05-0.15)	(0.05-0.13)	(0.30-5.91)	(0.08-6.37)	
○	*Bacteroidaceae*	0.14ᵃ (0.07-1.67)	0.24ᵃ (0.10-1.13)	0.07ᵇ (0.04-0.13)	0.09ᵇ (0.04-0.11)	3.08ᶜ (0.21-5.85)	0.53ᶜ (0.07-6.03)	<0.001
▪	*Bacteroides*	0.14ᵃ (0.07-1.67)	0.24ᵃ (0.10-1.13)	0.07ᵇ (0.04-0.13)	0.09ᵇ (0.04-0.11)	3.08ᶜ (0.21-5.85)	0.53ᶜ (0.07-6.03)	<0.001
○	*Porphyromonadaceae*	0.007ᵃ (0.003-0.349)	0.013ᵃ (0.006-0.037)	0.003ᵇ (0.003-0.011)	0.006ᵇ (0.003-0.017)	0.071ᶜ (0.014-0.514)	0.063ᶜ (0.003-0.631)	<0.001
▪	*Parabacteroides*	0.007ᵃ (0.003-0.349)	0.013ᵃ (0.006-0.037)	0.003ᵇ (0.003-0.011)	0.006ᵇ (0.003-0.017)	0.071ᶜ (0.014-0.514)	0.063ᶜ (0.003-0.631)	<0.001
○	*Prevotellaceae*	0.19ᵃ (0.01-0.63)	0.36ᵃ (0.02-0.79)	0.009ᵇ (0.003-0.014)	0.006ᵇ (0.000-0.011)	0.10ᵃ (0.01-0.21)	0.14ᵃ (0.01-0.28)	<0.001
▪	*Prevotella*	0.194ᵃ (0.011-0.634)	0.363ᵃ (0.020-0.794)	0.009ᵇ (0.003-0.014)	0.006ᵇ (0.000-0.011)	0.100ᵃ (0.014-0.206)	0.137ᵃ (0.006-0.277)	<0.001
Firmicutes		30.29ᵃ (11.58-65.48)	27.64ᵃ (10.37-42.67)	91.84ᵇ (83.66-97.50)	90.75ᵇ (74.36-98.31)	26.21ᵃ (13.98-55.26)	47.08ᵃ (8.84-67.65)	<0.001
●	*Clostridia*	12.69ᵃ (4.63-33.88)	18.83ᵃ (6.76-35.33)	85.49ᵇ (81.13-95.00)	82.78ᵇ (70.84-96.36)	15.95ᵃ (7.95-44.59)	36.75ᵃ (2.44-45.25)	<0.001
−	*Clostridiales*	12.69ᵃ (4.63-33.88)	18.83ᵃ (6.76-35.33)	85.49ᵇ (81.13-95.00)	82.78ᵇ (70.84-96.36)	15.95ᵃ (7.95-44.59)	36.75ᵃ (2.44-45.25)	<0.001
○	*Clostridiales*; Other	0.25ᵃ (0.10-1.14)	0.31ᵃ (0.05-0.97)	0.06ᵇ (0.03-0.13)	0.05ᵇ (0.03-0.13)	0.79ᵃ (0.09-2.01)	0.54ᵃ (0.01-2.47)	<0.001
▪	Other	0.25ᵃ (0.10-1.14)	0.31ᵃ (0.05-0.97)	0.06ᵇ (0.03-0.13)	0.05ᵇ (0.03-0.13)	0.79ᵃ (0.09-2.01)	0.54ᵃ (0.01-2.47)	<0.001
○	*Clostridiaceae*	1.97ᵃ (1.32-6.98)	2.28ᵃ (1.56-11.82)	82.66ᵇ (78.23-92.91)	80.16ᵇ (68.32-94.47)	3.56ᵃ (1.23-22.91)	5.99ᵃ (1.12-24.29)	<0.001
▪	Other	0.77ᵃ (0.47-2.22)	0.75ᵃ (0.47-4.83)	66.32ᵇ (60.30-77.61)	57.64ᵇ (54.96-72.51)	0.67ᵃ (0.48-2.33)	0.82ᵃ (0.44-5.27)	<0.001
▪	___	0.83ᵃ (0.57-5.43)	1.40ᵃ (0.82-10.25)	8.52ᵇ (3.79-12.68)	7.67ᵇ (3.53-13.62)	2.71ᶜ (0.65-18.51)	4.76ᶜ (0.47-21.95)	<0.001
▪	*Clostridium*	0.16ᵃ (0.11-0.25)	0.16ᵃ (0.08-0.47)	9.97ᵇ (8.78-11.13)	9.20ᵇ (7.90-11.19)	0.15ᵃ (0.09-1.63)	0.19ᵃ (0.11-0.55)	<0.001
▪	SMB53	0.020ᵃ (0.009-0.151)	0.026ᵃ (0.014-0.389)	0.011ᵇ (0.009-0.026)	0.016ᵇ (0.011-0.077)	0.089ᶜ (0.014-0.740)	0.197ᶜ (0.009-0.817)	<0.001
○	*Lachnospiraceae*	6.71ᵃ (1.66-16.53)	9.12ᵃ (1.05-14.87)	1.40ᵇ (1.14-1.79)	1.46ᵇ (1.06-1.74)	7.92ᵃ (2.47-16.14)	14.56ᵃ (0.75-20.25)	<0.001
▪	Other	0.33ᵃ	0.68ᵃ	0.06ᵇ	0.06ᵇ	0.77ᵃ	0.78ᵃ	<0.001

