FEMS Microbiology Ecology, 91, 2015, fiv008

doi: 10.1093/femsec/fiv008
Advance Access Publication Date: 15 January 2015
Research Article

RESEARCH ARTICLE

Polyphenol-rich sorghum brans alter colon microbiota and impact species diversity and species richness after multiple bouts of dextran sodium sulfate-induced colitis

Lauren E. Ritchie[1], Joseph M. Sturino[1,2], Raymond J. Carroll[3], Lloyd W. Rooney[4], M. Andrea Azcarate-Peril[5] and Nancy D. Turner[1,2,*]

[1]Intercollegiate Faculty of Genetics, Texas A&M University, College Station, TX 77843-2253, USA, [2]Nutrition and Food Science Department, Texas A&M University, College Station, TX 77843-2253, USA, [3]Department of Statistics, Texas A&M University, College Station, TX 77843-3143, USA, [4]Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA and [5]Department of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7545, USA

*Corresponding author: Texas A&M University, Nutrition & Food Science Department, Room 214C, Cater Mattil, 2253 TAMU, College Station, TX 77843-2253, USA. Tel: 979-847-8714; E-mail: n-turner@tamu.edu
One sentence summary: The dysbiosis occurring during inflammatory bowel disease is improved by polyphenol-rich sorghum brans.
Editor: Julian R. Marchesi

ABSTRACT

The microbiota affects host health, and dysbiosis is involved in colitis. Sorghum bran influences butyrate concentrations during dextran sodium sulfate (DSS) colitis, suggesting microbiota changes. We aimed to characterize the microbiota during colitis, and ascertain if polyphenol-rich sorghum bran diets mitigate these effects. Rats ($n = 80$) were fed diets containing 6% fiber from cellulose, or Black (3-deoxyanthocyanins), Sumac (condensed tannins), or Hi Tannin black (both) sorghum bran. Inflammation was induced three times using 3% DSS for 48 h (40 rats, 2 week separation), and the microbiota characterized by pyrosequencing. The *Firmicutes/Bacteroidetes* ratio was higher in Cellulose DSS rats. Colonic injury negatively correlated with *Firmicutes*, *Actinobacteria*, *Lactobacillales* and *Lactobacillus*, and positively correlated with Unknown/Unclassified. Post DSS#2, richness was significantly lower in Sumac and Hi Tannin black. Post DSS#3 *Bacteroidales*, *Bacteroides*, *Clostridiales*, *Lactobacillales* and *Lactobacillus* were reduced, with no *Clostridium* identified. Diet significantly affected *Bacteroidales, Bacteroides, Clostridiales* and *Lactobacillus* post DSS#2 and #3. Post DSS#3 diet significantly affected all genus, including *Bacteroides* and *Lactobacillus*, and diversity and richness increased. Sumac and Hi Tannin black DSS had significantly higher richness compared to controls. Thus, these sorghum brans may protect against alterations observed during colitis including reduced microbial diversity and richness, and dysbiosis of *Firmicutes/Bacteroidetes*.

Keywords: short chain fatty acids; inflammatory bowel disease; Chao; Shannon–Weaver

Received: 5 November 2014; **Accepted:** 8 January 2015

INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), affects nearly 1.4 million people in the United States (Loftus 2004). Symptoms include abdominal cramping, constipation, abnormal bowel movements and patients are at an increased risk of colorectal cancer (Itzkowitz and Yio 2004). Although the etiology of IBDs is not fully known, dysbiosis of the native bacterial populations residing in the gastrointestinal (GI) tract have been identified as a contributing factor in the progression and severity of UC (Elson *et al.* 2005; Xavier and Podolsky 2007). Studies using gnotobiotic or knockout animals (e.g. TLR pathway) or experimental models that induce an inflammatory state in the bowel using dextran sodium sulfate (DSS) can help demonstrate the effect of the intestinal microbiota on health and disease states (Elson *et al.* 2005; Lee *et al.* 2010).

Previous studies have sought to elucidate alterations to the microbiota or identify which bacterial populations might be associated with the onset or recurrence of UC (Loftus 2004; Elson *et al.* 2005; Swidsinski *et al.* 2005; Xavier and Podolsky 2007). Some implicate an increase in pathogenic bacteria or a depletion of beneficial bacteria, such as lactic acid bacteria (Martin *et al.* 2004), yet it is becoming more apparent that no particular bacterial group can be implicated in the cause of UC. However, some studies report alterations in the ratio of the predominant bacterial phyla, *Firmicutes* and *Bacteroidetes*, in patients affected with both UC and CD compared to controls (Swidsinski *et al.* 2005; Sokol *et al.* 2006; Rajilić-Stojanović *et al.* 2011), and others note a reduction in bacterial diversity and species richness (Ott *et al.* 2004; Manichanh *et al.* 2006).

In recent years, diets containing bioactive compounds, such as polyphenols and tannins, have been identified as possible interventions for IBD due to their antimicrobial and antioxidant capacity (Larrosa *et al.* 2009). Bran isolated from some varieties of sorghum grain contain polyphenols, including 3-deoxyanthocyanins and condensed tannins, and have been characterized to have a higher antioxidant capacity compared to wheat bran, blueberries and pomegranates (Dykes and Rooney 2006; Burdette 2010). Diet impacts the colon luminal environment by affecting transit time and the production of microbial metabolites [short chain fatty acids (SCFA), e.g. butyrate] that alter luminal pH (Rastall *et al.* 2005; Walker *et al.* 2010). Bran from black and brown sorghum cultivars are known to alter rat fecal SCFA concentrations, which suggests possible changes in the intestinal microbiota (Turner *et al.* 2010). Furthermore, secondary plant metabolites similar to those found in sorghum bran are reported to differentially affect luminal bacterial populations (Okubo 1992; Chung, Lu and Chou 1998; Rastmanesh 2011).

In vitro studies have shown that tannins and other bioactive compounds can dramatically affect the survival of bacterial groups that typically reside in the GI tract (Ahn *et al.* 1998; Cueva *et al.* 2012). Moreover, numerous *in vivo* human and animal studies have also characterized how these compounds can promote the growth of certain health-promoting bacteria (i.e. *Bifidobacterium* and *Lactobacillus* spp.), while suppressing or eliminating other pathogenic bacteria such as *Clostridium perfringens* and *C. difficile* in the intestine (Okubo 1992; Smith and Mackie 2004). Hydrocaffeic acid, a polyphenol metabolite derived from colonic microbiota, produced anti-inflammatory effects including suppression of proinflammatory cytokine production (i.e. TNFα, IL-8, IL-1β) and reduction of oxidative DNA damage in rat colonic mucosa (Larrosa *et al.* 2009).

Based on the existing literature, we hypothesized that perturbations in the rat colonic environment occurring with DSS-induced colitis, including changes in microbiota, will be partially mitigated by feeding sorghum bran-containing diets. Therefore, the aim of this study was to determine the effect of diets containing sorghum bran with 3-deoxyanthocyanins, condensed tannins or both polyphenols on the intestinal microbiota. Furthermore, we aim to ascertain if sorghum bran diets can mitigate alterations to the microbiota during repeated inflammatory bouts produced by DSS challenge.

