Effect of Fermented Chub Mackerel Extract on Lipid Metabolism of Rats Fed Diets without Cholesterol

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ABSTRACT : The present study was conducted to evaluate the effect of fermented chub mackerel extract (FCME) on lipid metabolism in rats fed diets without cholesterol. Four week-old male rats were divided into three groups of 10 rats with 0, 1% or 2% FCME supplementation to the diets. Purified diets were used in the present study. Feed and water were fed *ad libitum*. FCME supplementation had no effect on the activities of acetyl-CoA carboxylase, fatty acid synthetase, and the content of free cholesterol, triglyceride and phospholipid in the liver ($p>0.05$). 1% FCME supplementation significantly increased serum triglyceride ($p<0.05$) and hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity ($p<0.05$) with no effect on serum total cholesterol, free cholesterol and phospholipid concentration. FCME supplementation significantly reduced serum LDL+VLDL-cholesterol ($p<0.01$) and atherogenic index ($p<0.01$) with no effect on HDL-cholesterol. The current study showed that FCME inclusion might reduce the risk of atherosclerosis in rats fed diet without cholesterol. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4 : 535-539)*

Key Words : Fermented Chub, Mackerel Extract, Lipid Metabolism, Rats

INTRODUCTION

Excessive fat accumulation in animals can cause many abnormalities of lipid metabolism such as fatty liver, hypertriglyceridemia, hypercholesterolemia and obesity (Tanaka, 1992). These abnormalities not only reduce the production and decrease the commodity value of animals as food, but they also appear to disturb normal metabolism of animals, and therefore it would reduce the health of animals.

Feeding fish meal and related products may have beneficial a effect on preventing hypertriglyceridemia and hypercholesterolemia (Bang and Dyerberg, 1976; Bang et al., 1980), and preventing cardiovascular disease (Crawford et al., 1989; Hirai et al., 1989). Fermented product has also been proven to reduce lipid accumulation in animals (Danielson et al., 1989; Santoso et al., 1995). Therefore, fish quality could be improved by fermentation and it was expected to enhance the lowering effect of fish on lipid metabolism. The beneficial effect of fermented chub mackerel extract (FCME) on lowering plasma lipid has been proven in growing chicks (Tanaka et al., 1990), broiler chicks (Tanaka et al., 1992) and rats fed high-cholesterol containing diet (Santoso et al., 2000a).

It was proven that cholesterol inclusion to the diet might change the response of animals to the diet. For instance, Santoso et al. (2000b) showed that tu-chung left meal in a diet reduced serum triglyceride in chickens fed a high-cholesterol containing diet, but it increased serum triglyceride when it was added to a diet without cholesterol. Therefore, the present study was conducted to evaluate the effect of FCME on lipid metabolism in rats fed a diet without cholesterol addition. Because of high peptides content, FCME was assumed to lower lipid concentration in serum.

MATERIALS AND METHODS

Four week-old male rats (body weight 110 ± 10 g) used in this experiment were purchased from Japan SLC Inc (Hamamatsu, Shizuoka, Japan). They were then weighed individually and divided into three groups based on weight. Thereafter, they were randomly distributed to three treatments with 10 rats assigned to each treatment. One group was the control with no additive, and two-treatment groups were given the purified diets supplemented with 1% or 2% FCME. The rats were raised to 7 weeks of age in individual cages in an air-conditional room (temperature 22 ± 2℃ with humidity 50 to 60%) with the light on from 08:00 to 20:00. Rats were fed a commercial nonpurified diet (type CE-2, Japan Clea) for a week before the initiation of the experiment with purified diets. The composition of experimental diets is shown in table 1. Feed consumption and individual body weight were determined weekly. Feed and water were provided for *ad libitum* consumption.

