White and dark kidney beans reduce colonic mucosal damage and inflammation in response to dextran sodium sulfate

Article in The Journal of nutritional biochemistry · March 2015
DOI: 10.1016/j.jnutbio.2015.02.003 · Source: PubMed

CITATIONS 3
READS 130

11 authors, including:

Karl Peter Pauls
University of Guelph
218 PUBLICATIONS 3,877 CITATIONS
See Profile

Lindsay Robinson
University of Guelph
70 PUBLICATIONS 1,496 CITATIONS
See Profile

Some of the authors of this publication are also working on these related projects:

Economics of Plant Breeding View project
White and dark kidney beans reduce colonic mucosal damage and inflammation in response to dextran sodium sulfate

Jennifer M. Monk\(^{a,b}\), Claire P. Zhang\(^{a,b}\), Wenqing Wu\(^{a}\), Leila Zarepoor\(^{a,b}\), Jenifer T. Lu\(^{a,b}\), Ronghua Liu\(^{a}\), K. Peter Pauls\(^{c}\), Geoffrey A. Wood\(^{d}\), Rong Tsao\(^{a}\), Lindsay E. Robinson\(^{b}\), Krista A. Power\(^{a,b,*}\)

\(^{a}\)Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph ON, Canada, N1G 5C9
\(^{b}\)Department of Human Health and Nutritional Sciences, University of Guelph, Guelph ON, Canada, N1G 2W1
\(^{c}\)Department of Plant Agriculture, University of Guelph, Guelph ON, Canada, N1G 2W1
\(^{d}\)Department of Pathobiology, University of Guelph, Guelph ON, Canada, N1G 2W1

Abstract

Common beans are a rich source of nondigestible fermentable components and phenolic compounds that have anti-inflammatory effects. We assessed the gut-health-promoting potential of kidney beans in healthy mice and their ability to attenuate colonic inflammation following dextran sodium sulphate (DSS) exposure (via drinking water, 2% DSS w/v, 7 days). C57BL/6 mice were fed one of three isocaloric diets: basal diet control (BD), or BD supplemented with 20% cooked white (WK) or dark red kidney (DK) bean flour for 3 weeks. In healthy mice, anti-inflammatory microbial-derived cecal short chain fatty acid (SCFA) levels (acetate, butyrate and propionate), colon crypt height and colonic Mucin 1 (MUC1) and Resistin-like Molecule beta (Relmβ) mRNA expression all increased in WK- and DK-fed mice compared to BD, indicative of enhanced microbial activity, gut barrier integrity and antimicrobial defense response. During colitis, both bean diets reduced (a) disease severity, (b) colonic histological damage and (c) increased mRNA expression of antimicrobial and barrier integrity-promoting genes (Toll-like Receptor 4 (TLR4), MUC1-3, Relmβ and Trefoil Factor 3 (TFF3)) and reduced proinflammatory mediator expression [interleukin (IL)-1β, IL-6, interferon (IFN)γ, tumor necrosis factor (TNF)α and monocyte chemoattractant protein-1], which correlated with reduced colon tissue protein levels. Further, bean diets exerted a systemic anti-inflammatory effect during colitis by reducing serum levels of IL-17A, IFNγ, TNFα, IL-1β and IL-6. In conclusion, both WK and DK bean-supplemented diets enhanced microbial-derived SCFA metabolite production, gut barrier integrity and the microbial defensive response in the healthy colon, which supported an anti-inflammatory phenotype during colitis. Collectively, these data demonstrate a beneficial colon-function priming effect of bean consumption that mitigates colitis severity.

Keywords: Colitis; Kidney beans; Inflammation; Dextran sodium sulfate; Colon health; Short-chain fatty acids

