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ARTICLE

Cecal and Colonic Responses in Rats Fed 5 or 30% Corn Oil Diets Containing Either 7.5% Broccoli Dietary Fiber or Microcrystalline Cellulose

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Growing evidence suggests that microbiota in the human gastrointestinal tract play a crucial role in mediating the effects of foods on colonic health and host metabolism. The large bowel ecosystem is known to be perturbed in humans and animals fed high-fat diets and conversely to be protected by fermentable oligosaccharides. We examined the ability of largely fermentable dietary fiber from broccoli (*Brassica oleracea* L. var. *italica*) and minimally fermented microcrystalline cellulose to buffer against the effects of high-fat intakes. The results showed that high fat lowered food intakes and therefore fiber intake by 27%. The addition of fermentable oligosaccharide to the diet was shown to be beneficial to some microbiota in cecum, altered cecal short-chain fatty acids, and increased the colon crypt depth and the number of goblet cells per crypt in high- and low-fat diets. Although, the fat level was the predominant factor in changes to the large bowel ecosystem, we have shown that broccoli fiber conferred some protection to consumption of a high-fat diet and particularly in terms of colon morphology.

KEYWORDS: Brassicaceae; carboxylic acids; goblet cells; high-fat diet; large bowel; microbiota

INTRODUCTION

The intestinal microbial community is a complex, diverse ecosystem consisting of more than 500 different microbial species (*1*), which plays an important role in maintaining human health via its impact on a range of host physiological and metabolic functions. Short-chain fatty acids (SCFAs), the end products of microbial fermentation, are important for a healthy colonic mucosa (*2*) and could favor mineral absorption (*3*). Additionally, the SCFA butyric acid is considered to be a major energy source for colonocytes (*4*), has a regulatory effect on the cell cycle (*5*), and plays a major role in the prevention of colitis (*6*). Non-digestible carbohydrates have been shown to provide positive health benefits by modulating the intestinal microbial composition and SCFA concentrations (*7*). Modification of intestinal microbiota may in turn influence the dynamics of goblet cells and the mucus layer of gastrointestinal (GI) epithelium via either release of bioactive factors or stimulation of the host immune system (*8*). The epithelium of the GI tract is covered by a mucus layer, which is synthesized and secreted by goblet cells and plays an important role in the innate immune system. Earlier studies have demonstrated the morphological characteristics of intestinal goblet cells and mucus layer in conventional and germ-free animal models (*9*, *10*). Certain bacterial groups, such as *Bifidobacterium* spp., are obligate mucin dwellers; therefore, integrity of the mucus layer has important implications for beneficial microbes. Breves et al. (*11*) reported a prebiotic effect of oligosaccharides modifying the intestinal tract functions in pigs through increased ileal goblet cells and thickness of the colonic mucosa.

Previous studies that have investigated the effects of fat intake on microbiota and host reactions in the large bowel showed that high-fat intakes diminished the protection provided by the colonic microbiota. Low-grade inflammatory response and metabolic disease has also been shown to be associated with a decrease in *Bifidobacterium* spp. in mice fed a high-fat diet compared to the control (*12*); when oligosaccharides (10% oligofructose) that are readily fermented undigestible carbohydrates were added to the high-fat diet, inflammatory response was normalized and *Bifidobacterium* spp. was restored (*13*).

Brassica vegetables, such as Brussels sprouts, have been shown to modulate the ecology of the gut environment (*14*) and reduce DNA damage in hepatocytes and colonocytes of rats (*15*). *Brassica* vegetables are also a source of fermentable dietary fiber; therefore, it is possible that the mechanism behind the protective effect of feeding oligosaccharides may also apply to *Brassica* vegetables. Given the current emphasis on vegetable-oil-rich diets as a healthy alternative to saturated fats, we have chosen to investigate the effects of diets rich in polyunsaturated fatty acids on the microbiota and associated host parameters in rats and examine the extent to which any effects could be modulated by the

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dietary fiber background. We used 30% corn oil as a high-fat treatment of relevance to human intakes and examined its effect in diets containing either broccoli fiber as a largely fermentable non-starch polysaccharide or cellulose, which is minimally fermented in the large bowel of rats. We hypothesized that fermentable vegetable fiber could play a role in protecting the gut microbiota and preserve the beneficial downstream effects, such as SCFA concentrations and changes to colon morphology, against the background of a high-fat diet.

