Original Article

The effect of dietary oils on cecal microflora in experimental colitis in mice

Azita Hekmatdoost,[1] Mohammad M Feizabadi,[2] Abolghasem Djazayery,[1] Abbas Mirshafiey,[3] Mohammad R Eshraghian,[4] Samira M Yeganeh,[2] Reza Sedaghat,[5] Kevan Jacobson[6]

Departments of [1]Human Nutrition, [3]Immunology and Pathobiology, and [4]Biostatistics and Epidemiology, School of Health, Tehran University of Medical Sciences, Tehran; [2]Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran; [5]Division of Pathology, Shahed University of Medical Sciences, Tehran, Iran; and [6]Division of Gastroenterology, BC Children's Hospital, Vancouver, Canada

Objectives: In spite of growing evidence indicating the benefits of probiotics, the effects of different dietary oils on intestinal microflora and probiotics have not been elucidated. This study aimed to examine the effects of different dietary oils on intestinal microflora in an experimental model of colitis.

Methods: Eight-week mice were fed isocaloric diets varying only in fat composition for 4 weeks. The oils used were fish oil, canola oil, safflower oil, and chow diet containing beef tallow. Colitis was induced by intracolonic administration of acetic acid on day 21. The inflammation and fecal microflora and serum lipid profiles were evaluated 1 week after induction.

Results: Inflammation was highest in the chow diet group followed by safflower, canola, and fish oil fed groups, respectively. The number of fecal *bacteroideceae* was greater, whereas the number of fecal *bifidobacteria* was lower in mice fed beef tallow than the other ones. In addition, fish oil reduced the plasma level of triacylglycerole significantly.

Conclusion: Polyunsaturated fatty acids (PUFAs) can affect intestinal microflora increasing the number of probiotics. PUFAs might be recommended in addition to probiotics for the prevention and/or maintenance treatment of colitis.

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The intestinal microflora is a complex and exactly balanced ecosystem of various bacteria. Some of these bacteria such as *bifidobacterium* and *lactobacilli* have beneficial effects for the body. They are involved in the prevention of infectious diarrhea,[1] protection of intestinal barrier system,[2,3,4] production of short-chain fatty acids for enterocytes, metabolism of carcinogenic substances and neurotoxic components,[5] vitamin synthesis, and stimulating of the immune system.[5] It is suggested that they have beneficial effects in attenuating some disorders such as certain allergies,[6–9] irritable bowel syndrome,[10,11,12] non-alcoholic steatohepatitis,[13] hypothalamic pituitary adrenal axis disturbances,[14] and infectious diseases.[1,15]

Recent studies indicate that probiotics can ameliorate inflammatory bowel disease and its related conditions such as arthralgia and colon cancer.[16,17,18] On the other hand, some studies have shown that fatty acids high in n-3 can attenuate inflammation of colitis;[19] but there is no study evaluating the interaction between these fatty acids and probiotics in intestinal inflammation. This study aimed to examine the effects of different dietary oils on the number of different cecal bacteria in experimental colitis.

Methods

Animals and diets

Six- to eight-week-old female BALB/C mice (18–20 g) were obtained from the Pasteur Institute (Tehran, Iran). Mice were raised in the animal care facility with a 12 h–12 h light-dark cycle with unlimited access to food and water. Mice were randomized into the following diet groups of 8 mice each: (a) standard chow diet containing beef tallow as its fat component, or (b) a semi-synthetic diet, with 20% energy from the following oils (Table 1): safflower oil (high in 18:2n-6), canola oil (high in 18:1n-9 and 18:3n-3) and fish oil (high in 20:5n-3, 22:6n-3). Experimental diets were produced in-house (Table 2). All diets were isocaloric and isonitrogenous. The diets were stored in airtight containers in a cold room at 4°C, and fed to the animals daily. Weight and mortality were monitored for 4 weeks.

