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WHEY FROM CULTURED SKIM MILK DECREASES SERUM CHOLESTEROL AND INCREASES ANTIOXIDANT ENZYMES IN LIVER AND RED BLOOD CELLS IN RATS

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ABSTRACT

The study was carried out to examine the effect of whey from bovine skim milk fermented with bifidobacteria and lactic acid bacteria on plasma cholesterol and antioxidant enzymes in rats. Rats were maintained for 6 wks on a purified diet (reference diet) and on the purified diet supplemented milk whey or whey from cultured skim milk with *Bifidobacterium longum* (*B. longum*), *Lactobacillus acidophilus* (*L. acidophilus*) or *Streptococcus salivarius subsp. thermophilus* (*S. thermophilus*). Diets containing the product with *S. thermophilus* resulted in the lowest concentration of plasma cholesterol. The activity of superoxide dismutase (SOD) in red blood cells (RBC) and the activity of catalase in liver were elevated on cultured product-diets compared with the reference diet; in addition, the activity of glutathione peroxidase (GSHPx) in RBC was higher on the *L. acidophilus* diet compared with the reference diet. Although there were no significant differences in the concentrations of thiobarbituric acid-reactive substances and α-tocopherol in plasma, plasma $d<1.063$ g/ml lipoprotein fractions prepared from rats fed on the *B. longum*- and *L. acidophilus*-diets were resistant to the oxidative stress induced by a transition metal ion when compared with those from rats fed on the reference diet. The non-fermented whey diet was not as effective in lowering plasma cholesterol and in increasing antioxidant enzymes as were the fermentation product diets. These results therefore suggest that wheys from cultured milk may exert spesific effects on hypercholesterolemia and oxidative stress.

KEY WORDS: Fermented milk, Antioxidant enzymes, Lactic acid bacteria, Cholesterol.

INTRODUCTION

Several investigators have reported that a fermented milk product with lactic

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acid bacteria appears to have an action to decrease serum cholesterol concentration (1, 2), and to improve such disorders as intestinal diseases (3) and hepatic encephalopathy (4). Our previous study (5) showed that whey preparations prepared from cultured milk by 19 *Lactobacillus* (2 species) and 20 *Bifidobacterium* (5 species) strains affected differently the secretion and synthesis of bile acids in primary cultured rat hepatocytes: type of strains of lactic acid bacteria also affected differently immune functions and tumor formation when their cells or their lysates were given to experimental animals (6,7). We also showed beneficial effects of lactic acid bacteria products on peroxide-mediated cell and tissue injuries in vitamin E deficient rats (8) and experimental cholestatic rats (9).

The aim of this study is hence to investigate in intact rats whether a diet containing wheys from cultured bovine skim milk with bifidobactera and lactic acid bacteria would be able to affect such oxidative parameters as activities of antioxidative enzymes (superoxide dismutase, glutathione peroxidase and catalase), levels of α-tocopherol and hydroperoxides, and *in vitro* susceptibility of plasma lipoproteins to an oxidative modification. In addition, hypocholesterolemic action of the fermentation products was examined.

MATERIALS AND METHODS

Preparation of milk whey and cultured milk products. Milk whey was prepared from a skim milk suspension acidified to pH 4.6 in order to remove the curd and the resulting whey preparation was liophilized and designated as non-fermented milk whey. The preparation of fermentation products were described in details elsewhere (10). Briefly, a medium composed of 120 g/l defated skim milk and 3 g/l yeast extract (Asahi Brewery Co., Tokyo, Japan) in deionized water was sterilized at 115°C for 20 min, and aliquots were inoculated separately with *Bifidobacterium longum* (*B. longum*, SBT 2928 and SBT 2933R), *Lactobacillus acidophilus* (*L. acidophilus*, SBT 2062) and *Streptococcus salivarius subsp. thermophillus* (*S. thermophillus*, SBT 1035), which are commonly used for preparing yoghurt. After incubating at 37°C for 16 hr, each medium was centrifuged and the resulted whey preparations were liophilized. The fermented milk products contained 15-19 mg of vitamin C/kg and 16-17 mg of vitamin B/kg. The non-fermented milk whey contained 69 mg of vitamin C/kg and 15 mg of vitamin B/kg. Amino acid compositions of the fermented products and non-fermented milk whey were essentially similar, and tocopherols were not detected in both whey products (9).