MATERIALS AND METHODS

Dietary Fiber Preparation. Broccoli (*Brassica oleracea* L. var. *italica*) stems were collected from growers in the Manawatu region (New Zealand) and were stored at 4 °C until processing. Broccoli fiber processing was carried out at the food technology pilot plant, Massey University (Palmerston North, New Zealand). A cold-water, aqueous maceration method was used to minimize the loss of total non-starch polysaccharide and pectin. The broccoli fiber was freeze-dried and milled through 1 mm mesh fitted to a grinding mill and stored at room temperature until further use. The freeze-dried dietary fiber preparation contained more than 95% dietary fiber, determined as the dry weight remaining after pepsin and pancreatin digestion, and 80% ethanol precipitation.

Animals and Diets. Male, 9-week-old Sprague−Dawley rats were housed individually in raised stainless-steel cages with mesh floors. The rat feeding trial was carried out in a temperature-controlled room (22 ± 1 °C), with a humidity of 60 ± 5%, an air exchange of 12 times/h, and a 12 h light/dark cycle. Experimental procedures used in this study were approved by AgResearch Grasslands Animal Ethics Committee, Palmerston North, New Zealand. The rats were randomly allocated into experimental groups (n = 16), high-fat diet with cellulose, low-fat diet with cellulose, high-fat diet with broccoli, and low-fat diet with broccoli, and were fed for 17 weeks. One rat in the low-fat cellulose group died during the trial. Rats were given *ad libitum* access to water and experimental diets. Details of the components in experimental diets are shown in **Table 1**. The food intake and body weight of each rat was recorded every 7 days. At the end of the feeding trial, rats were euthanized by CO_2 asphyxiation. The cecum contents of each rat were collected, snap frozen in liquid nitrogen, and stored at −80 °C for microbial quantification and SCFA analysis. Colon tissue from rats was collected and fixed in 10% formalin for histological analysis.

Table 1. Composition of Experimental Diets (g/kg)[a]

ingredients	high-fat diet with cellulose	high-fat diet with broccoli	low-fat diet with cellulose	low-fat diet with broccoli
lactic casein	120	120	120	120
vitamin mix[b]	50	50	50	50
mineral mix[c]	50	50	50	50
sucrose	40	40	40	40
starch	365	365	615	615
corn oil	300	300	50	50
cellulose	75	0	75	0
broccoli	0	75	0	75
energy (kcal/kg)	4734.5	4734.5	3545.75	3545.75

[a] Diets were formulated to meet the dietary requirements of the laboratory rat (*25*). [b] The mixture contains the following components: (mg/kg diet) retinol acetate, 5.0; DL-α-tocopheryl acetate, 100.0; menadione, 3.0; thiamin hydrochloride, 5.0; riboflavin, 7.0; pyridoxine hydrochloride, 8.0; D-pantothenic acid, 20.0; folic acid, 2.0; nicotinic acid, 20.0; D-biotin, 1.0; *myo*-inositol, 200.0; choline chloride, 1500.0; (μg/kg diet) ergocalciferol, 25.0; cyanocobalamin, 50.0. [c] The mixture contains the following components: (g/kg diet) Ca, 6.29; Cl, 7.79; Mg, 1.06; P, 4.86; K, 5.24; Na, 1.97; (mg/kg diet) Cr, 1.97; Cu, 10.7; Fe, 424.0; Mn, 78.0; Zn, 48.2; (μg/kg diet) Co, 29.0; I, 151.0; Mo, 152.0; Se, 151.0.