Induction of colitis: After 3 weeks, mice were fasted for 24 h before induction of colitis. After ether anesthesia, a thin catheter was inserted 5 cm into the rectum, and 1 mL of 4% acetic acid (pH 2.3) was slowly infused into the rectal lumen. After 30 sec exposure, excess fluid was

Table 1: Fatty acid composition of the diets*

Fatty acids	*Safflower oil*	*Canola oil*	*Fish oil*
Palmitic acid (16:0)	7.2	5.2	21.9
Stearic acid (18:0)	2.2	1.8	5.7
Oleic acid (18:1n-9)	14.8	59.3	25.2
Linoleic acid (18:2n-6)	74.3	21.0	1.6
Arachidonic acid (20:4n-6)	0.0	0.0	2.5
Alpha linolenic acid (18:3n-3)	0.3	9.8	0.6
Eicosapantanoic acid (20:5-n3)	0.0	0.0	7.2
Docosahexanoic acid (22:6n-3)	0.0	0.0	24.6
18:2n-6:18:3n-3 ratio	256.0	2.1	2.7

Values are as g/100 g. *Fatty acids do not add up to 100, as not all fatty acids quantified are given.

withdrawn and the colon was flushed with 1 mL of phosphate-buffered saline (PBS). Following the enema, they were kept in cages, with free access to water and special diets for a week.

Tissue preparation: At the end of the 4-week experiment, all mice were sacrificed by exsanguinations under chloroform anesthesia, blood samples were centrifuged and plasma stored at -70ºC until they were analyzed. The cecum, spleen, kidney, and liver were removed and weighed. Furthermore, the first 4 cm of each colon, beginning at the anal verge, and cecal content were collected for the assessment of inflammation, and cecal microflora, respectively.

Histopathologic assessment: After macroscopic assessment using the method of Morris *et al*[20] full thickness of the first 1 cm of colonic tissues were fixed in 10% neutral buffered formalin. Sections (3 m) were cut and stained with hematoxylin and eosin. The histological damage was scored using the criteria described by Videla *et al*,[21] which include ulceration (0: none, 1: small ulcers <3 mm, 2: large ulcers >3 mm); inflammation (0: none, 1: mild, 2: moderate, 3: severe); depth of the lesion (0:none, 1: submucosa, 2: muscularis propria, 3: serosa); fibrosis (0: none, 1: mild, 2: severe).

RNA extraction and real-time-PCR: Colonic tissue that remained from the first 4 cm was transferred to TRIzol reagent (Gibco-BRL), frozen in liquid N_2, and stored at -70 °C until required. RNA was purified according to the manufacturer's instructions. Total RNA was treated with DNase I (Ambion) to remove any contaminating DNA. DNase I was removed with DNase inactivation reagent (Ambion) according to the manufacturer's instructions. cDNA was synthesized with Superscript II reverse transcriptase (Gibco-BRL) using 3 g of DNase-treated RNA. An aliquot (1/40) of the cDNA reaction was subject to PCR analysis according to the manufacturer's instructions for AmpliTaq Gold DNA polymerase (Perkin-Elmer). Quantitative real-time PCR was performed by using the Bio-Rad Laboratories MJ mini Opticon Real-Time PCR System. For chemokine analysis, the primers MIP-2F (5´-GCC AAG GGT TGA CTT CA-3´) and MIP-2R (5´-TGT CTG GGC GCA GTG-3´) or MCP-1F (5´-TAC TCA TTA ACC AGC AAG AT-3´) and MCP-1R (5´-TTG AGG TGG TGG TGG AA-3´) were used.

Table 2: Composition of diets*

Fatty acids	*Safflower oil*	*Canola oil*	*Fish oil*
Casein (vitamin-free)	19	19	19
Sucrose	22	22	22
Corn starch	38	38	38
Safflower oil	1	0	10
Canola oil	0	10	0
Fish oil	9	0	0
Vitamin mix (AIN-76)	1	1	1
Mineral mix (AIN-93)	5	5	5
Choline chloride	0.1	0.1	0.1
Methionine	0.3	0.3	0.3
Cellulose	5	5	5
SeO_2	0.084	0.084	0.084
$MnCl_2$	0.03	0.03	0.03

Values are as g/100 g. *The diet is so designed that protein, carbohydrates, and fat provide respectively 17.8%, 61.6%, and 20.6% of total calories.