MATERIALS AND METHODS

Animals and diets

Eighty (40-day-old) male Sprague-Dawley rats (Harlan Sprague-Dawley, Houston, TX, USA) were stratified by body weight and assigned to one of four experimental diets ($n = 20$ per diet). Experimental diets were formulated from purified ingredients and contained 6% dietary fiber from cellulose (control diet) or 6% dietary fiber from bran isolated from sorghum grains that contain 3-deoxyanthocyanins and no condensed tannins (Black bran), high levels of both 3-deoxyanthocyanins and condensed tannins (Sumac bran), or intermediate levels of 3-deoxyanthocyanins and high levels of condensed tannins (Hi Tannin). A complete description of the diets including antioxidant capacity, total polyphenol content and tannin content is reported elsewhere (submitted work from Lauren E. Ritchie, Stella S. Taddeo, Brad R. Weeks, Raymond J. Carroll, Linda Dykes, Lloyd W. Rooney, Nancy D. Turner). The proportions of soluble (6.0, 9.5, 11.7 and 6.4%) and insoluble fiber (94.0, 90.5, 88.3 and 93.6%) were similar among the diets (Cellulose, Black bran, Sumac bran and Hi Tannin bran, respectively).

After 21 days of experimental diets, half of the rats were exposed to three sequential DSS (MP Biomedicals, Irvine, CA, USA) treatments in their drinking water [3% (w/v) DSS for 48 h], with 14 days between each DSS exposure. Between DSS exposures, water was supplied to treated animals, and the remaining half of the animals (non-DSS controls) received water throughout the course of the study.

Body weight and food intake were routinely monitored. On day 82, animals were euthanized by CO_2 asphyxiation. A 1 cm segment was removed from the distal end of the colon and fixed in 70% ethanol solution prior to embedding in paraffin. The degree of inflammation and morphological injury caused by DSS exposure was assessed by hematoxylin and eosin staining as described previously (Jia *et al.* 2008).

Fecal sample collection and microbial DNA isolation

Fresh fecal samples were collected immediately upon defecation, placed in sterile cryotubes and then stored at −80°C. Samples collected after recovery from the second DSS treatment (day 47, $n = 9$ or 10 for Cellulose and Black, and/or Sumac and Hi Tannin DSS rats, respectively) and third DSS treatment (day 62, $n = 5$/diet for control rats and $n = 10$/diet for DSS rats) were used for microbial analyses. DNA was isolated using a FastDNA SPIN kit (MP Biomedicals, Solon, OH, USA) as described previously (Menon *et al.* 2013), and the purified DNA was stored at −80°C.

16S rRNA bacterial tag-encoded FLX amplicon pyrosequencing

16S rRNA amplicon sequencing was performed in the Microbiome Core Facility (University of North Carolina at Chapel Hill, NC, USA) as previously described (Devine *et al.* 2013). Briefly, initial amplification of the V1–V2 region of the bacterial 16S rRNA gene was performed on total DNA isolated from fecal samples. Master mixes for these reactions used the Qiagen Hotstar Hi-Fidelity Polymerase Kit (Qiagen, Valencia, CA, USA) with a forward primer composed of the Roche Titanium Fusion Primer A (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′), a 10-bp multiplex identifier (MID) sequence (Roche, Indianapolis, IN, USA) unique to each of the samples, and the universal primer for bacteria, 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Edwards *et al.* 1989). The reverse primer was composed of the Roche Titanium Primer B (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3′), the identical 10-bp MID sequence as the forward primer and the reverse bacteria primer 338R (5′-GCTGCCTCCCGTAGGAGT-3′) (Fierer *et al.* 2008), which span the V1–V2 hypervariable region of the bacterial 16S rRNA gene. Each sample was individually gel purified using the E-Gel Electrophoresis System (Life Technologies, Invitrogen). To ensure equal representation of each sample in the sequencing run, each barcoded sample was standardized by calculating equimolar amounts prior to pooling. Pooled samples of the 16S rDNA multiplexed amplicons were sequenced on a Roche 454 Genome Sequencer FLX Titanium instrument using the GS FLX Titanium XLR70 sequencing reagents and protocols.

Amplicon sequencing data analysis

Analysis of sequencing data was carried out using the QIIME pipeline (Caporaso *et al.* 2010). The combined raw sequencing data plus metadata describing the samples were de-multiplexed and filtered. Next, data were denoised using Denoiser software using standard parameters (Reeder and Knight 2010). Sequences were grouped into operational taxonomic units (OTUs) at a 97% level to approximate species-level phylotypes using Uclust (Edgar 2010). OTU sequences were aligned and OTU tables containing the counts of each OTU in each sample were used to calculate mean species diversity of each sample (alpha diversity) and the differentiation among samples (beta diversity). Alpha and beta diversity measures were used to calculate the Chao species richness estimate and Shannon–Weaver diversity index for each OTU. To evaluate the similarities between bacterial communities, a combination of Unifrac significance, principal coordinate analysis (PCoA) using Fast Unifrac (Lozupone, Hamady and Knight 2006) and network analysis (Ley *et al.* 2008) were performed to compare samples based on sample time and treatment.

Statistical analysis

Data were analyzed using two-way analysis of variance including variables of diet and DSS exposure in SAS 9.1 (SAS Institute, Inc.) considering a *P*-value of <0.05 as significant. On completion of all analyses, the uncorrected *P*-values were submitted to a single multiple-testing correction using the Benjamini and Hochberg false discovery rate method. Unadjusted *P*-values are presented in the tables and text with an asterisk indicating where results remained significant after adjusting for multiple comparisons. Relationships between microbial populations and colonic injury score were assessed by calculating Pearson's product moment correlation coefficient.

RESULTS

Body weight and experimental diet intake

Only minor differences in food intake or body weight were observed. Control rats fed Hi Tannin had higher body weights than control rats fed Cellulose at four time points (day 39, day 42, day 56 and day 60). The only difference in food intake occurred prior to the first DSS treatment (DSS#1) at day 39, where rats fed Cellulose DSS consumed less than rats fed Sumac DSS ($P < 0.05$).

Multivariate analysis of bacterial populations

Day 62 control rats (not exposed to DSS) feces enabled determination of the potential changes in microbiota that occurred from exposure to DSS within a diet group. The DSS-treated rat samples from day 47 and day 62 allowed the determination of how repeated bouts of inflammatory challenge impacted the microbiota, and to determine if there were differences in the response due to diet.

PCoA of weighted and unweighted UniFrac results (Hamady, Lozupone and Knight 2010) revealed distinct clustering of samples based on the experimental diet for both time points (Fig. 1A). Variation of data points along PC2 (10.3%) indicated clear clusters and therefore distinct differences in bacterial communities between rats fed the Cellulose, Black, Sumac and Hi Tannin black bran diets. The Hi Tannin black bran diet, which contains both condensed tannins and 3-deoxyanthocyanins, resulted in bacterial communities that clustered near both Black bran (contains 3-deoxyanthocyanins) and Sumac bran (contains condensed tannins) diets. Additionally, rats fed bran diets that contained condensed tannins (i.e. Sumac and Hi Tannin black bran) had bacterial communities that clustered together. Spatial relation of data points along PC3 (6.28%) revealed that rats consuming the Sumac bran diet had samples with the least amount of variation compared to other experimental diets. Furthermore, PCoA analysis revealed differences between samples collected after DSS#2 (day 47) and DSS#3 (day 62) (Fig. 1B), suggesting that the bacterial communities were sensitive to diet and the number of DSS exposures.

Microbial taxonomic structure analysis

Phylogenetic classification of OTUs revealed two predominant phyla, *Bacteroidetes* and *Firmicutes*, for all experimental diets post DSS#2 and post DSS#3 (Fig. 2). The phyla *Actinobacteria* and *Proteobacteria* were also represented, but proportions did not exceed 5% for any experimental group at either time point. We observed a significant diet effect for all phyla at both time points (Table S1, Supporting Information). For each experimental diet at both time points, there were OTUs that did not match any known sequences in the RDP database, and the proportion of OTUs classified as 'Unknown' or 'Unclassified' was elevated in all experimental diets post DSS#3 compared to DSS#2 ($\geq$2-fold increase).