Bacterial Strains. The bacterial reference strains used in this study are shown in **Table 2**. Bacterial strains were grown at 37 °C under anaerobic conditions in gas jars with a GasPak System (Oxoid, Adelaide, Australia) in de Man−Rogosa−Sharpe (MRS) broth (Oxoid) for *Lactobacillus reuteri*, MRS broth supplemented with 0.05% cysteine (Sigma-Aldrich, Sydney, Australia) for *Bifidobacterium adolescentis*, and brain heart infusion broth (Oxoid) for *Bacteroides fragilis*, *Enterococcus faecalis*, and *Clostridium perfringens*. After overnight incubation, the bacterial cell count was determined using a Neubauer hemocytometer to generate standard curves for real-time polymerase chain reaction (PCR) quantification.

DNA Extraction. Total DNA was extracted from the preweighed cecum content, which was homogenized using 0.1 mm zirconia−silica beads (BioSpec Products, Inc., Bartlesville, OK) in ASL buffer from QIAamp DNA Stool Mini Kit (Qiagen, Melbourne, Australia). Subsequently, DNA isolation was carried using QIAamp DNA Stool Mini Kit according to the instructions of the manufacturer. Genomic DNA was isolated from the reference bacterial strains using QIAamp DNA Mini Kit (Qiagen), with a modified protocol for Gram-positive cell lysis. The DNA concentration was quantified using a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Real-Time PCR Quantification (qPCR). The oligonucleotide primers and optimized PCR conditions used in this study to quantify cecum microbiota are shown in **Table 2**. Real-time PCR was carried out using a LightCycler 480 instrument, plates, and consumables (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed in triplicate with a reaction volume of 20 μL consisting of 10 μL of LightCycler 480 SYBR Green I Master Mix, 0.5 μM primers, and 5 μL of DNA template or water. Melting curve analysis was performed from 60 to 95 °C (0.1 °C/s) with continuous fluorescence acquisition. Agarose gel electrophoresis was used to confirm the specificity of PCR products. Standard curves were created using a 10-fold dilution series [from 1×10^9 to 1×10^4 colony forming units (CFU)/mL] from genomic DNA of the bacterial strains.

A calibration run was first carried out including the whole standard curve, but in subsequent qPCR runs, only one of the standard dilutions was included. The second-derivative method for determining the crossing point (Cp) of the amplification curve was used, as recommended by the manufacturer (Roche Diagnostics). This method gives the Cp value as the point of greatest change in the slope of the curve and is considered more reliable than the "threshold" method employed traditionally with qPCR. When the second-derivative method is selected, a nonlinear calibration curve is created rather than linear calibration employed elsewhere. The efficiency values obtained are therefore not directly comparable to those derived from the usual linear regression. To calibrate the data of rat samples between qPCR runs, a single standard was used to adjust the intercept of the curve alone. This assumes that the shape of a calibration curve and efficiency remain constant between the qPCR runs.

SCFAs. A gas chromatography (GC) method was used to analyze the SCFAs (acetic, butyric, formic, lactic, propionic, and succinic acids) in

Table 2. Oligonucleotide Primers Used for Real-Time PCR Quantification

target bacteria	reference bacterial strain	primer sequence (5′ → 3′)	annealing temperature (°C)	reference
Bacteroides−*Prevotella*−*Porphyromonas* group	*B. fragilis* NZRM 964	F: GGTGTCGGCTTAAGTGCCAT R: CGGA(C/T)GTAAGGGCCGTGC	63	*26*
Bifidobacterium spp.	*B. adolescentis* ATCC 15703	F: TCGCGTC(C/T)GGTGTGAAAG R: CCACATCCAGC(A/G)TCCAC	63	*26*
C. perfringens group	*C. perfringens* ATCC 13124	F: ATGCAAGTCGAGCGA(G/T)G R: TATGCGGTATTAATCT(C/T)CCTTT	55	*26*
Enterococcus spp.	*E. faecalis* AGR 991	F: CCCTTATTGTTAGTTGCCATCATT R: ACTCGTTGTACTTCCCATTGT	64	*26*
Lactobacillus spp.	*L. reuteri* DPC 16	F: CGATGAGTGCTAGGTGTTGGA R: CAAGATGTCAAGACCTGGTAAG	60	*27*