1. Introduction

As a whole food, common beans (Phaseolus vulgaris) contain high amounts of dietary fiber, starch, protein, vitamins and minerals, and phenolic compounds (e.g., flavonoids, anthocyanins, flavonol glycosides, tannins and phenolic acids)\(^{[1–3]}\) with antioxidant activity\(^{[2,4–6]}\). Collectively, beans exert health benefits in connection with obesity\(^{[7,8]}\), diabetes mellitus\(^{[9]}\), cardiovascular disease\(^{[8]}\) and cancer\(^{[10–13]}\). Further, bean consumption has been shown to improve aspects of gut health including beneficial changes in the gut microbiota profile and activity\(^{[14–16]}\). Microbial metabolism of nondigestible bean components, such as resistant starch, soluble fiber and nonstarch polysaccharides, produces an array of gut-health-promoting bioactives including short-chain fatty acids (SCFAs), namely, acetate, propionate and butyrate\(^{[15,16]}\), as well as phenolic metabolites\(^{[6,17,18]}\). Specifically, butyrate functions in the colon to support commensal bacterial growth\(^{[19]}\) and provides an energy source for epithelial cells\(^{[20–22]}\), increases mucus secretion via stimulating Mucin 2 (MUC2) expression\(^{[23]}\) and exerts anti-inflammatory effects via modulating inflammatory signaling pathway activation and enhancing gut barrier integrity\(^{[20,22,24–28]}\). Moreover, bean-derived phenolic compounds also promote gut health via enhancing gut barrier integrity\(^{[29,30]}\), modulating the microbiota community structure and activity\(^{[31–34]}\), attenuating immune responses\(^{[35–39]}\) and reducing oxidative stress\(^{[39,40]}\). Collectively, the diverse gut-health-associated targets of bean-derived bioactives may be beneficial in numerous chronic diseases including inflammatory mucosal pathologies.

Ulcerative colitis is a form of inflammatory bowel disease (IBD) that is associated with microbial dysbiosis\(^{[41–45]}\), compromised gut barrier integrity and function (i.e., altered permeability, tight junction protein expression, toll-like receptor signaling, antimicrobial peptide production, goblet cell function)\(^{[46–50]}\), defects in mucosal immune and inflammatory responses\(^{[50,51]}\), and elevated oxidative stress
In line with the gut-health-promoting potential of common beans, we recently demonstrated that dietary supplementation with cooked common beans (navy and black bean varieties) reduced the severity of the colitis-associated in induced colitis, the objectives of this current study were (a) to determine the effect of dietary supplementation of cooked common beans on aspects of colonic health in unchallenged healthy mice, (b) to determine the gut-health-promoting potential of bean diets on the severity of the colitis-associated inflammatory phenotype and (c) to determine the influence of phenolic compound levels and profiles on the gut-health-promoting potential of common beans.

With a specific focus on the potential priming effects of bean diets on the promotion of gut health and subsequent attenuation of injury-induced colitis, the objectives of this current study were (a) to determine the effect of dietary supplementation of cooked common beans on aspects of colonic health in unchallenged healthy mice, (b) to determine the gut-health-promoting potential of bean diets on the severity of the colitis-associated inflammatory phenotype and (c) to determine the influence of phenolic compound levels and profiles on the gut-health-promoting potential of common beans.

2. Materials and methods

2.1. Preparation of bean flours, diets and experimental design

White kidney (Yeti) and dark kidney beans (AC Calmont) were provided by Dr. Ali Navabi, AAFC/University of Guelph, Joint Bean Breeding Program. Whole beans were soaked overnight in room temperature distilled water and cooked in a slow cooker (Hamilton Beach, Southern Pines, NC) on high with 0.3% (w/v) baking powder (Kraft, Canada Inc., Don Mills, ON, Canada) for 7 h. Both cooked beans and the remaining cooking water were homogenized into a bean paste and freeze-dried by the Guelph Food Technology Centre (Guelph, ON, Canada) to produce bean flour. Proximate analyses and crude fiber content of bean flours were analyzed by Maxxam Analytics (Mississauga, ON, Canada), and three isocellular experimental diets (basal diet (BD), BD supplemented with 20% white kidney bean flour (WK) and BD supplemented with 20% dark kidney bean flour (DK) were prepared by Harlan Laboratories (Madison, WI) in accordance with the AIN-93G diet formulation with corn oil substituted for soybean oil and cellulose increased from 5% to 7% (Table 1). The 20% bean flour supplementation level is an achievable and physiologically relevant level of intake in humans [9,68,69]. The soluble fiber content in the BD, WK and DK diets were analyzed by Maxxam Analytics and determined to be 0.4, 1.9 and 1.7 g/100 g dry weight, respectively.

