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

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Abstract

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

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

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

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

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

Introduction

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

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

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

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

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

Materials and methods

Chemicals

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

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

Animals and diets

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

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

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

Sample treatments

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

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

Quercetin measurement by LC-MS/MS

Total quercetin derivatives, sum of deconjugated quercetin and methylquercetin, were determined using LC-MS/MS system [25] (TSQ Quantum Access Max with Accela High Speed LC System, Thermo Fisher Scientific Inc., MA, USA) with an electrospray ionization (ESI) source with SRM mode. The analytical column was a 1.9 μm C18 column (Inertsustain Swift C18, 2.1 mm × 100 mm GL Sciences Inc., Tokyo, Japan) set at 40°C. The mobile phase was water, methanol, and formic acid (70:30:0.1, solvent A), and methanol with formic acid (100:0.1, solvent B). The ratio of solvent A and B was delivered through a linear gradient (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. Concentrations of total quercetin derivatives were calculated from the calibration curve of standard compounds.

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 µL) were mixed with 200 µL 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 µm 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. × 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 µM 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

Results

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

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

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

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

Total quercetin derivatives in plasma

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

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

Total quercetin derivatives in urine

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

Total quercetin derivatives in feces

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

Degradation of quercetin and methylquercetin aglycones

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

Discussion

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

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

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

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

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

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

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Fig. 1 Pools of organic acids in cecal contents of rats fed a sucrose-based AIN 93G (BA) diet with 0.7 % Q3GM (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Total organic acids (sum of succinic, lactic, acetic, propionic, iso-butyric and n-butyric acids). 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 <0.0002 for each of organic acid and total organic acids except 0.1664 for succinic acid.

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

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

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

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

Fig.1

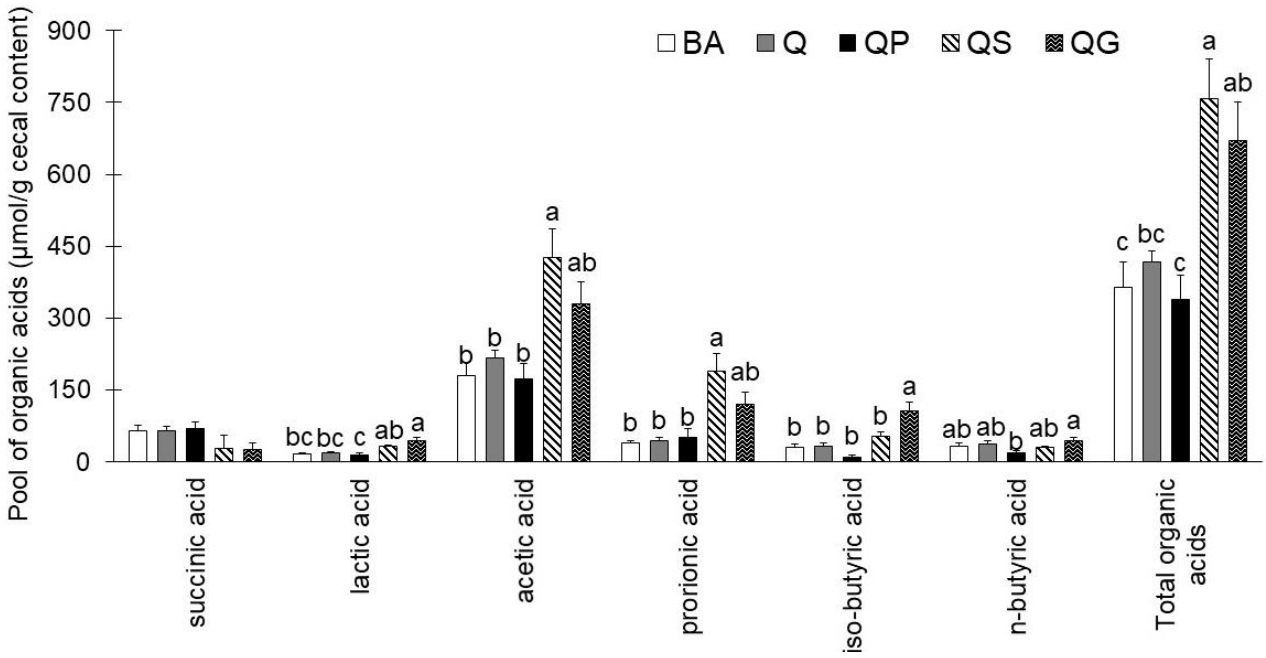
Pool of organic acids (μmol/g cecal content)
900
750
600
450
300
150
0
BA
Q
QP
QS
QG
bc bc c ab a
b b b a ab
b b b a ab
b b b b a
ab ab b ab a
c bc c a ab
succinic acid
lactic acid
acetic acid
prorionic acid
iso-butyric acid
n-butyric acid
Total organic acids

