Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing

Alison N. Beloshapka1, Scot E. Dowd2, Jan S. Suchodolski3, Jörg M. Steiner3, Laura Duclos4 & Kelly S. Swanson1,5,6

1Department of Animal Sciences, University of Illinois, Urbana, IL, USA; 2Research and Testing Laboratory, Lubbock, TX, USA; 3Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA; 4Nature’s Variety, Inc., Lincoln, NE, USA; 5Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA; and 6Division of Nutritional Sciences, University of Illinois, Urbana, IL, USA

Correspondence: Kelly S. Swanson, Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801. Tel: 00+1+217 333 4189; fax: 00+1+217 333 7861; e-mail: ksswanso@illinois.edu

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Abstract
Our objective was to determine the effects of feeding raw meat–based diets with or without inulin or yeast cell wall extract (YCW) on fecal microbial communities of dogs using 454 pyrosequencing. Six healthy female adult beagles (5.5 ± 0.5 years; 8.5 ± 0.5 kg) were randomly assigned to six test diets using a Latin square design: (1) beef control; (2) beef + 1.4% inulin; (3) beef + 1.4% YCW; (4) chicken control; (5) chicken + 1.4% inulin; and (6) chicken + 1.4% YCW. Following 14 days of adaptation, fresh fecal samples were collected on day 15 or day 16 of each period. Fecal genomic DNA was extracted and used to create 16S rRNA gene amplicons, which were subjected to 454 pyrosequencing and qPCR. Predominant fecal bacterial phyla included Fusobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. Beef-based diets increased ($P < 0.05$) Escherichia, but decreased ($P < 0.05$) Anaerobiospirillum vs. chicken-based diets. Inulin decreased ($P < 0.05$) Enterobacteriaceae. Inulin increased ($P < 0.05$) Megamonas vs. control. Inulin also decreased ($P < 0.05$) Escherichia vs. YCW. YCW increased ($P < 0.05$) Bifidobacterium vs. inulin and control and inulin increased ($P < 0.05$) Lactobacillus vs. YCW. Although a few changes in fecal microbiota were observed with inulin or YCW consumption, a strong prebiotic effect was not observed.

Introduction
Many of today’s pet owners are choosing raw meat–based diets to feed their dogs. Veterinarians have stated that one of their biggest health concerns with feeding such diets is the potential risk of bacterial contamination (LeJeune & Hancock, 2001). Contamination may come directly from the food itself or from fecal shedding of the animal (Ngaage et al., 1999; Sato et al., 2000; Joffe & Schlesinger, 2002; Behravesh et al., 2010). Even though dogs evolved eating raw meat and are often coprophagous, case reports of gastrointestinal upset have been reported in dogs fed raw meat (Cantor et al., 1997; Morley et al., 2006; Lefebvre et al., 2008). However, our recent study was the first to evaluate commercially available raw diets (Beloshapka et al., 2012). Therefore, more research studying dogs fed raw meat–based diets is needed.

Given the microbial concerns of raw meat–based diets, the addition of prebiotics or fermentable fibers that may beneficially alter the gut microbiota may be valuable. Inulin is a long-chain fructan (10–60 units) derived from chicory root. It is not digestible by mammalian enzymes and therefore reaches the colon to be fermented by intestinal microorganisms. Yeast cell wall extracts (YCW) are derived from Saccharomyces cerevisiae. Yeast cell wall extracts are moderately fermentable substrates that contain a mixture of carbohydrates and proteins. They are rich in mannans, which are believed to prevent adherence of bacteria expressing type-1 fimbriae to the intestinal wall (Ofek et al., 1977; Neesen et al., 1986).
High-throughput sequencing of the 16S rRNA gene, as is done with 454 pyrosequencing, is a DNA-based technique that allows for comprehensive evaluation of the gastrointestinal microbial community, providing a broad scope of the microbiome that is impossible using traditional assays. In a previous study, we evaluated the effects of inulin or YCW on total tract apparent macronutrient digestibility, fecal characteristics, fecal fermentative end-products, blood cell populations, serum metabolite concentrations, and nitrogen (N) balance in adult canines fed raw meat diets (Beloshapka et al., 2012). The purpose of the current study was to evaluate the fecal microbial communities of these dogs using 454 pyrosequencing.