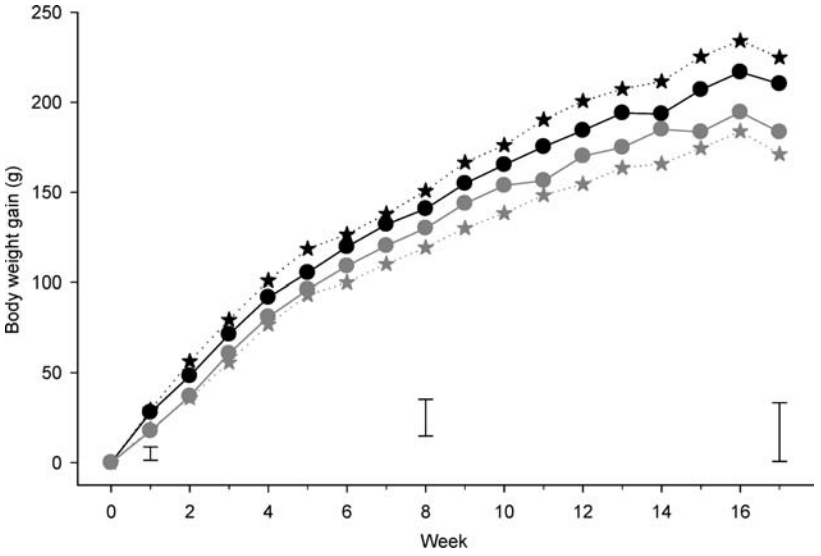

Figure 1. Effect of experimental diets on body weight gain in rats fed for 17 weeks. Mean body weight gain of rats from 0 to 17 weeks. High-fat diet with cellulose (black star), high-fat diet with broccoli (black circle), low-fat diet with cellulose (gray star), and low-fat diet with broccoli (gray circle). Error bars are least significant difference (LSD) between two means at the 5% level [degrees of freedom (df) = 59] for weeks 1, 8, and 17 only.

Table 3. Effect of Experimental Diets on the Body Weight Gain and Food Intake over 17 Weeks

	high-fat diet with cellulose	high-fat diet with broccoli	low-fat diet with cellulose	low-fat diet with broccoli	LSD[a] (df = 60)
body weight gain (g)	224.8	210.3	171.1	183.4	32.5
food intake (g/week)	110.0	110.7	151.2	150.7	11.1

[a] LSD = least significant difference between two means at the 5% level. df = degrees of freedom.

cecal contents of each rat (*16*). Briefly, cecal samples were homogenized with 0.01 M phosphate-buffered saline followed by 2-ethylbutyric acid as the internal standard. After centrifugation (3000*g* for 5 min at 4 °C), extraction of SCFAs was carried out by mixing the supernatant with hydrochloric acid and diethyl ether. After centrifugation (3000*g* for 10 min at 4 °C), the upper diethyl ether phase was collected and then derivatized by adding *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA, Sigma-Aldrich). Samples were kept at room temperature for 48 h, which were then analyzed using a Shimadzu GC-17A GC system fitted with a HP-1 (Agilent J&W) GC column (10 m × 0.53 mm × 2.65 μm). Three runs of the GC were required to analyze the samples from all 63 rats, with rats for each diet spread across the three runs and duplicate samples from a rat used within a run. Six concentrations of a cocktail of the target SFCAs were used as calibration standards and included within each run. Results of linear regressions for these standards were used to convert peak areas for the rat samples into SFCA concentrations. There were technical problems with one of the runs; therefore, the data from this run were discarded. Therefore, in the final data set, there were data between 7 and 11 rats only per diet (37 rats in all).