Fig.2

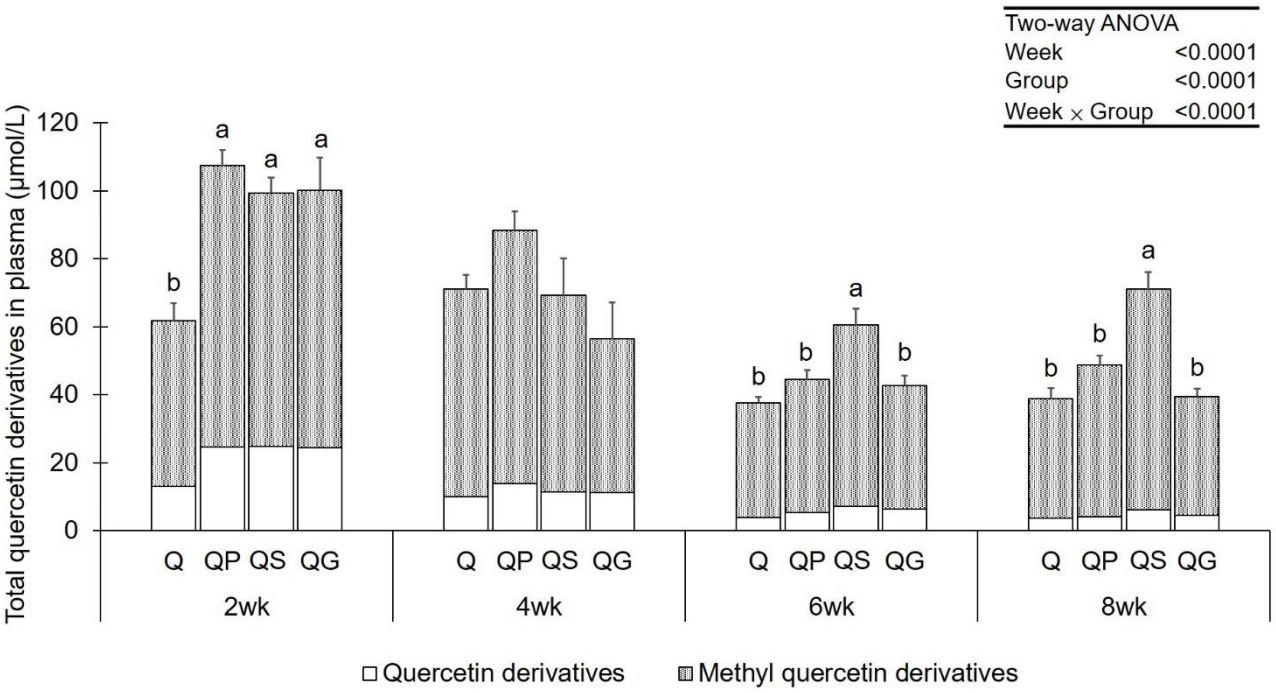
Two-way ANOVA
Week <0.0001
Group <0.0001
Week × Group <0.0001
Total quercetin derivatives in plasma (μmol/L)
120
100
80
60
40
20
0
a
a
a
b
b
b
b
b
a
b
b
b
a
b
Q QP QS QG
2wk
Q QP QS QG
4wk
Q QP QS QG
6wk
Q QP QS QG
8wk
Quercetin derivatives
Methyl quercetin derivatives

