



Title	Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived from soybean is most effective after long-term feeding in rats
Author(s)	Trakooncharoenvit, Aphichat; Tanaka, Seiya; Mizuta, Erika; Hira, Tohru; Hara, Hiroshi
Citation	European Journal of Nutrition, 59(4), 1389-1398 https://doi.org/10.1007/s00394-019-01992-9
Issue Date	2019
Doc URL	http://hdl.handle.net/2115/85218
Rights	The final publication is available at link.springer.com
Type	article (author version)
File Information	Final manuscript.pdf



[Instructions for use](#)

1 **Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived**
2 **from soybean is most effective after long-term feeding in rats**

3

4 Aphichat Trakooncharoenvit¹, Seiya Tanaka¹, Erika Mizuta¹, Tohru Hira^{1,2} and Hiroshi Hara^{1,2}

5

6 ¹ Graduate School of agriculture, Hokkaido University, Sapporo 060-8589, Japan

7 ² Research faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

8

9 Hiroshi Hara

10 E-mail: hara@chem.agr.hokudai.ac.jp

11 **Abstract**

12 **Purpose** To investigate the effects of water-soluble dietary fibers (pectin, soybean fiber, and
13 guar gum) on the bioavailability of quercetin glucoside mixture (Q3GM) comprising quercetin-
14 3-*O*-glucoside (Q3G, 31.8%) and its glucose adducts.

15 **Methods** Male Wistar/ST rats were fed test diet containing 0.7% Q3GM with or without 5%
16 of each dietary fiber for 8 weeks. Total quercetin derivatives were evaluated with liquid
17 chromatograph tandem mass spectrometry (LC-MS/MS) as total quercetin derivatives after
18 enzymatic deconjugation in plasma, urine, and fecal samples on week 2, 4, 6 and 8. Quercetin
19 glucuronides excreted in feces were also measured.

20 **Results** Fiber feeding elevated cecal weight and reduced cecal pH, indicative of cecal
21 fermentation promotion. Changes in plasma and urinary quercetin levels revealed three phases
22 of quercetin metabolism, including cumulative, transient, and stable phases. On week 2, total
23 quercetin derivatives were higher in plasma samples from three fiber-fed groups than those
24 control groups; however, urinary excretion increased in fiber-fed groups on week 4. Soybean
25 fiber upregulated plasma and urinary quercetin levels on week 6 and 8. Intestinal degradation
26 of quercetin by bacteria, calculated from differences between aglycone ingestion and sum of
27 urinary and fecal excretion, was suppressed after dietary fiber supplementation especially in
28 pectin fiber, which may partly contribute to the increase in quercetin bioavailability. Fecal
29 quercetin glucuronide excretion was high in soybean fiber-fed rats, suggestive of the reduction
30 of β -glucuronidase in colon.

31 **Conclusion** Water-soluble dietary fibers, especially soybean fiber, enhanced quercetin
32 bioavailability after chronic feeding and may promote beneficial effects of quercetin on disease
33 prevention.

34
35 **Keywords:** Quercetin; Water-soluble dietary fiber; Bioavailability; Fermentation; Degradation

36 **Introduction**

37 Quercetin is an abundant natural flavonoid belonging to the class of flavonol. It is usually
38 present in fruits and vegetables in glycosylated forms (sugar-bound) such as quercetin-3-*O*- β -
39 *d*-glucoside (Q3G). Several studies have demonstrated the biological activities of quercetin on
40 prevention of diseases such as colon cancer, cardiovascular diseases, and diabetes [1-4]. The
41 bioavailability of quercetin and its glycosides is low, limiting their beneficial effects [5].
42 Furthermore, the bioavailability of quercetin varies depending on coexisting food ingredients.

43 There are two mechanisms related to quercetin glucoside absorption in the intestine. In
44 the first mechanism, lactase-phlorizin hydrolase cleaves sugar moieties from quercetin
45 glucosides [6] and the resulting aglycone is absorbed into intestinal epithelial cells by simple
46 diffusion. In the second absorptive pathway, quercetin glucosides are transported via a glucose
47 transporter [7-8] and the absorbed quercetins are conjugated as glucuronides and sulfates, or
48 methylated in the epithelial cells. Quercetin derivatives are further conjugated and methylated
49 in the liver. These quercetin conjugates in the blood are excreted into the urine and bile.
50 Excreted quercetin conjugates into the bile are reabsorbed in the intestine. Urinary excretion
51 under stable condition (after saturation with quercetin in the body) reflects the intestinal
52 absorption rate of quercetin [9]. The unabsorbed quercetin glucosides and aglycones in the
53 small intestine flow into the cecum and colon, wherein quercetin is absorbed into the
54 epithelium or broken down by the intestinal bacteria [10-12]. The degradation of quercetin
55 aglycone in the large intestine may be the reason for the low bioavailability of quercetin [13,14].
56 Conjugated forms of quercetin excreted into the bile also reach into the large intestine, wherein
57 these molecules are usually degraded by the bacterial β -glucuronidase. The high activity of this
58 enzyme is known to increase the risk of colon cancer [15,16].

59 Dietary fibers are non-digestible polysaccharides that prevent the onset of several
60 metabolic diseases through physicochemical properties and intestinal fermentation. Being

61 resistant to the digestive enzymes in the small intestine, dietary fibers increase the fermentation
62 and production of organic acids, mainly short-chain fatty acids (SCFAs), in the large intestine
63 [17,18]. Clinical studies have demonstrated that the consumption of dietary fibers attenuates
64 severe increases in blood glucose, cholesterol, and triglyceride after a meal and lowers the risk
65 of disease development [19,20]. In recent studies, the bioavailability of quercetin glycosides
66 was shown to be increased by co-ingested saccharides, including non-digestible
67 oligosaccharides, depending on the intestinal bacterial metabolism [21-23]. These studies
68 suggest that water-soluble dietary fibers fermented by intestinal bacteria promote
69 bioavailability of dietary flavonoids. However, the effects of chronic feeding of water-soluble
70 dietary fibers on quercetin bioavailability have not been studied.

71 In this study, we used pectin and guar gum as representative water-soluble and
72 fermentable dietary fibers. We also included soybean fiber, a fermentable fiber with low
73 viscosity. We hypothesized that the continuous ingestion of dietary fibers may increase
74 quercetin bioavailability by promoting intestinal fermentation and that the viscous property of
75 fibers affects the intestinal absorption of quercetin.

76

77 **Materials and methods**

78 **Chemicals**

79 Quercetin-3-*O*-glucoside mixture (Q3GM, kindly supplied by San-Ei Gen F.F.I., Inc.,
80 Osaka, Japan) comprised quercetin-3-*O*-glucoside (Q3G, 31.8%) and mono (23.3%), di
81 (20.3%), tri (9.7%), tetra (6.8%), penta (4.3%), hexa (2.6%) and hepta (1.1%) glucose adducts
82 of Q3G with α -1,4-linkages, which was quantified by LC-MS/MS analyses described below
83 by using individual standard compounds [23]. We used Q3GM because absorption of this
84 quercetin glucosides is higher than Q3G (natural compound) [21] and already used as a food
85 additive with granted generally recognized as safe (GRAS) status in Japan. Soybean fiber, a

86 highly fermentable fiber with low viscosity, was kindly donated by Fuji oil Co., Ltd (Osaka,
87 Japan). Pectin from citrus and guar gum were purchased from Wako Pure Chemical Industries,
88 Ltd (Osaka, Japan) and Tokyo chemical industry Co., Ltd (Tokyo, Japan), respectively. As
89 deconjugating enzymes, β -glucuronidase ($\geq 100,000$ units/mL) and sulfatase ($\geq 2,000$
90 units/mL) from *Helix pomatia* extract (Cat. No. S9751) and sulfatase (10-20 units/mL) from
91 *Aerobacter aerogenes* extract (Cat. No. S1629) were purchased from Sigma Aldrich (Saint
92 Louis, USA). We used two sources of sulfatases because we found that insufficient hydrolysis
93 of some sulfate conjugates by *Helix pomatia* enzyme. Other reagents were provided by Wako
94 unless specified.

