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Raw Chinese Yam (*Dioscorea opposita*) Promotes Cecal Fermentation and Reduces Plasma Non-HDL Cholesterol Concentration in Rats

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Summary We examined the effects of raw Chinese yam (*Dioscorea opposita*), containing resistant starch (RS), on lipid metabolism and cecal fermentation in rats. Raw yam (RY) and boiled yam (BY) contained 33.9% and 6.9% RS, respectively. Male Sprague-Dawley rats were fed a cholesterol-free, control (C) diet supplemented with or without 15 and 30 g of RY or BY/100 g for 3 wk. Plasma total cholesterol concentrations in the tail vein of rats fed the 30% RY diet were significantly lower than in the C group throughout the feeding period. Compared with the C group, non-HDL concentrations in arterial plasma in the 30% RY group was significantly reduced. Liver cholesterol concentration in rats fed the 30% RY diet was significantly higher compared with those fed the C diet. Hepatic cholesterol 7α-hydroxylase mRNA and fecal bile acid excretion were significantly higher in the BY, but not the RY group, compared with the C group. Fecal cholesterol excretion in the 30% RY group was greater compared with the C group. Hepatic microsomal triacylglycerol transfer protein mRNA was significantly lower in the 30% RY group compared with the C group. Cecal pools of acetate, propionate and butyrate were 113–257%, 181–476% and 410–789% greater in the RY group compared with the C group. These results suggest raw yam is effective as a source of RS and facilitates production of short chain fatty acid (SCFA), especially butyrate, in the rat cecum. In addition, RY has a plasma-cholesterol lowering effect, possibly due to the inhibited release of VLDL.

Key Words Chinese yam, resistant starch, cholesterol, fermentation, rats

Yams, the edible tubers of various species of the genus *Dioscorea*, are important in the diets of many countries in Asia and Africa and are also widely used in most parts of the world because of the carbohydrate they provide. Yam is composed mainly of starch with small amounts of proteins, lipids and most vitamins; it is also very rich in minerals. Chinese yam, *Dioscorea opposita*, has been used as a herbal medicine as well as a food in Japan (*1*), Korea and China, and it is assumed Chinese yam has some physiological and pharmacological actions (*2*, *3*). However, few studies have examined the properties of Chinese yam. Chinese yam is high in starch and, in Japan, is unique in that it is generally consumed raw. Raw starches are digested slowly in the alimentary tract, resulting in the delivery of starch to the large intestine. These starches are referred to as resistant starch (RS) and act as dietary fibre-like compounds. RS has a hypolipidemic effect and a promoting effect on colonic microbiota and fermentation.

RS is classified into 4 types: RS1 (physically inaccessible or digestion-resistant starch), RS2 (raw granular starch), RS3 (retrograded starch) and RS4 (starches that have been chemically modified to resist digestion). Many studies have reported on the physiological effects of RS2 and RS3, but not RS1 or RS4. The effects of RS2 and RS3 are similar. Although raw high amylose cornstarch, banana starch and potato starch have been used as RS2 sources in most studies reporting physiological effects, other sources of RS2 have not been examined yet. In addition, it is rare to consume large amounts of RS2 in a typical meal, because most foodstuffs containing large amounts of starch are usually cooked. However, in Japan, Chinese yam is one of the few sources of RS2 which is available in an ordinary meal in Japan.

RS, including RS2 and RS3, reduces plasma cholesterol and triacylglycerol (TAG) concentrations in rats (*4*, *5*) and humans (*6*) and promotes the production of short chain fatty acid (SCFA), especially butyrate, in rodents (*7*, *8*) and humans (*9*). SCFA production in the large intestine contributes to inhibited hepatic cholesterol synthesis (*10*) and could suppress the proliferation of colon cancer cells (*11*). These effects are assumed to prevent the development of coronary heart disease and colon cancer. Sufficient amounts of RS are not easily consumed in a habitual diet; therefore, raw Chinese yam could be a valuable source of RS.

