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Diets enriched with cranberry beans alter the microbiota and mitigate colitis severity and associated inflammation

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Abstract

Common beans are rich in phenolic compounds and nondigestible fermentable components, which may help alleviate intestinal diseases. We assessed the gut health priming effect of a 20% cranberry bean flour diet from two bean varieties with differing profiles of phenolic compounds [darkening (DC) and nondarkening (NDC) cranberry beans vs. basal diet control (BD)] on critical aspects of gut health in unchallenged mice, and during dextran sodium sulfate (DSS)-induced colitis (2% DSS wt/vol, 7 days). In unchallenged mice, NDC and DC increased (i) cecal short-chain fatty acids, (ii) colon crypt height, (iii) crypt goblet cell number and mucus content and (iv) *Muc1*, *Klf4*, *Relmβ* and *Reg3γ* gene expression vs. BD, indicative of enhanced microbial activity and gut barrier function. Fecal 16S rRNA sequencing determined that beans reduced abundance of the Lactobacillaceae (*Ruminococcus gnavus*), Clostridiaceae (*Clostridium perfringens*), Peptococcaceae, Peptostreptococcaceae, Rikenellaceae and Pophyromonadaceae families, and increased abundance of S24-7 and Prevotellaceae. During colitis, beans reduced (i) disease severity and colonic histological damage, (ii) increased gene expression of barrier function promoting genes (*Muc1-3*, *Relmβ*, and *Reg3γ*) and (iii) reduced colonic and circulating inflammatory cytokines (IL-1β, IL-6, IFNγ and TNFα). Therefore, prior to disease induction, bean supplementation enhanced multiple concurrent gut health promoting parameters that translated into reduced colitis severity. Moreover, both bean diets exerted similar effects, indicating that differing phenolic content did not influence the endpoints assessed. These data demonstrate a proof-of-concept regarding the gut-priming potential of beans in colitis, which could be extended to mitigate the severity of other gut barrier-associated pathologies.

Keywords: Cranberry beans; Inflammation; Colitis; Microbiota; Short-chain fatty acids

1. Introduction

Gut health comprises both host and microbial factors including a functional intact mucosal epithelial barrier and a balanced gut microbiota composition [1]. A symbiotic relationship between the gut microbiota and the host immune system preserves the integrity of the intestinal barrier and minimizes inappropriate inflammatory responses; however, dysfunction or imbalances promote the development of chronic gut-related diseases such as inflammatory bowel disease (IBD), obesity and colon cancer [1–5]. In particular, dysfunctional host–microbiota interactions underlie the pathogenesis of ulcerative colitis and Crohn's disease (CD), two forms of IBD associated with microbial dysbiosis [6–8], resulting in compromised gut barrier integrity and function (i.e., alterations in barrier permeability, tight junction protein expression, toll-like receptor signaling, antimicrobial peptide production and goblet cell function) [9–12], defects in mucosal inflammatory responses [13,14] and elevated oxidative stress [15–18]. Dietary strategies are being studied for their effectiveness in reducing colitis severity, as well as preventing disease relapse [19–21], since common pharmaceutical interventions for IBD patients result in side effects, failure to respond and eventually resistance [22,23]. Moreover, a complex interplay between the specific dietary components and the host–microbiota exists, thereby impacting host mucosal barrier integrity, immune function and subsequent disease susceptibility [24].

Common beans (*Phaseolus vulgaris*) contain high levels of phenolic compounds, dietary fiber, starch and protein [25–27]. Microbial fermentation of bean components produces an array of gut health-promoting bioactives including phenolic metabolites [25,28,29] and short-chain fatty acids (SCFA), namely, acetate, propionate and butyrate [30,31], which collectively improve aspects of gut heath including beneficial changes in

Abbreviations: AAE, ascorbic acid equivalent; BD, basal diet; CD, Crohn's disease; DAI, disease activity index; DC, darkening cranberry; DSS, dextran sodium sulfate; H&E, hematoxylin and eosin; IBD, inflammatory bowel disease; NDC, nondarkening cranberry; OTU, operational taxonomy unit; RDP, ribosomal database project; SCFA, short-chain fatty acids

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the gut microbiota profile and activity [30–35]. Phenolic compounds can promote gut health by modulating host mucosal barrier integrity [36,37], attenuating colitis-associated inflammation [38–41] and oxidative stress [41,42]. SCFA, specifically butyrate, functions in the colon to support commensal bacterial growth [43], provide an energy source for epithelial cells [44,45] and exert anti-inflammatory effects via down-regulating inflammatory signaling pathway activation and cytokine production and enhancing gut barrier integrity [44–50]. Additionally, SCFA enemas have been used as an effective treatment in colitis [46,51,52]. Furthermore, a recent study demonstrated that beans can also modulate the microbiota community composition [53], and therefore, can modify both the microbiota and host tissues in the healthy unchallenged state, which could exert a protective effect within the gut microenvironment that influences disease susceptibility.

