



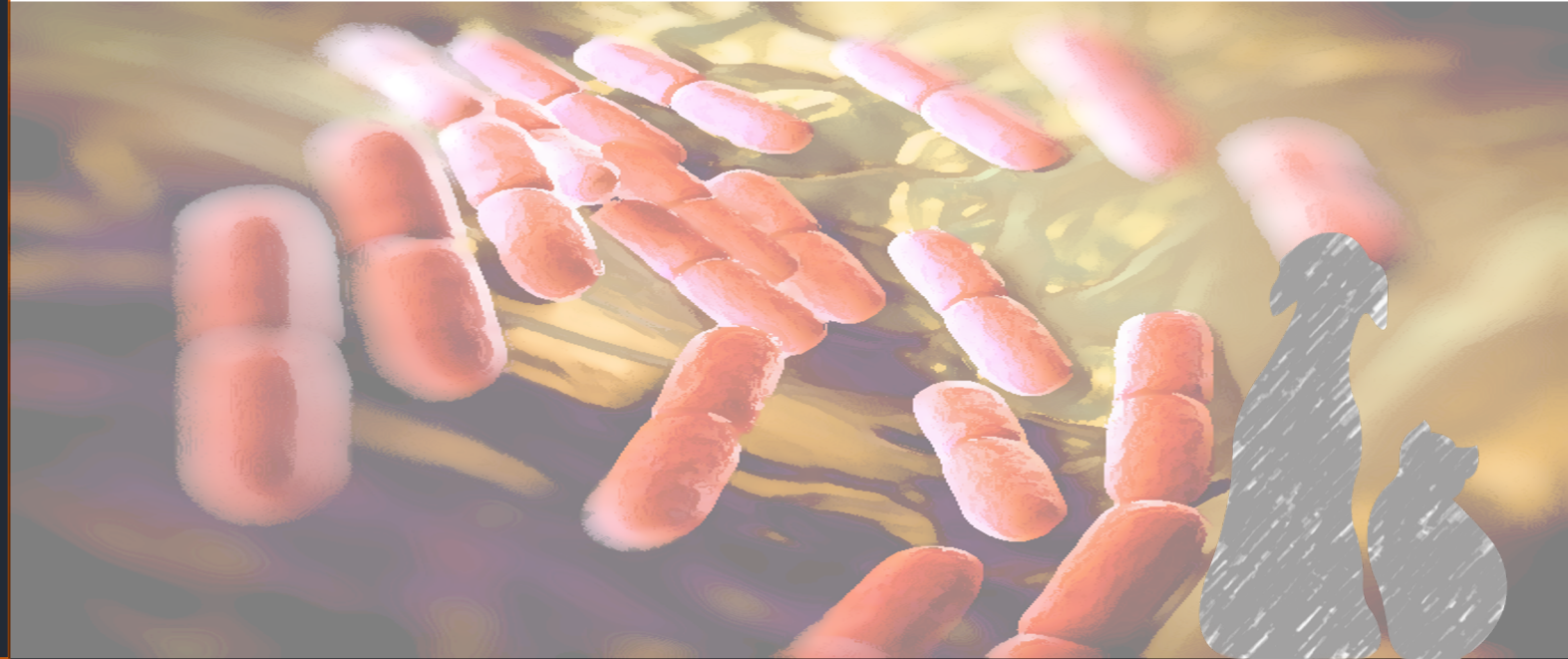
Maria R. C. de Godoy, Ph.D.
Associate Professor

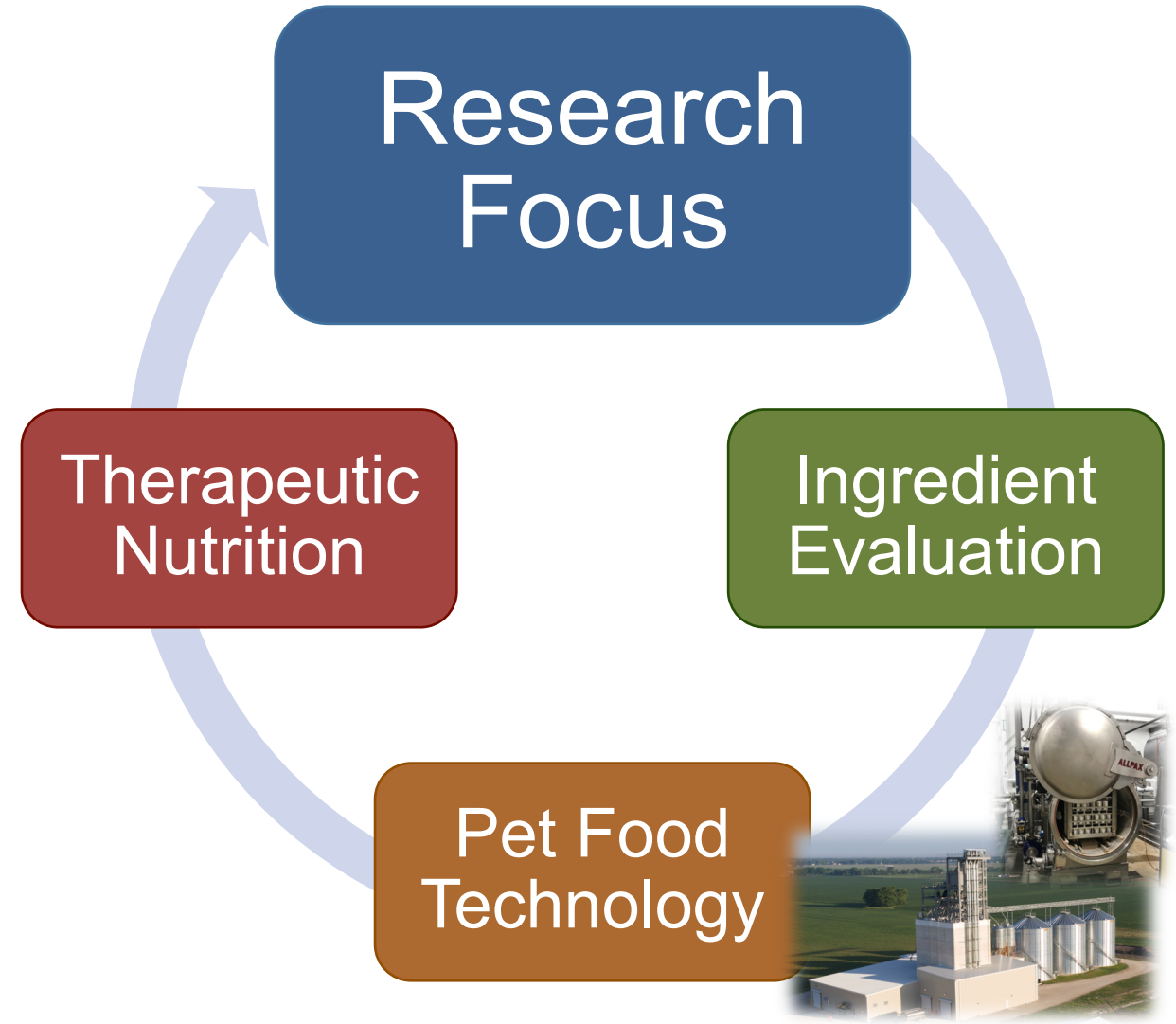
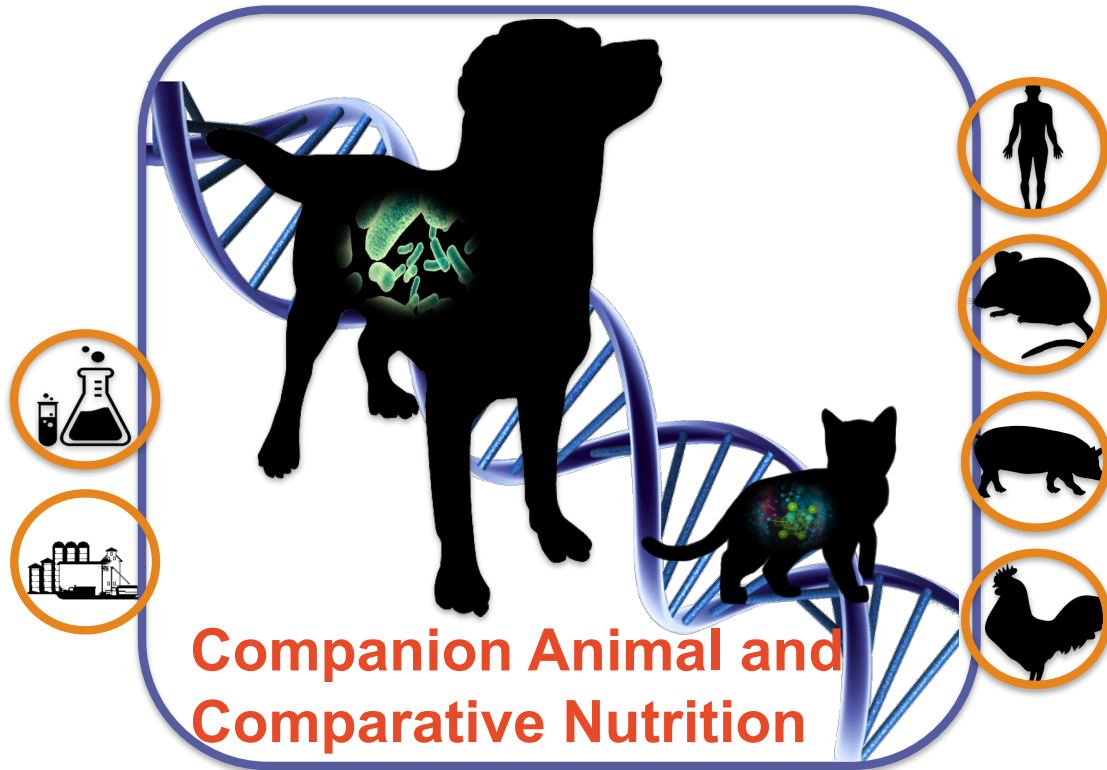
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Impact of dietary protein on intestinal and host health

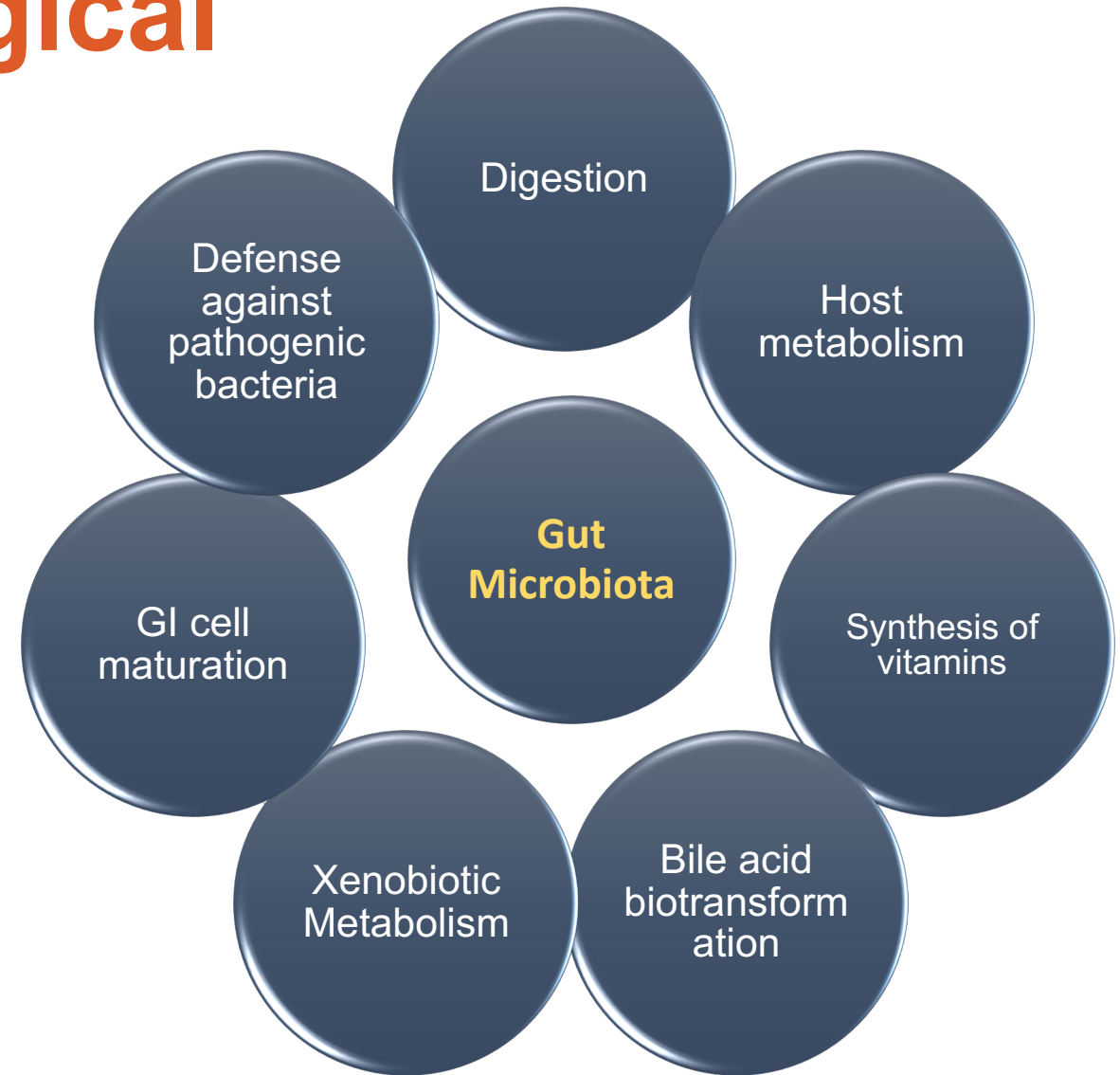




Role of the physiological gut microbiota

Intestinal microbiota:

- Influence host metabolism
- Protect against pathogens
- Aid in the development of immune system
- Affect physiological functions of the host



Role of the physiological gut microbiota

Intestinal microbiota:

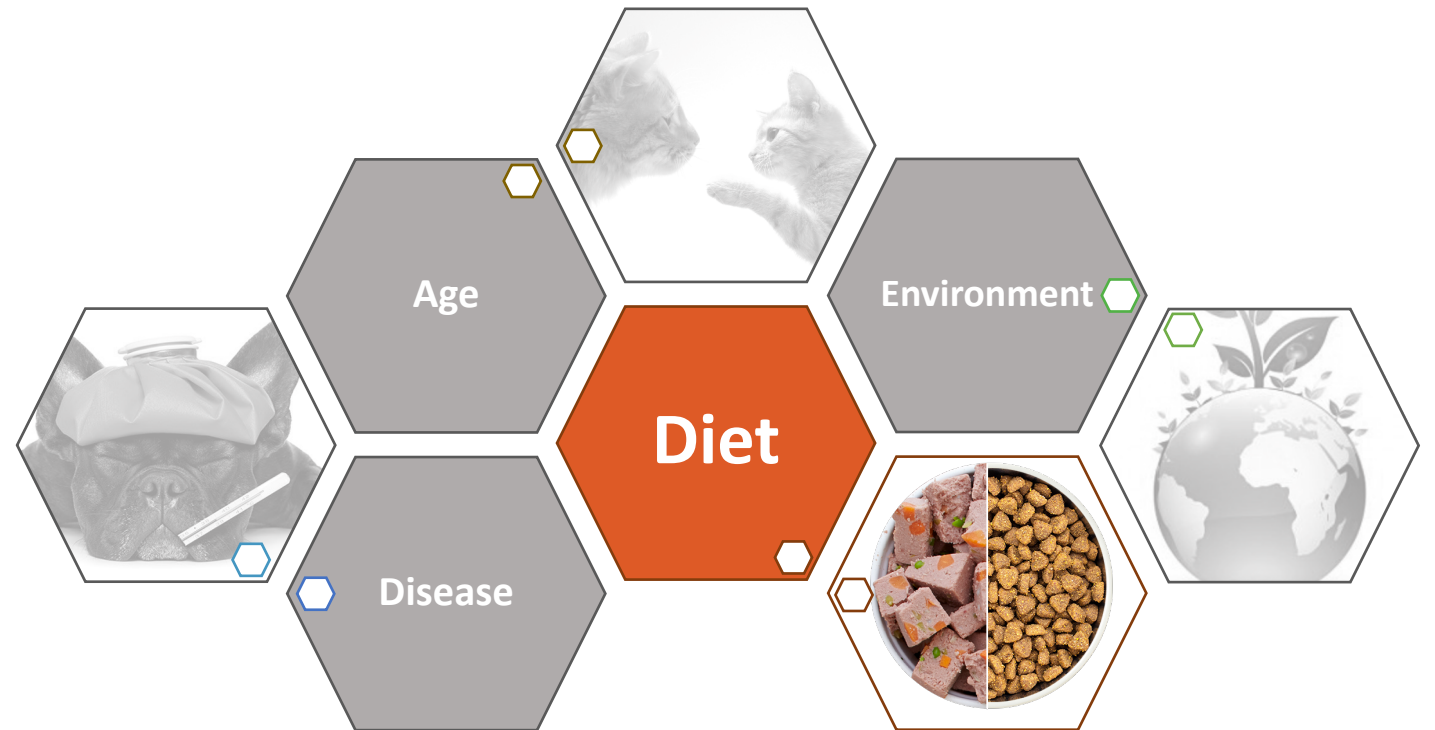
- Composition is stable in adult healthy animals
- Influenced by many factors



Role of the physiological gut microbiota

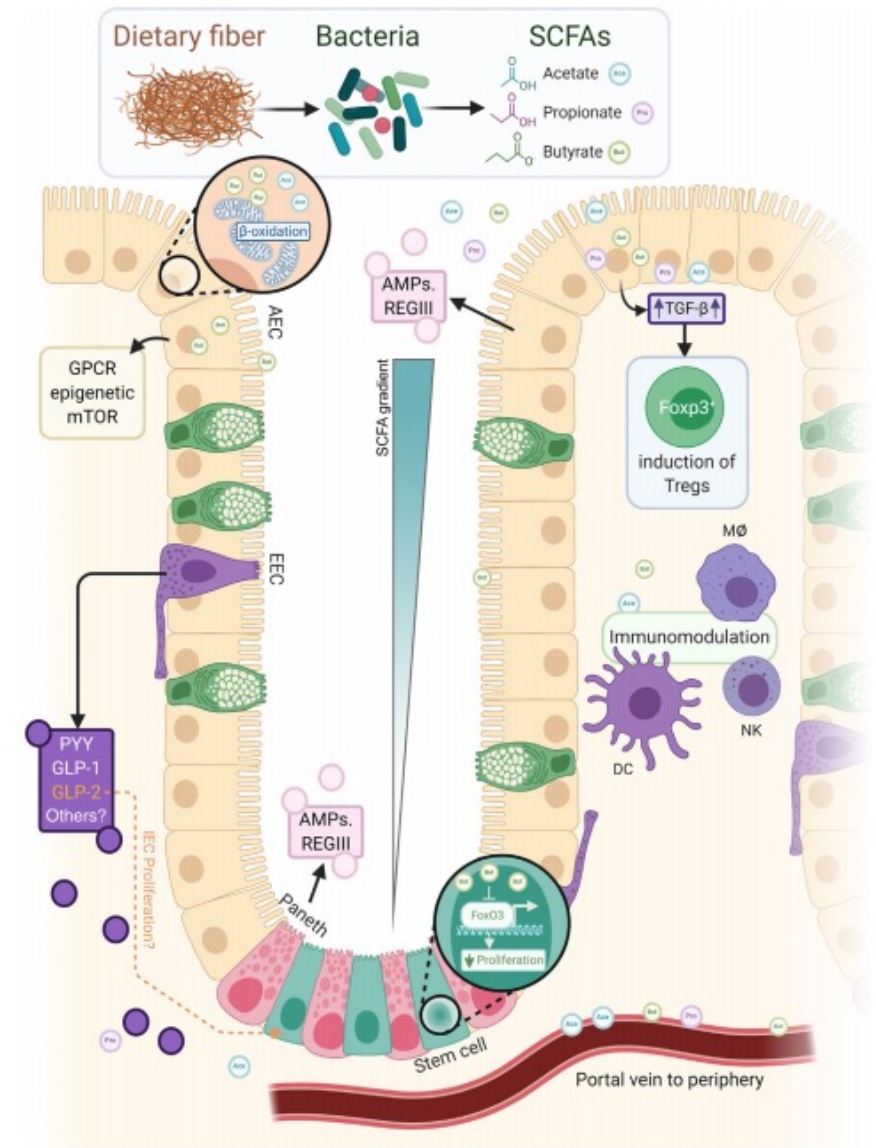
Intestinal microbiota:

- Composition is stable in adult healthy animals
- Influenced by many factors



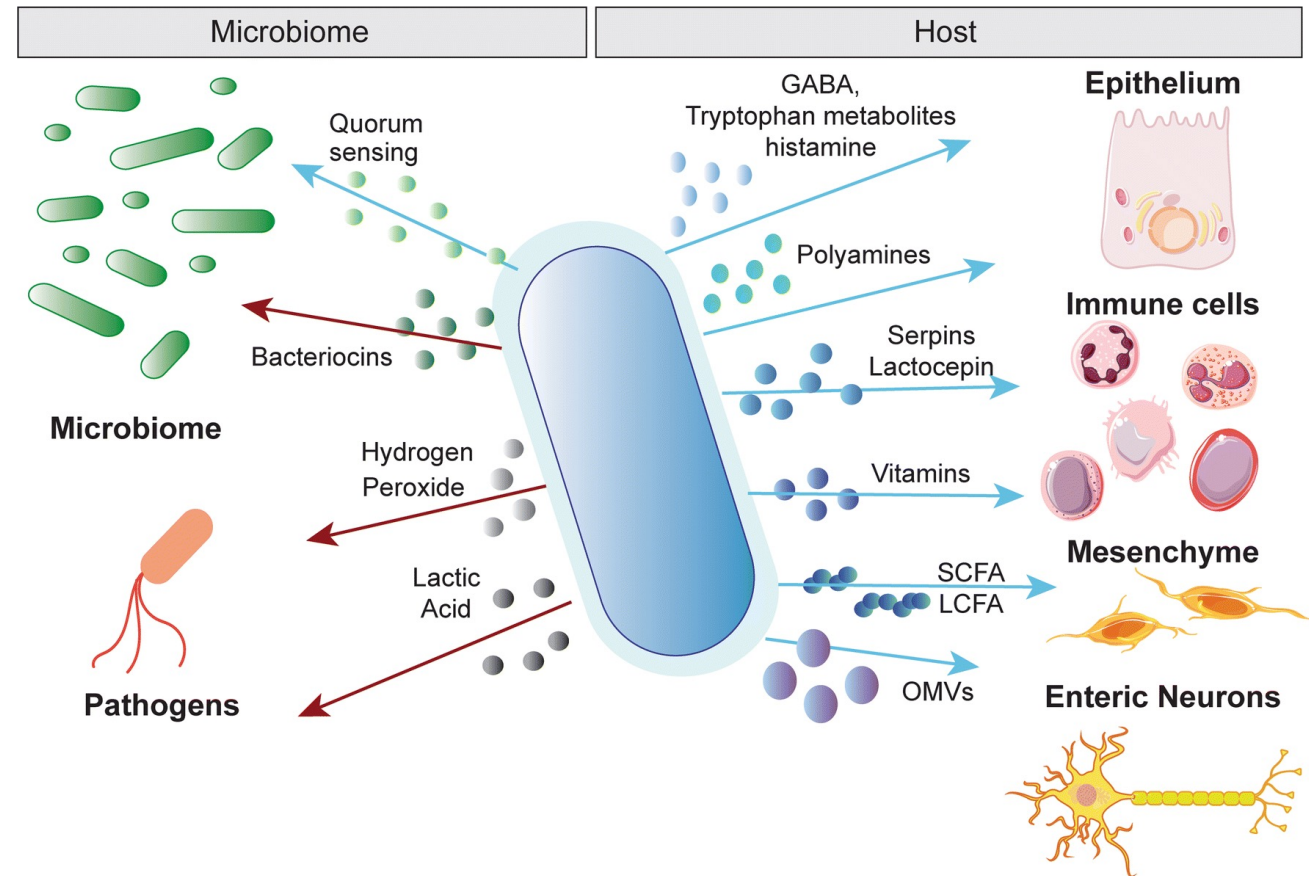
Role of the physiological gut microbiota

- Bacteria metabolic activities produce energy and substrates
- Fermentation of carbohydrates
 - Production SCFAs leads to a reduction of intestinal pH value
 - Supports growth, proliferation, and differentiation of epithelial intestinal cells



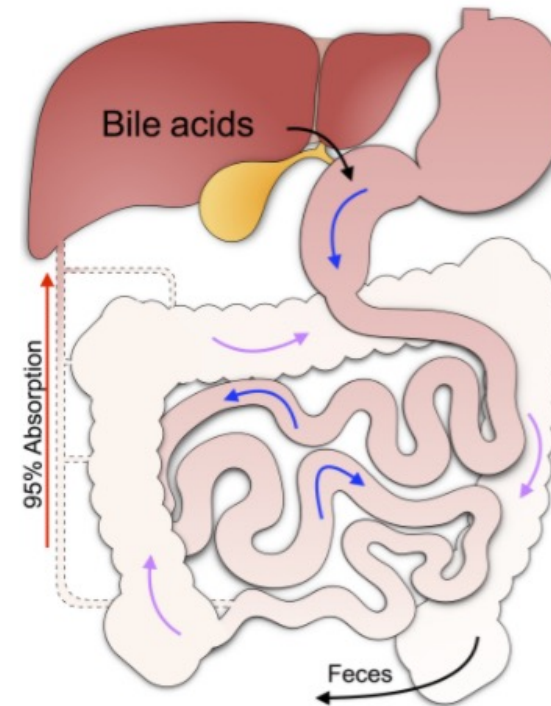
Role of the physiological gut microbiota

- Generation of microbial-derived metabolites
 - Modulate intra and inter-kingdom interactions
 - Impact host health

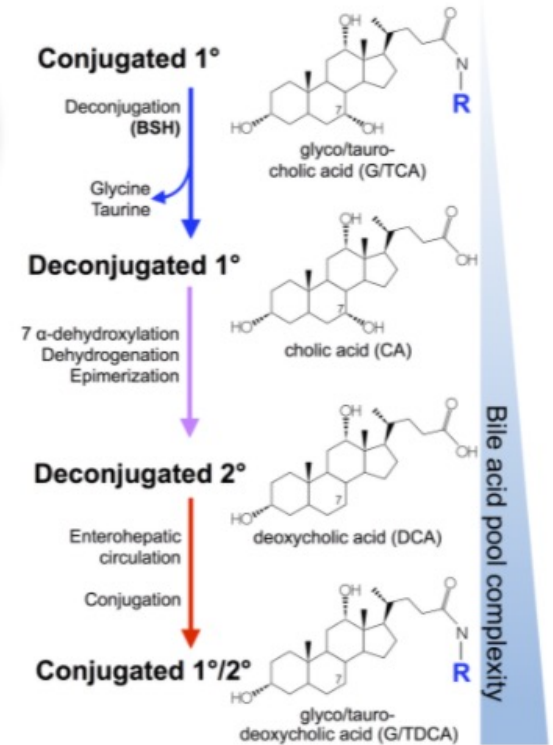


Role of the physiological gut microbiota

- Transformation both of primary bile acids (cholic acid and chenodeoxycholic acid) and secondary bile acids (deoxycholic and lithocholic)
- Essential in the digestion/ absorption of dietary fats and liposoluble vitamins in the gut
- Important role in mucosal defense and have anti-inflammatory properties
- Gut dysbiosis or inflammation can impair bile acid metabolism

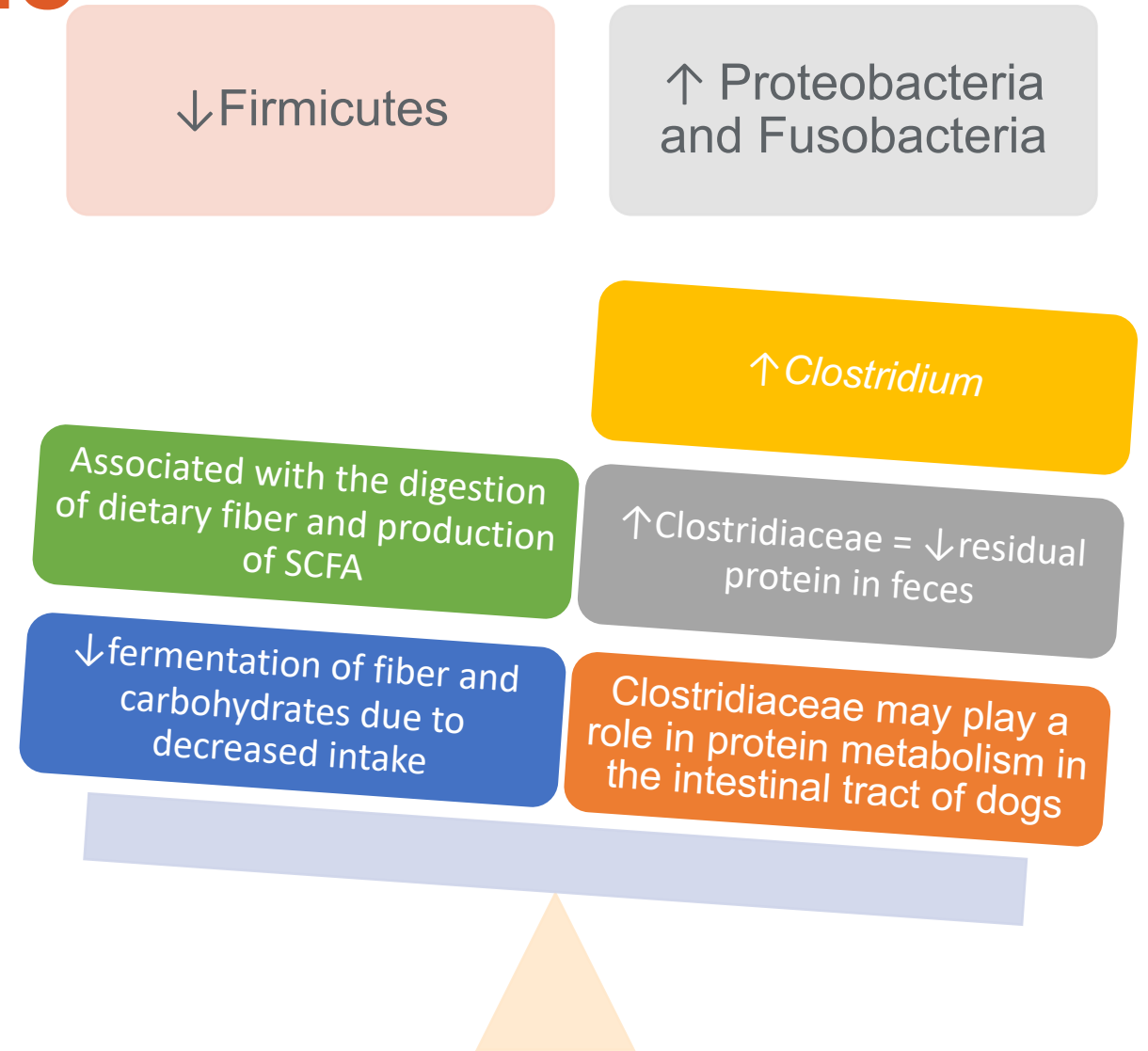


doi: <https://doi.org/10.1371/journal.ppat.1007581.g001>

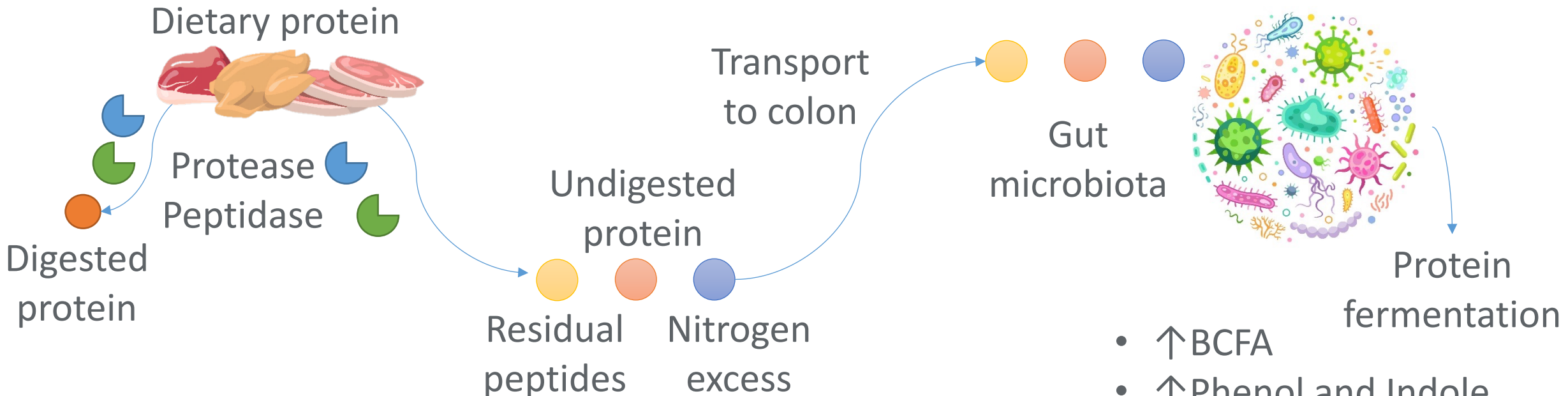


Effect of protein on the gut microbiota

↓ carbohydrates + ↑ proteins =



Protein metabolism by gut microbiota



- ↑ Fusobacteria and Proteobacteria
- ↓ Firmicutes (Ruminococcaceae and *Faecalibacterium* genus)
- ↑ abundance of *Clostridium hiranonis*, *Clostridium perfringens*, *Clostridium ramosum*

- ↑ BCFA
- ↑ Phenol and Indole
- ↑ Sulphur-containing compounds
- ↑ Ammonia and amines
- ↑ Putrefactive compounds



Hesta et al. (2003); Zentek et al. (2003); Kuda et al. (2014); Butteiger et al. (2016); Singh et al. 2017; Ma et al. (2017); Li et al. (2017)

Let's examine a few examples!



Dietary format alters fecal bacterial populations in the domestic cat (*Felis catus*)Emma N. Bermingham¹, Wayne Young¹, Sandra Kittelmann², Katherine R. Kerr³, Kelly S. Swanson^{3,4}, Nicole C. Roy^{1,5} & David G. Thomas⁶¹Food Nutrition & Health, Food & Bio-based Products Group, AgResearch Grasslands, Palmerston North 4442, New Zealand²Rumen Microbiology, Animal Nutrition & Health Group, AgResearch Grasslands, Palmerston North 4442, New Zealand³Division of Nutritional Sciences, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA⁴Department of Animal Sciences, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA⁵The Riddet Institute, Massey University, Palmerston North 4442, New Zealand⁶Centre of Feline Nutrition, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North 4442, New Zealand**Dry diet (33% CP) vs. Wet Diet (42% CP)****Table 1.** Macronutrient profile of commercially available Association of American Feed Control Officials (AAFCO)-tested maintenance diets fed to domestic short-hair cats (*Felis catus*).

Component	Dry diet ¹	Wet diet ²
Dry matter (DM; % as is)	89.20	23.03
Crude protein (% DM)	32.91	41.87
Crude fat (% DM)	11.05	42.39
Ash (% DM)	8.28	8.81
Crude fiber (% DM)	1.88	1.62
NFE ³ (% DM)	45.88	5.31
Gross energy (kcal/g DM)	4.80	6.66
Metabolizable energy ⁴ (ME; kcal/g DM)	3.70	5.25

¹Ingredient list of dry diet (from pack): corn and corn protein; rice flour; meat products and meat derived from poultry, fish, lamb, and tuna; digest of poultry; chicken fat; palm stearine; dicalcium phosphate; salt; vitamins.

²Ingredient list of wet diet (from pack): meat byproducts and meat derived from lamb, beef, chicken and mutton; vegetable protein; gelling agent; minerals; emulsifier; coloring; vitamins + taurine.

³Nitrogen-free extract calculated by difference (100 – crude protein – crude fat – crude fiber – ash).

⁴Determined using modified Atwater factors of crude protein (3.5 kcal ME/g DM), crude fat (8.5 kcal ME/g DM), NFE (3.5 kcal ME/g DM).

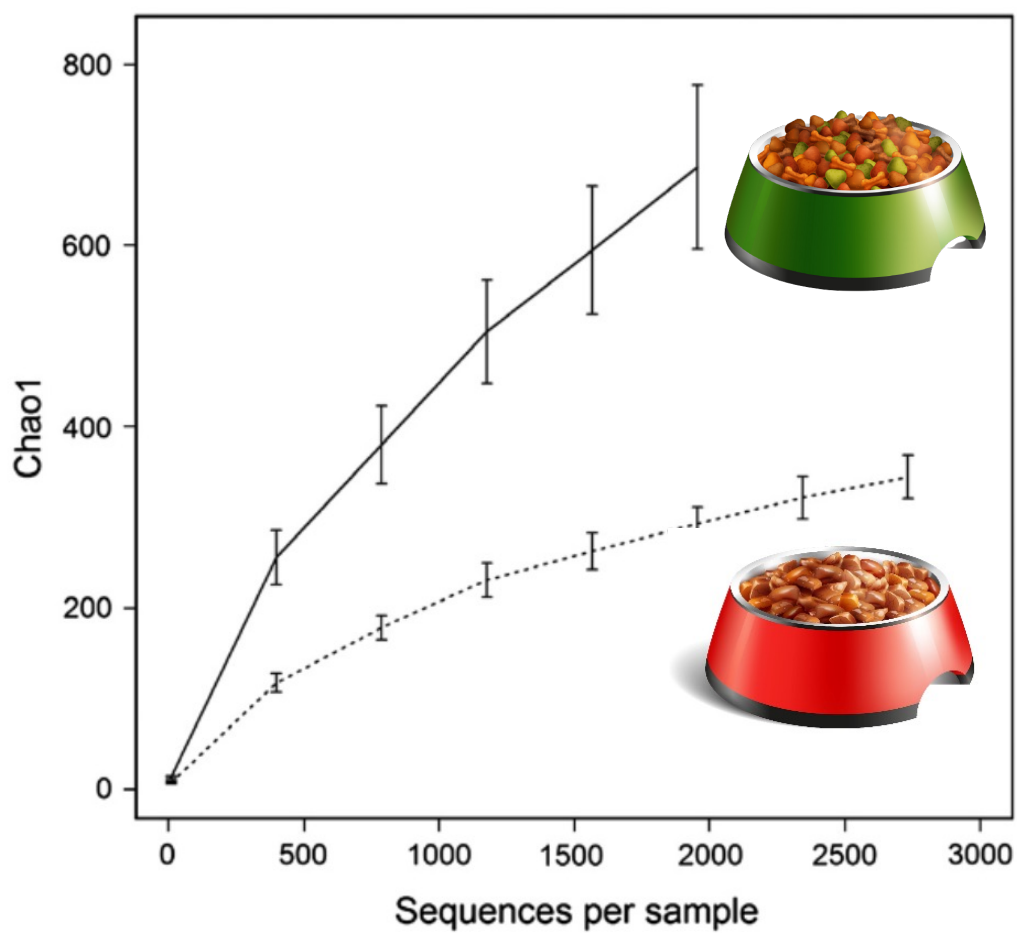


Figure 1. The effects of short-term exposure to a wet or dry diet on faecal microbial diversity. The rarefaction curve indicates the faecal microbiota CHAO1 diversity index (Chao1 index at 97% sequence identity cut-off) observed over the number of sequences sampled between cats fed wet (—) and dry (---) diets. Data are reported as means \pm SEM ($n = 16$ cats per treatment).

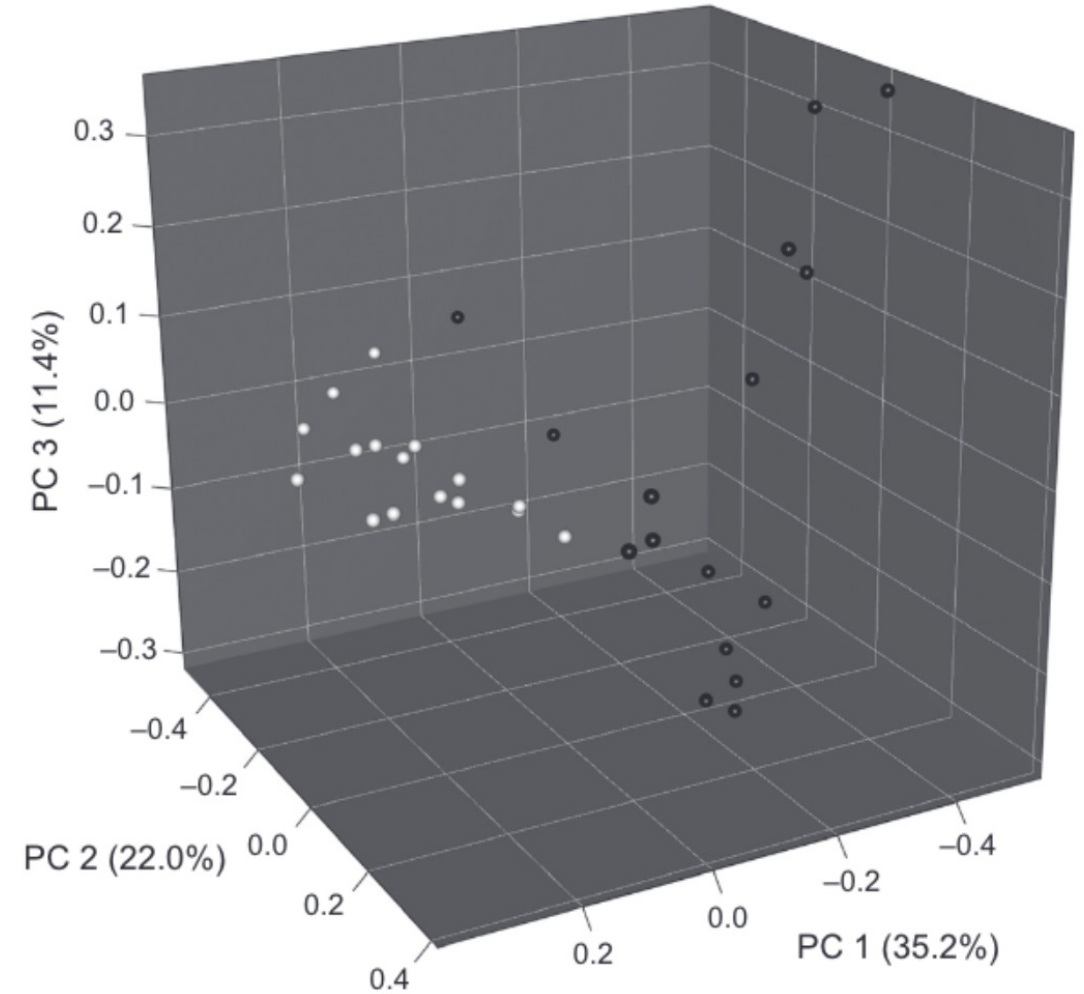


Figure 2. Principal Coordinate Analysis plot of weighted Unifrac phylogenetic distances showing the similarities between bacterial communities of cats fed dry (white) or wet (black) diets. Axes indicate percentage of variation explained by each principal coordinate.

Table 3. The effects of short-term exposure to a wet or dry diet on fecal bacterial phyla (% of total reads) in adult domestic short-hair cats (*Felis catus*). *P*-value indicates significance of Kruskal–Wallis rank sum test, and *q*-value indicates false discovery rate multiple-testing adjusted *P*-value.

