Journal of Functional Foods 64 (2020) 103625

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Red lentil supplementation reduces the severity of dextran sodium sulfate-induced colitis in C57BL/6 male mice

Daniela Graf[a,b,c,1], Jennifer M. Monk[a,b,1], Wenqing Wu[a], Hannah R. Wellings[b], Lindsay E. Robinson[b], Krista A. Power[a,b,d,*]

[a] Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, ON N1G 5C9, Canada
[b] Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada
[c] Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institute, Karlsruhe, Germany
[d] School of Nutrition Sciences, University of Ottawa, Ottawa, ON K1H 8L1, Canada

ARTICLE INFO

Keywords:
Lentils
Pulses
Colitis
Inflammation
Cytokines
Dextran sodium sulfate

ABSTRACT

Lentils (*Lens culinaris L.*) are a protein-rich plant food, also enriched in fibre and phenolic compounds that may reduce intestinal-associated disease risk. Male C57Bl/6 mice were pre-fed a basal diet (BD) or isocaloric 20% red lentil-supplemented diet (LD) for 3 weeks and acute colitis was induced via dextran sodium sulfate (DSS, 2% w/v in drinking water) for 5 days. LD-fed mice exhibited reduced (i) clinical symptoms, (ii) colon histological damage, and (iii) colonic pro-inflammatory cytokine levels. Additionally, biomarkers of improved colon epithelial barrier integrity and mucosal repair mediators were increased in LD mice (e.g. colonic *IL-22, Relmβ*, and *occludin* expression, and serum lipopolysaccharide binding protein). Collectively, the severity of the DSS-induced acute colitis phenotype in mice was attenuated by red lentil dietary supplementation, indicating that lentils may serve as a potential adjuvant dietary therapy in patients with colitis-associated diseases to help limit colonic inflammation and restore barrier function.

1. Introduction

The colon is comprised of both host and microbial components including the epithelial and mucus barriers, the mucosal immune system, and the colonic microbiome (Bischoff, 2011). Alterations in components of the colonic microenvironment can drive microbial dysbiosis, impair barrier integrity, and initiate colonic and systemic inflammation (Natividad & Verdu, 2012). Homeostasis within the colonic microenvironment is essential for this complex organ to evade and mount protective immune responses against damaging luminal antigens and pathogens, and effectively promote timely epithelial repair processes to maintain mucosal barrier integrity (Gersemann, Wehkamp, & Stange, 2012; Scaldaferri, Pizzoferrato, Gerardi, Lopetuso, & Gasbarrini, 2012). Non-digestible dietary components (e.g. fibres, phytochemicals, and phytoestrogens) are important modulators of the colonic microenvironment, most often these effects are driven by their microbial-derived metabolites. For example, consumption of non-digestible carbohydrates (i.e. soluble fibres, oligosaccharides, resistant starch) results in the formation of microbial-derived short chain fatty acids (SCFAs; acetate, propionate, and butyrate) that can modulate intestinal health and colonic physiological functions (Macfarlane & Macfarlane, 2012; Rios-Covian et al., 2016). Specifically, butyrate has been shown to influence colonic mucosal function by providing energy for epithelial cells (Hamer et al., 2008; Roediger, 1980); inhibit histone deacetylase activity thereby contributing to the anti-carcinogenic effects of SCFAs (Hamer et al., 2008), enhancing epithelial and mucus barrier integrity (Burger-van Paassen et al., 2009), and exerting anti-inflammatory effects via down-regulating activation of inflammatory signalling pathways and cytokine production (Butzner, Parmar, Bell, & Dalal, 1996; Di Sabatino et al., 2005; Hamer et al., 2008; Roediger, 1980; Scheppach et al., 1992; Segain et al., 2000; Steinhart, Hiruki, Brzezinski, & Baker, 1996). Additionally, SCFA enemas have been used as an effective treatment in colitis (Butzner et al., 1996; Cummings, 1997; Harig, Soergel, Komorowski, & Wood, 1989). Food-derived phenolic

Abbreviations: BD, basal diet; DSS, dextran sodium sulfate; DAI, Disease Activity Index; IBD, Inflammatory bowel disease; LD, lentil diet; LBP, lipopolysaccharide binding protein; Reg3γ, regenerating islet-derived protein-3-gamma; Relmβ, resistin-like molecule beta; SCFA, short-chain fatty acid

* Corresponding author at: School of Nutrition Sciences, University of Ottawa, Roger Guindon Hall, Room 3260, Canada.
E-mail addresses: daniela.graf@mri.bund.de (D. Graf), jmonk02@uoguelph.ca (J.M. Monk), wenqing.wu@canada.ca (W. Wu), hwelling@uoguelph.ca (H.R. Wellings), lrobinso@uoguelph.ca (L.E. Robinson), Krista.Power@uottawa.ca (K.A. Power).
[1] indicates co-first authors.

https://doi.org/10.1016/j.jff.2019.103625
Received 12 February 2019; Received in revised form 30 September 2019; Accepted 8 October 2019
Available online 01 November 2019

compounds (e.g. lignans, flavonoids), and their microbial-derived secondary metabolites have also demonstrated diverse effects on the colonic microenvironment including altering the microbial community structure (Anhe et al., 2015; Duenas et al., 2015; Roopchand et al., 2015), enhancing intestinal barrier integrity (Suzuki & Hara, 2011; Ulluwishewa et al., 2011), attenuating oxidative stress (Giusti, Caprioli, Ricciutelli, Vittori, & Sagratini, 2017; Palla, Iqbal, Minhas, & Gilani, 2016; Sung & Park, 2013; Xu, Yuan, & Chang, 2007; Yao et al., 2010), and reducing colonic inflammation (Koh et al., 2018; Palla et al., 2016; Pandurangan, Mohebali, Norhaizan, & Looi, 2015). Thus, increased consumption of foods enriched in both fermentable dietary fibre and phenolic compounds may promote colon health.