All experimental procedures were approved by the institutional animal care committee (University of Guelph; animal use protocol # 180867) in accordance with the guidelines of the Canadian Council of Animal Care. Five-week-old male C57BL/6 mice were purchased from Charles River (Portage, MI) housed 3–4/cage as described previously [61] and acclimated to the BD for 1 week prior to random assignment to one of three dietary groups (BD, WK and DK). All mice were fed their respective diets for 3 weeks and allowed ad libitum access to food and water during the study. Diet intake levels and body weight gain did not differ between dietary groups (Table 1). After 3 weeks of dietary intervention, fresh feces were collected, snap frozen and stored at −80°C for later analysis of phenolic content and antioxidant activity. A subset of healthy mice (n=4/diet) was terminated, and their tissues were processed as described below. All other mice were switched to the BD 1 day prior to colitis induction (n=9–12/original dietary group) via the addition of 2% (w/v) DSS (MP Biomedicals; MW 36,000–50,000) to the drinking water for 7 days, whereas age-matched controls (BD) were given ad libitum access to fresh drinking water. An experimental design schematic is shown in Supplemental Fig. 2. Having all mice consume the BD during DSS exposure removes the confounding effects of experimental diet/DSS interactions [65].

<table>
<thead>
<tr>
<th>Ingredient (g/kg)</th>
<th>BD</th>
<th>WK</th>
<th>DK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>377</td>
<td>290</td>
<td>286</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70.0</td>
<td>66.6</td>
<td>67.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>70.0</td>
<td>15.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Mineral mix, AIN-93G-MX (94046)</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Vitamin mix, AIN-93-VX (94047)</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TBHQ, antioxidant</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Protein (% kcal)</td>
<td>19.2</td>
<td>19.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>63.2</td>
<td>63.3</td>
<td>63.3</td>
</tr>
<tr>
<td>Fat (% kcal)</td>
<td>17.6</td>
<td>17.5</td>
<td>17.5</td>
</tr>
</tbody>
</table>

2.2. Colon histopathology and immunohistochemistry

Paraffin embedded colon Swiss rolls were sectioned (5 μm) and stained with hematoxylin and eosin (H&E) (Sigma-Aldrich, St. Louis, MO) for histological scoring of colitis severity. From proximal to distal colon, a crypt erosion score (1–6) was assigned based on the criteria previously described [61] with each score multiplied by the percentage of colon area affected (such that the most damaged colon would have a maximum score of 600) and presented as the histological damage score. Furthermore, using Alcian blue/Nuclear Fast Red (Sigma-Aldrich)-stained colon Swiss rolls, the proportion of the colon mucosa found to be hypersecreting mucins was measured. In healthy (unchallenged) BD- and bean-fed mice, the lengths of at least 10 properly oriented crypts at 400x magnification were measured within the proximal colon. All histological assessments were conducted using a BX51 microscope (Olympus America, Inc.) equipped with an Olympus DP72 Digital Camera System. The colon erosion scoring method was also validated by correlating the colon erosion scores with colon weight/length ratios, which are a well-known biomarker of colonic inflammation [72,73].

In DSS-exposed mice (n=8–9/dietary group), colonic epithelial apoptosis was assessed by detection of active caspase-3 (cleaved caspase-3, ASPT) by immunohistochemistry (Cell Signaling Technology, Danvers, MA) as described previously [61]. For the quantification of apoptosis, Swiss roll sections were viewed blindly (400x magnification) using a BX51 microscope (Olympus Canada, Inc.) equipped with an Olympus DP72 Digital Camera system. The numbers of positive cells (brown staining) within crypt-containing epithelium were counted throughout the entire colon and expressed as the number of positive cells/mm² crypt-containing epithelium.

2.3. Colon mRNA expression

Colon RNA was isolated by Triozl/chloroform extraction and purified using the RNeasy kit (Qiagen, Toronto, ON, Canada). Samples were DNase treated using the RNeasy-Free DNase kit (Qiagen), and RNA quality was assessed using the Experion RNA Analysis kits (Bio-Rad, Mississauga, ON, Canada). RNA (1 μg) was converted to cDNA using High-Capacity CDNA Reverse Transcription kit (Applied Biosystems, Forest City, CA), and real-time polymerase chain reaction (PCR) analysis was performed using a 7900HT Fast Real-Time PCR system (Applied Biosystems). Data were analyzed using the...
3. Results

3.1. Phenolic content and antioxidant potential of the experimental diets and feces

The WK and DK diets contained detectable phenolics and flavonoids (Table 2) as evidenced by elevated TPC and TFC levels compared to BD, with the greatest levels in the DK diet, potentially due to the anthocyanidin content (specifically pelargonidin and cyanidin), which was not present in the WK diet (Supplemental Fig. 3) as seen previously [2]. Additionally, fecal samples collected following 3-week consumption of experimental diets indicated that bean-fed mice also had higher colonic levels of antioxidant phenolics and flavonoids, with the highest TPC, TFC and ORAC levels found in fecal samples collected from DK-fed mice (Table 2).

