

Effects of Soybean Milk-Intake on Serum Cholesterol of Rats

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During cholesterol loading, the effect of addition to rat diet of soybean milk (20%) and 20% equivalents of soybean milk on serum and liver lipid metabolism was examined.

No effects on rat body weight and cecal weight were noted. Liver weight increased with cholesterol loading but the increase was inhibited by addition of soybean milk. Retroperitoneal fat increased in the soybean milk lipid extract group but decreased in the soybean milk group. In particular, in another group, (the switchover group), which showed an increase in serum cholesterol and which was subsequently given soybean milk-supplement diet, fat markedly decreased.

The increase in total serum cholesterol was inhibited by increasing the amount of soybean milk, soybean milk lipid extract and defatted soybean milk content but this inhibition was most notably seen in the soybean milk group compared with the other group.

Serum triglyceride decreased in the soybean milk, defatted soybean milk and switchover groups.

Liver cholesterol increased with cholesterol loading and this increase was not inhibited in the present test.

Fecal neutral steroid showed an increase in groups treated with soybean milk, soybean milk lipid extract and defatted soybean milk.

INTRODUCTION

In recent years, various proteins ^{1) 2)}, peptides ³⁾, dietary fibers ^{4) 5)} and multivalent unsaturated fatty acids ^{6) 7) 8) 9)} have been reported to decrease serum cholesterol. There are a number of reports especially on soybean protein ^{10) 11) 12)}, peptides ^{13) 14)} and unsaturated fatty acid ^{15) 16)}, but the effect of soybean milk as a processed soybean food

has not been elucidated. C. J. H. Woodward and K. K. Carrol ¹⁷⁾ reported that the hydrophilic property and digestibility of soybean protein decreases on heat treatment. Therefore, there may be a degeneration of the protein during preparation of soybean milk, resulting in changes in the bioavailability of proteins from soybean, and its effect on serum cholesterol may be affected. Therefore, by feeding rats with a high cholesterol

diet, inhibition of increases in serum cholesterol by soybean milk were investigated to evaluate soybean milk as a processed food of soybean from the perspective of improving levels of serum cholesterol.

METHOD

1. Experimental animal

The experimental animals used were DONRYU strain male rats, 4 weeks of age. After 2-weeks of preliminary feeding with pellet diet CE2 (Clea Japan Inc.), animals were assigned to 9 groups of 12 animals each and were individually housed in cages for 8 weeks. Room temperature was maintained at $23 \pm 3^\circ$ and lighting was given in a 12 hour light/dark cycle.

2. Enriched diet sample

Soybean lipids were extracted from freeze-dried soybean milk (soy-bean milk FD) with hexane. Defatted soybean milk is the residue after the extraction of lipids from soybean milk FD with Hexane. The composition of each sample was shown in Table 1.

3. Experimental diets

The compositions of the diets used for the experiment were shown in Table 2. Experimental design was shown in Fig. 1. A base diet containing no cholesterol as an additive (base diet group), a high cholesterol diet containing 1% cholesterol as an additive (control group), a high cholesterol diet to which 20% soybean milk FD was further added

Table 1 Composition of soybean milk powder(SM) and defatted soybean milk powder(DSM). (Treated by freeze dry)

	SM (%)	DSM (%)
Protein	46.7	53.7
Total lipids	26.3	0.6
Moisture	3.1	7.6
Suger		
Sucrose	8.6	
Raffinose	0.8	
Stachyose	4.9	

(soybean milk group), a diet containing 17.4% defatted soybean milk as an additive (defatted soybean milk group) and a diet containing 4.2% lipid extracted from soybean milk as an additive (soybean milk lipid group) were used, and animals were reared with these diets for 8 weeks. The amounts of defatted soybean milk and lipid extracted from soybean milk added to the diets corresponded to the contents in 20% soybean milk from Week 5 (switched group). When each sample was added to a high-cholesterol diet, the amounts of casein and lard were adjusted so that the protein content in the diet was 20% and the lipid content was 10%. Animals had free access to these diets during the period.