Materials and methods

Animals and diets

All animal care and study procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation. An in-depth description of animal procedures is described by Beloshapka et al. (2012). Briefly, six spayed female, healthy adult beagle dogs (mean ± SD age: 5.5 ± 0.5 years; mean ± SD BW: 8.5 ± 0.5 kg) were used. A 3 × 2 factorial in a 6 × 6 Latin square design with 21-days periods was conducted. Dogs were fed twice daily (8:00 and 17:00) to maintain BW throughout the study. Fresh water was offered ad libitum. Six raw diets, based on animal meats and organs, eggs, fruits, and vegetables, were formulated to contain approximately 25–30% crude protein (CP) and 45–50% fat on a dry matter (DM) basis: (1) beef control; (2) beef + 1.4% inulin dry matter basis (DMB); Orafti HP, BENEOL Group, Tienan, Belgium; (3) beef + 1.4% YCW (DMB); (4) chicken control; (5) chicken + 1.4% inulin (DMB); and (6) chicken + 1.4% YCW (DMB) [complete dietary description is provided in Table S1 (Supporting Information) and Beloshapka et al. (2012)]. The diets were truly raw and were not treated for pathogens [i.e. high pressure processing (HPP) was not used]. Diets were kept frozen and thawed under refrigeration in storage containers 2 days prior to feeding. Subsamples of all six thawed diets were submitted to the University of Illinois Veterinary Medicine Diagnostic Laboratory for general cultures, all of which came back negative or below detectible limits.

Sample collection

Following a 14-day adaptation period, one fresh fecal sample was collected within 15 min of defecation on either day 15 or day 16 of each period. The fresh feces were weighed and aliquoted into sterile cryogenic vials (Nalgene, Rochester, NY) and frozen at −80 °C until DNA extraction.

Microbial analyses

Genomic DNA was extracted using the repeated bead beater method described by Yu & Morrison (2004) with a DNA extraction kit (QIAamp DNA Stool Mini Kit, Qiagen, Valencia, CA). Once the DNA was eluted, all samples were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer’s instructions. Extracted DNA was quantified using a spectrophotometer (NanoDrop ND-1000, Nano-Drop Technologies, Wilmington, DE). Genomic DNA quality was assessed using agarose gel electrophoresis.

Genomic DNA was diluted to 20 ng μL⁻¹ in preparation for 454-pyrosequencing. Bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) based upon the V4-V6 region of the 16S rRNA gene was performed as described previously (Dowd et al., 2008). The 16S rRNA universal Eubacterial primers 530F (5′-GTG CCA GCM GCN GCG G) and 1100R (5′-GGG TTN CGN TCG TTG) were used to amplify the 600-bp region. In preparation for FLX-Titanium sequencing (Roche, Nutley, NJ), DNA fragment size and concentration were accurately measured using DNA chips under a Bio-Rad Experion Automated Electrophoresis Station (Bio-Rad Laboratories, Hercules, CA) and a TBS-380 Fluorometer (Turner Biosystems, Sunnyvale, CA). A four-region 454 sequencing run (40 tags per quarter) was performed on a GS PicoTiterPlate (PTP) using the Genome Sequencer FLX System according to manufacturer’s instructions (Roche).

Because Bifidobacterium and Lactobacillus are often used as probiotics or are the target taxa of prebiotics, including inulin, quantitative polymerase chain reaction (qPCR) was used to measure their abundance as described by Garcia-Mazcorro et al. (2012). Briefly, fecal samples were extracted using a commercial DNA extraction kit according to the manufacturer’s instructions (Zymo Research Corp., Irvine, CA). SYBR-based reaction mixtures (total 10 μL) containing 5 μL of SsoFast EvaGreen supermix (Bio-Rad Laboratories), 1.6 μL of water, 0.4 μL of each primer (final concentration: 400 nM), and 2 μL of DNA (1 : 10 or 1 : 100 dilution) were used. The following primers were used to measure Bifidobacterium: forward (5′-3′): TCGCGTCTGGTGTTGAAAG; reverse (5′-3′): CCACATCAGCRTCAC (Malinen et al., 2005). The following primers were used to measure Lactobacillus: forward (5′-3′): AGCAGATGGAATCCTTCCA; reverse (5′-3′): CACCGCTACACATGGAG (Walter et al., 2001). PCR conditions
component analysis (PCA) was performed using JMP soft-

were 95 °C for 2 min and 40 cycles at 95 °C for 5 s and

10 s at 60 °C (Bifidobacterium) or 58 °C (Lactobacillus).

