Hypcholesterolemic Effect of Katsuobushi, Smoke-Dried Bonito,
Prevents Ovarian Hormone Deficiency-Induced
Hypercholesterolemia

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Summary The purpose of this study was to examine whether katsuobushi, smoked-dried
bonito (KB), which is a traditional Japanese food, prevents ovarian hormone deficiency-
induced hypercholesterolemia. In experiment 1, ovariectomized rats (OVX-rats) were fed a
purified diet containing casein or KB. Compared with the casein diet, the KB diet reduced
the plasma cholesterol concentration and apparent protein digestibility, and increased the
fecal dry weight and fecal bile acid excretion. In experiment 2, OVX-rats were fed one of the
following four diets: casein diet containing corn oil or fish oil (CA/CO or CA/FO), or a diet
containing the digested or undigested fraction of KB after treatment with microbial protease
(KBE or KBR). KBR contains mainly two components: oil and protease-undigested protein
of KB origin. In comparison with the CA/CO diet, the KBE diet did not affect the plasma and
liver lipids concentrations, apparent protein digestibility nor fecal bile acid excretion. How-
ever, the KBR and CA/FO diets reduced the plasma cholesterol and triacylglycerol (TAG)
concentrations and the liver total lipid and TAG concentrations, but increased the liver total
and esterified cholesterol concentrations. The KBR diet increased fecal bile acid excretion
and fecal dry weight, whereas the CA/FO diet did not. Thus, the preventive effect of KB on
the ovarian hormone deficiency-associated increase in plasma cholesterol concentration
appears to be mediated by an increase in bile acid excretion through a promoted secretion of
bile acids by the binding of bile acids to resistant proteins.

Key Words ovariectomized rat, katsuobushi (smoke-dried bonito), plasma cholesterol,
fecal bile acid

Dietary fats and cholesterol have been among the
most studied nutrients with regard to the plasma cho-
lesterol concentration. However, food also contains
other nutrients such as dietary proteins that have
important physiological and metabolic effects on cho-
lesterol metabolism (1). In general, animal proteins are
considered to be hypercholesterolemic compared with
plant proteins (2, 3). Among studies that compared ani-
mal proteins and plant proteins, most have focused on
the comparison of soybean protein and milk casein.
Therefore, this concept has been based on the compar-
isn of a limited number of proteins. Several reports
have indicated that the quality and quantity of dietary
protein affect the plasma cholesterol concentration (3–
8). The plasma cholesterol concentration of rabbits fed
cod protein was at an intermediate level and did not dif-
er from that of rabbits fed casein or soy protein (9). Dif-
f erent fish proteins in the diet have different effects on
the plasma cholesterol concentration (10). However, lit-
tle is known about the effect of various types of fish
proteins on the plasma cholesterol concentration.

Processed bonito, which is called katsuobushi in Ja-
pinese, is a popular ingredient in many prepared foods in
Japan. Bonito is called katsu in Japanese. There are
two types of katsuobushi in Japan that differ in the
method of preparation: one type, named arabushi, is
produced by boiling followed by a smoke-drying treat-
ment, and the second type, named karebushi, is pro-
duced from arabushi by fermentation by Aspergillus spe-
cies. Sliced katsuobushi is called kezuribushi. Kezuri-
bushi is the form of katsuobushi that is used in the
kitchen when preparing Japanese dishes and is sold in
grocery stores. However, to date, there have been no
studies on the effect of katsuobushi on the plasma cho-
lesterol concentration.
Menopause, whether natural or surgically induced, is associated with elevated concentrations of circulating total cholesterol and low density lipoprotein-cholesterol, placing postmenopausal women at greater risk for coronary heart disease (CHD) (11-14). Estrogen replacement therapy (ERT) in postmenopausal women reduces the risk of CHD, in part, by modulating plasma cholesterol. However, ERT and cholesterol-lowering pharmacological agents may be accompanied by side effects.

Therefore, in this study, we examined the effect of katsuobushi on the plasma cholesterol concentration in ovariectomized (OVX) rats. We used arabushi as the katsuobushi source.