Pulses (beans, chickpeas, lentils, and peas) are important legumes worldwide, providing excellent dietary sources of protein, fibre, micronutrients, and phytochemicals (Brummer, Kaviani, & Tosh, 2015; Campos-Vega, Loarca-Piña, & Oomah, 2010; Chen, McGee, Vandemark, Brick, & Thompson, 2016; Rebello, Greenway, & Finley, 2014). Using the dextran sodium sulfate (DSS) mouse model for acute colitis-associated epithelial damage, we have demonstrated the colon health promoting potential of diets supplemented with cooked pulses, namely common beans (i.e. navy, black, kidney and cranberry bean varieties) and chickpeas, for up to three weeks prior to a DSS challenge (Monk et al., 2018, 2015, 2016, 2018). In these studies, we observed that pre-feeding dietary pulses beneficially primed the colonic microenvironment, which lead to an attenuation of the ensuing inflammatory response and colonic epithelial damage induced by DSS (Monk et al., 2018, 2015, 2016, 2018). Thus far, the potential for dietary lentils to similarly enhance colon health and attenuate DSS-induced epithelial barrier damage and inflammation has not been investigated.

Lentils (*Lens culinaris L.*) are a type of dietary pulse that exhibits a range of human health promoting effects, including anti-oxidant, anti-diabetic, and cardio-protective effects (Ganesan & Xu, 2017; Mudryj, Yu, & Aukema, 2014). With respect to intestinal health, lentils have been shown to improve laxation (Stephen, Dahl, Sieber, van Blaricom, & Morgan, 1995), reduce colorectal cancer cell proliferation *in vitro* (Xu & Chang, 2012) and reduce colonic aberrant crypt foci number in a rodent colon carcinogenesis model (Faris, Takruri, Shomaf, & Bustanji, 2009). However, the role of lentils in modulating colitis-associated disease processes are not known. Recently, we have demonstrated in mice that feeding cooked lentil supplemented diets for 3 weeks could modify the colonic microenvironment by increasing fecal SCFA levels, microbial abundance of SCFA-producing species (*Prevotella*, *Roseburia* and *Dorea*), and colonic mRNA expression of tight and adherens junctions components (Graf et al., 2019). Mechanistically, the intestinal-health promoting effects of lentils may be attributed to the products of microbial fermentation (e.g. SCFAs) from non-digestible lentil carbohydrate fractions (soluble fibre, resistant starch, oligosaccharides) (Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008; de Almeida Costa, da Silva Queiroz-Monici, Pissini Machado Reis, & de Oliveria, 2006; Hernandez-Salazar et al., 2010; Johnson, Thavarajah, Combs, & Thavarajah, 2013; Stephen et al., 1995; Tosh & Yada, 2010). Lentils have also been shown to contain a diverse profile of phytochemicals categorized into phenolic acids, flavanols, flavonols, soyasaponins, phytic acid and condensed tannins (Duenas, Hernandez, & Estrella, 2002; Xu & Chang, 2012; Zhao, Du, Wang, & Cai, 2014; Zou, Chang, Gu, & Qian, 2011), that exhibit enhanced anti-oxidant activity (Hernandez-Salazar et al., 2010; Xu & Chang, 2012; Zhao et al., 2014; Zou et al., 2011). Importantly, when compared to other pulse types, lentils contain lower levels of non-digestible carbohydrates, including total dietary fibre (Chen et al., 2016), resistant starch (Brummer et al., 2015), soluble fibre (Chen et al., 2016), and galacto-oligosaccharides (Chen et al., 2016), but higher concentrations of phytochemicals such as phenolic compounds (Oomah, Patras, Rawson, Singh, & Compos-Vega, 2011; Silva-Cristobal, Osorio-Díaz, Tovar, & Bello-Pérez, 2010; Zhao, Du, Wang, & Cai, 2014), and therefore, may be expected to exert differential effects on colitis severity as demonstrated previously with beans and chickpeas (Monk et al., 2018; Monk et al., 2018). The objective of this study was to determine the effectiveness of lentil supplemented diets to attenuate the severity of colitis-associated epithelial damage and inflammation, in a mouse model of acute colitis.

2. Material and methods

2.1. Preparation of lentil flour and diets

Red lentils (CDC Maxim) were weighed in 200 g batches for cooking, wherein they were placed in a colander and rinsed under deionised water for 1 min. The excess water was drained and lentils were placed in a pot with 500 ml of deionised water, brought to a boil and allowed to simmer for 30 min until tender. Once cooked, lentils were cooled, blended and freeze dried to produce a cooked lentil powder with uniform particle size achieved by sifting it through a brass wire sieve with a 1 mm pore size (VWR, Mississauga, ON, Canada) (Monk et al., 2017). Lentil powder macronutrient and insoluble and soluble fibre content was analyzed by Maxxam Analytics (Mississauga, Ontario, Canada) (27.6% protein, 47.9% available carbohydrates, 1.6% fat, 14.3% insoluble fibre, and 3.4% soluble fibre). Diet compositions were based on the AIN-93G diet formulation with minor modifications including the substitution of soybean oil with corn oil and an increase in the cellulose content (from 5% to 7%). Two isocaloric diets were prepared by Envigo (Madison, WI, USA), the control basal diet (BD) and the lentil diet (LD) which consisted of the BD supplemented with 20% red lentil powder (by weight, Table 1). According to the USDA National Nutrient Database, this level of pulse intake is equivalent to a 20% of energy intake, thereby confirming that the 20% lentil supplementation level utilized in this study represents an intake level that is achievable through the diet, as seen previously with beans and chickpeas (Monk et al., 2017, 2015, 2016, 2017).

2.2. Experimental design

All experimental procedures were approved by the institutional animal care committee (University of Guelph; animal use protocol #3130) in accordance with the guidelines of the Canadian Council of Animal Care. Five-week old C57BL/6 male mice were purchased from Charles River (Portage, MI, USA) and housed as described previously (Monk et al., 2017; Zhang et al., 2014) and a diagram of the experimental timeline is provided in Supplementary Fig. 1. Mice were acclimated to the BD for 1 week prior to random assignment to either the BD or LD, which were consumed for 3 weeks (pre-feeding period prior to colitis onset). After 3 weeks of dietary intervention a subset of mice

Table 1
Experimental diet composition.[1]

Ingredients (g/kg of diet)	Diets	
	BD	LD
Lentil flour	0	200
Casein	200	138
L-Cystine	3	3
Corn starch	377.5	277.2
Maltodextrin	132	132
Sucrose	100	100
Corn oil	70	67.5
Cellulose	70	34.8
Mineral Mix (AIN-93G-MX)	35	35
Vitamin Mix (AIN-93G-VX)	10	10
Choline bitartrate	2.5	2.5
TBHQ	0.014	0.014

All diets are isocaloric and contained 19.2% protein, 17.6% fat and 63.2% carbohydrate (as percent kcal).