		(0.05-0.73)	(0.07-1.50)	(0.04-0.11)	(0.03-0.09)	(0.27-1.28)	(0.05-1.97)	
▪	*Blautia*	4.75[a] (1.16-10.51)	6.16[a] (0.42-8.29)	0.58[b] (0.48-0.69)	0.60[b] (0.46-0.73)	4.49[a] (1.39-12.61)	10.91[a] (0.36-14.02)	0.002
▪	*Coprococcus*	0.016[a] (0.011-0.066)	0.027[a] (0.011-0.091)	0.014[b] (0.009-0.031)	0.013[b] (0.003-0.023)	0.050[c] (0.014-0.117)	0.086[c] (0.000-0.203)	0.002
▪	*Dorea*	0.52[a] (0.20-1.40)	0.54[a] (0.16-0.96)	0.35[a] (0.30-0.47)	0.33[a] (0.24-0.44)	0.69[b] (0.31-1.41)	1.01[b] (0.18-2.23)	0.014
▪	*Roseburia*	0.011[a] (0.003-0.151)	0.013[a] (0.003-0.040)	0.007[a] (0.000-0.020)	0.009[a] (0.000-0.023)	0.031[b] (0.026-0.160)	0.040[b] (0.003-0.280)	<0.001
▪	*[Ruminococcus]*	0.92[a] (0.17-5.49)	1.14[a] (0.24-3.84)	0.25[b] (0.20-0.38)	0.28[b] (0.18-0.35)	0.74[a] (0.21-1.20)	1.19[a] (0.11-2.05)	0.002
○	*Peptococcaceae*	0.15[a] (0.06-0.40)	0.20[a] (0.03-0.71)	0.06[b] (0.02-0.09)	0.06[b] (0.03-0.09)	0.23[a] (0.04-1.68)	0.34[a] (0.01-1.67)	0.015
▪	*Peptococcus*	0.15[a] (0.06-0.40)	0.20[a] (0.03-0.71)	0.06[b] (0.02-0.09)	0.06[b] (0.03-0.09)	0.23[a] (0.04-1.68)	0.34[a] (0.01-1.67)	0.014
○	*Ruminococcaceae*	1.22[a] (0.25-3.55)	1.97[a] (0.14-9.85)	0.25[b] (0.18-0.51)	0.22[b] (0.17-0.38)	0.82[a] (0.16-4.79)	0.58[a] (0.11-2.54)	<0.001
▪	*Faecalibacterium*	0.010[a] (0.000-0.146)	0.031[a] (0.003-0.077)	0.003[b] (0.000-0.009)	0.001[b] (0.000-0.011)	0.006[a] (0.000-0.063)	0.011[a] (0.000-0.091)	0.016
▪	*Oscillospira*	0.029[a] (0.009-0.163)	0.033[a] (0.011-0.057)	0.127[b] (0.066-0.320)	0.100[b] (0.023-0.174)	0.083[b] (0.026-0.434)	0.160[b] (0.006-0.543)	<0.001
▪	*Ruminococcus*	0.017[a] (0.006-0.031)	0.017[a] (0.006-0.040)	0.009[b] (0.006-0.017)	0.010[b] (0.003-0.014)	0.034[c] (0.011-0.160)	0.037[c] (0.003-0.054)	0.002
○	*Veillonellaceae*	1.03[a] (0.13-4.81)	0.97[a] (0.54-4.88)	0.07[b] (0.03-0.12)	0.08[b] (0.03-0.16)	0.26[c] (0.08-2.63)	0.17[c] (0.08-3.91)	<0.001
▪	*Megamonas*	0.11[a] (0.01-0.22)	0.21[a] (0.02-2.38)	0.02[b] (0.00-0.05)	0.02[b] (0.01-0.06)	0.04[a] (0.02-0.35)	0.05[a] (0.00-0.28)	0.009
▪	*Megasphaera*	0.86[a] (0.08-4.68)	0.72[a] (0.08-2.42)	0.04[b] (0.02-0.07)	0.05[b] (0.02-0.08)	0.23[c] (0.05-2.20)	0.09[c] (0.04-3.58)	<0.001
▪	*Phascolarctobacterium*	0.009[a] (0.003-0.029)	0.009[a] (0.000-0.069)	0.000[b] (0.000-0.006)	0.006[b] (0.000-0.017)	0.011[a] (0.003-0.063)	0.003[a] (0.000-0.046)	0.004
●	*Erysipelotrichi*	0.81[a] (0.21-15.32)	1.05[a] (0.18-5.59)	0.28[b] (0.21-0.36)	0.30[b] (0.22-0.37)	0.89[a] (0.21-6.46)	0.78[a] (0.24-12.78)	0.008
–	*Erysipelotrichiales*	0.81[a] (0.21-15.32)	1.05[a] (0.18-5.59)	0.28[b] (0.21-0.36)	0.30[b] (0.22-0.37)	0.89[a] (0.21-6.46)	0.78[a] (0.24-12.78)	0.005
○	*Erysipelotrichaceae*	0.81[a] (0.21-	1.05[a] (0.18-	0.28[b] (0.21-	0.30[b] (0.22-	0.89[a] (0.21-	0.78[a] (0.24-	0.006