Taxon	Dry (<i>n</i> = 16)	Wet (<i>n</i> = 16)	Pooled SEM	<i>P</i> -value	<i>q</i> -value
Actinobacteria	16.5	0.1	3.8	0.011	0.068
Bacteroidetes	8.7	15.9	4.7	0.010	0.062
Firmicutes	73.6	57.6	7.7	0.090	0.539
Fusobacteria	0.3	23.1	2.9	<0.000	<0.000
Proteobacteria	0.4	1.1	0.2	0.008	0.047
Unclassified bacteria	0.5	2.1	0.2	<0.000	0.002

Wet diet:

- ↓ *Lactobacillus*,
Megasphaera, and
Olsenella

RESEARCH ARTICLE

Open Access



A diet change from dry food to beef induces reversible changes on the faecal microbiota in healthy, adult client-owned dogs

Kristin M. V. Herstad^{1*}, Karina Gajardo², Anne Marie Bakke², Lars Moe¹, Jane Ludvigsen³, Knut Rudi³, Ida Rud⁴, Monika Sekelja^{3,5} and Ellen Skancke¹

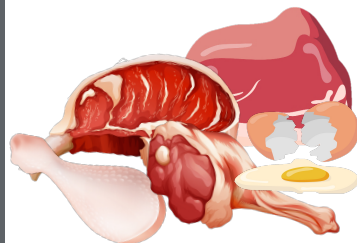


Table 2 Ingredients and nutrient composition of the rations during the seven-week dietary intervention study

	Rations				
	CD	LMB	MMB	HMB	MB
Ingredients, % of fresh weight in ration					
CD	100	61	34	15	-
MB	-	39	66	85	100
Nutrient composition, g/100 g DM					
Crude protein	27.1	32.5	38.9	46.2	55.3
Crude lipid	16.3	21.0	26.7	33.1	41.2
NFE	48.3	39.1	28.1	15.6	0
Crude fibre	1.2	1.0	0.7	0.4	0
Fibre (NSP)	10.4	8.4	6.1	3.4	0
Ash	7.0	6.4	5.6	4.7	3.5
ME (MJ/100 g DM)	1.80	1.93	2.09	2.28	2.50
DM in ration, as fed	92.2	69.5	53.8	42.7	34.0

Abbreviations and diet codes: *CD* commercial dry food (Felleskjøpet's Labb adult), *DM* dry matter, *HMB* high minced beef, *LMB* low minced beef, *MB* minced beef (retail sourced, Norway), *ME* metabolizable energy, *MJ* megajoules, *MMB* moderate minced beef, *NFE* nitrogen-free extract, *NSP* non-starch polysaccharides



Table 3 Median ♦ faecal pH, water, consistency, diversity index and observed species from the dietary intervention study

	Diet periods					Signed-Ranks test	
	CD1	LMB	MMB	HMB	CD2	<i>p</i> -values	
						CD1 vs. HMB ¹	CD2 vs. HMB ¹
pH ²	6.51 [6.22–7.07]	6.55 [6.2–6.77]	6.67 [6.46–6.91]	6.72 [6.66–7.03]	6.49 [6.03–6.83]	0.016*	0.063**
Water (%)	46 [39–64.6]	45.2 [40.3–67.6]	46.6 [40.8–62.6]	46.6 [40.5–68.6]	50.22 [40.3–68.6]	0.7	0.9
Consistency score	2.5 [2.2–3]	2,9 [2–3]	2.5 [2.3–3.5]	2.5 [2.5–3]	2.6 [2–3]	>0.9	0.6
Shannon diversity index	4.4 [3.38–5.06]	4.42 [3.76–4.85]	4.36 [3.09–4.7]	4.27 [3.15–4.76]	4.49 [3.22–4.72]	0.03*	0.08**
Observed species	73 [49–102]	74 [48–90]	77 [48–98]	79 [46–104]	78 [50–90]	0.57	0.55

♦Maximum and minimum values are provided in brackets.

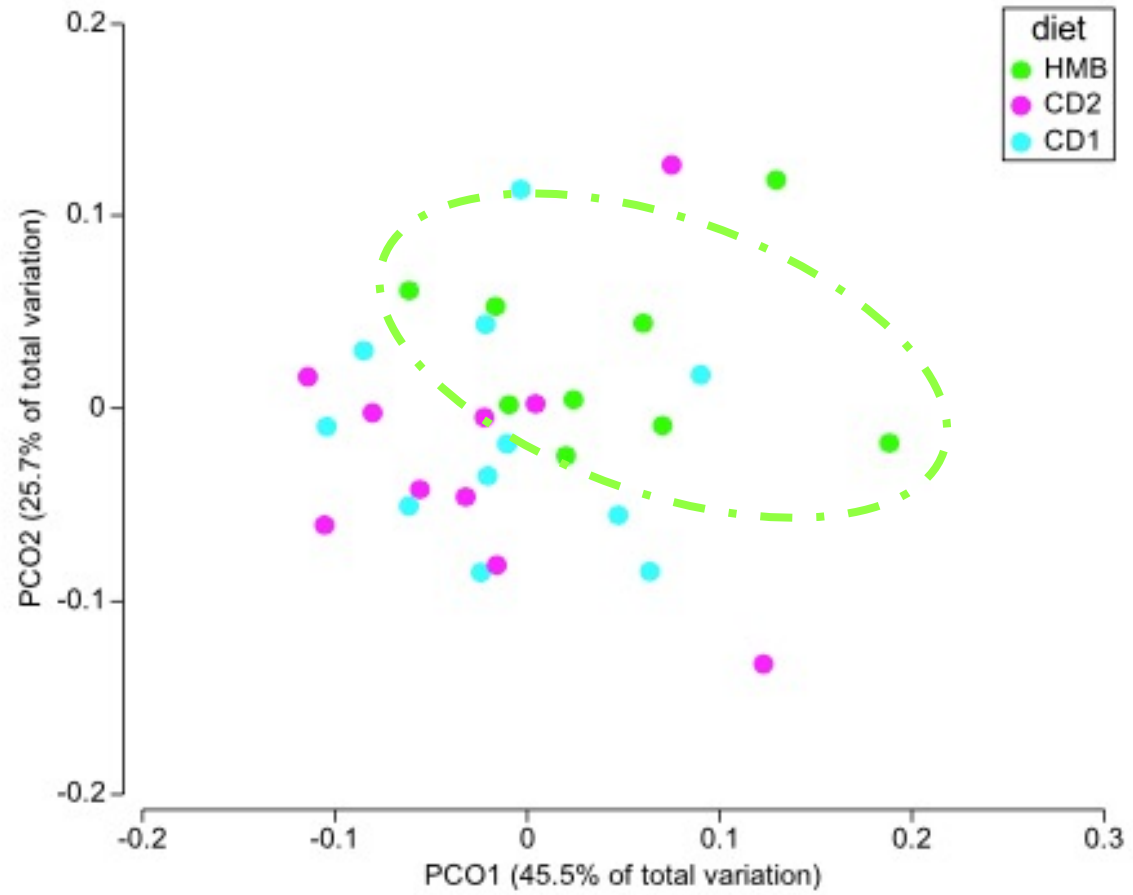
Abbreviations and explanation: The diet periods were as follows: CD1 for week 1 and 2, during which all dogs were acclimated to commercial dry food (CD; Felleskjøpet's Labb adult), followed by incremental substitution of the CD diet with minced beef – LMB low minced beef for week 3, MMB moderate minced beef for week 4, and HMB high minced beef for week 5 – and finally, CD2 for week 6 and 7, during which the dogs were reintroduced to the CD diet.

¹Wilcoxon-matched sign rank test without correction for multiple comparisons. *P*-value for CD1 vs. HMB was determined for 9 dogs and *P*-value for CD2 vs. HMB was determined for 8 dogs.

²*P*-values for faecal pH was determined for seven dogs (CD1 vs. HMB) and five dogs (CD2 vs. HMB), due to missing values.

*Considered statistically significant; ** Considered a trend

Fecal Microbial diversity differed in dogs fed HMB

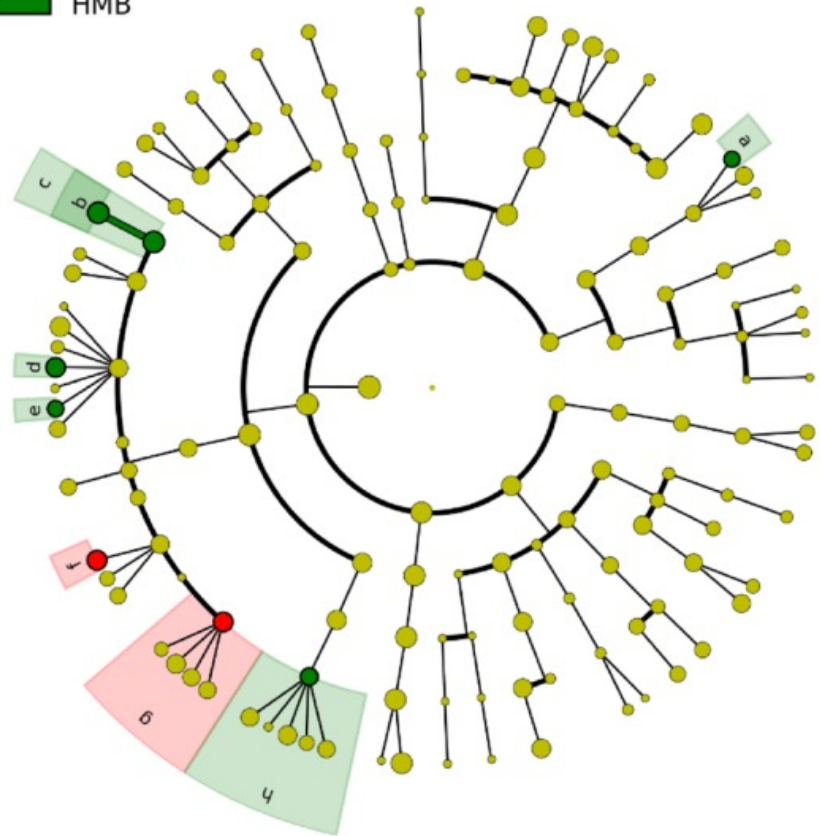
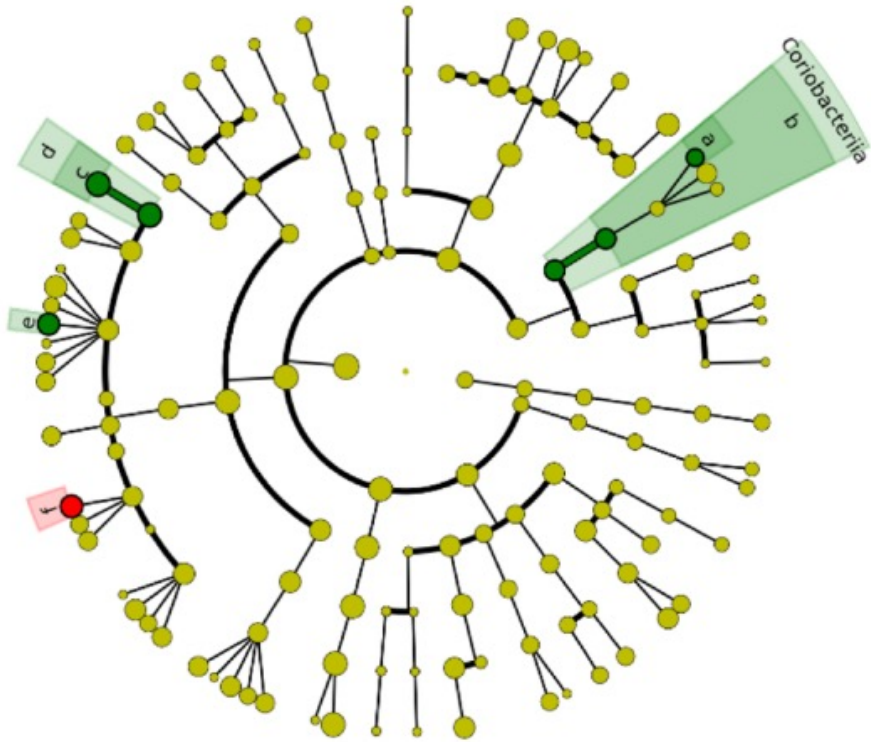


CD1
HMB

a: *Slackia*
b: *Coriobacteriales*
c: *Clostridiaceae* Other
d: *Clostridiaceae*
e: *Dorea*
f: *Faecalibacterium*

CD2
HMB

a: *Slackia*
b: *Clostridiaceae* Other
c: *Clostridiaceae*
d: *Dorea*
e: *Roseburia*
f: *Faecalibacterium*
g: *Veilonellaceae*
h: *Erysipelotrichaceae*



RESEARCH ARTICLE

The fecal microbiome and metabolome differs between dogs fed Bones and Raw Food (BARF) diets and dogs fed commercial diets

Milena Schmidt^{1*}, Stefan Unterer¹, Jan S. Suchodolski², Julia B. Honneffer², Blake C. Guard², Jonathan A. Lidbury², Jörg M. Steiner², Julia Fritz³, Petra Kölle¹

¹ Clinic of Small Animal Medicine, LMU University of Munich, Munich, Germany, ² Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, United States of America, ³ Napfcheck, small animal nutrition consultation, Munich, Germany

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BARF (44% CP) vs Dry Diet or Dry and Wet Diet (30% CP)

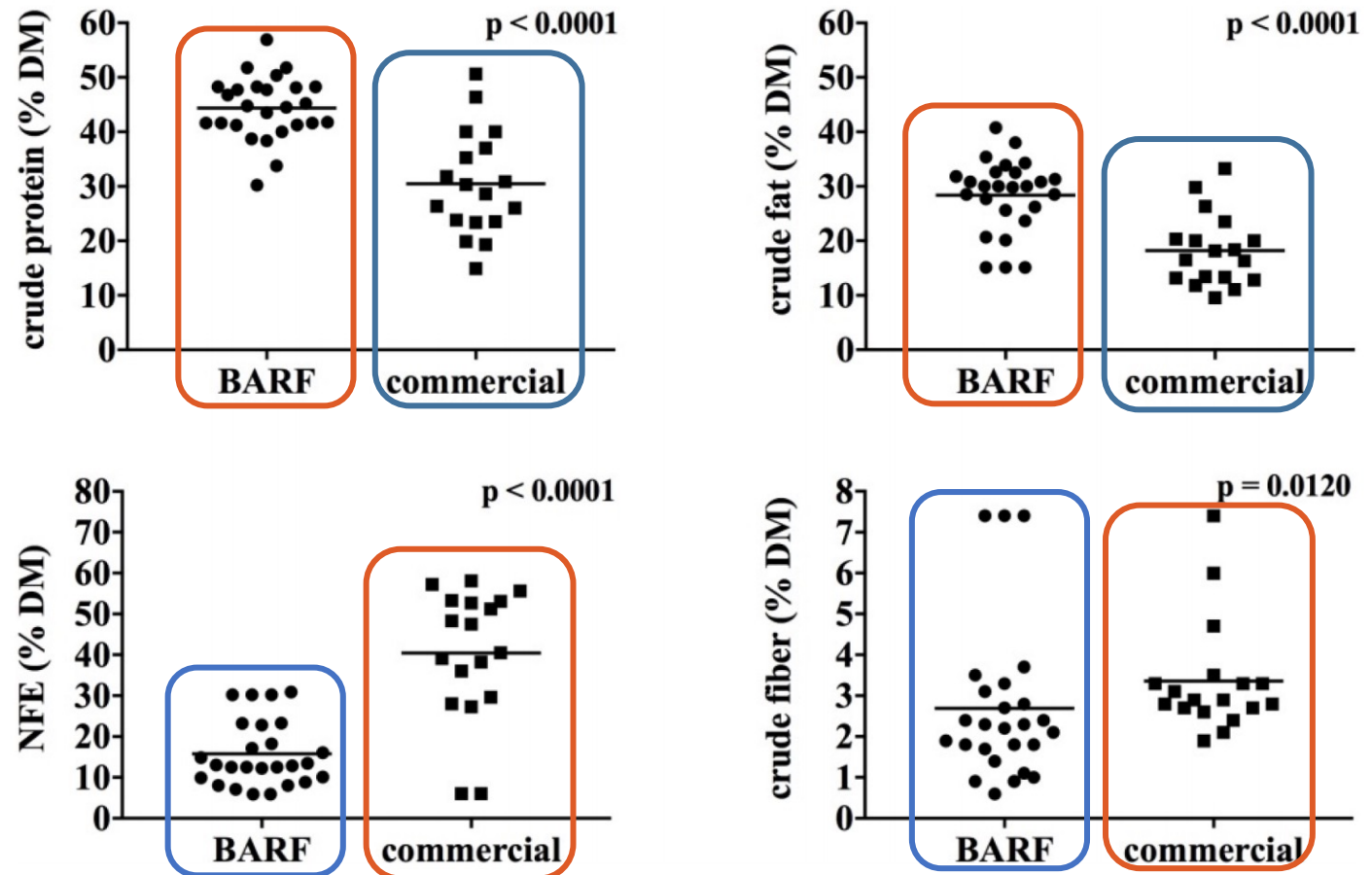


Fig 1. Intake of crude protein, fat, fiber, and NFE (Nitrogen-Free Extract) between both groups. The BARF dogs were fed a significantly higher amount of protein and fat and lower amount of NFE and fiber.

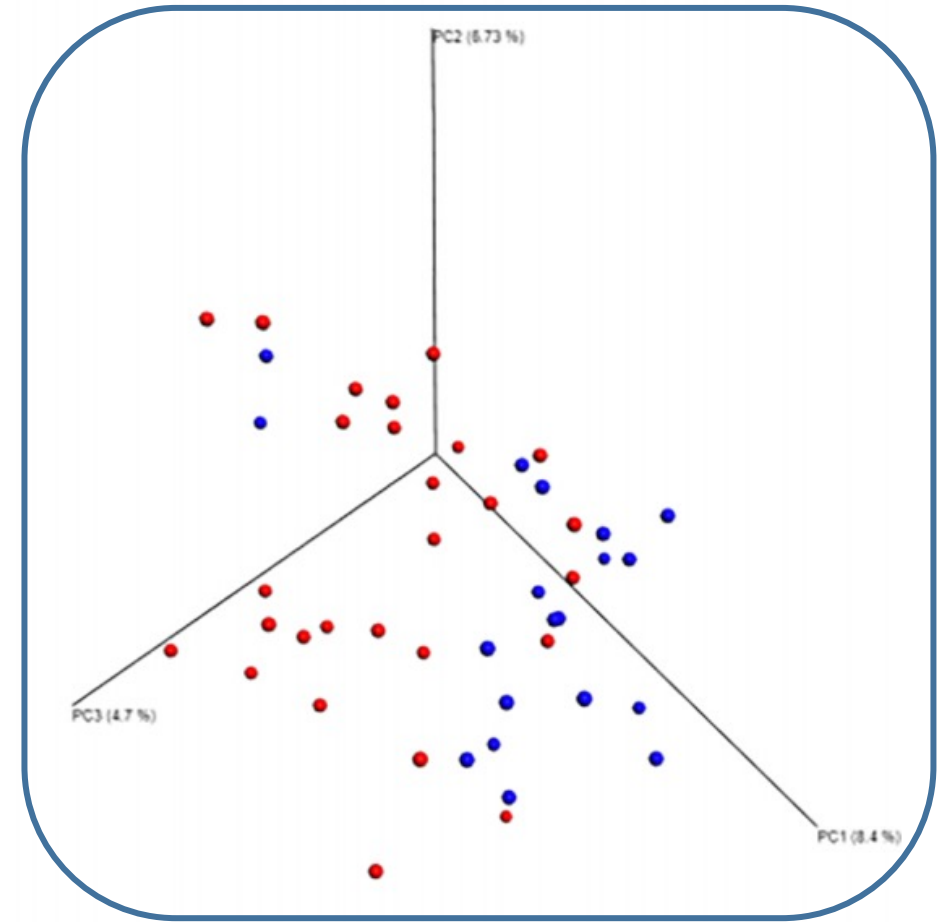
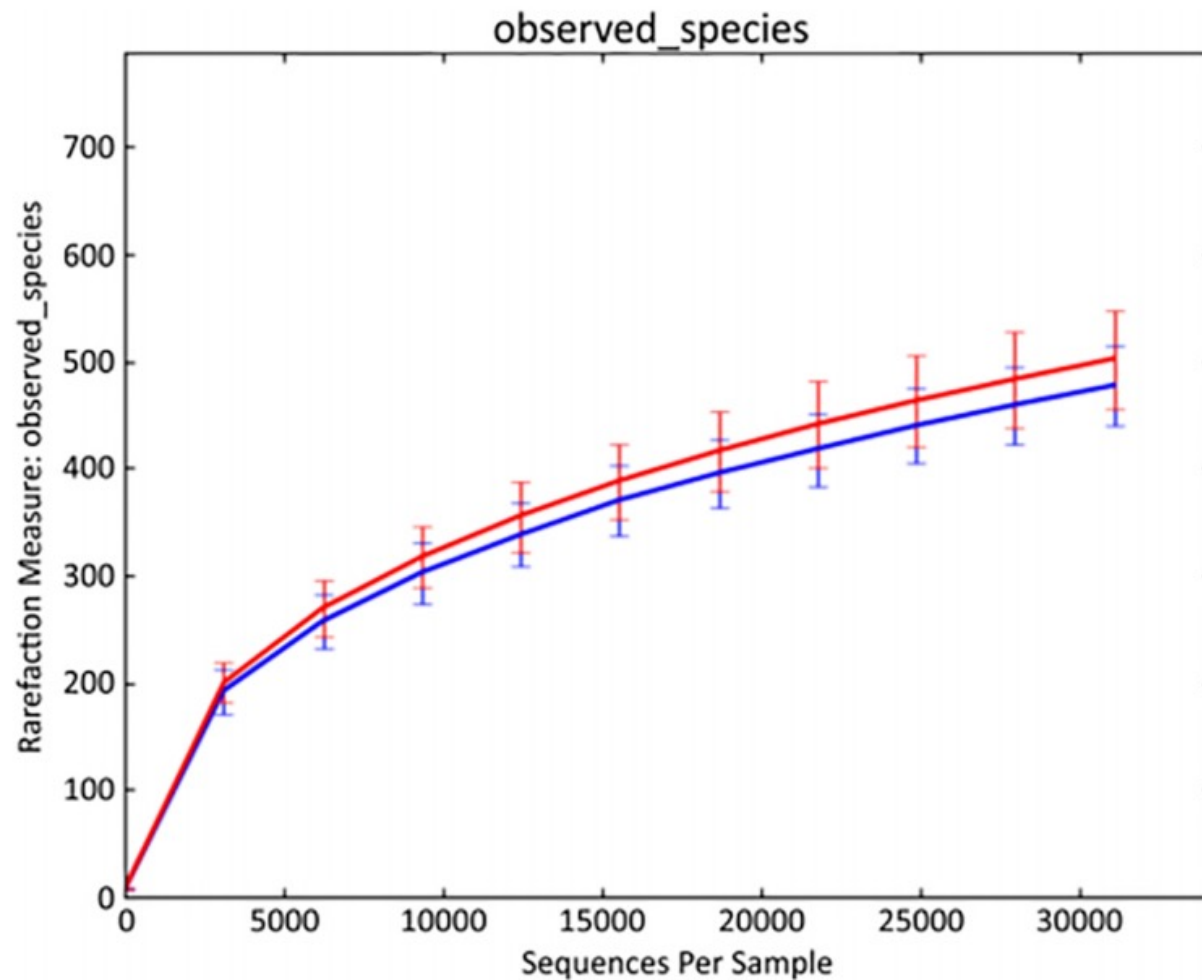


Fig 2. Bacterial diversity measures. (a) Alpha diversity: Rarefaction analysis of 16S rRNA gene sequences in raw and commercially fed dogs. Lines represent the mean of each group (red = BARF, blue = commercial), the standard deviation is shown by error bars. No significant difference in observed species was seen between BARF and commercially fed dogs. (b) Beta diversity: Principal coordinates analysis (PCoA) plot showing clustering of microbial communities from feces of raw and commercially fed dogs (red = BARF, blue = commercial). The closer the items, the more similar is the microbial community of the samples. Consequently, the microbiome of BARF dogs differs from the microbiome of commercially fed dogs (ANOSIM; $p < 0.01$).

