Effects of Fish Protein Concentrate and Lipids Derived from the Muscle of Stromateid Fish, *Cubiceps gracilis* on Growth and Liver Lipids in Rats

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Fish protein concentrate (FPC) and lipids were prepared from the muscle of the stromateid fish, *Cubiceps gracilis*, to investigate their nutritive effects on rats. The FPC was found to rather promote the growth of rats with the protein efficiency ratio 3.84, which is higher than that of casein, 3.26. The muscle lipid represented mainly by diacyl glyceryl ethers (DAGE's) showed a nutritional value comparable to that of cottonseed oil. The rats fed with a DAGE diet accumulated in their liver lipid detectable amounts of DAGE's which were similar in composition to those of the muscle lipid of *C. gracilis*.

Recent fishing efforts toward deep sea have led to a discovery of a variety of fish species which possess wax esters or diacyl glyceryl ethers (DAGE's) as the major lipids^{1,2)}. Since ingestion of wax esters in quantity causes severe diarrhea and seborrhea in humans some of the wax ester-rich fish have been prohibited from selling as food. The safety of DAGE's as food has not yet been confirmed, but it may be possible to use these fish as the raw materials for fish protein concentrate (FPC) because their DAGE content could be reduced by solvent extraction during the preparation process.

In our studies DAGE's and FPC were prepared from the stromateid fish, *Cubiceps gracilis*, and fed to rats to observe their effects on growth and liver lipids of the animals.

Materials and Methods

Materials

Specimen of *C. gracilis* were caught by a mid-water trawler of Nihon Suisan Co., Ltd., off New Zealand and kept frozen until used for extraction.

Preparation of FPC

FPC was prepared by the method of POWER³⁾. A 500 g portion of the minced muscle was extracted with 1.5 l of hot 2-propanol below 80°C for 1 h.

The mixture was centrifuged and the residue was extracted twice as above. The residue thus obtained was dried *in vacuo* at room temperature and powdered by a crusher.

Analysis of FPC

Determination of moisture, ash, lipid, and protein in FPC was carried out by the A.O.A.C. method⁴⁾. Amino acids were determined by a Hitachi amino acid analyzer KLA-5 after hydrolysis in 6 N HCl at 110°C for 24 h.

Characterization of the Lipids in 2-Propanol Extract

The 2-propanol extract was partitioned between diethyl ether and water. The ether layer was analyzed for lipid by the method reported previously^{5,6)}. The lipid extracted from the FPC by the A.O.A.C. method⁴⁾ was analyzed in the same manner. The lipid recovered from 2-propanol extract was used as DAGE's in feeding experiments.

Feeding Test

Male rats of the Wistar strain weighing 63.0-76.5 g were kept on control diet (Table 1) for one week before feeding test. Five rats in each group were fed with 4 different diets (Table 1) for 4 weeks. They were kept in an air-conditioned room at 23°C and with 60% relative humidity during experiment.

Body weight of each rat was recorded everyday

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Table 1. Composition of diets

Ingredient	Control diet (%)	FPC diet (%)	DAGE diet (%)	FPC-DAGE diet (%)
FPC	—	10	—	10
Casein	10	—	10	—
Cottonseed oil	8	8	—	—
DAGE's ^{*1}	—	—	8	8
Salt mixture USP ^{*2}	5	5	5	5
Vitamin mixture ^{*3}	1	1	1	1
Cellulose	1	1	1	1
Water	5	5	5	5
Sucrose	70	70	70	70

^{*1} Ether-soluble lipids from 2-propanol extract.

^{*2} Consists of 139.3 g NaCl, 0.79 g KI, 389.0 g KH₂PO₄, 57.3 g MgSO₄ anhydride, 381.4 g CaCO₃, 27.0 g FeSO₄·7H₂O, 4.01 g MnSO₄·H₂O, 0.548 g ZnSO₄·7H₂O, 0.477 g CuSO₄·5H₂O and 0.023 g CoCl₂·6H₂O.

^{*3}

	mg/100 g
Vitamin A (dry, stabilized)	2000 (IU)
Vitamin D (dry, stabilized)	200 (IU)
Vitamin E (dry, stabilized)	10
Menadione	0.5
Choline	200
p-Aminobenzoic acid	10
Inositol	10
Niacin	4
Ca D-pantothenate	4
Riboflavin	0.8
Thiamine·HCl	0.5
Pyridoxine·HCl	0.5
Folic acid	0.2
Biotin	0.04
Biotin B ₁₂	0.003
Glucose to make	1000

during a period of 4 weeks. Protein efficiency ratios (PER) were calculated according to the method of A.O.A.C.⁽⁷⁾.