	15.32)	5.59)	0.36)	0.37)	6.46)	12.78)	
▪ *Bulleidia*	0.019[a] (0.006-0.194)	0.006[a] (0.000-0.123)	0.003[b] (0.000-0.009)	0.004[b] (0.000-0.011)	0.016[a] (0.000-0.431)	0.009[a] (0.003-0.014)	0.015
▪ *[Eubacterium]*	0.26[a] (0.08-6.60)	0.22[a] (0.04-1.17)	0.07[b] (0.05-0.12)	0.08[b] (0.03-0.13)	0.27[a] (0.08-2.94)	0.33[a] (0.10-6.63)	<0.001
Proteobacteria	0.39[a] (0.24-3.03)	0.48[a] (0.29-4.74)	7.09[b] (1.28-15.46)	8.34[b] (0.85-24.53)	0.43[a] (0.28-0.57)	0.43[a] (0.21-0.89)	<0.001
● *Gammaproteobacteria*	0.27[a] (0.16-2.98)	0.34[a] (0.18-4.63)	7.04[b] (1.23-15.32)	8.30[b] (0.83-24.39)	0.25[c] (0.14-0.37)	0.31[c] (0.18-0.37)	<0.001
– *Aeromonadales*	0.01[a] (0.00-0.07)	0.02[a] (0.01-0.04)	0.03[b] (0.02-0.07)	0.03[b] (0.01-0.05)	0.01[a] (0.00-0.03)	0.01[a] (0.01-0.04)	0.010
○ *Succinivibrionaceae*	0.009[a] (0.003-0.069)	0.021[a] (0.006-0.043)	0.033[b] (0.017-0.071)	0.030[b] (0.014-0.051)	0.014[a] (0.003-0.029)	0.014[a] (0.006-0.040)	0.013
▪ *Anaerobiospirillumi*	0.007[a] (0.003-0.060)	0.017[a] (0.006-0.029)	0.031[b] (0.017-0.071)	0.030[b] (0.014-0.051)	0.014[a] (0.003-0.023)	0.011[a] (0.006-0.037)	0.002
– *Enterobacteriales*	0.26[a] (0.15-2.92)	0.32[a] (0.14-4.62)	6.99[b] (1.19-15.30)	8.25[b] (0.81-24.34)	0.23[c] (0.13-0.34)	0.28[c] (0.17-0.33)	<0.001
○ *Enterobacteriaceae*	0.26[a] (0.15-2.92)	0.32[a] (0.14-4.62)	6.99[b] (1.19-15.30)	8.25[b] (0.81-24.34)	0.23[c] (0.13-0.34)	0.28[c] (0.17-0.33)	<0.001

Metabolites of known biological import with profiles that significantly differed over time.