4. Measurement of each organ weight and method of analysis

During the rearing period, body weight and food consumption were, in principle measured every day, and mean values for each

Table 2 Composition of expermental diets

	A	B	C	D	E
Casein	20.0	20.0	10.66	10.66	20.0
α -Potato starch	58.55	57.55	53.19	49.59	57.55
Refined lard	10.0	10.0	4.74	9.9	5.8
Salt mix.	4.0	4.0	4.0	4.0	4.0
Vitamin mix.	2.0	2.0	2.0	2.0	2.0
Cellurose	5.0	5.0	5.0	5.0	5.0
Sodium Cholte	0.25	0.25	0.25	0.25	0.25
Coline chlorid	0.2	0.2	0.2	0.2	0.2
Cholesterol	—	1.0	1.0	1.0	1.0
SM diet	—	—	20.0	—	—
DSM diet	—	—	—	17.4	—
LESM diet	—	—	—	—	4.2
energy (Kcal/100g)	414.4	410.4	403.3	405.4	408.6

A : Base diet

B : High cholesterol diet (Control)

C : Contorol+Soybeen milk powder (SM) 20%

D : Contorol+Defatted soybeen milk powder (DSM) 17.4%

E : Contorol+Lipid extract soybeen milk powder (LESM) 4.2%

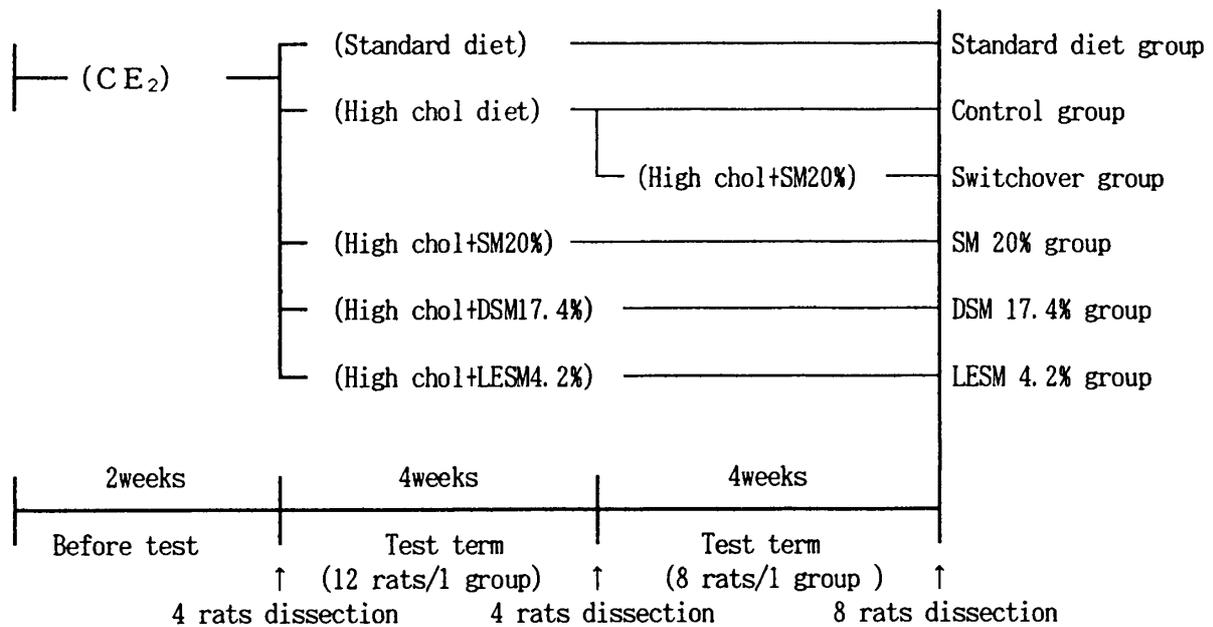


Fig. 1. Experimental plan

group were calculated. Feces were collected once every 2 weeks, weighed after drying, then stored at -20°C .