95

96 **Animals and diets**

97 Male Wistar/ST rats, weighing about 120-140 g (5 weeks old) purchased from Japan SLC,
98 Inc. (Hamamatsu, Japan), were housed in an individual stainless-steel cage with wire-mesh
99 bottom. Rats were maintained in a controlled environment (12 h light/dark period, relative
100 humidity 40-60%, and temperature $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with ad libitum access to diet and water. Rats
101 were assigned to five groups ($n = 7$ rats/group) after 1-week acclimation with a sucrose-based
102 diet based on AIN 93G formulation [24]. Five groups of rats were fed with different diets as
103 follows; sucrose-based AIN 93G diet for basal diet group (BA): control diet (sucrose-based
104 AIN 93G diet mixed with 0.7% Q3GM) for control group (Q): control diet mixed with 5% of
105 each test fiber which are pectin or soybean fiber or guar gum for QP, QS, and QG group,
106 respectively. In the diet component, sucrose is replaced by supplemented Q3GM and cellulose
107 is replaced by the test fibers for fiber treatment groups. The percentage (0.7%) of Q3GM used
108 in the present study was equivalent to 0.5% of quercetin aglycone, as our previous study [21].
109 Body weight and food consumption of rats were measured daily. Tail vein blood was collected
110 at 10.00 - 11.30 a.m. on week 2, 4, 6 and 8 after feeding test diets. Urine and feces were

111 collected for 2 and 3 days, respectively, in the same week before blood collection day to
112 evaluate quercetin bioavailability. For urine collection, 0.05% sodium azide was added to
113 prevent degradation of quercetin metabolites by bacterial growth. Collected urine samples were
114 filled up to 100 mL, and 15 mL of samples were stored at -40°C . Fecal samples were stored at
115 -80°C . At the end of feeding period, rats were anesthetized with sodium pentobarbital injection
116 (Somnopenyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan) and killed by withdrawal of the
117 aortic blood followed by exsanguination after overnight fasting. The liver and epididymal fat
118 pad were collected and weighed. The cecum and its contents were collected for measurements
119 of pH, SCFAs, and other organic acids.

120 This study was approved by the Hokkaido University Animal Committee, and the animals
121 were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of
122 Hokkaido University.

123

124 **Sample treatments**

125 Plasma or diluted urine sample (100 μL) was acidified with 10 μL of 0.58 mol/L acetic
126 acid and treated with 7.5 μL of each deconjugation enzyme, β -glucuronidase and sulfatase [23].
127 Enzyme treated-sample was incubated at 35°C for 1 h and naringenin (2 $\mu\text{mol/L}$ as an internal
128 standard) was added to all samples. The mixture was centrifuged ($9,300 \times g$ at 4°C for 5 min)
129 and the supernatant was collected; the precipitate was re-extracted with methanol. The
130 supernatant was loaded onto a C18 cartridge (Oasis HLB, Waters Co. LTD, Milford, MA,
131 USA) and eluted with 1 mL methanol. After drying and reconstitution in a 50% methanol
132 solution, sum of quercetin and methylquercetin after treatment of deconjugation enzymes (total
133 quercetin derivatives) were measured by liquid chromatography tandem mass spectrometry
134 (LC-MS/MS).

135 Fecal samples were freeze dried and milled and their dry weights measured. Powdered
136 fecal samples (0.1 g) were suspended in 1 mL of 80% methanol solution and treated with 100
137 μL of 10 $\mu\text{mol/L}$ naringenin as internal standard [23]. Mixtures were homogenized using
138 ultrasonic homogenizer (VP-050, Saitama, Japan) and incubated at 60°C for 1 h before
139 centrifugation (2,300 $\times g$ for 10 min at 4°C). Methanol (1 mL) was added to the precipitate and
140 homogenized with Teflon homogenizer (42W, ULTRAS homogenizer, Taitec Co. Ltd.,
141 Nagoya, Japan). The mixture was centrifuged to precipitate insoluble materials, including
142 proteins. The entire procedure was repeated twice. The obtained supernatant was evaporated
143 and the concentrations of total quercetin derivatives were measured as the same as plasma and
144 urine extracts.

145

146 **Quercetin measurement by LC-MS/MS**

147 Total quercetin derivatives, sum of deconjugated quercetin and methylquercetin, were
148 determined using LC-MS/MS system [25] (TSQ Quantum Access Max with Accela High
149 Speed LC System, Thermo Fisher Scientific Inc., MA, USA) with an electrospray ionization
150 (ESI) source with SRM mode. The analytical column was a 1.9 μm C18 column (Inertsustain
151 Swift C18, 2.1 mm \times 100 mm GL Sciences Inc., Tokyo, Japan) set at 40°C. The mobile phase
152 was water, methanol, and formic acid (70:30:0.1, solvent A), and methanol with formic acid
153 (100:0.1, solvent B). The ratio of solvent A and B was delivered through a linear gradient
154 (90:10 for 1 min; 90:10-20:80 for 8 min; 90:10 for 1 min) at a flow rate of 0.2 mL/min.
155 Concentrations of total quercetin derivatives were calculated from the calibration curve of
156 standard compounds.

157

158 **Cecum treatment for pH and SCFAs measurement**

159 Frozen cecum samples were thawed and cecal contents and tissues were separated and
160 individually weighed. Cecum contents were diluted with four times weight of deionized water,
161 and the pH value measured with a handy pH meter (B-211 twin waterproof pH, Horiba, Ltd.,
162 Kyoto, Japan). Diluted samples (700 μ L) were mixed with 200 μ L of 25 mM crotonic acid
163 (internal standard) in 50 mM sodium hydroxide (NaOH), and centrifuged. The supernatants
164 were mixed with the same volume of chloroform and centrifuged again. The supernatant was
165 filtrated through a 0.2 μ m membrane, and concentrations of organic acids, including SCFAs,
166 were measured with high-performance liquid chromatography (HPLC) constructed with two
167 shim-pack SCR-102H (8 mm I.D. \times 300 mm L Shimadzu Corp.) and conductivity detector
168 CCD-6A (polarity: +, response: slow, temperature: 45°C; Shimadzu Corp.). The flow rate of
169 the mobile phase (5 mM aqueous *p*-toluenesulfonic acid) and buffer (5 mM *p*-toluenesulfonic
170 acid solution containing 100 μ M ethylenediaminetetraacetic acid [EDTA] and 20 mM Bis-
171 Tris) were 0.8 mL/min. The concentration of each organic acid was calculated from peak area
172 of chromatogram and the calibration curve of each standard organic acid corrected by the
173 internal standard.

174

175 **Statistics**

176 Data are presented as means with standard errors of the mean (SEM). Statistical
177 significance was assessed using one-way analysis of variance (ANOVA) with Tukey-Kramer's
178 test. Two-way ANOVA was carried out to verify the effect of the duration of feeding test (week
179 i.e., weeks 2, 4, 6, and 8) and diet treatment (group i.e., Q, QP, QS, and QG) on values of
180 plasma, urine, feces quercetin aglycone as well as quercetin aglycone degradation. $P < 0.05$
181 was considered as significant. Statistic calculation was performed by JMP version 13.0 (SAS
182 Institute Inc., Cary, NC).

