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Dahyun Hwang, Jong-Ho Koh, Jiyun Lee, Youngshik Kim, Young-Hee Lim

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Research paper**Title:**

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

Authors:

Dahyun Hwang^a, Jong-Ho Koh^b, Jiyun Lee^a, Youngshik Kim^c, Young-Hee Lim^{a,d,*}

Authors Affiliations:

^a *Department of Public Health Science (BK21 PLUS program), Graduate School, Korea University, Seoul, Republic of Korea*

^b *Department of Bio-Food Analysis, Bio-campus, Korea Polytechnics College, Nonsan, Chungnam, Republic of Korea*

^c *Department of Plant and Food Sciences, Sangmyung University, Cheonan, Chungnam, Republic of Korea*

^d *Department of Laboratory Medicine, Korea University Guro Hospital, Seoul, Republic of Korea*

* Corresponding author at: Department of Public Health Science, Graduate School, Korea University, Seoul 136-703, Republic of Korea. Tel.:+82 2 940 2815; fax: +82 2 940 2819.

E-mail address: yhlim@korea.ac.kr (Y.H. Lim).

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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.

Keywords:

Gut histomorphology

Propionic acid

Lactic acid bacteria

Short chain fatty acids

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

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, Cao, Song, Diao, Zhou, & Zhang, 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 and analyzed in the serum of rats. Morphological and physiological changes in the intestine of rats fed different CTSDs were also investigated.

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 Cyclote, 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, 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 $1,000 \times g$ 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 $105\text{ }^{\circ}\text{C}$ for 1 h. The fecal water content (%) was calculated by:

$$\text{Water content (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100$$

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 $37\text{ }^{\circ}\text{C}$, 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 $1,000 \times g$, 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 SUPELCOWAXTM 10 column with a free fatty acid phase (SUPELCO, Bellefonte, PA, USA) of 30 m \times 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 \times 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.

2.8. Statistical analysis

All statistical analyses were performed using SPSS version 12.0 for Windows (SPSS, Chicago, IL, USA). Values were expressed as a mean \pm SD (standard deviation) of three independent experiments performed in triplicate. The differences among groups were evaluated by analysis of variance (ANOVA), followed by Duncan's multiple range tests. A level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of CTSDs on food efficiency ratio in rats

Rats fed different CTSDs exhibited a significant decrease ($p < 0.05$) in food intake and body weight compared with the control (Supplementary Table 4). However, the body weight gain was higher in the 12% supplemented CTSD than that in the 6% supplemented CTSD. The relative liver weights of rats were similar between groups. Although all diets had the same amount of protein, lipid, carbohydrate and fiber, the FERs in all rats fed CTSDs significantly decreased ($p < 0.05$) compared with the control, and the FERs of the juice powder diet groups were higher than those of the cake powder diet groups. The FERs of rats fed different CTSDs were 8–34% lower than that of the control.

3.2. The changes of fecal pH and water content

Before feeding rats with different CTSDs (0 day), the pH and water content of feces were approximately 7.46 and 43.56%, respectively, with no differences among the CTSD groups (Supplementary Table 3). Overall, the pH of feces significantly decreased ($p < 0.05$), and the water content increased ($p < 0.05$) in rats fed CTSD for 14 days compared with the control. Specifically, the pH of the cake supplementation group (B group) was less than 7.0. The water content of feces increased 1.25–1.42-fold after 28 days of CTSD feeding compared with those of feces at 0 day, whereas the water content of feces of the control group showed 1.12-fold increase. The water content of all fecal specimens from rats fed different CTSDs,

except 6%-A1 and -A2 groups and the control group, was greater than 50.0%. The pH of most feces specimens from rats fed CTSDs was acidic (approximately 6.9) after 28 days, whereas there was no change in the pH of feces from the control with experiment time.

3.3. The cecal contents and wall weights

To evaluate the effects of the different CTSDs on the cecal environment, we measured the cecal contents and wall weights. The weights of the cecal content and wall increased in rats fed CTSDs compared with the control (Table 1). The cecal water content was higher in rats fed CTSDs than that of the control. The cecal pH in rats fed CTSDs was not different from that of the control. However, total cecal, cecal wall and cecal content weights significantly increased ($p < 0.05$) in all rats fed CTSDs in a dose-dependent manner compared with the control. The cecal wall weight in rats fed CTSDs increased 1.18–1.53-fold compared with the control.