Fig.3

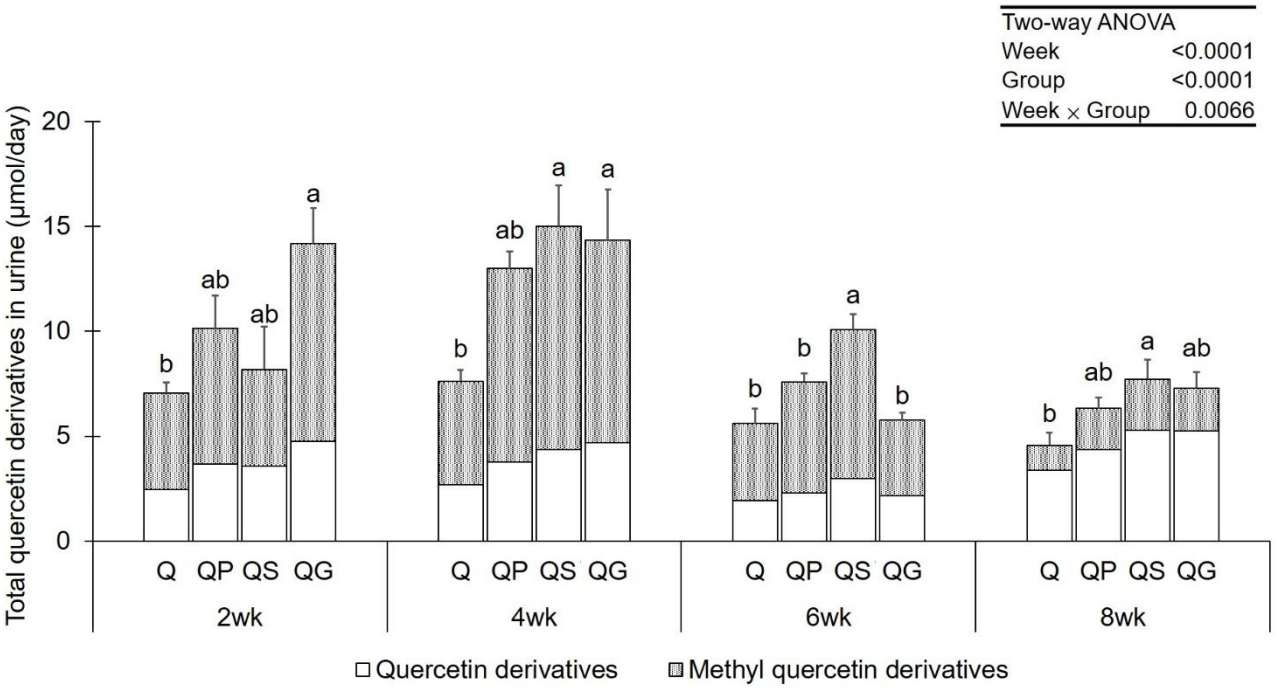
Two-way ANOVA
Week <0.0001
Group <0.0001
Week × Group 0.0066
Total quercetin derivatives in urine (μmol/day)
20
15
10
5
0
b
ab
ab
a
b
ab
a
a
b
b
a
b
b
ab
a
ab
Q QP QS QG
2wk
Q QP QS QG
4wk
Q QP QS QG
6wk
Q QP QS QG
8wk
Quercetin derivatives
Methyl quercetin derivatives

Fig.4

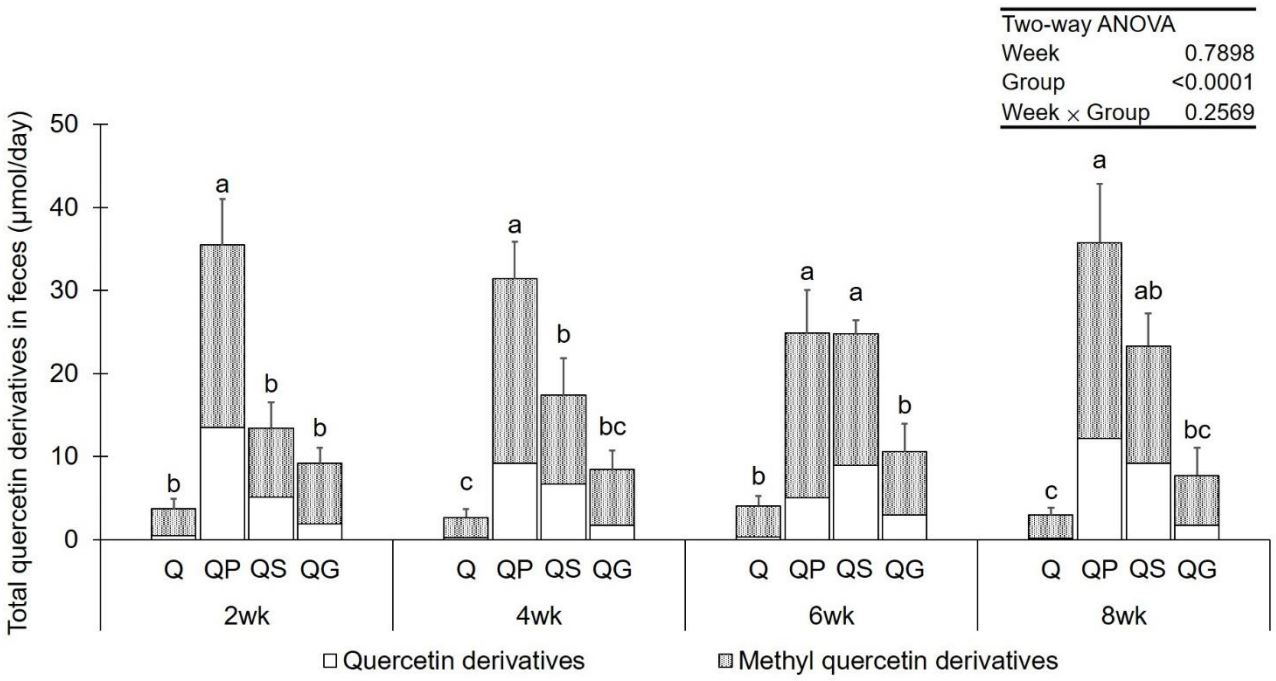
Two-way ANOVA
Week 0.7898
Group <0.0001
Week × Group 0.2569
Total quercetin derivatives in feces (μmol/day)
50
40
30
20
10
0
a
b
b
b
a
b
bc
c
a
a
b
b
a
ab
bc
c
Q QP QS QG
2wk
Q QP QS QG
4wk
Q QP QS QG
6wk
Q QP QS QG
8wk
Quercetin derivatives
Methyl quercetin derivatives