Diet and animals. Four wk old male Sprague-Dawley rats, (Seiwa Experimental Animals Co., Fukuoka, Japan), maintained on a commercial non-purified diet (NMF, Oriental Yeast Co., Tokyo, Japan) in a temperature-controlled room (22-25°C) with a 12 hr-light/12 hr-dark cycle, were arranged in five groups with 6 rats each. A reference diet was formulated according to the AIN-76™ formula (11) as shown in Table 1. To the reference diet was added 100 g/kg of either non-fermented milk whey or fermented milk whey with *B. longum, L. acidophilus* or *S. thirmophilus* at the expense of sucrose. All the diets were adjusted to give the same content of protein and lactose, hence the diets contained approximately 16.8 g protein and 317 Kcal for 100 g diet. The rats were raised on these diets and demineralized water for 6 wk. The body weight and food intake were measured every other day and feces were collected for the last 3 days.

Rats were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. Blood was collected in heparin coated tubes and centrifuged at 1,000 *g* for 10 min to separate plasma and red blood cells (RBC). The erythrocyte lysates (20%, v/v) were prepared by lysing aliquots of washed

TABLE 1

Composition of Experimental Diets (g/100 g diet)

Ingredients	Reference	Non-fermented whey	Fermented milk whey
Milk whey*	-	10	-
Fermented milk whey*	-	-	10
Casein**	20	19	19
Safflower oil	10	10	10
Vitamin mixture#	1	1	1
Mineral mixture#	3.5	3.5	3.5
Choline bitartrate	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3
Cellulose	5	5	5
α-Corn starch	15	15	15
Lactose	7	-	2.5
Sucrose	to 100	to 100	to 100

*Milk whey and fermented milk whey preparations per 100 g dry weight contained 0.38-0.42 g of crude fat, 7.2-8.3 g of protein, 12.1-13.2 g of ash and 0.01-0.02 mg of copper. The milk whey and the fermented milk wheys contained 0.14 and 5.2 -5.6 mg zinc/100 g, respectively. The whey from the milk fermented with *L.acidophilus* contained 0.33 mg iron/100 g; the other fermented milk wheys and the milk whey did 0.11-0.17 mg/100 g. The milk whey and the fermented milk wheys contained 70 and 40-50 g lactose per 100 g, respectively. ** A product composed of about 84% protein from Wako Pure Chemical Industries, Osaka, Japan. #AIN-76™ vitamin and mineral mixtures purchased from Seiwa Experimental Animal Co. Fukuoka, Japan.

RBC with deionizedwater (12). The liver was excised and the mitochondria were isolated as described previously (13). The rest of the liver as will as a portion of the RBC lysates and plasma were kept at -70°C until analysis. All aspects of the experiment were approved by Kyushu University Animal Policy and Welfare Committee.

Preparation of plasma lipoproteins and oxidative modifications of d<1.063 g/ml lipoprotein fractions. Plasma lipoproteins were prepared according to the method described previously (14). Fresh plasma containing 2.69 mmol/l Na_2-EDTA was adjusted the density (d) to 1.063 g/ml with KBr, and two lipoprotein fractions (d<1.063 g/ml and d>1.063 g/ml) were isolated by spinning at 1.6×10^6 g for 18 hr at 10°C with ultracentrifuge (Beckman, Palo Alto, CA, USA). The top layer containing d<1.063 g/ml lipoproteins was harvested by tube slicing and then both top and bottom (d>1.063 g/ml lipoproteins) layers were dialyzed extensively against phosphate buffered saline containing 10 μmol/l Na_2-EDTA. Transition metal ion-dependent peroxidation of plasma d<1.063 g/ml lipoproteins was induced by adding 1 mmol/l ascorbic acid and 0.1 mmol/l $FeSO_4 \cdot 7H_2O$ (15). Incubation was carried out in water bath at 37°C with constant shaking. The samples were removed at appropriate intervals and the oxidation reaction was terminated by adding final concentration of 20 μmol/l butylated hydroxytoluene and 2 mmol/l Na_2-EDTA. Lipid peroxidation was evaluated by thiobarbituric acid (TBA) assay (16).