We observed a significant effect of DSS on both the *Firmicutes* and *Bacteroidetes* phyla ($P = 0.0006$ and $P < 0.0001$, respectively) in samples collected post DSS#3. Thus, ratios of these phyla (% *Firmicutes* in a given experimental group /% *Bacteroidetes* in the same group) were calculated to characterize relative proportion of these predominant bacterial groups. The ratio of *Firmicutes* to *Bacteroidetes* was higher in Cellulose DSS rats post DSS#2 and post DSS#3 compared to Black, Sumac and Hi Tannin black bran DSS rats (Fig. 2).

Further phylogenetic analysis of OTUs revealed three predominant bacterial orders (*Bacteroidales*, *Clostridiales* and

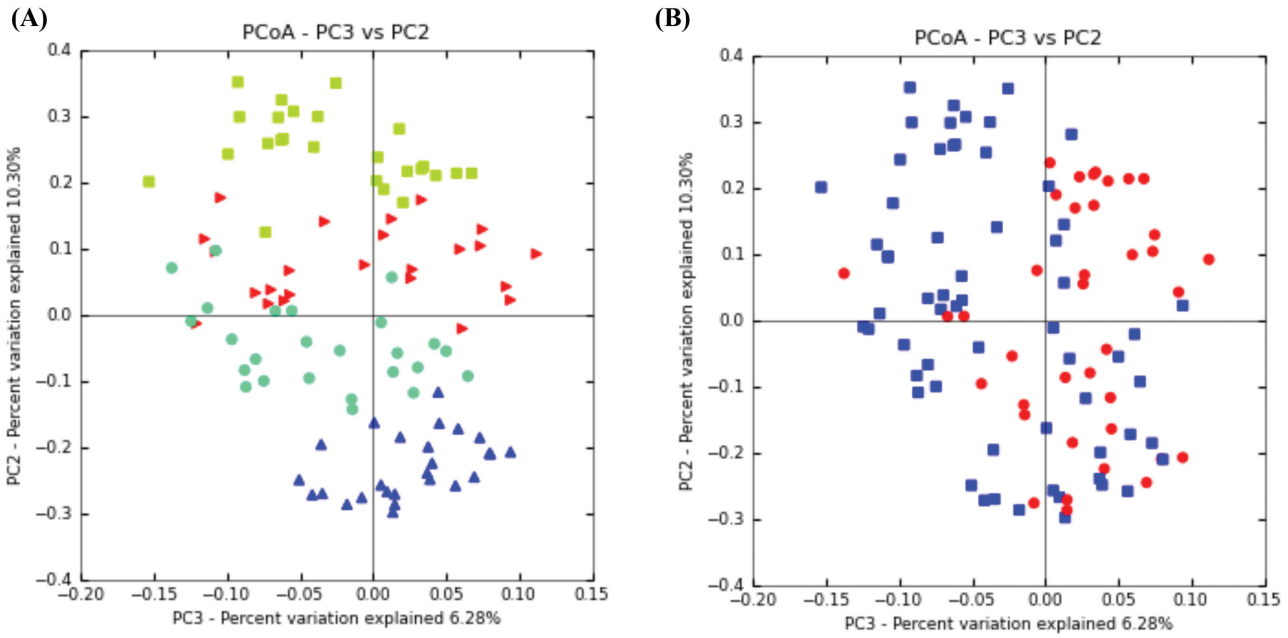

Figure 1. (**A**) PCoA plot of samples from both time points. Green squares (Cellulose), red triangles (Black bran), light blue circles (Hi Tannin black bran) and blue triangles (Sumac bran) illustrate differences in rat fecal bacterial populations due to experimental diets. (**B**) PCoA plot of amples post DSS#2 and DSS#3. Blue squares (post DSS#3) and red circles (post DSS#2) illustrate differences in rat fecal bacterial populations following DSS treatment over time.

Lactobacillales) and two genus (*Bacteroides* and *Lactobacillus*) in all collected samples (Figs 3 and 4, respectively). We observed significant diet effects post DSS#2 for bacteria in the *Bacteroidales* ($P < 0.0001$), *Clostridiales* ($P = 0.0205$) and *Lactobacillales* orders ($P = 0.0417$) (Table S2, Supporting Information), and for all characterized bacterial genus except *Coprobacillus* and *Parasutterella* (Table S3, Supporting Information). At the genus level, the confidence level for numerous OTUs was below 90% similarity to known sequences in the Ribosomal Database Project (RDP) database, and have been marked accordingly (Table S3, Supporting Information). Sumac DSS rats had significantly higher proportion of OTUs classified as *Bacteroidales*, and Hi Tannin black DSS rats had significantly higher proportions of *Lactobacillales* compared to the Sumac DSS rats, with the other two diets being intermediate ($P < 0.05$). These trends remained significant at the genus level as well (i.e. *Bacteroides* and *Lactobacillus*).

Post DSS#3, proportions of OTUs classified in the predominant bacterial orders (i.e. *Bacteroidales*, *Clostridiales* and *Lactobacillales*) were reduced in all DSS-treated rats compared to post DSS#2 for all experimental diets (Fig. 3), with the largest reduction observed in the *Clostridiales* order (56–79% reduction). A similar trend was observed at the genus level for both *Bacteroides* and *Lactobacillus*, with proportions remaining the same or reduced following DSS#3 for all diets. Additionally, we observed significant diet, treatment and interactive effects for both *Bacteroidales* and *Bacteroides* ($P < 0.0001$, $P < 0.0001$, and $P < 0.0005$, respectively), *Clostridiales* (Diet $P = 0.0005$, and DSS $P < 0.0001$, respectively) and *Turicibacter* (Diet $P = 0.0001$) at this time point (Tables S2 and S3, Supporting Information), which all remained significant after adjusting for multiple comparisons. In Sumac control rats, we observed a significantly higher proportion of OTUs classified as *Bacteroidales* and *Bacteroides*, and a significantly higher proportion of *Clostridiales* in Hi Tannin control rats compared to all other diets ($P < 0.05$). We observed a significant effect of diet and treatment for OTUs classified in the *Lactobacillales* order ($P < 0.001$ and 0.0377, respectively), and a significant diet effect for all classified genus at this time point (i.e. *Bacteroides*, *Lactobacillus*, *Turicibacter* and *Parasutterella*), which remained significant after adjusting for multiple comparisons. Cellulose controls had a significantly higher proportion of OTUs classified as *Lactobacillales* ($P < 0.05$) compared to all other groups, with a similar trend at the genus level (*Lactobacillus*) as well. Additionally, DSS-treated rats fed Cellulose, Sumac and Hi Tannin black bran diets had lower proportions of both *Lactobacillales* and *Lactobacillus* compared to their diet-matched controls, a difference that was only significant for the Cellulose rats.

When samples from rats fed Sumac and Hi Tannin diets and treated with DSS were compared to their diet-matched controls, we observed a significant reduction in numerous bacterial orders following recovery from DSS#3 (Fig. 3). Sumac DSS rats had significantly lower proportions of *Bacteroidales*, *Bacteroides*, *Clostridiales*, *Parasutterella* and *Burkholderiales*, and Hi Tannin DSS showed lower proportions of *Bacteroidales*, *Bacteroides*, *Clostridiales*, *Turicibacter* and *Erysipelotrichales* compared to their diet-matched controls ($P < 0.05$) (Tables S2 and S3, Supporting Information). In contrast, Black bran DSS rats had higher abundance of *Lactobacillales*, *Lactobacillus* and *Burkholderiales*, and Cellulose DSS rats had higher abundance of *Bacteroidales*, *Clostridiales* and *Erysipelotrichales* compared to their diet-matched controls.

