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# Effects of soya milk and Bifidobacterium-fermented soya milk on plasma and liver lipids, and faecal steroids in hamsters fed on a cholesterol-free or cholesterol-enriched diet

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The effects of freeze-dried soya milk (SM) and Bifidobacterium-fermented soya milk (FSM) on plasma and liver lipids, and faecal steroid excretion were estimated in hamsters fed on a cholesterol-free or cholesterol-enriched diet. Hamsters fed on the cholesterol-free diet containing 300 g FSM/kg had lower levels of plasma VLDL + LDL cholesterol than the animals fed on the control diet. SM in the diet produced a similar pattern without significant differences. In the cholesterol-enriched diet group, SM and FSM decreased the levels of plasma total cholesterol and VLDL + LDL-cholesterol. SM and FSM decreased the plasma triacylglycerol level in both the cholesterol-free and -enriched diet groups. The liver total cholesterol contents in the SM and FSM groups were lower than that in the control group, for hamsters fed on the cholesterol-free diet. The liver triacylglycerol content was not modified by SM or FSM in hamsters fed on either the cholesterol-free or -enriched diet. SM and FSM increased the total bile acid excretion and the proportion of cholesterol entering the cholic acid biosynthesis pathway in both the cholesterol-free and -enriched diet groups. SM and FSM did not affect neutral steroid excretion in the cholesterol-free or -enriched diet group. There was an inverse relationship between VLDL + LDL-cholesterol and faecal bile acid excretion in hamsters fed on the cholesterol-free ($r -0{\cdot}670$, $P < 0{\cdot}01$) and cholesterol-enriched ($r -0{\cdot}761$, $P < 0{\cdot}001$) diets respectively. These results indicated that SM had an anti-atherogenic effect, and that this effect was not diminished by prior fermentation.

**Soya milk: Bifidobacterium: Cholesterol: Bile acids**

The potential role of dietary soya in the prevention and treatment of chronic diseases, in particular, heart disease and cancer, has been recognized for a long time. Soyabean protein (Sugano & Koba, 1993), isoflavonoids (Sharma, 1978), phospholipids (Knuiman *et al.* 1989), saponins (Potter *et al.* 1993), and phytate (Jariwalla *et al.* 1990) have been investigated in a search for the active component responsible for the anti-atherogenic effect of soya. Some experiments suggested that the amino acid profile of proteins and other non-protein components present in soya may be partially responsible for the hypocholesterolaemic effect (Huff *et al.* 1977; Nagata *et al.* 1982; Anthony *et al.* 1996; Balmir *et al.* 1996; Potter *et al.* 1996). However, the mechanism and component responsible for the effect remain unclear.

Many types of soya food are consumed throughout the world. A new class of soya food is being developed to reduce soya's bean-like flavour, for incorporation into human foods. Soya milk is an aqueous extract of whole soyabeans. However many people find the taste of soya milk undesirable. Soya milk contains soyabean protein and isoflavones, which are thought to have an anti-atherogenic effect. The combination of soyabean protein and minor components may be important (Anthony *et al.* 1996). The fermentation of soya results in compositional changes in isoflavones, phytate and saponins (Anderson & Wolf, 1995). *Bifidobacterium breve* YIT 4065, which is used as a commercial fermented milk starter in Japan, is suitable for the fermentation of soya milk. *Bifidobacterium breve* YIT 4065 produces lactate and acetate, which could change the

**Abbreviations:** AI, atherogenic index; FSM, freeze-dried fermented soya milk; SM, freeze-dried soya milk.

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physicochemical character of soyabean protein, and cause the release of aglycones from isoflavone glucosides by β-glucosidase (*EC* 3.2.1.21) (Ishikawa *et al.* 1997).

Terpstra *et al.* (1991) showed that the hamster is a useful animal model for studying the effects of dietary proteins on lipid metabolism in the presence and absence of dietary cholesterol. Hamsters are known to have similar responses to human subjects with respect to dietary influences on blood lipids. An ethanol extract of isolated soyabean protein, which contains isoflavones, caused a more sensitive response to blood lipids in hamsters than in rats (Balmir *et al.* 1996).

To determine the effect of the fermentation of soya milk on lipid metabolism, we investigated the levels of cholesterol and triacylglycerol in plasma and liver, and faecal steroids in hamsters fed on a cholesterol-free or cholesterol-enriched diet with soya milk fermented with *Bifidobacterium breve* YIT 4065.

