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Dietary Chicory Inulin-Rich Meal Exerts Greater Healing Effects than Fructooligosaccharide Preparation in Rats with Trinitrobenzenesulfonic Acid-Induced Necrotic Colitis

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The aim of this study was to compare the effects of chicory root inulin-rich meal (containing a polyphenolic fraction as well) and pure fructooligosaccharides (FOS) on necrotic colitis induced with trinitrobenzenesulfonic acid (TNBS) in Wistar rats. Both chicory preparations significantly reduced the pH value of colonic digesta and favourably lowered the caecal activity of bacterial β-glucuronidase as well as the caecal concentration of putrefactive short-chain fatty acids in comparison to the control TNBS rats. In addition, they enhanced the production of total short-chain fatty acid (SCFA pool) and concentration of anti-inflammatory propionic acid in the caecal digesta. Nevertheless, only dietary chicory meal favourably increased the total SCFA concentration and thus decreased the pH value of caecal digesta. The increased caecal SCFA production may explain the observed greater reduction in mucosal necrosis and increased glandular mucosal regeneration in rats fed a diet with chicory root meal. Both chicory preparations beneficially regulated physiological parameters in the lower part of the rat intestinal tract after TNBS-induced colitis, however the dietary treatment with chicory meal showed stronger reduction of mucosal disturbances caused by colitis. Those beneficial effects might be related to the higher polymerization of inulin *vs*. FOS and to the presence of biologically active compounds in the meal, *i.e*., phenolic compounds, which had a strong impact on intestinal microbial activity and thus indirectly alleviated mucosal disturbances caused by colitis.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract whose etiology has not yet been fully elucidated [Motavallian-Naeini *et al*., 2012]. IBD is a multifactorial intestinal disorder that involves interactions among the immune system, genetic susceptibility and environmental factors, especially the intestinal microbiota [Witaicenis *et al.*, 2010]. The incidence of IBD is continually increasing, and it has become a worldwide healthcare problem [Zhang & Li, 2014]. There is evidence suggesting that the appropriate modulation of intestinal microbial activity regulates intestinal physiology and immunological function, which may assist in the prevention and treatment of IBD [Lara-Villoslada *et al.*, 2006].

Well-known dietary ingredients that modulate the activity of the intestinal microbiota are nondigestible carbohydrates. Some studies have shown that a diet enriched in dietary fibre successfully maintains the remission of ulcerative colitis in humans and experimental animal models because of an associated increase in the luminal production of short-chain fatty acids (SCFA) [Zhong *et al.*, 2000; Rodríguez-Cabezas *et al.*, 2002]. It has also been reported that dietary fructooligosaccharides (FOS) have a beneficial effect on intestinal inflammation in a rat model of colitis induced by trinitrobenzenesulfonic acid (TNBS); the applied dietary constituents reduced the extent of the damage and promoted epithelial healing [Cherbut *et al.*, 2003]. These and other authors suggested that the main beneficial effects were associated with the end products of FOS fermentation, such as lactic acid and SCFA, as well as with the acidification of the luminal contents [Kosmala *et al*., 2015]. More complex nondigestible dietary carbohydrates that also modulate the activity of the microbiota in the gastrointestinal tract are inulin-type fructans [Juśkiewicz *et al*., 2011a]. Some studies have shown that dietary supplementation with these compounds beneficially stimulates the numbers of *Bifidobacteria* [Fotschki *et al.*, 2014] and *F. prausnitzii* [Ramirez-Farias *et al.*, 2009], as well as the production of SCFA [Fotschki *et al.*, 2014; Kosmala *et al*., 2014].

Chicory is a rich source of dietary fibres such as inulin and FOS, which have health-promoting properties on consum-

