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Effects of Water-Soluble Carbohydrate Concentrate from Chinese Jujube on Different Intestinal and Fecal Indices

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This study was to investigate the effects of water-soluble carbohydrate concentrate (WSCC) prepared from Chinese jujube (*Zizyphus jujuba*) on different intestinal and fecal indices in a hamster model. WSCC contained carbohydrates (771 g kg^{-1} of WSCC) including glucose, fructose, pectin polysaccharide, and hemicellulose. The administration of WSCC (5.0 and 15 g kg^{-1} of diet) effectively ($P < 0.05$) shortened gastrointestinal transit time (by 34.2–57.3%), reduced cecal ammonia (by 58.1–60.3%), elevated total short-chain fatty acid concentrations in cecum (3–4-fold), increased fecal moisture (147–170%), reduced daily fecal ammonia output (by 31.9–75.8%), and decreased the activities of β-D-glucuronidase (by 73.0–73.8%), β-D-glucosidase (by 58.2–85.7%), mucinase (by 46.2–72.6%), and urease (by 31.9–48.7%) in feces. This study suggested that adequate consumption of jujube WSCC (at least 5.0 g kg^{-1} of diet or 40 mg day^{-1}) might exert favorable effects on improving the gastrointestinal milieu and reducing the exposure of intestinal mucosa to toxic ammonia and other detrimental compounds.

KEYWORDS: Bacterial enzyme; carbohydrate; Chinese jujube; *Zizyphus jujuba*; intestinal function; short-chain fatty acid

INTRODUCTION

Some studies have demonstrated that the consumption of certain carbohydrates such as polysaccharides and oligosaccharides might help influence the growth and enzyme activities of some intestinal microflora, support normal intestinal structure, and decrease the risk of gastrointestinal disease (*1–3*). Inside the hindgut and feces, the activities of some bacterial enzymes (e.g., β-glucosidase, β-glucosidase, mucinase, and urease) catalyze a broad range of metabolic transformations, resulting in the production of toxic, carcinogenic, or mutagenic effects in the colon (*4–6*). The measurement of these bacterial enzymes in the intestinal lumen and feces might hence be used as a biomarker to evaluate the change in intestinal function, health, and integrity. Short-chain fatty acids (SCFAs) (e.g., acetate, propionate, and butyrate) play an important role in maintaining the health and integrity of the colonic epithelium (*7*) and, hence, provide a useful clue to reflect the changes in intestinal environment and health.

Chinese jujube (*Zizyphus jujuba*) is a popular fruit native to China or Syria and is widely distributed in the warmer parts of Europe, southern Asia, Africa, and Australia. In addition to their culinary uses, dried Chinese jujubes have traditionally been used for medicinal purposes, with the fruit being made into paste, soup, puree, syrup, and confection that were supposed to be consumed for digestion enhancement and general health maintenance (*8*). Depending on different recipes and dietary purposes, the general method of consumption is to cook ~50 g of dried jujubes (~20 pieces of whole fruit, 2–2.5 cm in length) in water to prepare a hot soup or drink. Our preliminary study has revealed that the dried extract (~82.4 g kg^{-1} of dried jujube, DW) obtained from the jujubes was high in water-soluble carbohydrate (~77% by weight on a moisture-free basis). As limited scientific literature about the physiological effects of jujube extract on the intestinal function and health is available, further studies on the jujube extract would be useful to clarify its physiological benefits and also provide a clue to substantiate its traditional dietary and therapeutic uses.

The objective of this in vivo studywas to investigate the effects of the water-soluble carbohydrate concentrate (WSCC) prepared from Chinese jujubes on the activities of some colonic bacterial enzymes, SCFA concentrations in the cecal content, and also certain biochemical parameters in the intestinal tract and feces. On the scientific basis, the potential effects of the jujube extract on maintaining or improving the gastrointestinal milieu were discussed.