3.4. The production of SCFAs

The concentrations of fermentative end-products (SCFAs) in cecal contents are shown in Table 2. The concentration of cecal propionic acid significantly increased ($p < 0.05$) 1.23–1.50-fold in rats fed different CTSDs, and the concentrations of acetate and butyrate decreased compared with the control. The concentration of propionic acid increased in a

dose-dependent manner in all rats compared with the control, except in the B2 group. Although the ratio of SCFA changed, the total amount of SCFA was similar among all groups and not different from the control.

3.5. *Histomorphological changes*

We investigated histological changes at 4 weeks in rats fed with different CTSDs. To determine the thickness of cecum and colon wall, we performed HE staining and measured the thickness of the tunica muscularis externa, submucosa and mucosa using MetaMorph software (Table 3). The images of the cecum and colon are shown in Fig. 1A. The thickness of the cecum and colon significantly increased ($p < 0.05$) in a dose-dependent manner in all rats compared with the control, except in the B2 group. Specifically, the tunica mucosa of 12%-A1, A2 and B1 and 6%-B2 groups was 2-fold thicker than that of the control. The 12% supplemented group showed a better effect on the increase of the thickness of the cecum and colon than the 6% supplemented group, except in the B2 group. To detect acid mucin production in mucosa, alcian blue staining was performed, and the total area of acid mucin was measured using image software. The production of acid mucin significantly increased ($p < 0.05$) in rats fed different CTSDs compared with the control (Fig. 1B). In the 6%-B2 group, the area increased more than 3-fold compared with the control. In addition, the area of acid mucin increased in a dose-dependent manner in all rats, except in the B group. Different CTSDs showed profound effects on the production of acid mucin. The lengths of the small intestine and colon increased significantly ($p < 0.05$) in rats fed different CTSDs compared

with the control (Table 4). The gut length was longer in the 6% supplemented group than that of the 12% supplemented group.

3.6. The lactic acid bacteria level in the intestine

To measure the lactic acid bacteria level, fecal specimens were homogenized in saline and serially diluted. Fifty μL of each dilution was plated in triplicate on MRS agar, and the colony forming unit (cfu) was determined. The results were presented as the mean \log_{10} cfu/g of fecal sample. The lactic acid bacteria level in all fecal specimens from rats fed different CTSDs, except in the A1 group, increased compared with the control (Table 5). The lactic acid bacteria level increased in a dose-dependent manner in all rats fed CTSDs, except in the B2 group.

4. Discussion

Tomatoes have many beneficial advantages on gut health because they are rich in nutrients, such as lycopene and high in fiber. Lycopene was abundant in both A (juice) and B (cake) samples, and heat treatment increased its amount. Lycopene in fresh tomatoes exists mostly as an all-*trans* isoform, which has a lower bioavailability than the *cis* isoform. However, isomerization of the all-*trans* form to the *cis* form occurs during heat processing, which improves the bioavailability of lycopene. Even though there are no studies on the relationship

between lycopene and gut health, lycopene has several health benefits such as the prevention of prostate (Ilic & Misso, 2012) and breast cancer risk (Sato, Helzlsouer, Alberg, Hoffman, Norkus, & Comstock, 2002), and cardiovascular disease (Sesso, Liu, Gaziano, & Buring, 2003). Lycopene can regenerate the small intestine previously altered in morphology and function by exposure to gamma radiation by attenuating oxidative stress (Saada, Rezk, & Eltahawy, 2010). The effect of lycopene on the improvement of gut health requires further study. In general, high fiber foods have a lower energy density and require a long mastication time to breakup fibers (Haber, Heaton, Murphy, & Burroughs, 1977). Furthermore, fiber retains water. The water content was higher in the feces and cecum of rats fed CTSDs than in that of the control. Thus, a CTSD might improve constipation symptoms.