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ers [Żary-Sikorska *et al*., 2016]. From all parts of the chicory plant (peel, seed, leaf), the highest content of inulin was determined in root [Jurgoński *et al*., 2011]. Inulin consists of linear chains of β-2,1-linked fructosyl units terminating at the reducing end with a glucose residue attached *via* a sucrose-type linkage. In nature, inulin is the second most abundant storage carbohydrate after starch [Singh *et al.*, 2016]. Inulin is often used as a substrate to produce high purity FOS preparations [Ganaie *et al.*, 2014]. Despite the differences in the degree of polymerization between inulin and FOS (inulin>FOS), both nondigestible carbohydrates have been termed 'prebiotics' [Gibson *et al.*, 1995] and have been found to beneficially stimulate the growth and/or activity of the microbiota, thus affecting various physiological functions and ultimately having health-promoting impacts on consumers [Ganaie *et al.*, 2014]. Nevertheless, the different degrees of polymerization of nondigestible carbohydrates have considerable effects on the specific activity of the microbiota and intestinal immune functions [Żary-Sikorska & Juśkiewicz, 2008]. Moreover, unprocessed dietary fibre occurs as fibre–phenolic complexes, which present more health-promoting effects to consumers than the highly purified preparations of dietary fibre [Fotschki *et al.*, 2015]. The chicory root is also a source of phenolic compounds, such as caffeoylquinic acids (CQAs) more specifically mono- and di-CQAs isomers [Jurgoński *et al*., 2011]. Zorrilla *et al.* [2014] showed that a polyphenolic-enriched almond extract can effectively improve epithelial barrier function and ameliorate colonic inflammation in a rat model of TNBS-induced colitis. Therefore, the present study with Wistar rats tested the hypothesis that dietary supplementation with unprocessed inulin-rich chicory root meal is more effective in reducing intestinal inflammation in a rat model of TBNS-induced colitis than are equivalents of highly purified chicory FOS.

MATERIALS AND METHODS

Chicory preparations

In this study, two kinds of chicory preparations were used: meal from chicory roots obtained from industrial processing and a commercial preparation of fructooligosaccharide produced *via* the enzymatic hydrolysis of chicory inulin (Raftilose®P95, ORAFTI, Belgium). The raw material used to produce the meal was industrially produced chicory semolina (ZPC Cykoria S.A., Poland) dried with a convective dryer at a temperature below 70°C. The dried chicory material was disaggregated in a ball grinder and sieved through a screen with a mesh diameter of 0.8 mm. The commercial dietary FOS preparation consisted of 96.4% dry matter, 0.83% ash, 0.6% glucose, and 95% short-chain FOS. The chicory root meal consisted of 96.7% dry matter, 5.87% crude protein, 1.81% crude fat, 3.32% ash, 6.25% cellulose, 4.30% monosaccharides, 75% fructans (15% oligofructose and 60% inulin), and 0.13% phenolic compounds. The determination of the proximate chemical composition was performed according to the method described in the previous studies [Jurgoński *et al.,* 2011; Wang *et al.,* 2018]. Total phenolic content was determined with the Folin–Ciocalteu's phenol reagent [De Pascual-Teresa *et al*., 2000]. Absorbance was measured at 720 nm wavelength and chlorogenic acid was used as a standard (Sigma, Poznan, Poland).

Animals and experimental design

The rats were used in compliance with the European Guidelines for the Care and Use of Laboratory Animals [Directive 2010/63/EU], and the animal protocol was approved by the local institutional animal care and use committee (Permission No. 32/2012; Olsztyn, Poland). To determine the number of animals in group, the sample size estimation was done according to the approaches proposed by Dell *et al.* [2002]. The rats were housed individually. The selection of the animals and their maintenance over the experiment followed common regulations. The environment was controlled, with a 12-h light-dark cycle, a temperature of 21±1°C, a relative humidity of 50±5%, and 20 air changes/h. The experiment lasted 28 days and was conducted on 32 male Wistar rats aged 7 weeks and weighing 205.6±12.9 g. The rats were divided into 4 groups. Each group was fed a modified version of the semipurified diet recommended for laboratory rodents. All experimental diets were similar in terms of dietary ingredients with the exception of the phenolic content and fibre source (Table 1). Healthy control rats and control rats with induced colitis were fed diets with dietary cellulose (groups C and CC, respectively), and the rest of the rats with induced colitis were fed diets with either a dietary FOS preparation or chicory root meal (groups CCF and CCM, respectively). The rats had free access to tap water and the semipurified diets *ad libitum*, which were prepared and then stored at 4°C in hermetic containers until the end of the experiment.

Assessment of colonic condition

At the beginning of the experiment, all animals were fasted overnight and, except for the untreated control group, were rendered colitic by the method originally described by Morris

TABLE 1. Composition of experimental diets (%).