**MATERIALS AND METHODS**

Test materials. Two hundred sixty grams of powdered katsuobushi (KB) supplied from Marutomo Co. Ltd. (Ehime, Matsuyama, Japan) was suspended in a 5 L container containing 1 L of distilled water. Fourteen grams of mixture of end-type protease from Bacillus licheniformis and endo- and exo-type protease from Aspergillus oryzae was added to 100 g of arabushi. This suspended solution was incubated at 52°C for 17 h. We used two kinds of protease to achieve as much enzyme resolution of arabushi as possible. After incubation, the enzymatic reaction was stopped by placing in a water bath of 85°C for 20 min the container containing the suspended solution. The resulting solution was then filtered through the filter cloth (pore size 100 μm; Lion Co., Ltd., Tokyo, Japan) of a two-sheet pile. The filtrate was mixed with an equivalent weight of dextrin as the weight of the solid in the filtrate, and then freeze-dried. The resulting product was called KBE. The residue was dried at 80°C for 15 h, and the resulting product was called KBR. The yield of KBR from KB was 42.3%. The protein content was determined by the Kjeldahl method (15), with an N-to-protein conversion factor of 6.25. The concentration of lipids was determined by the Soxhlet method. The amount of moisture was determined as the loss in weight after drying at 105°C for 24 h. The ash content was determined by the direct ignition method (550°C overnight). The chemical compositions of KB, KBE and KBR are shown in Table 1. Unfortunately, we did not measure the levels of fatty acids in KB, KBE and KBR. However, the levels of major fatty acids in the edible part of KB were as follows (mg/100 g): palmitic acid, 352; stearic acid, 171; oleic acid, 209; linoleic acid, 21; eicosapentaenoic acid (EPA), 97; and docosahexaenoic acid (DHA), 558 (16). The amino acid compositions of casein, KBE and KBR were determined with the use of an amino acid analyzer (model JLC-555/ V, Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan) (Table 2) (17).

Animals and diets. This study was approved by the Laboratory Animal Care Committee of Ehime University. The rats were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Ehime University.

Six-month-old retired breeder Wistar female rats (Nippon SLC, Shizuoka, Japan) were used in this study. The rats were acclimated by feeding a commercial solid diet (MF Oriental Yeast Co., Ltd., Osaka, Japan) for 7 d. Rats were housed in individual cages with screen bottoms of stainless steel in a room maintained at 23 ± 1°C with a 12-h light/12-h dark cycle (light, 0700-1900 h). After acclimation, a bilateral ovariectomy (OVX) was performed under anesthesia by intraperitoneal injection of sodium pentobarbital (Nembutal®, 30 mg/kg body weight; Abbott, North Chicago, IL, USA). Rats were fed a commercial solid diet (MF, Oriental Yeast Co., Ltd.) during the 7-d recovery period. After recovery, the OVX-rats were divided into two dietary groups in Experiment 1 and four dietary groups in Experiment 2 (6 rats per group) on the basis of body composition.

<table>
<thead>
<tr>
<th>Table 1. Chemical composition of casein, KB, KBE and KBR.</th>
<th>Table 2. Amino acid composition of casein, KBE and KBR.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Casein</strong></td>
<td><strong>KBE</strong></td>
</tr>
<tr>
<td>Moisture</td>
<td>106</td>
</tr>
<tr>
<td>Protein</td>
<td>862</td>
</tr>
<tr>
<td>Lipids</td>
<td>15</td>
</tr>
<tr>
<td>Ash</td>
<td>17</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>20</td>
</tr>
</tbody>
</table>

1 KB, the powder of smoke-dried bonito.  
2 KBE, the digested fraction of KB after treatment with microbial proteases; the digested fraction was mixed with the same amount of dextrin as the amount of solid in the extract, and then spray-dried.  
3 KBR, the undigested fraction of KB after treatment with microbial proteases.  
4 Carbohydrate = 1,000 - (Moisture + Protein + Lipids + Ash).