[1] BD, basal diet; LD, lentil diet; TBHQ, tertiarybutylhydroquinone.

(n = 9/dietary group) were sacrificed and pre-DSS cecum content was collected for SCFA analyses. Cecum contents were isolated, weighed and stored at −80 °C. All remaining LD fed mice were switched to consume the BD one day (24 h) prior to the initiation of colitis, and remained on BD for the duration of the study. The rationale for having all mice consume the BD during colitis is to remove the confounding effects of experimental diet/DSS interactions that have been reported elsewhere (Piazzi et al., 2014), and to more accurately mimic Inflammatory Bowel Disease (IBD) patient consumption patterns of highly fermentable foods, such as beans during a period of active disease (MacDermott, 2007; Zallot et al., 2013) as seen previously with other pulse types (Monk et al., 2018, 2015, 2016). Acute colitis was induced via 5-day exposure to DSS (2% w/v in the drinking water, molecular weight 36,000–50,000; MP Biomedicals, Santa Ana, CA, USA) and mice were terminated by cervical dislocation at three time points (n = 12/dietary group/time point): (i) after acute colitis induction (5d DSS), (ii) 3 days following the removal of DSS from the drinking water (3d post-DSS), and (iii) 8 days following the removal of DSS from the drinking water (8d post-DSS). Water and diet intake were recorded daily during the experimental period in addition to criteria that constitute the Disease Activity Index (DAI) score, which is reflective of IBD clinical symptoms and includes body weight loss, stool consistency, and the presence of blood in the stool (Monk et al., 2015, 2016; Zhang et al., 2014). Blood was collected at sacrifice by cardiac puncture, allowed to clot for 30 min, centrifuged at 12,000 *g* for 4 min and stored at −80 °C. Colons were excised (from the ceco-colonic junction to the rectum), measured for length and weighed. Colons were opened longitudinally and one longitudinal half colon was Swiss-rolled and fixed in 10% buffered formalin solution for later histological analysis. The remaining colon tissue was snap frozen in liquid nitrogen and stored at −80 °C.

2.3. SCFA analysis

Gas chromatography was used to measure cecal SCFA concentrations (acetate, propionate and butyrate) as described previously (Monk et al., 2015; Power et al., 2016; Zhang et al., 2014).

2.4. Colon histopathology

Colon Swiss rolls were embedded in paraffin, sectioned (5 µm), placed on glass slides, deparaffinised and stained with Alcian Blue/nuclear fast red. From proximal colon through to the distal colon, a crypt erosion score (1–6) was assigned based on the criteria described previously (Zhang et al., 2014) with each score multiplied by the percentage of the colon area affected (such that the most damaged colon would have a maximum score of 600). This data is presented as the histological damage score as conducted previously (Monk et al., 2015, 2016; Power et al., 2016; Zhang et al., 2014). All images were captured using a BX51 microscope (Olympus America, Inc., Center Valley, PA, USA) equipped with an Olympus DP72 Digital Camera System.

2.5. Colon mRNA expression

Colon RNA and protein were isolated using the RNA/protein Purification Plus Kit (Norgen Biotek, Thorold ON, Canada) with total cellular protein quantified using the Pierce BCA Protein Assay Kit (Fisher Scientific). Total RNA (2 µg) was converted to cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) and relative mRNA expression of target genes were assessed by quantitative real-time PCR using Power SYBR Green PCR Master Mix (Applied Biosystems) and the 7900HT Fast Real-Time PCR system (Life Technologies Inc., Burlington, ON, Canada). Data were analyzed using the ΔΔCT method and normalized to the expression of the housekeeping gene *RPLP0*. Primer sequences have been validated and published previously (Monk et al., 2017, 2015, 2016; Power et al., 2016).

2.6. Colon transcription factor activation

Equal amounts of colon tissue protein (10 µg/sample/assay) were utilized to determine the activity of both NFκB p65 and STAT3 by measuring the ratio of activated (i.e. phosphorylated) to total protein levels of NFκB p65 and STAT3 using InstantOne ELISA kits as per the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA) with absorbance read at 450 nm using the PowerWave XS2 plate reader (BioTek, Winnooski, VT, USA). The activated forms of each transcription factor were phosphorylated-NFκB p65 (Ser 536) and phosphorylated-STAT3 (Tyr 705).

2.7. Colon cytokine and chemokine protein expression

An equal amount of colon tissue protein (25 µg/sample) was used to determine the concentration of TNFα, IL-6, IL-10, IL-17A, IL-18, IL-23 by multiplex using the ProcaraPlex mouse basic kit (Affymetrix) and the Bio-Plex 200 System equipped with Bio-Plex Manager, version 6.0 software (Bio-Rad, Mississauga, ON, Canada).

2.8. Serum lipopolysaccharide binding protein (LBP)

Serum levels of mouse lipopolysaccharide binding protein (LBP) were measured by ELISA as per the manufacturer's instructions (Hycult Biotech, Plymouth Meeting, PA, USA) with absorbance read at 450 nm (PowerWave XS2 plate reader, BioTek, VT, USA).

2.9. Statistics

Colon tissue histological damage scores were analyzed by two-way ANOVA (main effects: diet and colitis time point) and repeated measures Mixed-effect analysis model was used for the DAI analyses. All other data were analyzed using the Student's *t*-test and differences between dietary groups were considered significant with $P \leq 0.05$. All values are expressed as means ± SEM. Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Body weight, food intake and cecal SCFA concentrations prior to DSS exposure

During the three-week dietary intervention period prior to DSS exposure, there were no differences between dietary groups in food intake, initial or final body weight (Table 2). Cecal SCFA concentrations were measured from mice euthanized prior to colitis induction, which

Table 2
Pre-DSS body weight, food intake and cecal SCFA concentrations.[1]

	BD	LD
Initial Body Weight (g)	18.40 ± 0.31	18.42 ± 0.35
Pre-DSS Body Weight (g)	22.36 ± 0.30	22.51 ± 0.33
Food Intake (g/mouse/day)	2.49 ± 0.04	2.49 ± 0.18
Cecum SCFAs (µmol/g dry weight)		
Total SCFAs	54.42 ± 3.48	65.88 ± 3.23*
Acetate	27.84 ± 2.20	35.16 ± 1.83*
Propionate	11.20 ± 0.50	13.91 ± 0.56*
Butyrate	11.30 ± 0.85	13.70 ± 1.12*

[1] Data are mean values ± SEM. Body weight and diet intake data pre-DSS was collected for all mice from each time point combined (n = 45/dietary group). Cecal SCFAs were analyzed from a subset of mice sacrificed prior to DSS exposure (n = 9/dietary group). Data marked with an asterisk (*) differ as analyzed using the Student's *t*-test ($P \leq 0.05$). BD, basal diet; LD, lentil diet; SCFA, short chain fatty acid; DSS, dextran sodium sulfate.