Commercial FCME was obtained from Kanzaki Company, Ltd., Takamatsu, Japan. The main constituents of this extract are peptides with 20-50 chain-length amino acids. This product contains 39.6%

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moisture, 51.1% crude protein, 0.0% crude fat, 0.0% crude fiber, 8.7% crude ash and 0.6% nitrogen free extract (NFE). Amino acid profiles of FCME are published elsewhere (Santoso et al., 2000). At the end of the experiment, all rats were weighed, and five rats with similar body weights were selected from each treatment. Blood samples were drawn from the heart and removed from the liver under ether narcosis. Blood was then centrifuged at 2,500 rpm for 10 minutes. Serum obtained was stored and frozen at -30℃ until analysis of various lipid fraction concentrations. They were then sacrificed by decapitation. The liver was immediately removed and weighed. Livers were placed in an ice-cold saline to determine enzyme activities and contents of various lipid fractions.

Enzyme assay was prepared as previously described (Santoso et al., 1995). The activities of key enzymes in fatty acid synthesis and cholesterogenesis were measured. Acetyl-Coenzyme A carboxylase (E.C. 6.2.1.3) activity was assayed by $H^{14}CO_3$-fixation method (Qureshi et al., 1980). Fatty acid synthetase (FAS) activity was assayed by the 1-^{14}C-acetyl-CoA incorporation method (Hsu et al., 1965). 3-hydroxy-3-methylglutaryl-CoA reductase activity was assayed by the method of Shefer et al. (1973). The protein content of the solution used for enzyme assay was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. ACC and FAS activities were expressed as nanomole of substrate converted to product per minute per milligram of protein at 37℃. 3-hydroxy-3-methyl-glutaryl-CoA reductase activity was expressed as picomole of substrate converted to product per minute per mg protein at 38℃. Samples were analyzed in triplicate.

The lipid fractions were separated by thin-layer chromatography on silica gel chromarod using hexane-diethylether-formic acid (60:10:1) and hexane- benzene (1:1) as developing solvent and quantified by IATROSCAN - TH-10 TLC/FID Analyzer (Iatron Laboratories, Inc., Tokyo, Japan).

Concentrations of serum total cholesterol, HDL-cholesterol were measured with commercial kits (Cholesterol E Test Wako Kit and HDL-cholesterol E Test Wako Kit from Wako Junyaku Kogyo Co. LTD). The difference between the total cholesterol and HDL-cholesterol was assumed to be LDL+VLDL cholesterol (Nishizawa and Fudamoto, 1995). The same authors measured an atherogenic index using the following equation:

$$\text{Atherogenic index} = \frac{\text{Total cholesterol-HDL-cholesterol}}{\text{HDL-cholesterol}}$$

Treatment effects were assessed for all response variables using one-way ANOVA in which the overall treatment differences were represented by single orthogonal contrasts between control and treatment groups (Shinjo, 1990).

Table 1. Composition of experiment diets

Ingredients (%)	0% FCME	1% FCME	2% FCME
Corn starch	45	45	45
Sucrose	23.9	23.9	23.9
Soybean oil	3	3	3
Casein	23	22	21
Mackerel extract	0	1	2
Mineral mixture[1]	4	4	4
Vitamin A & D mixture[2]	0.1	0.1	0.1
Vitamin B mixture[3]	1	1	1
Total	100	100	100
Chemical composition			
Protein (%)	23.0	22.4	21.8
ME (kcal/kg)	3,751.6	3,733.6	3,715.6
ME-protein ratio	163.1	166.7	170.4

[1] Supplied 650.0 g $CaHPO_4$, 160.0 g NaCl, 140.0 g K_2CO_3, 32.7 g $MgCO_3$, 10.0 g $FeSO_4 \cdot 7H_2O$, 3.0 g $MnSO_4 \cdot H_2O$, 1.0 g $CoCl_2 \cdot 6H_2O$, 1.0 g $CuSO_4$, 2.0 g $ZnCO_3$, 0.1 g KI and 0.2 g NaF per 1 kg mixture.

[2] Supplied 0.10 g retinyl acetate, 0.00005 g cholecalciferol and 0.8995 g corn starch per 1 gram mixture.

