Effects of Fermented Chub Mackerel Extract on Lipid Metabolism of Rats Fed a High-Cholesterol Diet

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ABSTRACT : The present study was conducted to evaluate effect of fermented chub mackerel extract (FCME) on lipid metabolism of rats fed a diet supplemented with 1% cholesterol. Four week-old male rats were divided into three groups of 15 rats with 0, 0.1% or 0.2% FCME supplementation. In comparison with control, rats fed 0.2% FCME showed reduction of activities of acetyl-Coenzyme A carboxylase ($p<0.05$), 3-hydroxy-3-methyl glutaryl Coenzyme A reductase ($p<0.01$) and fatty acid synthetase ($p<0.01$). Rats fed 0.2% FCME also showed reductions in free cholesterol in the liver ($p<0.05$), and in concentrations of free cholesterol ($p<0.05$), LDL+VLDL-cholesterol ($p<0.05$), triglyceride ($p<0.01$) and phospholipid ($p<0.01$) in the plasma. Plasma HDL-cholesterol concentration was significantly ($p<0.05$) higher in treatment groups as compared with control group. Atherogenic index was also significantly lower in rats fed 0.1% or fed 0.2% supplement diet, whereas bile acid in feces was not significantly affected. The current study showed that 0.2% inclusion level of the fermented chub mackerel extracts might have hypolipidemic properties. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 4 : 516-520*)

Key Words : Fermented Chub, Mackerel Extracts, High Cholesterol Diet, Lipid Metabolism, Atherogenic Index

INTRODUCTION

The physiological actions of various nutrients are the subject of continuing interest, because of the widely held view that diet plays a major role in causation of coronary heart disease and in other chronic disorders (Grundy and Denke, 1990). Numerous studies have revealed that high intakes of dietary cholesterol cause severe hypercholesterolemia and atherosclerosis in many animals including non-human primates (Strong and McGill, 1967). This fact stimulated many investigators to reduce blood cholesterol caused by consuming high-cholesterol diets (Grundy and Denke, 1990).

It is well documented that fermented products would lower body fat accumulation in animals (Chah et al., 1975; Danielson et al., 1989; Imaizumi et al., 1992; Tanaka et al., 1990, 1992; Santoso et al., 1995). In previous papers (Tanaka et al., 1992), the authors showed that fermented chub mackerel promoted growth and reduce abdominal fat accumulation in growing chicks and broilers. Furthermore, fat of broiler thigh meat was also reduced. These investigations, however used diets without cholesterol supplementation. There is no study on effect of a fermented chub mackerel extract (FCME) inclusion on lipid metabolism of rat fed high-cholesterol diet. Therefore, the current study was conducted to evaluate the effect of this product on the lipid metabolism of rats fed a high-cholesterol diet. Because of high peptide contents, fermented chub mackerel extract was assumed to lower fat accumulation and the higher inclusion would result in more reduction of fat accumulation.

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MATERIALS AND METHODS

General procedure

Four-week old Wister line male rats (body weight 103±12 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 15 rats assigned to each treatment. One group was the control with no additive, and two treatment groups were given the purified diet supplemented with 0.1% or 0.2% fermented chub mackerel extract. The rats were raised to 7 weeks of age in individual floor 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 experiments with purified diets. The composition of experimental diets is shown in table 1. Our previous results showed that 1% inclusion of cholesterol to the diet was effective to increase cholesterol contents in the liver, plasma and intestine (Youn et al., 1993). Therefore, this level was used in the present study. Feed consumption and individual body weight were determined weekly. Feed and water were provided for *ad libitum* consumption.

Table 1. The composition of the experimental diets (g/kg dict)

Ingredients	Mackerel extract (%)		
	0	0.1	0.2
Corn starch	450.0	450.0	450.0
Sucrose	239.0	239.0	239.0
Soybean oil	17.5	17.5	17.5
Casein	230.0	220.0	210.0
Mackerel extract	00.0	10.0	20.0
Mineral mixture[1]	40.0	40.0	40.0
Vitamin A, D mixture[2]	1.0	1.0	1.0
Vitamin B mixture[3]	10.0	10.0	10.0
Cholesterol	10.0	10.0	10.0
Cholic acid	2.5	2.5	2.5
Total	1000.0	1000.0	1000.0
Chemical composition			
Protein (%)	23.0	22.5	22.0
ME (kcal/kg)	3,509.9	3,460.9	3,423.1
ME-protein ratio	152.6	153.8	155.6

[1] Supplied 650.0 g $CaHPO_4$, 160.0 g NaCl, 140.0 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 corn starch per 1 gram mixture.