Table 3
Mouse characteristics during the acute colitis experimental period.[1]

	5d DSS		3d post-DSS		8d post-DSS	
	BD	LD	BD	LD	BD	LD
Food Intake (g/mouse/day)	2.24 ± 0.10	2.39 ± 0.06	1.63 ± 0.13	1.83 ± 0.11	2.06 ± 0.08	2.10 ± 0.09
DSS/water Intake (g/mouse/day)	2.71 ± 0.09	2.69 ± 0.04	2.08 ± 0.79	2.14 ± 0.74	2.39 ± 0.68	2.40 ± 0.69
Colon W:L	2.03 ± 0.06	2.10 ± 0.12	2.64 ± 0.06	2.52 ± 0.07	2.53 ± 0.11	2.55 ± 0.13

[1] Data are mean values ± SEM (n = 12/dietary group at each experimental time point). Mice were sacrificed at 3 distinct time points during the acute colitis experimental period namely (i) 5 days DSS exposure (2% DSS, w/v) (n = 12/dietary group), (ii) 3 days post-DSS exposure (n = 12/dietary group) and (iii) 8 days post-DSS exposure (n = 12/dietary group). BD, basal diet; LD, lentil diet; DSS, dextran sodium sulfate; W:L ratio, weight:length ratio.

showed that LD-fed mice had significantly greater SCFA concentrations (total SCFA, acetate, propionate and butyrate) compared to BD as shown in Table 2.

3.2. DAI and colitis-associated tissue damage

Food and water intake did not differ between dietary groups at any point during the DSS cycle, indicating an equal degree of DSS exposure between dietary groups (Table 3). Additionally, macroscopic biomarkers of colitis severity, including the colon weight:length ratio (Charpentier et al., 2012) did not differ between dietary groups (Table 3).

DAI parameters represent colitis-associated clinical symptoms, which include body weight loss, stool consistency and the presence of stool blood. As shown in Fig. 1 A, the severity of the colitis symptoms (i.e. DAI) induced by 5 days of DSS exposure reached the maximal effect three days after the removal of DSS from the drinking water (i.e. at the 3d post-DSS time point). Interestingly, while there was no difference in DAI score between dietary groups for the first 4 days of DSS exposure, on day 5 the LD pre-fed mice exhibited a lower DAI score compared to BD mice, making the 5d DSS exposure time point critical for subsequent investigation of the colitis inflammatory phenotype. A similar outcome was also apparent at day 8 of the experiment (i.e. 3d post-DSS), wherein the BD pre-fed group exhibited a higher DAI score (i.e., more severe colitis-associated clinical symptoms) compared to the mice pre-fed LD, thereby making this another critical time point for subsequent