We aimed to examine the physiological effects of raw Chinese yam on plasma lipid concentrations and cecal

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Table 1. Composition of raw and boiled Chinese yam powder.[1]

	RY	BY
Composition (%, w/w)		
Carbohydrate	83.4	86.7
Resistant starch	33.9	6.9
Protein	10.1	8.1
Lipid	0.6	0.4
Ash	4.7	2.9
Dietary fiber	5.1	8.9
Soluble	1.9	1.8
Insoluble	3.2	7.1
Moisture	1.2	1.9

[1] RY, raw yam; BY, boiled yam.

fermentation. In the present study, we examined the effects of raw Chinese yam on plasma lipid concentration and cecal SCFA concentration compared to boiled Chinese yam in rats.

MATERIALS AND METHODS

Preparation of yam. Yam was supplied by JA Obihiro Kawanishi. The yam was peeled, sliced and immediately freeze-dried, or boiled for 1 min and freeze dried. Lyophilized samples were powdered as raw yam powder (RY) and boiled yam powder (BY). Carbohydrate, protein, lipid, ash, moisture and RS contents in RY and BY are shown in Table 1.

Animals and diets. The study was approved by Nayoro City University Animal Use Committee, and the animals were maintained in accordance with guidelines for the care and use of laboratory animals, Nayoro City University.

Male Sprague-Dawley rats weighing 80–90 g (4 wk old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed in individual cages with screen bottoms of stainless steel in a room maintained at 23±1°C with lighting from 0700 to 1900 h. Rats were acclimated by feeding a 20% casein control diet, free of cholesterol for 10 d, before feeding test diets. The basic composition of the control diet was (g/kg): casein, 200; α-cornstarch, 529.5; sucrose, 100; soybean oil, 70; AIN-93G mineral mix (*12*), 35; AIN-93 vitamin mix (*12*), 10; L-cystine, 3; choline bitartate, 2.5; and cellulose, 50.

After the acclimation period, rats were divided into 5 groups of six, based on body weight and plasma cholesterol concentration and were administered test diets for 21 d. Each group was fed the control diet supplemented with or without 150 or 300 g of RY or BY per kg. RY and BY were substituted for α-cornstarch. Body weight and food intake were recorded daily, and feces were collected during the last 3 d, lyophilized and weighed. We determined the total plasma cholesterol concentration in blood samples collected from the tail vein on day 0, 3, 7, 10, 14 and 21. After 21 d, rats were deprived of food for 2 h before sacrifice, to avoid the change in cholesterol metabolism due to prolonged fasting. The rats were anesthetized with Nembutal (sodium pentobarbital, 50 mg/kg body wt.) and blood was removed by abdominal aorta puncture into a centrifuge tube via a catheter. Plasma was separated by centrifugation at 1,200 ×*g* for 20 min, 4°C. The liver was removed, weighed, immediately frozen in liquid nitrogen and stored at −80°C until being analyzed for cholesterol and mRNA of cholesterol 7α-hydroxylase (CYP7A1), hydroxymethylglutaryl-CoA reductase (HMG-CoA-r), LDL receptor (LDL-R), Apo B, microsomal TAG transfer protein (MTP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. The epididymal fat pad and testes were removed, blotted, and weighed. The cecum was removed and weighed, and the pH of the cecal contents was measured with a pH meter (model F-52 equipped with a pH microsensor (9669–10D), Horiba, Kyoto, Japan). The cecal content was collected and stored at −40°C in air-tight tubes until being analyzed for SCFA. The cecal wall was blotted and weighed after rinsing in an ice-cold saline solution.

Biochemical analyses. The lipoprotein fractions were analyzed enzymatically to determine the concentrations of total cholesterol, lipoprotein cholesterol, esterified and non-esterified cholesterol, TAG, phospholipids and non-esterified fatty acids using an autoanalyzer (model AU-5400, Olympus, Tokyo, Japan).

To measure total liver cholesterol and TAG, liver lipids were extracted with chloroform/methanol (2 : 1, v/v) according to the method of Folch et al. (*13*). Aliquots of this extract were dried under a nitrogen stream, and the residue obtained was mixed with isopropanol containing 10% (v/v) Triton X-100 (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) or isopropanol. These mixtures were used to determine the cholesterol and TAG concentrations using a commercial kit (Cholesterol E-test, Wako) and the method of Fletcher (*14*), respectively. In a preliminary study, 20 μL of isopropyl alcohol containing 100 g Triton X-100/L did not affect the enzymatic reactions in a cholesterol assay (data not shown).