Four animals at the end of the preliminary rearing period, 4 animals from each group 4 weeks after the start of the study, and 8 animals from each group at the end of the study were autopsied under ether anesthesia, and blood samples were collected from abdominal aorta. The liver and fat from the posterior abdominal wall and cecum were isolated and weighed. The liver was stored at -80°C . Methods of analysis are described below.

(1) Serum lipid

Collected blood was centrifuged at 3000 rpm for 10 min to separate serum. Total cholesterol concentrations were measured using a kit based on the cholesterol oxidase-DAOS method (Cholesterol E Test, WAKO), HDL-cholesterol concentrations using a kit based on the phosphotungstic acid-magnesium salt precipitation method (HDL-Cholesterol E Test WAKO), triglycerides using a kit based on the OPO-DAOS method (Triglyceride E Test, WAKO), and phospholipids using a kit based on the choline oxidase-phenol method (Phospholipid B Test, WAKO).

(2) Lipids in liver

Lipid was extracted from the liver by Folch's method ¹⁸⁾, and total cholesterol and total lipids were determined. A 2g sample of liver, accurately weighed, was minced and 40ml chloroform/methanol (2:1) was added. It

was refrigerated overnight for extraction, and the filtrate was separated into a chloroform layer and methanol layer by adding 0.9% sodium chloride solution. This partition was repeated 3 times. Then anhydrous sodium sulfate was added to the chloroform layer to remove water and the chloroform solution was diluted to 50 ml and used as the sample solution. For measurement of total lipids, the constant weight of a container was previously measured and a 2 ml portion of the sample solution was transferred into the container. It was then weighed after completely evaporating the solvent.

Total cholesterol was measured on a 0.04 ml sample. After evaporating the solvent, 0.02 ml 10% polyoxyethyleneoctylphenyl ether/isopropanol was added and total cholesterol was measured using a kit based on the cholesterol oxidase-DAOS method (Cholesterol E Test WAKO).

(3) Steroids in feces

Steroids in the feces were extracted in accordance with the method described by Grundy et al ^{19) 20)}. Neutral steroids were determined by the ferric chloride method ²¹⁾, and bile acid using a kit based on oxygen colorimetry (Total Bile Acid Test WAKO). To exactly 100 mg of freeze-dried feces, 10 ml ethanol was added and refluxed for 60 min, then centrifuged at 3000 rpm for 10 min to separate the ethanol extract. This extraction procedure was repeated 3 times. Then ethanol was removed, 4 ml 1.25 N sodium hydroxide solution added, and it was autoclaved at 120°C for 3 hours for saponification.

Then ethyl ether was added and the ether layer was separated. It was diluted with ether to 20 ml and used as the sample for analysis of neutral steroids.

The lower layer was acidified with hydrochloric acid using 1% Congo red solution as an indicator, then ethyl ether was added for partition, the upper layer was recovered, diluted with ethyl ether to 20 ml and used as a sample for analysis of bile acid. For measurement of neutral steroids, 1 ml portion of the extract was taken and evaporated, and the residue was dissolved in 6 ml acetic acid. 4 ml ferric chloride solution were added to the solution, it was mixed well, cooled for 5 min and allowed to stand for 10 min. Absorption of the solution at 550 nm was measured to determine the amount of neutral steroids.

For the measurement of bile acid, a 1 ml portion of the extract was taken and evaporated, and the residue was dissolved in 2 ml 0.1 M tris-HCl buffer. A 0.02 ml portion of this

solution was subjected to evaluation of total bile acid.

RESULTS and DISCUSSIONS

1. Body weight and tissue weight

Body weights and tissue weights were shown in Table 3. There was no significant difference in the final body weight between the groups, and differences in growth were not observed. Therefore, it was considered that each of the samples added to the diet did not inhibit the growth of the animal.