	Group[1]		
	C and CC	CCF	CCM
Main components	90	89.4	86.05
Fibre sources			
Cellulose	10.0	–	–
FOS preparation	–	10.6	–
Chicory root meal	–	–	13.5
Calculated content			
Protein	15.50	15.50	15.50
Fibre	10.00	10.07	10.12
Total phenolic compounds	–	–	0.018

[1]C, healthy control rats fed dietary cellulose; CC, control rats with induced colitis fed a diet with dietary cellulose; CCF; rats with induced colitis fed a diet with a dietary fructooligosaccharide preparation; CCM, rats with induced colitis fed a diet with chicory root meal; FOS, fructooligosaccharide preparation (Raftilose®P95, ORAFTI, Belgium).

et al. [1989]. Briefly, the rats were anesthetised with isoflurane and given 10 mg of TNBS (Sigma, Poznań, Poland) dissolved in 0.25 mL of 50% ethanol (v/v) by means of a Teflon cannula inserted 8 cm into the anus. The cannula was left in place for 1 min to ensure that the solution was not immediately expelled by the rat. Rats in the noncolitic group were intracolonically administered 0.25 mL of phosphate-buffered saline (PBS) instead of TNBS. The rats were kept in a head-down position for an additional 30 s and returned to their cages.

At the termination of the experiment, the rats were anaesthetized with sodium pentobarbital according to the recommendations for euthanasia of experimental animals. After laparotomy, the caecum and colon were removed and weighed. Subsequently, the colon was flushed with PBS and dried, and the tissue was immersed for 7 days in a 10% solution of buffered formalin. Colonic segments were embedded in paraffin blocks. Paraffin sections (1–2 μm) were cut with a Reichert's microtome and tissue fragments were passed through increasing concentrations of alcohol solutions, acetone and xylene (de-waxed). The preparations were stained with hematoxylin and eosin (H&E; Merck, Darmstadt, Germany) according to the method described by Fischer *et al.* [2008]. The tissue sections were evaluated and images taken by standard light microscopy using a computer program for image analysis, B-cell, and Olympus BX50 microscope with a digital camera (Olympus Co., Tokyo, Japan).

A semiquantitative score was used to evaluate severity of histological lesions in the inflamed colon (Histopathological Colitis Score, HCS) [Engel *et al.,* 2008]. The HCS featured the parameters inflammation extent, crypt architecture, edema, and infiltration with inflammatory cells, with a maximum of 12 points (Table 2). The cross-sectioned segments from the colon were scored by investigators, blinded to the experimental groups tested.

TABLE 2. Histopathological Colitis Score (HCS).

Feature	Description	Score
Inflammation extent	none	0
	mucosa	1
	mucosa + submucosa	2
Damage in crypt architecture	none	0
	regeneration	1
	destruction	2
Hyperemia / Edema	without	0
	mild	1
	moderate	2
	severe	3
Infiltration with inflammatory cells	without	0
	mild	1
	moderate	2
	severe	3

(ulceration and/or crypt abscess respectively +1)

These features describe inflammation criteria to determine colitis severity [Engel *et al.,* 2008].

Analytical procedures

The individual feed consumption and final body weight of the rats were determined. Samples of the caecal and colonic digesta were collected, and their pH was immediately measured using a microelectrode and a pH/ION meter (model 301; Hanna Instruments, Vila do Conde, Portugal). In the fresh caecal digesta, the dry matter was determined by drying to constant weight at 105°C, whereas the ammonia concentration was determined by the microdiffusion method in Conway's dishes [Hofirek & Haas, 2001].

After storage of the caecal digesta at −70°C, SCFA concentrations were measured using a gas chromatography system (Shimadzu GC-2010, Kyoto, Japan) with a capillary column (SGE BP21, 30 m × 0.53 mm; SGE Europe Ltd., Milton Keynes, UK), as previously described [Jurgoński *et al*., 2015]. The concentrations of the caecal putrefactive SCFA (PSCFA) were calculated as the sum of isobutyric, isovaleric, and valeric acids. All SCFAs analyses were performed in duplicate. Standards of acetic, propionic, butyric, isobutyric, isovaleric, and valeric acids were obtained from Sigma Co. (Poznań, Poland). They were used to plot calibration curves and calculate individual fatty acid concentration in the digesta.

