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Cherry tomato supplementation increases the area of the intestinal mucosa and the number of muscle layers in rats

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ABSTRACT

Tomatoes act as prebiotics in the gut. The effects of cherry tomatoes on gastrointestinal health have not yet been studied. Four cherry tomato supplementation diets (CTSDs) were prepared from the juice and cake of fresh and processed (heat-treated) cherry tomatoes. The contents of the gut and histological changes in the cecum and intestine were analyzed at 4 weeks in rats fed CTSDs. The lactic acid bacteria level in fecal contents of rats fed CTSDs increased compared with the control. The gut length was longer in rats fed CTSDs than that in control animals. In addition, the cecal propionate level significantly increased ($p < 0.05$), and acetate and butyrate levels decreased compared with control animals, however, regardless of the type of CTSD, the total concentration of short chain fatty acids (SCFAs) in all rats fed different CTSDs was similar with the control. The thicknesses of the mucosa and muscle of the cecum and colon increased in rats fed CTSDs compared with the control. CTSDs increased the area of the mucosa and the number of muscle layers in the intestine and cecum of rats, which strengthened the barrier function and promoted gastrointestinal health.

1. Introduction

The colon and cecal wall consist of mucosa, submucosa and muscle layers. The mucus layer plays an important role as a physical–chemical barrier in the intestinal epithelium. Dietary fiber has beneficial effects on gut health by stimulating the production of short chain fatty acids (SCFAs) and changing the composition of the microbiota and the physiology of the colon (Hijova & Chmelarova, 2007). Probiotics improve the barrier function of the intestinal mucosa and facilitate its repair (Resta-Lenert & Barrett, 2003; Sun et al., 2013). SCFAs, such as acetate, butyrate and propionate, are produced from fibers by microbial fermentation in the intestine, and they can stimulate the secretion of colonic mucus, whereas lactate and succinate do not stimulate colonic mucus secretion (Shimotoyodome, Meguro, Hase, Tokimitsu, & Sakata, 2000). Different fibers possess different physical properties and chemical structures, and these characteristics are important factors in the efficient fermentation of fiber by bacteria. Depending on the fiber sources and the intestinal microbiota composition, the SCFA concentrations are variable (Stewart, Savarino, & Slavin, 2009), and different fiber sources may affect human health differently. Fibers can bypass digestion and absorption in the small bowel and undergo fermentation in the colon, thereby enhancing SCFA production that has been associated with the reduced risk of some diseases, including irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease and cancer (Floch & Hong-Curtiss, 2001; Jenkins, Kendall, & Vuksan, 1999).

Tomato is a highly consumed vegetable worldwide. Dried tomatoes reduce serum cholesterol. Specifically, a glycoside, esculeogenin A, in tomato significantly decreases cholesterol, triglycerides (TG) and low-density lipoprotein (LDL), and reduces atherosclerotic lesions in ApoE (apolipoprotein E) deficient mice (Fujiwara et al., 2007). Tomatoes contain a high amount of fiber, oligosaccharides and polysaccharides, which act as prebiotic compounds in the gut environment (Bornet, Brouns, Tashiro, & Duvillier, 2002). Nonetheless, there is limited information on the effects of cherry tomatoes (*Solanum lycopersicum*) on gastrointestinal health. The aim of the present study was to evaluate the effects of cherry tomato supplementation (CTS) on modulating gastrointestinal health in rats. To investigate the beneficial effects of different CTSDs on gastrointestinal health, we prepared four types of CTSDs according to their composition. The changes in health indices in the cecum and colon in rats fed CTSDs for 4 weeks were observed. Morphological and physiological changes in the intestine of rats fed different CTSDs were also investigated.

Abbreviations: CTSD, cherry tomato supplementation diet; MRS, deMan, Rogosa and Sharpe; AB, alcian blue; HE, hematoxylin and eosin; CFU, colony forming unit; BHT, butylated hydroxytoluene; IBS, irritable bowel syndrome.

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2. Materials and methods

2.1. Preparation and analysis of tomato samples

Cherry "Yoyo" tomatoes harvested from Chungcheongnamdo, South Korea were used in this study. Fresh and processed (heat-treated at 80 °C for 15 min) cherry tomato samples were separated into juice and cake after processing with a pulper (AG-5500, Angel Juicer Co., Pusan, Korea). Fresh and heat-treated tomato juices were designated as A1 and A2, respectively. To increase the absorption of high molecular nutrients in tomato cakes, the tomato cake was treated with 1% Viscozyme® L (Novozymes Inc., Copenhagen, Denmark) (54:1, v/v), reacted in a 50 °C water bath for 4 h with shaking at 200 rpm, freeze-dried, and ground using a grinder (1093 Cyclotec, Foss Tecator AB, 1093, Höganäs, Sweden) to yield 40–80 mesh size particles. Fresh and heat-treated tomato cakes were designated as B1 and B2, respectively. The chemical composition of the cherry tomato powder was determined by the standard AOAC method (Cunniff P. AOAC International, 1997). Lycopene was extracted from the cherry tomato powder using a mixed solvent (hexane:acetone:methanol = 50:25:25, v/v/v) in the presence of butylated hydroxytoluene (BHT) as an antioxidant. The hexane layer containing lycopene was separated from the mixture and recovered several times. The optical density was measured at 470 nm using a spectrophotometer (UV/VIS Lambda35, Perkin Elmer, Waltham, MA, USA). The total lycopene content was calculated from the optical density, which was based on a standard curve generated by a lycopene standard (Sigma, St Louis, MO, USA) (Koh, Kim, & Oh, 2010). The chemical composition of the tomato samples is shown in Supplementary Table 1.