3.2. Kidney bean diets increase cecal microbial fermentation and improve biomarkers of colon barrier function in healthy unchallenged mice

In healthy WK- and DK-fed mice, both cecum size and cecal SCFA concentrations of acetate, propionate and butyrate were increased compared to BD (P < 0.05, Fig. 1A & B). Additionally, bean-fed mice had increased crypt height (Fig. 1C, P < 0.05) indicative of an improved gut barrier which is evident in representative images of Alcian-blue-stained colon sections (Fig. 1D).

Colonic mRNA expression of MUC1, an epithelial membrane-bound mucin that helps protect the mucosal epithelial barrier [79], was up-regulated by both kidney bean diets compared to BD (Table 3), whereas MUC3 expression was unaltered and MUC2 expression was down-regulated in the WK group relative to both BD and DK. Gene expression of Beln, which promotes mucosal barrier integrity via up-regulating mucin secretion and exerts colonic immunoregulatory effects [79], was increased by both kidney bean diets versus BD, with the highest expression level detected in the WK group. Trefoil Factor 3 (TFF3), Toll-like Receptor (TLR2 and TLR4) expression was unaffected by diet. Further, as expected, healthy unchallenged colon inflammatory cytokine gene expression was low in all dietary groups and did not differ between dietary groups (Table 3).

3.3. Prefeeding WK and DK bean diets reduces DSS-induced colitis severity and colonic damage

Compared to age-matched healthy controls (BD), all DSS-exposed groups exhibited an increased DAI score (P < 0.05). However, prefeeding both the WK and DK bean diets prior to DSS exposure reduced the overall DAI score from day 4 to 6 of the DSS cycle compared to BD+DSS (P < 0.05, Fig. 2A), consistent with reduced clinical symptoms of weight loss severity, detection of stool blood and loss of stool consistency. Moreover, colon weight:length ratio, a gross biomarker of colonic inflammation [73], was increased in the BD+DSS group compared to healthy controls (BD); however, this effect was attenuated by both kidney bean diets which reduced the colon weight:length ratio compared to BD+DSS (P < 0.05) but did not differ from each other (Fig. 2B). Conversely, although colonic MPO levels were increased in all DSS-exposed mice compared to healthy controls (P < 0.05), the kidney bean diets did not improve this biomarker of immune cell infiltration (Fig. 2C). Diet and water intake was reduced in mice exposed to DSS towards the end of the DSS cycle; however, this reduction was delayed in mice prefed bean diets (Supplemental Fig. 4).

The severity of colonic histological damage induced by DSS was assessed in Swiss rolls stained with H&E and Alcian blue/Nuclear Fast Red. Mice consuming bean diets had less colonic damage and a greater proportion of mucus-secreting crypts compared to BD-fed mice following 7-day exposure to DSS (Fig. 3A). Representative images of Alcian-blue-stained Swiss rolls are shown in Fig. 3B, in which ulcers

### Table 2

<table>
<thead>
<tr>
<th>Phenolic content and antioxidant potential of diet and feces, a</th>
<th>BD</th>
<th>WK</th>
<th>DK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC (mg GAEl/g)</td>
<td>0.48±0.02b</td>
<td>1.34±0.06b</td>
<td>1.16±0.03b</td>
</tr>
<tr>
<td>TFC (mg CE/g DW)</td>
<td>0.0±0.04</td>
<td>0.27±0.013b</td>
<td>0.40±0.007b</td>
</tr>
<tr>
<td>ORAC (μM TE/g)</td>
<td>0.0±0.0</td>
<td>19.96±2.72b</td>
<td>23.08±0.82b</td>
</tr>
<tr>
<td><strong>Feces</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC (mg GAEl/g)</td>
<td>0.32±0.03a</td>
<td>0.63±0.02b</td>
<td>1.1±0.06b</td>
</tr>
<tr>
<td>TFC (mg CE/g DW)</td>
<td>0.17±0.01a</td>
<td>0.40±0.03b</td>
<td>0.67±0.04b</td>
</tr>
<tr>
<td>ORAC (μM TE/g)</td>
<td>5.01±0.46a</td>
<td>9.63±0.74b</td>
<td>26.50±1.70b</td>
</tr>
</tbody>
</table>

