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Blood Properties of Cultured Yellow Tail, *Seriola quinqueradiata* TEMMINCK and SCHLEGEL

I Effect of Disease Caused by Anchovy, *Engraulis japonica* HOUTTUYN, as Diet.

Kenji NANBA, Heisuke NAKAGAWA, Shiro MURACHI, Mitsu KAYAMA and Tatsuo NAKAMURA*

Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama;
(Figs. 1-3, Tables 1-3, Photo. 1)

A disease of cultured yellow tail fed on anchovy alone for a long time has been reported by several groups of workers[1)2)3)4)5)]. Untill recently there have been differences of opinions among them on the factor contained in anchovy which causes this disease: KIMURA[1)] (1963) and OKA[4)] (1967) stated that it might be the toxicity of peroxide originated from polyunsaturated fatty acid, while KITAJIMA *et al.*[5)] (1970) suggested vitamin B_1 deficiency caused by thiaminase as the reason. All of them, however, agreed to the finding of a serious liver damage in the sick fish by means of both gross pathological and histopathological examinations. The present investigation, therefore, was undertaken in order to confirm the effect of the disease on the blood properties of yellow tail and in an attempt to make this factor clear.

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MATERIAL AND METHODS

The material for this study were yearling yellow tails collected from the coast of the Japan Sea near Yamaguchi Prefecture in June 1972. Untill the beginning of the experiment, these fish were fed on sand launce (*Ammodytes personatus*

* *Yamaguchi Prefectural Gaikai Fisheries Experimental Station, Nagato, Yamaguchi Pref.*

GIRARD) and were reserved in a floating net (4×4×3 m) set up at the shore adjacent to the Yamaguchi Prefectural Gaikai Fisheries Experimental Station. This stock was divided into two groups on the 10th of July, 1972.

Each group consisted of 200 fish also kept in other floating nets (2×2×3 m) set up at the same shore. One group was fed on anchovy and was maintained as experimental group. Another group was fed on sand launce for control.

Both groups were given an amount of 8–18 % of their body weight of the diets, twice a day. The experiment was carried out from 18th July, 1972 through 6th September, 1972. Water temperature during this period was between 23.8°C and 28.3°C. Twenty fish were sampled at random out of each group, their standard length and body weight were measured individually every ten days. After the measurements, they were returned to their own nets.

For the blood sampling, 10 fish from each group were killed at each sampling. This sampling was performed on the 18th July, 22nd August and 6th September. Before sampling the fish were anesthetized in 1:20,000 tricanesulphonate (MS–222, Sandoz). Blood was collected from the Cuvierian duct with a 2m*l* disposable plastic syringe and a 23 gauge disposable needle, rinsed in advance with a stable heparin anticoagulant solution (Anticlot, Clinton Laboratories) to prevent blood coagulation. Immediately after the blood sampling, the fish was dissected and anatomical observations of various organs, such as liver, kidney, spleen and digestive tract were made. Particularly the liver was removed and was weighed on a torsion balance in order to see the relationship between liver weight and standard length.

The blood sample thus collected was used for determination of hemoglobin concentration and hematocrit value. The rest of the sample was centrifuged for 10 minutes at 2,000 rpm in order to obtain the plasma for the determination of total plasma protein concentration, A/G ratio and total plasma cholesterol concentration. The plasma also was used for electrophoretic analysis of the protein and thin-layer chromatography of the lipid.

The details of the methods for blood analysis are as follows.

Hemoglobin concentration

Hemoglobin concentration was determined by using Spencer's Hb-meter (American Optical Corporation). The application of this instrument to fish blood was studied by MATSUZATO *et al.*[6] (1971).

Hematocrit value

A small amount of the blood drawn into a capillary tube was centrifuged for 5 minutes at 12,000 rpm in a Microhematocrit Centrifuger (Kokusan H25–D). The

value of hematocrit was then measured by means of a special type of scale designed for the measurement of hematocrit value.

Total plasma cholesterol concentration

Total plasma cholesterol concentration was determined by ZAK-HENLY's[7)8)] method.