## Materials and methods

### *Preparation of soya milk and fermented soya milk*

Crude soya milk from Shikokukakouki Co. Ltd (Tokushima, Japan) was used as the starting material for fermented soya milk. *Bifidobacterium breve* YIT 4065 was obtained from the collection of the Culture Collection Research Laboratory of Yakult Central Institute for Microbiological Research (Tokyo, Japan). A seed culture prepared anaerobically in the soya milk was freshly added to the soya milk at 10 ml/l and fermented statically at 37° for 30 h. The titratable acidity, pH and viable cell counts of the fermented soya milk were 0·825 %, 4·84 and $4·04 \times 10^9$ colony forming units/ml respectively. The original unfermented soya milk and the fermented soya milk were freeze-dried and milled until the products passed through a 0·84 mm sieve (20 mesh). The crude protein, crude fat and ash content levels in the freeze-dried soya milk (SM) were 431, 227 and 55 g/kg respectively. The crude protein, crude fat and ash content levels in the freeze-dried fermented soya milk (FSM) were 429, 233 and 57 g/kg respectively.

### *Animals and diets*

Thirty-six male Golden Syrian hamsters (Japan S.L.C. Co., Shizuoka, Japan) were obtained at the age of 5 weeks. The animals were fed on a commercial non-purified solid diet (MF; Oriental Yeast, Tokyo, Japan) for 2 d and the powdered MF diet for 5 d. After this 7 d adaptation period, the hamsters were randomly divided into six groups of six each of similar mean body weights. The composition of the cholesterol-free basal diet was (g/kg): casein 200, maize oil 10, lard 90, cellulose 50, vitamin mixture 20, mineral mixture 40, choline bitartrate 2, and α-maize starch 588. The composition of the cholesterol-enriched basal diet was (g/kg): casein 200, maize oil 10, lard 90, cellulose 50, vitamin mixture 20, mineral mixture 40, choline bitartrate 2, cholesterol 5, and α-maize starch 583. The vitamin and mineral mixtures were those of AIN-76 (American Institute of Nutrition, 1977). The control mixture contained 431 g casein and 227 g maize oil/kg instead of the protein and oil in soya milk, and 342 g sucrose/kg. Hamsters were fed on one of the following six diets for 7 d: (1) a cholesterol-free basal diet that contained 300 g control mixture/kg, (2) a cholesterol-free basal diet that contained 300 g SM/kg, (3) a cholesterol-free basal diet that contained 300 g FSM/kg, (4) a cholesterol-enriched basal diet that contained 300 g control mixture/kg, (5) a cholesterol-enriched basal diet that contained 300 g SM/kg or, (6) a cholesterol-enriched basal diet that contained 300 g FSM/kg. The final compositions of the diets are shown in Table 1. The animals were allowed free access to food and water. Food consumption was recorded every 2 or 3 d. The hamsters were housed individually in stainless-steel wire-bottomed cages in a room with controlled lighting (lights on 08.30–20.30 hours), temperature (24 ± 2°), and humidity (60 ± 5 %). The animals were maintained in accordance with the guidelines of the Ethical Committee for Animal Experiments of Yakult Central Institute. After 5 d adaptation to the experimental diet, food intake and body weight were recorded, and faeces were collected for 2 d. The faeces were lyophilized and then stored at −20° until analysis of neutral steroids and bile acids. On the seventh day of the experimental diet period, the hamsters were anaesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal®, Abbott Laboratories,

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

| | Cholesterol-free diet | | | Cholesterol-enriched diet | | |
|---|---|---|---|---|---|---|
| | Control | Soya milk | Fermented soya milk | Control | Soya milk | Fermented soya milk |
| Soya milk | – | 30 | – | – | 30 | – |
| Fermented soya milk | – | – | 30 | – | – | 30 |
| Casein | 26·94 | 14 | 14 | 26·94 | 14 | 14 |
| Maize oil | 7·51 | 0·7 | 0·7 | 7·51 | 0·7 | 0·7 |
| Lard | 6·3 | 6·3 | 6·3 | 6·3 | 6·3 | 6·3 |
| Cellulose | 3·5 | 3·5 | 3·5 | 3·5 | 3·5 | 3·5 |
| Vitamin mixture* | 1·4 | 1·4 | 1·4 | 1·4 | 1·4 | 1·4 |
| Mineral mixture* | 2·8 | 2·8 | 2·8 | 2·8 | 2·8 | 2·8 |
| Choline bitartrate | 0·14 | 0·14 | 0·14 | 0·14 | 0·14 | 0·14 |
| Cholesterol | – | – | – | 0·35 | 0·35 | 0·35 |
| Sucrose | 10·25 | – | – | 10·25 | – | – |
| α-Maize starch | 41·16 | 41·16 | 41·16 | 40·81 | 40·81 | 40·81 |

* The vitamin and mineral mixtures were those of AIN-76 (American Institute of Nutrition, 1977).