[3] Supplied 0.083 g thiamine-HCl, 0.233 g riboflavine, 0.833 g niacin, 0.75 g Ca-pantothenate, 0.1 g 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 corn starch 47.599 g per 100 g mixture.

Commercial fermented mackerel extracts 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% 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 fermented mackerel extract are presented in table 2. It is rich glutamic acid, glycine, aspartic acid, lysine, arginine, leucine, alanine and proline.

At the end of experiment, all rats were weighed. Ten rats with relatively similar body weight were selected for each treatment and then sacrificed by decapitation. Blood samples were collected from which plasma was extracted later. The liver was immediately removed and weighed. Livers were placed in an ice-cold saline to determine enzyme activities and contents of various lipid fraction. Concentration of various lipid fractions of plasma was also determined.

Enzyme assay

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-CoA carboxylase (*E.C.* 6.2.1.3) activity was assayed by $H^{14}CO_3$-fixation method (Qureshi et al., 1980). Fatty acid synthetase was assayed by 1-^{14}C-acetyl-CoA incorporation method (Hsu et al., 1965). 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase was assayed (*E.C.* 1.1.1.34) by the method of Shefer et al. (1972). The protein content of the solution used for enzyme assay was determined by the method of Lowry et al. (1951) using bovine plasma albumin as a standard. These enzyme activities are expressed as nano or picomole of substrate converted to product per minute per mg protein at 37℃. Samples were analyzed in triplicate and they were accepted if the differences were less than 1%.

Table 2. Amino acid composition of fermented mackerel extract (g/100 g chub)

Amino acids	
Arginine	3.23
Lysine	4.17
Histidine	2.83
Phenylalanine	1.63
Tyrosine	1.25
Leucine	3.31
Isoleusine	1.69
Methionine	1.25
Valine	2.42
Alanine	3.96
Glycine	5.19
Proline	3.06
Glutamic acid	7.50
Serine	2.37
Threonine	2.17
Aspartic acid	4.65
Tryptophan	0.33
Cystine	0.3
Hydroxyproline	0.82
Free cysteine	0.02

Lipid composition and atherogenic index

Venous blood was taken with a heparinized syringe and then centrifuged at 2,500 rpm for 10 min. Plasma obtained was stored and frozen at -30℃ until analysis of various lipid fraction concentrations. The lipid fractions (esterified and free cholesterols, triglyce- ride and phospholipid) were separated by thin layer chromatography on silica gel chromarod using hexane-diethylether-formic acid (60:10:0.1) and hexane-benzene (1:1) as developing solvent and quantified by IATROSCAN TH-10 TLC/FID Analyzer (Iatron Laboratories Inc., Tokyo, Japan) (Santoso et al., 1995).

Concentrations of total plasma 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:

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

Fecal bile acid

Fecal bile acid was measured with a commercial kit (Total bile acid Wako Kit from Wako Junyaku Kogyo Co. Ltd).

Statistical analysis

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 (Yoshida, 1975). Where appropriate, regression analysis was used to assess the statistical significance of the correlation between variables.

RESULTS

Body weight gain, feed intakes and liver weight of rats fed FCME (fermented chub mackerel extract) were not significantly different from the control ($p<0.05$). Feed intake was 19.3, 19.4 and 19.2 g/day/rat; body weight gain was 6.98, 7.06 and 7.03 g/day/rat; and liver weight were 5.57, 5.77 and 5.58 g/100 g body weight for the control, 0.1% or 0.2% groups, respectively.

Table 3 shows the effect of FCME on activities of lipogenic-related enzymes and HMG-CoA reductase in the liver of male rats. Activities of acetyl-CoA carboxylase ($p<0.05$), fatty acid synthetase ($p<0.01$) and HMG-CoA reductase ($p<0.05$) were significantly lower in rats fed the diet with 0.2% fermented chub mackerel extract compared with control group. However, the activities of these enzymes were not significantly different from the control in rats fed 0.1%.