A melt curve analysis was performed under the following

conditions: 1 min at 95 °C, 1 min at 55 °C, and 80 cycles

of 0.5 °C increments (10 s each). TaqMan® reaction

mixtures (total 10 μL) contained 5 μL of TaqMan® Fast

Universal PCR master mix (2x), No AmpErase® UNG

(Applied Biosystems), 1 μL of water, 0.4 μL of each pri-

mer (final concentration: 400 nM), 0.2 μL of the probe

(final concentration: 200 nM), and 2 μL of DNA (1 : 10

or 1 : 100 dilution). The PCR conditions were 95 °C for

20 s, 40 cycles at 95 °C for 5 s, and 10 s at the optimized

annealing temperature provided above.

**Data analysis**

Prior to analysis, sequences shorter than 200 bp, sequences

with ambiguous base calls, sequences with quality average

< Q25, and sequences with homopolymers < 5 bp were

removed. Sequences were depleted of any nonbacterial

ribosomal sequences and chimeras using Black Box

Chimera Check software (s2c2) (Gontcharova et al., 2010).

To determine the identity of bacteria in the remaining

sequences, they were first queried using BLASTn against a

database of high-quality bacterial 16S rRNA gene sequences

derived from NCBI (Dowd et al., 2005). Using a.NET and

C# analysis pipeline, the resulting BLASTn outputs were

compiled and validated using taxonomic distance methods,

and data reduction analysis was performed as described

previously (Acosta-Martinez et al., 2008). Sequences were

identified as follows: Species (> 97%), Genus (95–97%),

Family (90–95%), Order (85–90%), Class (80–85%), and

Phylum (< 80%).

Pyrosequencing data presented as percentage of

sequences at each taxonomic level were analyzed using the

MIXED procedure of SAS (version 9.2, SAS Institute

Inc., Cary, NC), evaluating the main effects of protein,

fiber, and the interaction between protein and fiber. Data

were analyzed using the type 3 test of the MIXED proce-

dure. Means were separated using a protected least

squares difference with a Tukey adjustment. A \( P < 0.05 \)

was accepted as being statistically significant. Principal

component analysis (PCA) was performed using JMP soft-

ware of SAS. The qPCR data were expressed as log

amount of DNA (fg) for each particular bacterial group

per 10 ng of isolated total DNA (Suchodolski et al.,

2012).

**Results**

A total of 358 693 sequences were obtained from the cur-

rent data set. Sequences are available at the NCBI

sequence read archive (http://www.ncbi.nlm.nih.gov/

Traces/sra/) under accession numbers SAMN01889107–

SAMN01889148. There were an average of 5016 reads/

sample. Evaluation of 4000 randomly selected sequences

from all samples was used to provide diversity estimates.

Mean operational taxonomical unit (OTU) estimates for

97% similarity as evaluated by rarefaction analysis are as

follows: beef control = 1289, beef + inulin = 1087 beef +

YCW = 1139, chicken control = 1076, and chicken +
inulin = 1249; chicken + YCW = 1211.

Predominant fecal bacterial phyla present in all dogs

included Fusobacteria, Firmicutes, Bacteroidetes, Proteo-
bacteria, and Actinobacteria (Table 1). Together,

Fusobacteria and Firmicutes made up about 75–80% of

bacterial sequences, with Bacteroidetes, Proteobacteria,

and Actinobacteria contributing to only about 10–15%,

5%, and 2–3% of sequences, respectively. Principal

component analysis based on all phyla data, separated

by fiber source, (Fig. 1) provided a visual representa-
tion of the data. There was no clear separation due to

treatment. ANOSIM was used for testing the signifi-
cance of distance matrices. There was no significance

between the samples, including the analysis for fiber,

period, protein, and the interaction of protein x fiber.

This figure only presents the data at the phylum level,

but clustering was not observed at any taxa level (e.g.

family, genus).

Predominant fecal bacterial families included Fusobac-
teriaeae, Clostridiaceae, and Bacteroidaceae (Table 2).