Analysis of Lipid Composition of the Rat Liver

On the last day of feeding test the rats were sacrificed, and the pooled liver specimen from each group was analyzed for lipid composition by a Florisil column chromatographic method or by TLC as reported previously^{6,8)}.

Results and Discussion

Recovery and Properties of FPC

The recovery of FPC amounted to 20% of the total fish muscle. *C. gracilis* FPC contained 82.3% of protein and 5.1% of moisture; these values resemble those of FPC's prepared from usual fish⁹⁾. Its lipid and ash contents scored 10.7% and 5.8% respectively. Although 19.0% of the lipids in raw material was excluded during preparation of FPC, the lipid content was still high as compared to FPC products from other

fish species⁹⁾. Amino acid composition of *C. gracilis* FPC (Table 2) indicates that it contains basic amino acids 1.5–2.5 times as much as those in usual fish muscle^{9–11)}. It should be noted that the content of histidine is 4 times higher than that in FPC's from herring, hadock or anchovy¹²⁾.

Table 2. Amino acid composition of FPC prepared from the stromateid fish

Amino acids	Content (g/100 g protein)
Glycine	6.29
Alanine	7.90
Valine	7.68
Leucine	10.21
Isoleucine	3.96
Serine	4.98
Threonine	5.50
Methionine	1.26
Aspartic acid	11.15
Glutamic acid	17.34
Tyrosine	3.96
Phenylalanine	5.22
Proline	4.06
Tryptophan*	2.47
Arginine	9.73
Lysine	13.85
Histidine	10.75

* Determined by the method of GOODWIN and MORTON¹⁶⁾.

Lipid Composition of FPC and of the Fat Soluble Fraction of 2-Propanol Extract

As shown in Table 3, *C. gracilis* FPC contained 10.7% of lipids; the content of lipids in 2-propanol extract was 19.0%. Both lipids possess properties similar to each other (Table 3).

Table 3. Properties of lipids in FPC and the 2-propanol extract of the stromateid fish

Components	FPC (%)	DAGE's* (%)
Lipid content	10.7	19.4
Unsaponifiables content in lipid	24.8	26.4
In unsap. { Hydrocarbons	0.7	0.4
{ Sterols	1.2	2.7
{ GE's	83.7	86.9
{ Unknown	5.9	3.1

* Ether-soluble lipids from 2-propanol extract.

Growth of Rats

The weight gain, food intake, and protein efficiency ratio are seen in Table 4. Rats in each group showed normal appetite during feeding experiment and no abnormal signs in appearance and in internal organs except for one rat in DAGE

Table 4. Body weight and protein efficiency ratio (PER)

Group	Initial body weight (g)	Weight gain (g)	Food intake (g)	Protein intake (g)	PER
Control	63.0	88.0	312.9	28.2	3.13
	67.5	95.5	308.9	27.8	3.44
	68.0	96.0	297.9	26.8	3.58
	68.5	83.0	306.3	27.5	3.01
	75.5	88.5	315.3	28.4	3.12
Mean	68.5	90.2	308.3	27.7	3.26
FPC	65.0	93.5	307.1	25.3	3.77
	69.0	105.0	314.5	25.9	4.06
	64.0	93.5	305.9	25.2	3.71
	72.0	99.5	305.3	25.1	3.96
	74.0	96.5	315.8	26.0	3.71
Mean	68.8	97.6	309.7	25.5	3.84
FPC-DAGE	71.5	92.5	315.1	25.9	3.57
	65.0	85.5	275.4	22.7	3.77
	66.5	84.0	286.2	23.6	3.57
	67.0	86.5	303.5	25.0	3.46
	76.5	84.5	302.0	24.9	3.40
Mean	69.3	86.6	296.4	24.4	3.55
DAGE	68.0	87.0	308.6	30.2	2.88
	68.5	88.5	303.6	29.7	2.98
	70.5	86.5	301.8	29.5	2.93
	70.5	80.0	308.6	30.2	2.65
	75.0	94.0	317.8	31.1	3.03
Mean	70.5	87.2	308.1	30.1	2.89

diet group that lost its hairs in the hind limb but recovered by the 23rd day. Rats in FPC and FPC-DAGE diet groups gave PER's of 3.84 and 3.55, respectively, which are greater than that of control group, 3.26. This suggests that *C. gracilis* FPC is superior to casein as a protein source. In contrast to the control group, a casein diet containing DAGE gave a lower PER of 2.89. It is not clear whether this is due to the depression of growth of rats by DAGE's.