Caecal fermentation processes were analysed based on the activities of selected bacterial enzymes (α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase), as measured by the rate of release of *p*-nitrophenol or *o*-nitrophenol from the respective nitrophenyl glucosides, according to a previously described method [Juśkiewicz *et al.*, 2011b]. The following substrates were used: *p*-nitrophenyl-α-D-glucopyranoside (for α-glucosidase), *p*-nitrophenyl-β-D-glucopyranoside (for β-glucosidase), *p*-nitrophenyl-α-D-galactopyranoside (for α-galactosidase), *o*-nitrophenyl-β-D-galactopyranoside (for β-galactosidase), and *p*-nitrophenyl-β-D-glucuronide (for β-glucuronidase). To measure the activities of enzymes secreted by bacterial cells into the caecal environment, a reaction mixture containing 0.3 mL of a substrate solution (5 mM) and 0.2 mL of a 1:10 (v/v) dilution of the caecal sample in 100 mM phosphate buffer (pH 7.0) after centrifugation at 4°C, 7211 × g (MPW-350R, MPW Med. Instruments, Warsaw, Poland) for 15 min was prepared. The samples were incubated (10-min, 37°C) and then mixed with 2.5 mL of 0.25 M cold sodium carbonate to stop the reaction. The absorbance was measured at 400 nm for *p*-nitrophenol and at 420 nm for *o*-nitrophenol (Spectrophotometer Unicam Helios α, Thermo Fisher Scientific Polska Sp. z o.o., Warsaw, Poland). The enzymatic activity was expressed as μmol product formed per hour per g of fresh digesta. All analyses were performed in duplicate.

Statistical analysis

The results were examined statistically using one-way analysis of variance, and significant differences between groups were determined with Duncan's multiple range test at a significance level of $P \leq 0.05$. Calculations were made using STATISTICA 12.0 software (StatSoft Corp., Kraków, Poland).

TABLE 3. Body weight, diet intake, and basic indices of the distal intestine in rats with TNBS-induced colitis.

	Group[1]			
	C	CC	CCF	CCM
Initial body weight (g)	206±4	205±3	205±4	206±3
Final body weight (g)	309±7[a]	264±4[b]	279±4[b]	279±8[b]
Diet intake (g/4 weeks)	557±15[a]	477±13[bc]	456±13[c]	500±9[b]
Feed conversion ratio (g/g)	5.50±0.26[b]	8.61±0.79[a]	6.53±0.57[b]	7.35±0.82[ab]
Caecum				
Tissue mass[2]	0.27±0.01[c]	0.32±0.02[c]	0.61±0.02[a]	0.43±0.02[b]
Digesta mass[2]	0.83±0.04[c]	0.92±0.04[bc]	1.83±0.18[a]	1.21±0.14[b]
pH of digesta	6.82±0.06[a]	6.81±0.06[a]	6.33±0.15[ab]	6.21±0.14[b]
Dry matter of digesta (%)	23.9±0.53[a]	24.3±0.53[a]	18.4±0.93[b]	16.4±0.40[c]
Ammonia in digesta (mg/g)	0.36±0.02	0.38±0.02	0.38±0.03	0.34±0.02
Colon				
Mass with digesta[2]	0.99±0.04[b]	3.54±0.49[a]	1.09±0.10[b]	1.01±0.10[b]
pH of digesta	6.80±0.13[ab]	7.06±0.06[a]	6.59±0.15[b]	6.44±0.06[b]

Values are expressed as the mean ± standard error of the mean; TNBS, trinitrobenzenesulfonic acid. [1]C, healthy control rats fed a diet with dietary cellulose; CC, control rats with induced colitis fed a diet with dietary cellulose; CCF, rats with induced colitis fed a diet with a dietary fructooligosaccharide preparation; CCM, rats with induced colitis fed a diet containing chicory root meal. [2]g/100 g body weight. [a-c]Values with different superscript letters within a row are significantly different at P≤0.05.

RESULTS AND DISCUSSION

In the present study, after 28 days of the experiment, the animals administered TNBS exhibited the overt typical clinical signs of colitis: lesser weight gain related to a decrease of diet intake and an increase in the feed conversion ratio (C *vs*. CC; P<0.05) (Table 3). Similar signs were observed by Gonçalves *et al.* [2013] in Wistar rats with TNBS-induced colitis. Compared to the untreated animals (C group), the induction of colitis in the CC group was manifested by a significant increase of the colon mass with digesta (Table 3) and activity of caecal bacterial *β*-glucosidase (P<0.05; Table 3). The results obtained in the present study showed that diet enrichment with chicory preparations, especially chicory root meal, partly reduced these typical clinical signs of the TNBS--induced inflammatory process.