Analyses. Blood samples were analyzed for hemoglobin by cyanomethemoglobin method (Cyanomethemoglobin-Test, Wako Pure Chemical Industries, Osaka,

Japan). Freshly prepared RBC lysates were treated with chloroform-ethanol as described previously (17) and assayed for the activities of glutathione peroxidase (GSHPx, EC 1.11.1.9) (18), superoxide dismutase (SOD, EC 1.5.1.1.) (19) and catalase (EC 1.11.1.6) (20). RBC lysates were also analyzed for TBA reactive substances (TBARS) as described previously (21). Aliquots of the post-mitochondrial fraction were treated with Drabkin's reagent to remove hemoglobin as described previously (22), and assayed for the activities of GSHPx, SOD and catalasc. Mitochondrial TBARS were measured as described previously (23).

Liver and plasma total lipids were extracted by sodium dodecyl sulfate (SDS) procedure (24), and their α-tocopherol content was measured by high-performance liquid chromatography (Waters 600E, Japan Millepore, Tokyo, Japan) according to the method described previously (25) with slight modifications using a Zorbax SIL column (4.6 mm x 25 cm; Rockland Technologies, USA) and a mobile phase mixture composed of n-hexane:dioxan:isopropanol (985:10:5, v/v). Plasma and the lipoprotein lipids were enzymatically determined with commercially available kits (Cholesterol C-test and Triglyceride-test, Wako Pure Chemical Industries, Osaka, Japan). Liver and plasma lipids were also extracted by the method of Folch *et al.*(26) and were subjected for determining chemically cholesterol (27), triacylglcerols (28) and phospholipids (29). Fatty acid composition of plasma and liver total lipids, and fecal steroids were determined as described previously (30).

Statistical analysis. All data were expressed as mean±SEM, and statistical differences were determined by Duncan's multiple range test (31).

RESULTS AND DISCUSSION

Type of diets did not affect the body weight gain (g) for 6 weeks: reference diet 279±16; *B. longum*-diet 276±11; *L. acidophilus*-diet 259±12; *S. thermophilus*-diet 273±8; non-fermented milk whey-diet 287±8. Food efficiency was also comparable among the groups: reference diet 23.5±0.5; *B. longum*-diet 22.4±0.5; *L. acidophilus*-diet 22.3±0.8; *S. thermophils*-diet 22.9±0.7, non-fermented whey-diet 22.7±0.4. Relative liver weight (g/100g body weight) did not differ among the groups: reference diet 3.9±0.1; *B. longum*-diet 3.9±0.1, *L. acidophilus*-diet 3.9±0.1; *S. thermophilus*-diet 3.8±0.1 and non-fermented milk whey-diet 3.8±0.1.

Table 2 shows concentrations of plasma and liver lipids. Concentration of cholesterol in plasma as well as the d>1.063 g/ml lipoprotein fractions was the lowest in rats fed on the diet containing the cultured product with *S. thermophilus*; however, the *S. thermophilus*-diet did not lower the cholesterol in the d<1.063 g/ml fractions compared with the reference diet. These results are in agreement with Rao *et al.* who reported that rats fed milk fermented with *S. thermophilus* resulted in a reduction of plasma cholesterol (2); in contrast, Grunevald showed that rats received milk fermented with *L. acidophilus* lowered serum cholesterol levels (32). Type of diets, however, did not affect the concentration of hepatic cholesterol (Table 2), and the fecal excretion of bile acids and neutral sterols (data not shown). Concentration of plasma triacylglycerols was similar among the groups; but, the hepatic triacylglycerols were lowered in rats on the *B. longum*-and *S. thermophilus*-diets compared with the reference diet. Concentration of hepatic phospholipids was not affected by the type of diets. These results therefore confirmed again our previous observation that cultured milk products with lactic acid bacteria influenced cholesterol metabolism (5), although the mechanism of hypocholestrolemic action by fermented milk products is still remained unsolved. *L. acidophilus*- and *S. thermophilus*-diets resulted in a hepatic fatty acid

TABLE 2

Plasma and Liver Lipids in Rats Fed on Fermented and Non-Fermented Milk Whey Diets