Median (range) peak height of metabolites in feces collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,[+] that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. [+]Feces from one cat (synbiotic group) unavailable at days 631-633. *P-values adjusted based on the Benjamini and Hochberg False discovery rate (fdr). Profiles that do not share a common superscript letter differed significantly (fdr-adjusted $P < 0.05$) based on post-hoc analysis.

	Baseline		Days 26-28		Days 631-633		Fdr-adjusted P-value*
	Placebo	Synbiotic	Placebo	Synbiotic	Placebo	Synbiotic	
Short chain fatty acid metabolites							
3,4-dihydroxyphenylacetic acid	589[a] (317-1,201)	521[a] (221-1,577)	741[a] (263-1,130)	594[a] (126-1,522)	197[b] (66-1,436)	199[b] (63-643)	<0.001
3-hydroxyphenylacetic acid	3,350[a] (582-12,188)	2,860[a] (55-11,401)	65[b] (36-94)	57[b] (26-239)	554[c] (77-3,578)	275[c] (124-758)	<0.001
4-hydroxyphenylacetic acid	24,894[a] (10,957-34,200)	24,248[a] (12,980-73,736)	693[b] (473-6,935)	3,268[b] (378-139,343)	48,775[a] (10,270-133,645)	47,322[a] (837-128,116)	<0.001
2,3-dihydroxybutanoic acid	167[a] (119-347)	193[a] (67-240)	83[b] (18-157)	108[b] (25-268)	216[a] (97-356)	128[a] (63-376)	<0.001
2-hydroxybutanoic acid	12,514[a] (3,401-161,892)	10,110[a] (626-61,119)	38,839[b] (26,636-58,406)	38,680[b] (18,670-76,553)	3,846[c] (1,170-13,209)	3,082[c] (623-13,862)	<0.001
4-aminobutyric acid	2,002[a] (1,665-12,898)	2,573[a] (1,189-11,658)	112,677[b] (72,970-190,000)	156,808[b] (56,870-393,600)	26,744[c] (3,966-89,927)	20,753[c] (437-60,568)	<0.001
2,4-diaminobutyric acid	1,758[a] (568-3,102)	1,119[a] (184-3,865)	210[b] (140-350)	254[b] (109-590)	3,095[c] (1,652-8,818)	3,017[c] (765-6,398)	<0.001
3-phenyllactic acid	5,539[a] (781-31,484)	5,796[a] (438-32,942)	9,109[b] (7,089-11,018)	12,813[b] (7,617-21,692)	6,537[a] (1,217-19,572)	9,052[a] (331-16,813)	<0.001
Lactic acid	152,579[a] (24,216-1,034,220)	120,122[a] (10,433-1,403,821)	392,894[b] (269,519-1,091,298)	308,949[b] (129,795-638,477)	27,338[c] (11,859-149,087)	35,473[c] (6,439-111,809)	<0.001
P-hydroxylphenyl-lactic acid	3,698[a] (1,153-6,357)	4,503[a] (581-12,311)	3,590[a] (2,472-6,688)	4,760[a] (2,300-5,993)	1,654[b] (478-5,019)	1,431[b] (405-2,022)	<0.001
Propane-1-3-diol	1,108[a] (426-15,239)	1,900[a] (272-5,779)	12,212[b] (3,684-26,363)	12,461[b] (4,117-20,201)	359[c] (174-1,203)	341[c] (99-502)	<0.001
3,3-hydroxyphenyl propionic acid	76,962[a] (18,783-212,470)	45,560[a] (865-240,661)	789[b] (88-1,348)	648[b] (188-1,431)	77,032[a] (666-167,768)	27,581[a] (140-134,885)	<0.001
3-(4-hydroxyphenyl)-propionic acid	65,664[a] (28,474-90,967)	56,101[a] (4,122-141,022)	4,255[b] (350-16,751)	6,447[b] (415-16,156)	28,844[c] (4,747-68,675)	14,394[c] (12,923-79,425)	<0.001
Pyruvic acid	7,118[a] (1,844-19,684)	7,900[a] (3,534-12,585)	7,744[a] (3,418-20,148)	9,650[a] (1,509-48,246)	915[b] (456-3,736)	1,588[b] (489-2,230)	<0.001