1, 2, 3 See Table 1.
weight: the KB and C groups in Experiment 1, and the CA/CO, KBE, KBR and CA/FO groups in Experiment 2. The compositions of the experimental diets are shown in Table 3. The total volume of protein in all six diets was adjusted to equivalent levels. The major fatty acids contained in corn oil (Nihon Shokuhin Kako Co. Ltd., Shizuoka, Japan) and fish oil (Tama Biochemical Co. Ltd., Kanagawa, Japan) were as follows (g/100 g): palmitic acid, 11.2 and 10.6; stearic acid, 2.1 and 2.7; oleic acid, 34.6 and 9.5; linoleic acid, 50.2 and 0.7; EPA, 0 and 7.6; and DHA, 0 and 47.3, respectively. Fish oil contains vitamin E at a level of 0.3 g/100 g. The diets were freshly prepared each week and stored at 4°C until required. The O VX-rats were given free access to the respective diet and water for 28 d. Food intake was recorded daily for each rat in the morning before replenishing the diet. The body weight was measured every 7 d.

Sampling and analytical procedures. Before the O VX-rats were sacrificed, feces were collected on the final 3 d of the experimental period from each O VX-rat. The feces were freeze-dried, weighed and milled. The level of nitrogen (N) in diets and feces was analyzed in duplicate for each collection according to the Kieldahl method (15). The apparent digestibility of protein (N×6.25) was calculated by measuring the N content in the diet and feces. A blood sample was collected from the neck at midnight from fed rats into a blood collection tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) that contained heparin as an anticoagulant. The plasma was separated by centrifugation at 1,400 ×g at 4°C for 15 min, and was stored at −50°C until analysis. After blood collection, the liver was immediately removed, washed with cold saline (9 g NaCl/L), blotted dry on filter paper, weighed, and stored at −50°C until analysis.

The levels of cholesterol, triacylglycerol (TAG) and phospholipids (PL) in the plasma were enzymatically determined with commercial kits (Cholesterol E Test Wako, Triglyceride E-Test Wako and Phospholipids C-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The level of liver total lipids was determined gravimetrically after extraction by the method of Folch et al. (19). The levels of liver triacylglycerol, total cholesterol and free cholesterol were also measured. Lipids were extracted from 500 mg of liver with chloroform : methanol (2 : 1, v/v) according to the method of Folch et al. (19). After extraction, the volume of the lipid solution was adjusted to 20 mL with the same solution of chloroform : methanol (2 : 1, v/v). One milliliter of this extract was dried under a nitrogen stream, and the obtained residue was mixed with 100 µL isopropyl alcohol containing 100 g Triton X-100/L (Wako Pure Chemical Industries). Thirty microliters of this mixture was mixed with 3 mL of aqueous enzyme solution according to the standard procedure of the assay kit (Triglyceride E-Test Wako, Cholesterol E-Test Wako and F cholesterol E-Test Wako, Wako Pure Chemical Industries), and the triacylglycerol and cholesterol concentrations were determined colorimetrically. The level of esterified cholesterol was defined as the difference between the total cholesterol and free cholesterol concentrations. In a preliminary study, 30 µL of isopropyl alcohol containing 100 g Triton X-100/L did not affect the enzymatic reactions (data not shown).

Fecal steroids were extracted with a mixture of chloroform : methanol (1 : 1, v/v) at 70°C for 60 h (20). The level of fecal total bile acids was determined enzy-
Table 4. Body mass gain, food intake, plasma lipids, liver lipids and apparent protein digestibility in ovariec-
tomized rats fed each experimental diet for 28 d (Experi-
ment 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>C diet</th>
<th>KB diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass gain, g/28 d</td>
<td>38±4</td>
<td>33±5</td>
</tr>
<tr>
<td>Food intake, g/28 d</td>
<td>277±7</td>
<td>291±5</td>
</tr>
<tr>
<td>Plasma lipids, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.18±0.17a</td>
<td>3.16±0.18b</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>1.68±0.12</td>
<td>1.35±0.12</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3.73±0.18</td>
<td>3.23±0.21</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>7.49±0.31</td>
<td>7.83±0.33</td>
</tr>
<tr>
<td>Liver lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipids, mg/g</td>
<td>80.3±10.3</td>
<td>74.7±9.2</td>
</tr>
<tr>
<td>Cholesterol, µmol/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16.9±1.3</td>
<td>20.2±1.0</td>
</tr>
<tr>
<td>Free</td>
<td>10.1±0.9</td>
<td>11.4±0.7</td>
</tr>
<tr>
<td>Ester</td>
<td>6.8±0.4</td>
<td>8.8±0.6</td>
</tr>
<tr>
<td>Triacylglycerol, µmol/g</td>
<td>75.9±6.8</td>
<td>68.8±9.3</td>
</tr>
</tbody>
</table>
| Apparent protein diges-
   tibility2, %             | 98.0±0.5a | 86.8±0.4b |
| Fecal output              |        |         |
| Dry weight, g/3 d         | 1.72±0.06a | 4.23±0.18b |
| Bile acids, µmol/3 d      | 40.2±1.2a | 144.0±12.3b |
| µmol/g feces              | 23.4±0.9a | 43.5±4.8b  |