As a control for a constitutively expressed gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified with the oligonucleotides CR127 (5´-ACA TCA TCC CTG CAT CC-3´) and CR128 (5´-GGA TGG AAA TTG TGA GG-3´). PCR was conducted with IQ SYBR Green Supermix (Bio-Rad), using cycling conditions of 95 °C for 5 min and 35 cycles of 95 °C for 30 sec, followed by 55 °C for 30 sec and 72 °C for 30 sec. To calculate the relative gene expression, the Pfaffl method was used, as outlined in the Gene Ex Macro software by Bio-Rad.

Analysis of cecal microflora. A cecal sample (0.1 g) was suspended in 9.9 mL of anerobic diluent, and then a decimal dilution series from 10^{-2} to 10^{-8} was prepared. Aliquots of 0.05 mL were inoculated with five selective agar media: Bacteriodes Bile Esculin Agar and Brucella blood agar (DIFCO, USA) supplemented with vitamin K, hemin and gentamicin for Bacteroidaceae, Rogosa agar (Merck, Germany) for *Lactobacilli*, *Bifidobacter*ium agar (HiMedia, Mumbai, India) for *Bifidobacterium* spp., KF Streptococcus agar for enterococci (Merck, Germany), and Mac Conkey medium for *Enterobacteriaceae*). The plates were incubated at 37ºC for 72 h in an anerobic jar. The anerobic condition was provided by *Anoxamat* system (Mart, The Netherlands). Microbial groups were identified based on colonial morphology, Gram-staining, hydrolysis of bile esculin, catalase reactions, cellular morphologies, and spore formation. Gram-negative rods were identified as *Bacteriodes fragilis* group when they hydrolyzed esculin, grew in presence of kanamycin, vancomycin, colistin and bile salts, produced negative

Table 3: Organ weights in mice fed different dietary oils

	Fish oil		*Canola oil*		*Safflower oil*		*Chow diet*	
	g	*g/100 g BW*	*g*	*g/100 g BW*	*g*	*g/100 g BW*	*g*	*g/100 g BW*
Liver	1.65 (0.3)	4.7 (0.34)	1.69 (0.26)	4.6 (0.4)	1.67 (0.23)	4.25 (0.4)	1.66 (0.22)	4.5 (0.4)
Kidney	0.6 (0.06)	1.6 (0.1)	0.63 (0.06)	1.62 (0.1)	0.62 (0.07)	1.61 (0.12)	0.6 (0.05)	1.61 (0.11)
Spleen	0.1 (0.01)	0.26 (0.02)	0.1 (0.02)	0.25 (0.02)	0.1 (0.01)	0.25 (0.01)	0.1 (0.01)	0.25 (0.02)
Cecum	0.07 (0.01)	0.2 (0.02)	0.07 (0.01)	0.2 (0.01)	0.08 (0.01)	0.2 (0.01)	0.08 (0.01)	0.2 (0.01)

Values are mean (SD). BW -- body weight

reaction in nitrate test and lacked motility. The number of bacteria was expressed as $\log_{10}$ of colony forming units (CFU) per gram found in cecal content.

Statistical analysis

Data were expressed as the mean and SD for each group (eight mice). Statistical analysis was performed with an ANOVA test followed by posthoc Tukey test, whenever the difference was significant. A p value <0.05 was considered significant.

Results

The body and organ weights were similar in all the groups, (Table 3).

*Inflammation findings***.** Both macroscopic and microscopic pathology scores were highest in the chow diet group followed by safflower, canola, and fish oil fed groups, respectively (Table 4). The gene expression of the macrophage inflammatory protein (MIP)-2, as well as the monocyte chemoattractant protein (MCP)-1 through quantitative real-time PCR. Both of them were expressed at their highest level in chow diet group followed by safflower, canola, and fish oil fed groups, respectively (Table 5).

Cecal microflora: The mean (SD) for log bacterial counts are summarized in Table 4. The number of *bacteroideceae* in mice fed the chow diet was higher than in other groups (p<0.05). The number of *bifidobacteria* in mice fed the chow diet was significantly lower than in those fed polyunsaturated fatty acids (PUFAs) (p<0.05).