MATERIALS AND METHODS

Preparation of Jujube WSCC. Dried Chinese jujube sample was obtained from Ko Da Pharmaceutical Co., Ltd. (Taiwan). The seed and

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Table 1. Proximate Composition[a] of the Jujube WSCC[b]

proximate composition	g kg^{-1} of WSCC (dry wt)
protein	47.6 ± 6.5
ash	37.7 ± 0.1
polysaccharide fraction	201 ± 22.8
disaccharide fraction	
sucrose	tr[c]
monosaccharide fraction	
glucose	274 ± 2.3
fructose	296 ± 20.4
total crude saponin	46.0 ± 4.5
total polyphenols	32.0 ± 1.4

[a] Means ± SD of triplicate determinations. [b] Moisture content of the WSCC was 305 ± 4.0 g kg^{-1} WSCC. A small amount of unknown fraction in the extract (~65.7 g kg^{-1} WSCC) refers to some certain oligosaccharides and bioactive compounds. [c] Trace amount (<0.1).

Table 2. Monomeric Sugar Components of the Water-Soluble Polysaccharide Fraction in the Jujube WSCC[a]

monomeric sugar component[b]	g kg^{-1} of polysaccharide fraction
rhamnose	21.2 ± 9.5
arabinose	34.4 ± 4.5
xylose	29.7 ± 16.8
mannose	15.6 ± 0.7
galactose	43.1 ± 2.3
noncellulosic glucose	161 ± 17.2
uronic acid	589 ± 48.1

[a] The content of polysaccharide fraction in the jujube WSCC was 201 g kg^{-1} WSCC. [b] Means ± SD of duplicate determinations.

pulp of the jujube samples were found to be 94 ± 4 and 906 ± 4 g kg^{-1} of dried jujube, respectively. Following the traditional practice, jujube samples were split by hand to expose the pulp and seed, then mixed with distilled water at a ratio of 100 g L^{-1}, and boiled for 2 h with continuous stirring. A jujube puree was then obtained and filtered by cheesecloth while hot. The thick puree was dried by lyophilization and kept in a desiccator until use.

Carbohydrate Analysis. According to the method of Mondal et al. (*9*), the content of water-soluble polysaccharide in the WSCC was quantified by the precipitation with an addition of 4-fold volumes of 95% ethanol. After centrifugation at 4024*g* for 10 min, the precipitated polysaccharide fraction was collected, dried, and weighed. The amount of free glucose in the supernatant was determined enzymatically using a commercially available glucose assay kit (Megazyme K-GLUC, Wicklow, Ireland). Disaccharides in the supernatant were analyzed by HPLC using the method of Da Costa Leite et al. (*10*) with slight modifications. WSCC (0.5 g) was dissolved in 5 mL of distilled water, followed by ethanol precipitation. After centrifugation at 1006*g* for 10 min, the supernatant was filtered through a 0.45 μm membrane filter and analyzed for sugar components. Fructose and sucrose were used as standards. The HPLC system consisted of a pump (L-6200A, Hitachi, Tokyo, Japan) and a refractive index detector (Bischoff 8110, Leonberg, Germany) equipped with an Ultrasep ES 100 NH2 column (250 × 4 mm, particle size = 6 μm; Bischoff, Leonberg, Germany) using acetonitrile/water (88:12, v/v) as mobile phase. An injection volume of 20 μL sample and a flow rate of 0.6 mL min^{-1} were used in the analysis. Using the method as described by Chau and Huang (*11*) with slight modifications, the water-soluble polysaccharide fraction obtained after centrifugation was hydrolyzed by 2 M H_2SO_4 at 35 °C for 60 min followed by boiling for 60 min. The noncellulosic sugar profile of the polysaccharide was then determined and quantified. Uronic acid was also determined spectrophotometrically according to AOAC method 45.4.11 (*12*) using D-galacturonic acid monohydrate as reference.