Fig.5

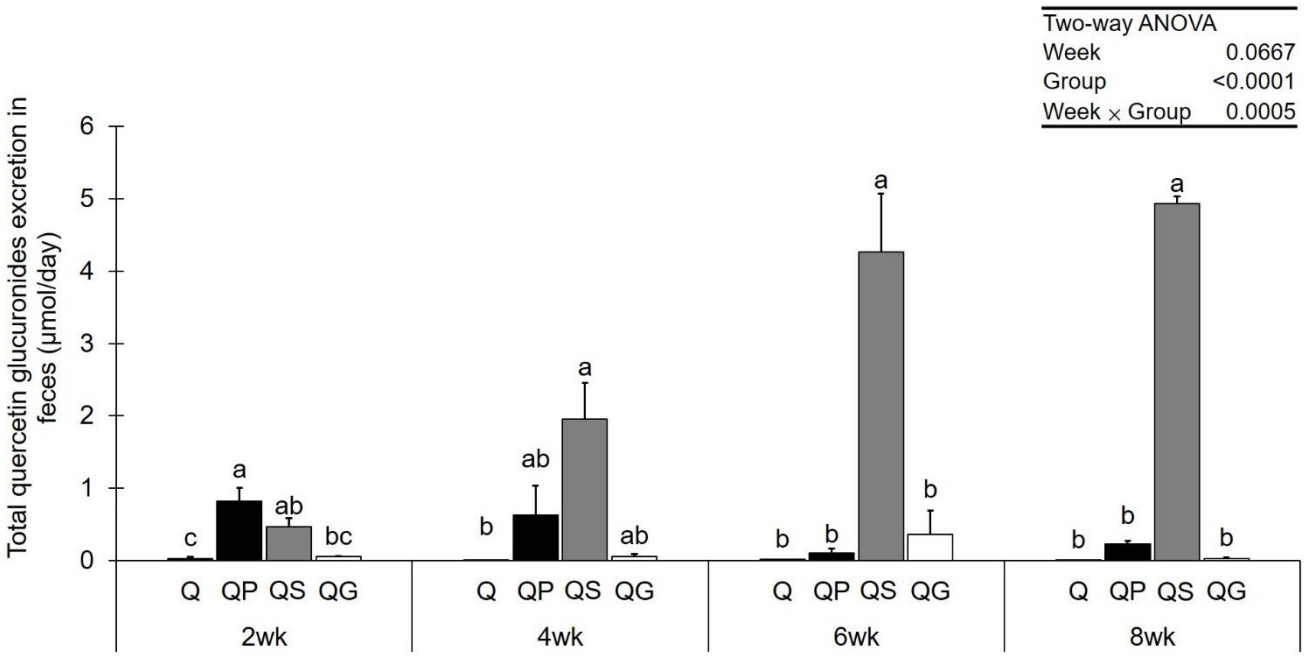
Two-way ANOVA
Week 0.0667
Group <0.0001
Week × Group 0.0005
Total quercetin glucuronides excretion in feces (μmol/day)
0
1
2
3
4
5
6
c
a
ab
bc
b
ab
a
ab
b
b
b
a
b
b
b
a
b
Q QP QS QG
2wk
Q QP QS QG
4wk
Q QP QS QG
6wk
Q QP QS QG
8wk

Table 1 Initial body weights, final body weight, average food intake, liver and epidydimal fad 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*** (%)
Week2				
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}
Week4				
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}
Week6				
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}
Week8				
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 – (degraded aglycone/ingested aglycone x 100)
*** Degraded aglycone (%) = degraded aglycone/ingested aglycone x 100