Colon Histology. A section of colon tissue from each rat was embedded in a paraffin block and processed in a routine manner to generate 5 μm thick paraffin sections, which were stained with hematoxylin and eosin and alcian blue for microscopic examination. A total of 30 colon crypt depths were measured using Image-Pro Plus analysis software (version 4.1) at 20× magnification in a bright field microscope (model Axiophot, Carl Zeiss, Inc., Gottingen, Germany). Goblet cell counts were determined using the same image of the histological section by counting 30 crypts.

Statistical Analysis. Rat body weights, food intake, SCFAs, colon crypt depth, and goblet cell count were analyzed with analysis of variance (ANOVA). Data for the initial body weights were analyzed separately from the final body weights. In addition, changes in body weight for each rat during the 17 week trial were analyzed. In the SFCA data, there were unequal numbers of samples for each diet; therefore, the main (overall) effects of fat and fiber were assessed both before and after adjusting for the other factor (fiber and fat, respectively). For colon morphology data, the variations between rats and between samples of each rat were included separately within the analysis. All analyses included factorial contrasts for the fat level × fiber type interaction.

Bacterial cell numbers estimated by qPCR were analyzed according to the approach described in Murray et al. (*17*). Briefly, estimated bacterial cell numbers were analyzed with a hierarchical generalized linear model approach (*18*). The fat level, fiber type, and their interaction (fat level × fiber type) were included as fixed effects with a Poisson distribution and logarithmic link, and rat−rat differences were included as random effects with a γ distribution and logarithmic link. The log of sample weights was included in the analysis as an offset to allow for the results to be presented as the number of bacterial cells per gram of cecum content. Comparisons between the experimental diets were made using deviances (*18*). Estimated mean bacterial cell numbers were obtained on the $\log_{10}$ scale along with 95% confidence limits. All analyses were carried out with GenStat, 12th edition (VSN International, Hemel Hempstead, U.K.).

RESULTS AND DISCUSSION

Body Weight and Food Intake. At baseline, there were no significant differences ($p = 0.214$ for all of the diets) in body weight between the experimental groups (data not shown). After 17 weeks, the body weight of rats in the experimental groups did not differ significantly ($p = 0.346$ for all of the diets) (data not shown). However, the body weight gain differed significantly ($p = 0.001$) between rats fed high- and low-fat diets (**Figure 1**), with no significant difference between rats fed cellulose compared to broccoli ($p = 0.865$ for the fiber, and $p = 0.250$ for the fat × fiber interaction) (**Table 3**). Rats fed high-fat diets gained an average of 40 g more than those fed low-fat diets. After week 17, there were no significant differences ($p > 0.05$ for the fiber and fat × fiber interaction) in the food intake between diets with cellulose and broccoli. However, rats fed low-fat diets consumed significantly more food compared to those fed high-fat diets ($p < 0.001$).

Cecum Microbiota. High-fat intakes increased some bacterial groups and decreased others, but the direction of changes was

Table 4. Real-Time PCR Quantification of Cecum Microbiota in Rats Fed Experimental Diets[a]

	diet	cellulose	broccoli
Bacteroides−Prevotella−Porphyromonas group	high-fat	9.00 (8.91−9.10)	8.93 (8.84−9.02)
	low-fat	9.07 (8.97−9.16)	8.97 (8.88−9.06)
Bifidobacterium spp.	high-fat	7.09 (6.71−7.47)	7.15 (6.76−7.54)
	low-fat	6.30 (5.89−6.70)	6.70 (6.31−7.09)
C. perfringens group	high-fat	6.22 (5.84−6.60)	6.55 (6.17−6.92)
	low-fat	5.68 (5.28−6.07)	5.15 (4.77−5.53)
Enterococcus spp.	high-fat	5.26 (5.02−5.50)	5.13 (4.89−5.36)
	low-fat	5.23 (4.99−5.48)	5.66 (5.42−5.89)
Lactobacillus spp.	high-fat	6.23 (6.00−6.45)	6.62 (6.40−6.84)
	low-fat	6.72 (6.49−6.95)	6.92 (6.70−7.14)

[a] Estimated mean $\log_{10}$ CFU/g of cecum content (95% confidence intervals).