183

184 **Results**

185 **Food intake, body weight, and organ and dry fecal weights**

186 Final body weight in QP group was lower than that reported in other groups, while daily
187 food intake reduced following ingestion of dietary fibers without any significant difference
188 (Table 1). Relative wet weight (grams per 100 g body weight) of the livers showed no change
189 following dietary fiber treatment, but that of epididymal fat pad was lower in QS group than
190 control group.

191 The weights of cecum tissues and cecum contents were significantly higher in QS and QG
192 groups, but not in QP group, than basal and control groups. Maximum weight was reported for
193 the samples from QS group (Table 2). Values of pH of the cecum contents were lower for
194 samples from QP, QS, and QG groups than those from basal and control groups, indicative of
195 the promotion of cecal fermentation by respective water-soluble fibers. Cecal pool of total
196 organic acids, which is the sum of all acids shown in Fig. 1, and that of acetic and propionic
197 acids were higher in QS group than other groups.

198 Dry weights of feces (Table 3) collected on week 2, 4, 6, and 8 showed lower values in
199 QP, QS, and QG groups than basal and control groups, suggesting that dietary fibers added to
200 diets were similarly degraded by intestinal bacterial fermentation.

201

202 **Total quercetin derivatives in plasma**

203 Total quercetin derivatives are shown in Fig. 2. Concentrations of methylquercetin
204 derivatives were higher than those of quercetin derivatives. On week 2, feeding Q3GM group
205 with individual water-soluble fibers elevated the concentrations of total quercetin derivatives.
206 From 2 to 6 weeks, plasma total quercetin derivatives gradually decreased in all groups. On
207 week 6 and 8, all groups of rats showed steady levels of total quercetin derivatives, with the
208 highest value reported for QS group. Two-way ANOVA results revealed that both duration of

209 feeding and diet treatment influenced the plasma concentrations of total quercetin derivatives
210 and showed significant interaction (week \times group: $P = < 0.0001$).

211

212 **Total quercetin derivatives in urine**

213 In the urine samples collected on week 2, the level of total quercetin derivatives was
214 significantly higher in QG group than in control group. Both QS and QG groups had higher
215 values than control group on week 4 (Fig. 3). From week 4 to 8, urinary levels of total quercetin
216 gradually decreased in all groups. Urinary quercetin level in QS group was higher than that in
217 all other groups on week 6 and 8. The fluctuation in total quercetin excretion was influenced
218 by both feeding period and dietary treatment, as per two-way ANOVA results, and showed
219 significant interactions (week \times group: $P = 0.0066$).

220

221 **Total quercetin derivatives in feces**

222 Fecal excretion of total quercetin derivatives was higher in QP group than control group
223 through 8-week feeding period, while ingestion of soybean fiber (QS group) increased the
224 excretion of total quercetin from week 4 to 8 (Fig. 4). QG group showed increased tendency of
225 fecal total quercetin throughout the experiment. According to two-way ANOVA results,
226 dietary treatment, but not feeding period, influenced the fecal total quercetin derivatives. As
227 shown in Fig. 5, quercetin glucuronides in feces were almost undetectable in control (Q) group,
228 but were detectable in QP and QS groups. In particular, total glucuronide levels (quercetin
229 glucuronides and methylquercetin glucuronides) gradually increased in QS group and were
230 higher than the other groups.

231

232 **Degradation of quercetin and methylquercetin aglycones**

233 We found that the amounts of quercetin derivatives excreted in urine and feces samples
234 were lower than the levels of ingested quercetin, suggestive of the degradation of large amounts
235 of quercetin aglycones by intestinal bacteria (Table 4). The amount of quercetin and
236 methylquercetin aglycone degraded was calculated by subtracting sum of aglycone excreted
237 into urine and feces from total aglycone ingested. The differences between ingested and
238 degraded aglycone is remaining aglycone survived from degradation by intestinal bacteria. The
239 percentages of remaining aglycone was approximately two times higher in water-soluble fiber
240 groups than the control group, and the values in QP group were the highest throughout the
241 experiment (approximately five times higher than the control group). The degradation ratio
242 was lower in all fiber groups than control group and the lowest value was reported for QS
243 group. These differences were maintained during the 8-week feeding period, which was
244 supported by the absence of any significant interaction between period and dietary treatment
245 as analyzed with two-way ANOVA ($P = 0.8832$).

246

247 **Discussion**

248 We observed the effects of three water-soluble dietary fibers on the bioavailability of a
249 quercetin glucoside in rats in an 8-week feeding study by monitoring the changes in the
250 concentrations of quercetin derivatives in the plasma, urine, and feces samples. The results of
251 plasma and urine quercetin levels suggest three phases in quercetin metabolism, including
252 cumulative (~week 2), transient (week 4), and stable (week 6-8) phases. The daily intake of
253 quercetin glucoside with individual water-soluble fibers for 2 weeks increased the plasma
254 concentrations of total quercetin derivatives, suggestive of the enhanced intestinal absorption
255 of the ingested quercetin. However, urinary excretion of total quercetin derivatives, which
256 usually reflects the bioavailability of ingested flavonoids, failed to increase in QP and QS
257 groups. On week 2, quercetin absorption may have increased after water-soluble fiber feeding

258 and the absorbed quercetin accumulated in the blood and tissues in these groups. Mullen *et al.*
259 have reported that accumulation of radio-labelled quercetin in tissues [26]. In contrast, urinary
260 excretion of quercetin derivatives in QG-fed rats largely increased on week 2. Quercetin in the
261 body was probably saturated in QS group earlier than other fiber groups due to higher
262 absorption of quercetin. The greater effect of guar gum for absorption of quercetin glycosides
263 is not known, however, we speculate fermentation of guar gum is faster than pectin and soybean
264 fiber on week 2. On week 4, urinary excretion of quercetin in QP, QS, and QG groups tended
265 to be or was largely increased, but the increased plasma quercetin concentrations disappeared
266 on week 6 except in QS group. These results suggest that week 4 is a transient phase of
267 increased total quercetin derivatives. From week 6 to 8, levels of plasma quercetin and urinary
268 excretion reduced but stabilized, with significantly higher levels observed in QS group than
269 BA group. On week 6 of all groups, the reduction of quercetin derivatives in both plasma and
270 urinary levels suggests the reduction of quercetin bioavailability that possibly be associated
271 with the increasing degradation of quercetin aglycone. However, we found increase in fecal
272 excretion of quercetin from week 4 to week 6 in QS group. We speculated that the reduction
273 of quercetin in plasma and urinary excretion on week 6 is associated with suppression of
274 intestinal absorption of quercetin by prolonged feeding of Q3GM by a feedback mechanism.

275 In recently, some intestinal bacteria catabolize are able to convert quercetin aglycone to
276 phenolic acids such as 3-(3,4-dihydroxyphenyl) propionic acid and 3,4-dihydroxy-
277 phenylacetic acid through ring scission process [27,28]. These degraded products are known
278 to have strong anti-oxidative properties and also effective for prevention of many diseases,
279 which depends on anti-oxidative ability [29,30]. However, quercetin has more various actions
280 independent of anti-oxidative properties, for example strong interaction with cell-signaling
281 proteins [31]. Some researches have been reported that phenolic acids possessed a low ability
282 to suppress the carcinogenesis compared with quercetin aglycone [32,33]. However, adaptation

283 of intestinal bacteria to long-term feeding of quercetin may increase the levels of the enzymes
284 that decompose quercetin aglycone mainly in the cecum [34,35]. In this experiment, we
285 observed an increase in the fecal excretion of quercetin derivatives following pectin (QP) and
286 soybean fiber (QS) diet feeding, observed probably through the suppression of quercetin
287 degradation. We calculated the remaining aglycone survived from bacterial degradation as
288 shown in Table 5. The amount of remaining aglycone, the restoration rates of quercetin
289 aglycone through the avoidance of bacteria degradation in rats, largely increased in QP and QS
290 groups on week 6 and 8. A possible explanation for this observation is that dietary fibers were
291 used as energy source instead of quercetin by cecal bacteria [36,37]. *Bacteroidetes* spp. are
292 known to upregulate the expression of several genes related to carbohydrate-metabolizing
293 enzymes and may use ingested fibers as substrate [38]. The suppression of bacterial
294 degradation of quercetin aglycone may be responsible for the constant high levels of quercetin
295 in plasma and urinary excretion in QS group. In QP group, no increase in quercetin levels in
296 plasma and urine was observed for the maximum amount of available aglycone. The high
297 viscosity property of pectin in the intestinal lumen possibly suppressed quercetin absorption in
298 the small intestine, and this property might also contribute to the low bioavailability in guar
299 gum-fed rats during the late phases.