Lipids	Reference	*B. longum*	*L. acidophilus*	*S. thermophilus*	Non-fermented whey
Plasma lipids (mg/dl)					
Cholesterol	111± 7.0[a]	106± 4.9[a]	105± 6.6[a]	89± 4.4[b]	112± 5.4[a]
Triacylglycerols	137±14.3	117± 9.4	143±22.1	107±21.9	102±15.1
Phospholipids	155± 4.5[a]	102±22.9[b]	134± 9.1[ab]	121± 8.0[b]	131±10.9[ab]
Lipoprotein cholesterol (mg/dl)					
d>1.063 g/ml	103±1.6[ac]	98.3±0.3[a]	98.8± 2.4[a]	80.4± 1.3[b]	106± 3.5[c]
d<1.063 g/ml	7.4 ± 0.1[ab]	7.4±0.5[ab]	6.3± 0.5[a]	8.4± 0.4[b]	6.8± 0.2[a]
Liver lipids (mg/g)					
Cholesterol	2.3 ± 0.2	2.2±0.2	2.0± 0.2	2.4± 0.4	2.0± 0.2
Triacylglycerols	4.9 ± 0.7[a]	4.0±1.0[ab]	2.2± 0.2[b]	2.1± 0.3[b]	3.3± 0.8[ab]
Phospholipids	20.8 ± 1.1	23.4±4.0	16.8± 0.9	22.4± 2.0	21.7± 1.9

Values are the means ± SEM for six rats per group.
[a, b, c] Means within a row with unlike superscripts are significantly different at $P < 0.05$.

composition with a higher proportion of arachidonic acid (20:4n-6) and a lower proportion of oleic acid compared with the reference diet (Table 3). The results noted on liver fatty acid composition suggests that fermentation products would affect an activity of Δ6-desaturase, a rate limiting enzyme for synthesis of arachidonic acid from linloleic acid (33). The non-fermented milk whey and cultured products however did not affect the plasma fatty acid composition except a lower proportion of docosahexaenoic acid (22:6n-3) in rats on the *B. longum*-diet.

TABLE 3

Proportion of Unsaturated Fatty Acids in Liver and Plasma in Rats Fed on Fermented and Non-Fermented Milk Whey Diets

Fatty acids	Reference	*B. longum*	*L. acidophilus*	*S. thermophilus*	Non-fermented whey
			(Weight %)		
Liver					
18:1	17.2± 1.6[a]	14.6± 1.5[ab]	13.7± 0.7[ab]	12.6± 0.6[b]	15.4± 1.5[ab]
18:2n-6	26.7± 2.6	27.3± 1.8	24.7± 1.8	25.8± 1.7	23.8± 1.8
20:4n-6	13.4± 0.7[a]	16.0± 1.7[ab]	18.3± 0.8[b]	18.7± 0.9[b]	16.7± 1.7[ab]
22:6n-3	2.4± 0.3	2.6± 0.3	2.9± 0.2	3.2± 0.3	3.2± 0.4
Plasma					
18:1	11.0± 0.7	10.6± 0.6	10.3± 1.1	10.0± 1.1	8.7± 2.0
18:2n-6	29.3± 1.6	26.7± 0.7	17.9± 2.7	27.0± 2.9	21.1± 4.6
20:4n-6	25.4± 1.4	25.2± 0.8	26.5± 3.9	28.0± 4.0	24.0± 5.5
22:6n-3	1.4± 0.2[a]	0.7± 0.0[b]	1.1± 0.3[ab]	0.9± 0.2[ab]	0.9± 0.2[ab]

Values are the means ± SEM for six rats per group.
[a, b] Means within a row with unlike superscripts are significantly different at $P < 0.05$.

Table 4 shows activities of antioxidative enzymes in the RBC and the liver. Rats fed on diets containing the cultured products had a higher SOD activity in RBC than did those fed on the reference and non-fermented milk whey diets. The

TABLE 4

Activities of Antioxidative Enzymes and Concentrations of TBARS and α-Tocopherol in Red Blood Cells, Plasma and Liver in Rats Fed on Fermented and Non-Fermented Milk Whey Diets.