Table 4. Effect of the Jujube WSCC on the Gastrointestinal Transit Time, Fecal Moisture Content, and Fecal Dry Weight

diet group[a]	gastrointestinal transit time[b] (h)	fecal dry wt[b] (g day^{-1})	fecal moisture content[b] (g kg^{-1} of feces)
control	10.1 ± 0.07w	0.83 ± 0.12w	269 ± 22.5w
low dose	9.34 ± 0.20w	1.00 ± 0.12w	280 ± 47.5w
medium dose	6.65 ± 0.39x	0.88 ± 0.06w	394 ± 26.9y
high dose	4.31 ± 0.01y	0.99 ± 0.05w	458 ± 28.2z

[a] The low-, medium-, and high-dose diets were prepared by mixing 1.7, 5.0, and 15 g of the WSCC into 1 kg of the control diet (basal formula), respectively. [b] Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$).

Proximate Analysis. Crude protein content was calculated by multiplying the nitrogen content obtained from a CHN-OS rapid element analyzer (Heraeus F002, Hanau, Germany) by a factor of 6.25. Moisture was determined by drying the sample to a constant weight at 105 °C. Total crude saponin in the WSCC was determined according to the method of Kwon et al. (*13*) with slight modifications. Briefly, 5 g of WSCC was mixed with 50 mL of distilled water and then extracted two times with 50 mL of water-saturated *n*-butanol. After separation, the butanol fraction was evaporated at 55 °C, dried, and weighed quantitatively. The level of total polyphenols in the WSCC was measured spectrophotometrically at 750 nm using the Folin−Cioculteau method (*14*). The results were expressed as gallic acid equivalents.

Antimicrobial Assay. The antimicrobial activity of the WSCC was determined by the agar well diffusion method as described by Ahmad and Beg (*15*) with slight modifications. The test organisms including *Bacillus cereus* BCRC 10603, *Escherichia coli* BCRC 13086, *Listeria monocytogenes* BCRC 14848, *Salmonella choleraesuis* BCRC 12948, and *Staphylococcus aureus* BCRC 15211 were obtained from the Bioresources Collection and Research Center (BCRC), Taiwan. On Mueller−Hinton agar plates was spread 0.1 mL of bacterial strain suspension (10^5 CFU mL^{-1}). Wells of 8 mm diameter were punched into the agar medium and filled with 100 μL of WSCC (0.17, 0.5, and 1.5 g mL^{-1}) and solvent blank. The plates were incubated for 18 h at 37 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Diets and Experimental Design. According to the AIN-93 M diet formulation (*16*), a basal formula (control diet) was prepared by mixing the following ingredients including casein (140 g kg^{-1}), cellulose (50 g kg^{-1}), sucrose (100 g kg^{-1}), corn starch (621 g kg^{-1}), soybean oil (40 g kg^{-1}), choline bitartrate (2.5 g kg^{-1}), L-cystine (1.8 g kg^{-1}), AIN-93 M vitamin mix (10 g kg^{-1}), and AIN-93 M mineral mix (35 g kg^{-1}). These ingredients were obtained from ICN Nutritional Biochemicals (Cleveland, OH).

For pharmaceutical and intestinal health maintenance purposes of an adult (~60 kg), the traditional practice of Chinese medicine practitioners to use jujube is usually up to ~50 g of dried fruit per day (i.e., ~4 g of extract, ~20 pieces of whole fruit, 2–2.5 cm in length). Using a conversion factor of 0.01 between human and hamsters (*3*), the dried extract intake per day by hamsters was estimated to be ~40

Table 3. Effect of the Jujube WSCC on Food Intake,[a] Body Weight Gain,[a] and Organ Weight[a] of Hamsters

			organ wt (g kg^{-1} of body wt)		
diet group[b]	food intake (g day^{-1})	wt gain (g day^{-1})	small intestine	cecum	colon + rectum
control	7.68 ± 0.13w	0.96 ± 0.20w	13.7 ± 1.30w	6.40 ± 1.00w	13.2 ± 3.43w
low dose	7.33 ± 0.23w	0.77 ± 0.07w	13.5 ± 1.10w	6.60 ± 1.10w	9.10 ± 2.30w
medium dose	7.87 ± 0.23w	0.82 ± 0.12w	13.5 ± 0.40w	7.00 ± 2.10w	10.7 ± 2.30w
high dose	7.67 ± 0.00w	0.84 ± 0.25w	14.1 ± 1.60w	8.30 ± 1.00w	12.4 ± 0.05w