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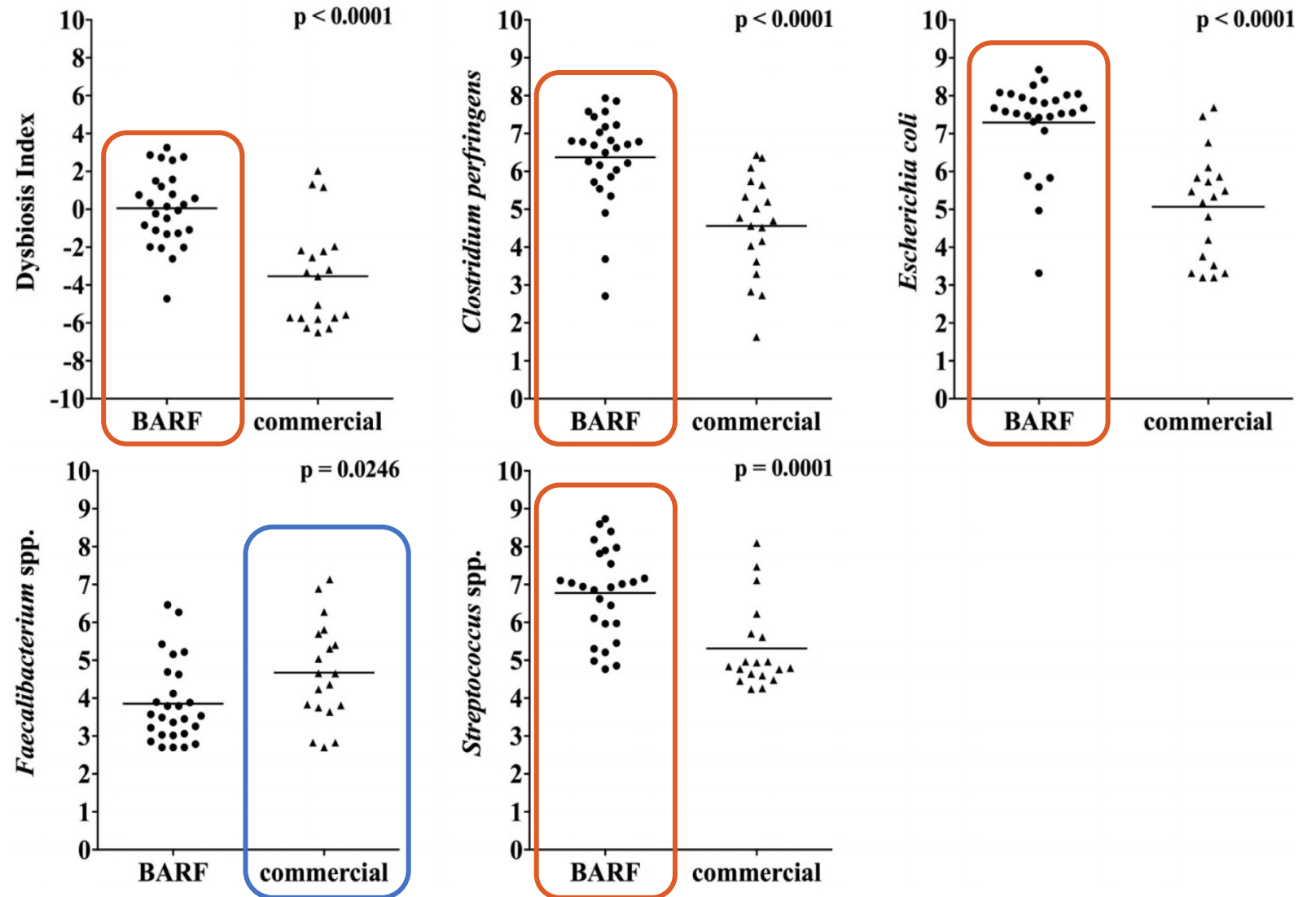


Fig 3. Fecal abundance of selected bacterial taxa between BARF and commercially fed dogs based on qPCR. The BARF dogs showed a higher abundance of *Clostridium perfringens*, *E. coli* and *Streptococcus*, while commercially fed dogs showed a higher abundance of *Faecalibacterium*. The Dysbiosis Index was significantly higher in the BARF group.

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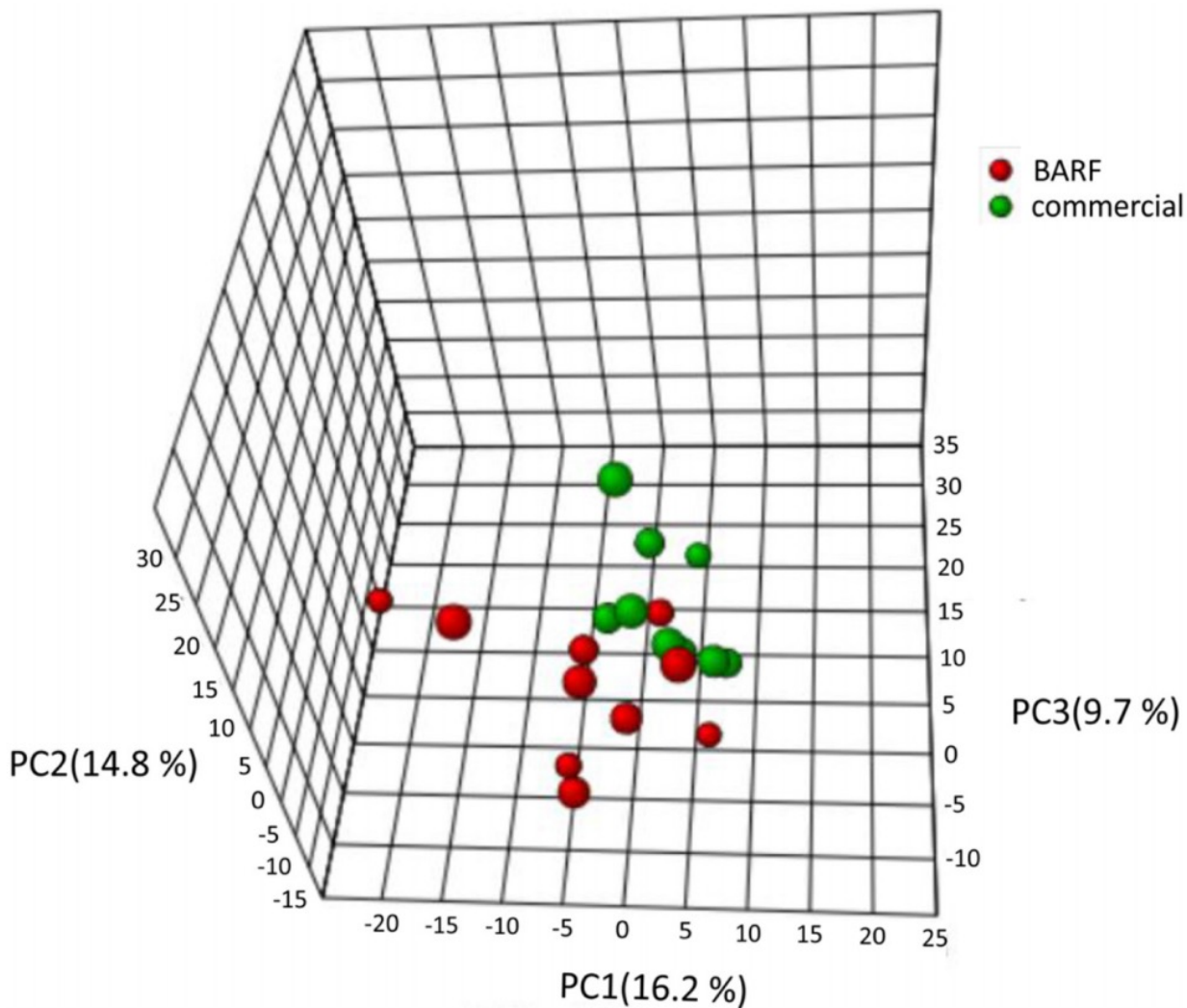


Fig 4. PCA plot of the fecal metabolome showing clustering of samples based on BARF versus commercially fed diets. The BARF (red) and commercially (green) fecal samples were used to detect differences between the two diets. The PCA plot showed a clustering between both groups.

Schmidt et al., 2018

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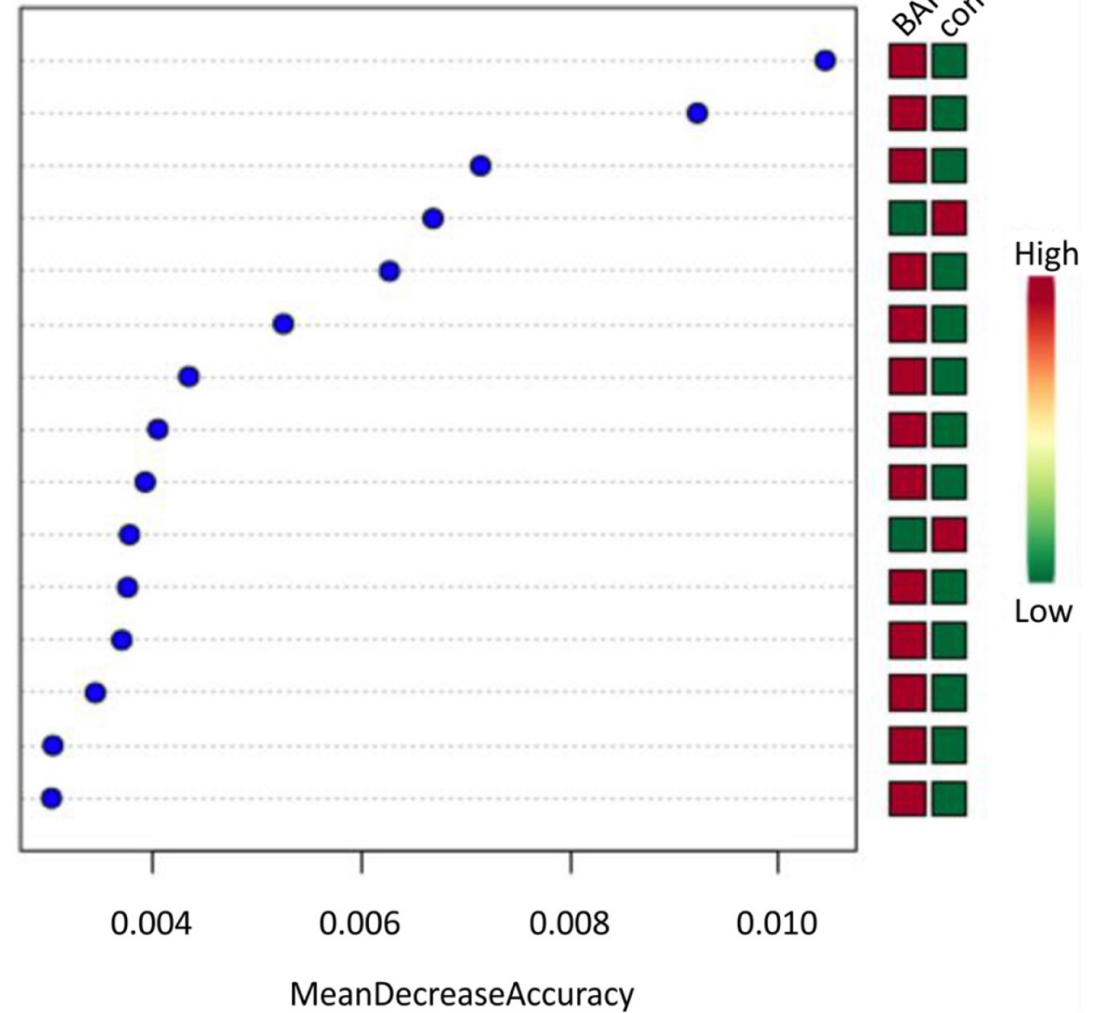
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isomaltose
 4-hydroxybutyric acid
 4-aminobutyric acid
 phosphoethanol
 gluconic acid
 propane-1,3-diol NIST
 homoserine
 sucrose
 isothreonic acid
 tocopherol alpha
 fucose
 vanillic acid
 2-hydroxybutanoic acid
 butane-2,3-diol NIST
 N-acetyl-D-mannosamine



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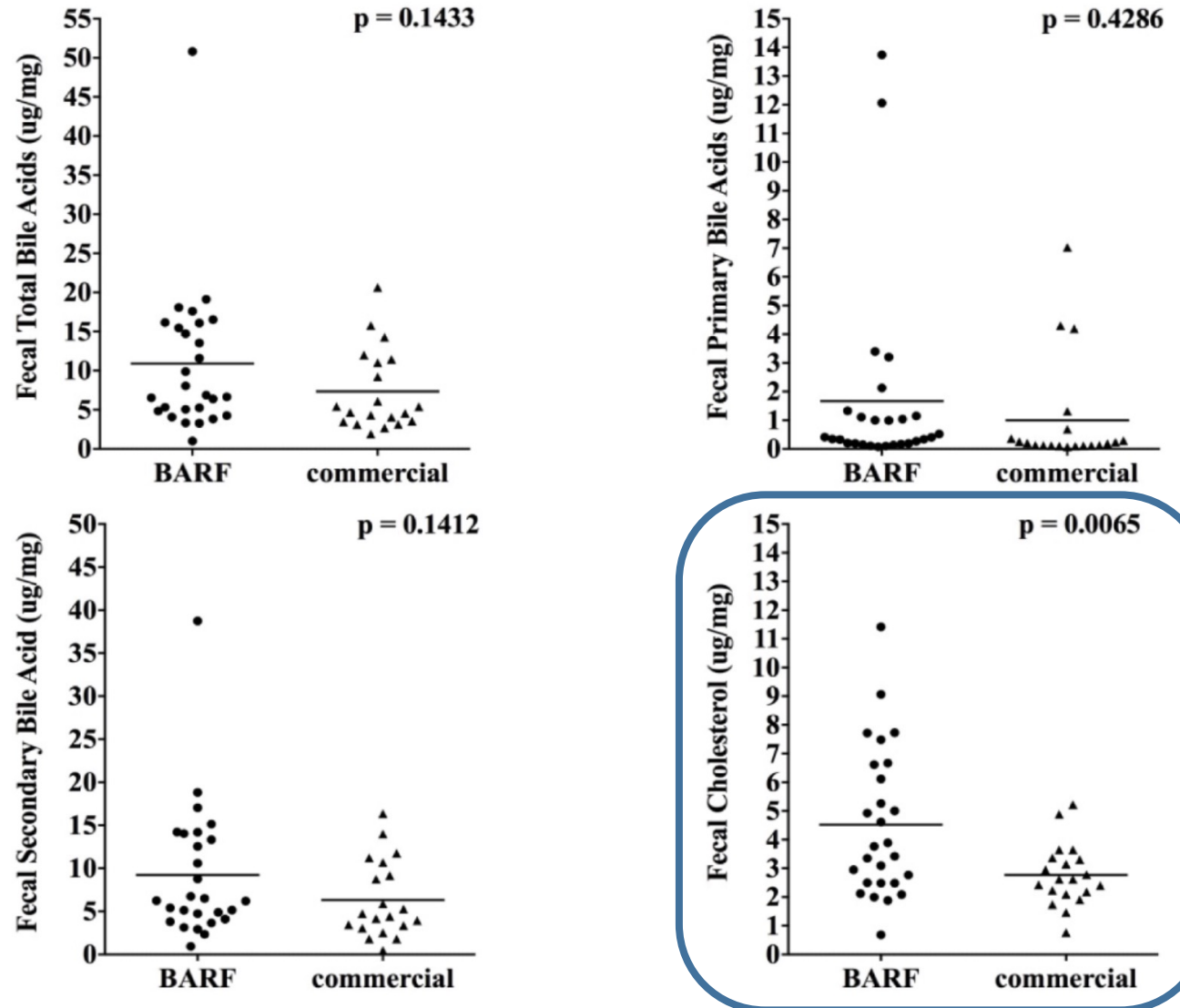


Fig 7. Total, primary, secondary fecal bile acids and cholesterol of BARF and commercially fed dogs. There was no significant difference in total, primary, and secondary bile acids between fecal samples of both groups, but BARF dogs had a higher abundance of cholesterol in their feces.

Schmidt et al., 2018

Commercial food (CF) vs. BARF diet (BARF)

Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features

Giulia Alessandri,^{1†} Christian Milani,^{2†} Leonardo Mancabelli,² Marta Mangifesta,² Gabriele A. Lugli,² Alice Viappiani,³ Sabrina Duranti,² Francesca Turrone,^{2,4} Maria C. Ossiprandi,^{1,4} Douwe van Sinderen⁵ and Marco Ventura^{2,4*}

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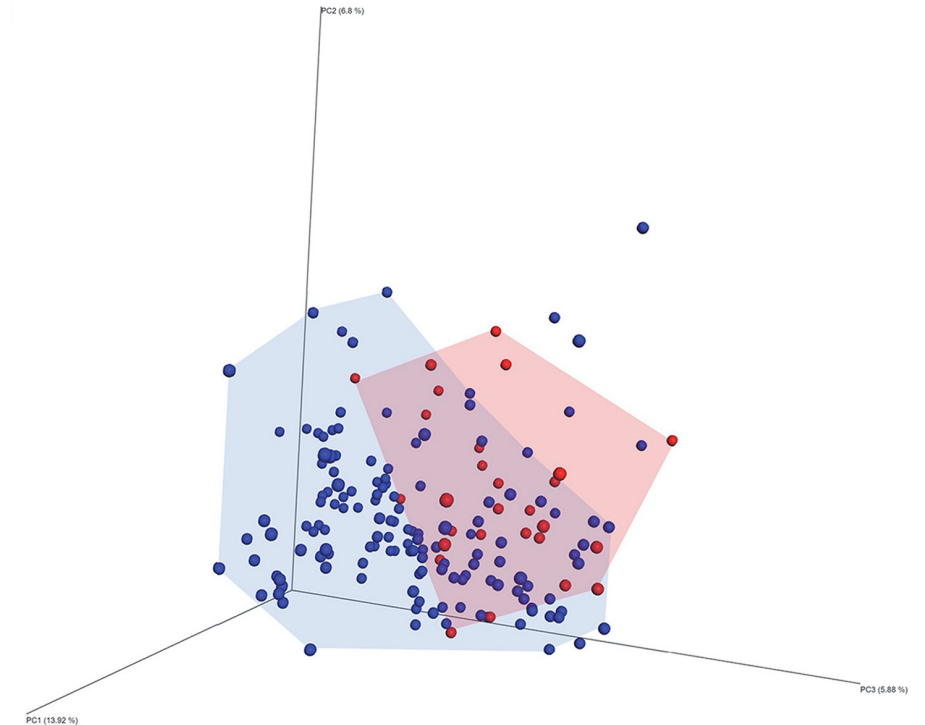
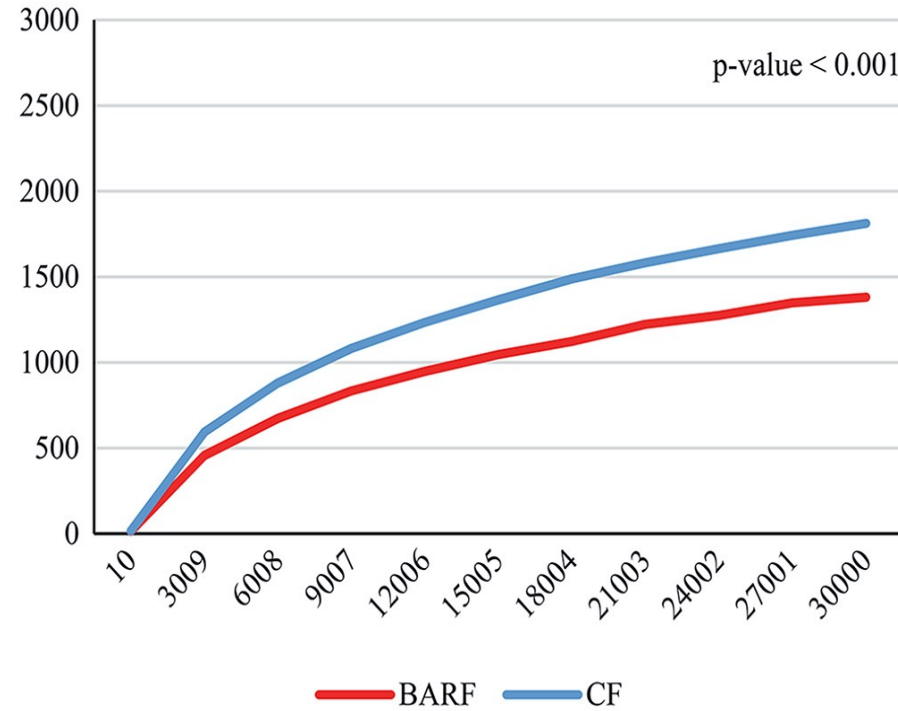
⁴Microbiome Research Hub, University of Parma, Parma, Italy.