Liver weight generally increased when cholesterol was added to the diet. These increases, however, were significantly reduced in the soybean milk group compared to the control group.

No differences in cecal weight were observed between groups.

There was no difference in the amount of

Table 3 Terminal body weight, liver weight, retroperitoneal fat weight and cecal weight

Group	Terminal body weight (g)	liver weight (g)	retroperitoneal fat weight (g)	cecal weight (g)
Standard	430.9 ± 24.9	13.5 ± 0.7 ^a	6.9 ± 1.5 ^a	3.6 ± 0.3
Control	430.0 ± 15.3	22.3 ± 2.0 ^b	6.0 ± 1.4 ^{ac}	3.7 ± 0.7
+SM 20%	420.0 ± 31.5	19.9 ± 0.9 ^c	5.6 ± 1.4 ^{ac}	3.5 ± 0.8
+DSM 17.4%	423.8 ± 10.2	21.2 ± 2.6 ^b	6.1 ± 1.0 ^b	3.5 ± 0.2
+LESM 4.2%	447.0 ± 29.1	23.6 ± 2.2 ^{bc}	7.7 ± 0.7 ^{ac}	3.1 ± 0.7
Switchover	425.7 ± 19.4	25.2 ± 1.2 ^b	5.1 ± 0.7 ^c	3.5 ± 0.8

* Results were expressed as means ± SE of 8 rats.

* Means not followed by a common letter were significantly different (p, 0.05)

fat on the posterior abdominal wall with or without the addition of cholesterol, though there was an increased amount in the soybean milk-extracted lipid group and less in the switched group.

2. Serum lipids

Results were shown in Table 4 and Fig. 2 and 3. Serum T-Chol levels in the control group increased significantly compared to the base diet group, while the soybean milk group showed a significant decrease. The levels in the defatted soybean milk group and soybean milk-extracted lipid group were comparable to the level in the base diet group, and there was a significant difference compared to the control group. In the switched group, remarkable decreases were observed from week 4 to week 8 with a significant difference from the control group. In this study, to investigate the effect of soybean milk itself against elevated blood cholesterol levels, not only cholesterol

was added to the diet but casein as a protein source and lard as a fat were also used. In addition, to investigate the effect of substituting soybean protein with casein and soybean oil with lard, the defatted soybean milk group and the soybean milk-extracted lipid group were employed. When soybean milk, defatted soybean-milk and soybean milk-extracted lipid were added to the diet, cholesterol levels decreased compared to the control group, and these are considered to inhibit increases in cholesterol level.

Since there were a number of reports indicating that soybean protein ^{10) 11) 12)} and unsaturated fatty acid ^{15) 16)} inhibit increases in cholesterol levels, any increases in cholesterol levels in the defatted soybean milk-extracted lipid group were expected to be inhibited. However, decreases in T-Chol in the soybean milk group were greater than decreases observed in the defatted soybean milk group and the soybean milk-extracted

Table 4 Total cholesterol(T-Chol), HDL-cholesterol rate(HDL-Chol), tryglyceride(TG) and phospholipids(PL) in serum.

group	T-Chol (mg/dl)	HDL-Chol (%)	TG (mg/dl)	PL (mg/dl)
Standard	61.7 ± 8.8 ^a	75.1 ± 6.5 ^a	50.4 ± 8.3 ^a	99.5 ± 8.8 ^a
Control	81.3 ± 12.5 ^b	48.0 ± 4.7 ^b	45.1 ± 7.6 ^{ac}	99.9 ± 15.2 ^{ac}
+SM 20%	43.2 ± 11.3 ^d	59.3 ± 13.8 ^b	36.3 ± 4.7 ^b	71.2 ± 7.4 ^b
+DSM 17.4%	58.8 ± 12.2 ^{ac}	58.4 ± 16.8 ^b	31.7 ± 2.5 ^d	77.1 ± 8.1 ^c
+LESM 4.2%	56.5 ± 10.1 ^{ac}	70.1 ± 11.6 ^a	39.2 ± 5.0 ^c	81.4 ± 8.5 ^d
Switchover	46.4 ± 6.3 ^d	62.9 ± 3.0 ^c	25.3 ± 3.0 ^e	69.2 ± 6.7 ^e

* Results were expressed as means ± SE of 8 rats.