a After 3 weeks of dietary intervention, fresh fecal samples were collected and analyzed for TPC, TFC and ORAC. Values are mean±SEM; n=8–10/dietary group for feces, and the analysis of each diet was completed in triplicate. Values not sharing a lowercase letter differ (P < 0.05). Data were analyzed by one-way ANOVA followed by SNK post hoc analysis.
are clearly evident in BD-fed DSS-exposed mice and increased mucus secretion is evident in bean-fed DSS-exposed mice. The level of cellular apoptosis was also measured in DSS-exposed colons through detection of cleaved caspase-3 by immunohistochemistry. As shown in Fig. 3A, there were no differences between dietary treatment groups in the level of apoptosis within the colon epithelium (P > 0.05).

3.4. Prefeeding WK and DK bean diets reduces colon inflammatory mediator gene and protein expression levels

Following DSS exposure, the colon mRNA expression profile was modified by both the kidney bean diets compared to the expression levels in the BD+DSS group (Table 4). TLR2 mRNA expression did not differ between dietary groups, whereas TLR4 gene expression was up-regulated by both kidney bean diets compared to BD+DSS, as seen previously with bean supplementation and the DSS colitis model [61]. MUC1 and MUC2 colonic mRNA expression was increased in DSS-treated WK and DK groups compared to BD+DSS (P < 0.05), whereas MUC3 expression was increased in only the WK+DSS group. Additionally, Relmβ and TFF3 mRNA expression was increased by both DSS-treated kidney bean dietary groups compared to BD+DSS (P < 0.05). Colon mRNA expression of IL-6 and IL-1β were reduced by prefeeding both kidney bean diets compared to BD+DSS, with a more dramatic reduction apparent in the DK group for IL-1β (P < 0.05). Colon TNFα and monocyte chemotactant protein (MCP)-1 mRNA expression levels were differentially affected by the kidney bean diets, evidenced by reduced relative expression levels to BD+DSS in the WK and DK groups, respectively (P < 0.05). Conversely, both bean diets increased colonic mRNA expression of the anti-inflammatory cytokine IL-10 in response to DSS exposure, with higher fold-change levels detected in the WK group (P < 0.05).

Representative colon tissue protein levels of critical inflammatory (IL-6 and IL-1β) and anti-inflammatory (IL-10) cytokines were assessed in DSS-treated mice, in part, to confirm the gene expression outcomes. For all cytokines assessed, the colon tissue protein levels mirrored the gene expression outcome. Specifically, both kidney bean DSS-treated groups reduced IL-6 and IL-1β levels compared to BD+DSS but did not differ from each other (P < 0.05, Fig. 4A & B).

Similarly, colon tissue levels of IL-10 were increased by both kidney bean diets compared to BD+DSS (P < 0.05, Fig. 4C).

3.5. Biomarkers of systemic inflammation are reduced by prefeeding WK and DK bean diets

As expected, DSS exposure increased all circulating cytokine levels in the BD+DSS group compared to healthy age-matched controls (Fig. 5). Prefeeding the WK and DK bean diets attenuated DSS-induced circulating levels of IL-1β, TNFα, IFNγ and IL-17A compared to BD+DSS (P < 0.05). Further, only the WK+DSS group exhibited a reduction in the circulating concentration of IL-6 and IL-10 compared to BD+DSS (P < 0.05). Spleen hypertrophy, a well-accepted biomarker of systemic inflammation in response to DSS [70,71,80,81], was attenuated by prefeeding both kidney-bean-supplemented diets compared to BD+DSS, an effect that is in agreement with our previous findings (Fig. 6A) [61]. Further, the DSS-induced reduction in serum anti-oxidant levels (ORAC) was reverted only by prefeeding the WK diet (P < 0.05, Fig. 6B).