There is a relationship between diet and gastrointestinal growth in rats (Younoszai, Adedoyin, & Ranshaw, 1978). For example, fiber induced the growth of the small intestine and colon in both length and weight. Enlargement of the cecum is common in rats fed dietary fiber. Gastrointestinal growth may be influenced by diverse factors such as prolonged cecal residence of the fiber, increased bacterial mass, or increased bacterial end-products (Eastwood, 1992). Therefore, it is possible that fiber in CTSDs affected gut elongation and cecum enlargement in the present study. By histological analysis, the thickness of the tunica muscularis externa, submucosa and mucosa significantly increased ($p < 0.05$). When soluble fiber reaches the small intestine, it absorbs water to create a large, jelly-like mass, which makes gut muscle layer enlarged due to increased physical work required to propel the viscous gut contents (Judd & Truswell, 1985) and gastric distention increases (Saltzman et al., 2001). The acidification of colon contents stimulates mucus synthesis and secretion (Kleessen, Hartmann, & Blaut, 2003). In addition, acidic mucins such as the protective sulfomucins

increased in the intestines of rats fed fiber. Sulfomucins have important effects on the gut mucosal barrier and in the maintenance of gut health (Kleessen, Hartmann, & Blaut, 2003). In the present study, the acid mucin area in rats fed CTSDs also greatly increased compared with the control. These results are consistent with the increase in propionic acid in cecal contents of rats fed different CTSDs. Therefore, CTSDs clearly increase mucosal layers and muscle in the rat intestine, which might be due to the increase in the concentration of propionic acid produced from dietary fiber. Although the intestinal mucus dramatically increased with the increase in propionic acid in rats fed CTSDs, the concentrations of other SCFAs such as butyric acid and acetic acid decreased compared with the control. A relationship may exist between the mucosal thickness and the propionic acid concentration because the levels of total SCFA were similar but the concentrations of propionic acid were higher in rats fed CTSDs compared with the control. The effect of mucus on the role of propionic acid in the intestine of rats fed CTSDs requires further study. The dietary fiber, pectin, has been shown to induce significant elongation of the small and large intestines, enlarge the ileum muscle and increase the mucosal area in the colon (Stark, Nyska, & Madar, 1996). Different CTSDs elongated the lengths of the small intestine and colon and significantly increased ($p < 0.05$) the muscularis externa, submucosa, and mucosa area in the colon and cecum, especially in the submucosa area. Except for the increase in colon length, cellulose feeding does not induce significant morphological changes (Stark, Nyska, & Madar, 1996). This indicates that the chemical composition of the dietary fiber may play an important role in the structural modification of the intestine and cecum. In addition, SCFA products vary according to fermentation bacteria and the chemical composition of the fiber, which might affect morphological changes and result in physiological and pathological changes in the intestine.

Acetate, propionate and butyrate are the predominant SCFAs in the gut, and they are produced by microbiota in the colon and the distal small intestine from resistant starch, dietary fiber, and other low-digestible polysaccharides during fermentation (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). The composition of SCFA was different depending on the chemical and physical structure of the fiber. The rate and amount of SCFA produced also depended on the species and amounts of microbiota in the colon. SCFA affects gastrointestinal morphology and function such as the energy supply to the intestinal mucosa, pH and stimulation of sodium and water absorption (Scheppach, 1994). The concentration of propionic acid in cecal content significantly increased ($p < 0.05$) compared with the control. Interestingly, the total concentration of SCFA of all rats fed CTSDs was similar with the control. This means that a similar concentration of SCFA was produced from a similar amount of carbohydrates, regardless of the source of fiber, whereas the composition of SCFA varied according to the source.

Fermentable fibers as prebiotics increase the mass of health-promoting bacteria such as *lactobacilli* and *bifidobacteria* in the colon (Roberfroid, 2005). The feces of rats fed different CTSDs contained a higher level of lactic acid bacteria than the control fed a normal diet, especially in the feces of rats fed the heat-treated CTSD, which might have contained more beneficial factors such as lycopene than fresh CTSD. The cecum in rodents is a major site of fermentation. Probiotics effectively reduced intestinal permeability and alleviated stress-induced intestinal mucosa damage in rats probably because of improved immune function in the intestine (Sun, Cao, Song, Diao, Zhou, & Zhang, 2013). The concentration of lactic acid bacteria increased in rats fed different CTSDs, which may have promoted mucus secretion, resulted in the reduction of intestinal permeability, and improved mucosal barrier integrity.