	Reference	*B. longum*	*L. acidophilus*	*S. thermophilus*	Non-fermented whey
RBC					
Catalase[1] (KU/g Hb*)	4.88 ± 0.75^{ab}	5.97 ± 0.86^{a}	4.11 ± 0.79^{ab}	4.64 ± 0.49^{ab}	3.82 ± 0.16^{b}
SOD[2] (KU/g Hb)	1.41 ± 0.05^{a}	1.61 ± 0.06^{b}	1.66 ± 0.03^{b}	1.62 ± 0.05^{b}	1.37 ± 0.06^{a}
GSHPx[3] (U/g Hb)	29.1 ± 0.01^{ac}	32.2 ± 0.01^{ab}	35.1 ± 0.02^{b}	30.2 ± 0.02^{ac}	28.3 ± 0.01^{c}
TBARS (nmol MDA#/g Hb)	14.7 ± 0.70	15.3 ± 0.89	14.3 ± 0.61	16.3 ± 1.40	16.6 ± 0.52
Liver					
Catalase[1] (KU/g)	0.93 ± 0.71^{a}	1.37 ± 0.88^{b}	1.18 ± 0.52^{bc}	1.26 ± 0.51^{bc}	1.01 ± 0.40^{ac}
SOD[2] (KU/g)	0.48 ± 0.36	0.43 ± 0.28	0.40 ± 0.55	0.34 ± 0.52	0.36 ± 0.66
GSHPx[3] (KU/g)	23.5 ± 1.9	24.1 ± 1.0	24.2 ± 1.1	24.2 ± 1.4	24.4 ± 1.1
α-Tocopherol (μg/g liver)	23.7 ± 1.5^{a}	21.0 ± 0.5^{ab}	20.1 ± 0.5^{ab}	17.4 ± 2.1^{b}	16.9 ± 2.1^{b}
Mitochondria TBARS (nmol MDA/g liver)	32.7 ± 1.51	31.3 ± 1.02	29.7 ± 1.72	32.5 ± 2.2	30.9 ± 1.21
Plasma					
TBARS (μmol MDA/l)	12.5 ± 1.21	11.6 ± 1.31	12.5 ± 0.58	12.6 ± 1.52	13.5 ± 1.12
α-Tocopherol (μgl/ml)	7.51 ± 0.54	7.80 ± 0.89	7.83 ± 0.70	7.90 ± 0.83	8.22 ± 0.66
Lipoproteins α-Tocopherol (μgl/ml)					
d>1.063 g/ml	4.24 ± 0.25	4.47 ± 0.16	4.47 ± 0.33	4.32 ± 0.21	4.94 ± 0.52
d<1.063 g/ml	3.22 ± 0.11	3.35 ± 0.14	3.32 ± 0.12	3.45 ± 0.11	3.28 ± 0.11

Values are the means ± SEM for six rats per group.

[a, b, c] Means within a row with unlike superscripts are significantly different at $P < 0.05$.

* Hb= hemoglobin

MDA= malondialdehyde

[1] 1 unit (U) is equivalent to the amount of enzyme which liberates half the peroxide oxygen from a hydrogen solution of any concentration in 100 sec at 25 °C.

[2] 1 unit (U) of activity is equivalent to the activity of 1 μg purified bovine erythrocyte superoxide dismutase in the riboflavin-o-dianisidine photo-oxidation system.

[3] 1 unit (U) is equivalent to 1 μmol NADPH oxidized/min.

catalase activity in RBC was higher in rats fed on the *B. longum*-diet than in those fed on the non-fermented milk whey-diet. The activity of GSHPx in RBC was higher in rats fed on the *L. acidophilus*-diet than in those fed on the reference and non-fermented milk whey-diets. Because the AIN-76™-based diets supplied approximately 0.11 mg selenium/kg diet which is more than an adequate selenium requirement for rats (0.04 mg Se/kg diet) (34), higher GSHPx activity on the *L. acidophilus*-diet seemed not to be affected by selenium content among the diets. Furthermore, rats fed on the cultured products had a higher activity of hepatic catalase compared with those fed on the reference; in addition, the activity in *B. longum* group was higher than that in non-fermented whey group diet. The activities of the hepatic SOD and GSHPx were not affected by the type of diets.