Table 3

<table>
<thead>
<tr>
<th>Colon mRNA expression in healthy mice. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>IL-1β</td>
</tr>
<tr>
<td>TNFα</td>
</tr>
<tr>
<td>MCP-1</td>
</tr>
<tr>
<td>IL-10</td>
</tr>
<tr>
<td>TLR2</td>
</tr>
<tr>
<td>TLR4</td>
</tr>
<tr>
<td>MUC1</td>
</tr>
<tr>
<td>MUC2</td>
</tr>
<tr>
<td>MUC3</td>
</tr>
<tr>
<td>Relmβ</td>
</tr>
<tr>
<td>TFF3</td>
</tr>
</tbody>
</table>

*Colon mRNA expression after 3 weeks of dietary intervention. For each gene, data were normalized to the housekeeping gene RPLP0. Data are means±S.E.M., and P values are shown. n=4/dietary group. Values not sharing a lowercase letter differ (P < 0.05).

Fig. 1. Effect of kidney bean diets on (A) SCFA concentrations, (B) cecum size and (C) colon histomorphology in healthy mice. Values are means±S.E.M., and all data were analyzed by one-way ANOVA followed by Fisher’s Least Significant Difference (LSD) post hoc test (n=4/dietary group). Bars not sharing a lowercase letter differ (P < 0.05). SCFA concentrations are expressed as μmol/g cecum content. Representative images (40×) of colon sections stained with Alcian blue/Nuclear Fast Red highlighting the increased crypt height in the bean-fed mice are shown (scale bar=50μm).
4. Discussion

The overall objective of the current study was to determine if mice fed 20% cooked kidney bean diets, prior to colitis induction, would experience less clinical symptoms and colonic inflammation when later exposed to 2% DSS to induce colitis. Collectively, we demonstrated that mice consuming bean-supplemented diets for 3 weeks had improved colon health and mucosal barrier integrity which exerted a priming effect, such that the severity of mucosal damage and inflammation produced in response to DSS was attenuated. These current findings are somewhat in contrast to our previous findings, where mice fed bean-supplemented diets, both prior to and during DSS exposure, had reduced mucosal and systemic inflammation; however, they also displayed an increase in colonic mucosal histological damage and apoptosis [61]. This may indicate that the timing of bean consumption may have a critical impact on the resulting colitis-associated phenotype.

In the current study, mice were fed bean-supplemented diets for 3 weeks prior to DSS exposure only, at which time all mice were given BD during colitis induction via 7 days of DSS exposure. This experimental design is relevant as IBD patients report experiencing gastrointestinal discomfort when consuming highly fermentable foods such as beans [66] and report a perception that these foods could induce disease relapse and, therefore, avoid the consumption of fiber-rich foods [67].

In colitic mice exposed to DSS for 7 days, prefeeding both WK and DK diets resulted in reduced severity of colitis-associated clinical symptoms as the DAI score was reduced from day 4 to 6 of the DSS cycle compared to BD+DSS (Fig. 2A). Moreover, the degree of colitis-associated mucosal damage was reduced by prefeeding the bean diets prior to DSS exposure (Fig. 3), which is in contrast to previous outcomes wherein colon histological damage scores were increased when bean diets were consumed during DSS exposure [61]. The magnitude of the local inflammatory response was also attenuated by

<table>
<thead>
<tr>
<th>A)</th>
<th>BD+DSS</th>
<th>WK+DSS</th>
<th>DK+DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological Damage Score</td>
<td>338.3±10.8a</td>
<td>291.0±14.5b</td>
<td>292.5±7.7b</td>
</tr>
<tr>
<td>% Mucification</td>
<td>18.7±3.8a</td>
<td>36.5±4.1b</td>
<td>29.8±2.7b</td>
</tr>
<tr>
<td>Apoptosis (# positive cells/mm²)</td>
<td>0.039±0.010</td>
<td>0.023±0.004</td>
<td>0.029±0.006</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of prefeeding bean diets on (A) DAI, (B) colon weight:length ratio and (C) colon MPO levels. Values are means±S.E.M. Data were analyzed by one-way ANOVA followed by SNK post hoc test. Bars not sharing a lowercase letter differ (P≤.05). n=5 (BD) and n=8–11/DSS-treated dietary group. MPO data were log-transformed.