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Chicago, IL, USA), 25 mg/kg body weight. Food was withheld for 4 h before death. Blood was collected from the aorta ventralis into tubes containing EDTA and then separated by centrifugation at 2000 *g* for 15 min at 4°. The livers were perfused *in situ* with saline (9 g NaCl/l), removed, weighed, and then kept in plastic bags at −20° until analysis of liver lipids.

### *Analytical methods*

*Diet.* Crude protein, diethyl ether extract (crude lipid), and crude ash analyses of SM and FSM were carried out by the method of the Association of Official Analytical Chemists (1990). Isoflavones in the SM and FSM diets were analysed by HPLC as follows. For the measurement of free isoflavones, 0·1 g sample was extracted with 4 ml methanol (80 ml/l). HCl (4 M, 2 ml) and 1 ml flavone (4 μg/ml; Tokyo Kasei Co., Tokyo, Japan) as an internal standard were added to 2 ml of the extract. For measurement of total isoflavones, 0·1 g sample was extracted with 4 ml methanol (80 ml/l). The extract (2 ml) was hydrolysed with 2 ml 4 M-HCl at 100° for 30 min. The mixture was cooled to room temperature and then 1 ml flavone (4 μg/ml) was added as an internal standard. Free and total isoflavone were analysed by HPLC (LC Module I; Waters, Millipore Japan, Tokyo, Japan) equipped with a YMC-Pack C4 column (YMC Co., Kyoto, Japan) and a u.v. detector (260 nm). The flow rate of the mobile phase (acetate (100 ml/l)–methanol, 73 : 27 v/v) was maintained at 2·0 ml/min. The column temperature was 50°. Daidzein (Sigma Chemical Co., St Louis, MO, USA) and genistein (Seikagaku Corporation Co., Tokyo, Japan) were used as standard substances. Daidzin and genistin were estimated as total and free isoflavones.

*Plasma lipids.* Plasma total cholesterol and triacylglycerol concentrations were measured enzymically with commercial kits (Determiner TC555; Kyowa Medics, Tokyo, Japan and Triglyceride G Test Wako; Wako Junyaku, Osaka, Japan respectively). Plasma HDL-cholesterol was measured after precipitation with heparin–Mn reagent and the supernatant fraction was assayed for cholesterol with a commercial kit (HDL Cholesterol Test Wako, Wako Junyaku). VLDL-cholesterol plus LDL-cholesterol was calculated as the difference between total cholesterol and HDL-cholesterol. The atherogenic index (AI) was calculated as the VLDL + LDL-cholesterol : HDL cholesterol ratio.

*Liver lipids.* Liver lipids were extracted by the method of Folch *et al.* (1957). The liver total cholesterol and triacylglycerol concentrations were measured as described earlier.

*Faecal neutral steroid excretion.* Faeces were lyophilized and any hair and food attached to the faeces was removed. Homogenized faecal matter (30–70 mg), with 5 α-cholestane as an internal standard, was saponified and extracted according to the method of Grundy *et al.* (1965). The solvent was evaporated under $N_2$ gas and the residue was dissolved in 500 μl chloroform. N, O-bis(trimethylsilyl)trifluoroacetamide (250 μl, BSTFA; Pierce Chemical Co., Rockford, IL, USA) and 5 μl trimethylchlorosilane (Pierce Chemical Co.) were added for silylation at 80° for 2 h. A GC system (model 5890; Yokogawa-Hewlett-Packard, Tokyo, Japan) equipped with a 30 m × 0·32 mm i.d. capillary glass column coated with SPB-1 FS (Supelco, Belfonte, PA, USA) was used for the analysis. The initial conditions were as follows: oven, 220°; injector, 220°; flame ionization detector (FID), 280°; and carrier gas flow rate, 0·8 ml/min. Cholesterol, cholestanol, coprostanol, epicoprostanol, cholestanone and coprostanone, obtained from GL Science Inc. (Tokyo, Japan), and β-sitosterol, stigmasterol and campesterol, obtained from Tama Biochemical Co. (Tokyo, Japan), were used as standard substances.

*Faecal bile acid excretion.* Lyophilized faeces (40–60 mg) with 200 μl 1 mM-5β-pregnan-3α, 17α, 20α-triol were extracted with 5 ml ethanol at 80°. The ethanol was removed under $N_2$ gas, and then the residue was dissolved in methanol and passed through a 0·45 μm filter (C3 LH; Millipore Japan, Tokyo, Japan). The bile acids were analysed by HPLC by the method of Ishimoto (1986). The standard substances used were cholic acid, glycocholic acid, taurocholic acid, chenodeoxycholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, lithocholic acid, glycolithocholic acid, taurolithocholic acid, ursodeoxycholic acid, glycoursodeoxycholic acid and tauroursodeoxycholic acid from JASCO (Tokyo, Japan).