300 Supplementation with fibers, especially soybean fiber, largely and gradually increased the
301 fecal excretion of quercetin and methylquercetin glucuronides. This finding indicates that
302 soybean fiber suppressed the deconjugation of quercetin glucuronides. Cermak et al. [39]
303 reported the transformation of the conjugated metabolites of quercetin to aglycone by certain
304 strains of *Pediococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and
305 *Bacteroides* spp. Rowland et al. [40] also found a significant increase in β -glucuronidase
306 activity in rats fed with 5% pectin diet, consistent with the results of the present study that
307 glucuronide levels in feces reduced from week 4 to 6 and 8 in pectin group (QP). In QS group,

308 fecal glucuronide levels were higher than those in other groups and further increased after long-
309 term dietary fiber ingestion. Bacterial β -glucuronidase activity in the large intestine is one of
310 the strong inducers of colon cancer [41]. Ingested soybean fiber may suppress the proliferation
311 of β -glucuronidase-producing bacteria or inhibit β -glucuronidase activity in the large intestine.
312 Therefore, feeding soybean fiber may serve as a strategy to prevent colon cancer.

313 In the present study, we hypothesized that ideal dietary fibers may enhance quercetin
314 bioavailability by increasing cecal fermentation and suppressing quercetin degradation. Pectin
315 showed the highest ability to suppress bacterial degradation of quercetin aglycone and seemed
316 to be the best candidate for enhancing blood levels of quercetin, but it was true only in the early
317 phase of feeding (week 2). We revealed that soybean fiber is the most effective fiber to enhance
318 quercetin bioavailability and blood levels among three water-soluble and fermentable dietary
319 fibers for a long-term feeding studied herein. Soybean fiber is known to increase mucosal
320 surface area with the expansion of cecal wall [42], consistent with the increase in cecal contents
321 and tissue weight observed in the present study. Soybean fiber has relatively low viscosity as
322 compared with pectin and guar gum [43,44] that may have contributed to the prolonged effects
323 on quercetin bioavailability.

324 In conclusion, we found that water-soluble dietary fibers, especially soybean fiber,
325 enhanced quercetin glycoside bioavailability following long-term feeding of rats. Duration of
326 feeding is an important factor to modulate these abilities of dietary fibers. The findings of the
327 present study suggest that dietary fibers promote the actions of quercetin on prevention of
328 diseases.

329

330

331

332

333 Reference:

- 334 1. Gee JM, Hara H, Johnson IT (2002) Suppression of Intestinal Crypt Cell Proliferation
335 and Aberrant Crypt Foci by Dietary Quercetin in Rats. *Nutr Cancer*, 43:193-201.
336 https://doi.org/10.1207/s15327914nc432_10
- 337 2. Vessal M, Hemmati M, Vasei M (2003) Antidiabetic effects of quercetin in
338 streptozocin-induced diabetic rats. *Comp Biochem Physiol C Toxicol Pharmacol*
339 135:357-364. [https://doi.org/10.1016/s1532-0456\(03\)00140-6](https://doi.org/10.1016/s1532-0456(03)00140-6)
- 340 3. Chen S, Jiang H, Wu X, Fang J (2016) Therapeutic effects of quercetin on inflammation,
341 obesity, and type 2 diabetes. *Mediators Inflamm* 1-5.
342 <https://doi.org/10.1155/2016/9340637>
- 343 4. Li Y, Yao J, Han C et al (2016) Quercetin, inflammation and immunity. *Nutrients* 8:
344 167. <https://doi.org/10.3390/nu8030167>
- 345 5. Guo Y, Bruno RS (2015) Endogenous and exogenous mediators of quercetin
346 bioavailability. *J Nutr Biochem* 26:201-210.
347 <https://doi.org/10.1016/j.jnutbio.2014.10.008>
- 348 6. Day AJ, Gee JM, Dupont M et al (2003) Absorption of quercetin-3-glucoside and
349 quercetin-4'-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase
350 and the sodium-dependent glucose transporter. *Biochem Pharmacol* 65:1199-1206.
351 [https://doi.org/10.1016/S0006-2952\(03\)00039-X](https://doi.org/10.1016/S0006-2952(03)00039-X)
- 352 7. Wolfram S, Blöck M, Ader P (2002) Quercetin-3-glucoside is transported by the
353 glucose carrier SGLT1 across the brush border membrane of rat small intestine. *J Nutr*
354 132:630-635. <https://doi.org/10.1093/jn/132.4.630>
- 355 8. Chabane MN, Ahmad AA, Peluso J et al (2009) Quercetin and naringenin transport
356 across human intestinal Caco-2 cells. *J Pharm Pharmacol* 61:1473-1483.
357 <https://doi.org/10.1211/jpp/61.11.0006>

- 358 9. Vries JH, Hollman PC, Meyboom S et al (1998) Plasma concentrations and urinary
359 excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for
360 dietary intake. *Am J Clin Nutr* 68:60-65. <https://doi.org/10.1093/ajcn/68.1.60>
- 361 10. Manach C, Morand C, Demigné C et al (1997) Bioavailability of rutin and quercetin in
362 rats. *FEBS Lett* 409:12-16. [https://doi.org/10.1016/s0014-5793\(97\)00467-5](https://doi.org/10.1016/s0014-5793(97)00467-5)
- 363 11. Keppler K, Hein E, Humpf H (2006) Metabolism of quercetin and rutin by the pig
364 caecal microflora prepared by freeze-preservation. *Mol Nutr Food Res* 50:686-695.
365 <https://doi.org/10.1002/mnfr.200600016>
- 366 12. Marín L, Miguélez EM, Villar CJ et al (2015) Bioavailability of dietary polyphenols
367 and gut microbiota metabolism: Antimicrobial properties. *Biomed Res Int* 1-18.
368 <https://doi.org/10.1155/2015/905215>
- 369 13. Thilakarathna S, Rupasinghe H (2013) Flavonoid bioavailability and attempts for
370 bioavailability enhancement. *Nutrients* 5:3367-3387.
371 <https://doi.org/10.3390/nu5093367>
- 372 14. Rich G, Buchweitz M, Winterbone M et al (2017) Towards an understanding of the low
373 bioavailability of quercetin: A study of its interaction with intestinal lipids. *Nutrients*
374 9:111. <https://doi.org/10.3390/nu9020111>
- 375 15. Shiau S, Chang GW (1983) Effects of dietary fiber on fecal mucinase and β -
376 glucuronidase activity in rats. *J Nutr* 113:138-144. <https://doi.org/10.1093/jn/113.1.138>
- 377 16. Maruti SS, Chang J, Prunty JA et al (2008) Serum β -glucuronidase activity in response
378 to fruit and vegetable supplementation: A controlled feeding study. *Cancer Epidemiol*
379 *Biomarkers Prev* 17:1808-1812. <https://doi.org/10.1158/1055-9965.EPI-07-2660>
- 380 17. Andoh A, Tsujikawa T, Fujiyama Y (2003) Role of dietary fiber and short-chain fatty
381 acids in the colon. *Curr Pharm Des* 9:347-358.
382 <https://doi.org/10.2174/1381612033391973>