[a] Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [b] The low-, medium-, and high-dose diets were prepared by mixing 1.7, 5.0, and 15 g of the WSCC into 1 kg of the control diet (basal formula), respectively.

mg day^{-1}. While the daily food intake of hamsters was in general around 8 g, the jujube-containing diets were prepared by adding different amounts of dried extract (i.e., 1.7, 5.0, and 15 g extract kg^{-1} of diet) into the basal formula in this study. The three test diets, namely, low-, medium-, and high-dose diets, were prepared by mixing 1.7, 5.0, and 15 g of the dried extract into 1 kg of the basal formula, respectively.

The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. Thirty-two male Golden Syrian hamsters (6 weeks old) weighing 105 ± 2.0 g were obtained from the National Laboratory Animal Center of Taiwan. After an acclimation period of 1 week, animals were divided into eight weight classes of four each. The four diets were then randomly allocated to one of the four animals in each weight class. They were housed (in pairs) in screen-bottomed, stainless steel cages in a room maintained at 24 ± 1 °C, with a 12 h light/dark cycles, and had free access to food and water. For the determination of gastrointestinal transit time (*17*), the animals were fed a colored diet, in which a small amount of carmine was added, after fasting for 14 h. The excretion of colored feces was observed at every 30 min intervals. The time for the colored feces to be seen was regarded as the gastrointestinal transit time. After that, the animals were fed the control and test diets for 30 days. Food intakes and body weights were recorded daily. Feces were collected, weighed, and analyzed for moisture content daily. Some of the fecal samples left unused were stored at −20 °C for further use. At the end of experiment, animals were anesthetized by isoflurane (Halocarbon Laboratories, River Edge, NJ) after deprivation of food for 16 h. After laparotomy, the small intestine, cecum, and large intestine were collected. Chyme inside the small intestine and cecal content were removed. These organs and cecal content were weighed and immediately frozen at −80 °C for further analysis.

Determination of Cecal Ammonia. Cecal ammonia was determined using the methods as described by Ebihara and Nakamoto (*18*). Ammonia contents in the deproteinized solution of cecal content were determined spectrophotometrically at 630 nm using ammonia standard solution (Wako Pure Chemical Industries, Osaka, Japan) as a standard. The cecal ammonia content was expressed as micromoles of ammonia per gram of wet matter of cecal content.

Determination of Fecal Moisture and Ammonia. Fecal moisture content was determined by drying the fecal sample to constant weight in a 105 °C air oven. According to the method of Shiau and Chang (*5*), ammonia in the fresh fecal sample was extracted with cold 0.01 M phosphate buffer (pH 7.2) (1:150, w/v) for 30 min, followed by centrifugation at 1006*g* for 10 min. Fecal ammonia content in the supernatant was then determined according to the method of Okuda and Fujii (*19*).

Determination of Bacterial Enzyme Activities in Feces. Following the method of Shiau and Chang (*5*), bacterial enzymes in the fresh fecal samples collected in the last three days of the experimental period were extracted with 0.1 M phosphate buffer (pH 7.2, 1:150, w/v) and centrifuged at 1006*g* for 10 min. The bacterial enzyme activities in the supernatant were then determined. Protein in the supernatant was determined using a commercially available protein assay kit (catalog no. 500-0006, Bio-Rad, Hercules, CA).