The experimental groups of animals with TNBS-induced colitis (CCF and CCM) consumed less food and thus gained significantly less in weight than the rats from group C (Table 3). Among the rats treated with TNBS, the dietary intake differed between the CCF and CCM groups (CCF<CCM; P<0.05). Animals fed the diet with FOS utilised the diet significantly better than the control group animals with induced colitis (CC group; P<0.05). The highest relative caecal tissue mass was noted in the rats fed dietary FOS, and the values of that parameter decreased as follows among the groups: CCF>CCM>CC. Dietary inclusion of the FOS preparation resulted in a significant increase in the caecal digesta mass in comparison to other groups (P<0.05). The concentrations of caecal digesta dry matter in the CCF and CCM groups *vs*. those of the C and CC (P<0.05) groups, were respectively reduced.

The administration of experimental diets to the rats with TNBS-induced colitis caused positive effect by decreasing *β*-glucuronidase activity in the caecal digesta (CCF, CCM vs. CC; P<0.05) and compensating the increase of the *β*-glucosidase during the inflammation processes caused by TNBS (Table 4). Bacterial enzymes, such as *β*-glucuronidase, may exert toxic, carcinogenic or mutagenic effects in the colon [Klewicka *et al*., 2009]. Roberton *et al.* [1982] suggested that the most important factor in the modulation of *β*-glucuronidase activity in the rat large bowel is bile flow. Dietary fibre increases peristalsis, and thus greater quantities of bile are transported to distal segments of the gastrointestinal tract. Some studies reported that diet with dietary fibres and polyphenols may reduce the activity of such enzymes as *β*-glucuronidase and *β*-glucosidase [Lahouar *et al.*, 2012; Kosmala *et al.*, 2017]. Juśkiewicz *et al.* [2011b] also examined the effects of the chicory dietary polyphenolic fraction and found favourable changes in the activity of bacterial *β*-glucuronidase in the faeces and caecal digesta of rats.

In comparison to the dietary treatment with cellulose, dietary inclusion of the commercial FOS preparation as well as chicory root meal exerted a positive effect by lowering the pH value in the colonic digesta (Table 3). It is noteworthy that the acidification of the digesta promotes positive microbiota proliferation and decreases the growth of pathogenic bacterial species [Topping & Clifton, 2001]. In the CCM group, this dietary preparation lowered the percentage of dry matter and the pH value in the caecum (Table 3), indicating more intensive fermentation of indigestible components ahead

TABLE 4. Microbial enzyme activities and short-chain fatty acid (SCFA) concentrations, profile, and pool in the caecal digesta.

	Group[1]			
	C	CC	CCF	CCM
Enzyme activity (μmol/h/g digesta)				
α-Glucosidase	10.9±1.16[b]	11.6±3.11[b]	47.0±6.16[a]	30.2±5.59[a]
β-Glucosidase	5.29±0.82[c]	13.1±1.8[a]	7.57±0.80[bc]	9.48±1.21[ab]
α-Galactosidase	6.28±1.17[c]	10.9±1.80[bc]	55.6±8.50[a]	31.0±5.73[ab]
β-Galactosidase	24.0±1.80[c]	27.6±1.85[bc]	222±21.45[a]	128±11.33[ab]
β-Glucuronidase	18.8 ±2.8[ab]	30.4±4.7[a]	8.69±1.46[b]	8.63±2.43[b]
SCFA concentration (mmol/g digesta)	91.8±7.1[b]	88.9±6.6[b]	98.3±9.9[b]	141±9[a]
Acetic acid	66.4±5.94	63.0±5.1	53.1±5.7	71.5 ±5.1
Propionic acid	14.4±1.23[b]	14.9±1.2[b]	38.0±6.1[a]	61.3±8.0[a]
Butyric acid	8.89±0.74[a]	8.14±0.70[a]	5.59±0.42[b]	6.86±0.81[ab]
PSCFA[2]	2.16±0.20[ab]	2.79±0.29[a]	1.60±0.54[b]	1.16±0.29[b]
SCFA profile (%)				
Acetic acid	72±1.3[a]	71±1.2[a]	55±1.3[b]	51±3.4[b]
Propionic acid	16±0.6[b]	17±0.5[b]	37±2.1[a]	43±3.5[a]
Butyric acid	10±1.2[a]	9±0.8[a]	6±0.9[b]	5±0.7[b]
SCFA pool (mmol/100 g body weight)	77.1±8.4[b]	86.4±5.5[b]	169±11[a]	173±24[a]

