Effects of Rice Starch-Isoflavone Diet or Potato Starch-Isoflavone Diet on Plasma Isoflavone, Plasma Lipids, Cecal Enzyme Activity, and Composition of Fecal Microflora in Adult Mice

Motoi TAMURA1,*, Kazuhiro HIRAYAMA2, Kikuji ITOH2, Hiramitsu SUZUKI1 and Kazuki SHINOHARA1

1 National Food Research Institute, Tsukuba 305-8642, Japan
2 Laboratory of Veterinary Public Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan

(Received November 12, 2001)

Summary The effects of rice starch-isoflavone diet or potato starch-isoflavone diet on plasma concentration of isoflavones, plasma lipids, cecal enzyme activity, and intestinal microflora were studied. Male 15-wk-old mice were fed a rice-starch-based or potato-starch-based diet supplemented with isoflavones for 4 wk, and plasma samples, cecal contents, and feces were collected individually. Plasma equol concentration was significantly higher in the potato-isoflavone diet group than in the rice-isoflavone diet group, but no significant difference was observed in plasma daidzein or genistein concentrations. Plasma total cholesterol concentration was higher in the potato-isoflavone diet group, but no significant difference was observed in plasma triglyceride concentration. Both cecal β-glucuronidase and β-glucosidase activities were significantly higher in the rice-isoflavone diet group. The number of bifidobacteria was significantly higher in the potato-isoflavone diet group. These results indicate that different types of starches have different influences on plasma isoflavone and suggest that the influences might be through the change of host physiology and/or the metabolism and composition of intestinal microflora.

Key Words rice starch, potato starch, cecal enzyme activity, isoflavone, plasma lipids

Much attention has recently been focused on the isoflavones because of their possible ability to prevent cancer and other chronic diseases (1, 2). Their bioavailability in rats is reported (3). Among isoflavones, genistein and daidzein are present in soy food products as glycosides. Intestinal microbial enzymes can hydrolyze glycosides of plant origin. Furthermore, equol is a metabolite of daidzein produced by intestinal microflora (4) and is known to be much more estrogenic than daidzein is (5). Thus intestinal microflora seems to play an important role in the effects of isoflavones on the host.

Different types of starch affect the composition of fecal microflora and short-chain fatty acid production (6). Resistant starch is utilized by intestinal microflora (6) and seems to affect the cecal and colonic functions (7). It is contained in rice starch and potato starch, but little information is available about the influence of the source of starch in the diet on the metabolism of dietary isoflavones. The present study was undertaken to investigate the effects of the diet containing different types of starch supplemented with the soybean extract rich in isoflavones on the plasma concentration of isoflavones, plasma lipids, cecal bacterial enzyme activities, and composition of fecal microflora.

MATERIALS AND METHODS

Materials. Daidzein, genistein, and Fujiflavone P-40 were purchased from Fujicco (Kobe, Japan). Fujiflavone P-40 is a soybean extract with a high isoflavone content. The contents of daidzein and daidzein derivatives in Fujiflavone P-40 are 24.5%, and genistein and genistein derivatives are 6.4%. Equol was purchased from Extrasynthese (Genay, France). β-Glucuronidase type H-5, p-nitrophenyl-β-D-glucopyranoside, and p-nitrophenyl-β-D-glucuronide were purchased from Sigma (St. Louis, Mo., USA).