According to the method of Goldin and Gorbach (*4*), the β-D-glucuronidase activity (micromoles of phenolphthalein liberated per minute per milligram of fecal protein) was determined by the amount of phenolphthalein released from 0.01 M phenolphthalein β-glucuronide (no. P0501, Sigma Chemical Co., St. Louis, MO). The β-glucosidase activity (nanomoles of nitrophenol produced per minute per milligram of fecal protein) was determined by the content of nitrophenol released from 1 mM 4-nitrophenyl β-D-glucopyranoside (no. N7006, Sigma). Mucinase activity (micromoles of reducing sugar released per minute per milligram of fecal protein) was estimated by the amount of reducing sugar released from porcine gastric mucin (no. M1778, Sigma) using the method of Shiau and Chang (*5*). Urease activity (nanomoles of ammonia released per minute per milligram of fecal protein) was determined by the contents of ammonia released from 0.01 M urea (no. U0631, Sigma) using the methods as described by Okuda and Fujii (*19*) and Ling et al. (*20*).

Determination of SCFA Concentrations in Cecal Content. According to the method of Whitehead et al. (*21*) with slight modifications, the SCFA concentrations in the cecal content were extracted and analyzed. Cecal content (0.3 g) was slurried in cold saline (0.9%, w/w) and centrifuged at 1006*g* for 10 min. Using isocaproic acid as an internal standard, the SCFA in the supernatant was extracted with diethyl ether, and 1 μL of the ether layer was assayed by a packed column (GP10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb) using a gas chromatograph (Hitachi G-5000) equipped with a flame ionization detector. The conditions were as follows: oven temperature, initially held at 80 °C for 3 min, raised to 130 °C at a rate of 2 °C min^{-1}; injector temperature, 200 °C; detector temperature, 250 °C; gas flow rate, 20 mL min^{-1} (carrier gas, nitrogen).

Statistical Analysis. All determinations expressed as mean ± SD were analyzed by one-way analysis of variance using the Statistical Analysis System. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In this study, the level of WSCC prepared from the Chinese jujube was about 82.4 g kg^{-1} of dried fruit. **Table 1** reveals that the jujube WSCC is high in water-soluble carbohydrates including polysaccharide and monosaccharide fractions (201 and 570 g kg^{-1}, respectively) and contains only small amounts of protein (47.6 g kg^{-1}) and ash (37.7 g kg^{-1}). The polysaccharide, disaccharide, and monosaccharide fractions accounted for 77.1% of extract by weight. Chemical analyses showed that fructose was the major sugar in the WSCC (up to 32.9% by weight) followed by glucose (30.4% by weight), hence explaining the sweetness of the jujube extract. It was also found that the WSCC contained a small amount of total saponin (46.0 g kg^{-1}) and total polyphenols (32.0 g kg^{-1}). **Table 2** reveals that the polysaccharide fraction mainly consists of several monomeric sugars including uronic acid, noncellulosic glucose, galactose, arabinose, and xylose at different levels (58.9, 16.1, 4.3, 3.4, and 3.0% by weight, respectively). These monomeric sugars accounted for 894 g kg^{-1} of polysaccharides. In general, the sugar components of pectic polysaccharides are galacturonic acid, rhamnose, arabinose, and galactose, whereas those of hemicelluloses are glucose, xylose, and mannose (*22*). This monosaccharide profile implied that the polysaccharides in the WSCC were mainly composed of pectic polysaccharide followed by hemicellulose (i.e., xyloglucan).

During the experimental period, all animals remained healthy and active. After 30 days of feeding, no significant changes in the average food intake (7.33–7.87 g day^{-1}) and body weight gain (0.77–0.96 g day^{-1}) were observed in the hamsters among the four diet groups (**Table 3**). Moreover, there were no significant variations in the weights of small intestine (13.5–14.1 g kg^{-1} bw), cecum (6.40–8.30 g kg^{-1} bw), and colon plus rectum (9.10–13.2 g kg g^{-1} bw) of hamsters among the four diet groups neither (**Table 3**). These results indicated that the consumption of jujube WSCC at different levels (1.7 to 15 g kg^{-1} diet) did not result in apparent changes in the body weight gain as well as the growth of digestive tract.