Total plasma protein concentration and A/G ratio

Total plasma protein concentration and plasma albumin concentration were determined by the Biuret's method and the BCG (bromcresol green) method[9)] respectively. Plasma globulin concentration could be obtained by subtracting plasma albumin concentration from that of the total plasma protein. A/G ratio was then calculated from the concentrations of both plasma albumin and plasma globulin.

Electrophoretic analysis of the plasma proteins

Plasma 8μl was spotted on a cellulose acetate film (Sartorius Membranfilter) of 7 cm in length and 3 cm in width, and the electrophoresis was carried out in a field strength of 0.8 mA per cm width for 45 minutes in veronal buffer of pH 8.6 and ion strength 0.06. The cellulose acetate film was stained with ponceau 3R for the protein fraction, and with Schiff's reagent after preliminary ozonization for the lipid containing fraction according to the method of KOHN[10)] (1961).

A quantitative analysis of the electrophoretic pattern was measured by using a Ozumor Densitometer 82 (Asuka Kogyo Co.) after an application of decahydronaphthalene to make the film transparent.

Thin-layer chromatography of plasma lipid

The lipid was extracted from plasma by the method of BRIGH and DYER[11)] (1959) using methanol and chloroform, then the extract was concentrated under nitrogen stream.

The lipid class composition was examined by thin-layer chromatography on a plate covered with silicagel G (E. Merck, Darmstadt). The solvent system used was petroleum ether-ether-acetic acid (85:15:1, v/v/v).

The plates were developed by spraying 70 % sulfuric acid saturated with potassium dichromate and charring at 120°C. Then the plates were applied for densitometory to the Ozumor Densitometer 82.

RESULTS AND DISCUSSION

The results obtained on the growth rate and the accumulated mortality from the two groups during the course of the experiment are presented in Fig. 1. Mortality in both groups for the first 10 days could not have been caused by the disease

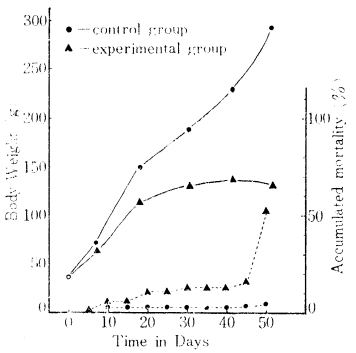

Fig. 1 Growth rate and accumulated mortality of yellow tail in the control and experimental groups during the course of experiment. —— Growth rate, ····· Accumulated mortality

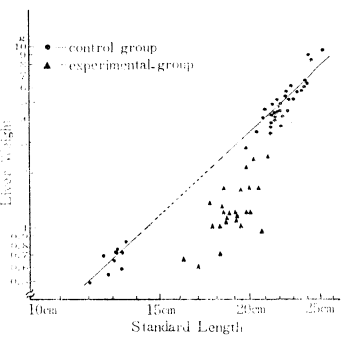

Fig. 2 The relationship between liver weight and standard length of yellow tail

Table 1. Blood Properties of Yellow Tail

Treatment	Body Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Plasma Protein (g/dl)	A/G	Cholesterol (mg/dl)
			Initial			
	37.9±7.00	44.0±5.23	7.5±0.82	5.2±0.59	——	262±64.4
			35 days			
Control	197±19.4	38.5±6.43	7.7±0.95	5.9±0.66	0.76±0.19	440±84.9
Experiment	113±21.3	31.4±5.68	6.5±1.67	4.1±0.84	0.74±0.27	294±96.4
			50 days			
Control	286±55.1	53.7±11.31	11.6±2.38	7.0±0.9	0.38±0.08	475±67.1
Experiment	136±42.6	44.3±11.64	9.9±2.19	3.9±1.3	0.55±0.18	229±165

because no symptoms of the disease could not be found nowhere. Most of the fish in the experimental group did not accept the diet readily from the 10th day and their growth rate began to slow down. After about 20 days, some of them exhibited such symptoms of the disease as abnormal swimming, change of body color, and atrophy of liver. Then mortality started on the 20th day and sharply increased after 45 days. It reached about 50% of the experimental group on the 50th day. The dead fish always showed the remarkable change of body color, severe atrophy of the liver and sometimes protrusion of the eye-balls.