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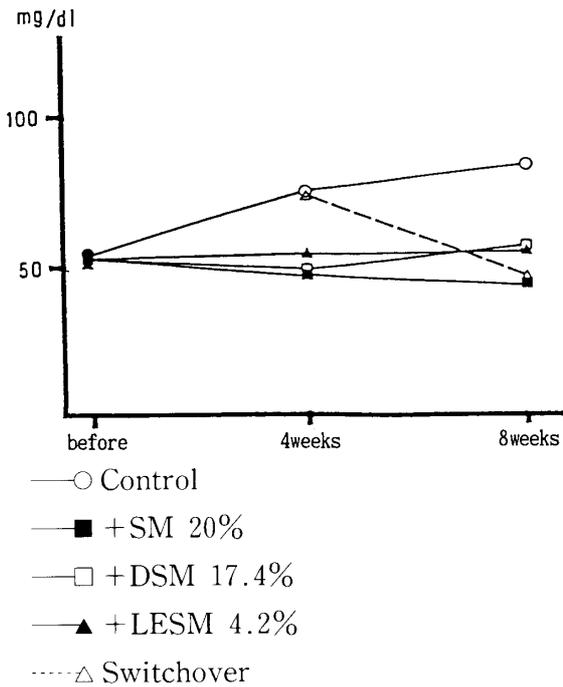


Fig. 2. Total cholesterol in serum.

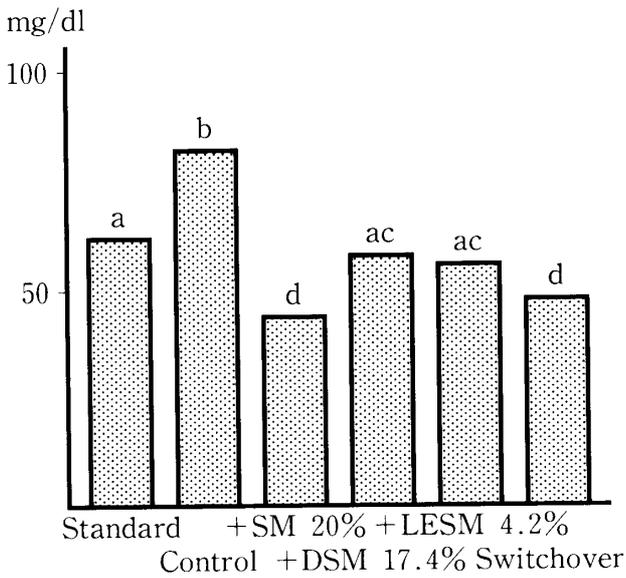


Fig. 3. Terminal total cholesterol in serum.

lipid group, and it was suggested that the inhibitory effect on increases in serum cholesterol levels was potentially produced by proteins, lipids and sugars in soybean milk.

The switched group was employed to investigate the effect of soybean milk on rats with