Recently, we have shown that bean-derived bioactives exert beneficial effects in mitigating the severity of colitis-associated inflammation and tissue damage in C57Bl/6 mice [54,55]. Specifically, prefeeding a kidney bean flour supplemented diet prior to colitis induction exerted a colon priming effect that altered critical aspects of gut barrier function, thereby predisposing these mice a to less severe physiological response to dextran sodium sulfate (DSS)-induced colitis [54,55]. In this current study, we set out to determine if the beneficial effects of kidney beans could be expanded to additional bean varieties [i.e., nondarkening (NDC) and darkening (DC) cranberry beans] as well as to expand our knowledge on the mechanisms of action of bean effects on gut health. Therefore, our objectives were to assess the ability of cranberry beans to (i) beneficially modify the healthy unchallenged mouse colon, which would induce protective effects against DSS-induced colitis, and if this effect was related to the level of bean phenolics, and (ii) to advance our understanding of the mechanisms of action by assessing both the host tissue biomarkers related to mucosal barrier integrity, microbial defence, and inflammation, as well as the colonic microbial community structure and activity in response to bean diet consumption.

2. Materials and methods

2.1. Preparation of bean flours, diets and experimental design

DC beans (CBX9148 cultivar) and NDC beans (CBX9151 cultivar) were provided by the University of Guelph, Bean Breeding Program, and representative whole bean images are shown in Supplementary Fig. 1. DC and NDC bean flours were produced as described [55] and proximate analyses were performed and crude and soluble fiber content was analyzed by Maxxam Analytics (Mississauga, ON, Canada). Three isocaloric experimental diets were prepared by Harlan Laboratories (Madison, WI, USA) in accordance with the AIN-93G diet formulation with corn oil substituted for soybean oil and cellulose increased from 5% to 7% (Table 1): basal diet (BD), BD supplemented with 20% DC bean flour and BD supplemented with 20% DC bean. The 20% bean flour supplementation level is an achievable and physiologically relevant level of intake in humans [56–58]. The soluble fiber content in BD, NDC and DC diets were determined to be 0.4, 2.0 and 1.6 g/100 g dry weight, respectively.

All experimental procedures were approved by the institutional animal care committee (University of Guelph; animal use protocol #10R067) 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, USA), housed 3–4/cage as described previously [55] and acclimated to the BD for 1 week prior to random assignment to one of three dietary groups (BD, NDC and DC). All mice were fed their respective diets for 3 weeks and allowed *ad libitum* access to food and water. After 3 weeks, fresh feces were collected, snap frozen and stored at −80°C for microbiota profiling. A subset of healthy untreated mice (n=4/dietary group) that were housed in different cages were terminated and their colons excised (from the cecocolonic junction to the rectum), measured for length, weighed and flushed with sterile phosphate-buffered saline (PBS) prior to removing 0.5 cm of the proximal colon which was formalin fixed for histological assessment, and the remaining tissue was snap frozen in liquid nitrogen and stored at −80°C. Cecum and cecum contents were also weighed and stored at −80°C. All other mice were switched to the BD 1 day prior to colitis induction via the addition of 2% (wt/vol) DSS (MW 36,000–50,000; MP Biomedicals, Santa Ana, CA, USA) to the drinking water for 7 days (n=9–12/original dietary group), with the exception of age-matched BD-fed healthy controls (BD) were given *ad libitum* access to fresh drinking water instead of DSS. This experimental design, wherein all mice consume the BD during DSS exposure, more accurately mimics IBD patient consumption patterns of highly fermentable foods, such as beans, during a period of disease activity [59,60] and minimizes the confounding effects of experimental dietary constituent and DSS interactions [61].