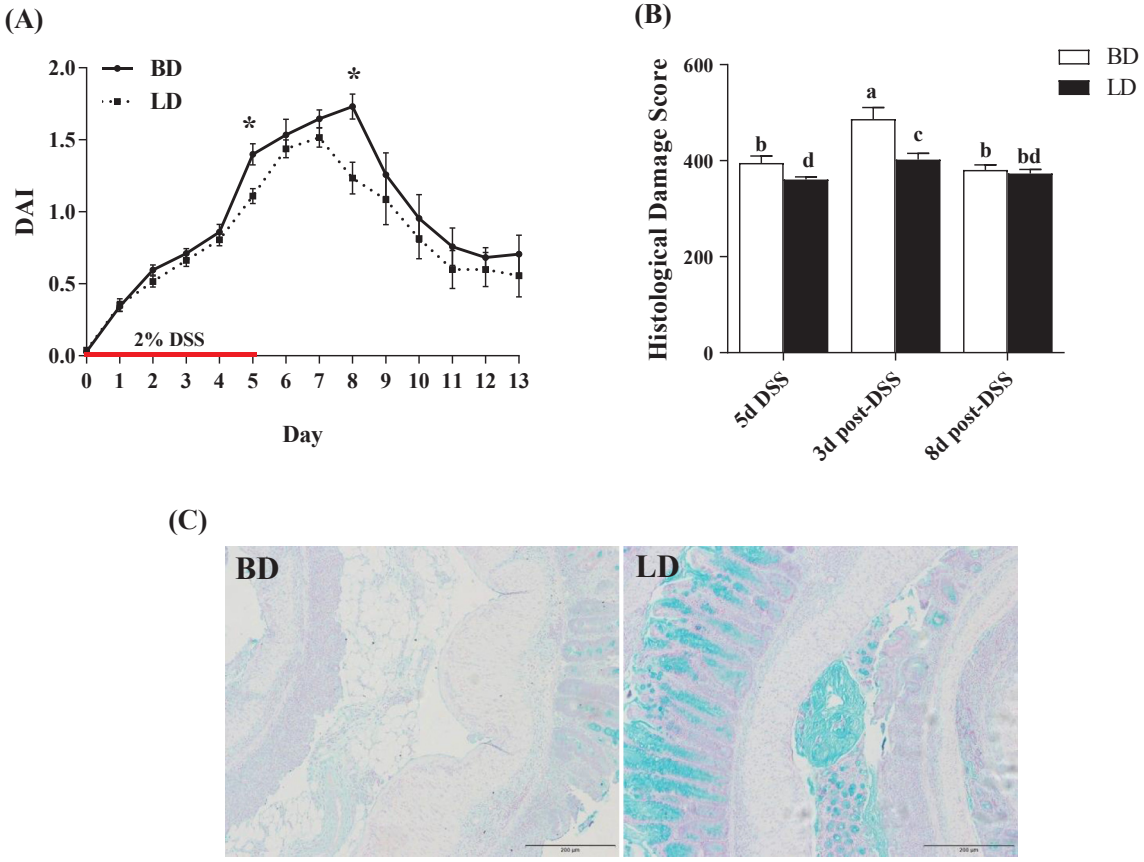

Fig. 1. Effect of LD pre-feeding on acute colitis Disease Activity Index (DAI) (A), colon histological damage score (B) and representative images of colon Swiss rolls (C). (A) DAI data were analyzed using repeated measures mixed-effects model followed by Bonferroni's multiple comparisons test [*denotes $P < 0.05$); n = 36/group from days 0–5 (all mice exposed to DSS for 5d); n = 24/group from days 5–8 (all mice exposed to 5d DSS plus water for 3d post-DSS); n = 12/group from days 8–13 (all mice exposed to 5d DSS plus water for 8d post DSS)]; (B) Alcian blue/nuclear fast red stained colon Swiss rolls were analyzed for histological damage scores. Bars represent mean histological damage score ± SEM (n = 12/dietary group at each time point); differences between groups were analyzed by two-way ANOVA (diet $P = 0.031$; colitis time point $P = 0.001$; interaction $P < 0.0001$); bars without a common letter differ significantly ($P < 0.05$). (C) Representative images of Alcian blue/nuclear fast red stained colon Swiss rolls from the time point 3d post-DSS at 10X magnification (scale bar = 200 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

investigation of the colitis inflammatory phenotype.

DSS-induced histological damage was assessed throughout the entire length of the colon at three time points in the acute colitis cycle: (i) after 5d DSS exposure (day 5 of the experiment), (ii) 3d post-DSS (day 8 of the experiment), and (iii) 8d post-DSS (day 13 of the experiment), and similar to the longitudinal pattern observed during the DAI assessment (Fig. 1A), the histological damage score was the highest three days after the removal of DSS from the drinking water (i.e. at the 3d post-DSS time point) in BD-prefed mice (Fig. 1B). LD pre-fed mice exhibited reduced DSS-induced colonic tissue damage, which was apparent at both the 5d DSS and 3d post-DSS time points, compared to BD (Fig. 1B). These two time points coincided with the reduction in DAI clinical symptoms observed in the mice pre-fed LD compared to BD (Fig. 1A). At the third time point assessed, 8d post-DSS exposure, there was no difference in either histological damage or DAI score between dietary groups, and therefore, we did not conduct further analyses at this time point and focussed only on the degree of inflammation in samples collected at the 5d DSS and 3d post-DSS acute colitis time points.

3.3. Effect of LD pre-feeding on the acute colitis inflammatory phenotype assessed after 5d DSS exposure

Inflammatory mediator mRNA expression in the inflamed colon after 5 days DSS exposure is shown in Fig. 2A. LD pre-fed mice exhibited reduced colon mRNA expression of *IL-6*, *MCP-1* and *IL-1β*, whereas there was no difference in *TNFα* or *IFNγ* expression compared to BD (Fig. 2A). Interestingly, colon mRNA expression of *IL-22*, which plays a role in epithelial barrier protection, maintenance of barrier integrity and promotion of epithelial restitution processes (i.e. epithelial wound closure) (Brand et al., 2006; Mizoguchi, 2012) was increased in LD pre-fed mice, whereas *IL-27* expression, which exhibits similar barrier integrity promoting effects did not differ between dietary groups. Conversely, mRNA expression of *IL-13*, the negative regulator of epithelial wound healing that has been shown to induce apoptosis and delay epithelial restitution processes (Ahdieh, Vandenbos, & Youakim, 2001; Heller et al., 2005; Schulzke et al., 2009) was reduced in LD pre-fed mice versus BD (Fig. 2A). Colon protein expression of TNFα and IL-6 were both reduced in LD pre-fed mice, whereas there was no difference between dietary groups in the protein expression of IL-10, IL-17A, IL-18 or IL-23 (Fig. 2B). Colon tissue inflammatory transcription factor activation status (ratio of phosphorylated to total protein expression) demonstrated a reduction in STAT3 activation in LD pre-fed mice, whereas NFκB p65 activation did not differ between dietary groups (Fig. 2C). Serum LBP levels, which have been shown to contribute to the intestinal and systemic inflammatory response to lipopolysaccharide (LPS) in a dose-dependent manner (Richter et al., 2012), were reduced in LD pre-fed mice compared to BD (Fig. 2D).

3.4. Effect of LD pre-feeding on the acute colitis inflammatory phenotype assessed at 3d post-DSS exposure

In the trajectory of the acute colitis response, after 3d post-DSS exposure colon protein expression of TNFα was sustained, but still