Fecal lipids were extracted from the lyophilized feces using 40 vol of 0.5 M ethanolic KOH at 80°C for 2 h (*15*). Neutral sterol was extracted from prepared fecal lipid samples with *n*-hexane. Cholesterol and coprostanol were measured after trimethylsilyl derivatization using a gas chromatography system (GC-17A, Shimadzu, Kyoto, Japan) equipped with CP-Sil 8 CB (0.25 mm×25 m, 0.12 μm; Agilent Technologies, Tokyo, Japan) (*15*). Total bile acid was enzymatically analyzed using the 3α-hydroxysteroid dehydrogenase assay (EC 1.1.1.50) of Sheltawy and Losowsky (*16*) and lithocholic acid as a standard.

After homogenization of the cecal contents, cecal organic acids (acetate, propionate, *n*-butyrate and succinate) were measured (*17*) using a HPLC system (LC-10A, Shimadzu) equipped with a Shim-pack SCR-102H column (8 mm×30 cm, Shimadzu) and an electroconductivity detector (CDD-6A, Shimadzu). Briefly, ~300 mg of cecal contents were homogenized in 2 mL of 10 mM NaOH and then centrifuged at 10,000 ×*g* for

Table 2. Primer sequences used in mRNA quantification by real-time RT-PCR.[1]

Gene	Forward (5′→3′)	Reverse (5′→3′)
CYP7A1	CAACTGAATGACCTGCCGGTACTA	GGAACCGTCCTCAAGATGGAGA
HMG-CoA-r	ACGTTCACCCTTGACGCTCTG	AGTTGGCAAGCACGGACATACA
LDL-R	ACCCAGAGCCATCGTAGTGGAC	TGGAGTTTGGAATCAACCCAATAGA
Apo B	GGGTGAGGCTGTACGTACTGGAA	CCTTTGGTAATGGCAGCTTTGAA
MTP	CGACCCTGTCAGTGTGGTGAA	TGAACCAGAAATGTCAATGGCTAGA
GAPDH	GACAACTTTGGCATCGTGGA	ATGCAGGGATGATGTTCTGG

[1] CYP7A1, cholesterol 7α-hydroxylase; HMG-CoA-r, hydroxymethylglutaryl-CoA reductase; LDL-R, LDL receptor; Apo B, apolipoprotein B; MTP, microsomal TAG transfer protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

15 min. The supernatant obtained was applied to HPLC analysis.

Real-time PCR analysis of CYP7A1, HMG-CoA-r, LDL-R, Apo B and MTP. Total RNA was extracted from the liver using an RNeasy Mini Kit (QIAGEN, Tokyo, Japan) and reverse-transcribed using a Reverse Transcriptase kit (PrimeScript RT reagent Kit, Takara Bio Inc., Otsu, Japan). The primers for rat CYP7A1, HMG-CoA-r, LDL-R, Apo B, MTP and GAPDH were designed by TaKaRa Perfect Real Time Support System (Takara); the sequences are listed in Table 2. GAPDH was used as an endogenous control. The primers were purchased from Takara Bio Inc. Real-time PCR was carried out in triplicate for each sample on a Stratagene Mx3000P (Stratagene) using a SYBR Premix Ex Taq kit (Takara). For each sample, results were normalized to GAPDH.

Statistical analysis. Experimental data were statistically analyzed by one-way ANOVA, followed by Tukey-Kramer's post-hoc test. Pearson's correlation coefficient was used to evaluate correlations between dependent variables. Differences with p-values of 0.05 or less were considered significant. Data are presented as means± SE unless otherwise indicated. All statistical analyses were performed using SAS JMP software (version 8.0.1; Tokyo, Japan).

RESULTS

Body weight gain and food intake did not differ between groups (control, 157±4 and 429±16 g/21 d; 15RY, 134±10 and 402±16 g/21 d; 30RY, 132±8 and 394±18 g/21 d; 15BY, 147±6 and 415±15 g/21 d; 30BY, 154±10 and 419±22 g/21 d). In addition, energy intake did not differ between groups (control, 6.95±0.26 MJ/21 d; 15RY, 6.48±0.28 MJ/21 d; 30RY, 6.27±0.28 MJ/21 d; 15BY, 6.67±0.23 MJ/21 d; 30BY, 6.69±0.35 MJ/21 d). Figure 1 shows the time course of changes in plasma cholesterol concentration in rats fed diets containing raw and boiled yam. After 3 d feeding, plasma cholesterol concentrations were significantly lower ($p<0.05$) in rats fed the 30% RY diet compared with those fed the control diet; the lower values were maintained over 21 d. However, significant reductions in plasma cholesterol were not observed in rats fed the other diets (15% RY, 15% BY and 30% BY).