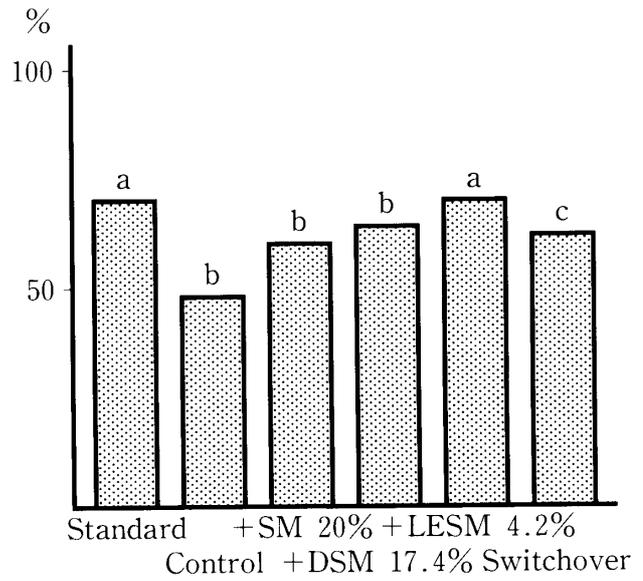


Fig. 4. Terminal HDL-cholesterol rate in serum.

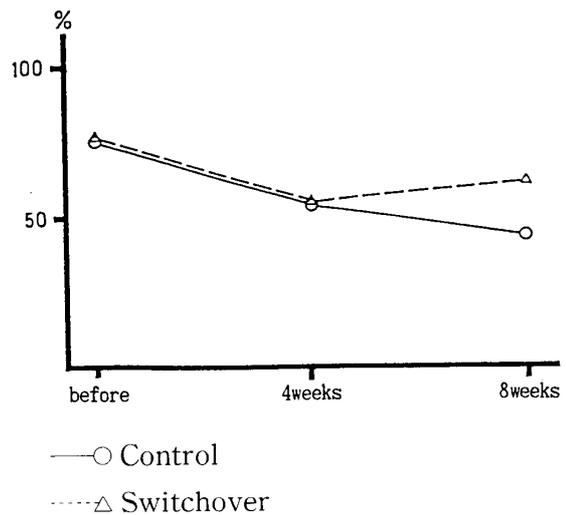


Fig. 5. HDL-cholesterol rate in serum.

high serum T-Chol, and decreases in serum cholesterol caused by consumption of soybean milk was confirmed.

HDL-Chol levels significantly decreased in all groups receiving a cholesterol-enriched base diet. Then the ratio of (HDL-Chol)/T-Chol, which indicates the quality of cholesterol in blood, was calculated. The results were shown in Fig. 4 and 5. In contrast, the

control group showed gradual decreases to 48.0% in week 8. No significant difference was observed between the soybean milk-extracted lipid group and the base diet group. The soybean milk group, defatted soybean milk group and the switched group showed significant decreases compared to the base diet group, but significant increases compared to the control group. The base diet group, which received no cholesterol as an additive, showed no changes in its HDL ratio over time, and the quality of blood cholesterol was not aggravated in spite of the increases in total cholesterol levels. In contrast, the soybean milk group, defatted soybean milk group and soybean milk lipid group showed decreases in their HDL ratio. The amount it decreased was, however, smaller compared to the control group, and they were considered to have a lower level of serum cholesterol while maintaining the quality of the cholesterol. In addition, in the switched group, there was tendency for the rate of

HDL-Chol to increase when soybean milk was dosed, and decreases in serum cholesterol levels by soybean milk were accompanied by improvement in the quality of the cholesterol.

Triglyceride and phospholipid levels, as shown in Table 4, decreased significantly in the soybean milk group, defatted soybean milk group and soybean milk-extracted lipid group compared to the base diet group and the control group. Triglyceride levels decreased remarkably in the switched group. In consideration of the fact that fat on the posterior abdominal wall tended to decrease in the switched group, it is suggested that soybean milk is associated with the improvement of lipid metabolism.

3. Lipids in the liver

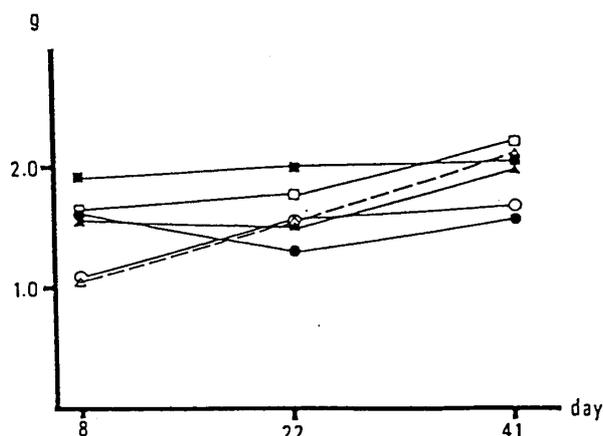
Results of analysis of the liver were shown in Table 5. The amount of T-Chol in the liver increased remarkably following addition of cholesterol, though there was no difference