A strong correlation was observed between the liver weight and the standard body length in both groups, i. e. the liver weight increased with the standard body length (Fig. 2). The liver weight corresponding to the standard body length in

the experimental fish was lower than that in the control fish. According to this finding, the liver atrophy of the fish in the experimental group could be determined quantitatively.

Results of the determinations on blood properties of the two groups are shown in Table 1. The observed differences in the blood properties of the two groups are as follows. Hemoglobin concentration and hematocrit value of fish in the experimental group were slightly lower in averages than those of the control group, on the 35th and 50th day. Those differences, however, were not significant because of their standard deviations.

SNIESZKO[12] (1960) found out that the use of a microhematocrit was a simple, accurate and rapid method for detection of ordinary anaemia in fish. In the present examination, however, comparison of observed hematocrit value between the two groups did not reveal the anaemia of yellow tail in the experimental group.

Total plasma cholesterol concentration of fish in the control group increased with their growth from 262 ± 64.4 mg/d*l* at the initial of experiment to 440 ± 84.9 mg/d*l* on the 35th day and later on still increased slowly. SAKAGUCHI *et al.*[13] (1969) obtained the higher concentration of total serum cholesterol from yellow tail fed on sand launce for 93 days which was 643 mg/d*l* on the 70th day and 738 mg/d*l* on the 93rd day.

On the other hand, in the experimental group they were almost unchanged and were remarkably lower than those in the control group.

Total plasma protein concentrations of fish in the control group increased during the period of experiment, while in the experimental group the reverse happend. The decrease of total plasma protein in the experimental group was accompanied by the condition of disease. Therefore, there was a difference of 3.1 g/d*l* on an average on the 50th day. The decrease of A/G ratio of both groups was observed also. The average ratio for A/G in the experimental group was higher than that of the control group. The remarkable decrease of the total plasma protein concentration in the experimental group seemed to be due primarily to a decrease of the globulin fraction, as concluded from the results of A/G ratio.

The results above described, suggested the necessity of further examinations on the pattern of plasma lipid and plasma protein of fish in both groups. The protein and the lipid in the plasma sampled after 50 days, accordingly, were analyzed by using electrophoresis and the thin-layer chromatography respectively. Results of the analyses were as follows.

Cellulose acetate electrophoresis separated the plasma protein of yellow tail in

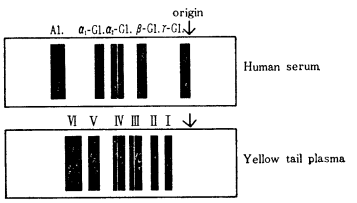

Fig. 3 Schematic electrophoretic diagram on cellulose acetate film of yellow tail plasma as compared to normal human serum. Veronal buffer pH 8.6, a current intensity 0.8 mA/cm for 45 minutes.

Table 2. Protein distribution in yellow tail plasma

	Control		Experiment	
Total protein	7.0±0.9 g/dl		3.9±1.3 g/dl	
A/G	0.38±0.08		0.55±0.18	
Fraction	%	g/dl	%	g/dl
I	2.8±0.9	0.20±0.06	5.6±5.2	0.22±0.20
II	13.7±3.9	0.96±0.27	16.4±4.0	0.64±0.16
III	33.0±3.0	2.31±0.21	24.4±6.2	0.95±0.24
IV	17.9±5.3	1.25±0.37	17.4±4.3	0.68±0.17
V	23.5±6.6	1.65±0.46	27.6±8.8	1.08±0.34
VI	9.4±4.4	0.66±0.31	8.6±5.8	0.34±0.23

the control group into six majar fractions. The typical electrophoretic pattern of healthy yellow tail was shown with that of normal human serum in Fig. 3. The variation of protein fractions depending upon the sex was not taken into consideration in the present study. The nomenclature of protein fractions obtained from yellow tail plasma was so difficult that the zones of six fractions were designated by numbering as shown in Fig. 3. Plasma fractions III and IV consisted of two sub-fractions which showed similar electrophoretic mobility and indistinct separation from each other. Data concerning the percentage and the concentration of six fractions were presented in Table 2. Only slight differences in the plasma protein composition between two groups were detected by electrophoretic analysis. The electrophoretic pattern of the control group differed from the experimental group, especially on plasma fraction III. The lipid staining-band of fish in the control group was found in plasma fraction IV, VI, and origin (Photo. 1).