Animals and diets. Male mice of the Crj:CD-1 (ICR) strains (5 wk old) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). They were housed in suspended stainless-steel cages with wire mesh bottoms in a room kept at 24±0.5°C and a relative humidity of 65%, with lighting of alternating 12 h periods of light and dark. The animals were fed a commercial nonpurified diet (type MF, Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum. At the age of 15 wk, the mice were randomly divided into two groups of five animals each, and the MF diet was replaced with the rice or potato starch based diet supplemented with isoflavones for 4 wk. The composition of each diet is shown in Table 1. Total starch content was 45.55 g/100 g diet. The rice-isoflavone diet contained 25 g raw rice starch and 20.55 g raw cornstarch. The potato-isoflavone diet contained 25 g raw potato starch and 20.55 g raw corn-
starch. The raw cornstarch was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan); raw rice starch from Shimada Chemical Industry Co., Ltd. (Jyotsu, Japan); and raw potato starch from Nissin Flour Milling Co., Ltd. (Tokyo, Japan); and raw potato starch from Shimada Chemical Industry Co., Ltd. (Jyoetsu, Japan); and raw potato starch from Shimada Chemical Industry Co., Ltd. (Jyoetsu, Japan). Fujiflavone P-40 was obtained from Mitsuoka et al. (12, 13) and Itoh and Mitsuoka (14). The eating of the diet by the mice was good, Fujiflavone P-40 was used for the supplementation of isoflavones. Because the raw cornstarch was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan); raw rice starch from Shimada Chemical Industry Co., Ltd. (Jyotsu, Japan); and raw potato starch from Nissin Flour Milling Co., Ltd. (Tokyo, Japan). Fujiflavone P-40 was incorporated at the expense of an equivalent weight of cornstarch of 0.25%. Body weight and food consumption were measured during the experimental period.

**Sampling.** After the experimental diet-feeding, the feces were collected individually. We did not fast the mice before dissection. The mice were then anesthetized with diethyl ether and the blood was withdrawn from the abdominal aorta into heparinized tubes. We then centrifuged the blood samples and stored the plasma at $-40^\circ C$ until HPLC analysis for isoflavones and plasma lipids. Cecal contents were collected and stored at $-40^\circ C$ until the measurement of cecal enzyme activities, and feces were immediately processed for the bacteriological procedures.

**Analysis of plasma isoflavones.** We measured isoflavones in the plasma samples by the method of Piskula et al. (8) with modification. Specifically, we spiked 200 µL of plasma with 50 µm flavone for use as an internal standard and added 200 µL of β-glucuronidase type H-5 solution in 0.2 M sodium acetate buffer, pH 5.0 (3,500 units of β-glucuronidase and 193 units of sulfatase). We next incubated the mixture at 37°C in a shaking water bath for 2 h, then treated it with 3,600 µL of methanol/acetic acid (100/5, v/v), vortexed it for 30 s, sonicated for 30 s, vortexed it again for 30 s, and centrifuged it for 15 min at 4°C and 800×g. The supernatants were transferred to the eggplant type flask and evaporated completely by a rotary evaporator. We then dissolved the sample with the 80% methanol at the same volume of plasma. For HPLC analysis, we injected 20 µL of each preparation into a 250×4.6 mm Capcell Pak C18-5 µm column (Shiseido, Tokyo, Japan). We performed elution at a flow rate of 1 mL/min with the following linear gradient: A, methanol/acetic acid (95:5, v/v); B, water/acetic acid (95:5, v/v); and A (by vol) at 30% for 10 min, from 30% to 70% in 35 min, and from 70% to 30% in 5 min. A JASCO MD-1515 Photodiode array detector (JASCO Co., Ltd., Tokyo, Japan) was used to quantify spectral data from 200 to 400 nm for each peak.

**Measurement of plasma lipid concentrations.** The total plasma cholesterol was determined by the methods of Allain et al. (9). Plasma triglyceride was determined by the methods of Spayd et al. (10).

**Measurement of enzyme activity.** We measured enzyme activities as previously described (11). A 1:100 cecal suspension was prepared in prereduced 0.1 M phosphate buffer (pH 7.0), and nonbacterial debris was removed by centrifugation at 700×g for 2 min. The supernatant fluids was used immediately for β-glucosidase and β-glucuronidase assays. β-Glucosidase activity was measured with p-nitrophenyl-β-D-glucopyranoside as a substrate. β-Glucuronidase activity was measured with p-nitrophenyl-β-D-glucuronide.