Diversity and species richness comparisons

To analyze the effects of diet and DSS-induced inflammation on bacterial species richness and diversity, sequences from each sample (approximately 1508) were used to perform alpha and beta diversity analyses and to calculate the Chao species richness estimate and the Shannon–Weaver diversity index. We observed a significant diet effect on both species richness and diversity post DSS#3 ($P < 0.0001$), with rats fed Black bran showing a significantly higher species richness ($P < 0.05$) compared to

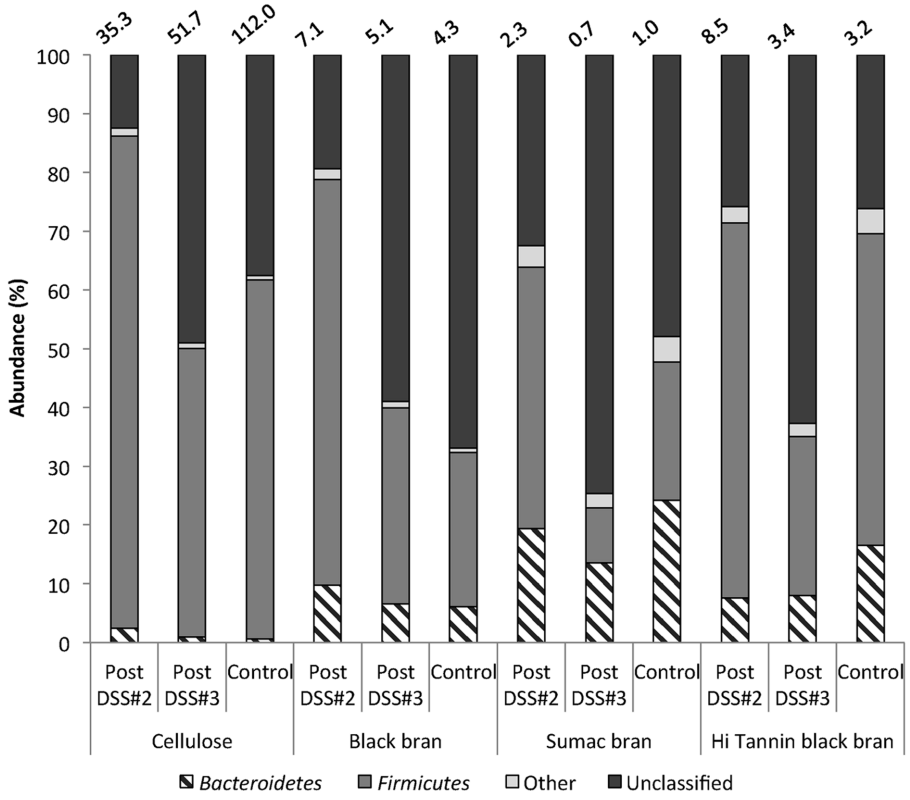

Figure 2. Phylogenetic classification of OTUs at the phylum level in fecal samples from control rats (day 62) and DSS-treated rats (on day 47—post DSS#2, and on day 62—DSS#3) for all diets. The *Firmicutes/Bacteroidetes* ratio is reported above each column.

animals fed Sumac and Hi Tannin black bran diets, and a significantly higher diversity index ($P < 0.05$) compared to all other controls (Table 1). Sumac control rats had lower species richness and diversity indices compared to the Cellulose and Black bran fed rats. Following DSS#2, we observed no significant differences in bacterial diversity between Cellulose, Black, Sumac or Hi Tannin black bran DSS rats. Sumac bran DSS rats had a significantly lower species richness compared to Cellulose, Black and Hi Tannin black bran DSS rats ($P < 0.05$) at this time point. DSS-treated rats following DSS#2 had lower species richness and diversity indices compared to their diet-matched (non-DSS) controls, yet post DSS#3 there was a numerical increase in diversity (13.6–25%) and species richness (39–62%) compared to post DSS#2 for Cellulose and Sumac and Black, Sumac, and Hi Tannin black bran DSS rats (Table 1).

Post DSS#3, experimental diet and DSS treatment had an interactive effect on species richness and diversity ($P < 0.0001$ and $P = 0.0003$, respectively), in which Cellulose and Black bran DSS rats had a significantly lower Chao score compared to their diet-matched controls ($P < 0.05$). Sumac bran DSS rats had significantly higher richness ($P = 0.0199$) and numerically higher diversity index compared to Sumac controls, and Hi Tannin black bran DSS rats had significantly higher richness and diversity indices ($P < 0.001$ and $P = 0.013$, respectively) compared to Hi Tannin black bran controls at this time point (Table 1).

DSS-induced injury of distal colon

All DSS-treated rats had elevated colonic injury compared to their diet-matched controls, with significantly higher injury scores observed in Hi Tannin black bran DSS rats compared to Hi Tannin black control rats ($P = 0.0072$) (submitted work from Lauren E. Ritchie, Stella S. Taddeo, Brad R. Weeks, Raymond J. Carroll, Linda Dykes, Lloyd W. Rooney, Nancy D. Turner). DSS-treated rats fed bran diets had significantly higher injury scores compared to Cellulose DSS rats ($P < 0.05$). Cellulose controls had the lowest injury scores, which were significantly lower than Black and Sumac bran controls ($P < 0.05$). (submitted work from Lauren E. Ritchie, Stella S. Taddeo, Brad R. Weeks, Raymond J. Carroll, Linda Dykes, Lloyd W. Rooney, Nancy D. Turner).

In order to determine if there were relationships between the bacterial taxa characterized in fecal samples collected post DSS#3 and colon injury, we ran correlational analyses. In this UC model, significant correlations were observed for four phylogenetic groups (Table 2). At the phylum level, both *Firmicutes* and *Actinobacteria* were negatively correlated with colonic injury ($P = 0.003$ and $P = 0.019$). Within the *Firmicutes* phylum, *Clostridiales*, *Erysipelotrichales*, *Lactobacillales* and *Lactobacillus* negatively correlated with colonic injury, and the relationship reached significance for both *Lactobacillales* and *Lactobacillus* ($P = 0.045$ and $P = 0.05$). The abundance of OTUs classified as 'Unknown' or 'Unclassified' (OTUs that did not match any known sequences in the RDP database) were both positively correlated with colonic injury (both $P = 0.003$, Table 2).

DISCUSSION

The intestinal microbiota of mammals is composed of trillions of bacteria and over 400 individual species have been identified thus far (Rajilić-Stojanović, Smidt and De Vos 2007). These