- 383 18. Koh A, Vadder FD, Kovatcheva-Datchary P et al (2016) From dietary fiber to host
384 physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* 165:1332-1345.
385 <https://doi.org/10.1016/j.cell.2016.05.041>
- 386 19. Lattimer JM, Haub MD (2010) Effects of dietary fiber and its components on metabolic
387 health. *Nutrients* 2:1266-1289. <https://doi.org/10.3390/nu2121266>
- 388 20. Threapleton DE, Greenwood DC, Evans CE et al (2013) Dietary fibre intake and risk
389 of cardiovascular disease: Systematic review and meta-analysis. *Bmj* 347.
390 <https://doi.org/10.1136/bmj.f6879>
- 391 21. Matsukawa N, Matsumoto M, Shinoki A et al (2009) Nondigestible saccharides
392 suppress the bacterial degradation of quercetin aglycone in the large intestine and
393 enhance the bioavailability of quercetin glucoside in rats. *J Agric Food Chem* 57:9462–
394 9468. <https://doi.org/10.1021/jf9024079>
- 395 22. Shinoki A, Lang W, Thawornkuno C et al (2013) A novel mechanism for the promotion
396 of quercetin glycoside absorption by megallo α -1,6-glucosaccharide in the rat small
397 intestine. *Food Chem* 136:293-296. <https://doi.org/10.1016/j.foodchem.2012.08.028>
- 398 23. Tanaka S, Shinoki A, Hara H (2016) Melibiose, a nondigestible disaccharide, promotes
399 absorption of quercetin glycosides in rat small intestine. *J Agric Food Chem* 64:9335-
400 9341. <https://doi.org/10.1021/acs.jafc.6b03714>
- 401 24. Reeves PG, Nielsen FH, Fahey GC (1993) AIN-93 purified diets for laboratory rodents:
402 final report of the American institute of nutrition ad hoc writing committee on the
403 reformulation of the AIN-76A rodent diet. *J Nutr* 123:1939-1951.
404 <https://doi.org/10.1093/jn/123.11.1939>
- 405 25. Tanaka S, Oyama M, Nishikawa M et al (2018) Simultaneous collection of the portal
406 and superior vena cava blood in conscious rats defined that intestinal epithelium is the

407 major site of glucuronidation, but not sulfation and methylation, of quercetin. *Biosci*
408 *Biotechnol Biochem* 82:2118-2129. <https://doi.org/10.1080/09168451.2018.1515615>

409 26. Mullen W, Rouanet JM et al (2008) The bioavailability of [2-14C] quercetin-4'-
410 glucoside in rats. *J Agric Food Chem* 56:12127-12137.
411 <https://doi.org/10.1021/jf802754s>

412 27. Aura A, Oleary KA, Williamson G et al (2002) Quercetin derivatives are deconjugated
413 and converted to hydroxyphenylacetic acids but not methylated by human fecal flora in
414 vitro. *J Agric Food Chem* 50:1725-1730. <https://doi.org/10.1021/jf0108056>

415 28. Marín L, Miguélez EM, Villar CJ et al (2015) Bioavailability of dietary polyphenols
416 and gut microbiota metabolism: Antimicrobial properties. *Biomed Res Int* 1-18.
417 <https://doi.org/10.1155/2015/905215>

418 29. Raneva V, Shimasaki H, Ishida Y et al (2001) Antioxidative activity of 3,4-
419 dihydroxyphenylacetic acid and caffeic acid in rat plasma. *Lipids* 36:1111-1116.
420 <https://doi.org/10.1007/s11745-001-0821-6>

421 30. El-Ansary, A., Shaker, G., Siddiqi, N. J., & Al-Ayadhi, L. Y. (2013). Possible
422 ameliorative effects of antioxidants on propionic acid / clindamycin - induced
423 neurotoxicity in syrian hamsters. *Gut Pathogens* 5. [https://doi.org/10.1186/1757-4749-](https://doi.org/10.1186/1757-4749-5-32)
424 5-32

425 31. Suzuki T, Hara H (2009) Quercetin enhances intestinal barrier function through the
426 assembly of zonula occludens-2, occludin, and claudin-1 and the expression of
427 claudin-4 in caco-2 cells. *J Nutr* 139:965-974. <https://doi.org/10.3945/jn.108.100867>

428 32. Skrbek S, Rüfer CE, Marko D et al (2009) Quercetin and its microbial degradation
429 product 3,4-dihydroxyphenylacetic acid generate hydrogen peroxide modulating their
430 stability under in vitro conditions. *J. Food Nutr* 48:129-140.

- 431 33. Kern M, Fridrich D, Reichert J et al (2007) Limited stability in cell culture medium and
432 hydrogen peroxide formation affect the growth inhibitory properties of delphinidin and
433 its degradation product gallic acid. *Mol Nutr Food Res* 51:1163-1172.
434 <https://doi.org/10.1002/mnfr.200700004>
- 435 34. Wu GD, Chen J, Hoffmann C et al (2011) Linking long-term dietary patterns with gut
436 microbial enterotypes. *Science* 334:105-108. <https://doi.org/10.1126/science.1208344>
- 437 35. Moco S, Martin FJ, Rezzi S (2012) Metabolomics view on gut microbiome modulation
438 by polyphenol-rich foods. *J Proteome Res* 11:4781-4790.
439 <https://doi.org/10.1021/pr300581s>
- 440 36. Hervert-Hernández D, Goñi I (2011) Dietary polyphenols and human gut microbiota:
441 A review. *Food Reviews International*, 27:154-169.
442 <https://doi.org/10.1080/87559129.2010.535233>
- 443 37. Palafox-Carlos H, Ayala-Zavala JF, González-Aguilar GA (2011) The role of dietary
444 fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *J*
445 *Food Sci* 76. <https://doi.org/10.1111/j.1750-3841.2010.01957.x>
- 446 38. Flint HJ, Scott KP, Duncan SH et al (2012) Microbial degradation of complex
447 carbohydrates in the gut. *Gut Microbes* 3:289-306. <https://doi.org/10.4161/gmic.19897>
- 448 39. Cermak R, Breves G, Lüpke M et al (2006). In vitro degradation of the flavonol
449 quercetin and of quercetin glycosides in the porcine hindgut. *Arch Anim Nutr* 60:180-
450 189. <https://doi.org/10.1080/17450390500467695>
- 451 40. Rowland IR, Mallett AK, Wise A (1983) A comparison of the activity of five microbial
452 enzymes in cecal content from rats, mice, and hamsters, and response to dietary
453 pectin. *Toxicol Appl Pharmacol* 69:143-148. [http://doi.org/10.1016/0041-](http://doi.org/10.1016/0041-008X(83)90130-8)
454 [008X\(83\)90130-8](http://doi.org/10.1016/0041-008X(83)90130-8)

- 455 41. Kim D, Jin Y (2001) Intestinal bacterial β -glucuronidase activity of patients with colon
456 cancer. Arch Pharm Res 24:564-567. <http://doi.org/10.1007/BF02975166>
- 457 42. Hara H, Onoshima S, Nakagawa C (2010) Diffructose anhydride III promotes iron
458 absorption in the rat large intestine. Nutrition 26:120-127.
459 <https://doi.org/10.1016/j.nut.2009.05.024>
- 460 43. Takahashi T, Maeda H, Aoyama T et al (1999) Physiological effects of water-soluble
461 soybean fiber in rats. Biosci Biotechnol Biochem 63:1340-1345.
462 <https://doi.org/10.1271/bbb.63.1340>
- 463 44. Rideout T (2008) Guar gum and similar soluble fibers in the regulation of cholesterol
464 metabolism: Current understandings and future research priorities. Vasc Health Risk
465 Manag 4:1023-1033. <https://doi.org/10.2147/VHRM.S3512>

466
467

468 **Fig. 1** Pools of organic acids in cecal contents of rats fed a sucrose-based AIN 93G (BA) diet
469 with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Total
470 organic acids (sum of succinic, lactic, acetic, propionic, iso-butyric and n-butyric acids). Values
471 are mean \pm SEM (n = 7) and not sharing a common letter differ significantly ($P = < 0.05$) by
472 Tukey-Kramer's post hoc test. All P -values of one-way ANOVA were < 0.0002 for each of
473 organic acid and total organic acids except 0.1664 for succinic acid.