Fecal Veillonellaceae (member of Clostridiales and

Firmicutes) were increased (\( P = 0.02 \)) in dogs fed the
diets containing inulin [means: control = 1.45%; inu-

lin = 9.19%; YCW = 3.46%]. Fecal Enterobacteriaceae

(member of Enterobacteriales and Proteobacteria), how-
ever, were decreased (\( P = 0.04 \)) in dogs fed the diets

containing inulin (means: control = 0.56%; inulin = 0.25%;

YCW = 0.90%). Fecal Succiniviribionaceae (member of

Aeromonadales and Proteobacteria) were increased

(\( P < 0.01 \)) in dogs fed the chicken-based diets (3.28%)

compared with dogs fed the beef-based diets (1.18%).

Fecal Enterobacteriaceae were increased (\( P = 0.01 \)) in
dogs fed the beef-based diets (0.83%) compared with dogs fed

the chicken-based diets (0.31%).

Predominant bacterial genera included Fusobacterium,

Cetobacterium, Clostridium, and Bacteroides (Table 1),

with all bacterial genera presented in Table S2. Many gen-

era were impacted by the protein source or the inclusion

of inulin or YCW. Dogs fed diets containing inulin had

increased (\( P = 0.02 \)) fecal Megamonas and decreased

(\( P = 0.03 \)) fecal Escherichia when compared with dogs fed

the control or YCW diets. Dogs fed chicken-based diets

had increased (\( P < 0.01 \)) fecal Anaerobiospirillum, but
decreased (\( P = 0.01 \)) fecal Escherichia when compared

with dogs fed beef-based diets.
Predominant bacterial species included *Fusobacterium varium*, *Roseburia intestinalis*, and *Clostridium saccharolyticum* (Table 2). Dogs fed diets containing inulin had decreased fecal *Bacteroides eggerthii* \((P < 0.01)\), *Fusobacterium mortiferum* \((P = 0.01)\), and *F. varium* \((P = 0.04)\) and increased fecal *Megamonas hypermegale* \((P = 0.02)\) when compared with dogs fed the control or YCW diets. Dogs fed diets containing YCW had decreased \((P = 0.05)\) fecal *Clostridium celerecrescens* when compared with dogs fed the control or inulin diets. Dogs fed beef-based diets had increased \((P = 0.04)\) fecal *Bacteroides vulgatus* and decreased \((P = 0.01)\) *Bacteroides coprocola* when compared with dogs fed chicken-based diets.

The abundance of *Bifidobacterium*, as measured by qPCR, was greater \((P = 0.02)\) in dogs fed YCW (4.23 log DNA/10 ng total DNA) compared with those fed inulin (3.26 log DNA/10 ng total DNA) or the control diet (3.24 log DNA/10 ng total DNA). The abundance of *Lactobacillus*, as measured by qPCR, was greater \((P = 0.05)\) in dogs fed inulin (4.36 log DNA/10 ng total DNA) compared with those fed YCW (3.83 log DNA/10 ng total DNA). Intermediate *Lactobacillus* concentrations were present in dogs fed the control diet (3.91 log DNA/10 ng total DNA) and not different than those fed inulin and or YCW.

![Fig. 1](https://academic.oup.com/femsec/article-abstract/84/3/532/579061)

**Table 1.** Prominent bacterial phyla and genera (expressed as percentage of total sequences) in feces from dogs fed raw chicken- or beef-based diets with or without the addition of inulin or yeast cell wall (YCW).