Increased intestinal permeability might be related to several diseases such as human type 2 diabetes (Horton, Wright, Smith, Hinton, & Robertson, 2013) and irritable bowel syndrome (IBS) (Dunlop et al., 2006). Therefore, we may assume that an increase in the intestinal mucosa layer by a CTSD might alleviate such diseases by building the thick physical barrier, which inhibits the translocation of antigens in intestine.

The data in the present study showed an improvement in gut health in a dose-dependent manner in all rats fed different CTSDs, except the B2 group. Rats fed a 12% supplemented diet containing B2 showed less effective growth of lactic acid bacteria, production of propionic acid, an increase of the mucosa and muscle layer, and an increase of the amount of acid mucin than rats fed a 6% supplemented diet containing B2. The 12% supplemented diet containing B2 included 0.6% cellulose, which compensated for the dietary fiber of the other groups. It is not clear whether this amount of cellulose may have affected our results. The concentration of SCFA varied according to the species of bacteria and the presence of undigested carbohydrate in the intestine. In the present study, CTSDs yielded higher concentrations of propionic acid than butyric acid and acetic acid produced by the intestinal microbiota of rats. Although the species of bacteria are unknown, bacterial fermentation products of CTS, especially propionic acid, showed beneficial effects on intestinal health by increasing the mucus layer, which is important for barrier function.

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Figure legend

Fig. 1. Histomorphology of the colon wall of rats fed with a CTSD for 4 weeks.

(A) Hematoxylin and eosin staining ($\times 100$) and (B) the area of acid mucin. (a) A1-6%, (b) A1-12%, (c) A2-6%, (d) A2-12%, (e) B1-6%, (f) B1-12%, (g) B2-6%, (h) B2-12% and (i) control.

Table 1

Total weight, weight of contents and wall, water contents and pH of the cecum in rats fed different CTSDs for 4 weeks.

Group	Weight (g/100 g b.w.)			Water contents (%)	pH
	Cecal total	Cecal wall	Cecal contents		
A1-6%	0.88 ± 0.04 ^{ac}	0.21 ± 0.02 ^a	0.68 ± 0.03 ^{abcd}	77.54 ± 0.08 ^{ab}	7.26 ± 0.09 ^{ac}
A1-12%	1.05 ± 0.04 ^b	0.26 ± 0.03 ^b	0.80 ± 0.06 ^b	79.64 ± 1.98 ^{abc}	7.05 ± 0.09 ^a
A2-6%	0.83 ± 0.11 ^{ac}	0.19 ± 0.02 ^{ac}	0.61 ± 0.10 ^{ac}	78.16 ± 0.90 ^{ab}	7.47 ± 0.12 ^b
A2-12%	0.82 ± 0.10 ^{ac}	0.19 ± 0.01 ^{ac}	0.63 ± 0.08 ^{acd}	79.42 ± 0.81 ^{abc}	7.25 ± 0.23 ^{ac}
B1-6%	0.95 ± 0.06 ^{bc}	0.20 ± 0.02 ^{ac}	0.75 ± 0.07 ^{bcd}	80.30 ± 1.89 ^{bc}	7.30 ± 0.08 ^{bc}
B1-12%	0.98 ± 0.18 ^{bc}	0.22 ± 0.05 ^a	0.77 ± 0.13 ^{bd}	82.15 ± 3.50 ^c	7.25 ± 0.07 ^{ac}
B2-6%	0.82 ± 0.15 ^{ac}	0.20 ± 0.01 ^{ac}	0.63 ± 0.13 ^{acd}	80.25 ± 1.40 ^{bc}	7.19 ± 0.05 ^{ac}
B2-12%	0.94 ± 0.10 ^{bc}	0.22 ± 0.01 ^a	0.72 ± 0.10 ^{abcd}	80.35 ± 0.48 ^{bc}	7.20 ± 0.05 ^{ac}
Control	0.77 ± 0.02 ^a	0.17 ± 0.01 ^c	0.59 ± 0.03 ^a	77.14 ± 0.16 ^a	7.49 ± 0.10 ^b

^{abcd} Values in the same column not sharing a common superscript are significantly different ($p < 0.05$) from each other.

Table 2

Concentration of short chain fatty acid (SCFA) in cecal contents of rats fed different CTSDs for 4 weeks.