Succinic acid	236,185[a] (14,243-708,079)	88,426[a] (882-1,122,477)	41,400[a] (25,669-96,357)	48,077[a] (7,555-107,389)	8,572[b] (3,020-65,677)	9,082[b] (1,337-65,270)	<0.001
Bile acids							
Cholic acid	109,466[a] (1,898-200,811)	59,483[a] (423-220,771)	11,303[b] (113-76,704)	27,391[b] (286-89,000)	9,511[b] (240-77,925)	8,907[b] (3,191-95,296)	0.04
Deoxycholic acid	3,295[a] (77-28,703)	1,843[a] (109-43,646)	136[b] (45-5,010)	352[b] (27-3,531)	11,544[a] (150-111,319)	8,762[a] (687-309,022)	0.01
Tryptophan metabolites							
Indole-3-acetate	13,713[a] (7,780-94,057)	8,832[a] (3,110-125,618)	841[b] (541-1,795)	1,051[b] (448-11,089)	15,203[a] (6,967-22,835)	27,026[c] (17,204-168,850)	<0.001
Indole-3-lactate	80,846[a] (34,669-127,305)	62,189[a] (7,536-128,029)	6,751[b] (2,893-11,123)	7,133[b] (4,437-22,490)	15,933[b] (773-44,962)	5,311[b] (836-54,443)	<0.001
Tryptophan	190,390[a] (132,057-340,750)	173,956[a] (106,947-276,525)	120,418[b] (95,182-158,327)	153,738[b] (19,076-294,367)	20,219[c] (3,724-138,699)	35,369[c] (5,707-174,485)	<0.001
Sphingolipid metabolites							
Cellobiose	54,905[a] (14,235-120,530)	92,176[a] (5,399-111,969)	28,177[ab] (6,974-261,178)	10,182[b] (2,284-14,269)	2,513[b] (249-26,017)	5,248[b] (753-24,528)	<0.001
D-erythro-sphingosine	7,341[a] (4,159-39,450)	8,433[a] (2,795-14,346)	10,575[a] (1,003-22,798)	3,460[a] (620-20,251)	2,778[b] (869-6,781)	21,981[a] (438-49,033)	<0.001
Isopentadecanoic acid	16,537[b] (8,419-47,233)	19,852[a] (6,576-44,308)	32,099[b] (1,673-766,411)	5,119[b] (1,627-17,766)	25,695[b] (7,307-84,652)	65,848[c] (21,866-707,919)	<0.001
Polyamines							
Putrescine	325,567[a] (97,835-1,380,413)	129,926[a] (44,868-363,883)	38,927[b] (27,370-102,805)	83,581[a] (28,669-207,557)	60,980[a] (11,066-212,790)	201,011[a] (6,192-485,041)	0.01
Antioxidants / antimicrobials							
3,4-dihydroxyhydrocinnamic acid	20,308[a] (11,158-79,529)	19,951[a] (9,793-36,089)	6,608[b] (3,079-14,086)	7,699[b] (4,398-16,818)	28,082[a] (1,849-40,617)	26,438[a] (1,485-61,532)	<0.001
3,4-dihydroxycinnaminic acid	4,356[a] (1,269-10,007)	6,229[a] (1,990-25,507)	2,543[a] (1,178-16,989)	2,955[a] (1,481-18,549)	1,468[b] (281-6,545)	760[b] (161-4,120)	<0.001
3,4-dihydroxybenzoic acid	4,141[a] (1,918-12,984)	4,062[a] (2,054-13,140)	2,114[b] (1,533-3,328)	2,489[b] (1,694-4,578)	13,395[c] (2,783-26,592)	6,790[c] (518-26,569)	<0.001

4-hydroxybenzoate	6,538[a] (3,972-27,743)	11,523[a] (4,231-17,030)	1,035[b] (665-1,411)	1,201[b] (619-1,940)	16,773[c] (8,232-23,719)	16,643[c] (420-49,060)	<0.001

Metabolic pathways that differed significantly over time.