**Bacteriological procedures.** Bacteriological procedures were essentially the same as those described by Mitsuoka et al. (12, 13) and Itoh and Mitsuoka (14). We introduced the sample into an anaerobic chamber after its weighing and prepared serial 10-fold dilutions of feces. The diluted samples were spread on the surfaces of the 11 selective and 4 nonselective agar media. Bacteria were identified at the levels of genus or family based on colony form, Gram stain, cell morphology, and growth under aerobic conditions. Bacterial numbers were expressed as log10 of viable bacteria per wet weight of feces (g).

**Statistics.** The data were expressed as the mean±SE. The Student’s t-test was used to determine the statistical significance of the differences between mean values.

**Results**

**Body weight, food consumption, and cecal contents**

No differences occurred in the final body weights (g±SE) of mice in either the rice-isoflavone diet group (44.7±1.0) or the potato-isoflavone diet group (47.2±1.2). A significant difference, however, was noted in the food consumption between the rice-isoflavone diet group (5.2±0.2) and the potato-isoflavone diet group

---

**Table 1. Compositions of the experimental diets.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Rice-isoflavone (%)</th>
<th>Potato-isoflavone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Rice starch</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>Potato starch</td>
<td>—</td>
<td>25</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20.55</td>
<td>20.55</td>
</tr>
<tr>
<td>Sucrose</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mineral mixture‡</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Soybean extract + high content</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>of isoflavones</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Obtained from Oriental Yeast. Composition (mg/kg diet): retinol acetate, 3.44; cholecalciferol, 0.05; α-tocopherol, 100; menadione, 104; thiamin HCl, 24; riboflavin, 80; pyridoxine·HCl, 16; cyanocobalamin, 0.01; ascorbic acid, 600; biotin, 0.4; folic acid, 4; calcium pantothenate, 100; p-aminobenzoic acid, 100; niacin, 120; inositol, 120; choline chloride, 4,000; and cellulose powder, 14,624.

‡ Obtained from Oriental Yeast. Composition (mg/kg diet): calcium phosphate, dibasic, 5,824; potassium phosphate, monobasic, 10,288; sodium phosphate, monobasic, 3,740; sodium chloride 1,864; calcium lactate, 14,036; ferric citrate, 1,272; magnesium sulfate, 2,868; zinc carbonate, 44; manganese sulfate, 48; cupric sulfate, 12; and potassium iodide, 4.
Table 2. Plasma concentrations of daidzein, equol, and genistein of the mice fed the rice-isoflavone and potato-isoflavone diets (μM).

<table>
<thead>
<tr>
<th></th>
<th>Rice-isoflavone (n=5)</th>
<th>Potato-isoflavone (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>0.81±0.35</td>
<td>0.30±0.18</td>
</tr>
<tr>
<td>Equol</td>
<td>5.95±1.21</td>
<td>9.77±0.72*</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.05±0.01</td>
<td>0.07±0.03</td>
</tr>
</tbody>
</table>

Values are means±SE. *Significantly different (p<0.05) from the rice-isoflavone diet group.

Table 3. Plasma concentration of cholesterol and triglyceride (mg/dL) and enzyme activities of cecal contents (μmol/h/g cecal contents) of the mice fed the rice-isoflavone and potato-isoflavone diets.

<table>
<thead>
<tr>
<th></th>
<th>Rice-isoflavone (n=5)</th>
<th>Potato-isoflavone (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>144.5±6.6</td>
<td>196.1±13.3*</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>101.1±25.1</td>
<td>87.1±10.5</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>66.9±14.6</td>
<td>23.2±4.2*</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>162.5±35.1</td>
<td>47.3±9.0*</td>
</tr>
</tbody>
</table>

Values are means±SE. *Significantly different (p<0.05) from the rice-isoflavone diet group.

(5.8±0.2) (p<0.05). The cecal contents in the potato-isoflavone diet group (0.31±0.03) were significantly higher than in the rice-isoflavone diet group (0.12±0.03) (p<0.05).