ACKMAN *et al.*¹³⁰ obtained a similar result in feeding experiment of FPC prepared from the white barracudina, *Paralepis rissol Krøyeri*; 2.81 for PER of FPC and 2.50 for that of casein. On the contrary, KINUMAKI *et al.*¹⁴⁰ found that fish meal prepared from the flesh of *Centrolophus niger*, one of the stromateid fish, showed a PER as low as 0.78. KINUMAKI *et al.*¹⁴⁰ reported that no difference occurred in growth of rats fed with either soybean oil or lipids obtained from the *C. niger* muscle by hot benzene extraction. However, KANEDA *et al.*¹⁴¹ observed that DAGE's obtained by molecular distillation from liver oils of the shark, *Chimaera barbouri*, were well below soybean oil in nutritive value for rats, though less hazardous

to test animals than wax esters.

Liver Lipids

The rats in both DAGE and FPC-DAGE groups were found to contain more lipids in the liver than the control group, as shown in Table 5.

Table 5. Liver lipids of rats fed with 4 diets*

	Control group	DAGE group	FPC-DAGE group	FPC group
Mean liver weight (g)	4.4	5.4	5.4	5.7
Lipid content in liver (%)	3.0	4.7	5.2	3.9
Unsaponifiables content in lipid (%)	3.3	3.4	14.9	tr.
GE contents in unsaponifiables (%)	0	20.9	14.6	tr.

* See Table 1.

From these results, it is apparent that rats accumulate lipids in the liver from diet; more lipids are accumulated from higher lipid containing-diet. This is also true for DAGE's. A great quantity

Fig. 1. Thin layer chromatograms of lipids obtained from FPC and rat liver, and 2-propanol extract from stromateid muscle.

- 1: Acetone soluble lipids in FPC.
- 2: 2-Propanol extract from stromateid muscle.
- 3: Acetone soluble liver lipids of control diet group.
- 4: Acetone soluble liver lipids of FPC diet group.
- 5: Acetone soluble liver lipids of DAGE diet group.
- 6: Acetone soluble liver lipids of FPC-DAGE diet group.

of glyceryl ethers (GE's) ranging from 15 to 20% of unsaponifiables were accumulated in the liver of rats fed with the diet containing DAGE's. On the other hand, only a trace of DAGE's was detected in the liver of FPC group by Florisil column chromatography, although FPC contained considerable amounts of DAGE's. No DAGE was detected in the liver of the control group by TLC (Fig. 1). The GE composition of liver lipids from DAGE and FPC-DAGE groups showed a similarity to each other (Table 6). Liver lipids exhibited GE composition resembling those of lipids from FPC and 2-propanol extract except for $C_{18:0}$ that was contained in smaller percentages. This indicates that $C_{18:0}$ component may be metabolized more rapidly than other components in the rat liver.

It comes to a conclusion that fish such as *C. gracilis* having DAGE's as the major lipids in the muscle may be acceptable as a material for FPC. However, further examination on their toxicity is required since IIDA¹⁾ reported that the muscle of *Stromateus maculatus* with a large quantity of DAGE's might cause diarrhea in humans.

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Table 6. Glyceryl ether compositions of the lipids of FPC (A), the 2-propanol soluble lipids from stromateid muscle (B), and the acetone soluble lipids from livers of rat fed with two different diets (C)

GE*	A	B	C	
			DAGE diet group	FPC-DAGE diet group
12:0 br	—	—	0.3	1.3
13:0 br	—	—	0.2	2.1
13:1	—	—	0.1	tr.
14:0 br	—	—	0.2	2.6
14:0	3.3	4.8	—	—
14:1	0.1	0.4	0.6	0.6
15:0 br	0.1	0.1	1.3	tr.
15:0	1.6	1.7	1.0	3.6
15:1	—	—	—	0.5
16:0 br	0.4	0.1	0.7	0.4
16:0	34.9	33.5	24.8	20.3
16:1	3.8	7.4	2.4	1.8
17:0 br	1.2	1.3	0.7	1.6
17:0	1.0	2.3	—	4.9
17:1	1.2	1.5	—	—
18:0 br	1.7	0.7	1.2	1.5
18:0	2.6	3.3	6.4	9.6
18:1	44.6	41.1	54.9	38.8
18:2	1.0	—	—	—
19:0	—	—	—	3.0
19:1	0.6	—	—	—
20:0 br	—	—	5.0	—
20:1	2.0	0.3	—	6.8
21:0	—	1.3	—	—

* Indicated by carbon chain length and double bond of the alkyl moiety.

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