Table 5. Estimation of SCFAs in Cecum of Rats Fed Experimental Diets[a]

	high-fat diet with cellulose	high-fat diet with broccoli	low-fat diet with cellulose	low-fat diet with broccoli	mean LSD[b]	df[c]
acetic	25.79	51.61	36.71	49.73	9.11	33
butyric	6.08	8.55	7.83	8.63	1.64	33
formic	13.66	19.16	7.51	18.15	8.87	32
lactic	2.47	1.61	2.37	2.99	2.87	32
propionic	6.09	7.25	7.83	9.03	1.46	33
succinic	4.79	4.62	5.24	5.19	0.44	31

[a] The SCFAs are expressed as μmol/g of cecum content. [b] Mean LSD = mean least significant difference between two means at the 5% level. Actual LSD for particular comparisons vary because of the varying numbers of samples per treatment, from 93.3 to 107.7% of the mean LSD. [c] df = degrees of freedom associated with the LSD; these vary because outliers were omitted in some cases.

Table 6. Effect of Experimental Diets on Colon Morphology in Rats Fed for 17 Weeks

	high-fat diet with cellulose	high-fat diet with broccoli	low-fat diet with cellulose	low-fat diet with broccoli	LSD[a] (df = 59)
crypt depth (μm)	145.13	158.54	162.78	176.39	20.60
goblet cells/crypt	11.73	15.71	17.37	20.39	1.78

[a] LSD = least significant difference between two means at the 5% level. df = degrees of freedom.

generally the same in both broccoli fiber and cellulose groups (**Table 4**). Low-fat diets significantly increased ($p = 0.01$) the *Bacteroides−Prevotella−Porphyromonas* group compared to high-fat diets. In contrast, a low-fat diet decreased *Bifidobacterium* spp. compared to a high-fat diet for both fiber types (cellulose and broccoli) ($p = 0.002$). The *Bifidobacterium* spp. were higher in rats fed broccoli diets compared to cellulose. However, the differences did not vary between cellulose and broccoli ($p = 0.23$ for the fiber, and $p > 0.3$ for the fat × fiber interaction). The decrease of *Bifidobacterium* spp. in response to fat in the present study was in contrast with the findings by Cani et al. (*12*). The high-fat diet led to an increase in the *C. perfringens* group compared to the low-fat diet, in both cellulose and broccoli diets ($p = 0.023$ for the fat × fiber interaction). In comparison to low-fat diet, high-fat diet did not affect *Enterococcus* spp. in cecal contents of rats fed cellulose but led to a decrease in rats fed broccoli fiber ($p = 0.019$ for the fat × fiber interaction). *Lactobacillus* spp. were lower with a high-fat diet compared to a low fat diet ($p = 0.001$); this difference did not vary significantly between broccoli and cellulose ($p > 0.3$ for the fat × fiber interaction). The *Lactobacillus* spp. in rats fed the broccoli diet were higher than those fed the cellulose diet ($p = 0.009$).