*Organic acids.* The organic acid contents of the SM and FSM were determined by the method of Kikuchi & Yajima (1992).

### *Statistical analysis*

The results are expressed as means and pooled SD. The means were compared using STATISTICA software (StatSoft, Inc., OK, USA), by ANOVA and subsequent Tukey's HSD comparisons after logarithmic transformation to stabilize the variance, if the variance was significant (Bartlett test) (Zar, 1984). The difference was considered to be statistically significant when $P$ was less than 0·05.

## Results

### *Chemical compositions of soya milk and fermented soya milk*

The lactic, acetic and formic acid contents of the SM were 0·2, 12·1 and 0·4 μmol/g respectively; and those of the FSM were 225·7, 187·1 and 20·7 μmol/g respectively. The isoflavone composition of each diet is shown in Table 2. The SM and FSM diets contained the same amount of total isoflavone. The FSM diet contained five and sixty times higher quantities of daidzein and genistein than the SM diet respectively.

### *Growth and digestibility*

In the hamsters that were fed on the cholesterol-free diet, no significant differences in the final body weight, body-weight

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**Table 2.** Isoflavone concentrations in the hamster diets (μg/g diet)

| Diet | Daidzein | Genistein | Daidzin* | Genistin* | Total |
|---|---|---|---|---|---|
| Cholesterol-free diet | | | | | |
| Soya milk | 13·2 | 3·2 | 214·0 | 324·8 | 555·2 |
| Fermented soya milk | 64·0 | 213·7 | 161·6 | 111·6 | 550·9 |
| Cholesterol-enriched diet | | | | | |
| Soya milk | 12·1 | 3·3 | 207·1 | 310·6 | 533·1 |
| Fermented soya milk | 63·9 | 213·5 | 159·1 | 106·0 | 542·4 |

* Values are presented as the amounts of aglycones.

gain or food efficiency ratio were detected (Table 3). The food intake was greater in the hamsters fed on SM or FSM than in the control diet group. The apparent digestibility of DM was lower in the SM and FSM groups than in the control group.

In the animals that received the cholesterol-enriched diet, no significant differences in the final body weight were observed (Table 3). The body-weight gain, food intake and food efficiency ratio were greater in the hamsters fed on SM or FSM than in the control group. However, the apparent digestibility of DM was lower in the SM and FSM groups than in the control group.

### *Plasma lipid concentrations*

Among hamsters fed on the cholesterol-free diet, the plasma total cholesterol level was not affected by the diet (Table 4). The plasma triacylglycerol level was approximately 50% lower than the control level in the hamsters fed on SM or FSM. The FSM diet increased the HDL-cholesterol level and decreased the VLDL + LDL-cholesterol level by 25%, and consequently decreased the AI value. The HDL-cholesterol level in the SM group was not significantly different from that in the controls. The VLDL + LDL-cholesterol level in the SM group was lower than that in the controls, but not significantly. The control group had the highest AI value and the FSM group the lowest value; the level in the SM group was intermediate but was not significantly different from the control and FSM groups.

Among hamsters fed on the cholesterol-enriched diet (Table 4), the plasma total cholesterol level was about 15% lower in both the SM and FSM groups than in the control group. The plasma triacylglycerol level was about 75% lower than that of the controls in hamsters fed on SM or FSM. Both SM and FSM increased the HDL-cholesterol level and decreased the VLDL + LDL-cholesterol level, and consequently decreased the AI value.

### *Liver lipid contents*

Among hamsters fed on the cholesterol-free diet, the wet and dry liver weights were not different in the three groups (control, SM and FSM) (Table 5). The liver total cholesterol contents in the SM and FSM groups were lower than that in the control group. The liver triacylglycerol content was not affected by SM or FSM.