<table>
<thead>
<tr>
<th>Item</th>
<th>Beef</th>
<th>Chicken</th>
<th>Pooled SEM</th>
<th>Protein</th>
<th>Fiber</th>
<th>Protein*Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inulin</td>
<td>YCW</td>
<td>Control</td>
<td>Inulin</td>
<td>YCW</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>38.98</td>
<td>50.01</td>
<td>47.05</td>
<td>51.54</td>
<td>29.16</td>
<td>40.77</td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>30.52</td>
<td>16.12</td>
<td>29.86</td>
<td>36.46</td>
<td>18.41</td>
<td>22.42</td>
</tr>
<tr>
<td>Cetobacterium</td>
<td>8.45</td>
<td>33.89</td>
<td>17.19</td>
<td>15.08</td>
<td>10.74</td>
<td>18.34</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>37.87</td>
<td>37.17</td>
<td>32.58</td>
<td>27.35</td>
<td>52.49</td>
<td>34.42</td>
</tr>
<tr>
<td>Clostridium</td>
<td>16.91</td>
<td>12.54</td>
<td>13.97</td>
<td>17.12</td>
<td>21.70</td>
<td>13.11</td>
</tr>
<tr>
<td>Roseburia</td>
<td>7.43</td>
<td>4.21</td>
<td>4.54</td>
<td>4.31</td>
<td>5.35</td>
<td>5.88</td>
</tr>
<tr>
<td>Allobaculum</td>
<td>3.78</td>
<td>5.98</td>
<td>5.68</td>
<td>1.34</td>
<td>4.80</td>
<td>2.76</td>
</tr>
<tr>
<td>Turicibacter</td>
<td>3.76</td>
<td>2.32</td>
<td>2.74</td>
<td>1.38</td>
<td>0.77</td>
<td>4.15</td>
</tr>
<tr>
<td>Megamonas</td>
<td>1.67a</td>
<td>5.31b</td>
<td>2.44a,b</td>
<td>0.57a</td>
<td>12.64b</td>
<td>3.61a,b</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>1.05</td>
<td>2.58</td>
<td>0.86</td>
<td>0.77</td>
<td>1.63</td>
<td>1.22</td>
</tr>
<tr>
<td>Ruminococcus</td>
<td>0.43</td>
<td>1.52</td>
<td>0.33</td>
<td>0.37</td>
<td>0.82</td>
<td>0.67</td>
</tr>
<tr>
<td>Succinispira</td>
<td>0.15</td>
<td>0.05</td>
<td>0.21</td>
<td>0.15</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>Anaerovorax</td>
<td>0.09</td>
<td>0.21</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Pseudoramibacter</td>
<td>0.07</td>
<td>0.13</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Succinivibrio</td>
<td>0.03</td>
<td>0.01</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>14.95</td>
<td>6.45</td>
<td>12.79</td>
<td>14.61</td>
<td>11.91</td>
<td>18.24</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>14.63</td>
<td>6.30</td>
<td>12.04</td>
<td>14.03</td>
<td>11.29</td>
<td>17.97</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>5.59</td>
<td>4.05</td>
<td>5.83</td>
<td>5.56</td>
<td>5.07</td>
<td>5.70</td>
</tr>
<tr>
<td>Acidovorax</td>
<td>2.06</td>
<td>2.07</td>
<td>2.50</td>
<td>1.46</td>
<td>0.41</td>
<td>1.05</td>
</tr>
<tr>
<td>Anaerobiospirillum</td>
<td>1.45</td>
<td>0.78</td>
<td>1.29</td>
<td>2.82</td>
<td>3.64</td>
<td>3.39</td>
</tr>
<tr>
<td>Escherichia</td>
<td>0.93ab</td>
<td>0.29a</td>
<td>1.69ab</td>
<td>0.29ab</td>
<td>0.33a</td>
<td>0.45ab</td>
</tr>
<tr>
<td>Simplicispira</td>
<td>0.025</td>
<td>0.020</td>
<td>0.022</td>
<td>0.002</td>
<td>0.005</td>
<td>0.017</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>2.54</td>
<td>2.26</td>
<td>1.45</td>
<td>0.89</td>
<td>1.30</td>
<td>0.80</td>
</tr>
<tr>
<td>Collinsella</td>
<td>1.87</td>
<td>1.66</td>
<td>0.49</td>
<td>0.70</td>
<td>0.97</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Means not sharing common superscript letters differ \((P < 0.05)\).
Discussion

It has been well established that the number and/or activity of the gastrointestinal microbiota can be manipulated by diet, but little data have been provided using high-throughput molecular techniques in dogs, especially in those consuming raw meat-based diets. Therefore, this study was conducted to gain more knowledge about how
gastrointestinal microbial populations of healthy dogs are impacted by raw meat–based diets with or without the addition of inulin or YCW. This is the first study that takes a comprehensive look at the fecal microbiome of dogs fed raw meat–based diets. All dogs remained healthy throughout the study. However, the results of the current study highlight some of the effects of protein source, inulin, and YCW and continue to provide insight into how diet impacts the gastrointestinal microbiota as well as potential health implications.