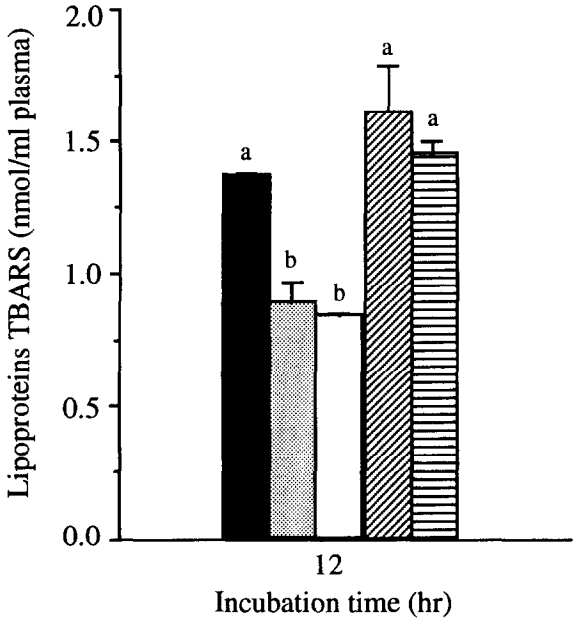

Figure 1. *In vitro* peroxidation of plasma d<1.063 g/ml lipoprotein fractions in rats fed fermented and non-fermented milk whey diets. (■), Reference; (▨), *B. longum*; (□), *L. acidophilus*; (▨), *S. thermophilus*; (▤), Non-fermented milk whey. TBARS after 12 hr incubation was subtracted from the initial values. Values are means with their standard errors represented by vertical bars. ^{a,b,c} Mean values for TBARS with unlike superscripts were significantly different at P <0.05.

Type of diets exert no significant effect on the concentration of TBARS in RBC, liver mitochondria and plasma nor on the level of α-tocopherol in the liver, plasma and plasma lipoprotein fractions; but, the concentration of liver α-tocopherol was lower in the *S. thermophilus* and non-fermented whey-diets than in the reference group.

Fig. 1 shows the transition metal ion-mediated *in vitro* peroxidation of plasma d<1.063 g/ml lipoprotein fractions. Peroxides estimated as TBARS increased very slowly until 8 hr incubation (data not shown). After 12 hr incubation, TBARS formation was lower in the d<1.063 g/ml lipoproteins from *B. longum* and *L. acidophilus*, groups but not from *S. thermophilus* group, than in those from the

reference diet or non-fermented milk whey groups. A property resistant to in vitro peroxidation of d<1.063 g/ml lipoproteins from *B. longum* and *L. acidophilus* groups is not attributed to the content of lipids used for the incubation as the reference and the cultured products diets did not result in differences in the concentration of cholesterol in d<1.063 g/ml lipoproteins and serum triacylglycerols, present mainly in d<1.063 g/ml lipoproteins, as shown in Table 2. Hence, it raises the possibility that the d<1.063 g/ml lipoproteins prepared from *B. longum* and *L. acidophilus* groups might contain an anti-oxidative substance(s) other than α-tocopherol (35), because the content of α-tocopherol and the fatty acid composition in plasma and the d<1.063 g/ml lipoproteins were similar among the groups. It however remains a possibility that feeding fermentation products to rats would cause of structure of d<1.063 g/ml lipoproteins resistant to *in vitro* peroxidation, because cholesterol content in human LDL has been reported to exert a variable effect on the susceptibility of LDL to oxidative modifications (36).

In summary, the present study showed that cultured products by bifidobacteria and lactic acid bacteria exerted divergent effects on oxidative parameters and plasma cholesterol concentration. Although some of the peroxidative changes brought by cultured products were also observed by non-fermented whey, the changes were more prominent in rats fed the cultured products than in those fed non-fermented whey. In order to extrapolate the present results to a case of humans, it would be needed to carry out further studies under an experimental condition to render animals hypercholesterolemic and susceptible to oxidative stress.

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REFERENCES

1. Richardson T. The Hypocholesteremic effect of milk-A review. J Food Prot 1978;41:226-235.

2. Rao DR, Chawan CB, Pulusani SR. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J Food Sci 1981;46:1339-1341.

3. Beck C, Necheles H. Beneficial effects of administration of *Lactobacillus acidophilus* in diarrhea and other intestinal disorders. Am J Gastro 1961;35:522-530.

4. Read AE, McCarthy CF, Heaton KW, Laidlaw J. *Lactobacillus acidophilus* (Enpac) in treatment of hepatic encephalopathy. Br Med J 1966;1:1267-1269.

5. Imaizumi K, Hirata K, Zommara M, Sugano M, Suzuki Y. Effects of cultured milk products by *Lactobacillus* and *Bifidobacterium* species on the secretion of bile acids in hepatocytes and in rats. J Nutr Sci Vit 1992;38:343-351.