**Table 3.** Body weights, food intake, and apparent digestibility of DM in hamsters fed on cholesterol-free or cholesterol-enriched diets containing soya milk or fermented soya milk*

(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA($P<$) |
|---|---|---|---|---|---|
| Cholesterol-free diet | | | | | |
| Initial body wt (g) | 95·6[a] | 95·3[a] | 94·9[a] | 6·1 | NS |
| Final body wt (g) | 107·1[a] | 110·9[a] | 107·9[a] | 6·6 | NS |
| Body wt gain (g/d) | 1·6[a] | 2·2[a] | 1·9[a] | 0·5 | NS |
| Food intake (g/d) | 7·2[a] | 8·0[a] | 7·9[a] | 0·7 | 0·05 |
| Food efficiency† | 0·23[a] | 0·28[a] | 0·23[a] | 0·05 | NS |
| DM digestibility (%) | 94·9[a] | 92·7[b] | 92·4[b] | 1·3 | 0·01 |
| Cholesterol-enriched diet | | | | | |
| Initial body wt (g) | 95·4[a] | 95·2[a] | 95·3[a] | 6·2 | NS |
| Final body wt (g) | 103·3[a] | 110·0[a] | 110·3[a] | 7·3 | NS |
| Body wt gain (g/d) | 1·1[b] | 2·1[a] | 2·1[a] | 0·6 | 0·001 |
| Food intake (g/d) | 6·5[b] | 7·7[a] | 8·1[a] | 0·8 | 0·001 |
| Food efficiency† | 0·17[b] | 0·27[a] | 0·27[a] | 0·07 | 0·01 |
| DM digestibility (%) | 95·1[a] | 92·9[b] | 93·3[b] | 1·3 | 0·01 |

[a,b] Mean values within a row with unlike superscript letters were significantly different, $P<0·05$ (Tukey's test).
* For details of diets, see Table 1.
† Food efficiency = body-weight gain/food intake.

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**Table 4.** Plasma lipid concentrations in hamsters fed on cholesterol-free or cholesterol-enriched diets containing soya milk or fermented soya milk‡
(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA ($P<$) |
|---|---|---|---|---|---|
| Cholesterol-free diet | | | | | |
| Total cholesterol (mmol/l) | 4·2[a] | 3·8[a] | 4·0[a] | 0·32 | NS |
| Triacylglycerol* (mmol/l) | 5·5[a] | 2·3[b] | 2·7[b] | 0·002 | 0·0001 |
| HDL-cholesterol (mmol/l) | 2·1[b] | 2·1[ab] | 2·4[a] | 0·26 | 0·05 |
| VLDL + LDL-cholesterol (mmol/l) | 2·1[a] | 1·7[ab] | 1·6[b] | 0·38 | 0·05 |
| Atherogenic index*† | 1·05[a] | 0·84[ab] | 0·66[b] | 0·12 | 0·05 |
| Cholesterol-enriched diet | | | | | |
| Total cholesterol (mmol/l) | 6·4[a] | 5·4[b] | 5·4[b] | 0·68 | 0·01 |
| Triacylglycerol* (mmol/l) | 9·2[a] | 2·1[b] | 2·2[b] | 0·004 | 0·0001 |
| HDL-cholesterol (mmol/l) | 2·1[b] | 2·7[a] | 2·5[a] | 0·30 | 0·0001 |
| VLDL + LDL-cholesterol† (mmol/l) | 4·3[a] | 2·7[b] | 2·8[b] | 0·003 | 0·001 |
| Atherogenic index*† | 2·07[a] | 1·03[b] | 1·13[b] | 0·15 | 0·0001 |

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P<0·05$ (Tukey's test).
* Means were compared after logarithmic transformation of the data. Pooled SD was based on the logarithmically transformed data.
† Atherogenic index = VLDL + LDL-cholesterol/HDL-cholesterol.
‡ For details of diets, see Table 1.

Among hamsters fed on the cholesterol-enriched diet, the mean dry liver weight in the FSM group was 15 % heavier than that in the controls. The liver cholesterol and triacylglycerol contents were not affected by diet (control, SM and FSM).

### *Faecal steroid excretion*

The total neutral steroid excretion values were similar in the control, SM and FSM groups, in both the cholesterol-free diet and cholesterol-enriched diet hamsters (Table 6). In the animals that received the cholesterol-free diet, none of the neutral steroids was affected by the diets. Hamsters in the SM and FSM diet groups excreted more cholesterol and epicoprostanol than the controls. The hamsters fed with SM or FSM excreted less coprostanol than those on the control diets, but not significantly.

Among the animals that were fed on the cholesterol-free diet, the total bile acid excretion was four- and fivefold higher in the hamsters fed on SM and FSM diets respectively, than in those on the control diets (Table 7). The proportion of deoxycholic acid in the SM and FSM groups was twofold higher than the control group value, and that of lithocholic acid in the SM and FSM groups was 30 % lower than that in the controls. Consequently, the proportion of bile acids originating from the cholic acid biosynthesis pathway in the SM and FSM groups was higher than that in the control group.