⁵APC Microbiome Institute and School of Microbiology, Bioscience Institute, National University of Ireland, Cork, Ireland.

shotgun metagenomics, highlighted that both bacterial composition and metabolic repertoire of the canine gut microbiota have evolved to adapt to high-protein or high-carbohydrates intake. Altogether, these data indicate that artificial selection and domestication not only affected the canine genome, but also shaped extensively the bacterial population harboured by the canine gut.

Introduction

The gastrointestinal (GI) microbial complex community of microorganisms has a crucial role in maintaining and (Suchodolski *et al.*, 2010, 2012) zation of the GI microbiota was culture-dependent methods, all



Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features

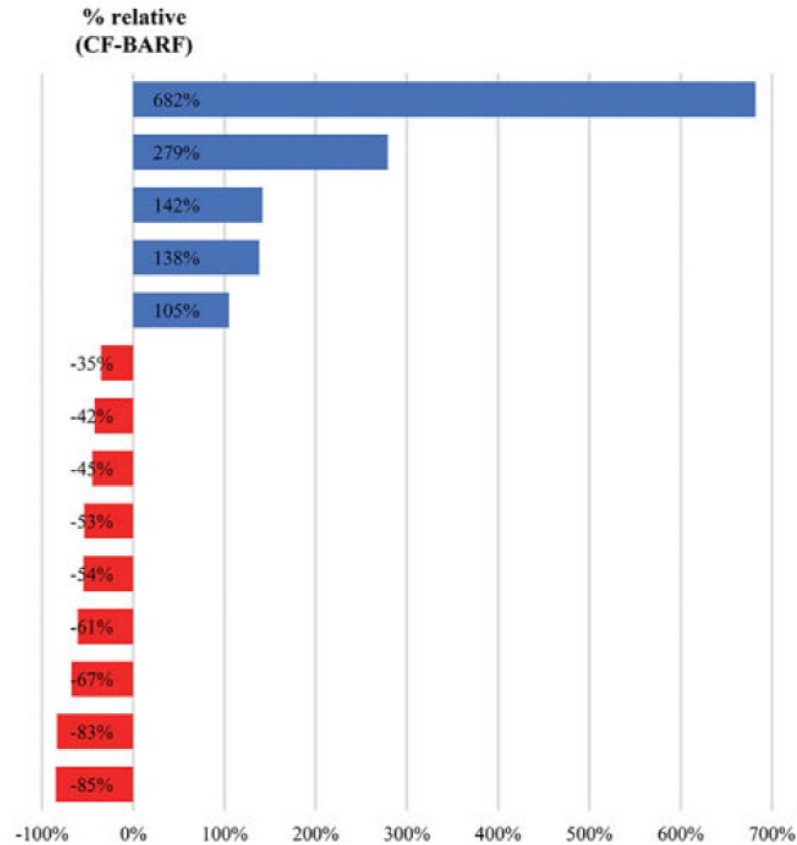
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Introduction

The gastrointestinal (GI) microbiota is a large and highly complex community of microorganisms that plays a crucial role in maintaining and promoting host health (Suchodolski *et al.*, 2010, 2012). Historically, characterization of the GI microbiota was performed by means of culture-dependent methods, allowing biochemical and



Phylum	Genus	BARF	CF	% absolute (CF-BARF)	p-value
Firmicutes	→ <i>Faecalibacterium</i>	0.71%	5.59%	4.88%	0.00005
Bacteroidetes	U. m. of Bacteroidales order	0.08%	0.29%	0.22%	0.02119
Proteobacteria	→ <i>Sutterella</i>	1.28%	3.08%	1.81%	0.00028
Bacteroidetes	→ <i>Prevotella 9</i>	6.44%	15.33%	8.90%	0.00639
Firmicutes	U. m. of Clostridiales order	0.02%	0.04%	0.02%	0.01669
Bacteroidetes	<i>Alloprevotella</i>	6.49%	4.23%	-2.27%	0.02399
Firmicutes	<i>Peptoclostridium</i>	0.11%	0.06%	-0.05%	0.04151
Firmicutes	U. m. of Peptostreptococcaceae family	0.15%	0.08%	-0.07%	0.04478
Fusobacteria	U. m. of Fusobacteriales order	0.14%	0.07%	-0.08%	0.00675
Actinobacteria	<i>Collinsella</i>	1.21%	0.55%	-0.66%	0.02994
Actinobacteria	<i>Bifidobacterium</i>	0.52%	0.20%	-0.31%	0.01099
Bacteroidetes	<i>Parabacteroides</i>	0.19%	0.06%	-0.13%	0.00600
Firmicutes	<i>Clostridium sensu stricto 1</i>	4.10%	0.68%	-3.41%	0.00000
Firmicutes	<i>Allobaculum</i>	2.04%	0.31%	-1.73%	0.00002

Apparent total-tract macronutrient digestibility, serum chemistry, urinalysis, and fecal characteristics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets¹

Kiley M. Algya,* Tzu-Wen L. Cross,[†] Kristen N. Leuck,* Megan E. Kastner,* Toshiro Baba,*
Lynn Lye,[‡] Maria R. C. de Godoy,^{*,†} and Kelly S. Swanson^{*,†,2}

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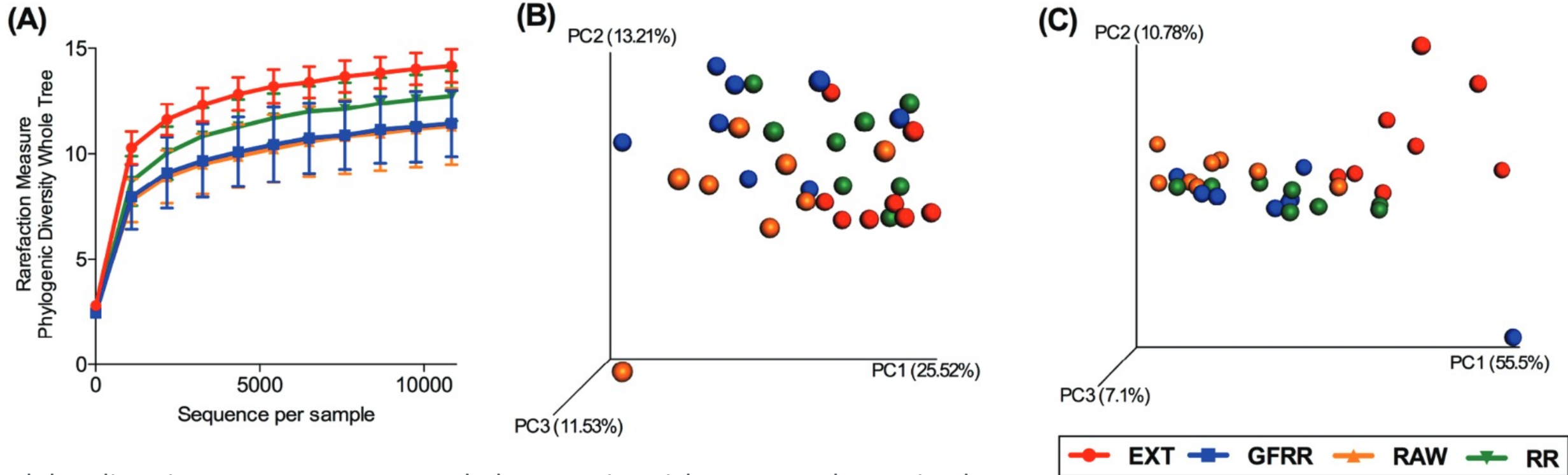
Table 1. Analyzed chemical and energy composition of dog diets tested

Item	Treatment			
	Extruded ¹	Grain-free roasted refrigerated ²	Raw ³	Roasted refrigerated ⁴
DM (%)	93.33	38.63	47.28	42.23
OM (%), DMB ⁵	92.47	88.58	93.23	89.51
CP (%), DMB	24.07	45.69	25.13	31.08
Acid-hydrolyzed fat (%), DMB	13.30	30.30	33.90	27.82
Total dietary fiber (%), DMB	9.60	7.28	6.94	11.84
GE ⁵ (kcal/g), as-is	4.58	2.32	3.07	2.39
GE (kcal/g), DMB	4.91	6.01	6.50	5.66
ME ⁵ (kcal/g), as-is	3.02	1.63	2.15	1.77
ME (kcal/g), DMB	3.24	4.22	4.55	4.19

Apparent total-tract macronutrient digestibility, serum chemistry, urinalysis, and fecal characteristics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets¹

Kiley M. Algya,* Tzu-Wen L. Cross,[†] Kristen N. Leuck,* Megan E. Kastner,* Toshiro Baba,*
Lynn Lye,[‡] Maria R. C. de Godoy,^{*,†} and Kelly S. Swanson^{*,†,2}

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Alpha diversity measures suggested that species richness was lower in dogs fed the high-moisture grain-free roasted refrigerated (GFRR) diet or raw (RAW) diet than dogs fed the extruded dry kibble (EXT) diet

Apparent total-tract macronutrient digestibility, serum chemistry, urinalysis, and fecal characteristics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets¹

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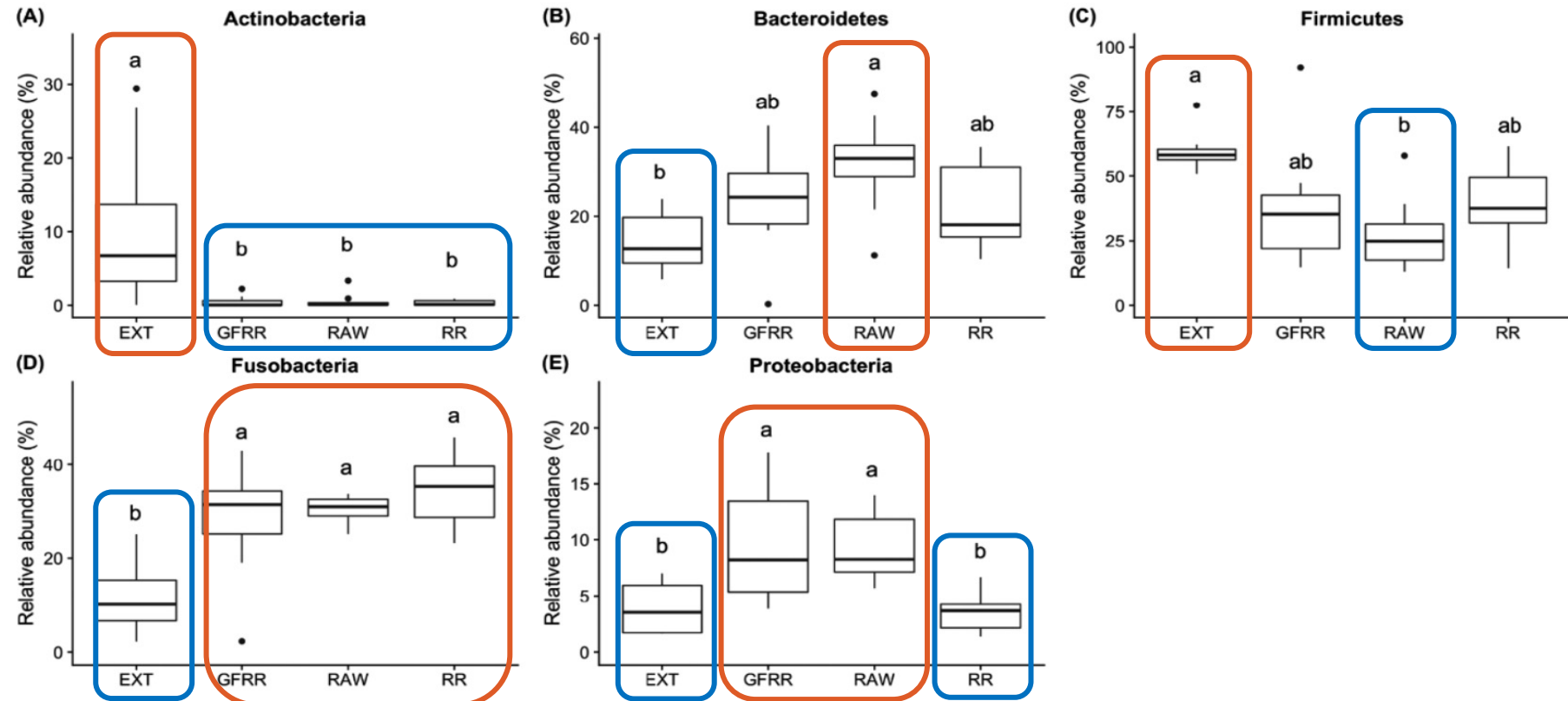


Figure 2. Relative abundances of differentially abundant microbial phyla ($P < 0.05$) in feces of dogs fed an extruded dry kibble (EXT), high-moisture roasted refrigerated (RR), high-moisture grain-free roasted refrigerated (GFRR), or raw (RAW) diet ($n = 8$ /treatment). False discovery rate corrected P values using the Benjamini–Hochberg method were calculated using Statistical Analyses of Metagenomic Profiles (STAMP) software, using ANOVA and a Tukey adjustment. Each box represents the 25% and 75% percentiles; error bars indicate 95% confidence interval of median.



Article

Varying Protein Levels Influence Metabolomics and the Gut Microbiome in Healthy Adult Dogs

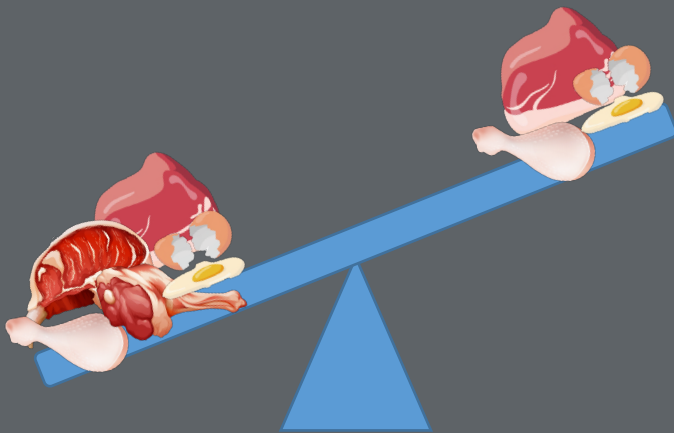
Eden Ephraim ^{1,*}, Chun-Yen Cochrane ¹ and Dennis E. Jewell ²

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High protein vs. low protein extruded diet

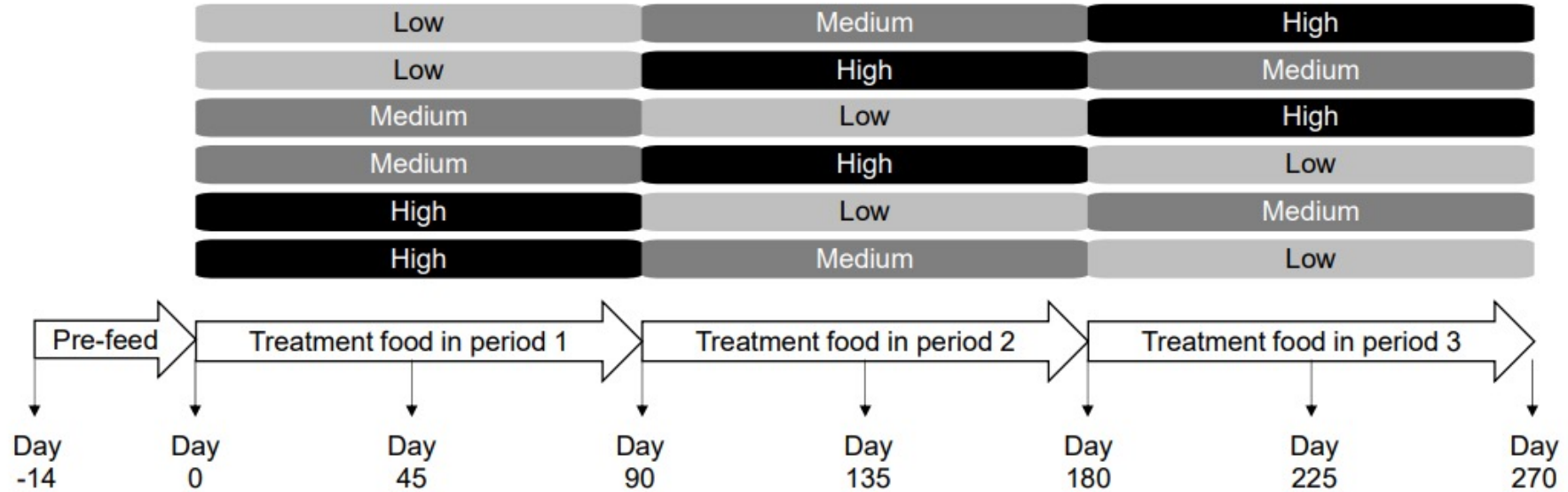


Figure 7. Study design and timeline in which dogs consumed the low, medium, and high protein foods in a Williams Latin Square sequence.

- High (25% dried chicken and 20% soybean protein) – CP 46%
- Medium (17% dried chicken and 3% soybean protein) – CP 25%
- Low (11% dried chicken) – CP 19%

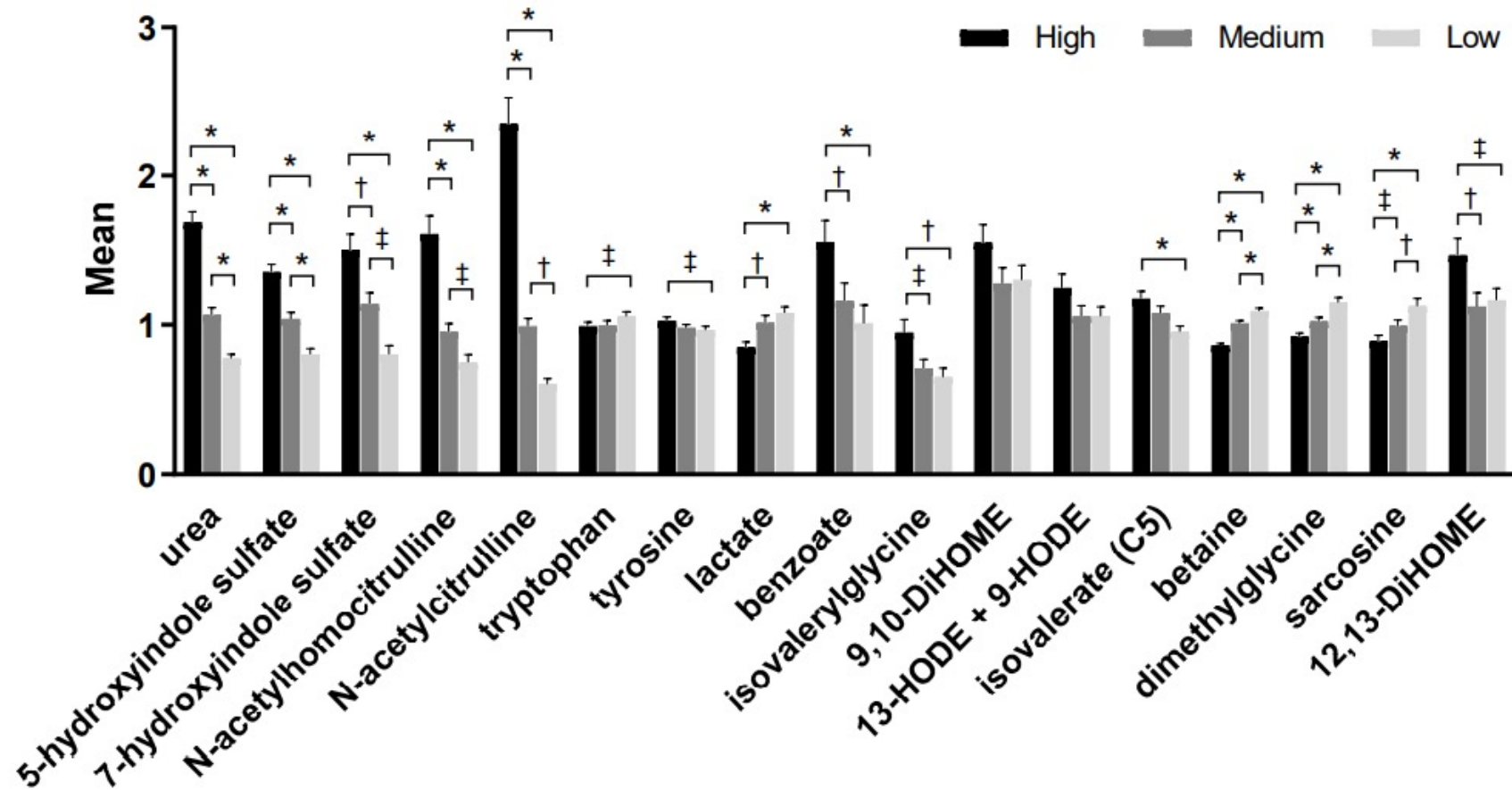
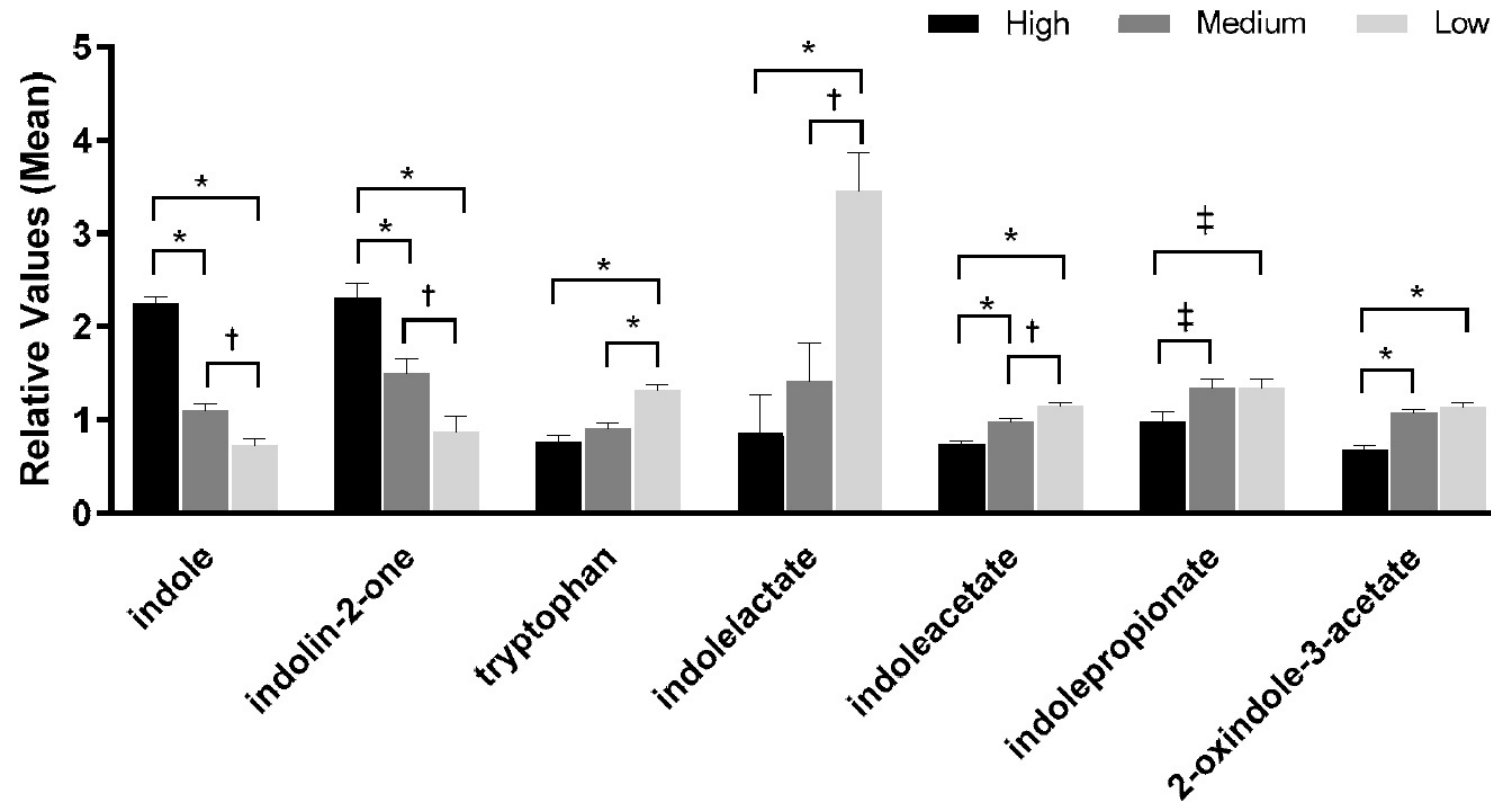