6. Kadooka Y, Fujiwara S, Hirota T. Effect of bifidobacteria cells on mitogenic responses of splenocytes and several functions of phagocytes. Milchwissenchaft 1991;46:626-630.

7. Fujiwara S, Kadooka Y, Hirota T. Mitogenic activity in skimmed milk culture supernatant of *Aspergillus oryzae* EF-08: Preparation of mitogenic and their

immunopotentiating effect in vivo. J Agr Food Chem 1992;40:1280-1286.

8. Kaizu H, Sasaki M, Nakajima H, Suzuki Y. Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. J Dairy Sci 1993;76:2493-2499.

9. Zommara M, Takagi H, Sakono M, Suzuki Y, Imaizumi K. Effect of milk whey and its fermentation products by lactic acid bacteria on mitochondrial lipid peroxide and hepatic injury in bile duct ligated rats. Biosci Biotech Biochem 1994;58:1213-1217.

10. Suzuki Y, Kaizu H, Yamauchi Y, Nakazato H. Effects of yoghurt and cultured milk made using *Bifidobacterium longum* on survival rate and serum constituents of ICR mice. J Jap Soc Food and Nutr 1991;44:177-182.

11. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1977;107:1340-1348.

12. Huang CJ, Fwu ML. Protein insufficiency aggravates the enhanced lipid peroxidation and reduced activities of antioxidative enzymes in rats fed diets high in polyunsaturated fat. J Nutr 1992;122:1182-1189.

13. Recknagel RO, Ghoshal AK. Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. Exp Mol Pathol 1966;5:413-426.

14. Havel RJ, Edar, HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345-1353.

15. Yoshida K, Terao J, Suzuki T, Takama K. Inhibitory effect of phosphatidylserine on iron-dependent peroxidation. Biochem Biophys Res Commun 1991;16:1077-1081.

16. Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 1992;202:384-389.

17. McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-6055.

18. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976;71:952-958.

19. Misra HP, Fridovich I. Superoxide dismutase: a photochemical augmentation assay. Arch Biochem Biophys 1977;181:308-312.

20. Luck H. Catalase. In: Bergmeyer HU, ed.Methods of Enzymatic Analysis. New York: Academic Press, 1963:885-894.

21. Brownlee NR, Huttner JJ, Panganamala RV, Cornwell DG. Role of vitamin E in glutathione-induced oxidant stress: methemoglobin, lipid peroxidation, and hemolysis. J Lipid Res 1977;18:635-644.

22 Paliga DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clinl Med 1967;70:158-169.

23. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues

by thiobarbituric acid test. Anal Biochem 1978;86:271-278.

24. Burton GW, Weeb A, Ingold KU. A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. Lipids 1985;20:29-39.

25. Tanabe K, Yamaoka M, Kato A. High performance liquid chromatography and mass spectra of tocotrienols in rice bran oils. Yukagaku 1981;30:116-118.

26. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957 ;226:497-506.

27. Sperry WM, Webb M. A revision of the Schoenheimer Sperry method for cholesterol determination. J Biol Chem 1950;187:97-106.

28. Fletcher MJ. A colorimetric method for estimating serum triglycerides. Clin Chem Acta 1968;22:393-397.

29. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem 1959;234:466-468.

30. Imaizumi K, Abe K, Kuroiwa C, Sugano M. Fat containing stearic acid increases fecal neutral steroid excretion and catabolism of low density lipoproteins without affecting plasma cholesterol concentration in animals fed a cholesterol-containing diet. J Nutr 1993;123:1693-1702.

31. Duncan DB. Multiple range and Multiple F testes. Biometrics 1955;11:1-42.

32. Grunevald KK. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J Food Sci 1982;47:2078-2079.

33. Huang YS, Koba K, Horrobin, DF, Sugano M. Interrelationship between dietary protein, cholesterol and *n*-6 polyunsaturated fatty acid metabolism. Prog Lipid Res 1993; 32:123-137.

34. Warner RG, Breuer Jr LH. Nutrient Requirements of the laboratory rat. In: Nutrient requirements of domestic animals. No. 10. Nutrient requirements of laboratory animals. Washington DC: National academy of sciences, 2nd ed. 1972;56-93.

35. Scaccini C, Nardini M, D'Aguino M, Gentili V, Di Felico M, Tomassi G. Effect of dietary oil on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. J Lipid Res 1992;23:627-633.

36. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93:189-199.

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