Values are expressed as the mean±standard error of the mean. The colitis has been induced by trinitrobenzenesulfonic acid (TNBS). [1]C, healthy control rats fed a diet with dietary cellulose; CC, control rats with induced colitis fed a diet with dietary cellulose; CCF, rats with induced colitis fed a diet with a dietary fructooligosaccharide preparation; CCM, rats with induced colitis fed a diet containing chicory root meal. [2] Short-chain fatty acids of putrefactive origin (isobutyric acid + isovaleric acid + valeric acid). [a-c]Values with different superscript letters within a row are significantly different at P≤0.05.

of the colon. In the examined preparations, only the feeding with the diet with chicory root meal was able to significantly elevate the caecal concentration of SCFAs, as the main fermentation products of the abundant microbiota (Table 4). The caecal SCFAs pool was found to be significantly increased upon dietary application of both tested preparations (CCF and CCM *vs*. C and CC; $P<0.05$). The SCFAs are essential for the constant repair of the colon epithelium [Sturm & Dignass, 2008] and exert anti-inflammatory and anticarcinogenic effects [Van der Beek *et al.*, 2017]. The secretory response to SCFAs, in combination with the contractile response, seems to act as a lubricant for the movement of luminal content in the colon [Yajima *et al.,* 2011]. In addition, dietary application of chicory preparations resulted in the highest caecal propionic acid concentration (CCF and CCM *vs*. C and CC, respectively; $P<0.05$). As a result, in comparison to control animals the application of both experimental diets (CCF, CCM) significantly increased percentage of propionic acid in the SCFA profile at the expense of acetic and butyric acids ($P<0.05$) (Table 4). Acetic and propionic acids are known to reduce the production of anti-inflammatory mediators such as TNF-*a* by human neutrophils. Propionic acid is also able to inhibit the expression of proinflammatory mediators [Vinolo *et al.*, 2011]; in addition it stimulates chloride secretion and exerts a range of health-promoting functions [Yajima *et al.*, 2011; Louis & Flint, 2017]. Jurgoński *et al.* [2011] found no significant difference in the production of butyric acid in the caecum of rats fed a diet supplemented with chicory root extract. In the present study, the caecal concentration of butyric acid, which is a major nutritive factor for colonic enterocytes, was reduced when the rats were feed CCF and CCM diets. The butyric acid exhibits a wide spectrum of cellular effects on the mucosa, such as enhanced differentiation and apoptosis. It is presumed that butyric acid exerts local anti-inflammatory effects [Hamer *et al.,* 2008]. In the studies performed on the isolated perfused rat colon, it has been shown that butyric acid increased the secretion of mucin [Barcelo *et al.,* 2000; Shimotoyodome *et al.,* 2000]. Putrefactive SCFAs are products of bacterial fermentation of undigested proteins [Cardona *et al.,* 2005]. Both experimental groups, in comparison to the CC group, exhibited significantly reduced production of PSCFAs (Table 4), which may suggest the less intensive anaerobic bacterial fermentation of polypeptides and amino acids.

The histological examination of the colonic tissue in rats not treated with TBNS showed normal morphology of the mucosa. Numerous small lumps of lymphocytes were found in the mucosa (Figure 1). The number of lumps appearing in the lymph mucosa was higher, and they had the characteristics of proliferated lymphocytes with few eo-