Analysis of plasma isoflavone metabolites

Plasma isoflavone metabolite concentrations are shown in Table 2. The plasma equol concentration was significantly higher in the potato-isoflavone diet group than in the rice-isoflavone diet group. But plasma daidzein was lower in the potato-isoflavone diet group, though the difference was not statistically significant. Plasma genistein concentration was low in both groups.

Measurement of plasma lipid concentrations and enzyme activity

Plasma total cholesterol concentration was higher in the potato-isoflavone diet than in the rice-isoflavone diet group. No significant difference, however, was observed in plasma triglyceride concentration between the two groups (Table 3). β-Glucosidase and β-glucuronidase activities were both significantly higher in the rice-isoflavone diet group than in the potato-isoflavone diet group (Table 3).

Bacteriological analysis of the intestinal microflora

The compositions of intestinal microflora are shown in Table 4. The number of bifidobacteria in the potato-isoflavone diet group was significantly higher than in the rice-isoflavone diet group. No significant difference was observed in other bacterial groups.

**DISCUSSION**

The plasma equol concentration was significantly higher in the potato-isoflavone diet group than in the rice-isoflavone diet group. Dietary daidzein is converted by intestinal flora to equol (4, 15). The ratio of equol to daidzein in the plasma of the potato-isoflavone diet group (145.5 on average) was higher than in the rice-isoflavone diet group (18.2 on average). The conversion rate of equol from daidzein might be greater in the potato-isoflavone diet group than in the rice-isoflavone diet group. It has been reported that the mean gastrointestinal transit time was significantly larger in the rats fed a potato starch diet than in those fed a high-amylose cornstarch diet (16). A slow gastrointestinal transit time might affect the plasma equol concentration by increasing the chance to be absorbed from gastrointestinal cells. In our experiment, the cecal contents were significantly heavier in the potato-isoflavone diet group than in the rice-isoflavone diet group. The heavier ceca also might increase the chance of absorption of equol from the large intestine, though we did not measure the transit time in the present study. Most isoflavones used in our experiment were isoflavone-glycosides. The hydrolysis of the isoflavone-glycosides were occurred, and the isoflavones were absorbed as aglycone in the intestine. Bacterial β-glucosidase would contribute to this hydrolysis. From this point, higher β-glucosidase in the rice-isoflavone diet group may contribute to the absorption of the isoflavones. However, there was no significant difference in the plasma daidzein and genistein. The bacterial degradation of isoflavone might be higher in the rice-isoflavone diet group in the intestine. A higher degradation of isoflavone might have canceled the effect of higher β-glucosidase on the absorption of isoflavone-glycosides in the rice-isoflavone diet group.

Intestinal microbial β-glucosidase is thought to be responsible for generating carcinogenic aglycon by hydrolyzing β-glucosides present in plants (17). Bacterial
\( \beta \)-glucuronidase is thought to hydrolyze glucuronide conjugates in the gut and to yield toxic and carcino-
genic substances (18–20). The results in this study indi-
cate that the potato-isoflavone diet is more effective by
reducing the cecal \( \beta \)-glucosidase and \( \beta \)-glucuronidase
activities compared with the rice-isoflavone diet.
Moreover, the number of bifidobacteria were signifi-
cantly higher in the potato-isoflavone diet group.
Bifidobacteria are believed to have beneficial effects on
the host (21, 22), and it is reported that the dietary ad-
mistration of Bifidobacterium longum significantly in-
hibits the incidence of colon adenocarcinomas (23).
Thus the potato-isoflavone diet might be more protec-
tive against carcinogenesis in comparison with the rice-
isoflavone diet.