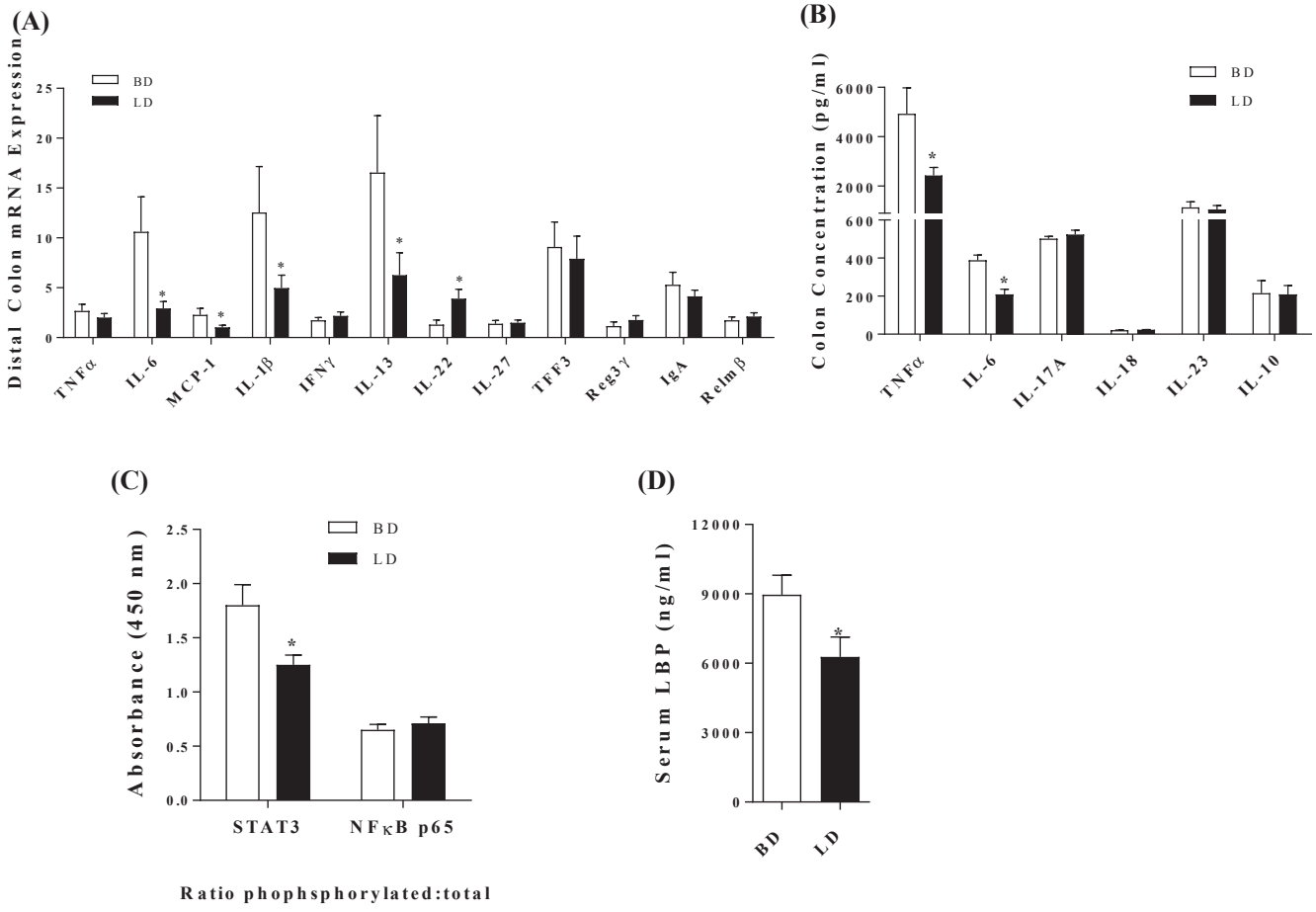

Fig. 2. Effect of LD pre-feeding on the 5d DSS acute colitis phenotype including colon mRNA expression (A), colon protein expression (B), colon transcription factor activation (C), and serum LBP concentration (D). Bars represent means ± SEM (n = 10–12/dietary group). Data for each gene was normalized to the expression of the housekeeping gene *Rplp0*. Transcription factor activation (ratio of phosphorylated to total protein expression) for NFκB p65 (Ser 536) and STAT3 (Tyr 705). All data were analyzed using the Student's *t*-test (*denotes $P < 0.05$). BD, basal diet; LD, lentil diet.

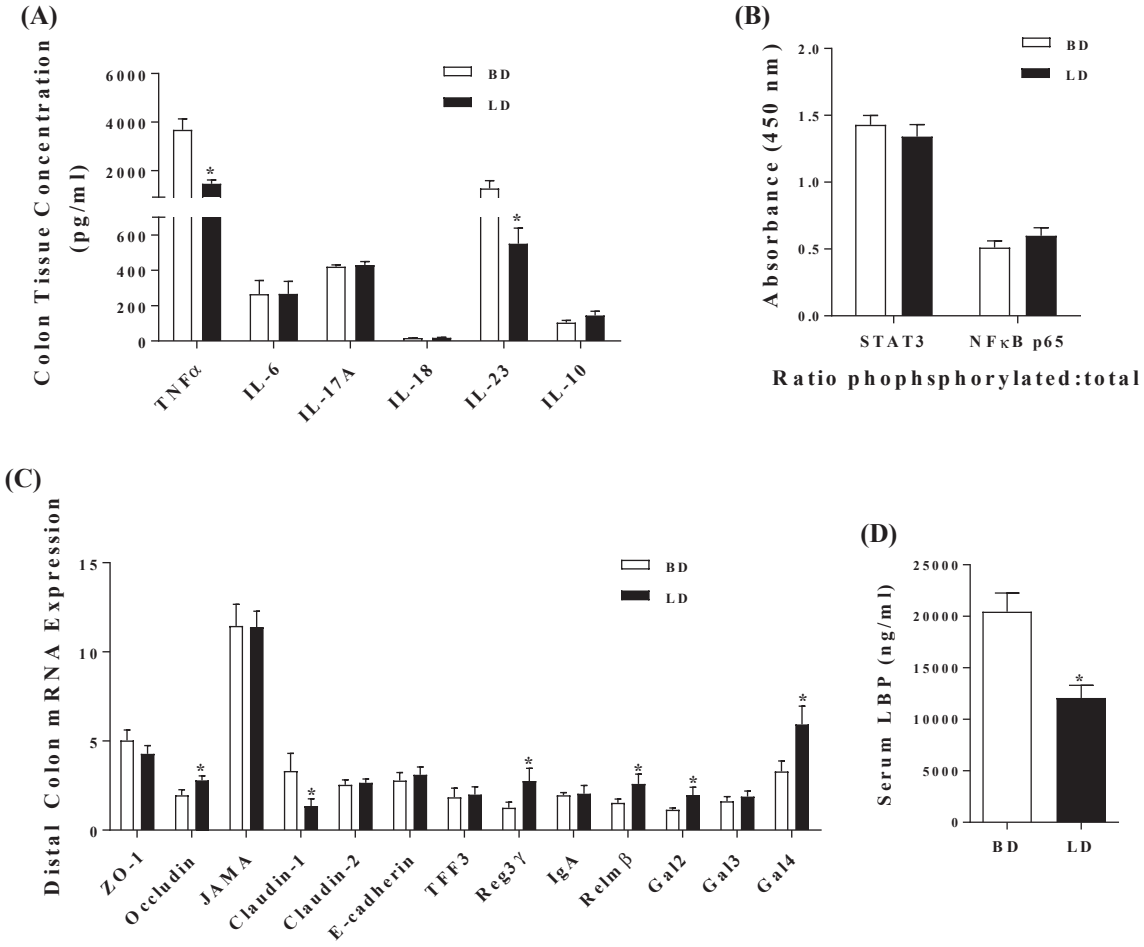

Fig. 3. Effect of LD pre-feeding on the 3d post-DSS acute colitis phenotype including colon protein expression (A), colon transcription factor activation (B), colon mRNA expression (C), and serum LBP concentration (D). Bars represent means ± SEM (n = 12/dietary group). Gene expression data was normalized to the expression of the housekeeping gene *Rplp0*. Transcription factor activation (ratio of phosphorylated to total protein expression) for NFκB p65 (Ser 536) and STAT3 (Tyr 705). All data were analyzed using the Student's *t*-test (*denotes $P < 0.05$). BD, basal diet; LD, lentil diet.