474

475 **Fig. 2** Plasma concentration of quercetin and methyl quercetin derivatives in the tail blood of
476 rats fed the sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean
477 fiber (QS) or guar gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter
478 differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

479

480 **Fig. 3** Urinary excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-
481 based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar
482 gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly
483 ($P < 0.05$) by Tukey-Kramer's post hoc test.

484

485 **Fig. 4** Fecal excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-
486 based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar
487 gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter differ significantly
488 ($P < 0.05$) by Tukey-Kramer's post hoc test.

489

490 **Fig. 5** Fecal excretion of quercetin and methyl quercetin monoglucuronide metabolites of rats
491 fed the sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean
492 fiber (QS) or guar gum (QG). Values are mean \pm SEM (n = 7) and not sharing a common letter
493 differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

Fig.1

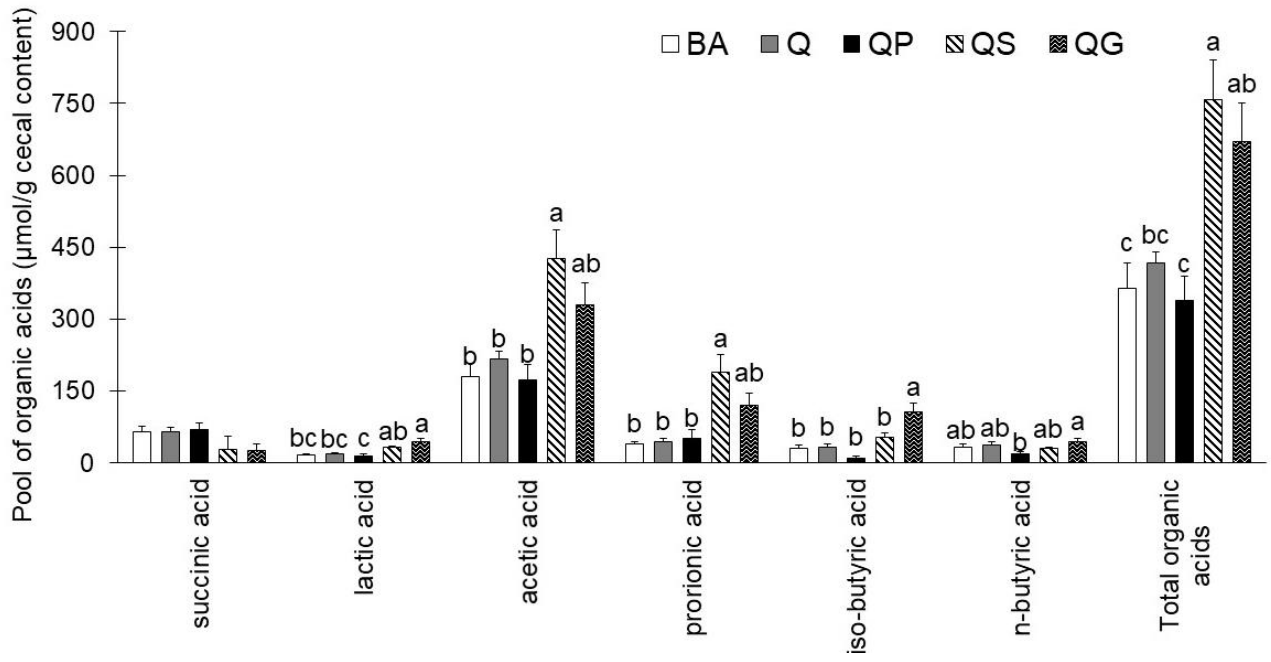


Fig.2

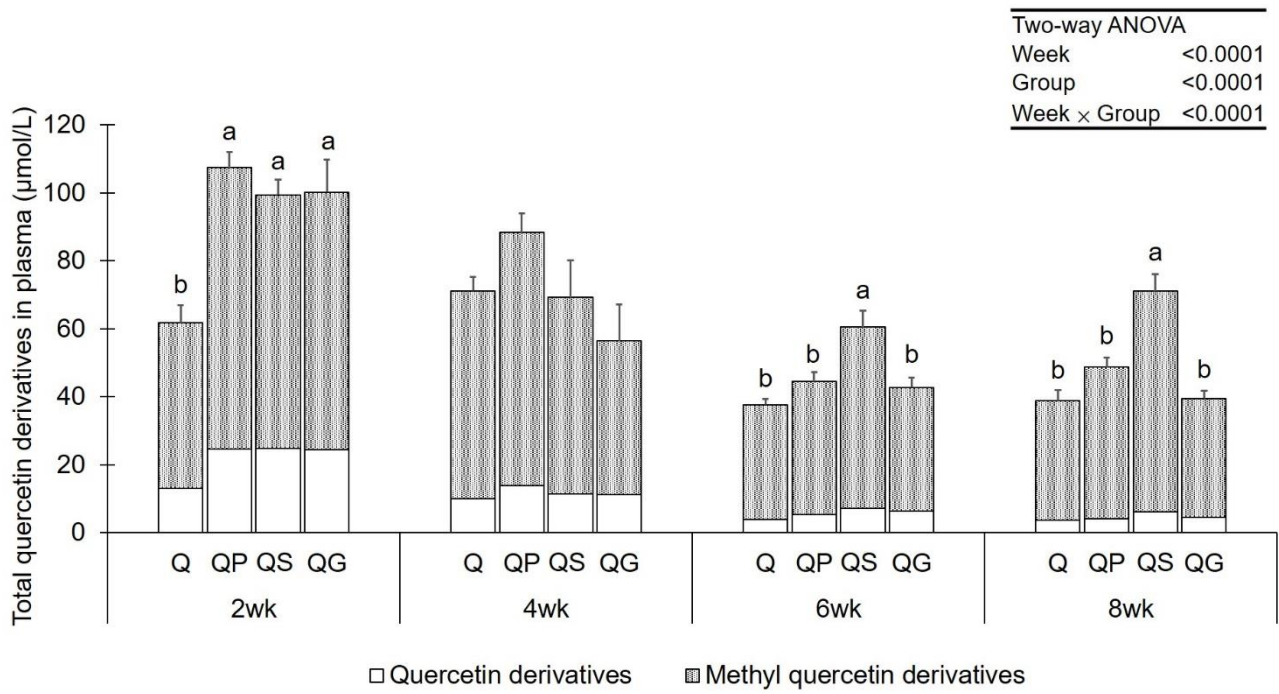


Fig.3

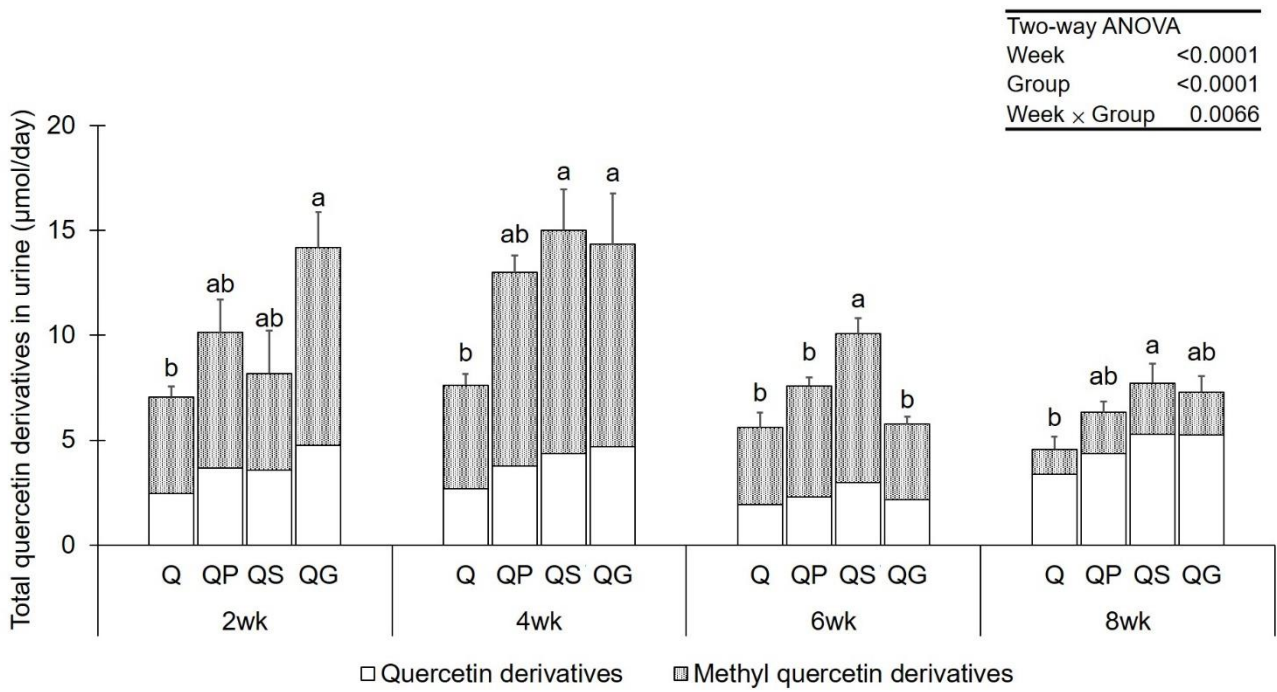


Fig.4

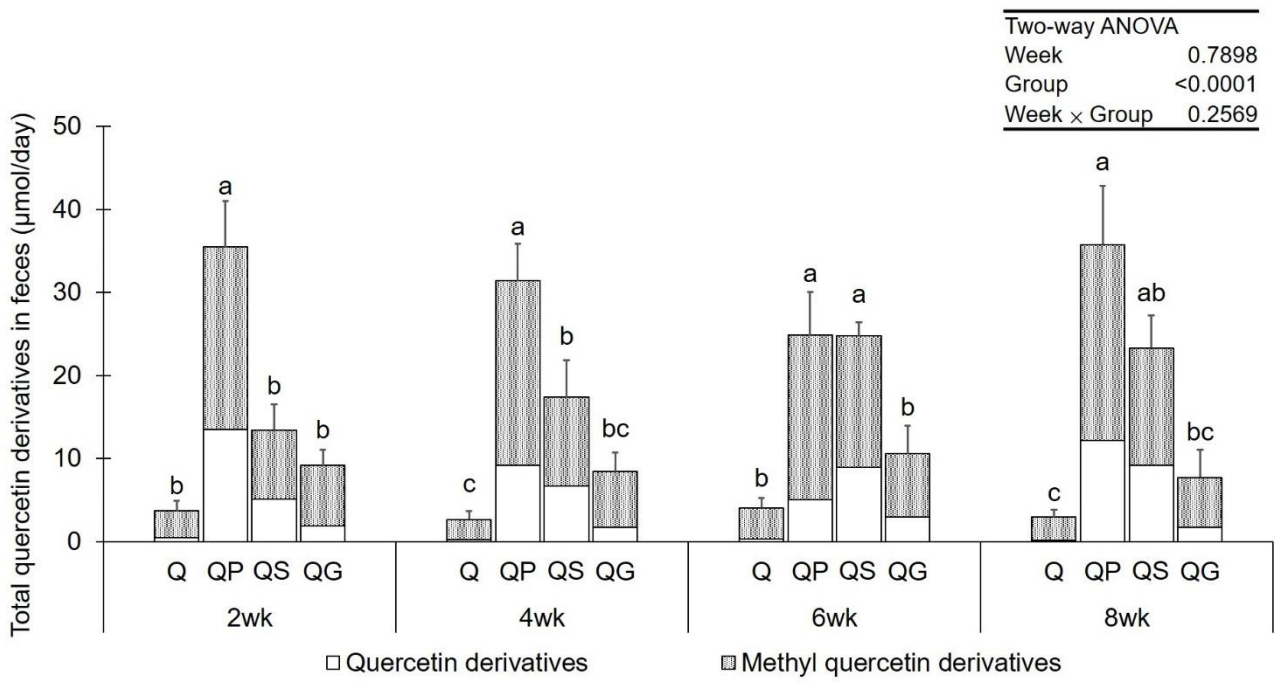


Fig.5

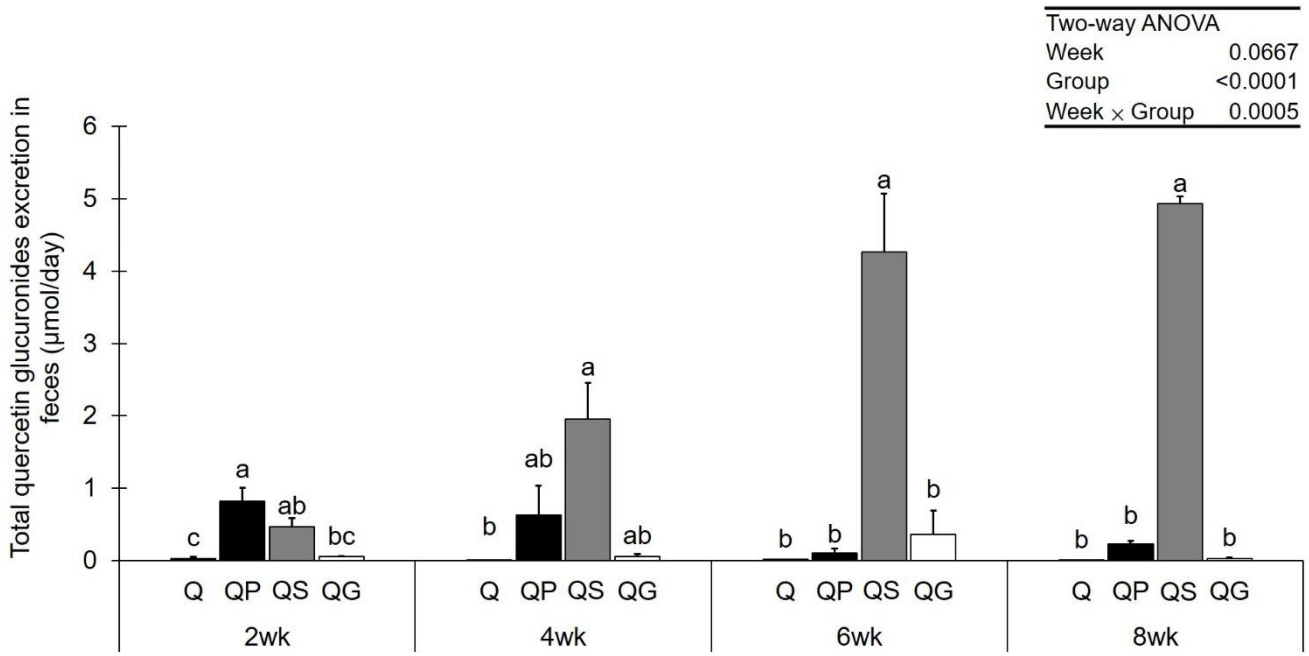


Table 1 Initial body weights, final body weight, average food intake, liver and epididymal fat pad weight of rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Group	Initial BW (g.)	Final BW (g.)	Food intake (g/day)	Liver (g/100 g BW)	Epididymal fat pad (g/100 g BW)
BA	162 ± 3	426 ± 11	19.46 ± 0.58	0.79 ± 0.02	1.51 ± 0.16 ^a
Q	162 ± 2	432 ± 14	19.53 ± 0.66	0.80 ± 0.01	1.31 ± 0.03 ^{ab}