[3] Supplied 0.083 g thiamine-HCl, 0.233 g riboflavine, 0.833g niacin, 0.75 g Ca pantothenate, 0.1 pyridoxine-HCl, 0.058 g folic acid, 15 g inositol, 1.667 g p aminobenzoic acid, 0.005 g biotin, 0.004 g cyanocobalamin, 33.333 g choline-HCl, 0.333 g menadione and 47.599 corn starch per 100 g mixture.

RESULTS

Body weight, body weight gain, feed intake, feed conversion ratio and liver weight of rats fed FCME were not significantly different (table 2). However, feed conversion ratio tended to be higher by 3.5% and 11.4% for 1% and 2% groups, respectively. Body weight gain tended to be higher by 3 % and 8.4% for 1% and 2% groups, respectively.

Table 3 shows the effect of FCME on activities of lipogenic-related enzymes and 3-hydroxy-3-methyl-glutaryl-CoA reductase in the liver of rats. FCME supplementation had no effect on the activities of acetyl-CoA carboxylase and fatty acid synthetase ($p<0.05$); 1% FCME supplementation significantly increased hepatic 3-hydroxy-3-methylglutaryl-CoA in the liver of rats. ($p<0.05$).

Table 4 shows the effect of FCME on hepatic and serum lipid fractions. FCME did not significantly influence hepatic triglyceride, free cholesterol and phospholipids. Serum triglyceride was increased in rats

fed 1% FCME ($p<0.05$), while serum free cholesterol and phospholipids were not significantly different. FCME supplementation significantly reduced serum LDL+VLDL-cholesterol and atherogenic index ($p<0.01$) with no effect on HDL-cholesterol.

DISCUSSION

The results show that 2% FCME inclusion in the diet increased feed conversion ratio by 11.4% as compared with the control. Fermented fish increases the dietary soluble-nitrogen content because the complex protein structure is degraded and also increases the level of free amino acids and short-chain peptides (Hassan and Heath, 1987). The main constituents of FCME are peptides with 20-50 chain-length amino acids. It is known that FCME is rich in glutamic acid, glycine, aspartic acid, lysine, arginine, leucine, alanine and prolin (Santoso et al., 2000a). Thus, these changes are expected to improve the nutritional values and the digestibility of FCME. This may explain the tendency to higher feed efficiency and body weight gain in rats fed 2% FCME. In addition, Tanaka et al. (1990, 1992) assumed that FCME might contain substances which promote growth. The present results disagree with the observation of Santoso et al. (2000a) who found that FCME inclusion had a little value for improving feed efficiency in rats fed cholesterol-containing diet. It was proven that cholesterol inclusion to the diet would produce lower feed conversion ratio (Santoso et al., 2000b).

FCME inclusion at 1% level produced higher serum triglyceride as compared with the control indicating that at this level FCME causes hypertriglyceridemia in rats. This result disagrees with the observation of Santoso et al. (2000a) who found that FCME inclusion reduced serum triglyceride of rats fed high-cholesterol containing diets. There was evidence that the response of animals to feed supplementation was also influenced by cholesterol addition to the diet. For instance, Santoso et al. (2000b) showed that tu-chung inclusion to the diet reduced serum triglyceride in chickens fed a high-cholesterol containing diet, but it increased serum triglyceride when it was added to the a diet without cholesterol. The present results show that hepatic fatty acid synthesis could not explain the enhanced serum triglyceride in rats fed a diet with 1% FCME inclusion, as indicated by no significant change in hepatic acetyl-CoA carboxylase and fatty acid synthetase activities. The current study shows that cholesterol synthesis may be increased in rats fed 1% FCME as indicated by higher 3-hydroxy-3-methylglutaryl-CoA reductase activity. Duane (1995) found that increased cholesterol synthesis is a primary abnormality which, in turn, results in enhancedbile acid synthesis. Interruption of the enterohepatic circulation of bile acid increases serum triglyceride by increasing production of very low density lipoprotein (Beil et al., 1982a; Grundy et al., 1971; Miettinen and Lempinen, 1977). Thus, an increase in serum triglyceride in the current study could partly be explained by higher bile acid synthesis. In addition, in mammalia including rats, fatty acid synthesis mainly occurs in adipose tissue (Tanaka et al., 1978). Therefore, fatty acid synthesis in adipose tissue should be determined to give additional information and explanation of higher serum triglyceride in rats fed FCME.