Total bile acid excretion was fivefold higher than the control group value in hamsters fed on the cholesterol-enriched diet with SM or FSM (Table 8). The proportion of deoxycholic acid in the SM and FSM groups was twofold higher than that in the control group, and lithocholic acid in the SM and FSM hamsters was 20 % lower than the control group level. Consequently, the proportion of bile acids

**Table 5.** Liver weights and liver lipid concentrations in hamsters fed on cholesterol-free or cholesterol-enriched diets containing soya milk or fermented soya milk*
(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA ($P<$) |
|---|---|---|---|---|---|
| Cholesterol-free diet | | | | | |
| Dry liver wt (g) | 1·65[a] | 1·69[a] | 1·69[a] | 0·13 | NS |
| Total cholesterol (mg/liver) | 33·2[a] | 20·4[b] | 22·3[b] | 8·2 | 0·01 |
| Triacylglycerol (mg/liver) | 56·7[a] | 47·9[a] | 48·9[a] | 7·5 | NS |
| Cholesterol-enriched diet | | | | | |
| Dry liver wt (g) | 1·76[b] | 1·98[ab] | 2·02[a] | 0·2 | 0·05 |
| Total cholesterol (mg/liver) | 120·9[a] | 134·9[a] | 135·5[a] | 12·9 | NS |
| Triacylglycerol (mg/liver) | 52·8[a] | 52·8[a] | 51·3[a] | 8·9 | NS |

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P<0·05$ (Tukey's test).
* For details of diets, see Table 1.

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**Table 6.** Faecal neutral steroid excretion (mg/2 d) in hamsters fed on cholesterol-free or cholesterol-enriched diets containing soya milk or fermented soya milk*
(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA ($P<$) |
|---|---|---|---|---|---|
| Cholesterol-free diet | | | | | |
| Cholesterol | 0·28 | 0·21 | 0·31 | 0·10 | NS |
| Cholestanol | 0·31 | 0·38 | 0·31 | 0·22 | NS |
| Coprostanol | 2·61 | 2·37 | 2·14 | 0·81 | NS |
| Epicoprostanol | 0·13 | 0·13 | 0·39 | 0·22 | NS |
| Total | 3·33 | 3·09 | 3·18 | 0·86 | NS |
| Cholesterol-enriched diet | | | | | |
| Cholesterol | 0·60$^{a}$ | 1·24$^{b}$ | 1·13$^{b}$ | 0·44 | 0·05 |
| Cholestanol | 0·25$^{a}$ | 0·64$^{b}$ | 0·39$^{ab}$ | 0·23 | 0·01 |
| Coprostanol | 9·34 | 7·78 | 7·41 | 1·67 | NS |
| Epicoprostanol | 0·00$^{a}$ | 1·38$^{b}$ | 1·15$^{b}$ | 0·90 | 0·01 |
| Total | 10·19 | 11·03 | 10·09 | 1·54 | NS |

$^{a,b}$ Mean values within a row not sharing a common superscript letter were significantly different, $P<0·05$ (Tukey's test).
* For details of diets, see Table 1.

originating from the cholic acid biosynthesis pathway in these SM and FSM groups was higher than that in the control group.

## Discussion

The aim of the present study was to determine the effect of soya milk, unfermented or fermented with Bifidobacterium, on plasma and faecal steroid excretion. SM and FSM diets decreased plasma VLDL + LDL-cholesterol and triacylglycerol levels, and remarkably enhanced faecal bile acid excretion. There was an inverse relationship between faecal bile acid excretion and plasma VLDL + LDL-cholesterol in both the animals fed on the cholesterol-free diet ($r -0·670$, $P<0·01$) and those fed on the cholesterol-enriched diet ($r -0·761$, $P<0·001$). Studies on rats (Nagata *et al.* 1982) and rabbits (Huff & Carroll, 1980) have also shown that soyabean protein increases faecal bile acid excretion. These findings, and the results of the present study, suggest that hepatic cholesterol metabolism could change in order to provide more cholesterol for bile acid synthesis.

The cholic : chenodeoxycholic acid ratio in the SM and FSM groups was greater than that in the controls. Two pathways of bile acid biosynthesis, cholesterol 7α-hydroxylase and mitochondrial 27 α-hydroxylase, have been described for mammalian liver (Russell & Setchell, 1992). Bile acid metabolism, especially the change in the cholic : chenodeoxycholic acid ratio in bile or faeces, has been associated with thyroid hormones (Jackson *et al.* 1993), diabetes (Uchida *et al.* 1985), oestrogen (Van Erpecum *et al.* 1991), cholesterol 7α-hydroxylase deficiency (Schwarz *et al.* 1996), and ageing (Uchida *et al.* 1978). Feeding soyabean protein alters hormone concentrations (Sanchez & Hubbard, 1991; Forsythe, 1995) and these may change bile acid metabolism. In rats, cholesterol feeding led to an increase in the chenodeoxycholic : cholic acid ratio (Uchida, 1992). However, cholesterol feeding did not affect this ratio in the hamsters in the present study (Tables 7 and 8). This difference between rats and hamsters may be due to different regulation of hepatic 7α-hydroxylase (Horton *et al.* 1995; Pandak *et al.* 1995) to dietary cholesterol. Cholic acid, but not chenodeoxycholic