Pathways that differed significantly over time by group based on fecal metabolite profiles in feces collected at baseline (days 5-7), at the conclusion of antibiotic administration (days 26-28), and after a 603 day washout (days 631-633) from 16 healthy cats, 8 per group,+ that received 150 mg clindamycin with either a placebo or synbiotic PO once daily for 21 days. +Feces from one cat (synbiotic group) unavailable at the days 631-633 timepoint. Total Cmpds = total compounds in pathway; * = amino acid metabolism, ° = sugar metabolism, † = short-chain fatty acid metabolism, ‡ = nucleotide metabolism.

		Total Cmpds	Hits	fdr-adjusted P-value	Pathway impact value
1	Linoleic acid metabolism	15	1	0.018	0.66
2*	Alanine, aspartate and glutamate metabolism	24	9	1.54E-10	0.59
3°	Galactose metabolism	41	16	2.00E-08	0.58
4*	Glycine, serine and threonine metabolism	48	9	4.69E-09	0.54
5*	Arginine and proline metabolism	77	16	1.54E-10	0.48
6*	Taurine and hypotaurine metabolism	20	4	1.37E-08	0.38
7°	Starch and sucrose metabolism	50	8	2.87E-08	0.36
8*	beta-Alanine metabolism	28	6	7.25E-09	0.34
9	Pyruvate metabolism	32	2	3.82E-06	0.32
10*	Phenylalanine metabolism	45	13	1.18E-08	0.27
11†	Pantothenate and CoA biosynthesis	27	6	2.77E-09	0.25
12*	Cysteine and methionine metabolism	56	8	4.43E-09	0.23
13	Nicotinate and nicotinamide metabolism	44	4	1.43E-08	0.23
14*	Lysine degradation	47	4	1.50E-02	0.23
15‡	Pyrimidine metabolism	60	9	6.20E-08	0.22
16	Arachidonic acid metabolism	62	1	1.01E-04	0.22
17	Glycerolipid metabolism	32	5	2.80E-08	0.22
18	Histidine metabolism	44	3	1.43E-08	0.21
19*‡	Amino sugar and nucleotide sugar metabolism	88	9	1.37E-08	0.21
20*	Tyrosine metabolism	76	10	1.96E-09	0.20

21	Aminoacyl-tRNA biosynthesis	75	18	2.84E-10	0.17
22†	Butanoate metabolism	40	7	9.61E-11	0.17
23*	Tryptophan metabolism	79	2	2.77E-09	0.16
24*	D-Glutamine and D-glutamate metabolism	11	2	3.82E-06	0.14
25	Inositol phosphate metabolism	39	1	2.60E-03	0.14
26	Citrate cycle (TCA cycle)	20	3	9.75E-06	0.12
27	Sulfur metabolism	18	4	1.59E-08	0.11
28°	Pentose phosphate pathway	32	5	8.38E-12	0.11
29‡	Purine metabolism	92	12	1.61E-10	0.11
30*	Phenylalanine, tyrosine and tryptophan biosynthesis	27	5	4.90E-09	0.11
31*	Lysine biosynthesis	32	2	3.07E-03	0.10
32	Glycolysis or Gluconeogenesis	31	4	2.12E-06	0.10
33	Sphingolipid metabolism	25	3	5.39E-04	0.09
34°	Pentose and glucuronate interconversions	53	7	7.42E-09	0.09
35	Glycerophospholipid metabolism	39	2	3.38E-07	0.09
36†	Propanoate metabolism	35	6	2.23E-06	0.09
37*	Valine, leucine and isoleucine biosynthesis	27	6	9.82E-07	0.09
38°	Fructose and mannose metabolism	48	3	4.94E-04	0.07
39	Nitrogen metabolism	39	10	2.77E-09	0.07
40*	Valine, leucine and isoleucine degradation	40	4	1.84E-04	0.06
41	Ubiquinone and other terpenoid-quinone biosynthesis	36	4	2.84E-10	0.05
42	Glyoxylate and dicarboxylate metabolism	50	4	1.18E-06	0.04
43	Caffeine metabolism	21	1	1.84E-04	0.03

44	Glutathione metabolism	38	5	3.28E-10	0.03
45	Ascorbate and aldarate metabolism	45	3	2.59E-08	0.02
46	Vitamin B6 metabolism	32	1	1.47E-05	0.02
47	Methane metabolism	34	2	1.30E-05	0.02