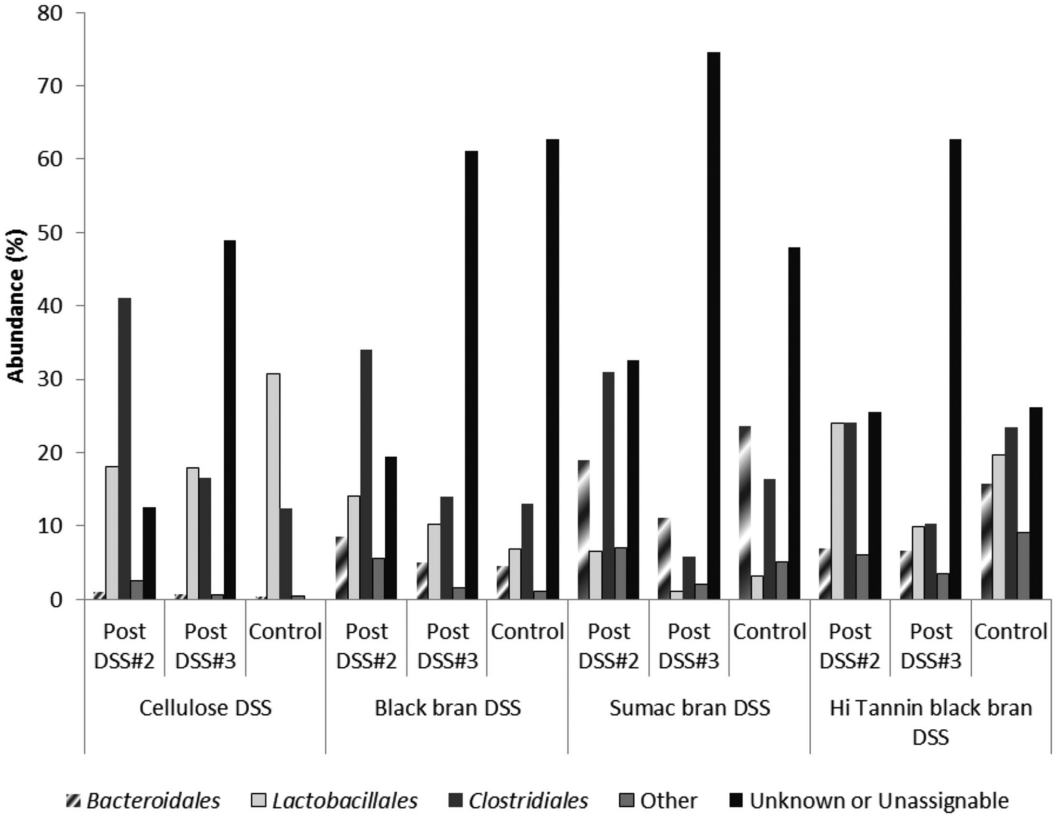

Figure 3. Phylogenetic classification of OTUs at the order level in fecal samples from control rats (day 62) and DSS-treated rats (on day 47—post DSS#2, and on day 62—DSS#3) for all diets.

bacterial populations provide numerous benefits to the host, including immune system development, epithelial barrier maintenance, and providing metabolic substrate for colonocytes (Louis and Flint 2009; Wells *et al.* 2010). It is now understood that perturbations to the microbiota are involved in the initiation of intestinal inflammation and recurrence of inflammatory bouts, and is an important factor in the etiology of IBD such as UC (Manichanh *et al.* 2006; Frank *et al.* 2007; Sepehri *et al.* 2007). Another environmental input that has been shown to affect the microbiota is diet composition, and recent focus has been put on bioactive compounds and their possible role in mitigating the deleterious effects of intestinal inflammation (Gibson *et al.* 2004; Smith and Mackie 2004; Louis *et al.* 2007; Larrosa *et al.* 2009; Biasi *et al.* 2011; Possemiers *et al.* 2011). Brans utilized in this study contain concentrated levels of 3-deoxyanthocyanins (Black bran), condensed tannins (Sumac bran) or a combination of these compounds (Hi Tannin black bran) (Dykes and Rooney 2006; Dykes, Rooney and Rooney 2013). Our previous work demonstrated that these diets can alter fecal SCFA concentrations, a microbial metabolite, suggesting that these dietary ingredients alter the composition of the microbiota and/or microbial metabolism.

In the present study, PCoA analysis of microbial taxa from fecal samples suggests that there are distinct bacterial communities in animals fed experimental diets containing Cellulose or bran isolated from Black, Sumac or Hi Tannin sorghums. Additionally, samples collected at different time points (post DSS#2 and DSS#3) also grouped in different quadrants of the PCoA plot, suggesting, as expected, that DSS treatment and colonic inflammation also modify the composition of the gut microbial populations. Little research has been done to understand how the bioactive compounds found in sorghum (i.e. 3-deoxyanthocyanins and condensed tannins) can alter the luminal environment, particularly the intestinal bacterial populations. Therefore, the aim of this work was to characterize alterations to the microbiota during DSS-induced colitis and determine if polyphenol-rich sorghum diets have the ability to mitigate the dysbiosis (e.g. decreased bacterial diversity and richness; elevated *Firmicutes/Bacteroidetes* ratio) associated with UC.

Although research has been done to understand the role of the microbiota in UC, it is becoming more apparent that no specific microbiome component can be identified as an etiologic agent (Nagalingam and Lynch 2012). However, an overall dysbiosis in the ratio of predominant bacterial populations (i.e. increased *Firmicutes* to *Bacteroidetes* ratio) have been observed in both the feces and colon biopsies of IBD patients and experimental models of obesity and UC (Sokol *et al.* 2006; Nagalingam, Kao and Young 2011; Rajilić-Stojanović *et al.* 2011). In our study, the *Firmicutes/Bacteroidetes* ratio observed in Cellulose fed animals (both control and DSS) was at least 10-fold higher than that of bran fed animals. These results could be in part due to the presence of cellulose fermenting bacterial species within this phylum (e.g. *Ruminococcus* spp.) (Chassard, Gaillard-Martinie and Bernalier-Donadille 2005). Previous studies testing the effect of condensed tannins *in vivo* also reported inhibition of Gram-positive bacteria (i.e. *Firmicutes* phylum), specifically inhibition of the *C. leptum* group, while other enteric bacteria (i.e. *Bacteroides fragilis* and *Bacteroides—Prevotella–Porphyromonas* groups) increased significantly (Smith and Mackie 2004). Our observations are similar to these reports, as animals fed a bran diet

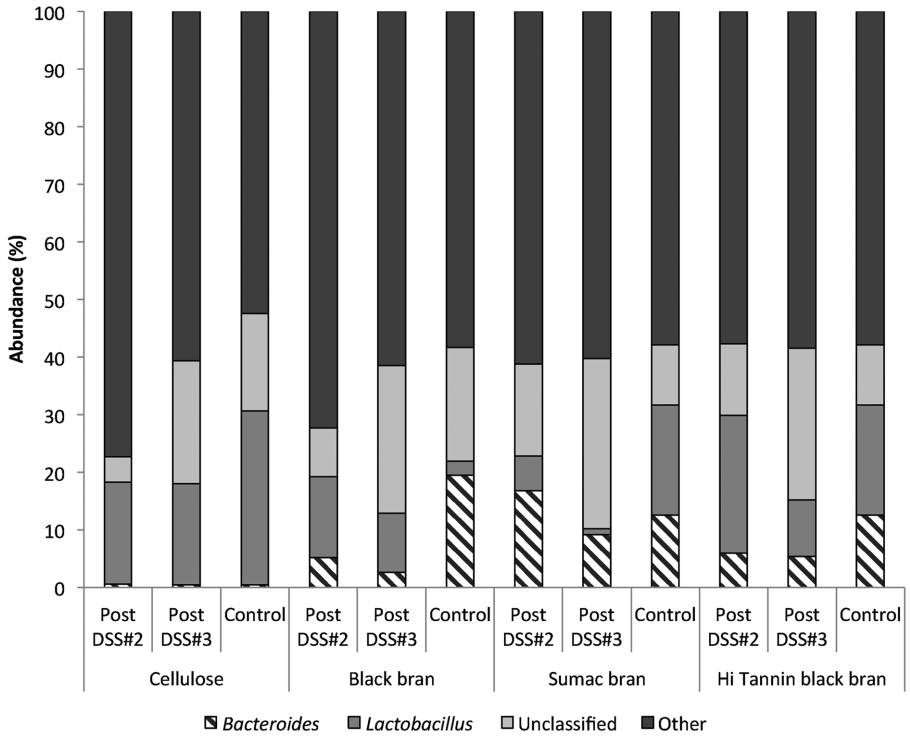

Figure 4. Phylogenetic classification of OTUs at the genus level in fecal samples from control rats (day 62) and DSS-treated rats (on day 47—post DSS#2, and on day 62—DSS#3) for all diets.

containing tannins (i.e. Sumac and Hi Tannin black) had higher proportions of both *Bacteroidales* and *Bacteroides* compared to Cellulose and Black bran fed animals. Other dietary compounds such as tea polyphenols and other flavonoids have been reported to alter the composition of the intestinal microbiota in rats and human subjects, including decreased proportion of *Clostridium* spp. compared to those that did not consume these polyphenols (Okubo 1992; Hanske 2005).