At the end of the experimental period, there was a tendency for total cholesterol concentrations in arterial plasma to be lower in rats fed the 30% RY diet compared with those fed the control diet, but the differences were not statistically significant ($p=0.0728$; Table 3). Plasma non-HDL cholesterol concentrations were significantly lower in rats fed the 30% RY diet compared with those fed the control diet. Compared with rats fed the control diets, plasma TAG concentrations in rats fed all diets, excluding the 15% BY diet, were significantly lower, and the dose-dependent supplementation of RY enhanced this reduction.

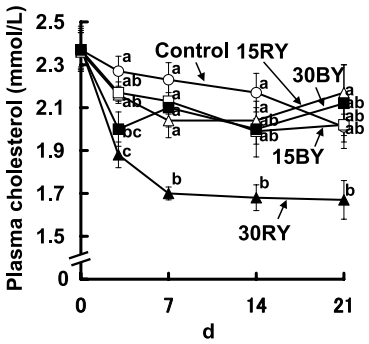

Fig. 1. Change in plasma cholesterol concentration in rats fed the raw yam (RY) and boiled yam (BY) diets. Each point is the mean of six rats; vertical bar represent SE. Values at each time point not sharing a common letter are significantly different ($p<0.05$). Statistical comparisons were made with Tukey-Kramer's post-hoc test. Control (○, rats fed the control diet); 15RY (△, rats fed the 15% RY diet); 30RY (▲, rats fed the control diet); 15BY (□, rats fed the control diet); 30BY (■, rats fed the control diet).

Epididymal fat pad weights were significantly lower in rats fed the 30% RY diet compared with those fed the control diet (Table 4). There were no significant differences in liver weights between the groups. Liver cholesterol concentrations were higher in rats fed the 30% RY diet compared with those fed the control diet (Table 4). Liver TAG levels did not differ among the groups.

Table 5 indicates fecal weight and bile acid excretion in rats fed RY and BY. The excretions of feces and cholesterol were significantly higher in rats fed the 30% RY diet compared with those fed the control diet. Only rats fed BY, but not RY, excreted significantly less coprostanol and more total fecal bile acids compared with those fed the control diet.

Table 3. Effect of raw and boiled yam on lipid concentration in arterial plasma.[1,2]

	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
Cholesterol					
Total (mmol/L)	1.96±0.10	1.86±0.11	1.55±0.09	1.87±0.05	1.90±0.14
HDL (mmol/L)	0.754±0.024	0.733±0.024	0.694±0.029	0.772±0.025	0.707±0.032
Non-HDL (mmol/L)	1.20±0.08^{a}	1.13±0.10^{ab}	0.854±0.067^{b}	1.10±0.04^{ab}	1.19±0.12^{ab}
Esterified (mmol/L)	1.77±0.09	1.73±0.10	1.47±0.08	1.62±0.16	1.78±0.13
Free (mmol/L)	0.185±0.033	0.133±0.012	0.073±0.012	0.254±0.131	0.121±0.014
Triacylglycerol (mmol/L)	1.34±0.10^{a}	0.649±0.116^{c}	0.497±0.093^{c}	1.20±0.17^{ab}	0.855±0.059^{bc}
Phospholipids (mmol/L)	0.233±0.008^{a}	0.198±0.011^{a}	0.162±0.006^{b}	0.217±0.009^{a}	0.207±0.009^{a}
Free fatty acids (mEq/L)	2.48±0.35	1.97±0.28	1.60±0.22	2.17±0.27	1.80±0.26

[1] RY, raw yam; BY, boiled yam.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.