1 Values are the means±SE, n=6. Values with different superscript letters in a row are significantly different.

2 Apparent digestibility of dietary protein (D) was calculated with the following equation: D=(I−F)×100/I, where I is N intake and F is the fecal N excretion. The amount of N ingested and excreted in feces was analyzed by the Kjeldahl method (see text).

matically by the 3α-hydroxysteroid dehydrogenase assay method of Sheltaway and Losowsky (21) using taurocholic acid as the standard.

Statistical analysis. Data were expressed as mean±SE. In Experiment 1, the statistical significance of differences between the two groups was evaluated by Student’s t-test, using a computer software package (StatView Version 4.5, Abacus Concept Inc., Berkeley, CA). In Experiment 2, the statistical significance of differences among the four groups was evaluated by one-way ANOVA, using a computer software package (StatView Version 4.5, Abacus Concept Inc.). Individual comparisons were made by the Tukey multiple comparison test using the Super ANOVA statistical software package (Abacus Concept Inc.). Differences were considered to be significant at p<0.05.

RESULTS

Experiment 1

The food intake, body mass gain, liver weight and liver lipids did not significantly differ between the KB and C groups (Table 4). The plasma cholesterol concentration and apparent protein digestibility were significantly lower in the rats fed the KB diet than in those fed the C diet. The dry weight of fecal output and fecal excretion of bile acids in the rats fed the KB diet were significantly greater than those in the rats fed the C diet.

Experiment 2

The food intake, body mass gain and liver weight did not significantly differ among the CA/CO, KBE, KBR, and CA/FO groups (Table 5). The plasma cholesterol, TAG and PL concentrations were significantly lower in the rats fed the KBR and CA/FO diets than in those fed the CA/CO and KBE diets.

The liver total lipids were significantly lower in the rats fed the KBR and CA/FO diets than in those fed the CA/CO and KBE diets. The liver total, free and esterified cholesterol concentrations in rats fed the KBR diet were significantly higher than those in rats fed the CA/CO and CA/FO diets, but did not significantly differ compared with those in rats fed the KBE diet. The liver triacylglycerol concentration of rats fed the KBR diet was significantly lower than that of rats fed the CA/CO and KBE diets, but did not significantly differ from that of rats fed the CA/FO diet.

The apparent protein digestibility was significantly lower in rats fed the KBR diet than in those fed the other three diets. The dry weight of fecal output and fecal excretion of bile acids in rats fed the KBR diet were significantly greater than those in rats fed the other three diets.

DISCUSSION

The KB and KBR diets each contain relatively large amounts of two components, fish oil and proteins that were not digested by the proteases. In rats, feeding a diet containing fish oil greatly reduced the plasma cholesterol concentration (22–24). In the present study, feeding the KB or KBR diet significantly reduced the plasma cholesterol concentration. Excretion of cholesterol and bile acids is a major factor in the regulation of plasma cholesterol. In vitro studies showed that undigested protein can bind bile acids (25, 26). Feeding the protease-undigested fraction of soybean protein reduced the plasma cholesterol concentration in rats by increasing fecal weight and fecal excretion of both acidic and neutral sterols (27). Rats that were fed the KB and KBR diets showed higher fecal output, because of lower digestibility of KB and KBR. The fecal bile acid excretion of rats fed the KB diet was 3.6 times higher than that of rats fed the C diet, and the fecal bile acid excretion of rats fed the KBR diet was 3.3 times higher than that of rats fed the CA/FO diet. The higher fecal excretion of bile acids in rats fed the KB or KBR diet may be associated with their low digestibility and their binding capacity to bile acids. KB and KBR may have properties similar to those of dietary fiber by virtue of their lower digestibility. KB and KBR seemed to lower the intestinal reabsorption of bile acids, and this may be one of the mechanisms through which they exert their hypcholesterolemic effects.