Table 4 shows the effect of fermented chub mackerel extract on contents of various lipid fractions in the liver and plasma. Contents of cholesterol ester, triglyceride and phospholipid in the liver were not affected by the treatments, whereas free cholesterol

Table 3. Effect of dietary mackerel extract on activities of lipogenic related enzymes in the liver of rats fed the high-cholesterol diet[1]

Variable	Treatment (%)		
	0	0.1	0.2
Acetyl-CoA carboxylase (nmol/min./mg protein) *	1.98 ± 0.13^a	1.95 ± 0.14^a	1.77 ± 0.12^b
Fatty acid synthetase (nmol/min./mg protein)**	1.90 ± 0.19^a	1.80 ± 0.17^a	1.53 ± 0.14^b
HMG-CoA reductase (pmol/min./mg protein)*	6.94 ± 0.36^a	6.38 ± 0.37^a	5.89 ± 0.45^b

[1] Values are presented as mean ± SD (n=5 each group); * $p<0.05$, ** $p<0.01$.

Table 4. Effect of dietary mackerel extract on contents of various lipid fractions in the liver and plasma[1]

Variable	Treatments (%)		
	0	0.1	0.2
Liver (mg/g)			
Cholesterol ester	46.62 ± 7.75	44.73 ± 7.63	41.73 ± 7.91
Triglyceride	69.94 ± 3.52	72.17 ± 4.30	73.32 ± 4.91
Free cholesterol**	2.50 ± 0.13^a	2.43 ± 0.23^a	2.24 ± 0.10^b
Phospholipid	35.17 ± 2.20	35.52 ± 1.24	36.77 ± 2.23
Serum (mg/100 ml)			
Cholesterol ester	134.4 ± 14.5	139.9 ± 11.3	137.1 ± 16.9
Triglyceride**	125.5 ± 4.9^a	125.6 ± 9.3^a	80.9 ± 9.4^b
Free cholesterol*	24.8 ± 4.50^a	21.9 ± 3.4^a	18.2 ± 4.2^b
Phospholipid**	285.6 ± 25.40^a	271.2 ± 29.9^a	204.3 ± 21.6^b
Total cholesterol	159.2 ± 21.8	161.8 ± 21.3	155.2 ± 20.3
HDL-cholesterol*	63.2 ± 11.4^a	77.8 ± 12.1^b	79.9 ± 10.9^b
LDL+VLDL-cholesterol	96.0 ± 23.1^a	84.0 ± 24.4^a	75.4 ± 21.1^b
Atherogenic index*	1.52 ± 0.25^a	1.08 ± 0.22^b	0.94 ± 0.25^b

[1] Values are presented as mean ± SD (n=5 each group); * $p<0.05$; ** $p<0.01$.

was significantly reduced in rats fed the diet containing 0.2% fermented chub mackerel extract ($p<0.05$). Cholesterol ester concentration in plasma was not affected, whereas concentrations of triglyceride ($p<0.01$), free cholesterol ($p<0.05$) and phospholipid ($p<0.01$) were significantly lower in rats fed the diet with 0.2% fermented chub mackerel extract as compared with the control. Total cholesterol concentration in the plasma was not significantly affected. In comparison to control group, HDL-cholesterol concentration was significantly higher ($p<0.05$) in both treatments, whereas LDL+VLDL-cholesterol concentration was significantly ($p<0.05$) reduced in rats fed the diet with 0.2%. Atherogenic index was significantly lower in rats fed the diet with 0.1% ($p<0.05$) or 0.2% ($p<0.05$) as compared with the control.

It was shown that fecal bile acid was not affected by the treatments. Bile acid content for each treatment was 110.3, 109.0 and 118.2 mol/day for control, 0.1% and 0.2 % fermented mackerel extract diet.