Gastrointestinal microbial populations vary greatly among host species and regions of the gastrointestinal tract. In many ways, the results of the current study were similar to previous studies (Suchodolski et al., 2008; Middelbos et al., 2010; Handl et al., 2011), but there were some interesting observations (i.e. high predominance of Fusobacteria; extremely low Faecalibacterium, etc.). Previous research has indicated that the major phyla present in the intestinal tract of dogs and cats are Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria (Suchodolski et al., 2008, 2009; Middelbos et al., 2010; Handl et al., 2011; Suchodolski, 2011; Swanson et al., 2011). Although Firmicutes and Bacteroidetes are predominant phyla present in the canine intestinal lumen, the dogs in the current study and those in a few other recent studies have had a much greater presence of Fusobacteria (Suchodolski et al., 2008; Middelbos et al., 2010). The high incidence of Fusobacteria may be due to species differences (i.e. dogs are prone to higher Fusobacteria abundance than humans or mice) or due to a bias that exists in the techniques used. Middelbos et al. (2010) highlighted the predominance of bacterial phyla in feces of dogs that were similar to those identified in this study, with 27–34% Bacteroidetes, 17–27% Firmicutes, 5–7% Proteobacteria, and 27–44% Fusobacteria. Suchodolski et al. (2008) generated sequences from clone libraries of samples from canine colon luminal content and concluded that Firmicutes were the most abundant phylum, representing about 40% of sequences, Bacteroidetes and Fusobacteria both represented nearly 30% of sequences in the colon, while Proteobacteria had a relatively low abundance, with only about 1.4% of sequences. The current study also included an evaluation of bacterial groups that are typically present at lower taxonomic levels. Many studies that utilize high-throughput assays only evaluate sequences at the phyla level. It is important to investigate differences that may exist at lower taxonomic levels as this may be a beneficial way in deciphering why certain bacterial groups occupy the gastrointestinal tract of dogs.

Protein source, whether due to amino acid balance, digestibility, or other factors, may alter the microbial balance within the intestinal tract of dogs. In the current data set, PCA analysis did not identify any great shifts due to diet, which may not be too surprising given that all dogs were healthy adults throughout the study. Many individual bacterial populations, however, were altered by protein source (i.e. beef or chicken). Because a control diet containing neither beef nor chicken was not tested, it is difficult to decipher what protein source caused these shifts. The bacterial family Coriobacteriaceae has been reported to affect the absorption of cholesterol as well as circulating serum cholesterol concentrations and has also been shown to be positively correlated with increased hepatic triglyceride concentrations in hepatic tissue in mice (Ridlon et al., 2006; Martinez et al., 2009; Claus et al., 2011). While high serum cholesterol concentrations are not as major of a clinical concern in dogs as they are in humans, especially with respect to cardiovascular disease, assessment of serum cholesterol along with that of serum triglyceride concentrations may provide insight into lipid metabolism. The beef-based diets contained more fat (63%) than the chicken-based diets (51%). Although serum cholesterol concentrations were not significantly different between dogs based on the protein source, serum triglyceride concentrations were numerically greater in dogs fed a beef-based diet than those fed a chicken-based diet (Beloshapka, 2011). Further research is necessary to determine whether and what role Coriobacteriaceae plays in host lipid metabolism.

Consuming prebiotics is an effective way to manipulate the gastrointestinal microbiota. In previous studies, fermentable fiber and prebiotics have been demonstrated to generate shifts in certain fecal bacteria, namely increased Lactobacillus spp. and Bifidobacterium spp. and decreased Clostridium perfringens and Escherichia coli (Jenkins et al., 1999; Rao, 1999; Flickinger et al., 2003; Middelbos et al., 2007). These bacteria have traditionally been used to assess prebiotic potential of novel carbohydrates. Due to the potential for an increased pathogen load in raw meat-based diets (based on general cultures; Strohmeyer et al., 2006), adding prebiotics, such as inulin or YCW may be of benefit. Not only do these microorganisms compete for nutrients and binding sites, but reduce gastrointestinal pH via short-chain fatty acid (SCFA) production and produce a variety of bacteriocins, both of which limit pathogen growth.