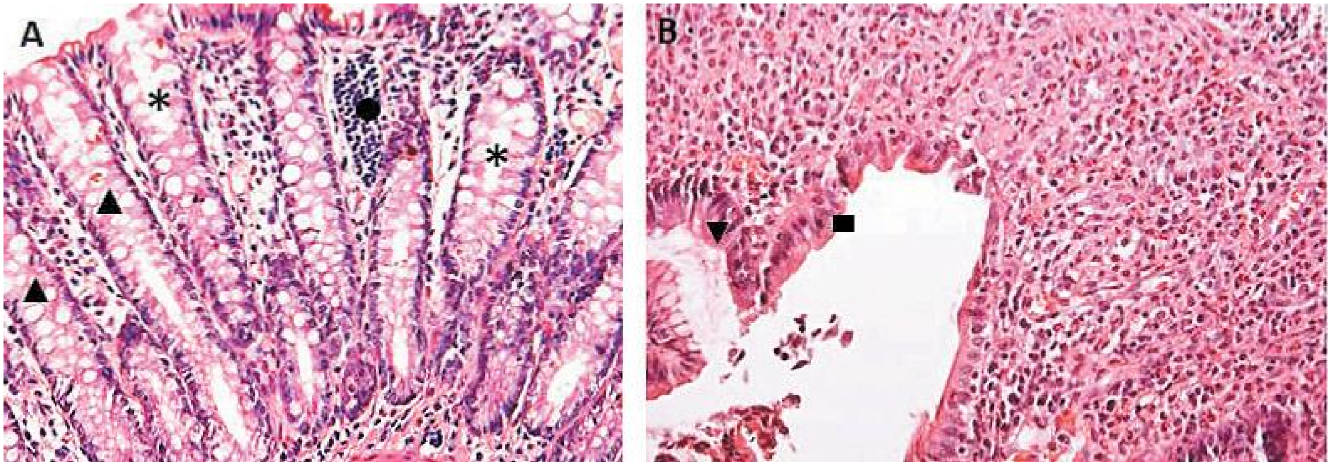

FIGURE 1. A – Example of colonic mucosa from the healthy control (C group) Wistar rat group; Goblet cells (black triangles); Intestinal glands in the crypts (black asterisks); Small clump of lymphocytes (black dot); B – Regeneration of mucosal glands in a rat from the colitic control group (CC group); Brush border (black square); Simple columnar epithelium (black arrowhead). The specimens were stained with hematoxylin and eosin. The original magnification was 40×.

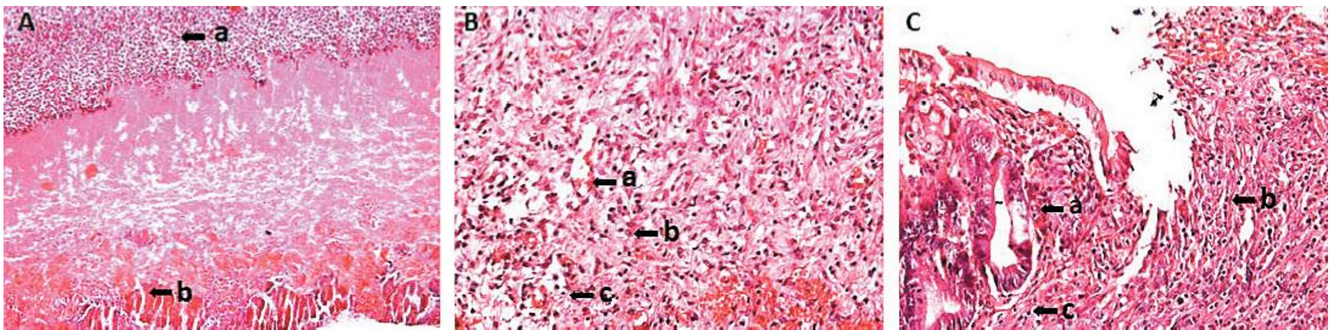

FIGURE 2. A – Necrosis of colonic mucosa and leukocyte infiltration in a rat from the colitic group fed a dietary fructo-oligosaccharide preparation (CCF group); a -leukocytes; b – necrotic changes; B – Thick layer of young connective tissue (granulation) with fibroblasts under the dead mucosa in a rat from the colitic group fed chicory root meal; a – erythrocytes, b – macrophages; c – lymphocytes (CCM group); C – Mucosal regeneration on the border of damage in a rat from the colitic group fed a diet containing chicory root meal (CCM group). Glandular cells are seen arising on young granulation tissue, in which eosinophils are present alongside fibroblasts; a – macrophages; b – eosinophils, c – lymphocytes. The specimens were stained with hematoxylin and eosin. The original magnification was 20× (A) and 40× (B, C).

sinophil cells. The administration of TNBS, which induces inflammation in rats, significantly increased the weight of the colon. This observed effect might be related to a higher level of edema and inflammatory processes. The administration of TNBS resulted in widespread mucosal necrotic inflammation in the entire colon in all animals. A similar effect was observed by Yue-Meng *et al.* [2011] in a nutritional study regarding the therapeutic effects of Peifeikang (a probiotic compound) on rats with experimental TNBS-induced colitis. The defensive response to the necrotic inflammation observed in all animals was leukocyte (neutrophil) infiltration. Neutrophil infiltration is one of the most prominent histological features in the inflamed colonic mucosa of colitis [Liu & Wang, 2011]. In the present study, the largest regenerative changes in the colon were found in the group fed chicory root meal. The lowest average thickness of tissue necrosis was observed in the animals from the CCM group, and this thickness increased among the groups as follows: CCM<CCF<CC ($P<0.05$) (Table 5). The observed beneficial effects might be related to the production of SCFA and to the regulation of leukocytes ability to migrate to loci of inflammation [Vinolo *et al.,* 2011]. The SCFAs regulate differentiation of mucosal T_{reg} cells, modulate Toll-like receptor 4 signalling, suppress the production of proinflammatory cytokines, and reduce the infiltration of colonic mucosa by leukocytes, thereby directly suppress the immune response and regulate colonic inflammatory processes [Van der Beek *et al.*, 2017]. Directly under the necrosis area in all groups treated with TNBS was a zone of leukocyte infiltration (Figure 2). Moreover, under the zone of leukocyte infiltration, the CCM group had a considerably smaller area of connective tissue with edema and infiltration of eosinophil cells and fibroblasts than the CC group had ($P<0.05$) (Figure 1 and 2, Table 5). The infiltration of cells with the ability to produce pro-inflammatory mediators or cytokines affects the formation and differentiation of the connective tissue [Debnath *et al.*, 2013]. Of great importance is the infiltration of eosinophil cells, which potentiates inflammatory processes such as the formation of young granulation tissue. In all animals treated with TNBS, there was an increase in new connective tissue, which was dominated by fibroblasts and numerous eosinophil cells as well as single plasmatic cells and mononuclear macrophages. In all