Some researchers have suggested that microflora
adapt to the entry of new substrates by changing their
floral metabolic activity (24, 25). Considerable differ-
ences are reported in the microbial utilization of resis-
tant starch, depending on its source and structure (6).
Macfarlane and Englyst (26) have shown that amy-
loytic bacteria belonging to the genera Bifidobacterium,
Bacteroides, Fusobacterium, and Butyrivibrio play a major
role in fermenting starch in the colon. A difference of
fermentability between the two diets in our study might
have caused the difference of floral composition. Raw
potato starch might be a more suitable substrate for the
bifidobacteria than raw rice starch. There also were sig-
dificant differences of bacterial enzyme activities be-
tween the two dietary groups. The change in the floral
composition and/or different fermentability of the two
diets might have caused the difference of metabolic
activity of the microflora. These factors probably affect
not only the enzymes tested in the present study, but
also the metabolic activity for the equol production.
Equol production in the gastrointestinal tract might be
greater in the potato-isoflavone diet group than in the
rice-isoflavone group.

Although both diets in the present study contained
the resistant starch, which is effective in lowering
plasma cholesterol (27), there was a significant differ-
ence in plasma total cholesterol concentration between
the two dietary groups. Because it is reported that
isoflavone without soy protein did not alter the serum
lipid profile (2), the difference of plasma cholesterol
isoflavone concentration, probably through influences
between the two dietary groups. The change in the floral
composition and/or different fermentability of the two
diets might have caused the difference of metabolic
activity of the microflora. These factors probably affect
not only the enzymes tested in the present study, but
also the metabolic activity for the equol production.
Equol production in the gastrointestinal tract might be
greater in the potato-isoflavone diet group than in the
rice-isoflavone group.

In summary, the present study suggests that different
types of starch in the diet might affect the plasma
isoflavone concentration, probably through influences
on the host physiology and/or the intestinal microflora.

REFERENCES
1) Kennedy AR. 1995. The evidence for soybean products
as cancer preventive agents. J Nutr 125: 733S–743S.
2) Yamakoshi J, Piskula MK, Izumi T, Tobe K, Saito M,
Kataoka S, Obata A, Kikuchi M. 2000. Isoflavone agly-
cone-rich extract without soy protein attenuates ath-
erosclerosis development in cholesterol-fed rabbits. J
Nutr 130: 1887–1893.
3) Uehara M, Ohta A, Sakai K, Suzuki K, Watanabe S,
Adlercreutz H. 2001. Dietary fructooligosaccharides
modify intestinal bioavailability of a single dose of
genistein and daidzein and affect their urinary excre-
4) Axelson M, Sjövall J, Gustafsson BE, Setchell KDR.
1984. Soya a dietary source of the non-steroidal oestro-
gen equol in man and animals. J Endocrinol 102:
49–56.
Urinary equol excretion with a soy challenge: influence
333–339.
M. 1997. Feeding resistant starch affects fecal and
cecal microflora and short-chain fatty acids in rats. J
Anim Sci 75: 2453-2462.
Consumption of raw potato starch alters intestinal function and
colonic cell proliferation in the rat. J Nutr 119:
1610–1616.
and genistein but not their glucosides are absorbed from the
9) Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC.
1974. Enzymatic determination of total serum choles-
10) Spayd RW, Bruschi B, Burdick BA, Dappen GM,
Eikenberry JN, Esders TW, Figueras J, Goodhue CT,
Multilayer film elements for clinical analysis: Ap-
of the activity of five microbial enzymes in cecal content
from rats, mice, and hamsters, and response to dietary
12) Mitsuoka T, Ohno K, Benyo Y, Suzuki K, Namba K.
Vergleich des neu entwickelten Verfahrens mit dem
bisherigen üblichen Verfahren zur Darmfloraanalyse.
verbesserte Methodik der qualitativen und quantita-
tiven Analyse der Darmflora von Menschen und Tieren.
14) Itoh K, Mitsuoka T. 1980. Production of gnotobiotic
actes from raw potato starch might play
some role in a higher plasma total cholesterol con-
centration in the potato-isoflavone diet group. The concen-
tration and composition of short-chain fatty acids in the
gastrointestinal tract and in portal blood should be
studied.

In summary, the present study suggests that different
types of starch in the diet might affect the plasma
isoflavone concentration, probably through influences
on the host physiology and/or the intestinal microflora.