A major advance in the understanding of cholesterol metabolism emerged from the observation that in rats the liver exhibits high rates of cholesterol synthesis, whereas nonhepatic tissues other than intestine show rates that are less than 5% of those in the liver (Balasubramaman et al., 1976). 1% inclusion

Table 2. Effects of fermented chub mackerl extract on feed intake, body weight gain and feed conversion ratio of rats

Variables	0% FCME	1% FCME	2% FCME
Body weight, g/rat	2669.9 ±6.2[1]	271.7 ±7.5	280.1 ±5.2
Body weight gain, g/day	7.47 ±0.79	7.70 ±1.03	8.10 ±0.24
Feed intake, g/day	18.9 ±1.7	18.9 ±1.2	18.4 ±2.2
Feed conversion ratio	2.53 ±0.05	2.46 ±0.06	2.27 ±0.05
Liver weight, g/100 g BW	4.79 ±0.35	4.87 ±0.17	4.67 ±0.29

[1] Mean±SD for 10 rats.

Table 3. Effects of fermented chub mackerel extract on the activities of acetyl-CoA carboxlase, fatty acid synthetase and 3-hydroxy-3-methylgluratyl-CoA reductase in rats[1]

Variables	0% FCME	1% FCME	2% FCME
Acetyl-CoA carboxylase (nmol/min/mg protein)	2.65 ±0.18	2.93 ±0.36	2.75 ±0.38
Fatty acid synthetase (nmol/min/mg protein)	2.35 ±0.93	2.74 ±0.78	2.64 ±0.79
3-hydroxy-3-methylglutaryl-CoA reductase (pmol/min/mg protein)	6.080 ±0.81	9.65 ±2.11*	6.35 ±0.99

[1] Mean±SD for 5 rats.* Significantly different ($p<0.05$) from the control group.

Table 4. Effects of fermented chub mackerel extract on contents of various lipid fractions in the liver and serum[1]

Variables	0% FCME	1% FCME	2% FCME
Liver (mg/g liver)			
Triglyceride	11.3 ± 3.8	9.7 ± 3.5	9.5 ± 3.9
Free cholesterol	1.4 ± 0.4	1.5 ± 0.3	1.5 ± 0.2
Phospholipids	29.6 ± 5.0	29.9 ± 3.4	27.8 ± 4.8
Serum (mg/100 ml)			
Triglyceride	85.9 ± 14.9	127.9 ± 19.2*	83.3 ± 18.1
Free cholesterol	20.1 ± 2.0	23.4 ± 3.4	23.4 ± 5.0
Phospholipid	162.6 ± 19.5	197.2 ± 31.9	176.8 ± 33.3
Total cholesterol	130.6 ± 16.1	133.9 ± 18.6	147.1 ± 28.1
HDL-cholesterol	113.1 ± 15.6	118.5 ± 19.5	134.6 ± 25.6
VLDL+LDL-cholesterol	21.3 ± 3.3	15.4 ± 2.2*	12.5 ± 2.0**
Atherogenic index	0.20 ± 0.03	0.13 ± 0.02**	0.10 ± 0.03**

[1] Mean ± SD for 5 rats.

* Significantly different ($p<0.05$) from the control group.