The plasma cholesterol concentration of rats fed the KBR diet was slightly higher than that of rats fed the CA/FO diet, although there was no significant difference. This may have resulted from a difference in the
quality of the fish oil in the KBR and CA/FO diets. It is thought that the fish oil in KBR was partly oxidized during the hydrolysis of KB with protease. Therefore, the slightly higher plasma cholesterol concentration in rats fed the KBR diet may have been due to oxidation of a portion of the lipids in KBR. Unfortunately, we had not measured the degree of oxidation in lipids in KBR.

In rabbits, when fish protein was combined with corn oil, the plasma cholesterol concentration of rabbits fed fish protein combined with corn oil was similar to that of rabbits fed casein (5, 28, 29). In the present study, the plasma cholesterol concentration of rats fed the KBE diet did not significantly differ from that of rats fed the CA/CO diet. A study has implicated the amino acid composition of the dietary protein as a factor affecting the plasma cholesterol concentration (29). Arginine counteracts the hypercholesterolemic effect of other essential amino acids (30). Two essential amino acids, lysine and methionine, were the most effective for increasing the plasma cholesterol concentration (31). It has been considered that a higher arginine:lysine ratio may lead to lower plasma cholesterol concentration (32). The contents of lysine and methionine in KBE were lower than those in casein. However, the ratio of arginine to lysine in KBE was lower than that in casein (0.235 vs. 0.402, respectively). On the other hand, the ratio of arginine to lysine in KB and KBR was higher than that in casein (0.599 vs. 0.402 and 0.666 vs. 0.402). When the apparent protein digestibility is taken into consideration, the ratio of arginine to lysine in KB, KBR, and casein were 0.520, 0.549 and 0.385, respectively. Therefore, the amino acid compositions of KB and KBR appear to be the primary factor responsible for its hypocholesterolemic effect, though the apparent protein digestibilities of KB and KBR were lower than that of casein.

The plasma concentrations of TAG in rats fed the KBR and CA/FO diets were significantly lower than that in rats fed the CA/CO diet. In this study, rats were sacrificed in the fed state. Therefore, the plasma TAG originated from the diet and from liver synthesis. Triacylglycerols from fish oil may be hydrolyzed less efficiently by pancreatic lipases than other TAGs (33). The digestion and absorption of fish oil were lower than those of corn oil in rats (34). The absorption of dietary 14-C-labeled triolein was lower in rats fed rat chow containing fish oil than in those fed rat chow containing soybean oil (35). This lowered intestinal absorption of dietary fish oil may have contributed to the reduced plasma TAG concentration in rats fed the KBR and CA/FO diets. However, in rats that were previously fed diets containing corn oil or fish oil, there was no difference in the intestinal absorption of dietary fish oil (36). In the liver, newly synthesized fatty acids are a good substrate for esterification into TAG and PL (37). However, the administration of n-3 polyunsaturated fatty acids (n-3 PUFA) such as EPA and DHA in rats has a hypotriglyceridemic effect in vivo, by inducing a lower fractional rate of secretion of newly synthesized TAG by the liver (38). Furthermore, it is known that dietary n-3 PUFA inhibit de novo hepatic fatty acid synthesis by regulating the transcription of genes encoding lipogenic enzymes (39). In the present study, the concentration of TAG in the liver was significantly lower in the rats fed the KBR and