**Table 7.** Faecal bile acid excretion in hamsters fed on cholesterol-free diets containing soya milk or fermented soya milk*
(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA ($P<$) |
|---|---|---|---|---|---|
| Total bile acid excretion (mmol/2 d) | 1·68$^{b}$ | 6·69$^{a}$ | 8·06$^{a}$ | 3·28 | 0·0001 |
| Bile acid composition (%) | | | | | |
| Ursodeoxycholic acid | 8·15$^{a}$ | 1·68$^{b}$ | 3·48$^{b}$ | 3·96 | 0·01 |
| Cholic acid | 0·11$^{b}$ | 3·88$^{a}$ | 4·07$^{a}$ | 1·94 | 0·0001 |
| Glycochenodeoxycholic acid | 0 | 0·08 | 0 | 0·12 | NS |
| Glycodeoxycholic acid | 0$^{b}$ | 0·68$^{a}$ | 0·91$^{a}$ | 0·51 | 0·001 |
| Taurodeoxycholic acid | 4·75$^{a}$ | 3·37$^{b}$ | 6·11$^{a}$ | 1·9 | 0·05 |
| Deoxycholic acid | 19·11$^{b}$ | 43·85$^{a}$ | 43·99$^{a}$ | 12·81 | 0·0001 |
| Lithocholic acid | 67·88$^{a}$ | 46·45$^{b}$ | 41·44$^{c}$ | 12·14 | 0·0001 |
| Cholic acid pathway bile acids† | 23·96$^{b}$ | 51·80$^{a}$ | 55·08$^{a}$ | 14·51 | 0·0001 |
| Chenodeoxycholic acid pathway bile acids‡ | 76·04$^{a}$ | 48·20$^{b}$ | 44·92$^{b}$ | 14·86 | 0·0001 |

$^{a,b,c}$ Mean values within a row not sharing a common superscript letter were significantly different, $P<0·05$ (Tukey's test).
* For details of diets, see Table 1.
† Cholic acid, glycodeoxycholic acid, taurodeoxycholic acid and deoxycholic acid.
‡ Ursodeoxycholic acid, glycochenodeoxycholic acid and lithocholic acid.

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**Table 8.** Faecal bile acid excretion in hamsters fed on cholesterol-enriched diets containing soya milk or fermented soya milk*
(Mean values for six hamsters and pooled standard deviation)

| | Control | Soya milk | Fermented soya milk | Pooled SD | ANOVA ($P<$) |
|---|---|---|---|---|---|
| Total bile acid excretion (mmol/2 d) | 1·73[b] | 7·73[a] | 8·95[a] | 3·5 | 0·0001 |
| Bile acid composition (%) | | | | | |
| Ursodeoxycholic acid | 6·14[a] | 1·38[b] | 1·34[b] | 2·43 | 0·0001 |
| Cholic acid | 0·73[b] | 3·77[a] | 4·38[a] | 0·10 | 0·0001 |
| Glycochenodeoxycholic acid | 0 | 0·18 | 0·04 | 0·15 | NS |
| Glycodeoxycholic acid | 0·46[b] | 1·27[a] | 0·97[ab] | 0·54 | 0·05 |
| Taurodeoxycholic acid | 4·49 | 3·62 | 4·39 | 1·71 | NS |
| Deoxycholic acid | 19·74[c] | 37·43[b] | 41·99[a] | 10·25 | 0·0001 |
| Lithocholic acid | 68·44[a] | 52·27[b] | 46·9[c] | 9·83 | 0·0001 |
| Cholic acid pathway bile acids† | 25·42[b] | 46·09[a] | 51·73[a] | 11·67 | 0·0001 |
| Chenodeoxycholic acid pathway bile acids‡ | 74·58[a] | 53·83[b] | 48·28[b] | 12·01 | 0·0001 |

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P<0·05$ (Tukey's test).
* For details of diets, see Table 1.
† Cholic acid, glycodeoxycholic acid, taurodeoxycholic acid and deoxycholic acid.
‡ Ursodeoxycholic acid, glycochenodeoxycholic acid and lithocholic acid.

acid promotes cholesterol absorption (Uchida *et al.* 1980). Therefore, an increase in the cholic acid pathway : chenodeoxycholic acid pathway ratio may promote cholesterol absorption. However, faecal cholesterol excretion was not affected in this study.