Figure 2. Selected metabolites from metabolomic analyses from serum samples of dogs fed the high, medium, and low protein foods. Data were rescaled to a median value of 1 and are presented as group means and standard errors. Significance was determined using one-way ANOVA followed by a post hoc Tukey's test. * $p \leq 0.001$; † $p \leq 0.01$; ‡ $p \leq 0.05$. DiHOME, dihydroxyoctadecanoic acid; HODE, hydroxyoctadecadienoic acid.

Fecal Indole Concentrations from fecal samples of dogs fed high, medium, and low protein diets



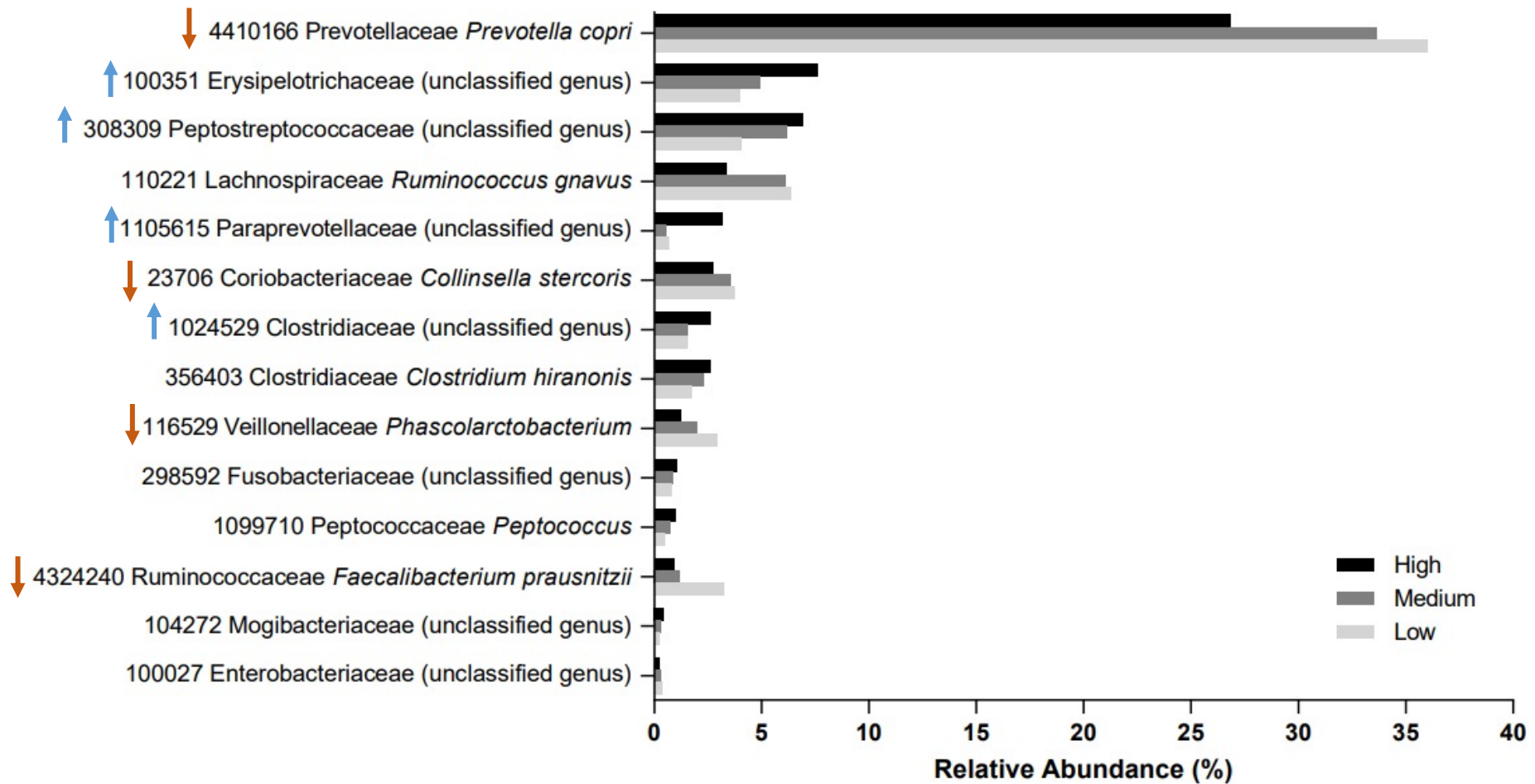


Figure 6. Relative abundance (percentage) of operational taxonomic units (OTU) that showed significant changes in fecal samples of dogs fed the high, medium, and low protein foods. Operational taxonomic unit number, family, and genus are shown (species also included where available).

ORIGINAL RESEARCH article

Front. Vet. Sci., 04 June 2021 | <https://doi.org/10.3389/fvets.2021.667642>



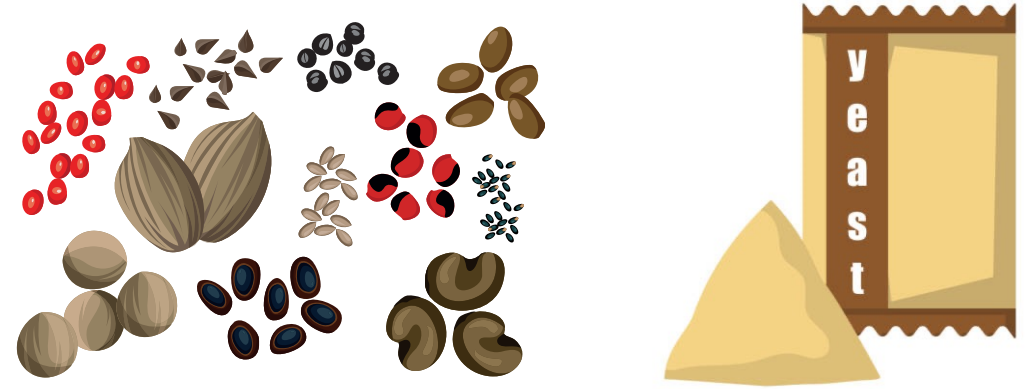
Use of Legumes and Yeast as Novel Dietary Protein Sources in Extruded Canine Diets

Lauren M. Reilly¹, Fei He¹, Sandra L. Rodriguez-Zas¹, Bruce R. Southey¹, Jolene M. Hoke², Gary M. Davenport² and Maria R. C. de Godoy²*

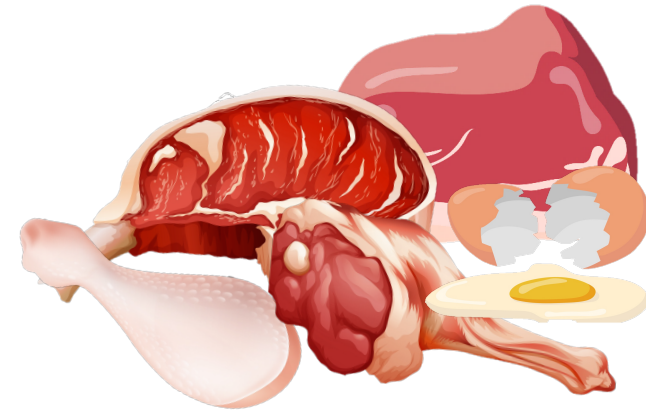
¹Department of Animal Sciences, University of Illinois, Urbana, IL, United States

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The popularity of plant-based protein sources has increased as consumer demand for grain-free and novel protein sources increase. Minimal research has been conducted as regards to use of legumes and yeast and their effects on acceptability and digestibility in canine diets. The objective of this study was to evaluate macronutrient apparent total tract digestibility (ATTD), gastrointestinal tolerance, and fermentative end-products in extruded, canine diets. Five diets were formulated to be isocaloric and isonitrogenous with either garbanzo beans (GBD), green lentils (GLD), peanut flour (PFD), dried yeast (DYD), or poultry by-product meal (CON) as the primary protein sources. Ten adult, intact, female beagles (mean age: 4.2 ± 1.1 yr, mean weight: 11.9 ± 1.3 kg) were used in a replicated, 5×5 Latin square design with 14 d periods. Each experimental period consisted of 10 d of diet adaptation, followed by 4 d of total fecal and urine collection. A fasted, 5 ml blood sample was collected at the end of each period and analyzed for serum metabolites and complete blood count. Serum metabolites were within normal ranges and all dogs remained healthy throughout the study. Fecal quality, evaluated on a 5-point scale, was considered ideal. Macronutrient ATTD was similar among dietary treatments, with diets highly digestible (>80%). Total fecal branched-chain fatty acid concentrations were highest ($P < 0.05$) for DYD ($23.4 \mu\text{mol/g}$) than GLD ($16.1 \mu\text{mol/g}$) and PFD ($16.0 \mu\text{mol/g}$) but not different ($P > 0.05$) than other treatments. The plant-based protein treatments had greater ($P < 0.05$) total fecal short chain fatty acid (SCFA) concentrations (average $627.6 \mu\text{mol/g}$) compared with CON ($381.1 \mu\text{mol/g}$). Fecal butyrate concentration was highest ($P < 0.05$) for DYD than all other dietary treatments ($103.9 \mu\text{mol/g}$ vs. average $46.2 \mu\text{mol/g}$). Fecal microbial communities showed *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, and *Proteobacteria* as abundant phyla. There was greater β -diversity for dogs fed DYD which differed from all other diets in both weighted and unweighted UNIFRAC analyses. Inclusion of these novel, plant-based, protein sources showed no detrimental effects on nutrient digestibility or fecal characteristics and represent viable protein



VS.



Diet Composition

Ingredient	Dietary Treatments		
	Control	Garbanzo Beans	Green Lentils
Garbanzo Bean	--	43.56	--
Green Lentil	--	--	44.65
Peanut Flour	--	--	--
Dried Yeast	--	--	--
Pea Protein	--	--	--
Chicken By-Product Meal	33.50	22.26	19.15
Rice	42.96	10.00	10.00
Poultry Fat	8.47	8.74	10.14
Corn	10.00	10.00	10.00
Dried Beet Pulp	2.50	2.50	2.50
Palatant	1.00	1.00	1.00

Vitamin and mineral premixes were added to make all diets complete and balanced

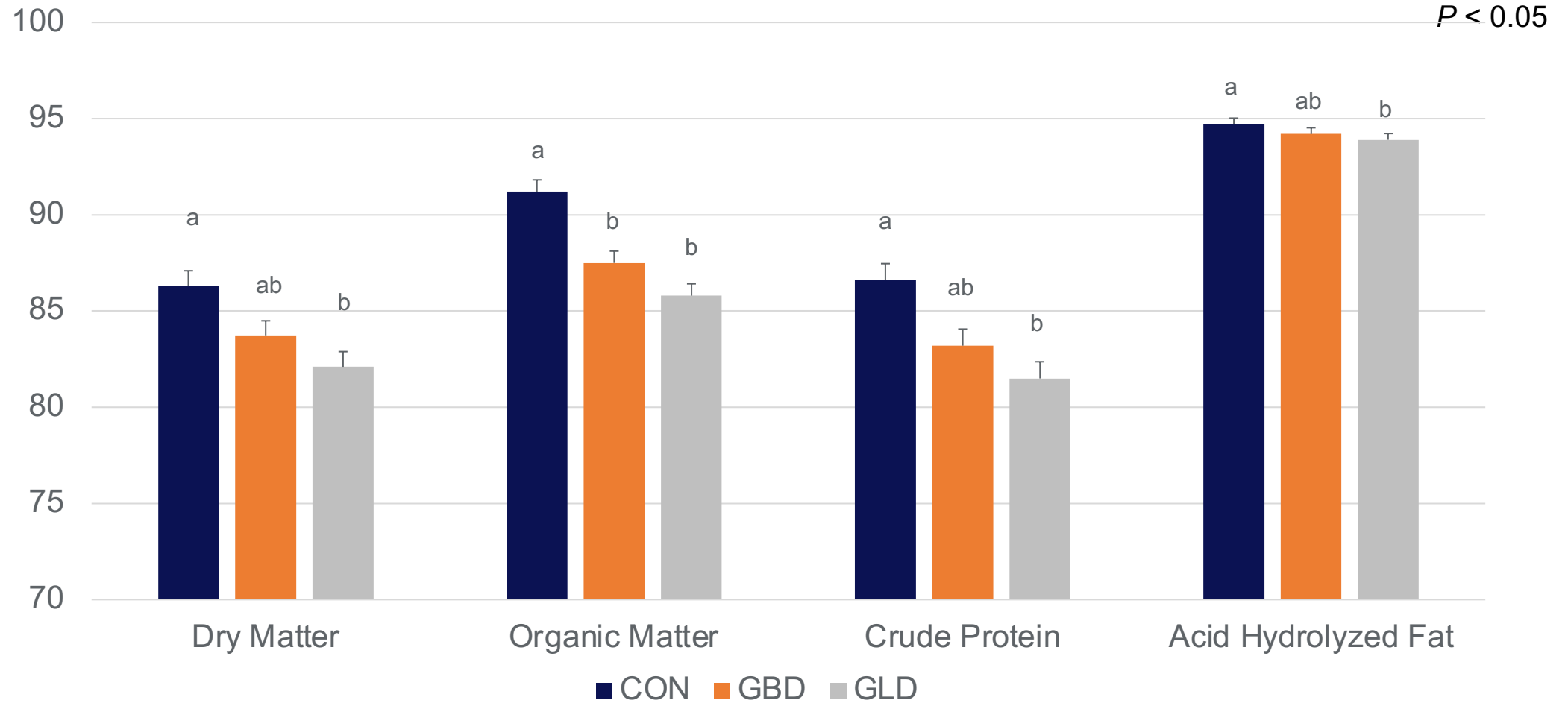
Reilly et al., 2021

Diet Proximate Analysis

Item	Dietary Treatment ¹		
	CON	GBD	GLD
Dry Matter (%)	91.8	92.5	92.2
	Dry Matter Basis		
Crude Protein (%)	31.2	26.9	27.4
Acid Hydrolyzed Fat (%)	15.9	17.3	14.5
Total Dietary Fiber (%)	8.7	11.9	11.2
- Soluble (%)	3.4	3.9	4.2
- Insoluble (%)	5.3	8.1	7.0
Ash (%)	7.2	7.0	7.1
Gross Energy (kcal/g)	5.1	5.0	5.0

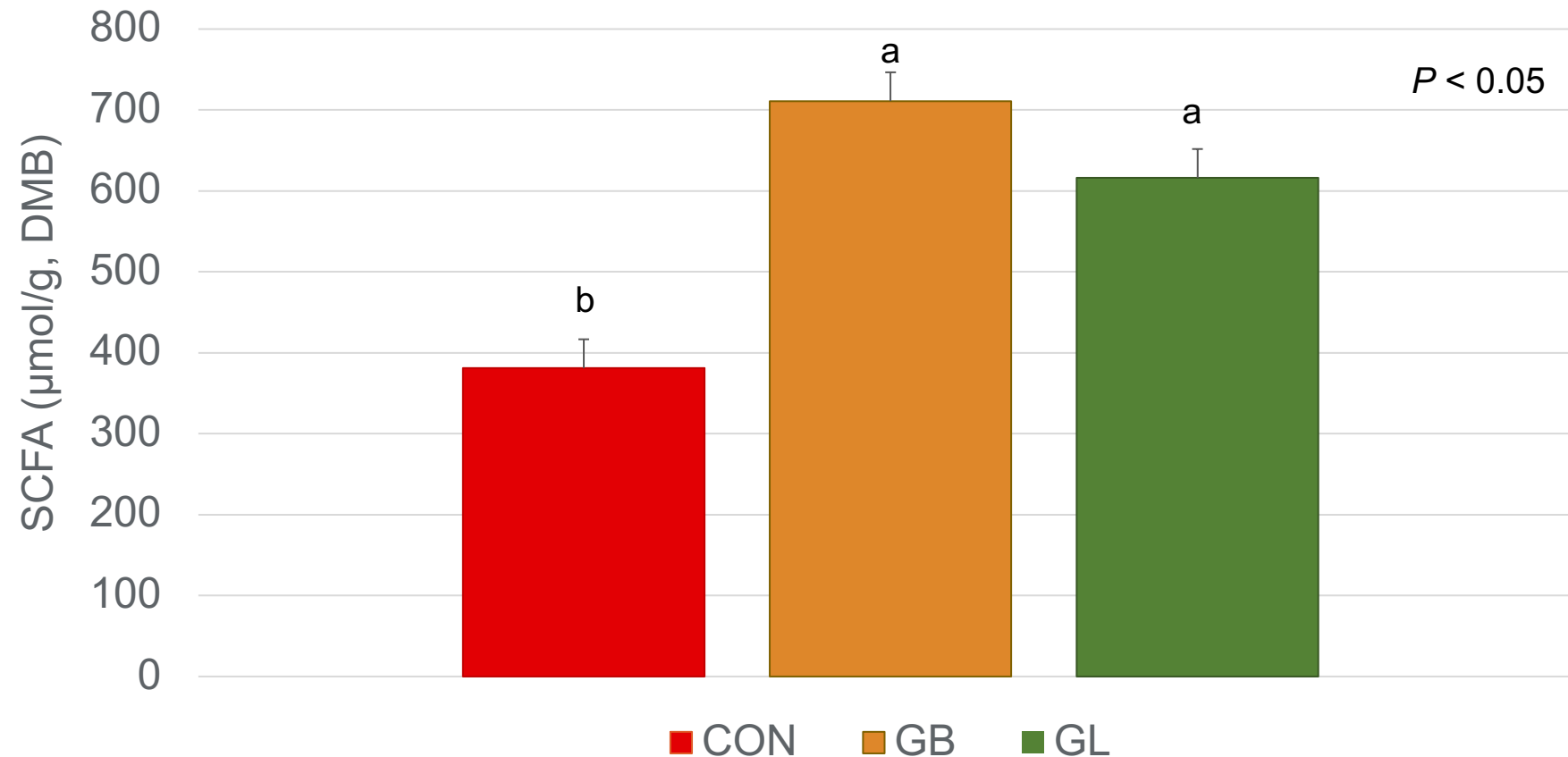
¹ CON = Control, GBD = Garbanzo Bean Diet, GLD = Green Lentil Diet,