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**Table 5.** Body mass gain, food intake, plasma lipids, liver lipids and apparent protein digestibility in ovariectomized rats fed each experimental diet for 28 d<sup>1</sup> (Experiment 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>CA/CO diet</th>
<th>KBE diet</th>
<th>KBR diet</th>
<th>CA/FO diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass gain, g/28 d</td>
<td>30±3</td>
<td>33±5</td>
<td>37±8</td>
<td>34±3</td>
</tr>
<tr>
<td>Food intake, g/28 d</td>
<td>265±7</td>
<td>291±5</td>
<td>266±11</td>
<td>273±11</td>
</tr>
<tr>
<td>Plasma lipids, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.83±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.73±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.63±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>1.58±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.00±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3.83±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.73±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.63±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>7.49±0.31</td>
<td>7.53±0.13</td>
<td>7.53±0.29</td>
<td>7.50±0.16</td>
</tr>
<tr>
<td>Liver lipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipids, mg/g</td>
<td>80.3±10.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.3±9.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.6±2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.0±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.9±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.2±1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.0±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free</td>
<td>10.3±0.9</td>
<td>9.9±0.4</td>
<td>12.9±0.5</td>
<td>12.0±0.6</td>
</tr>
<tr>
<td>Ester</td>
<td>5.7±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triacylglycerol, μmol/g</td>
<td>75.9±12.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.8±10.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>24.0±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.7±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apparent protein digestibility&lt;sup&gt;2&lt;/sup&gt;, %</td>
<td>96.0±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.8±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.5±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.8±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fecal output</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight, g/3 d</td>
<td>1.39±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.79±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bile acids, μmol/3 d</td>
<td>39.2±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.9±4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>224.0±16.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.0±6.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>μmol/g feces</td>
<td>28.1±1.0</td>
<td>33.2±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.3±5.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.9±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are the means±SE, n=6. Values with different superscript letters in a row are significantly different.

<sup>2</sup>See Table 4.
CA/FO diets than in rats fed the CA/CO diet. Therefore, the reduced plasma TAG concentrations in rats fed the KBR and CA/FO diets may have resulted from reductions in hepatic synthesis and secretion of TAG caused by fish oil.

The cholesterol content of the liver of rats fed fish oil was increased compared with that of rats fed corn oil (40, 41). In the present study, the total cholesterol content of the liver of rats fed the KB diet (158 ± 10 μmol) was higher than those of rats fed the C diet (126 ± 14 μmol). Moreover, the total cholesterol content of the liver of rats fed the KBR and CA/FO diets (175 ± 15 μmol and 158 ± 6 μmol, respectively) were higher than those of rats fed the CA/CO and KBE diets (121 ± 14 μmol and 106 ± 4 μmol, respectively). In agreement with a previous study (40, 41), the cholesterol content of the liver of rats fed a diet containing fish oil, i.e., the KB, KBR and CA/FO diets, was increased. Unfortunately, we had not measured the cholesterol content in KB and KBR. However, KB contains cholesterol as shown in the fifth edition of Standard Tables of Food Composition in Japan. Therefore, the increased cholesterol content in the liver of rats fed the KB and KBR may depend on the presence of cholesterol in the diets.

The liver is the key organ involved in cholesterol homeostasis and makes a significant contribution to cholesterol in the entire body. It takes up most of the circulating cholesterol and has the unique function of eliminating cholesterol from the body through secretion of bile acid and free cholesterol. The level of fecal total bile acid excretion of rats fed the KBR diet was 4.8 times higher than that of rats fed the CA/FO diet. Cholesterol synthesis is stimulated by enhanced fecal excretion of bile acids and neutral sterols. Therefore, the increased liver cholesterol concentration in rats fed the KBR diet compared with rats fed the other three diets may have occurred due to the greater synthesis of cholesterol to compensate for the enhanced fecal excretion of bile acids and neutral sterols.

In conclusion, KB and KBR showed a hypcholesterolemic effect. This effect was associated with higher fecal output and higher excretion of fecal bile acids. The undigested protein in KB and KBR may act synergistically to regulate cholesterol metabolism. The effect of undigested proteins seems to be similar to that of dietary fiber.

REFERENCES
23) Roach PD, Kambouris AM, Trimble RP, Topping DL,