By including inulin and YCW to the raw meat–based diets used in the current study, it was expected that there would be an increase in potentially beneficial bacteria, such as Lactobacillus spp. or Bifidobacterium spp. According to pyrosequencing in this study, however, Bifidobacterium were not detectable and Lactobacillus were present at < 0.05% of sequences. Thus, these bacteria appear to be underestimated using these 16S rRNA gene-based sequencing approaches, which have been previously observed (Dethlefsen et al., 2008; Ritchie et al., 2008).
Previous research has drawn attention to the potential for bias when attempting to quantify the number of sequences from Bifidobacteria using 454 pyrosequencing (von Wintzingerode et al., 1997; Garcia-Mazcorro et al., 2011; Handl et al., 2011). There are many potential sources of bias, including primer selection. We have used the pyrosequencing primers from this study in feline (Hooda et al., 2012a) and human (Hooda et al., 2012b) nutrition experiments previously. In both cases, Bifidobacterium were measured and at much higher levels (kittens: up to 20%; Hooda et al., 2012a; adult humans: 1.2–2.5%; Hooda et al., 2012b) than in the dogs of the current study.

Because of the potential biases with pyrosequencing, qPCR was also used in this study to measure the abundance of Bifidobacterium and Lactobacillus. Using qPCR, we observed an increased abundance of Lactobacillus in dogs fed inulin compared with YCW. In contrast, we unexpectedly observed an increased abundance of Bifidobacterium in dogs fed YCW, but not inulin, when compared with those fed the control diet. Even though small dietary changes were observed with YCW and inulin, all dogs had low fecal Bifidobacterium and Lactobacillus populations. Such low populations may have been due to the feeding of raw meat diets, which were highly digestible and contained very little carbohydrate-related ingredients and substrates for saccharolytic bacteria in the large bowel and feces. Other recent canine experiments using 16S rRNA gene-based assays in our laboratory, however, have also reported fecal Actinobacteria and Bifidobacteria populations near 1% of sequences or less in dogs fed extruded kibble diets that are more commonly fed (Xenoulis et al., 2008; Middelbos et al., 2010; Swanson et al., 2011). The current study and these previous studies indicate that these bacterial species previously used to evaluate prebiotic effects may not be as ostensive as once thought.

Given the fact that high-throughput molecular techniques are now available, a search for other health-promoting bacteria or conversely potentially pathogenic bacteria that may be useful indicators of gut health is needed. Although fecal abundance of Lactobacillus and Bifidobacterium species was unchanged with addition of either inulin or YCW according to pyrosequencing data, it did identify other bacterial shifts occurring in the fecal microbiota. For instance, the abundance of fecal Veillonellaceae was increased with the addition of inulin. Veillonella rely on the fermentation of organic acids, such as lactic acid, which has been most studied in the human oral cavity (Delwiche et al., 1985; Bernalier-Donadille, 2010; Periasamy & Kolenbrander, 2010). Using large intestinal digesta, Lin et al. (2011) associated this family of bacteria with the production of SCFA. These data agree with the current study with dogs fed inulin or YCW having increased fecal total SCFA (Beloshapka et al., 2012). Megamonas is a predominant member of the family of Veillonellaceae. In the current study, dogs consuming diets containing inulin had the highest sequence percentage of Megamonas. Hidaka et al. (2008) also reported that fructooligosaccharides (FOS) are utilized by Megamonas hypermegas. Based on these results, a greater emphasis on Veillonella, and its potential impact on gastrointestinal health, may be justified in the future.

Bacteroides is one of the most prevalent bacterial genera in the gastrointestinal tract of many mammalian species. Xu et al. (2007) highlighted the wide diversity of dietary polysaccharide utilization among the many species within this bacterial genus in the human gut ecosystem. In the current study, the abundance of most Bacteroides decreased with the addition of inulin to the diets, which is likely due to the utilization differences observed among bacterial species belonging to this genus (Sonnenburg et al., 2010).