A recent review documented the numerous changes observed in experimental models and patients with IBD, and no agreement has been reached on the association of specific microbial groups and UC (Nagalingam and Lynch 2012). Studies have documented elevated levels and enhanced epithelial adherence of pathogenic bacteria species (e.g. pathogenic *Escherichia coli* (*E.coli*)) following DSS-induced colitis (Heimesaat *et al.* 2007). In our study, we observed less than 5% of OTUs classified as *Proteobacteria* in control and DSS-treated animals at both time points. Plant polyphenols similar to those utilized in this study (i.e. gallic acid, methyl gallate, propyl gallate) have been observed to mitigate the effects of IBD and also been found to have inhibitory properties against potentially harmful bacterial species such as *C. perfringens, C. paraputrifficum, Eubacterium limosum, Bacteroides fragilis, Staphylococcus aureus* and *E. coli* in culture (Ahn *et al.* 1998; Chung, Lu and Chou 1998). This inhibitory effect could be potentially beneficial and one reason for the reduced abundance of *Proteobacteria* in our study (≤4.29%), which harbors numerous pathogenic bacteria species such as *Escherichia*, *Salmonella*, *Vibrio* and *Helicobacter.*

Previous studies in experimental models and patients with UC have also documented a depletion in bacterial species that provide benefit to the host such as species of *Bifidobacterium* and *Lactobacillus* (Sokol *et al.* 2006; Frank *et al.* 2007; Heimesaat *et al.* 2007). These species produce antimicrobial substances, compete with pathogens for epithelial and mucin-binding sites (Ljungh and Wadstrom 2006), and have been shown to attenuate symptoms and maintain remission of UC (Garcia Vilela *et al.* 2008). During an active disease state (following DSS#2), we observed a significantly higher proportion of *Lactobacillales* and elevated proportion of *Lactobacillus* in Hi Tannin black DSS rats compared to Cellulose, Black and Sumac bran fed DSS rats. However, following DSS#3, we observed reduced abundance of *Lactobacillales* and *Lactobacillus* in DSS-treated rats fed Black, Sumac and Hi Tannin black bran diets, which is similar to other reports in patients and experimental models of UC that document a suppression of lactic acid bacteria (Heimesaat *et al.* 2007). Previous studies have reported that hydrolysable tannins have minimal effect on growth of lactic acid bacteria, specifically *Bifidobacterium infantis* and *Lactobacillus acidophilus* (Ahn *et al.* 1998; Chung, Lu and Chou 1998), which parallels animals fed Hi Tannin black and treated with DSS having a higher abundance of both *Lactobacillales* and *Lactobacillus* post DSS#2. In this study, we observed virtually undetectable levels of OTUs classified in the *Actinobacteria* phylum (≤0.80%), and *Bifidobacterium* in particular, which may suggest that this group is not a major constituent of the microbiota in any of our experimental groups. However, the low abundance of *Bifidobacterium* observed in our study could be also due to the facts that no *Bifidobacterium*-specific primers were used, which have been shown essential to quantify abundance changes in this group (Ritchie *et al.* 2010; Davis *et al.* 2011).

Previous studies and our results indicate that the effects of polyphenols on microbiota vary substantially and may be dependent upon the polyphenolic structure of these compounds. Very little is known regarding microbial metabolism of the 3-deoxyanthocyanins and condensed tannins found in sorghum bran. In general, polyphenols are poorly absorbed and therefore available to be catabolized by the colonic microbiota. The

Table 1. Chao and Shannon–Weaver indices for rat fecal microbial populations in samples from all treatment groups post DSS#2 and DSS#3.[1]

		Cellulose		Black bran		Sumac bran		Hi Tannin black bran			
		DSS ($n = 9$)		DSS ($n = 9$)		DSS ($n = 10$)		DSS ($n = 10$)		Diet	
Post DSS#2 (day 47)	Chao	117.33 ± 4.78^{b}		124.74 ± 6.23^{b}		92.76 ± 2.12^{a}		112.98 ± 5.91^{b}		0.0005	
	Shannon–Weaver	4.00 ± 0.18^{a}		4.35 ± 0.16^{a}		4.17 ± 0.08^{a}		4.17 ± 0.12^{a}			
		Control ($n = 5$)	DSS ($n = 10$)	Control ($n = 5$)	DSS ($n = 9$)	Control ($n = 5$)	DSS ($n = 10$)	Control ($n = 5$)	DSS ($n = 9$)	Diet	DSS	Diet* DSS
Post DSS#3 (day 62)	Chao	207.32 ± 14.08^{d}	169.29 ± 5.45^{c}	222.91 ± 12.47^{d}	173.79 ± 14.27^{c}	118.27 ± 6.85^{a}	155.08 ± 6.87^{b,c}	133.45 ± 13.05^{a,b}	210.40 ± 9.17^{d}	<0.0001		<0.0001
	Shannon–Weaver	4.79 ± 0.33^{c,d}	4.86 ± 0.15^{c}	5.82 ± 0.04^{a}	5.13 ± 0.15^{b,c}	4.39 ± 0.14^{d}	4.78 ± 0.08^{c,d}	4.73 ± 0.16^{c,d}	5.50 ± 0.10^{a,b}	<0.0001		0.0003

Data are LS mean ± SEM.
[1]Means in a row without a common superscript (a, b, c, d) differ ($P < 0.05$).

Table 2. Correlation of intestinal microbiota and colonic injury score in rats after three bouts of DSS-induced colonic injury.[a]

Taxon			Pearson's correlation coefficient
Actinobacteria			−0.308[a]
Bacteroidetes			0.076
	Bacteroidales		0.049
		Bacteroides	0.033
Firmicutes			−0.387[a]
	Clostridiales		−0.123
	Erysipelotrichales		−0.096
		Turicibacter	−0.088
	Lactobacillales		−0.264[a]
		Lactobacillus	−0.258[a]
Proteobacteria			−0.012
	Burkholderiales		0.080
		Parasutturella	0.076
Unknown			0.382[a]
Unclassified			0.387[a]

[a]Indicates phylogenetic groups which proportion correlated significantly with injury score ($P < 0.05$).

microbial metabolism of polyphenols is extremely complex, and numerous microbial catalytic and hydrolytic enzymes have been identified (van Duynhoven *et al.* 2011); therefore, a complete description is beyond the scope of this paper. However, unlike other flavonoids, many anthocyanins do not undergo extensive metabolism and their structure is stabilized in acidic conditions (Crozier, Del Rio and Clifford 2010). Furthermore, condensed tannins such as those described in this study are large non-hydrolysable proanthocyanindins, with three or more polymerizations, that are not easily fractionated by water and tannases (Ree 2001). One study demonstrated that condensed tannins isolated from sorghum bran were only isolated in excrement and not absorbed in chickens (Jimenez-Ramsey *et al.* 1994). Furthermore, both animal and human studies have shown that anthocyanins from berries (i.e. bilberry and raspberry) are poorly absorbed in the small intestine and typically <0.1% of the quantities ingested are detected in urine within 24 h of consumption (Borges *et al.* 2007; Sakakibara *et al.* 2009). This implies that compounds similar to those found in this study may not be absorbed or metabolized and therefore concentrated in the intestinal lumen where they are able to impact the microbiota and/or be metabolized by them. Metagenome and metabolomics analyses may help elucidate the relationship of the microbiota and the bioactive compounds found in sorghum; however, due to the large number of genes and metabolites identified in these analyses it could be quite difficult to elucidate the biomolecular mechanisms involved in the observed beneficial effects.