Cecum SCFA Analysis. In the present study, acetic acid that is usually the most abundant SCFA (*19*) was higher ($p < 0.001$) in rats fed broccoli fiber compared to cellulose, in both high- and low-fat diets ($p > 0.1$ for the fat) (**Table 5**). Butyric acid levels were not strongly affected by fat levels in the diet ($p > 0.1$ for the fat, and $p = 0.052$ for the fat × fiber interaction), but butyric acid levels were generally higher with broccoli than with cellulose ($p = 0.011$). The bacterial groups that produce butyric acid are of interest because of their health-promoting properties. Butyric acid is a preferred energy source for the colonocytes (*4*). The increased butyric acid levels observed in this study could be due to the release from butyrate-producing bacteria or metabolic cross-feeding between the bacteria by converting lactate and acetate (*20*). Excess acetate in the large bowel can also stimulate bacteria possessing coenzyme A (CoA)-transferase (*21*), and through this route, acetate contributes as a co-substrate to the formation of butyrate.

Formic acid levels was higher in rats fed broccoli compared to cellulose ($p < 0.02$), but there was no difference between high- and low-fat diets ($p > 0.1$ for the fat). Lactic acid levels did not vary significantly between the experimental groups ($p > 0.47$ for all of the diets). Propionic acid levels were higher in low-fat diet than high-fat diet ($p = 0.003$) and were greater with broccoli than with cellulose ($p = 0.025$), with a similar effect of fat for both fiber types (cellulose and broccoli) ($p = 0.967$ for the fat × fiber interaction). Succinic acid levels did not vary significantly between broccoli and cellulose ($p > 0.5$ for the fiber and fat × fiber interaction), but there was a significant increase in the low-fat diet compared to the high-fat diet ($p = 0.002$).

Crypt Depth and Goblet Cells in Colon. High-fat diets reducing crypt depths and goblet cells per crypt compared to low-fat diets was observed, and broccoli fiber induced greater crypt depths and goblet cells per crypt than cellulose diets (**Table 6**). Colon crypt depth varied with the fat level, with crypts being on average 18 μm deeper in rats fed low-fat diets compared to those fed high-fat diets ($p = 0.017$). There was also a weaker effect ($p = 0.071$) associated with broccoli fiber, with crypts on average 13.5 μm deeper than in rats fed cellulose diets. Goblet cells per crypt were

significantly increased ($p < 0.001$) in diets with broccoli compared to cellulose (**Table 6**). Irrespective of fiber types, rats fed low-fat diets showed more goblet cells per crypt, with an average of 5 cells per crypt, than those fed high-fat diets ($p < 0.001$). The increase in goblet cells per crypt observed in rats fed broccoli may have increased the thickness of the mucous layer in the large bowel. McCullough et al. (*22*) demonstrated the effect of dietary fiber in increasing goblet cells, crypts per circumference, and crypt branching in the colon of rats. In the large bowel, fermentation of broccoli fiber resulting in increased levels of SCFAs could have played a mediatory role in stimulating the secretion of glucagon-like peptide-2 (*23*), which can promote intestinal cell proliferation and development of normal mucosal epithelium (*24*).

The findings of this study can be explained in terms of dietary fiber intakes and indirectly in terms of fat intakes, when one considers that animals were fed *ad libitum* and they adjusted their food intakes to compensate for the energy density of the diets (**Table 3**). The food intakes were about 110 g per week in rats on high-fat diets compared to slightly over 150 g per week for the rats fed low-fat diets. Therefore, food and dietary fiber intake was reduced by 27% in the high-fat diets. Many of the differences between high- and low-fat diets could therefore have been due to differences in dietary fiber intake as an indirect consequence of the effect of fat in the diet. In this experiment, cellulose was used as a minimally fermented dietary fiber; therefore, it is interesting to note that, in both high- and low-fat diets containing cellulose, appreciable quantities of SCFAs were observed. A possible explanation could be that significant cecal loading of undigested starch from the ileum occurred in rats fed all of the diets. The results suggest that future studies emphasizing the interaction of fat and fiber on colonic health should consider monitoring the progress of starch digestibility in the gut or feeding animals a non-fiber energy source that is completely digested.

In conclusion, data from the current study indicate that broccoli fiber as a fermentable source showed some beneficial effects on cecum microbiota and SCFAs, and enhanced the colon crypt depth and number of goblet cells per crypt.

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