DISCUSSION

The present study showed that fermented mackerel extract has antilipid properties but with little value in improving performance in rats. This fermented product was produced by fermentation of the enzymatically dissolved chub mackerel. Fermented fish increases the 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 fermented chub mackerel extracts are also peptides with 20-50 chain-length amino acids. Thus, these changes are expected to improve the digestibility of fermented fish and therefore it resulted in slightly higher body weight gain in treatment groups.

It seems that effect of fermented chub mackerel extract was more pronounced in lipid metabolism than in growth performance. Acetyl-CoA carboxylase is suggested as a rate-limiting enzyme in fatty acid synthesis. Therefore, reduced acetyl-CoA carboxylase activity in rats fed the 0.2% fermented mackerel diet would result in lower fatty acid synthesis (Brindley, 1991). This may explain the lower plasma triglyceride concentration in rats fed the 0.2% fermented mackerel diet. At low fatty acid availability the major flux from diacylglycerol is directed to the synthesis of phospatidyl-choline and phosphatidyletanolamine. However, the present study shows that plasma phospholipid concentration was also reduced as well as triglyceride. This indicated that the availability of fatty acid might be very low due to lower fatty acid synthesis.

The activity of hepatic HMG-CoA reductase, which is the rate-limiting enzyme for cholesterol synthesis was reduced in rats fed the 0.2% fermented mackerel diet. This observation was in agreement with previous results (Tanaka et al., 1992) using growing chicks and broiler chicks. However, fermented mackerel extract had no effect on total cholesterol in the liver and plasma. The possibility of lower cholesterol excretion is not favorable explanation for this phenomenon because bile acid content in excreta was not affected. It is known that other organs than liver there is significant synthesis of cholesterol (Lindsay and Wilson, 1965; Wilson, 1968). Therefore, it is possible that cholesterol synthesis in extrahepatic organs might contribute to total plasma cholesterol.

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., 1982; Spady and Dietschy, 1985; Mattson and Grundy, 1985; Fernandeg and McNamara, 1991). Therefore, an increase in plasma HDL- cholesterol with lower or normal LDL-cholesterol would have a beneficial impact on reducing the risk of atherosclerosis. The present study showed that fermented mackerel extract at 0.2% supplementation to a diet increased HDL-cholesterol concentration, with lower LDL-cholesterol in rats fed the high cholesterol diet. This result is in agreement with the observation of Tanaka et al. (1990) who found that mackerel extract at 0.2% resulted in an increase in plasma HDL-cholesterol in growing chicks. The importance of the findings is that this elevation arises from the increase in HDL-cholesterol itself combined with a lowering the concentration of LDL-cholesterol without any change in plasma total plasma cholesterol level. This is an important action and suggests a beneficial effect of mackerel extract on cholesterol metabolism. Higher mackerel extract supplementation would result a higher HDL-cholesterol level ($r^2=0.8426$, $p<0.01$) and lower atherogenic index. Lower atherogenic index indicated a decrease in the risk of atherosclerosis in rats fed fermented mackerel extract.

High plasma triglyceride appears to be an underlying cause of several putative lipid risk factors for coronary heart disease, for instance reduced concentrations of HDL-cholesterol (Ricahards et al., 1989). The present study also showed that there was a negative correlation between plasma triglyceride and HDL-cholesterol ($Y=57.6748-0.2179x$, $r^2=0.3730$, $p<0.05$; where Y=plasma HDL-cholesterol and x=plasma tri-glyceride).

The results of this study show further that the hypotriglyceremic and hypocholesterolemic effects of mackerel extract are dose-dependent, i.e. no decreases

in plasma triglyceride and free cholesterol concentrations were noted among group 2 (0.1% group) rats till the mackerel extract was increased to 0.2%.

It is known that polypeptides, such as macrocortin and chemotactic peptides, inhibit the activity of phospholipase A2 which release arachidonic acid from phospholipids (Blackwell et al., 1980; Hirato et al., 1980). Therefore, peptides contained in the fermented mackerel extracts seem to serve as a suppressor of fat accumulation and cholesterol synthesis. It is necessary to solve the functional mechanism of these effects of peptides derived from the fermented chub mackerel.

In conclusion, the present study showed that fermented chub mackerel extract inclusion at the level of 0.2% might lower the risk of atherosclerosis measured by atherogenic index, and might have hypolipidemic properties.

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