Table 4. Weight of liver, kidney and epididymal fat pad, and liver cholesterol in rats fed diets supplemented with raw or boiled yam.[1,2]

	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
Tissue weight					
Liver (g/100 g BW)	4.21±0.14	3.98±0.14	3.93±0.16	3.92±0.06	4.07±0.11
Epididymal fat pad (g/100 g BW)	1.50±0.13^{ab}	1.26±0.08^{bc}	1.09±0.04^{c}	1.63±0.05^{ab}	1.66±0.10^{a}
Testis (g/100 g body BW)	0.901±0.014	0.955±0.022	0.961±0.034	0.924±0.031	0.892±0.025
Liver cholesterol (μmol/g liver)	6.77±0.39^{b}	9.57±0.87^{ab}	11.0±1.5^{a}	7.39±0.88^{ab}	6.84±0.52^{b}
Liver triacylglycerol (μmol/g liver)	42.9±3.0	37.8±4.9	35.5±9.3	36.6±4.6	33.8±6.1

[1] RY, raw yam; BY, boiled yam; BW, body weight.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.

Table 5. Fecal weight and bile acid excretion in rats fed diets supplemented with raw or boiled yam.[1,2]

	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
Dry fecal weight (g/3 d)	5.06±0.27^{a}	6.09±0.37^{a}	8.81±0.63^{b}	5.12±0.18^{a}	6.09±0.40^{a}
Total bile acids					
Excretion (μmol/3 d)	30.5±5.6^{a}	33.8±4.0^{ab}	34.4±5.0^{ab}	56.7±5.7^{c}	51.1±3.8^{bc}
Concentration (μmol/g)	6.22±1.23^{ab}	5.75±0.88^{ab}	3.95±0.57^{a}	11.0±0.9^{c}	8.64±0.91^{bc}
Neutral sterol					
Cholesterol (μmol/3 d)	17.3±2.0^{b}	16.7±1.6^{b}	31.9±3.1^{a}	19.0±1.1^{b}	18.4±18.4^{b}
Coprostanol (μmol/3 d)	8.79±0.77^{a}	4.78±0.78^{b}	1.21±0.77^{c}	7.20±0.57^{ab}	8.34±0.41^{a}

[1] RY, raw yam; BY, boiled yam.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.

Table 6 shows the mRNA content of hepatic CYP7A1, HMG-CoA-r, LDL-R, apo B and MTP in rats fed RY and BY. The mRNA content of MTP in rats fed the 30% RY diet decreased to 70% of the control group. The administration of 15% BY diet significantly increased the mRNA content of hepatic CYP7A1 and the administration of 30% BY diet tended to increase hepatic CYP7A1 mRNA compared with those fed the control diet. Other hepatic mRNA contents did not differ between any of the groups.

Table 7 indicates pH and weight of the cecal contents and wall in rats fed RY and BY. There were significant increases in the cecal content caused by an intake of increasing doses of RY, whereas such increases were not

Table 6. Hepatic mRNA level in rats fed diets supplemented with raw or boiled yam.[1,2]

Gene	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
(Arbitrary units)					
CYP7A1	100±14[b]	119±27[ab]	178±24[ab]	229±41[a]	220±33[ab]
HMG-CoA-r	100±11	96.6±8.4	110±12	145±21	145±20
LDL-R	100±7	96.3±8.2	88.8±9.5	101±5	103±12
Apo B	100±4	96.1±5.5	98.8±13.6	99.7±3.4	111±5
MTP	100±4[ab]	75.0±7.3[bc]	68.8±8.6[c]	103±6[a]	109±7[a]

[1] RY, raw yam; BY, boiled yam; CYP7A1, cholesterol 7α-hydroxylase; HMG-CoA-r, hydroxymethylglutaryl-CoA reductase; LDL-R, LDL receptor; Apo B, apolipoprotein B; MTP, microsomal TAG transfer protein.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.

Table 7. Weight and pH of cecal content and cecal wall in rats fed diets supplemented with raw or boiled yam.[1,2]

	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
Cecal content					
pH	7.14±0.11[b]	6.64±0.17[ab]	6.15±0.19[a]	6.87±0.08[b]	6.79±0.07[b]
Weight (g)	3.12±0.27[a]	6.14±0.49[b]	11.1±0.63[c]	3.38±0.25[a]	4.08±0.28[a]
Cecal wall (g)	0.812±0.052[a]	1.13±0.12[a]	1.79±0.14[b]	0.874±0.028[a]	1.10±0.06[a]

[1] RY, raw yam; BY, boiled yam.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.