Fusobacteria is one of the most prominent bacterial phyla present in the canine gastrointestinal tract in many studies. Members of this phylum generally obtain energy through the fermentation of select carbohydrates and amino acids (Robrish et al., 1991; Ramezani et al., 1999; Duncan et al., 2002). While many consider F. varium to be a harmless bacterial species that is a common inhabitant of the gut microbial ecosystem, some research suggests that it is linked with ulcerative colitis (UC) in humans (Ohkusa et al., 2002). In the current study, all dogs remained healthy throughout the study. However, abundance of F. varium decreased when inulin was added to the diets, which may be indicative that a fermentable fiber can help modulate the gut microbial populations in individuals with UC.

Yeast cell wall extracts are moderately fermentable substrates that contain a mixture of carbohydrates and proteins, which are covalently linked to mannan (i.e. mannoproteins; Nguyen et al., 1998). The high content of mannans found in YCW is believed to prevent the adherence of bacteria expressing type-1 fimbriae to the intestinal wall, such as E. coli and Salmonella (Ofek et al., 1977; Neeser et al., 1986). Thus, adding YCW to canine raw meat-based diets may improve intestinal health and provide resistance against intestinal upset. Many bacterial shifts occurred at the genera and species level with the addition of YCW. Previous research has placed C. celerecrescens into Clostridium cluster XIVa, a group of gram-positive bacteria (Collins et al., 1994). In the current study, C. celerecrescens was decreased with the addition of YCW to the diets. Growth of C. celerecrescens abundance decreases considerably below pH 7.0 or above pH 8.0, with optimal growth near pH 7.0. The decrease in
C. celerecrescens observed in the current study correlates with the decreased fecal pH observed in these same dogs (Beloshapka et al., 2012). In the current study, the abundance of Clostridium saccharolyticum was decreased with the addition of YCW, which may indicate that YCW contains carbohydrate sources that are not utilizable by this species.

In the current study, dogs fed diets containing YCW had the highest percentage of sequences for Enterobacteriales, Enterobacteriaceae, and Escherichia. Because YCW is thought to act as a type-1 fimbrial analogue, it may be expected to have led to a decreased fecal abundance of Escherichia with consumption. Mucosal samples may be determined whether YCW consumption is an effective method for the prevention of pathogen adherence. The current study used dogs that remained healthy throughout the duration of the study. Xenoulis et al. (2008), who studied 10 dogs that had been diagnosed with inflammatory bowel disease (IBD) and nine healthy control dogs, observed that sequences from the Enterobacteriaceae family were more commonly identified in dogs with IBD than in control dogs (P = 0.01). The majority of these Enterobacteriaceae sequences were further classified as E. coli-type sequences. Given these data, there may benefit in adding YCW to the diet of dogs with IBD to limit the adhesion of pathogenic bacteria to the mucosal wall, but more research is necessary.

Conclusion
In conclusion, diet plays a major role in determining the microbial balance that exists within the gastrointestinal tract. This study characterized the microbial ecology of dogs consuming raw meat-based diets. All dogs remained healthy throughout the study, and the microbiome of the dogs was similar to that observed in dogs enrolled in other studies. Several changes in the canine fecal microbiota were observed with the addition of inulin or YCW to the raw meat-based diets. Although qPCR data demonstrated that YCW increased Bifidobacterium compared with inulin and control treatments and inulin increased Lactobacillus compared with YCW treatment, such changes were not observed in the pyrosequencing data. Therefore, more research is needed using a combination of next-generation sequencing technology with other molecular tools (e.g. qPCR and FISH) to not only characterize the canine gastrointestinal microbiota, but to also identify how diet, age, or genetics may affect the microbiota of an individual, to identify what differences in the microbiota exist throughout the gastrointestinal tract (i.e., proximal vs. distal colon, etc.), and to identify any microbial targets for gastrointestinal disease prevention or treatment.

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Statement
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References
Beloshapka AN (2011) Effects of inulin or yeast cell wall extract on nutrient digestibility and fecal fermentative end-product concentrations of healthy adult dogs fed raw diets. MS Thesis, University of Illinois, Urbana, IL.


Fecal microbial communities of dogs fed raw diets


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Ingredient and chemical composition of raw chicken-and beef-based diets with and without the inclusion of inulin or yeast cell wall (YCW).

Table S2. All bacterial genera (expressed as percentage of sequences) in feces of dogs fed raw chicken-and beef-based diets with or without the inclusion of inulin or yeast cell wall (YCW).