Group	Concentration of wet cecum contents ($\mu\text{mol/g}$)			
	Acetate	Propionate	Butyrate	Total
A1-6%	131.05 ± 2.92^a	22.29 ± 1.88^a	9.10 ± 3.04^a	162.44 ± 2.61^a
A1-12%	137.62 ± 1.78^{bc}	25.29 ± 1.10^b	12.17 ± 2.05^{bcd}	175.08 ± 1.64^b
A2-6%	134.65 ± 4.57^{bc}	25.53 ± 1.37^b	8.88 ± 1.07^a	169.06 ± 2.33^c
A2-12%	137.28 ± 6.06^{bc}	26.24 ± 1.04^b	10.08 ± 1.08^{ab}	173.60 ± 2.72^{bc}
B1-6%	135.02 ± 3.78^{bc}	25.54 ± 0.44^b	10.85 ± 0.32^{abc}	171.41 ± 1.51^{bc}
B1-12%	134.65 ± 3.03^{bc}	25.95 ± 1.12^b	12.89 ± 1.46^{cd}	173.49 ± 1.87^{bc}
B2-6%	137.79 ± 5.06^{bc}	27.08 ± 1.43^b	11.93 ± 0.60^{bcd}	176.80 ± 2.36^b
B2-12%	131.04 ± 0.27^a	26.54 ± 1.08^b	10.98 ± 0.54^{abc}	168.56 ± 0.63^c
Control	139.19 ± 2.37^c	18.11 ± 0.62^c	13.76 ± 0.24^d	171.06 ± 1.08^{bc}

^{abcd} Values in the same column not sharing a common superscript are significantly different ($p < 0.05$) from each other.

Table 3

Thickness of the tunica muscularis externa, tunica submucosa and tunica mucosa in the cecal wall and colon wall and the area of total acid mucin in the colon mucosa of rats fed different CTSDs for 4 weeks.

Group	Thickness of cecal wall (μm)			Thickness of colon wall (μm)			Area of acid mucin in mucosa (mm^2)
	muscularis externa	submucosa	mucosa	muscularis externa	submucosa	mucosa	
A1-6%	68.40 \pm 4.21 ^a	55.76 \pm 1.90 ^a	194.24 \pm 1.17 ^a	67.06 \pm 4.54 ^a	64.68 \pm 0.66 ^a	136.66 \pm 3.53 ^a	32.05 \pm 1.10 ^a
A1-12%	69.54 \pm 1.00 ^a	67.26 \pm 3.06 ^b	257.76 \pm 9.06 ^b	85.01 \pm 2.94 ^b	65.69 \pm 2.16 ^a	176.79 \pm 7.87 ^b	62.02 \pm 2.72 ^b
A2-6%	59.15 \pm 6.33 ^{bc}	72.72 \pm 5.19 ^{bc}	159.91 \pm 4.21 ^c	55.91 \pm 5.91 ^c	73.73 \pm 0.43 ^b	181.12 \pm 6.15 ^b	34.13 \pm 3.05 ^a
A2-12%	69.63 \pm 2.02 ^a	79.21 \pm 3.54 ^c	205.79 \pm 2.28 ^d	68.90 \pm 1.06 ^a	76.54 \pm 1.70 ^{bc}	204.36 \pm 6.51 ^c	57.54 \pm 0.82 ^c
B1-6%	79.71 \pm 7.34 ^d	94.36 \pm 7.30 ^d	181.24 \pm 4.20 ^e	67.68 \pm 2.33 ^a	74.63 \pm 5.84 ^{bc}	156.64 \pm 2.03 ^d	38.78 \pm 1.25 ^d
B1-12%	94.49 \pm 1.52 ^e	100.93 \pm 3.66 ^d	282.28 \pm 8.31 ^f	78.74 \pm 3.74 ^b	81.60 \pm 6.08 ^c	224.64 \pm 0.58 ^e	69.86 \pm 0.26 ^e
B2-6%	63.50 \pm 2.65 ^{ac}	72.71 \pm 4.67 ^{bc}	258.13 \pm 2.00 ^b	91.81 \pm 3.05 ^d	74.57 \pm 3.55 ^{bc}	202.10 \pm 3.74 ^c	71.50 \pm 0.64 ^e
B2-12%	57.67 \pm 3.82 ^{bc}	70.80 \pm 3.77 ^b	181.89 \pm 9.09 ^e	71.56 \pm 2.85 ^a	70.58 \pm 4.91 ^{ab}	194.06 \pm 3.11 ^f	45.70 \pm 2.79 ^f
Control	55.65 \pm 2.17 ^b	53.35 \pm 1.63 ^a	118.48 \pm 7.97 ^g	48.96 \pm 6.12 ^e	47.17 \pm 3.83 ^d	127.83 \pm 2.07 ^g	23.70 \pm 2.15 ^g

^{abcdetg} Values in the same column not sharing a common superscript are significantly different ($p < 0.05$) from each other.