Fig. 3. Effect of prefeeding bean diets on colonic histological damage, mucification and apoptosis and in DSS-exposed mice. (A) Values are means±S.E.M. Within a row, data not sharing a lowercase letter differ significantly (P≤.05). Data were analyzed by one-way ANOVA followed by SNK post hoc (n=8–11/group). (B) Representative images (10×, scale bar=200μm) of colon sections stained with Alcian blue/Nuclear Fast Red from DSS-exposed BD-fed (i), or prefed WK (ii) and DK (iii) mice; (→) denotes colon regions with increased ulceration and loss of crypts in the BD+DSS group, and (▲) denotes regions with increased production of mucus in colons of mice prefed the bean diets.
Table 4
Fold changes in colon mRNA expression following exposure to DSS for 7 d.a

<table>
<thead>
<tr>
<th>Gene</th>
<th>BD+DSS</th>
<th>WK+DSS</th>
<th>DK+DSS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>62.56±11.93a</td>
<td>33.27±7.40b</td>
<td>12.32±3.10b</td>
<td>.004</td>
</tr>
<tr>
<td>IL-6</td>
<td>154.58±3.69a</td>
<td>43.38±15.33b</td>
<td>32.41±7.82b</td>
<td>.034</td>
</tr>
<tr>
<td>TNFα</td>
<td>21.12±2.45a</td>
<td>11.95±1.62b</td>
<td>17.39±3.35b</td>
<td>.01</td>
</tr>
<tr>
<td>MCP-1</td>
<td>29.33±8.11a</td>
<td>19.17±4.48ab</td>
<td>11.81±3.05b</td>
<td>.046</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.40±0.10a</td>
<td>6.57±1.46b</td>
<td>2.51±0.29c</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TLR2</td>
<td>2.30±0.47</td>
<td>3.00±0.65</td>
<td>2.53±0.40</td>
<td>.31</td>
</tr>
<tr>
<td>TLR4</td>
<td>0.33±0.15a</td>
<td>1.44±0.37b</td>
<td>2.95±0.41b</td>
<td>.0003</td>
</tr>
<tr>
<td>MUC1</td>
<td>1.63±0.55a</td>
<td>9.07±2.41ab</td>
<td>3.87±0.17b</td>
<td>.0044</td>
</tr>
<tr>
<td>MUC2</td>
<td>0.65±0.18b</td>
<td>1.15±0.30b</td>
<td>1.94±0.88b</td>
<td>.05</td>
</tr>
<tr>
<td>MUC3</td>
<td>0.11±0.08b</td>
<td>2.62±0.20b</td>
<td>0.86±0.18b</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Relmα</td>
<td>1.87±0.31a</td>
<td>3.86±1.09b</td>
<td>4.68±0.67b</td>
<td>.02</td>
</tr>
<tr>
<td>TFF3</td>
<td>0.68±0.19a</td>
<td>1.42±0.20b</td>
<td>1.32±0.52b</td>
<td>.05</td>
</tr>
</tbody>
</table>

a Colon mRNA expression following DSS exposure. For each gene, data were normalized to BD healthy expression levels and expressed as fold changes. Data are means±S.E.M., and P values are shown. n=6–8/dietary group. Values not sharing a lowercase letter differ (P<.05).

prefeeding both bean diets, evidenced by a reduced colon weight:length ratio, a biomarker of colonic inflammation [73] (Fig. 2B), and reduced colon mRNA expression of IL-1β and IL-6 and increased mRNA expression of IL-10 versus BD+DSS (Table 4). Moreover, gene expression levels of IL-1β and IL-6 and IL-10 correlated with colon tissue protein levels (Fig. 4), wherein inflammatory cytokine tissue levels were reduced and IL-10 colonic levels were increased by both bean diets compared to BD+DSS. In addition to a reduction in the local inflammatory cytokine profile, prefeeding kidney bean diets prior to colitis induction also reduced circulating inflammatory cytokine levels (Fig. 5), similar to our previous findings [61]. These results indicate that the local and systemic anti-inflammatory effects of beans can persist for several days following discontinuation of bean consumption. This dietary approach to mitigating colitis-associated symptoms and severity may be useful if employed by IBD patients, wherein frequent bean consumption during periods of disease remission may attenuate the severity and perhaps duration of periods of disease relapse, although further studies are required.