** Significantly different ($p<0.01$) from the control group.

of FCME may increase the rate of hepatic cholesterol synthesis as indicated by higher hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity, and this may cause an increase in cholesterol content in the liver and/or serum. The present study however, shows no change in cholesterol content neither in the liver nor in the serum. A number of studies have documented increased bile acid synthesis in hypertriglyceridemia in association with increased cholesterol synthesis (Einarsson et al., 1974; Einarsson and Hellstrom, 1972; Beil et al., 1982b). If it is true, the higher bile acid synthesis in rats fed 1% FCME may partly explain no change in cholesterol content in the liver and serum. Another possible mechanism is a higher rate of steroidogenesis.

HDL-cholesterol is a major vehicle for transportation of cholesterol to tissue for steroidogenesis and it reverses cholesterol transport from peripheral tissue to the liver, where cholesterol is converted to bile acids. It was proven that HDL-cholesterol and/or LDL-cholesterol is a better indicator for estimating the occurrence of atherosclerosis than total cholesterol (Vega et al., 1962; Spady and Distschy, 1985; Mattson and Grunde, 1985). Therefore, a decrease in serum LDL-cholesterol with the tendency of higher HDL-cholesterol would have a beneficial impact on reducing the risk of atherosclerosis. This is an important action and suggests a beneficial effect of FCME on cholesterol metabolism. Our previous results (Santoso et al., 2000a) also showed that when FCME was supplemented to a diets containing high-cholesterol, an increase in serum HDL-cholesterol with lower LDL-cholesterol without any change in total cholesterol was observed. Higher FCME inclusion resulted in lower serum LDL-cholesterol ($r=-0.98$; $p<0.01$) and atherogenic index ($r=-0.97$; $p<0.01$). Lower atherogenic index may indicate decreases in the risk of atherosclerosis in rats fed FCME. This occurrence suggests that feeding FCME would be beneficial for the health of rats, i.e. lowering the risk of atherosclerosis, and may improve the growth of rats. In conclusion, FCME inclusion reduced the risk of atherosclerosis in rats as indicated by lower LDL-cholesterol and atherogenic index.

REFERENCES

Balasubramanian, S., J. L. Goldstein, J. R. Faust and M. S. Brown. 1976. Evidence for regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol synthesis in nonhepatic tissues of rat. Proc. Natl. Acad. Sci. 73:2564-2568.

Bang, H. O. and J. Dyerberg. 1976. Plasma lipid and lipoprotein pattern in Greenlandic West Coast Eskimos. Lancet 1: 1143-1146.

Beil, U., J. R. and S. M. grundy. 1982a. Effects of interruption of the enterohepatic circulation of bile acids on the trasport of very low density-lipoprotein triglycerides. Metab. Clin. Exp. 31:438-444.

Beil, U., S. M. Grundy, J. R. Course, and L. Zech. 1982b. Triglyceride and cholesterol metabolism in primary hypertriglyceridemia. Arterioclerosis 2:44-57.

Crawford, M. A., W. Doyle, P. Drury, K. Chebremeskel, L. Harbige, J. Leyton and G. Williams. 1989. The food chain for n-6 and n-3 fatty acids with special reference to animal products. In: Dietary 3 and 6 Fatty Acids: Biological Effects and Nutritional Essentiality (Ed. C. Galli and A. P. Simopoulos). Plenum Press, New York and London.

Danielson, A. D., E. P. Peo, Jr., K. M. Shahani, A. J. Lewis, P. J. Whalen and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus*