Table 4

Lengths of the small and large intestines of rats fed different CTSDs for 4 weeks.

Group	Small intestine (cm)	Large intestine (cm)
A1-6%	107.67 ± 3.51 ^{ab}	20.50 ± 1.00 ^a
A1-12%	103.00 ± 2.65 ^{abc}	19.75 ± 0.50 ^{ab}
A2-6%	109.67 ± 3.21 ^b	19.38 ± 0.75 ^{ab}
A2-12%	105.67 ± 2.08 ^{ab}	18.25 ± 0.50 ^{bc}
B1-6%	107.67 ± 4.16 ^{ab}	20.50 ± 1.29 ^a
B1-12%	101.67 ± 5.03 ^{ac}	18.75 ± 0.96 ^{bc}
B2-6%	105.00 ± 6.08 ^{abc}	18.88 ± 0.63 ^b
B2-12%	102.33 ± 4.04 ^{abc}	19.25 ± 0.50 ^{ab}
Control	98.00 ± 3.61 ^c	17.25 ± 1.89 ^c

^{abc} Values in the same column not sharing a common superscript are significantly different ($p < 0.05$) from each other.

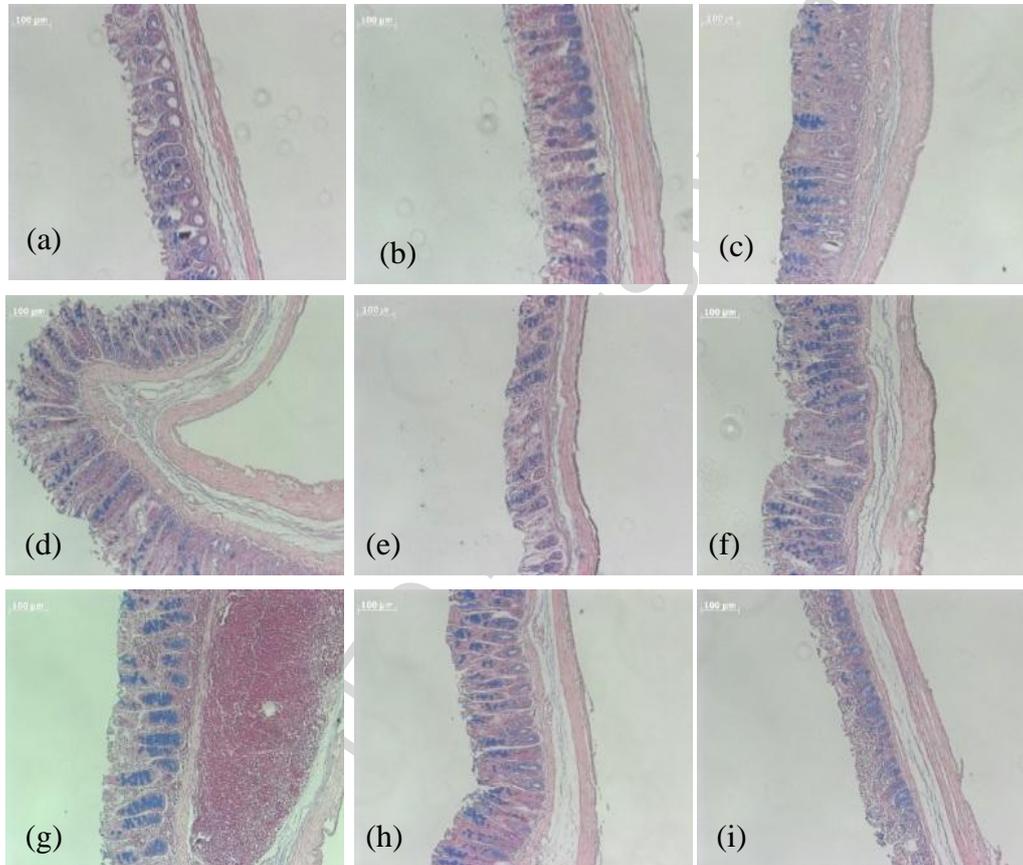
Table 5

Lactic acid bacteria analysis in fecal contents of rats fed different CTSDs for 4 weeks.