reduced in the LD group compared to BD (Fig. 3A). Additionally, IL-23 colon tissue protein levels were reduced in LD pre-fed mice compared to BD (Fig. 3A). TNFα production is downstream of NFκB transcriptional activity and in colitis the TNFα/NFκB axis induces inflammation and tissue damage (Wullaert, Bonnet, & Pasparakis, 2011), however, activation of the inflammatory transcription factors STAT3 and NFκB p65 did not differ between dietary groups at this time point (Fig. 3B). Interestingly, the serum LBP concentration was reduced in LD pre-fed mice versus BD (Fig. 3D), which coincided with reduced colon histological damage (Fig. 1B), and therefore, may be reflective of improved epithelial barrier integrity. To investigate this further, colon mRNA expression of tight junction complex components was quantified. *Occludin* expression was increased in LD pre-fed mice, although mRNA expression of *ZO-1*, *JAM-A*, *claudin-2* and *E-cadherin* did not differ between dietary groups and *claudin-1* expression was decreased (Fig. 3C). Gene expression of resistin-like molecule beta (*Relmβ*), which functions to promote mucosal barrier integrity and enhances barrier function by exerting immunoregulatory effects (Kim & Ho, 2010) and galectins (*Gal2* and *Gal4*), which function to promote cell migration and help initiate wound closure (Paclik, Lohse, Wiedenmann, Dignass, & Sturm, 2008; Parlato & Yeretssian, 2014), were all increased in LD pre-fed mice (Fig. 3C). Conversely, trefoil factor 3 (*TFF3*) expression, which is involved in mucosal injury repair (Poulsom, Begos, & Modlin, 1996) did not differ between dietary groups. Finally, evidence of enhanced antimicrobial responses during colitis in LD pre-fed mice was supported by increased colon mRNA expression of regenerating islet-derived protein-3γ (*Reg3γ*), which helps to control microbial interaction with the colonic mucosa (Vaishnava et al., 2011), whereas IgA expression did not differ between dietary groups (Fig. 3C). Collectively, these data demonstrate that within LD pre-fed mice the inflammatory response generated during DSS-induced acute colitis is attenuated while mRNA expression of genes related to restoration of colonic homeostasis and re-establishment of colonic tissue function are up-regulated.

4. Discussion

Previously we have shown that pre-feeding dietary pulses (common bean varieties and chickpeas) prior to a colitis challenge can beneficially prime the colon and reduce colitis severity (Monk et al., 2018, 2015, 2016, 2018). However, not all pulses are anticipated to exert the same effects within the colonic microenvironment given that they exhibit differences in their non-digestible carbohydrate and phenolic compound profiles (Chen et al., 2016; Silva-Cristobal et al., 2010), substrates for microbial production of bioactive metabolites (namely SCFAs and secondary phenolics, respectively), that influence intestinal health and colonic function (Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016). In this regard, we have observed in healthy unchallenged mice, that unlike the effects of different cooked common bean varieties on colonic microenvironment, cooked red lentils did not alter architectural changes to the colon epithelial barrier (e.g. crypt height, goblet cell number and mucus content) (Graf et al., 2019; Monk et al., 2017). Therefore, in the current study we assessed the effect of

pre-feeding a diet supplemented with 20% cooked red lentils on the DSS-induced acute colitis phenotype including disease clinical symptoms (DAI), colonic epithelial histological damage, biomarkers of colonic function, and inflammatory mediator production.

In the DSS-induced acute colitis model, DSS causes injury to the epithelial barrier and induces a secondary (or indirect) inflammatory response characterized by the production of a panel of pro-inflammatory cytokines (Alex et al., 2009; Atreya et al., 2000; Hernandez-Salazar et al., 2010; Kitajima, Takuma, & Morimoto, 1999; Li, Alli, Vogel, & Geiger, 2014; Low, Nguyen, & Mizoguchi, 2013; Maloy & Powrie, 2011) produced from multiple cellular sources including epithelial cells and diverse immune cell types. LD pre-fed mice exhibited reduced colonic epithelial histological damage and disease symptoms (DAI) (Fig. 1A and 1B) at both the 5d DSS and 3d post-DSS time points within the acute colitis disease trajectory. Furthermore, colon inflammatory mediator mRNA and protein expression was attenuated in LD pre-fed mice at both time points, although the effects were more pronounced after 5 days of DSS exposure (Figs. 2 and 3). Specifically, at the 5d DSS time point, LD pre-fed mice exhibited reduced colon protein expression of TNFα, IL-6 and reduced activation of STAT3 (Fig. 2A and 2B), mediators which promote colonic epithelial barrier damage and exacerbate colitis severity (Allocca, Jovani, Fiorino, Schreiber, & Danese, 2013; Kojouharoff et al., 1997; Lee et al., 2012; Li et al., 2010). In addition to the reduced mRNA expression of other inflammatory cytokines (*IL-1β* and *MCP-1*), LD pre-fed mice exhibited reduced expression of *IL-13,* which functions to antagonize epithelial wound healing (Ahdieh et al., 2001; Heller et al., 2005; Schulzke et al., 2009), and increased expression of *IL-22*, which promotes epithelial barrier protection, integrity and restitution processes (Brand et al., 2006; Mizoguchi, 2012). Three days after the removal of DSS from the drinking water (3d post-DSS), the intensity of the colitis response reached its maximum, which is reflected in the clinical symptoms comprising the DAI score and the degree of colon epithelial histological damage in the BD group (Fig. 1A and 1B). The improvements in these parameters in LD pre-fed mice coincided with reduced serum LBP concentrations (Fig. 2D and 3D), which is commonly used as a surrogate measure of inflammation (Branescu, Serban, Savlovschi, Dascalu, & Kraft, 2012; Heumann et al., 1995; Heumann, Gallay, Le Roy, & Glauser, 1995; Pal et al., 2015) and microbial translocation that is reflective of enhanced epithelial barrier permeability (Stehle et al., 2012). Furthermore, colon protein expression of TNFα and IL-23 were reduced in LD pre-fed mice (Fig. 3A), and antagonism of these colitis-associated inflammatory cytokines represents a clinically relevant treatment intervention, as seen in IBD with neutralizing cytokine therapeutic approaches (Cayatte et al., 2012; Colombel et al., 2007; Elson et al., 2007; Jacob, Targan, & Shih, 2016; Thomson, Gupta, & Freeman, 2012; Yen et al., 2006; Zhang et al., 2007). Interestingly, in support of the promotion of inflammation resolution and epithelial repair, LD pre-fed mice exhibited increased colon mRNA expression of *Relmβ* (which has been shown to promote epithelial barrier integrity and regulates colonic inflammation) (Hogan et al., 2006; Kim & Ho, 2010; Krimi et al., 2008; McVay et al., 2006) and galectins (*Gal2* and *Gal4*; Fig. 3C), which function to promote cell migration and help initiate wound closure (Paclik et al., 2008; Parlato & Yeretssian, 2014). Moreover, the tight junctional component *occludin*, which exhibits reduced expression in IBD patients (Gassler et al., 2001), was increased in LD pre-fed mice, along with the anti-microbial *Reg3*γ (Vaishnava et al., 2011), thereby indicating that some components of barrier defense are enhanced in LD pre-fed mice (Fig. 3C). Furthermore, *claudin-1* mRNA expression was reduced in LD pre-fed mice, which may reflect a beneficial outcome given that up-regulated claudin-1 expression is associated with intestinal inflammation and may increase the susceptibility to inflammation-associated colon tumorigenesis (Mees et al., 2009; Pope et al., 2014; Poritz, Harris, Kelly, & Koltun, 2011; Weber, Nalle, Tretiakova, Rubin, & Turner, 2008). Collectively, these data indicate that mice pre-fed LD, may be positioned sub-clinically to resolve the colitis-associated inflammatory response, restore colonic homeostasis and re-establish tissue function more effectively compared to BD control, however, longitudinal studies assessing epithelial repair post-DSS colitis are required.