Beans were supplemented to mice diets as a flour produced from whole cooked beans prepared using conventional cooking practices and at a level that is achievable and physiologically relevant in the human diet [9,68,69]. While these experimental diets represent a whole-food approach to reducing colitis severity, the complex matrix of whole foods makes it difficult to discern which dietary bioactive(s) may be responsible for the anti-inflammatory response to DSS-induced colitis. Beans are high in dietary fiber, starch, protein, vitamins and minerals and are phenolic compounds with antioxidant activity [2,4–6]. Despite differences in both the level and profile of the phenolic compounds between the WK and DK diets, very little difference in the colitis-associated phenotype were observed between WK- and DK-fed mice. This may indicate that the presence of additional DK-derived phenolic compounds, such as cyanidin and pelargonidin (Supplemental Fig. 3), did not strongly influence the colitis phenotype, apart from differential effects of the WK and DK diets on reducing colon mRNA expression of TNFα and MCP-1, respectively (Table 4). However, the contribution of phenolics to the anti-inflammatory effects of beans cannot be overlooked as they play a role in enhancing gut barrier integrity [29,30], modulating the microbiota community structure and activity [31–34], attenuating immune responses [35–39] and reducing oxidative stress [39,40].

Another candidate bioactive responsible for the bean-associated anti-inflammatory effects are the SCFAs (acetate, propionate and butyrate), especially butyrate which supports commensal bacterial growth [19] and provides an energy source for epithelial cells [20–22], increases mucus secretion via stimulating MUC2 expression [23] and exerts anti-inflammatory effects via modulating inflammatory signaling pathway activation and enhancing gut barrier integrity [20,22,24–28]. Moreover, increased production of microbial fermentation end products indicates the potential for bean diet-derived changes not only in the host tissues but also within the microbiota, potentially affecting both the microbial community structure and function. Further studies to discern the contribution of these bean-derived bioactives are required.

Regardless of source, beans consumed as a whole food exerted a colon-priming effect that persisted at least 7 days following their removal from the diet. This effect could be attributed to changes exerted within the host epithelium, as evidenced by the increased mRNA expression of MUC1 and Relmα in healthy unchallenged bean diet-fed mice (Table 3). Moreover, following DSS exposure, mRNA expression of both MUC1 and Relmα was further up-regulated (Table 4), indicating that the baseline colonic mucosal gene expression set point was altered by bean consumption in healthy mice, which translated to a beneficial and more rapid physiological response to a mucosal inflammatory challenge via DSS exposure. For example, the role of MUC1 in promoting mucosal barrier defense against bacterial infections [82] and modulating apoptosis-induced epithelial cell barrier turnover [83–85] could help to explain the decreased degree of DSS-associated mucosal histological damage. Further, increased Relmα expression may also help attenuate colitis-associated tissue damage and inflammation [86], consistent with its immunoregulatory role favoring noninflammatory adaptive responses to infection and to promote mucin-dependent barrier defense mechanisms [86,87]. In connection to this, both bean-fed groups exposed to DSS showed evidence of increased mucin-dependent barrier defense via increased colon mucus production (Fig. 3) and mRNA expression of MUC1 and MUC2 (Table 4), which coincided with up-regulated Relmα expression.

The colonic mucus layer is the first line of defense between the luminal contents and host tissues, thereby preventing pathogens and...
antigens from gaining access to the underlying epithelium, and dysfunction or loss of barrier integrity may predispose individuals to IBD [79,88–90]. In ulcerative colitis patients, goblet cell number and colonic expression of MUC2 and MUC3 are low [91]; however, bean consumption increased mRNA expression of MUC1, MUC2 and MUC3 (Table 4) and the degree of colonic mucin production in DSS-treated mice (Fig. 3). Additionally, DSS-associated TFF3 mRNA expression was increased by both bean diets, which plays a role in both mucosal protection and epithelial restitution [92,93], thereby helping to protect the epithelial barrier and promote healing from DSS-induced damage. Moreover, the combination of TFF3 and mucin together has been shown to be more effective in protecting epithelial cells compared to either component alone [94], and gene expression of both factors was increased by bean consumption in response to DSS. Collectively, our data demonstrate that dietary whole bean flour supplementation consumed in a manner that mimics IBD patient consumption patterns (i.e., avoided during disease relapse) [66,67] can improve critical aspects of normal gut health, function and epithelial barrier integrity. The resultant outcome is a colon-priming effect, whereby during colitis (mimicking disease relapse) the degree of mucosal damage and the ensuing inflammatory response are attenuated with coincident up-regulation of mucosal wound-healing-promoting genes. Therefore, increased bean consumption may represent a dietary strategy to improve gut health during times of disease remission and to reduce the severity of relapse in mucosal damage-associated pathologies.

Acknowledgements

Funding was provided by the Ontario Ministry of Agriculture and Food, Ontario Research Fund (RE-04-043) and Agriculture and Agri-Food Canada.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2015.02.003.
References