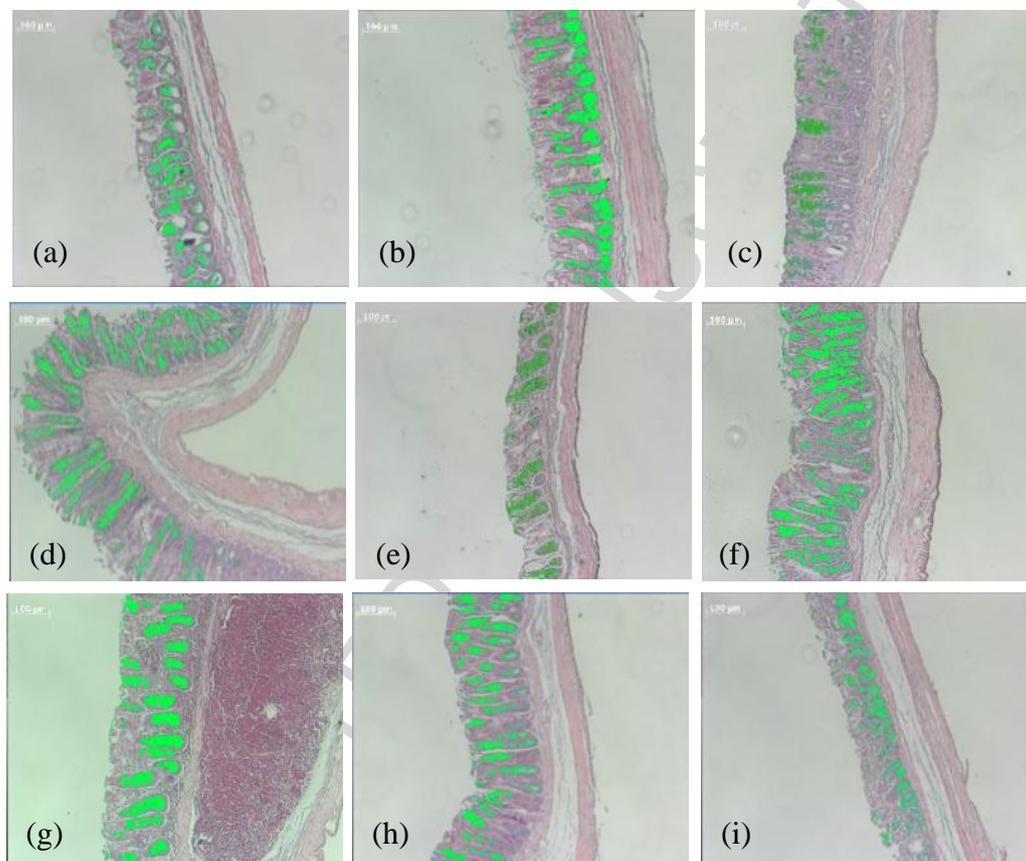
Group	Mean log ₁₀ of cfu/g of feces
A1-6%	8.74 ± 0.03 ^a
A1-12%	8.87 ± 0.02 ^{ab}
A2-6%	9.24 ± 0.01 ^c
A2-12%	9.44 ± 0.14 ^d
B1-6%	8.94 ± 0.15 ^{bc}
B1-12%	9.20 ± 0.06 ^{cf}
B2-6%	9.26 ± 0.01 ^c
B2-12%	9.05 ± 0.18 ^{ef}
Control	8.80 ± 0.02 ^{ab}

^{abcdef} Values in the same column not sharing a common superscript are significantly different ($p < 0.05$) from each other.

(A)

**Fig. 1A.**

(B)

**Fig. 1B.**

Highlights

- Cherry tomato supplementation increased lactic acid bacteria level in the gut.
- Cherry tomato supplementation significantly increased propionic acid level.
- Cherry tomato supplementation increased significantly gastrointestinal growth.
- Cherry tomato supplementation strengthened the barrier function in the gut.