Table 8. Effect of raw and boiled yam on cecal pools and concentrations of SCFAs and succinate.[1,2]

	Control	15RY[3]	30RY[3]	15BY[4]	30BY[4]
Pool					
Acetate (μmol/caecum)	128±14[a]	272±33[b]	457±52[c]	134±15[a]	181±18[ab]
Propionate (μmol/caecum)	42.0±3.9[a]	118±18[b]	242±28[c]	55.7±6.1[ab]	68.4±11.4[ab]
n-Butyrate (μmol/caecum)	29.8±2.4[a]	152±17[c]	265±22[d]	62.3±6.6[ab]	88.9±4.5[b]
Total[5] (μmol/caecum)	200±19[a]	543±53[b]	964±92[c]	252±25[a]	339±29[ab]
Succinate (μmol/caecum)	4.76±1.35[a]	96.3±65.2[a]	254±39[b]	7.45±2.11[a]	46.1±17.0[a]
Concentration					
Acetate (μmol/g)	41.1±3.8	44.0±3.8	41.1±3.7	39.2±2.5	44.0±1.5
Propionate (μmol/g)	13.5±0.8[a]	18.9±1.7[ab]	21.6±1.8[b]	16.2±0.9[ab]	16.3±2.1[ab]
n-Butyrate (μmol/g)	9.87±1.15[a]	24.9±1.9[c]	23.9±1.3[bc]	18.5±1.5[b]	22.1±1.4[bc]
Total[5] (μmol/g)	64.5±5.3[a]	87.8±3.5[b]	86.6±5.4[b]	73.9±3.1[ab]	82.5±2.1[b]
Succinate (μmol/g)	1.49±0.38[a]	14.8±9.9[ab]	22.6±2.8[b]	2.28±0.71[ab]	10.9±4.0[ab]
Molar ratio (Ace/Pro/But)	64/21/15	50/21/29	47/25/28	53/22/25	53/20/27

[1] RY, raw yam; BY, boiled yam; Ace, acetate; Pro, propionate; But, butyrate.
[2] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$). Data were analyzed with one-way ANOVA with Tukey's post-test.
[3] 15RY and 30RY, rats fed 15% and 30% raw yam diets, respectively.
[4] 15BY and 30BY, rats fed 15% and 30% boiled yam diets, respectively.
[5] acetate+propionate+*n*-butyrate.

observed in rats fed BY. Pools of acetate, propionate and *n*-butyrate were significantly higher in rats fed RY compared with those fed the control diet, whereas these increases were not observed in rats fed BY (Table 8). Pools of acetate, propionate and *n*-butyrate in rats fed the 30% RY diet were 257%, 476% and 789% greater ($p<0.05$) compared with those fed the control diet (Table 8).

DISCUSSION

Yam contains a large amount of starch, including amylose, which comprises about 30% of the whole yam and is not easily digested without cooking (*18*). The RS content (33.9%) in raw yams used in the present study was similar to the amylose content in yams from a study reported by Huang (*18*). Amylose gelatinized at a high temperature is easily digested. The content of RS was five times greater in RY compared with BY. The data indicates the amylose component of RS in Chinese yam becomes digestible when heated, even if heat treatment is brief. Many investigators have reported on the physiological effects of RS2; however, the sources of RS2 have been limited to raw starch from maize (*19*, *20*), banana (*21*) and potato (*22*). Only a few studies have examined the effects of RS2 from RY on cecal fermentation; a large amount of RS2 is supplied to the large intestine by consumption of RY, and is then used as a substrate for intestinal bacteria. RS enhances fermentation in the large intestine and contributes to an increased production of SCFAs, especially butyrate (*8*, *23*, *24*) and propionate (*25*). In the present study, the effects of both cooked and uncooked yams on cecal fermentation were compared in rats, and found to be different. Strong positive correlations between RS intake and the content of cecal SCFAs were observed (acetate, $r=0.9006$, $p<0.0001$; propionate, $r=0.9148$, $p<0.0001$; butyrate, $r=0.9452$, $p<0.0001$). Rats fed RY diets had a higher proportion of cecal butyrate compared to rats fed BY diets. These different effects of raw and boiled yams seem to be due to amounts of RS. RS has a hypertrophic effect on the large intestine, because butyrate is the predominant energy source for colonocytes (*26*, *27*). We also observed enlarged ceca in rats fed diets supplemented with RY. At the same time, greater cecal SCFA concentrations, especially of butyrate, were caused by RY feeding. Butyrate generated by fermentation seems to promote cell proliferation in the cecum. From these results, RY is suggested to be an effective source of RS for intestinal fermentation, resulting in an increased production of butyrate.