2.2. Animals

Male Sprague–Dawley (SD) rats (6 weeks old) were purchased from KOATECH (Gyeonggi-do, Pyeongtaek, Korea). They were housed and maintained at a constant temperature (24 ± 1 °C) and humidity (55%) with 12 h cycles of light and dark. Rats were fed AIN-93G (TestDiet, St. Louis, MO, USA) for 7 days prior to experiments to allow adaptation, with free access to water. All experimental procedures were approved by the Korea University Institutional Animal Care and Use Committee (Approval No. KUIACUC-2013-151) and performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996). Rats (n = 6) received pelleted CTSDs, which were prepared by Feedlab (Gyeonggi-do, Guri, Korea) to have the same chemical composition. Each CTSD was compensated to contain the same amount of protein (%), lipid (%) and crude fiber (%) using casein (protein), soybean oil (lipid), and cellulose (crude fiber). Corn starch was used to adjust the total amount of carbohydrate. The ingredients and composition of the CTSDs are shown in Supplementary Table 2. The control diet consisted of AIN-93G.

2.3. Measurement of body weight, food intake, food efficiency ratio (FER), and relative liver weight

Food intake was measured daily, and body weights were determined weekly. Daily food intake was calculated by subtracting the weight of the leftover food from the weight of the total amount of food provided and divided by the number of rats. The FER was calculated as the total gain of body weight divided by the total intake of food for 4 weeks. The relative liver weight per 100 g of total weight of each rat was calculated as the total liver weight divided by the body weight on the last day of the experiment and multiplied by 100.

2.4. Fecal analysis

Fecal analysis was performed three independent times. The first, second and third analyses were 0, 14 and 28 days, respectively, after rats received different CTSDs. Fecal specimens of 0.5 g were diluted with 5 mL of distilled water and centrifuged at 1000 ×*g* for 5 min. The pH of the supernatants was measured using a pH meter from Mettler Toledo (Greifensee, Switzerland). The water content of the fecal sample was measured using a drying oven set at 105 °C for 1 h. The fecal water content (%) was calculated by:

$$\text{Water content}(\%) = \frac{\text{Wwet} - \text{Wdry}}{\text{Wwet}} \times 100.$$

W_{wet} and W_{dry} represent the weights of the fecal samples before and after drying in the oven, respectively (Lee et al., 2009).

2.5. Enumeration of fecal lactic acid bacteria

The level of lactic acid bacteria was determined in fecal samples at 4 weeks after rats were fed different CTSDs. The overnight-fasted rats were anesthetized with 2% isoflurane, and the colon was removed. The fresh fecal samples were diluted 10-fold by weight in saline and homogenized. Lactic acid bacteria were anaerobically cultured on deMan, Rogosa and Sharpe (MRS) agar (BD Difco, Sparks, MD, USA) for 48 h at 37 °C, and colonies were counted at the end of the incubation period. Microbiological analyses were performed in triplicate.

2.6. Analysis of cecal contents and SCFA

After 4 weeks of experimental diet feeding, rats were fasted for 12 h and sacrificed under anesthesia with 2% isoflurane. Thereafter, the cecum was excised and weighed with and without its contents. Cecal contents of 0.5 g were used for the measurement of pH, and another identical amount was used for the determination of water content. These methods were similar with those used to analyze the feces. For the analysis of SCFA, the pH of the suspension was adjusted to 2–3 by adding 5 M HCl. The sample was then placed on a shaker for 1 h at 300 rpm (Zhao, Nyman, & Jonsson, 2006). Samples were centrifuged for 15 min at 1000 ×*g*, and the supernatants were filtered through a 0.2 μm syringe filter. After sample preparation, gas chromatography (GC) was performed using a VARIAN 3900 GC system (Vernon Hills, IL, USA) equipped with a flame ionization detector (FID). A fused-silica capillary SUPELCOWAX™ 10 column with a free fatty acid phase (SUPELCO, Bellefonte, PA, USA) of 30 m × 0.25 mm internal diameter (i.d.) coated with a 0.25 μm-thick film was used. Helium was supplied as the carrier gas at a flow rate of 1.0 mL/min. The initial oven temperature of 120 °C was maintained for 1.5 min, raised to 220 °C at 20 °C/min, and held for 5.0 min. Glass wool (SUPELCO) was inserted into the glass liner of the splitless injection port. The temperatures of the FID and the injection port were 240 °C and 200 °C, respectively. The injected sample volume for GC analysis was 1 μL, and the run time for each analysis was 11.5 min. The measurements were compensated using acetate, propionate, butyrate and isobutyric acid standards (Sigma, St. Louis, MO, USA). Isobutyric acid was used as the internal standard.

2.7. Histological analysis and measurement of the length of the small intestine and colon

For histological analysis, cecum and colon specimens were fixed in 10% formalin and embedded in paraffin. Paraffin blocks were cut at 5 μm, and cross-sections were stained with either hematoxylin and eosin (HE) or alcian blue (AB). After mounting with Canada balsam, the slides were observed under an optical microscope at 100× magnification, and images were analyzed using MetaMorph software (Molecular Devices, Sunnyvale, CA, USA). The lengths of the small intestine and colon were measured using a tapeline.