Alterations in other bacterial populations that are prevalent constituents of the commensal intestinal flora have been reported in experimental models and patients with UC, and both an elevation and suppression have been observed in species of the genera *Bacteroides* and *Clostridium* in feces and colonic tissue (Noor *et al.* 2010; Andoh *et al.* 2011). To understand how the microbiota was fluctuating during different stages of inflammation, we compared DSS-treated animals post DSS#2 to post DSS#3 and observed decreased proportions of *Bacteroidales*, *Clostridiales* and *Lactobacillales* in DSS animals for all experimental diets following DSS#3, with the highest reduction observed in the *Clostridiales* order (56–79%). At the genus level, we see

similar reductions in *Bacteroides* and *Lactobacillus*, with minimal OTUs classified within the *Clostridium* genus (<3% post DSS #2 and none post DSS#3). The *Clostridiales* and *Clostridium* taxa are of particular importance, as they harbor bacterial species that have the ability to produce important bacterial metabolites such as butyrate (Wiegel, Kuk and Kohring 1989). Although this order also harbors opportunistic pathogens, alterations to this particular population could be detrimental to colonic health as butyrate is not only the predominant fuel for colonocytes, but suppressed availability and uptake of this metabolite have been implicated in the etiology of IBD (Thibault *et al.* 2007; Vicky De *et al.* 2010). Observed differences could be due, in part, to the progression of inflammation or severity of epithelial barrier injury, as it has been previously reported that inflamed tissue harbors different bacterial populations than non-inflamed tissues from the same individual (Bibiloni *et al.* 2006; Frank *et al.* 2007; Sepehri *et al.* 2007). Additionally, previous studies have shown correlations between bacterial populations and disease severity, which can further elucidate the relationship between the microbiota and UC. In the present study, we report a negative correlation between colonic injury and numerous phylogenetic groups, including significant correlations to *Firmicutes*, *Actinobacteria*, and both *Lactobacillales and Lactobacillus*. Similarly, a previous study reported that the abundance of certain taxa in the *Firmicutes* phylum (i.e. *Clostridium* clusters IV and IX) was negatively correlated with disease symptom score (e.g. abdominal pain, distention) in patients with irritable bowel syndrome (Rajilić-Stojanović *et al.* 2011). In contrast to our results, another study reported a positive correlation between the abundance of cecal *E. coli* and histologic colon score in HLA-B27 transgenic rats (Onderdonk *et al.* 1998). Our results could differ from previous studies due to the experimental model utilized, tissue analyzed (i.e. feces versus cecal content and/or colonic tissue) and phylogenetic characterization techniques (e.g. FISH, T-RFLP, DGGE, microbiological culture). Further analysis, such as identifying closest neighbors to unclassified and unidentified OTUs, will further elucidate the significance of these bacterial groups and their relation to colonic injury in our experimental groups.

Although we observed a decrease in the predominant bacterial orders post DSS#3, there was no reduction in bacterial diversity and species richness at this time point, which has been previously reported in both patients with UC and DSS-induced colitis animal models (Ott *et al.* 2004; Heimesaat *et al.* 2007; Andoh *et al.* 2011; Nagalingam, Kao and Young 2011). Following the second DSS treatment, we observed a decrease in both species richness and diversity compared to diet-matched controls; however, the values of these indices were elevated following recovery from DSS#3. Furthermore, following all DSS exposures diseased animals fed brans containing condensed tannins (i.e. Sumac and Hi Tannin black bran) showed bacterial richness and diversity indexes higher than their diet-matched controls. This result parallels a previous study using denaturing gel electrophoresis (DGGE) that reported tannins derived from the *Acacia angustissima* shrub significantly increased murine fecal bacterial diversity (Smith and Mackie 2004). A reduction in bacterial diversity and species richness could be detrimental due to decreased colonization resistance, imbalances in microbial-host signaling through pattern recognition receptors such as Toll-like receptors (TLR), as well as allowing for pathogenic bacteria to thrive (Croswell 2009). These data suggest that even though the bacterial populations may be affected during repeated DSS exposure (i.e. a more active disease state), feeding bran-based diets, particularly those containing condensed tannins, may be useful to restore bacterial species richness and diversity.

CONCLUSIONS

To our knowledge, there are no studies that describe the effect of sorghum bran on the microbiota. We demonstrated that sorghum bran diets may be able to prevent an overall dysbiosis of predominant bacterial populations and decreased microbial diversity commonly associated with UC. Furthermore, we observed distinct differences among experimental diets suggesting that the presence of bioactive compounds like 3-deoxyanthocianins and condensed tannins may be a factor in these diets ability to alter the luminal environment and microbial populations. Additional analyses, such as those employing species-specific bacterial primers, could elucidate additional population changes not captured in this study. Future studies elucidating mechanisms by which these bioactive compounds affect the intestinal microbiota during a healthy and diseased state are warranted.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSEC online.

ACKNOWLEDGEMENTS

The authors appreciate the assistance with sample collection provided by Sujitta Raungrusmee and Adam Brum.

FUNDING

Research support from United Sorghum Checkoff Program (Roo31A-09 and HVM006-12), and the NIH/National Institute of Diabetes and Digestive and Kidney Diseases grant P30 DK34987. The work was also supported through a fellowship to Lauren Ritchie from the National Space Biomedical Research Institute through NCC 9-58 (EO01001) and Whole Systems Genomics at Texas A&M University.

Conflict of interest. None declared.

REFERENCES

Ahn YJ, Lee CO, Kweon JH, *et al.* Growth-inhibitory effects of Galla Rhois-derived tannins on intestinal bacteria. *J Appl Microbiol* 1998;**84**:439–43.

Andoh A, Imaeda H, Aomatsu T, *et al.* Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. *J Gastroenterol* 2011;**46**:479–86.

Biasi F, Astegiano M, Maina M, *et al.* Polyphenol supplementation as a complementary medicinal approach to treating inflammatory bowel disease. *Curr Med Chem* 2011;**18**:4851–65.

Bibiloni R, Mangold M, Madsen KL, *et al.* The bacteriology of biopsies differs between newly diagnosed, untreated, Crohn's disease and ulcerative colitis patients. *J Med Microbiol* 2006;**55**:1141–9.

Borges G, Roowi S, Rouanet JM, *et al.* The bioavailability of raspberry anthocyanins and ellagitannins in rats. *Mol Nutr Food Res* 2007;**51**:714–25.

Burdette A. Anti-inflammatory activity of select sorghum (Sorghum bicolor) brans. *J Med Food* 2010;**13**:879.

Caporaso JG, Kuczynski J, Stombaugh J, *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**:335–6.

Chassard C, Gaillard-Martinie B, Bernalier-Donadille A. Interaction between H2-producing and non-H2-producing

cellulolytic bacteria from the human colon. *FEMS Microbiol Lett* 2005;**242**:339–44.

Chung KT, Lu Z, Chou MW. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem Toxicol* 1998;**36**:1053–60.

Croswell A. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. *Infect Immun* 2009;**77**:2741–53.

Crozier A, Del Rio D, Clifford MN. Bioavailability of dietary flavonoids and phenolic compounds. *Mol Aspects Med* 2010;**31**:446–67.

Cueva C, Sánchez-Patán F, Monagas M, *et al*. In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and phenolic metabolites. *FEMS Microbiol Ecol* 2012;**83**:792–805.

Davis LMG, Davis Is, MartÃnez J, *et al*. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One* 2011;**6**:e25200.

Devine AA, Gonzalez A, Speck KE, *et al*. Impact of ileocecal resection and concomitant antibiotics on the microbiome of the murine jejunum and colon. *PLoS One* 2013;**8**:e73140.

Dykes L, Rooney LW. Sorghum and millet phenols and antioxidants. *J Cereal Sci* 2006;**44**:236–51.