SCFAs suppress hepatic cholesterol and fatty acid synthesis, resulting in the reduction of plasma cholesterol concentration in rats (*10*). Many investigators also report that RS decreases serum cholesterol and TAG concentrations, and increases pools of cecal SCFA in rats (*4*, *5*, *19*) and humans (*6*). In the present study, RY, but not BY, reduced total and non-HDL cholesterol and TAG concentrations in plasma, while pools of cecal SCFAs in rats fed the RY diet were greater compared with the control and BY diets (Fig. 1 and Table 8). Negative correlations between cecal propionate and non-HDL cholesterol in plasma were found ($p=0.0166$). However, RY feeding did not suppress the hepatic mRNA content of HMG-CoA-r. Younes et al. reported that the activity of hepatic HMG-CoA-r was increased twofold by RS (*28*). Therefore, RY may induce a plasma cholesterol-lowering effect independent of the inhibition of hepatic cholesterol synthesis by SCFAs. Further investigation is required to evaluate the direct effect of fermentation of RY in the large intestine on lipid metabolism.

High liver cholesterol concentrations were observed in rats fed the RY diet compared with rats fed the control and BY diets. The liver plays an important role in the release and uptake of cholesterol as well as its synthesis and degradation. In the present study, the mRNA content of hepatic MTP, which transfers lipids onto the newly synthesized apo B polypeptide and is involved in VLDL assembly and release (*29*), was inhibited by RY feeding. Plasma non-HDL cholesterol concentration was significantly lower in rats fed RY and correlated with hepatic MTP mRNA content in rats fed the control and RY diets ($r=0.588$, $p=0.010$, $n=18$). Hepatic LDL-R mRNA content was not affected by RY feeding. Therefore, a reduced plasma non-HDL-cholesterol concentration and an increased liver cholesterol concentration may be due to suppressed release of VLDL from the liver. In the present study, it remains unclear how the effect of RS is triggered. Marcil et al. reported that butyrate inhibits MTP expression in Caco-2 cells, but not hepatocytes (*30*). The presence of some SCFAs may also be related to the suppression of MTP expression and VLDL release in the liver.

In many previous studies, enhanced cholesterol catabolism has been reported to contribute to the reduction of plasma and liver cholesterol concentrations (*31–33*). In the current study, however, plasma and liver cholesterol concentrations did not correlate with the hepatic mRNA content of CYP7A1, which is a rate-limiting enzyme in bile acid synthesis ($p=0.9088$ and $p=0.5574$, respectively). The intake of BY, but not RY, increased fecal bile acid excretion, although RS in raw potato starch was effective in excreting bile acid into feces (*28*). This discrepancy may be due to the low ability of RS in yam and high ability of dietary fiber in BY to excrete bile acid. However, cholesterol excretion increased in rats fed the 30% RY diet. Levrat et al. reported that RS feeding also caused an increased excretion of cholesterol in feces (*34*). Therefore, increased excretion of cholesterol, but not bile acid, by RS at least partially contributes to the lowering of non-HDL cholesterol by RY feeding, through the inhibition of cholesterol absorption. In addition, it has been reported that RS reduces energy absorption leading to less abdominal fat deposit and lower serum TAG concentration (*35*). Reduced energy absorption may be involved in lowering plasma TAG levels and weight of epididymal fat pads by RY feeding.

In conclusion, raw yam is effective as a source of RS and facilitates the production of SCFAs, especially butyrate and propionate in rat cecum. In addition, raw yam may have a plasma-cholesterol lowering effect due to the inhibition of VLDL release. However, these results require further investigation to explain the relationship between cholesterol concentrations in plasma and liver and other factors (including fermentation products) accompanying cholesterol metabolism.

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