Table 5 Total lipids(T-lipid) and total cholesterol(T-Chol) in liver

group	T-Lipid (%)	T-Chol (mg)
Standard	3.3 ± 0.5 ^a	127.8 ± 17.9 ^a
Control	9.9 ± 0.5 ^b	1920.5 ± 312.4 ^{bc}
+SM 20%	10.4 ± 0.9 ^b	1761.2 ± 158.7 ^{bc}
+DSM 17.4%	9.4 ± 0.7 ^b	1792.8 ± 386.5 ^{bc}
+LESM 4.2%	8.9 ± 0.5 ^b	1666.8 ± 267.8 ^c
Switchover	10.5 ± 1.6 ^b	2110.7 ± 412.1 ^b

* Results were expressed as means ± SE of 8 rats.

* Means not followed by a common letter were significantly different (p, 0.05)



- Standard
- Control
- +SM 20%
- +DSM 17.4%
- ▲ +LESM 4.2%
- △ Switchover

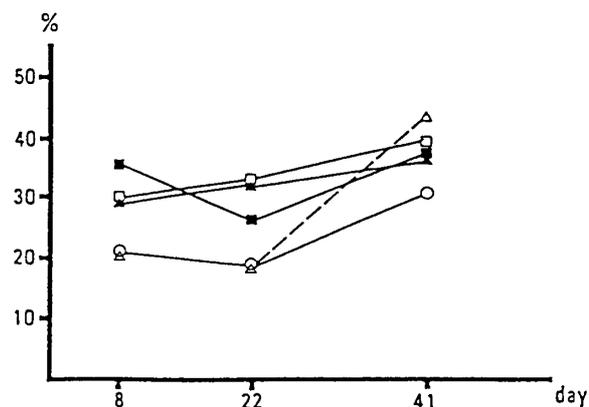
Fig. 6. Dry fecal weight.

between the groups receiving cholesterol-enriched diet. There was no difference in the percentage of total lipids in the liver between the groups receiving a cholesterol-enriched diet. As described, decreases in serum cholesterol levels were not accompanied by decreases in cholesterol in the liver and total lipids. In addition, no notable disorders in liver tissue were observed.

4. Steroids in feces

Results of the measurement of fecal excretion of steroids for each group were shown in Fig. 6 and 7.

Dry weight showed a tendency to increase compared to the control group in the soybean milk group, defatted soybean milk group, soybean milk-extracted lipid group and the



- Control
- +SM 20%
- +DSM 17.4%
- ▲ +LESM 4.2%
- △ Switchover

Fig. 7. Fecal neutral steroid/cholesterol intake

switched group. In addition, excretion of neutral steroids increased in the soybean milk group, defatted soybean milk group, soybean milk-extracted lipid group and the switched group compared to the control group. Especially in the soybean milk group, excretion on Day 8 was 1.7 times that in the control group.

There was no change in the tendency of excretion of bile acid as neutral steroids.

From these results, the inhibition of increases in serum cholesterol by soybean milk is indicated, and marked decreases in cholesterol were confirmed especially in animals with high serum cholesterol. Soybean milk was a kind of processed food of soybean. In this study, it was confirmed to have

a similar effect on serum cholesterol as soybean proteins and multivalent unsaturated fatty acid. Thus it was considered as an efficiently processed food in view of its effect on serum cholesterol. When soybean milk was evaluated as a processed food of soybean, similar potential effects of protein and lipids were expected and it was considered an efficient food for improving serum cholesterol.

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