Table 4 presents the effects of WSCC supplementation on gastrointestinal transit time. As compared to the control group (10.1 h), feeding with the medium- and high-dose diets significantly ($P < 0.05$) shortened the gastrointestinal transit time by 34.2 and 57.3%, respectively. The extract from some other foods or medicinal plants such as *Amomum villosum* Lour., *Rhazya stricta*, and *A. xanthioides* could also increase intestinal motility and decrease gastrointestinal transit time by 5.1–58% (*2, 23, 24*). As shown in **Table 4**, the average fecal dry weights (0.83–1.00 g day^{-1}) of hamsters were comparable among the four diet groups. However, the moisture contents in the feces with the medium- and high-dose diets were markedly ($P < 0.05$) higher (147–170%) than those with the control diet (269 g kg^{-1} of feces). It was inferred that the significant decrease in transit

Table 5. Effect of the Jujube WSCC on the Cecal Ammonia, Fecal Ammonia, and Daily Fecal Ammonia Output

diet group[a]	cecal ammonia[b] (μmol g^{-1} of cecal content)	fecal ammonia[b] (μmol g^{-1} of fresh feces)	daily fecal ammonia output[b,c] (μmol day^{-1})
control	2.67 ± 0.54w	44.3 ± 2.96w	50.5 ± 3.37w
low dose	2.28 ± 0.02x	23.9 ± 1.83x	33.2 ± 2.55x
medium dose	1.06 ± 0.29y	26.2 ± 1.90x	34.4 ± 3.49x
high dose	1.12 ± 0.02y	6.70 ± 0.48y	12.2 ± 0.88y

[a] The low-, medium-, and high-dose diets were prepared by mixing 1.7, 5.0, and 15 g of the WSCC into 1 kg of the control diet (basal formula), respectively. [b] Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [c] The fecal fresh weight per day with the low-, medium-, and high-dose diets were 1.39, 1.44, and 1.72 g day^{-1}, respectively.

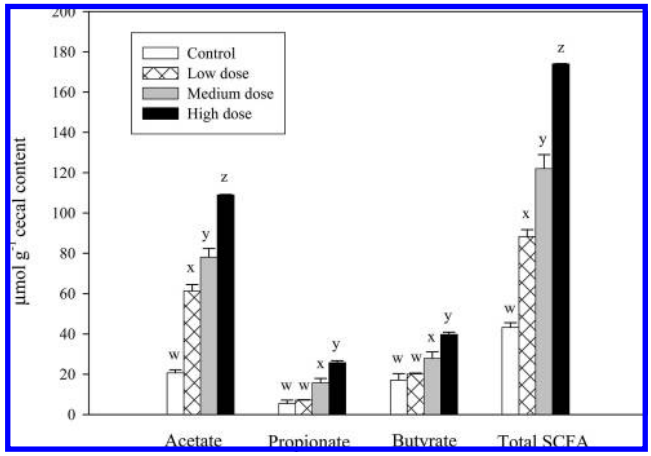

Figure 1. Effect of the jujube WSCC on different SCFA concentrations in the cecal contents. Values (means ± SD, $n = 8$) with different letters are significantly different ($P < 0.05$).

time reduced water reabsorption in the intestine lumen, hence leading to the increased moisture retention in feces. It was also reported that the consumption of fiber-rich seaweed could reduce the gastrointestinal transit time and then increase the fecal moisture content of some healthy subjects (*25*).