yogurt fed to mature boars. J. Anim. Sci. 67:966-974.
Duane, W. C. 1995. Abnormal bile acid absorption in familial hypertriglyceridemia. J. Lipid Res. 36:96-107.
Einarsson, K. and K. Hellstrom. 1972. The formation of bile acids in patients with three types of hyperlipoproteinamia. Eur. J. Clin. Invest. 2:225-230.
Einarsson, K., K. Hellstrom and M. Kallner. 1974. Bile acid kinetics in relation to sexm serum lipids, body weights, and gallbladder disease in patients with various types of hiperlipoproteinemia. J. Clin. Invest. 54:1301-1311.
Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. J. Lab. Clin. Med. 78:94-121.
Hassan, J. E. and J. L. Heath. 1987. Chemical and nutritive characteristics of fish silage produced by biological fermentation. Biol. Wastes 20:187-201.
Hirai, A., T. Terano, Y. Tamura and S. Yoshida. 1989. Eicosapentanoic acid and adult diseases in Japan: Epidemiological and clinical aspects. J. Int. Med. 225:69-76.
Hsu, R. Y., G. Wasson and J. W. Porter. 1965. The purification and properties of the fatty acid synthetase of pigeon liver. J. Biol. Chem. 240:3736-3746.
Lowry, O. H., N. J. Rosebrough, A. L. Fart and R. J. Randell. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265-275.
Mattson, F. H. and S. M. Grundy. 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acid on plasma lipids and lipoproteins in man. J. Lipid Res. 26:194-202.
Miettinen, T. A. and M. Lempinen. 1977. Cholestyramine and ileal by-pass in the treatment of familial hypercholesterolaemia. Eur. J. Clin. Invest. 7:509-514.
Nishizawa, N. and Y. Fudamoto. 1995. The evelation of plasma concentration of high-density lipoprotein cholesterol in mice fed with protein from proso millet. Biosci. Biotech. Biochem. 59:333-335.
Qureshi, A. A., W. C. Burger, N. Prentice, H. R. Bird and M. L. Sunde. 1980. Regulation of lipid metabolism in chicken liver by dietary cereals. J. Nutr. 110:388-393.
Santoso, U., M. Ishikawa and K. Tanaka. 2000a. Effects of fermented chub mackerel extract on lipid metabolism of rats fed a high-cholesterol diet. Asian-Aus. J. Anim. Sci. 13:516-520.
Santoso, U., S. Ohtani and K. Tanaka. 2000b. Tu-chung leaf meal supplementation reduced an increase in lipid accumulation of chickens stimulated by dietary cholesterol. Asian-Aus. J. Anim. Sci. 13(12):1758-1763.
Santoso, U., K. Tanaka and S. Ohtani. 1995. Effect of dried *Bacillus subtilis* culture on growth and lipogenic enzyme activity in female broiler chicks. Br. J. Nutr. 74:523-529.
Shefer, S., S. Hauser, L. Lapar and E. H. Mosbch. 1973. Regulatory effects of dietary sterols and bile acids on rat intestinal 3-hydroxy-3-methylglutaryl-CoA reductase. J. Lipid Res. 14:400-405.
Shinjo, A. 1990. First Course in Statistics. Laboratory of Animal Breeding, College of Agriculture, University of the Ryukyus. Japan.
Spady, D. K. and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low-density lipoprotein transport in hamster. J. Clin. Invest. 81:300-309.
Tanaka, K. 1992. Usefulness of a pantothenic acid derivate (pantethine) as a feed additive. Daiichi Vitamin News 8: 1-8
Tanaka, K., N. Akazaki, S. Ohtani and K. Shigeno. 1978. Effect of dietary fat level on lipid metabolism.. Comparison of growing chicks with rats. Res. Bull. Fac. Agr. Gifu Univ. 41:191-199
Tanaka, K., B. S. Youn, S. Ohtani and Sakaida. 1990. Effects of fermented product from chub mackerel on growth, and on lipogenesis and contents of various lipids in the liver of growing chicks. Jpn. J. Zootech. Sci. 61:1102-1106
Tanaka, K., B. S. Youn, U. Santoso, S. Ohtani and M. Sakaida. 1992 Effects of fermented product from chub mackerel extract on growth, and carcass composition, hepaticlipogenesis and on contents of various oipid fractions in the liver and the thigh muscle of broilers. Anim. Sci. Technol. 63:32-37.
Vega, G. L., F. Grosjek,. R. Wolf and S. M. Grundy. 1962. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J. Lipid Res. 23:811-822.