On the other hand, a cholesterol-lowering effect of soyabean protein without enhancement of bile acid excretion has been observed in monkeys (Jaskiewicz *et al.* 1987) and human subjects (Fumagalli *et al.* 1982). Soyabean protein and other non-protein components present in soyabeans may be partially responsible for the hypocholesterolaemic effect observed in some experiments (Huff *et al.* 1977; Nagata *et al.* 1982; Anthony *et al.* 1996; Balmir *et al.* 1996; Potter *et al.* 1996).

Soyabean protein containing isoflavones was reported to have favourable effects on plasma lipid and lipoprotein concentrations compared with alcohol-extracted soyabean protein (Anthony *et al.* 1996). Compositional changes in isoflavones, phytic acid, saponins and trypsin inhibitor occur during processing of soyabeans, and these components vary not only in quantity but also in availability (Anderson & Wolf, 1995). *Bifidobacterium breve* YIT 4065 has $\beta$-glucosidase activity and is therefore able to release aglycones from isoflavonoid glucosides (Ishikawa *et al.* 1997). In this study, 25 % of daidzin and 65 % of genistin released their aglycones on fermentation. The plasma genistein concentration at 2 h after dosing in genistein-treated rats was higher than that in soya extract-treated rats, although there were no significant differences at 8 h or later (King *et al.* 1996). Isoflavones have weak oestrogenic effects and their functioning as both oestrogen agonists and antagonists *in vitro* has been reported (Martin *et al.* 1978; Mathieson & Kitts, 1980). Oestrogen, when taken orally, increases the level of HDL (Schaefer *et al.* 1983; Applebaum-Bowden *et al.* 1989; Sacks & Walsh, 1990), and lowers that of LDL (Schaefer *et al.* 1983; Sacks & Walsh, 1990). It has been reported that post-menopausal women who take oestrogen generally have lower rates of cardiovascular disease than women of similar age who do not take it (Stampfer *et al.* 1991). Some reports suggest that isoflavones may be influential in the cholesterol-lowering effect of soya due to their oestrogen-like action (Potter, 1995; Anthony *et al.* 1996).

We found that fermented soya milk affected the plasma lipoprotein composition in animals fed on both cholesterol-enriched and cholesterol-free diets. Soya milk had a similar effect, but this was not significant in hamsters fed on the cholesterol-free diet. Most clinical trials have revealed a decrease in the serum or plasma cholesterol concentration when animal protein in the diet is replaced by soyabean protein, and the decrease is generally greater in hypercholesterolaemic than in normocholesterolaemic subjects (Carroll, 1991). The FSM diet also had a tendency to produce greater faecal bile acid excretion than the SM diet, but this was not significant.

*Bifidobacterium breve* YIT 4065 produces lactate and acetate, which can modify the physicochemical properties of soyabean protein. The viscosity of the fermented soya milk was very high (1573 mPa s), whereas that of the soya milk was 13·4 mPa s. The viscosity of a meal can regulate its physiological effect, such as the gastric emptying rate in man (Meyer *et al.* 1986). Carr *et al.* (1996) showed that both *ex vivo* viscosity and cholesterol absorption were significantly correlated with the plasma cholesterol concentration, emphasizing the importance of the small intestine in mediation of cholesterol metabolism. In the present study, *ex vivo* viscosity was not estimated. The DM digestibility of soya milk and fermented soya milk was lower (Table 3) and the undigested fraction of soyabean protein decreased the serum cholesterol level more than the original soyabeans, with faecal bile acid excretion (Sugano *et al.* 1990), suggesting that the undigested fraction may have had a physiological effect in the small intestine of the hamsters in this study.

Plant sterols can affect cholesterol absorption (Vahouny *et al.* 1983). In the present study, the total contents of plant sterols ($\beta$-sitosterol, stigmasterol and campesterol) in the SM, FSM and control diets were 0·5, 0·4 and 0·4 mg/g cholesterol-free diet respectively, and 0·4, 0·6 and 0·4 mg/g cholesterol-enriched diet. This difference might not have contributed to the plasma lipid findings. Phospholipids (Knuiman *et al.* 1989) and phytic acid (Jariwalla *et al.*

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1990) can reduce the plasma cholesterol level, but large amounts of phospholipids and phytic acid are required for the cholesterol-lowering effect.

In conclusion, soya milk had an anti-atherogenic effect, and this effect was not diminished by prior fermentation.

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