In **Table 5**, the inclusion of jujube WSCC (1.7–15 g kg^{-1} of diet) into the basal diet resulted in a significant ($P < 0.05$) reduction of cecal ammonia content from 2.67 to 1.12–2.28 μmol g^{-1} of cecal content (by 14.6–58.1%). For the fecal samples, the feeding of WSCC at three different doses also resulted in a significant ($P < 0.05$) reduction in ammonia contents (by 46.0–84.9%) compared with the fecal ammonia content of the control group (44.3 μmol g^{-1} of fresh feces). It was found that both the cecal and fecal ammonia contents decreased with increased consumption of WSCC. There was a correlation ($r = 0.74$; $P < 0.05$) between the cecal ammonia contents and the fecal ammonia contents. On the basis of the comparable daily fecal output among the four diet groups (0.83–1.00 g day^{-1}) (**Table 4**), the results demonstrated that the feeding of WSCC at three different doses could lead to a significant ($P < 0.05$) reduction in the daily output of fecal ammonia (by 31.9–75.8%) (**Table 5**). Some studies have shown that the ammonia level in the intestinal tract was related to the growth of intestinal microflora (*18, 26*). However, no apparent antimicrobial activity of the WSCC (0.17, 0.5, and 1.5 g/mL) against *B. cereus* BCRC 10603, *E. coli* BCRC 13086, *L. monocytogenes* BCRC 14848, *S. choleraesuis* BCRC 12948, and *S. aureus* BCRC 15211 was observed in this study. Although ammonia concentration could be decreased in the presence of saponin (~10–40 g kg^{-1} of extract) (*27*), it was speculated that the saponin in the jujube WSCC (46.0 g kg^{-1} of extract) (**Table 1**) might be one of the factors affecting the ammonia contents in cecum and feces.

Figure 1 presents the SCFA profiles in the cecal contents of hamsters fed different doses of jujube WSCC. The results demonstrated that the feeding of WSCC (1.7–15 g kg^{-1} of diet) effectively ($P < 0.05$) elevated the total SCFA concentrations by 2–4-fold (88.5–174 μmol g^{-1} of cecal content) as compared with the control group (44.6 μmol g^{-1} of cecal content). Some other authors have reported that the consumption of carbohydrate-rich fruits and vegetables such as lettuce, apple, and corn bran could also lead to an apparent increase in SCFA levels in cecum (*28–30*). Variations in the SCFA concentrations in hindgut are generally related to the consumption level of dietary carbohydrate as well as the extent of microbial fermentation (*31*). Thus, the significantly elevated SCFA levels with the WSCC-supplemented diets might be associated with the higher consumption of fermentable carbohydrates. In **Figure 1**, compared with the control group, the levels of acetate, propionate, and butyrate in the cecal contents with the medium-dose diet were significantly ($P < 0.05$) increased by 3.7-, 2.9-, and 1.6-fold, respectively, and those with the high-dose diet had a much higher value (up to 6.2-, 4.7-, and 2.3-fold, respectively). Grinder and Piland (*32*) have reported that chemical stimulation of mucosal cells by SCFAs (i.e., acetate, propionate, and butyrate) may trigger a peristaltic reflex and increase gut motility, hence shortening gastrointestinal tract transit time. Some other findings indicated that acetate could stimulate mucin secretion (*33*), propionate could affect lipid metabolism by decreasing de novo fatty acid synthesis (*34*), and butyrate as a preferred energy source for the mucosal cells might inhibit neoplastic changes in cancer cells (*35*).

Table 6 summarizes the activities of four bacterial enzymes in the feces of hamsters among all four diet groups. As the enzyme activities were measured per milligram of fecal protein, the comparable fecal dry weight (0.83–1.00 mg day^{-1}) and daily fecal protein excretion (2.13–3.11 mg day^{-1}) among different diet groups suggested that any changes in the fecal bacterial activities were basically related to the physiological effects of WSCC. As shown in **Table 6**, the initial β-glucuronidase and β-glucosidase activities in hamsters fed the control diet were 1.41 μmol of phenolphthalein liberated min^{-1} mg^{-1} of fecal protein and 136 nmol of nitrophenol produced min^{-1} mg^{-1} of fecal protein, respectively. The consumption of WSCC-supplemented diets resulted in significant ($P < 0.05$) decreases in the activities of both fecal β-glucuronidase (by 67.4–73.8%)

Table 6. Effects of the Jujube WSCC on the Activities[a] of Different Fecal Bacterial Enzymes

diet group[b]	β-D-glucuronidase[c]	β-D-glucosidase[c]	mucinase[c]	urease[c]
control	1.41 ± 0.07w	136 ± 2.88w	1.06 ± 0.07w	132 ± 41.6w
low dose	0.46 ± 0.07x	59.4 ± 0.00x	0.90 ± 0.22x	108 ± 11.7x
medium dose	0.37 ± 0.04x	56.9 ± 9.81x	0.57 ± 0.09y	89.9 ± 13.4y
high dose	0.38 ± 0.06x	19.4 ± 0.00y	0.29 ± 0.1z	67.7 ± 9.57y