Dykes L, Rooney WL, Rooney LW. Evaluation of phenolics and antioxidant activity of black sorghum hybrids. *J Cereal Sci* 2013;**58**:278–83.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Edwards U, Rogall T, Blocker H, *et al*. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* 1989;**17**:7843–53.

Elson CO, Cong Y, McCracken VJ, *et al*. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005;**206**:260–76.

Fierer N, Hamady M, Lauber CL, *et al*. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *P Natl Acad Sci USA* 2008;**105**:17994–9.

Frank DN, St. Amand AL, Feldman RA, *et al*. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *P Natl Acad Sci USA* 2007;**104**:13780–5.

Garcia Vilela E, De Lourdes De Abreu Ferrari M, Oswaldo Da Gama Torres H, *et al*. Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission. *Scand J Gastroentero* 2008;**43**:842–8.

Gibson GR, Probert HM, Loo JV, *et al*. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004;**17**:259–75.

Hamady M, Lozupone C, Knight R. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME J* 2010;**4**:17–27.

Hanske L. Xanthohumol does not affect the composition of rat intestinal microbiota. *Mol Nutr Food Res* 2005;**49**:868.

Heimesaat MM, Fischer A, Siegmund B, *et al*. Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis via Toll-like receptors 2 and 4. *PLoS One* 2007; **2**:e662.

Itzkowitz SH, Yio X. Inflammation and Cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol-Gastr L* 2004;**287**:G7–17.

Jia Q, Lupton JR, Smith R, *et al*. Reduced colitis-associated colon cancer in Fat-1 (n-3 fatty acid desaturase) transgenic mice. *Cancer Res* 2008;**68**:3985–91.

Jimenez-Ramsey LM, Rogler JC, Housley TL, *et al*. Absorption and distribution of 14C-labeled condensed tannins and related sorghum phenolics in chickens. *J Agr Food Chem* 1994;**42**: 963–7.

Larrosa M, Luceri C, Vivoli E, *et al*. Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Mol Nutr Food Res* 2009;**53**: 1044–54.

Larrosa M, Yañéz-Gascón MaJ, Selma MaV, *et al*. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *J Agr Food Chem* 2009;**57**:2211–20.

Lee I-A, Bae E-A, Hyun Y-J, *et al*. Dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid induce lipid peroxidation by the proliferation of intestinal gram-negative bacteria in mice. *J Inflamm* 2010;**7**:7.

Ley RE, Hamady M, Lozupone C, *et al*. Evolution of mammals and their gut microbes. *Science* 2008;**320**:1647–51.

Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. *Curr Issues Intest Microbiol* 2006;**7**:73–90.

Loftus EV, Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology* 2004;**126**:1504–17.

Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009;**294**:1–8.

Louis P, Scott KP, Duncan SH, *et al*. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 2007;**102**:1197–208.

Lozupone C, Hamady M, Knight R. UniFrac–an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* 2006;**7**:371.

Manichanh C, Rigottier-Gois L, Bonnaud E, *et al*. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006;**55**:205–11.

Martin HM, Campbell BJ, Hart CA, *et al*. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology* 2004;**127**:80–93.

Menon R, Watson SE, Thomas LN, *et al*. Diet complexity and estrogen receptor β status affect the composition of the murine intestinal microbiota. *Appl Environ Microb* 2013;**79**:5763–73.

Nagalingam NA, Kao JY, Young VB. Microbial ecology of the murine gut associated with the development of dextran sodium sulfate-induced colitis. *Inflamm Bowel Dis* 2011;**17**:917–26.

Nagalingam NA, Lynch SV. Role of the microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis* 2012;**18**: 968–84.

Noor S, Ridgway K, Scovell L, *et al*. Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota. *BMC Gastroenterol* 2010;**10**:134.

Okubo T. In vivo effects of tea polyphenol intake on human intestinal microflora and metabolism. *Biosci Biotech Bioch* 1992;**56**:588.

Onderdonk AB, Richardson JA, Hammer RE, *et al*. Correlation of cecal microflora of HLA-B27 transgenic rats with inflammatory bowel disease. *Infect Immun* 1998;**66**:6022–3.

Ott SJ, Musfeldt M, Wenderoth DF, *et al*. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004;**53**:685–93.

Possemiers S, Bolca S, Verstraete W, *et al*. The intestinal microbiome: a separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. *Fitoterapia* 2011;**82**:53–66.

Rajilić-Stojanović M, Biagi E, Heilig HGHJ, *et al*. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* 2011;**141**:1792–801.

Rajilić-Stojanović M, Smidt H, De Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. *Environ Microbiol* 2007;**9**:2125–36.

Rastall RA, Gibson GR, Gill HS, *et al*. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. *FEMS Microbiol Ecol* 2005;**52**:145–52.

Rastmanesh R. High polyphenol, low probiotic diet for weight loss because of intestinal microbiota interaction. *Chem Biol Interact* 2011;**189**:1–8.

Ree T. Tannins: classification and definition. *Nat Prod Rep* 2001;**18**:641–9.

Reeder J, Knight R. Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nat Methods* 2010;**7**:668–9.

Ritchie LE, Burke KF, Garcia-Mazcorro JF, *et al*. Characterization of fecal microbiota in cats using universal 16S rRNA gene and group-specific primers for Lactobacillus and Bifidobacterium spp. *Vet Microbiol* 2010;**144**:140–6.

Sakakibara H, Ogawa T, Koyanagi A, *et al*. Distribution and excretion of bilberry anthocyanins in mice. *J Agr Food Chem* 2009;**57**:7681–6.

Sepehri S, Kotlowski R, Bernstein CN, *et al*. Microbial diversity of inflamed and noninflamed gut biopsy tissues in inflammatory bowel disease. *Inflamm Bowel Dis* 2007;**13**:675–83.

Smith AH, Mackie RI. Effect of condensed tannins on bacterial diversity and metabolic activity in the rat gastrointestinal tract. *Appl Environ Microb* 2004;**70**:1104–15.

Sokol H, Seksik P, Rigottier-Gois L, *et al*. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm Bowel Dis* 2006;**12**:106–11.

Swidsinski A, Weber J, Loening-Baucke V, *et al*. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol* 2005;**43**:3380–9.

Thibault R, Thibault P, De Coppet K, *et al*. Down-regulation of the monocarboxylate transporter 1 is involved in butyrate deficiency during intestinal inflammation. *Gastroenterology* 2007;**133**:1916–27.

Turner N, Taddeo SS, McDonough CM, *et al*. Polyphenol-rich sorghum brans promote fecal water retention and alter short chain fatty acids in Sprague Dawley rats. *Cereal Foods World* 2010;**55**(4 Suppl.):A72–3.

van Duynhoven J, Vaughan EE, Jacobs DM, *et al*. Metabolic fate of polyphenols in the human superorganism. *P Natl Acad Sci USA* 2011;**108**:4531–8.

Vicky De P, Greet V, Paul JR, *et al*. T1795 Impaired butyrate oxidation in ulcerative colitis is due to decreased butyrate uptake and a defect in the oxidation pathway. *Gastroenterology* 2010;**138**:S-580.

Walker AW, Ince J, Duncan SH, *et al*. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2010;**5**:220–30.

Wells JM, Rossi O, Meijerink M, *et al*. Epithelial crosstalk at the microbiota–mucosal interface. *P Natl Acad Sci USA* 2010;**108**(Suppl. 1):4607–14.

Wiegel J, Kuk S-U, Kohring GW. Clostridium thermobutyricum sp. nov., a moderate thermophile isolated from a cellulolytic culture, that produces butyrate as the major product. *Int J Syst Bacteriol* 1989;**39**:199–204.

Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;**448**:427–34.