The main lipoprotein fraction agreed to plasma protein fraction IV and the others showed individually various intensity of staining. The distribution of lipoprotein in yellow tail plasma was different from that in human serum. In human serum there are four lipoprotein fractions, while in the case of the yellow tail plasma, at least three or four fractions reacted as lipoproteins. The greatest difference between the two groups was observed in the intensity of lipid-staining, i. e. the experimental group frequently lacked a lipoprotein corresponding to plasma fraction IV, moreover the intensity of lipid-staining was faint and its position on cellulose acetate film fluctuated among III, IV, V, and VI. GORDON[14] (1955) reported that the binding of unesterified fatty acid to serum lipoproteins increased their mobility. Therefore, the fluctuation of mobility of lipoprotein would depend upon variability of the composition of lipid conjugated with the protein. The probable decrease of

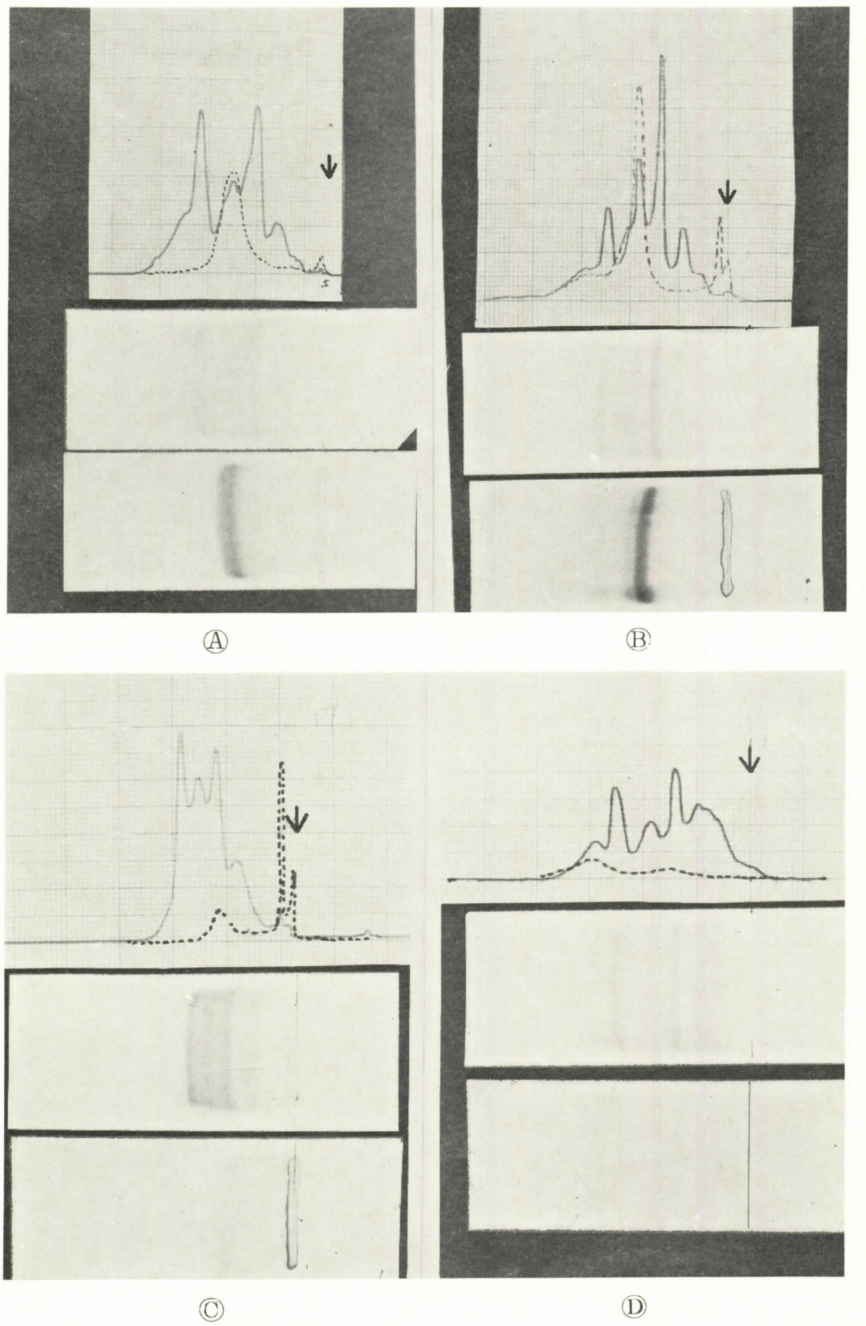

Photo 1 Electrophoretic pattern and their densitometry of yellow tail plasma obtained by staining with ponceau 3R and Schiff's reagent after ozonization. The arrow indicates the point of application of plasma. The solid line represents the densitometry of protein, and the dotted line of lipid.

A and B : control group, C and D : experimental group.

plasma lipid in the experimental group was suggested to be due to a lower degree of staining intensity of lipid and the decrement of total plasma cholesterol concentration. Therefore, it was evident that a marked decrease of lipoprotein occurred. The lipid found on a origin of a cellulose acetate film proved the possibility of the presence of a chylomicron-like substance in the plasma of the yellow tail.

The lipid band on the origin appeared in many samples of the control group, but only in a few of the experimental group.

By thin-layer chromatography the plasma lipids obtained from yellow tail of the control and the experimental groups were, respectively, fractionated into several lipid classes. The lipid of the healthy individuals was fractionated into cholesterol esters, wax esters, triglycerides, fatty acids, fatty alcohols, sterols (mainly cholesterol), partial glycerides, and phospholipids. The percentage composition of the lipid classes was computed by the densitometer, as shown in Table 3. An abundance of triglycerides was observed in the control group. However, in the expermental group it was noteworthy that wax esters showed relatively a high concentration. Concerning the lipid classes of the