TABLE 5. Effects of chicory root preparations on the histopathology of colitis induced in rats by TNBS.

	Group[1]		
	CC	CCF	CCM
Mucosal necrosis (μm)	262±7.8[a]	211±19.2[b]	145±10.7[c]
Leukocyte infiltration under the necrosis zone (μm)	144±15.0	131±14.7	114±15.5
Connective tissue with edema and infiltration of eosinophils, plasmatic cells and fibroblasts (μm)	416±13.0[a]	–	65.5±19.1[b]
Granulation tissue with eosinophilic infiltration (μm)	1124±34.5[a]	843±57.2[b]	1096±24.3[a]
Signs of glandular mucosa regeneration (no. of rats out of 8)	5	3	7
Histopathological Colitis Score (HCS)	10±2[a]	6±2[b]	4±1[b]

Values are expressed as the mean±standard error of the mean; TNBS, trinitrobenzenesulfonic acid. [1]CC, control rats with induced colitis fed a diet with dietary cellulose; CCF, rats with induced colitis fed a diet with a dietary fructooligosaccharide preparation; CCM, rats with induced colitis fed a diet containing chicory root meal. [a-c]Values with different superscript letters within a row are significantly different at P≤0.05. The scoring method for HCS is described in Table 2.

animals treated with TNBS, the largest thickness of the new connective tissue in the colon was observed in the rats fed cellulose and chicory meal (P<0.05) (CC>CCM; Table 4). Furthermore, the infiltration of lymphocytic and eosinophil cells in the muscularis and under the colonic adventitia was observed in all animals. In both preparations examined, the addition of chicory meal to the diet most effectively stimulated the regeneration of the glandular mucosa in the form of small foci or the renewal of intestinal epithelial glands (Figure 2). Also the value of Histopathological Colitis Score used to evaluate histological lesions in the inflamed colon presented more favourable effect of chicory meal than of FOS (CCM<CCF<CC; Table 5).

CONCLUSIONS

The present study showed that the meal from chicory roots and the commercial chicory preparation of fructooligosaccharides exerted positive changes in rats with TNBS-induced colitis. Both examined preparations significantly reduced the pH value of colonic digesta and favourably lowered the activity of bacterial *β*-glucuronidase as well as the production of putrefactive SCFAs in rats treated with TNBS. Nevertheless, only the dietary chicory root meal treatment favourably increased the SCFAs concentration and decreased the pH value of caecal digesta. Moreover, the morphological characteristics of the colon showed that the feeding with the diet containing chicory root meal most effectively reduced TNBS-induced colitis by reducing the level of mucosal necrosis and the field of granulation tissue with eosinophilic infiltration, as well as by increasing the signs of glandular mucosal regeneration (7/8 animals). The observed beneficial effects of chicory root meal might be related to the higher polymerization of inulin (*vs*. FOS) and to the presence of biologically active compounds, *e.g.*, phenolic compounds, which have a strong impact on intestinal microbial activity and thus indirectly alleviate colonic mucosal disturbances caused by colitis. Based on these results, the examined chicory products, especially chicory root meal, might be considered a valuable source of dietary fibre that regulates the intestinal physiological fermentative processes and thus reduces disorders associated with colitis. Further *in vivo* studies are needed to better understand the potential clinical relevance of chicory products in processes related to the abnormalities in the gut immune system associated with inflammatory bowel disease.

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