QP	162 ± 2	405 ± 15	18.31 ± 0.38	0.71 ± 0.01	1.34 ± 0.07 ^{ab}
QS	162 ± 2	425 ± 13	18.52 ± 0.56	0.73 ± 0.02	1.12 ± 0.07 ^b
QG	162 ± 2	432 ± 14	18.87 ± 0.53	0.78 ± 0.02	1.34 ± 0.05 ^{ab}

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test. P values for one-way ANOVA were 0.998 for initial body weight, 0.3802 for final body weight, 0.3417 for average food intake, 0.5201 for liver weight and 0.0396 for epididymal fat pad.

Table 2 Whole cecum weight, cecum tissue weight, content weight and pH value of the cecal's rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Group	Whole cecum weight (g/100 g BW)	Cecum tissue weight (g/100 g BW)	Content weight (g/100 g BW)	pH
BA	0.77 ± 0.01 ^c	0.23 ± 0.01 ^d	0.54 ± 0.07 ^c	6.7 ± 0.1 ^a
Q	0.82 ± 0.04 ^c	0.27 ± 0.01 ^c	0.55 ± 0.05 ^c	6.6 ± 0.1 ^a
QP	0.97 ± 0.07 ^{bc}	0.30 ± 0.01 ^{bc}	0.67 ± 0.12 ^c	6.1 ± 0.0 ^b
QS	3.35 ± 0.45 ^a	0.41 ± 0.01 ^a	2.94 ± 0.25 ^a	6.1 ± 0.1 ^b
QG	1.88 ± 0.15 ^b	0.35 ± 0.02 ^b	1.53 ± 0.12 ^b	6.1 ± 0.0 ^b

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test. All P -values of one-way ANOVA were <0.0001 .

Table 3 Total dry fecal weight of rats fed a sucrose-based AIN 93G diet (BA) with 0.7 % Q3GM (Q) with 5% pectin (QP) or water-soluble soybean fiber (QW) or guar gum (QG).

Group	Total dry fecal weight (g/3days)			
	Week 2	Week 4	Week 6	Week 8
BA	5.22 ± 0.36 ^a	5.46 ± 0.35 ^a	5.38 ± 0.25 ^a	5.51 ± 0.15 ^a
Q	4.93 ± 0.19 ^a	5.49 ± 0.30 ^a	5.64 ± 0.29 ^a	5.87 ± 0.24 ^a
QP	2.59 ± 0.26 ^b	3.23 ± 0.16 ^b	3.10 ± 0.24 ^b	3.53 ± 0.20 ^b
QS	2.00 ± 0.18 ^b	2.28 ± 0.15 ^b	3.31 ± 0.20 ^b	3.02 ± 0.19 ^b
QG	2.23 ± 0.14 ^b	3.23 ± 0.28 ^b	3.11 ± 0.39 ^b	3.42 ± 0.29 ^b

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test. All P -values of one-way ANOVA were <0.05 .