Pulses represent a novel dietary strategy to alter colonic physiological baseline function and mitigate colonic disease severity, given that lentils are rich sources of non-digestible and fermentable carbohydrates (Chung et al., 2008; de Almeida Costa et al., 2006; Hernandez-Salazar et al., 2010; Johnson et al., 2013; Stephen et al., 1995) and phenolic compounds (Duenas et al., 2002; Xu & Chang, 2012; Zou et al., 2011), which are metabolized into bioactive SCFA and secondary phenolic metabolites, respectively. Attenuation of the acute colitis phenotype has been reported previously with pulse pre-feeding prior to colitis onset with common bean varieties and chickpeas, which also demonstrated that the priming effects of dietary pulses on colonic function can persist at least for one week following their removal from the diet (Monk et al., 2018, 2015, 2016, 2018). Additionally, these effects are observed when pulses are consumed in a manner that mimics IBD patient consumption patterns of fermentable foods such as pulses (i.e. prior to disease relapse) (MacDermott, 2007; Zallot et al., 2013). After 3 weeks of LD supplementation (prior to colitis induction), cecal SCFA concentrations of acetate, propionate and butyrate were increased (Table 2). This is in line with our previous study in which red lentil consumption increased fecal SCFA and changed microbiota composition in healthy unchallenged mice (Graf et al., 2019). It is likely that this represents the dominant mechanism through which lentil consumption, prior to colitis onset, attenuates disease severity. SCFA, have been shown to enhance gut barrier integrity (Peng, Li, Green, Holzman, & Lin, 2009) and exert anti-inflammatory effects via down-regulating activation of inflammatory signalling pathways and cytokine production (Andoh, Bamba, & Sasaki, 1999; Butzner et al., 1996; Di Sabatino et al., 2005; Hamer et al., 2008; Macfarlane & Macfarlane, 2012; Roediger, 1980; Scheppach et al., 1992; Segain et al., 2000; Steinhart et al., 1996), as evidenced by the use of SCFA enemas as a colitis intervention (Butzner et al., 1996; Cummings, 1997; Harig et al., 1989). Additionally, lentil-derived phenolic compounds have been shown to exert anti-oxidant activity (Hernandez-Salazar et al., 2010; Xu & Chang, 2012; Zhao et al., 2014; Zou et al., 2011), which is beneficial within the context of DSS-induced colitis wherein oxidative stress has been shown to increase within the colonic tissue (Te Velde, Pronk, de Kort, & Stokkers, 2008; Tham, Whitin, & Cohen, 2002). Future studies are required to ascertain which lentil bioactive(s) underlie the improved colitis phenotype; however, given that lentils are commonly consumed as a whole food the results from this study demonstrate the translational potential for lentil consumption as a dietary approach to mitigate colonic inflammation and may be useful if employed by IBD patients. Frequent pulse consumption during periods of disease remission may prime colonic function such that remission duration is extended and the severity of periods of disease relapse are attenuated.

The translational potential of these findings to humans is supported by the utilization of an experimental design that includes the removal of the lentil diet prior to disease onset, which reflects IBD patient consumption patterns of fermentable foods, such as pulses, during periods of disease activity (MacDermott, 2007; Zallot et al., 2013), while concomitantly minimizing the confounding effects between DSS and dietary components consumed during active colitis, as seen previously (Geier, Butler, Giffard, & Howarth, 2007; Jia et al., 2011; Piazzi et al., 2014; Zarepoor et al., 2014). Additionally, the 20% lentil supplementation level utilized in this study represents a physiologically relevant and achievable intake level of dietary pulses in humans (Jenkins et al., 2012; Olmedilla-Alonso et al., 2013; Thompson et al., 2012), which mimics the highest intake level of Canadian pulse-consumers that is reported to be approximately 295 g/day of pulses as part of an average 2390 kcal/day diet (Mudryj et al., 2012). In humans the use of pulse flours (including lentils) as a means of dietary incorporation has been shown to exert beneficial post-prandial blood glucose responses

(Anderson et al., 2014), and thus, the incorporation of pulse flour as a "value-added" ingredient represents a useful dietary approach to increase and/or promote pulse consumption, particularly in low-consuming populations.

Ethics statement

ARRIVE guidelines have been followed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Christine Carey (Agriculture and Agri-food Canada) for assistance with cooking lentils and data entry, and Laurel McGillis, Danyelle Liddle and Amber Hutchinson (University of Guelph) for their assistance with tissue collection. This work was supported by the Agriculture and Agri-Food Canada-Pulse Science Cluster (AIP-CL03) and University of Ottawa. D.G. was supported by the German Research Foundation (GR 4449/2-1(2)).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2019.103625.

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