Discussion

We investigated the effect of fish oil, canola oil, safflower oil, and beef tallow (the usual fat component of chow diet) on some inflammatory indices, cecal microflora and biochemical parameters in an experimental model of colitis. As was expected, the oils high in omega-3 fatty acids (fish oil and canola oil) reduced inflammation. Our results also showed that all PUFAs could elevate the number of *bifidobacter*s, which are known as probiotics, and beneficial for the body. These findings are in line with some other studies. In Arctic charr, it was found that either fish oil or flax oil in the diet could increase lactic acid

Table 4: Inflammatory indexes and cecal microflora in mice fed diets containing different oils

	Fish oil	*Canola oil*	*Safflower*	*Chow diet*
MIP-2 fold expression	2 (0.89)	10.1 (0.99)	69.8 (2.6)	97.6 (5.27)
MCP-1 fold expression	2 (0.66)	2 (0.81)	6 (0.81)	10.2 (0.91)
Histopathology score	4 (0.66)	6 (0.66)	8 (1.41)	10.4 (1.17)
Bacteroidaceae	9.12 (0.3)	9.23 (0.2)	9.25 (0.3)	9.86 (0.3)†
Bifidobacteria	8.78 (0.2)	8.69 (0.2)	8.65 (0.2)	8.21 (0.3)†
Lactobacilli	8.56 (0.6)	8.53 (0.5)	8.53 (0.5)	8.46 (0.6)
Enterobacteriaceae	6.33 (0.7)	6.41 (0.7	6.3 (0.6)	6.12 (0.7)
Streptococci	6.75 (0.3)	6.72 (0.4)	6.64 (0.4)	6.7 (0.3)

Values are mean (SD). Bacterial counts are as log colony forming units per gram of fecal content
† p<0.05 as compared to other diet groups

Table 5: Comparison of effects of various oils on inflammation parameters

	MIP-2		*MCP-1*		*Histopathology*	
Parameter	*p value*	*95% CI**	*p value*	*95% CI*	*p value*	*95% CI*
Fish oil vs Canola oil	<0.05	-15.27 to -1.094	>0.05	-2.615 to 2.615	<0.05	-4.592 to -0.4079
Fish oil vs Safflower oil	<0.001	-74.09 to -59.91	<0.01	-6.615 to -1.385	<0.001	-6.986 to -2.802
Fish oil vs Chow diet	<0.001	-104.9 to -90.74	<0.001	-10.61 to -5.385	<0.001	-10.03 to -5.845
Canola oil vs Safflower oil	<0.001	-65.91 to -51.73	<0.01	-6.615 to -1.385	<0.05	-4.486 to -0.3016
Canola oil vs Chow diet	<0.001	-96.74 to -82.56	<0.001	-10.61 to -5.385	<0.001	-7.530 to -3.345
Safflower oil vs Chow diet	<0.001	-37.92 to -23.74	<0.01	-6.615 to -1.385	<0.01	-5.136 to -0.9516

MIP -- Macrophage inflammatory protein; MCP -- Monocyte chemoattractant protein
Tukey's multiple comparison test between various groups. *95% CI of difference

bacteria and *lactobacilli* in particular, whereas coconut oil did not show this effect on microflora.[22] An *in vitro* study showed that the PUFAs are toxic for microflora of the rumen, and the toxicity to bacterial growth was ranked as long-chain omega-3 fatty acids > short-chain omega-3 fatty acids > omega-6 fatty acids.[23]

Though some reports suggest different effects for different types of PUFAs,[24,25] we could not find any difference among different PUFAs. Therefore, we conclude that the effect of omega-3 fatty acids in attenuation of colitis is due to their anti-inflammatory properties, and is not related to their effect on intestinal microflora.

In conclusion, our results indicate no interaction between the effect of omega-3 fatty acids on inflammation reduction and intestinal microflora. However, all PUFAs had a beneficial effect on the intestinal microflora. So we recommend more studies in animals and humans to confirm the effect of PUFAs, particularly omega-3 fatty acids on intestinal microflora. The results might lead to replacement of special oils in dietary intake instead of consumption of probiotics or some medications.