[a] Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [b] The low-, medium-, and high-dose diets were prepared by mixing 1.7, 5.0, and 15 g of the WSCC into 1 kg of the control diet (basal formula), respectively. [c] Enzyme activities were as described under Materials and Methods.

and β-glucosidase (by 56.3–85.7%). The pectin component has been associated with the inhibition of colon carcinogensis by modulating the activity of β-glucuronidase (*36*). It was inferred that the reduced β-glucuronidase activity after consumption of the WSCC might be related to the considerable amounts of pectic polysaccharides in the extract (~118 g kg^{-1} of WSCC or above). In hindgut, β-glucuronidase and β-glucosidase synthesized by colonic bacteria could hydrolyze the conjugated products of detoxification and lead to the liberation of toxins, carcinogens, and drugs; therefore, the reduced activities of these enzymes were associated with decreased incidence of colon cancer (*4*). The reduced activities of these two bacterial enzymes were accordingly desirable.

Table 6 shows that the basal mucinase activities (1.06 μmol of reducing sugar released min^{-1} mg^{-1} of fecal protein) could be significantly reduced by the inclusion of WSCC at different doses (1.7, 5.0, and 15 g kg^{-1} of diet) (−15.1, −46.2, and −72.6%, respectively). Mucin can serve as a defense barrier layer on the intestinal mucosa to protect against toxic substances, enzymatic degradation, and most bacterial invasion (*37*). The presence of mucinase may hydrolyze the protective mucins and exposes the intestinal cells to carcinogenic substances (*5*). Therefore, the reduction in mucinase activity by feeding the WSCC-containing diets was desirable in maintaining a gel-like barrier protecting the colonocytes against bacteria and toxins. Furthermore, the urease activities (initially at 132 nmol of ammonia released min^{-1} mg^{-1} of fecal protein) were also markedly reduced by the feeding of WSCC at different concentrations (1.7, 5.0, and 15 g kg^{-1} of diet) (−18.2, −31.9, and −48.7%, respectively). In **Tables 5** and **6**, there is an apparent correlation ($r = 0.94$; $P < 0.05$) between the urease activity and fecal ammonia content. It might be due to the fact that about 20–25% of urea produced from amino acid degradation is hydrolyzed into ammonia by microbial urease in the gastrointestinal tract (*26*). However, the ammonia locally produced can damage the mucosal cell as well as enter the blood stream to cause harmful effects in animals (*26*). Our results suggested that the feeding of WSCC could effectively decrease the microbial urease activity as well as the harmful ammonia formed in the large intestine. Accordingly, it was speculated that adequate consumption of WSCC might help to promote the growth of beneficial microflora in the intestinal lumen and, meanwhile, suppress the growth of undesirable bacteria and therewith the harmful effects of their enzymes.

Our results demonstrated that the administration of jujube WSCC (5.0 and 15 g kg^{-1} of diet) effectively ($P < 0.05$) decreased gastrointestinal transit time, increased fecal moisture content, reduced cecal and fecal ammonia contents, increased different SCFA concentrations in cecum, and also decreased the activities of different colonic bacterial enzymes in feces. The changes of these cecal and fecal parameters might provide a concrete effect in maintaining intestinal health via reducing the exposure of intestinal mucosa to toxic ammonia and other harmful substances. On the basis of these findings, it was suggested that adequate consumption of the jujube extract (at least 40 mg day^{-1} for hamsters, ~4 g day^{-1} for human adults) might exert a favorable effect on maintaining or improving the gastrointestinal milieu and also provide a clue to substantiate its traditional therapeutic uses and dosage for intestinal health improvement.

ABBREVIATIONS USED

WSCC, water-soluble carbohydrate concentrate; SCFA, short-chain fatty acid.

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