Apparent Total Tract Digestibility



Reilly et al., 2021

Total Fecal Short-Chain Fatty Acid Concentrations

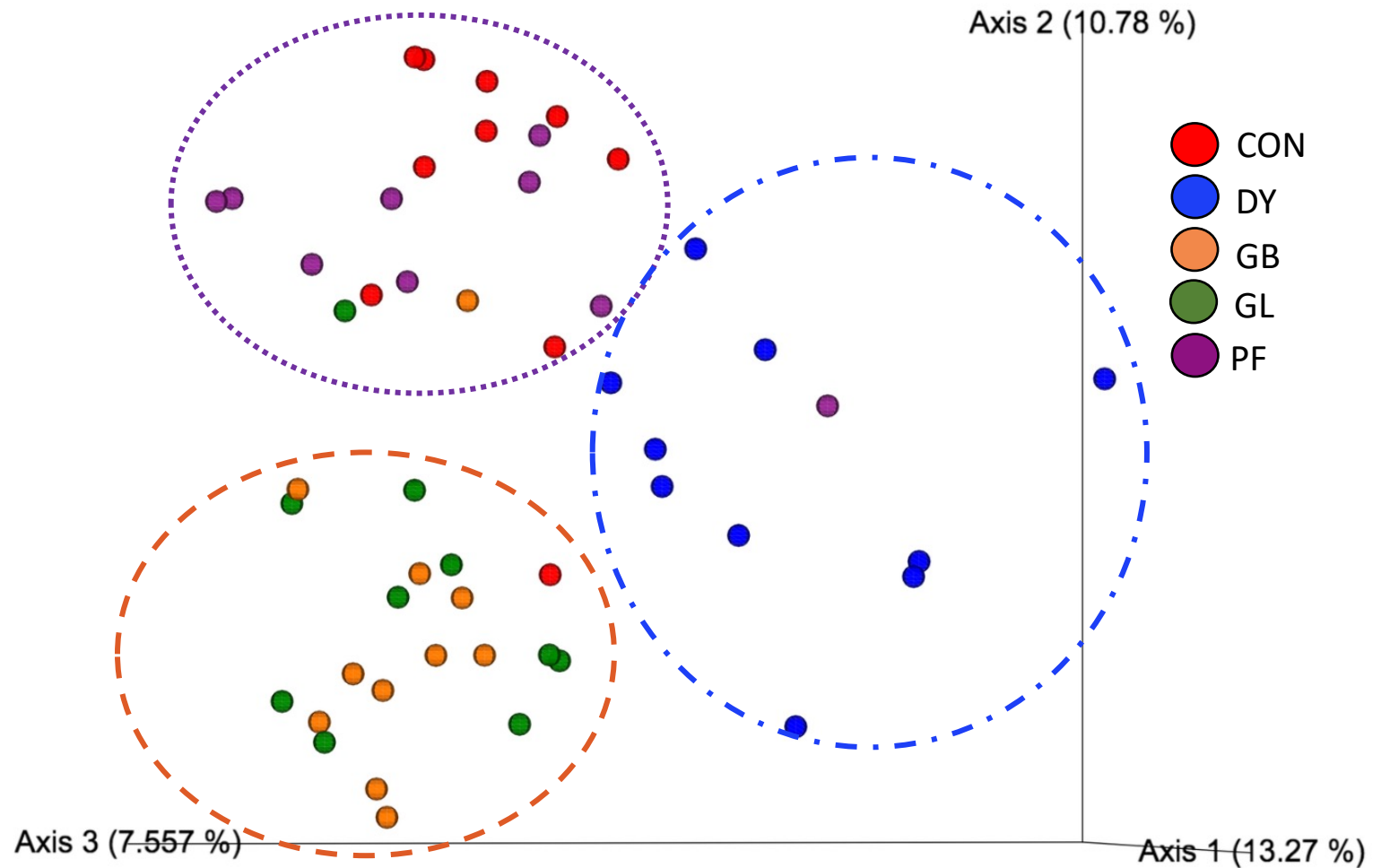


Reilly et al., 2021

^{a-b} Means with different superscripts differ at $P < 0.05$

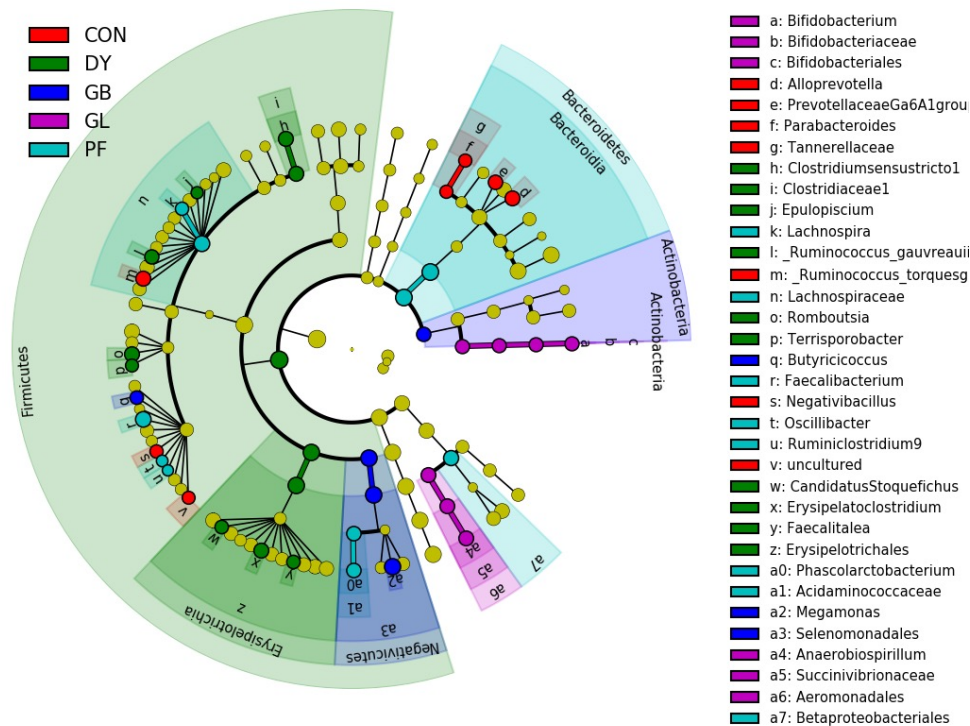
Diet Affects Microbial Abundance

Bray-Curtis Dissimilarity Analysis

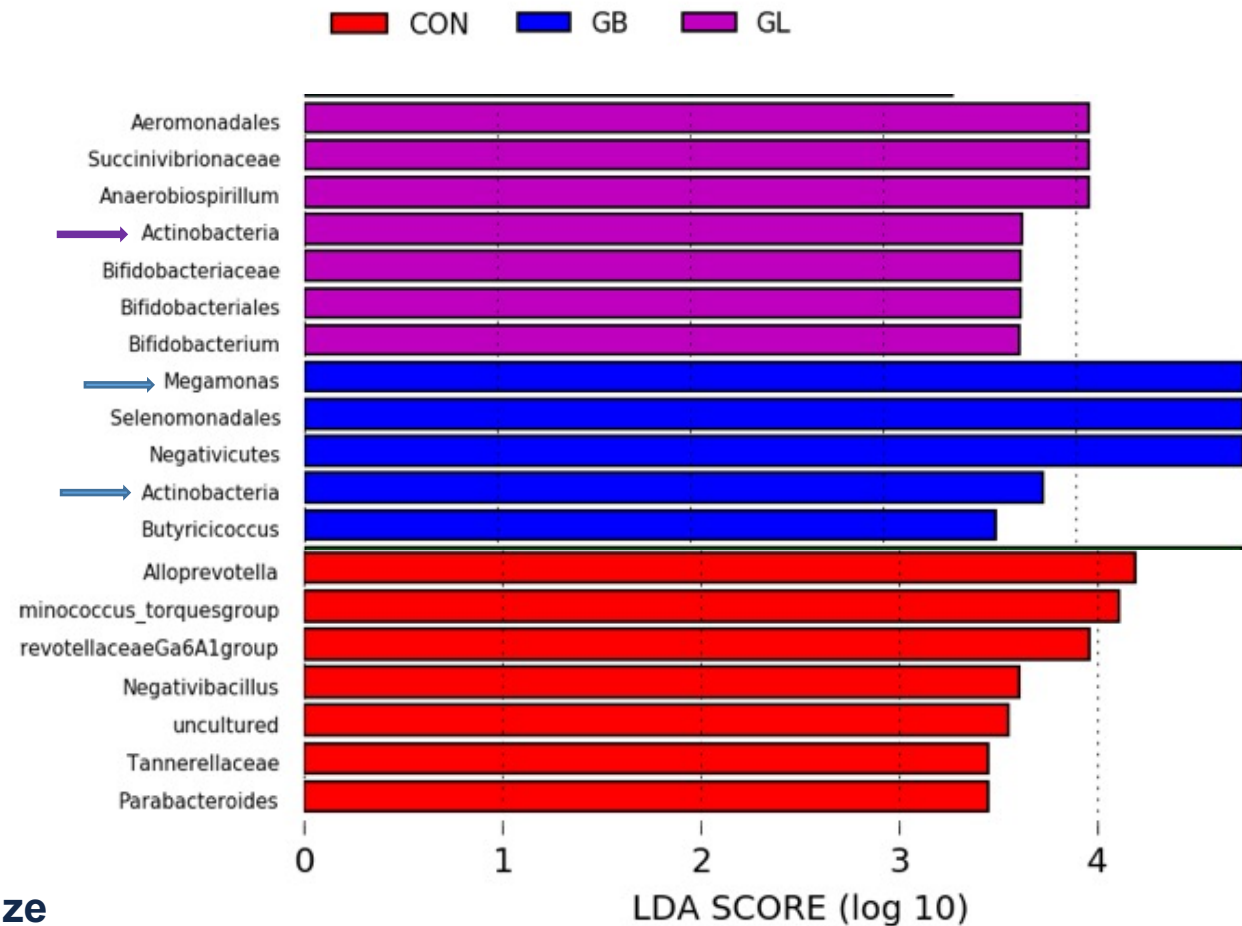


Reilly et al., 2021

Characterization of Fecal Microbiota of Dogs Fed Different Protein Sources



Linear Discriminant Analysis (LDA) Effect Size (LEfSe) Analysis



Longitudinal Assessment of taurine and amino acid concentration in dogs fed a green lentil diet



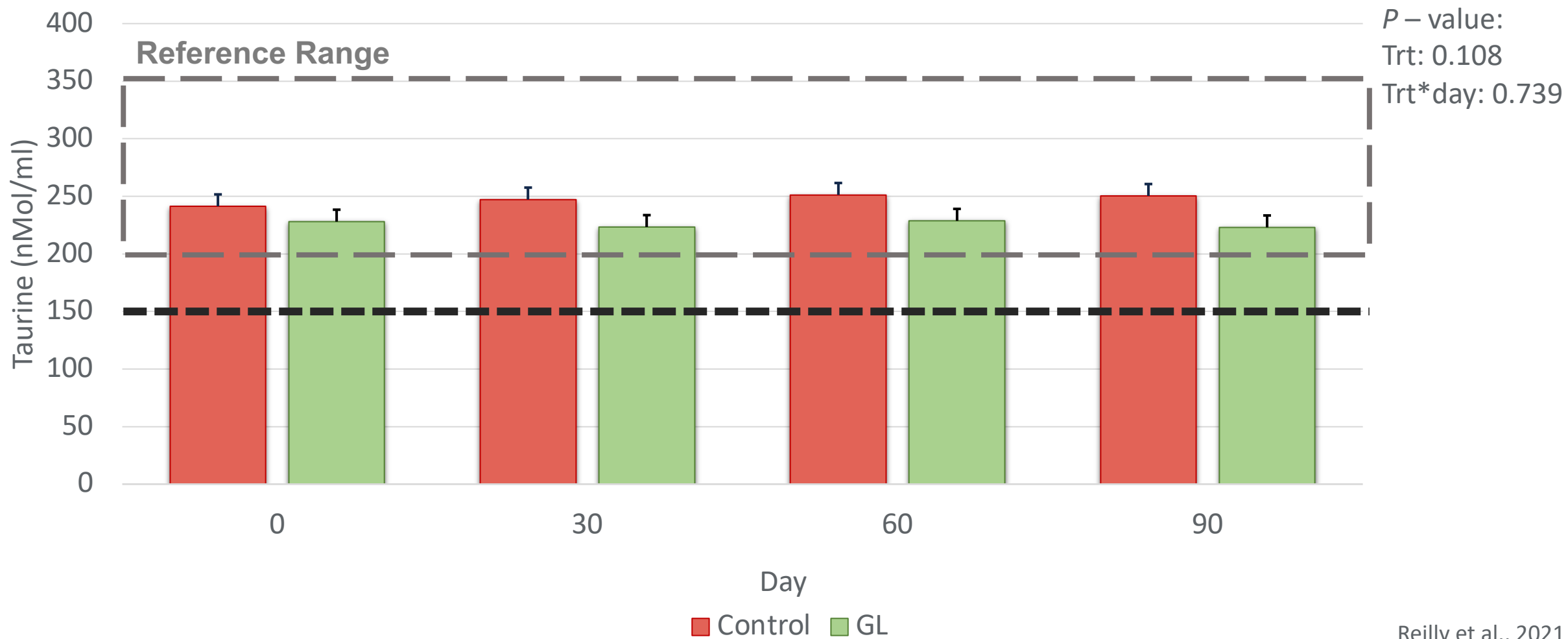
DCM and Grain-Free Diets

- FDA press release – July 2018
 - Associates DCM with grain-free diets
 - 273 reported cases between July 2018 – February 2019
 - 90% of dogs fed grain-free diet
 - 10% of dogs diets contained grains
 - Majority contained peas or lentils as a main ingredient
 - Boutique, Exotic, Grain-free (BEG) diets



FDA, 2019

Whole Blood Taurine Concentration



Reilly et al., 2021

Fecal Bile Acids

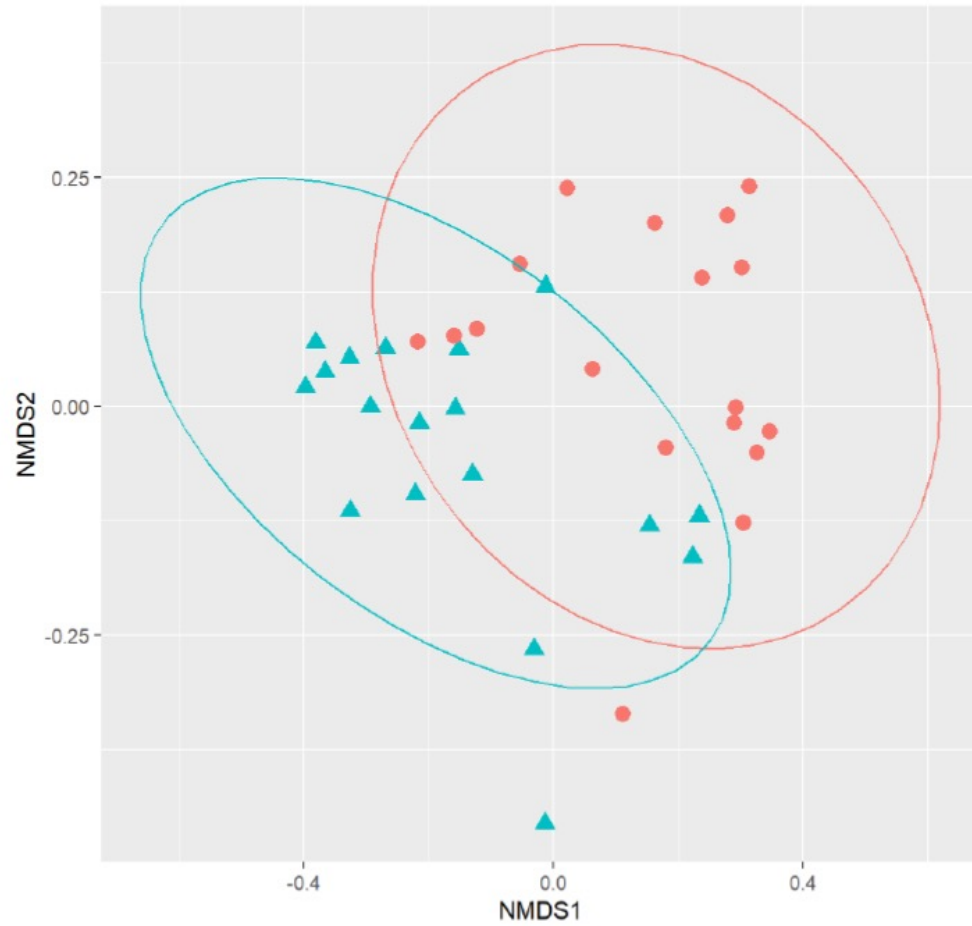
Bile Acid (µg/mg)	Day 30		Day 60		Day 90		SEM ²	Trt	P-Value	
	CON ¹	GLD ¹	CON	GLD	CON	GLD			Day	Trt*Day
Primary, total	0.46	0.71	0.46	0.66	0.41	0.51	0.063	0.0137	0.1014	0.4285
CA	0.21	0.38	0.21	0.34	0.18	0.28	0.046	0.0280	0.2354	0.4665
CDCA	0.27	0.38	0.25	0.32	0.23	0.26	0.031	0.0304	0.0550	0.3980
Secondary, total	3.30	3.46	7.02	4.41	3.15	3.15	1.088	0.4907	0.0164	0.2349
DCA	1.98	2.54	5.04	3.36	2.05	2.28	0.931	0.7708	0.0212	0.2938
LCA	1.16	0.76	1.86	0.86	0.96	0.71	0.183	0.0138	0.0087	0.0651
UDCA	0.15	0.16	0.15	0.17	0.14	0.16	0.009	0.0914	0.1633	0.4660
Total Bile Acids	3.77	4.81	7.66	5.06	3.56	3.65	1.154	0.6959	0.0217	0.1575

CA = Cholic acid, CDCA = Chenodeoxycholic acid, DCA = Deoxycholic acid, LCA = Lithocholic acid, UDCA = Ursodeoxycholic acid