References

1. Snelling AM. Effects of probiotics on the gastrointestinal tract. *Curr Opin Infect Dis* 2005;18:420–6.
2. Liopis M, Antolín M, Guarner F, Salas A, Malagelada JR. Mucosal colonisation with *Lactobacillus casei* mitigates barrier injury induced by exposure to trinitronbenzene sulphonic acid. *Gut* 2005;54:955–9.
3. Luyer MD, Buurman WA, Hadfoune M, Speelmans G, Knol J, Jacobs JA, *et al.* Strain-specific effects of probiotics on gut barrier integrity following hemorrhagic shock. *Infect Immun* 2005;73:3686–92.
4. Ewaschuk J, Endersby R, Thiel D, Diaz H, Backer J, Ma M, *et al*. Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. *Hepatology* 2007;46:841–50.
5. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH. Overview of gut flora and probiotics. *Int J Food Microbiol* 1998;41:85–101.
6. Björkstén B. Evidence of probiotics in prevention of allergy and asthma. *Curr Drug Targets Inflamm Allergy* 2005;4:599–604.
7. Verdú EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, *et al*. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006;55:182–90.
8. Bischoff S, Crowe SE. Food allergy and the gastrointestinal tract. *Curr Opin Gastroenterol* 2004;20:156–61.
9. Quigley EM, Flourie B. Probiotics and irritable bowel syndrome: A rationale for their use and an assessment of the evidence to date. *Neurogastroenterol Motil* 2007;19:166–72.
10. Drisko J, Bischoff B, Hall M, McCallum R. Treating irritable bowel syndrome with a food elimination diet followed by food challenge and probiotics. *J Am Coll Nutr* 2006;25:514–22.
11. Fanigliulo L, Comparato G, Aragona G, Cavallaro L, Iori V, Maino M, *et al*. Role of gut microflora and probiotic effects in the irritable bowel syndrome. *Acta Biomed* 2006;77:85–9.
12. Lirussi F, Mastropasqua E, Orando S, Orlando R. Probiotics for non-alcoholic fatty liver disease and/or steatohepatitis. *Cochrane Database Syst Rev* 2007;24:CD005165.
13. Gareau MG, Jury J, Macqueen G, Sherman PM, Perdue MH. Probiotic treatment of rat pups normalizes corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut* 2007;56:1522–8.
14. Ivec M, Botiæ T, Koren S, Jakobsen M, Weingartl H, Cencic A. Interactions of macrophages with probiotic bacteria lead to increased antiviral response against vesicular stomatitis virus. *Antiviral Res* 2007;75:266–74.
15. Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr* 2007;137:819S–24S.
16. de Moreno de LeBlanc A, Perdigón G. Reduction of beta-glucuronidase and nitroreductase activity by yoghurt in a murine colon cancer model. *Biocell* 2005;29:15–24.
17. Kruis W, Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, *et al*. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004;53:1617–23.
18. Innis SM, Jacobson K. Dietary lipids in early development and intestinal inflammatory disease. *Nutr Rev* 2007;65(12 Pt 2):S188-S193.
19. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989;96:795–803.
20. Videla S, García-Lafuente A, Antolín M, Vilaseca J, Guarner F, Crespo E, *et al*. Antitumor necrosis factor therapy in rat chronic granulomatous colitis: critical dose-timing effects on outcome. *J Pharmacol Exp Ther* 1998;287:854–9.
21. Ringø E, Bendiksen HR, Gausen SJ, Sundsfjord A, Olsen RE. The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of arctic charr, salvelinus alpinus (L.). *J Appl Microbiol* 1998;85:855–64.
22. Maia MR, Chaudhary LC, Figueres L, Wallace RJ. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie Van Leeuwenhoek* 2007;91:303–14.
23. Thompson L, Spiller RC. Impact of polyunsaturated fatty acids on human colonic bacterial metabolism: an in vitro and in vivo study. *Br J Nutr* 1995;74:733–41.
24. Kuda T, Enomoto T, Yano T, Fujii T. Cecal environment and TBARS level in mice fed corn oil, beef tallow and menhaden fish oil. *J Nutr Sci Vitaminol* (Tokyo) 2000;46:65–70.

Correspondence to: Dr Djazayery, Ph.D., Department of Human Nutrition, School of Health, Tehran University of Medical Sciences, Tehran, Iran. Fax: 98 (21) 8895 1404
E-mail: *djazayery@yahoo.com*

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