Table 4 Amount of quercetin aglycone degraded per day and rate (%) of remaining aglycone in rats fed test diet containing a sucrose-based AIN 93G diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG).

Group	Ingested aglycone (μmol/day)	Excreted aglycone* (μmol/day)	Remaining aglycone** (%)	Degraded aglycone*** (%)
<u>Week2</u>				
Q	195.91 ± 5.44 ^a	6.69 ± 1.67 ^b	3.42 ± 0.85 ^b	96.58 ± 0.85 ^a
QP	177.42 ± 7.46 ^b	33.65 ± 4.97 ^a	18.97 ± 2.82 ^a	81.03 ± 2.82 ^b
QS	171.72 ± 5.07 ^b	11.37 ± 2.64 ^b	6.65 ± 1.60 ^b	93.35 ± 1.60 ^a
QG	169.73 ± 3.60 ^b	12.77 ± 1.66 ^b	7.59 ± 1.07 ^b	92.41 ± 1.07 ^a
<u>Week4</u>				
Q	202.77 ± 7.75	6.22 ± 0.99 ^c	3.12 ± 0.53 ^c	96.88 ± 0.53 ^a
QP	187.39 ± 4.57	32.76 ± 3.43 ^a	17.63 ± 1.98 ^a	82.37 ± 1.98 ^c
QS	188.23 ± 5.54	17.10 ± 2.72 ^b	9.20 ± 1.57 ^b	90.80 ± 1.57 ^b
QG	189.62 ± 4.74	13.59 ± 2.88 ^{bc}	7.13 ± 1.52 ^{bc}	92.87 ± 1.52 ^{bc}
<u>Week6</u>				
Q	206.87 ± 7.51	6.23 ± 1.20 ^c	2.97 ± 0.53 ^c	97.03 ± 0.53 ^a
QP	193.36 ± 5.18	26.14 ± 3.59 ^a	13.52 ± 1.72 ^a	86.48 ± 1.72 ^c
QS	193.75 ± 5.71	19.40 ± 1.30 ^{ab}	10.08 ± 0.78 ^{ab}	89.92 ± 0.78 ^{bc}
QG	197.56 ± 5.22	11.38 ± 2.99 ^{bc}	5.84 ± 1.57 ^{bc}	94.16 ± 1.57 ^{ab}
<u>Week8</u>				
Q	209.19 ± 7.54	5.40 ± 1.02 ^b	2.55 ± 0.43 ^b	97.45 ± 0.43 ^a
QP	195.79 ± 4.80	34.28 ± 5.91 ^a	17.33 ± 2.86 ^a	82.67 ± 2.86 ^b
QS	197.60 ± 6.05	17.75 ± 2.29 ^b	9.13 ± 1.31 ^b	90.87 ± 1.31 ^b
QG	201.56 ± 5.02	10.03 ± 3.45 ^b	4.98 ± 1.71 ^b	95.02 ± 1.71 ^b
Two-way ANOVA				
Week	<0.0001	0.8679	<0.0001	0.6599
Group	0.0004	<0.0001	<0.0001	<0.0001
Week × Group	0.9299	0.4457	0.8832	0.4099

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P = < 0.05$) by Tukey-Kramer's post hoc test.

* Excreted aglycone (μmol/day) = sum of total aglycone excreted into urine and feces

** Remaining aglycone (%) = $100 - (\text{degraded aglycone} / \text{ingested aglycone} \times 100)$

*** Degraded aglycone (%) = $\text{degraded aglycone} / \text{ingested aglycone} \times 100$