Table 1
Macronutrient composition of the experimental diets[a]

Ingredient (g/kg)	BD	NDC	DC
NDC flour	0	200	0
DC flour	0	0	200
Casein	200	146	147
L-Cystine	3.0	3.0	3.0
Corn starch	377	281	277
Maltodextrin	132	132	132
Sucrose	100	100	100
Corn oil	70.0	66.8	67.0
Cellulose	70.0	24.0	26.0
Mineral Mix, AIN-93G-MX (94046)	35.0	35.0	35.0
Vitamin Mix, AIN-93-VX (94047)	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5
TBHQ, antioxidant	0.014	0.014	0.014
Protein (% kcal)	19.2	19.2	19.2
Carbohydrate (% kcal)	63.2	63.3	63.3
Fat (% kcal)	17.6	17.5	17.5

[a] Three experimental diets: BD, BD supplemented with 20% NDC bean flour (NDC) and BD supplemented with 20% DC bean flour (DC) were prepared in accordance with the AIN-93G diet formulation with corn oil substituted for soybean oil. Experimental diets were further analyzed for their soluble fiber content by Maxxam Analytics (Mississauga, ON, Canada) and determined to be 0.4, 2.0 and 1.6 g/100 g dry weight, for the BD, NDC and DC diets, respectively.

During the DSS cycle, water and diet intake were recorded daily, as well as criteria that constitute a Disease Activity Index (DAI), which included body weight loss, stool consistency and the presence of blood in the stool as described [55]. Mice were terminated on day 7 of the DSS cycle by cervical dislocation, blood was collected by cardiac puncture and serum was stored at −80°C. Colons were excised (from the cecocolonic junction to the rectum), measured for length, weighed and flushed with sterile PBS prior to removing 0.5-cm sections of the proximal and distal colon, which were snap frozen in liquid nitrogen and stored at −80°C. The remaining colon was Swiss-rolled and fixed in 10% buffered formalin solution for later histological analysis. Cecum and cecum contents were also weighed and stored at −80°C.

2.2. Phenolic compound and antioxidant analyses

Total phenolics were extracted from BD, NDC and DC experimental diets using the Microwave Assisted Extraction method as described [55,62]. Fecal phenolic compounds were extracted as described [63]. Diet and fecal total phenolic content (TPC) was determined using the Folin–Ciocalteu method [64], and expressed as mg gallic acid equivalent (GAE) per gram (g) of sample. Total flavonoid content (TFC) was measured as described [55,65] and expressed as mg catechin equivalent (CE) per g of sample. Antioxidant activity was measured using the ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays [64] and are expressed as μmol acetic acid equivalents (AAE) per g of sample and μmol Trolox equivalents (TE) per g of sample, respectively.

2.3. 16S rRNA gene library preparation and sequencing

Genomic DNA was extracted from feces using the QiaAmp DNA stool mini kit (Qiagen, Valencia, CA, USA). Sequencing libraries of the 16S V3–4 region were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation Guide Rev. B. Briefly, primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATC TAATCC-3′) [66] containing 5′ Illumina overhang adapter sequences (5′ TCGTCGGCAGCGT CAGATGTGTATAAGAGACAG and 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG, respectively) were used to amplify an ~550-base-pair (bp) fragment of the 16s rRNA V3–4 region. Each reaction containing 12.5 ng of template DNA, 200 nM each primer and 1× KAPA HiFi HotStart ReadyMix (VWR, Mississauga, ON, Canada) in a 25-μl volume was amplified under the following conditions: 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by 72°C for 5 min. Polymerase chain reaction (PCR) products were purified with Ampure XP beads (Beckman Coulter, Mississauga, ON, Canada) and sequencing adapters containing 8-bp indices were added to the 3′ and 5′ ends by PCR using the Nextera XT Index kit (Illumina, San Diego, CA, USA) in a 50-μl reaction containing 5-μl PCR amplicon, 5 μl each indexing primer, and 25 μl 2× KAPA HiFi HotStart ReadyMix under the following conditions: 95°C for 3 min, 8 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by 72°C for 5 min. Following a second purification with Ampure XP beads, the amplicons were quantified using the Quant-iT PicoGreen double-stranded DNA assay (Invitrogen/Life Technologies Inc., Burlington, ON, Canada) and equimolar ratios were pooled and combined with 5% equimolar PhiX DNA (Illumina) for sequencing on one flow cell of a MiSeq instrument, using the MiSeq 600-cycle v3 kit (Illumina).