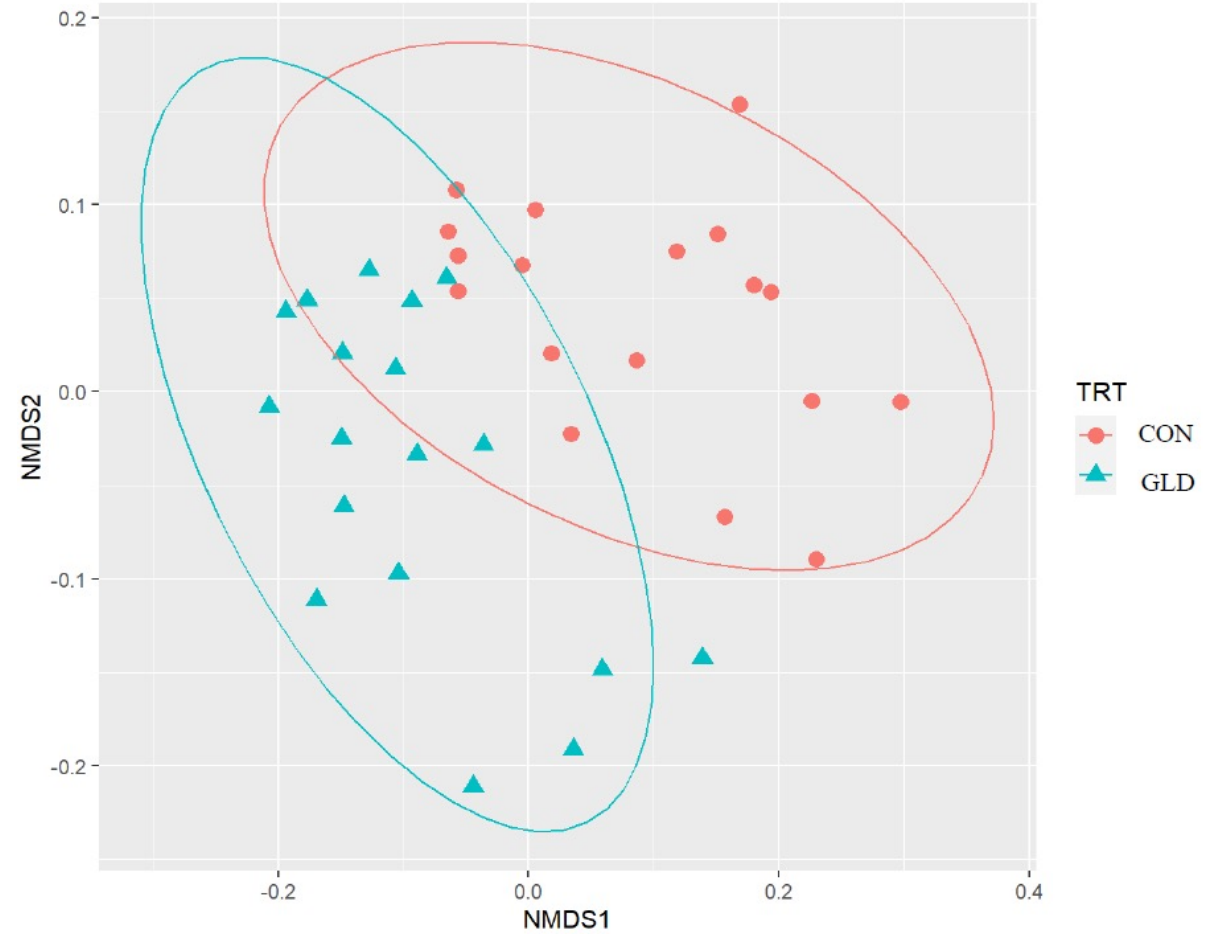
Reilly et al., 2021

Beta-Diversity - UniFrac

(a) Weighted



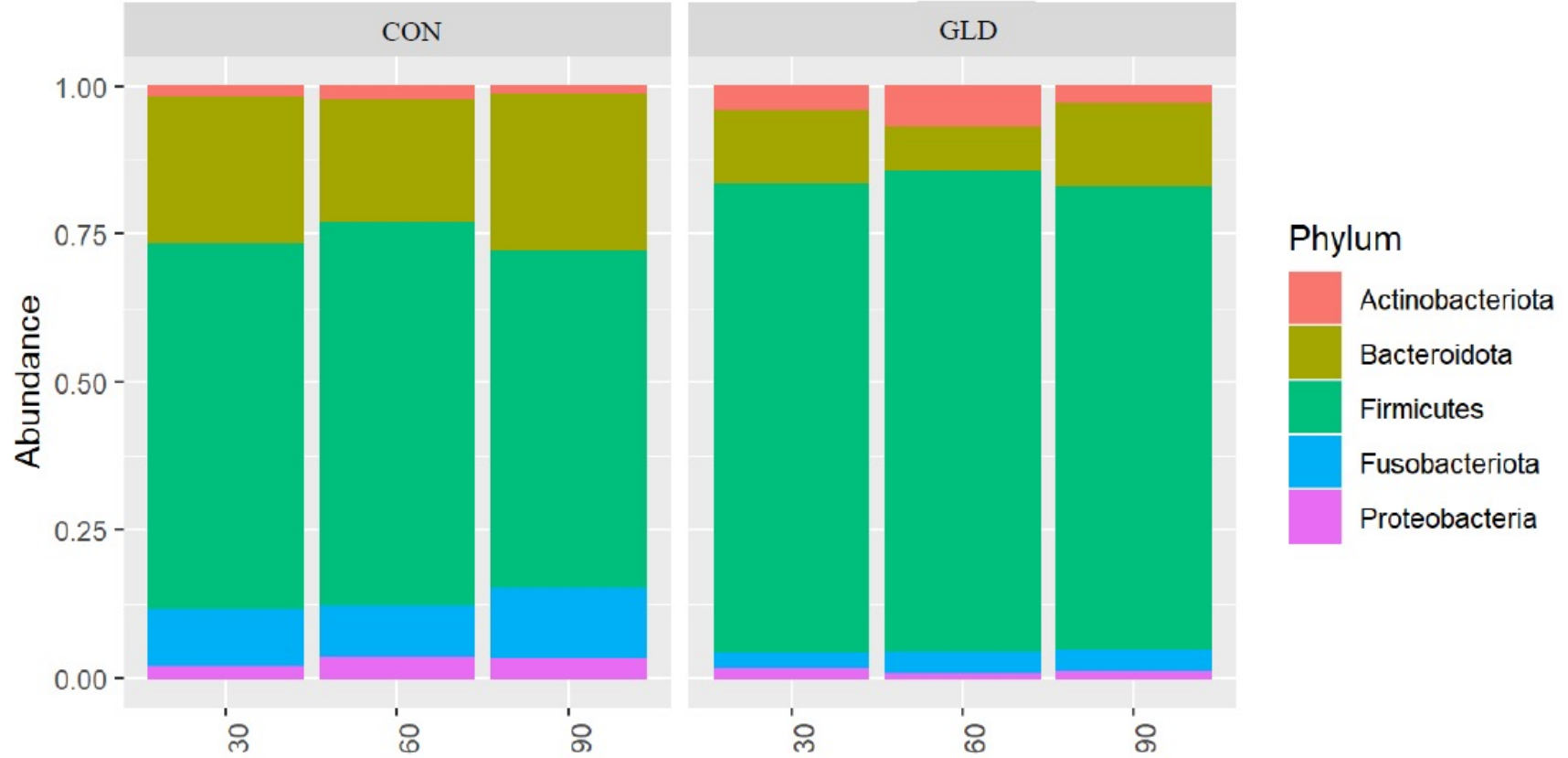
(b) Unweighted



Reilly et al., 2021

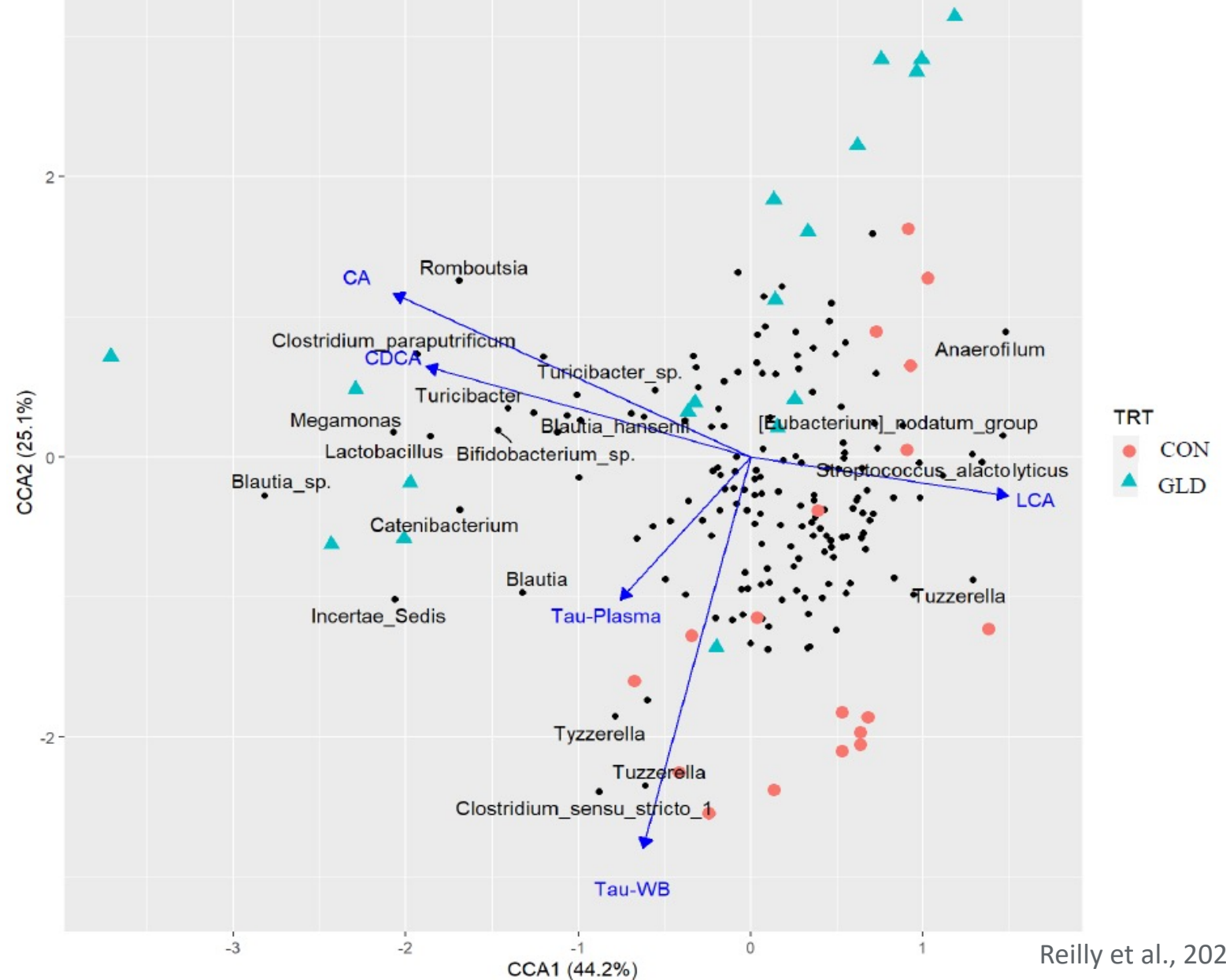
- 160 different taxa were identified
- Differential abundance showed 14 different taxa were increased in dogs fed GLD compared to CON
 - Bifidobacterium
 - Coriobacteriaceae
 - Lachnospiraceae

Microbial Composition - Phyla



- Canonical correspondence analysis (CCA) of taxa abundance constrained by CA, CDCA, LCA and taurine concentrations
- Dogs fed CON more strongly correlated with LCA
- Dogs fed CON more strongly correlated to whole blood taurine concentrations
- Dogs fed GLD were more strongly correlated with CA and CDCA

Taxa and Metabolic Variables



Reilly et al., 2021

Evaluation of cooked and raw garbanzo beans as main plant protein sources in extruded feline diets



Diet Composition

Ingredient	Dietary Treatments				
	0% Garbanzo Bean	7.5% Raw Garbanzo Bean	15% Raw Garbanzo Bean	30% Raw Garbanzo Bean	30% Cooked Garbanzo Bean
Cooked Garbanzo Bean	--	--	--	--	30.00
Raw Garbanzo Bean	--	7.5	15.00	30.00	--
Poultry BPM	38.39	36.56	35.14	30.79	30.79
Rice	30.00	25.64	19.46	8.27	8.27
Corn Gluten Meal	10.76	10.50	10.50	10.50	10.50
Poultry Fat	10.00	10.00	10.00	10.16	10.16
Corn	5.00	5.00	5.00	5.00	5.00
Dried Beet Pulp	2.50	2.50	2.50	2.50	2.50
Palatant	1.00	1.00	1.00	1.00	1.00

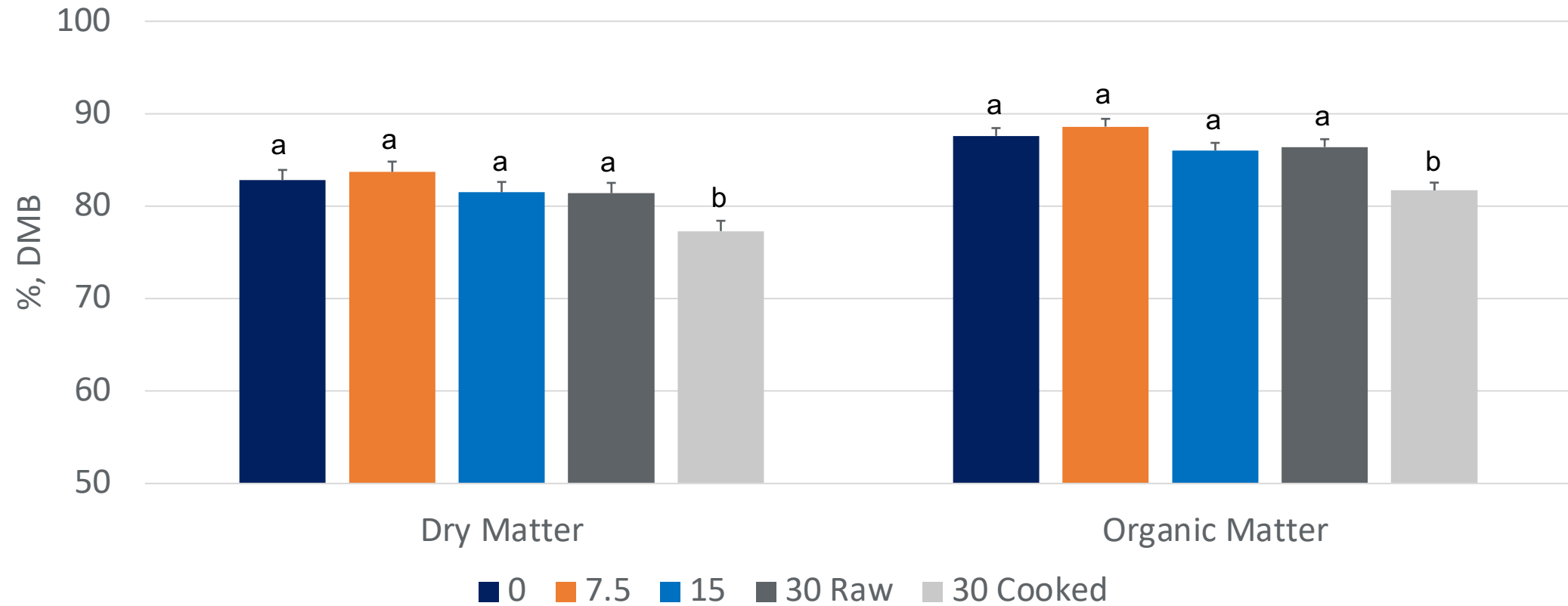
Vitamin and mineral premixes were added to make all diets complete and balanced

Reilly et al., 2021

Diet Proximate Analysis

Item	Dietary Treatment				
	0%	7.5%	15%	30% Raw	30% Cooked
Dry Matter (%)	93.4	92.1	92.8	91.9	94.1
	Dry Matter Basis				
Crude Protein (%)	40.6	37.4	37.3	35.8	37.5
A.H. Fat (%)	19.5	17.5	19.1	18.6	19.2
Ash (%)	8.2	8.2	7.3	8.3	6.9
Total Dietary Fiber (%)	11.9	9.1	11.4	11.3	13.7
- Soluble (%)	4.6	3.7	2.5	4.1	3.6
- Insoluble (%)	7.2	5.4	8.8	7.3	10.1
GE (kcal/g)	5.4	5.2	5.3	5.3	5.4

Apparent Total Tract Digestibility of Dry Matter and Organic Matter



^{a-b} Means within a group with different superscripts are significantly different at $P < 0.05$

Reilly et al., 2021

Fermentative End-Products

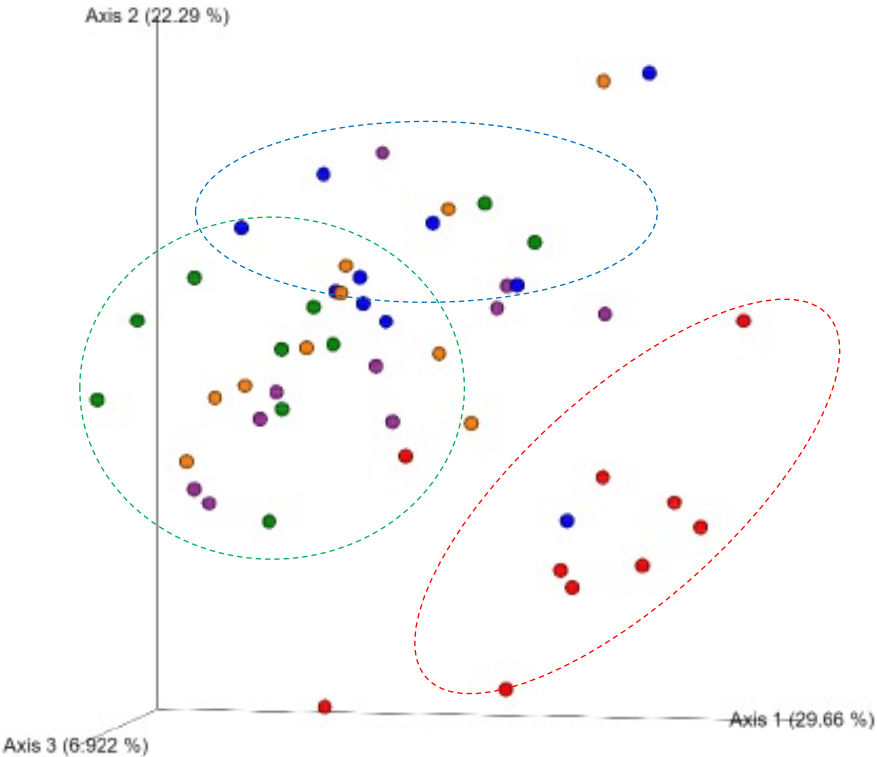
	Dietary Treatment					SEM
	0%	7.5%	15%	30% Raw	30% Cooked	
pH	6.68	6.44	6.47	6.59	6.28	0.112
SFCA ($\mu\text{mol/g}$, DMB)						
Acetate	251.5 ^c	308.4 ^{bc}	345.4 ^{abc}	373.6 ^{ab}	434.6 ^a	35.995
Propionate	74.9 ^d	121.9 ^c	133.6 ^{bc}	173.1 ^{ab}	206.8 ^a	12.601
Butyrate	35.2	43.6	48.9	44.1	41.1	4.789
Total SCFA	361.6 ^b	474.1 ^b	527.9 ^{ab}	590.7 ^{ab}	682.4 ^a	50.570

^{a-c} Means within a row with different superscripts are significantly different at $P < 0.05$

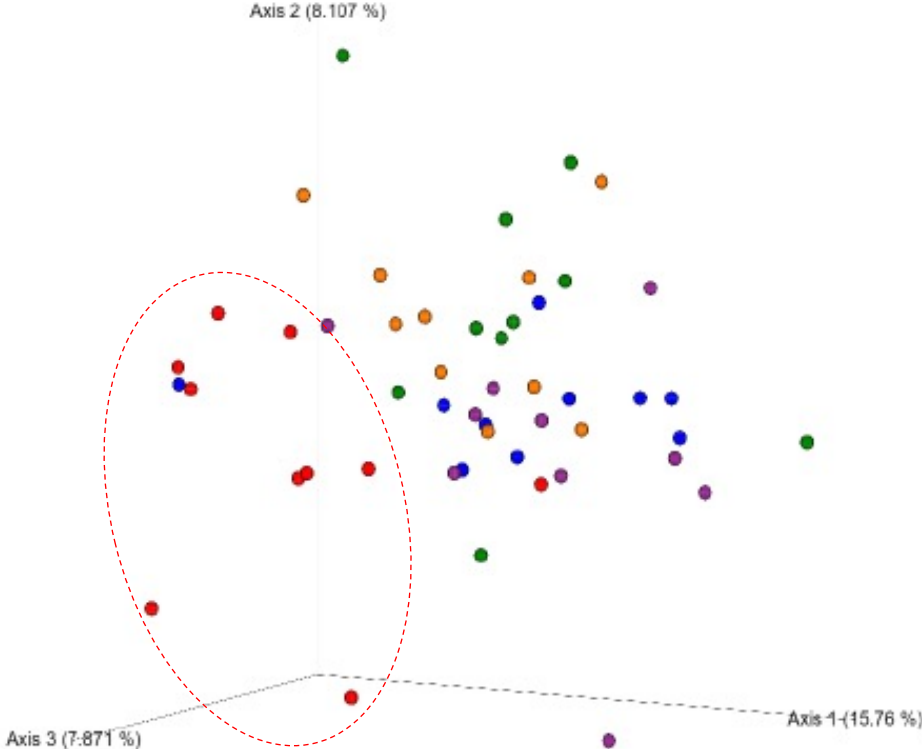
Reilly et al., 2021

Beta-Diversity - UniFrac

(a) Weighted



(b) Unweighted



● 0% ● 7.5% ● 15% ● 30% Raw ● 30% Cooked

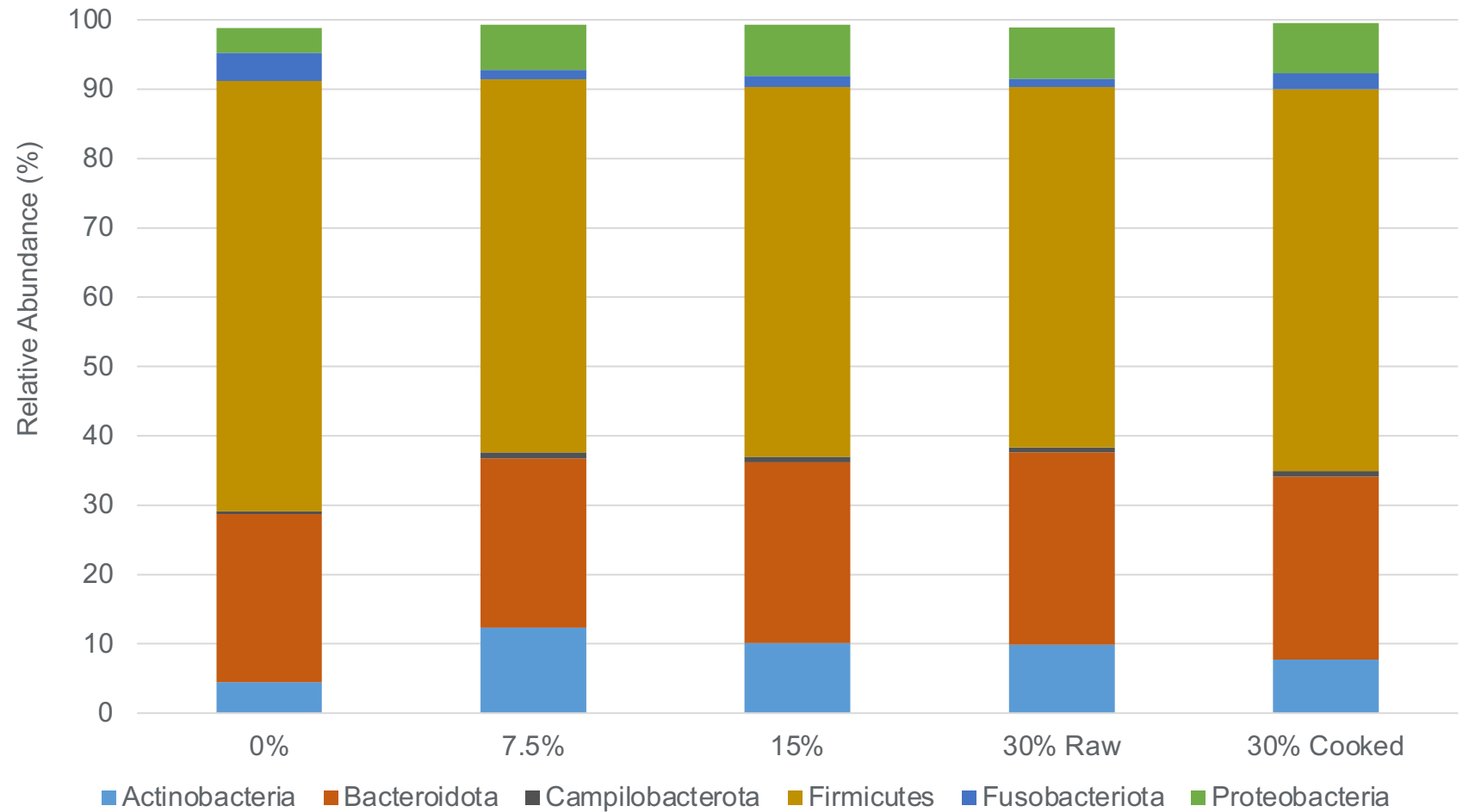
Reilly et al., 2021

- Identified 6 phyla, 40 families, and 70 genera

- Cats fed 0% GB had lower Actinobacteria than cats fed raw GB diets
 - Bifidobacteriaceae* and *Coriobacteriaceae*

- Cats fed 0% GB had higher Firmicutes and Fusobacteria and lower Proteobacteria compared to GB-containing diets

Microbial Composition - Phyla



Take-home message

- Diet has a significant effect on the modulation of gut microbiota and metabolites
- In general, high dietary protein leads to
 - ↑ Fusobacteria and Proteobacteria
 - ↓ Firmicutes (Ruminococcaceae and *Faecalibacterium* genus)
 - ↑ abundance of *Clostridium spp* and *Erysipelotrichaceae*
- Long term effects of high-protein diets on health of pet animals are unknown
- Most studies have evaluated the effect of diet format and changed multiple nutrients, ingredients, processing conditions
 - No direct inference to dietary protein is possible



Muito Obrigada!



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