experimental group, the proportions of triglycerides and phospholipids were characterized by low value, while wax esters and fatty alcohols had high values. Furthermore, triglycerides of the experimental group showed three separable fractions which seemed to depend upon the difference of unsaturation of fatty acids. The lipid composition of plasma was similar to that of the body lipid. In the lipid class compositions no remarkable difference was observed between sand launce diet and anchovy diet for yellow tail, yet a slight difference between their fatty acid compositions was observed.

Table 3. Plasma lipid composition of yellow tail

	Control	Experiment
phospholipids	15.3±3.4%	10.7±3.5%
partial glycerides	3.8±1.3	6.9±3.5
cholesterol	14.2±2.4	14.2±3.8
unknown	0	0.7±1.0
fatty alcohols	3.6±1.6	6.7±2.7
fatty acids	3.3±1.1	3.6±2.7
unknown	0.2±0.1	1.3±1.0
triglycerides	35.4±5.2	20.5±8.9
wax esters	8.0±2.2	17.1±7.0
cholesterol esters	16.3±3.6	18.3±4.0

The anchovy was relatively rich in such polyunsaturated fatty acids as eicosapentaenoic and docosahexaenoic acid.[15]

The interpretation of the decrease of plasma lipid in these pathological conditions was made difficult through the lack of basic information about fish plasma.

However, from the above mentioned experiment it seems to result that fish fed on anchovy will fall in diseases with respect to the plasma lipid and protein metabolism.

SUMMARY

Investigations have been carried out in view of verifying the effects of the disease caused by anchovy, *Engraulis japonica,* as a diet, on the blood properties of cultured yellow tail, *Seriola quinqueradiata.*

Yearling yellow tail were fed for 50 days with two different diets, i. e. one was anchovy as a experimental diet and the other was sand launce, *Ammodytes personatus,* as control.

The results can be summarized as follows:

i) Fish fed on anchovy showed a loss of appetite and a retarding growth after about 10 days; exhibited abnormal swimming patterns, change of body color, and atrophy of the liver after about 20 days; mortality mounted to about 50% at the end of 50 days while that of control group remained at 5% level. The dead fish showed a remarkable change of body color, severe atrophy of the liver, and protrusion of the eye-balls. The liver weight of fish fed on anchovy was smaller than that of control fish.

ii) Hemoglobin concentration and hematocrit value did not differ significantly in both groups.

iii) Total plasma cholesterol concentration of fish fed on anchovy stayed almost unchanged with their growth, while those of the controls increased.

iv) Total plasma protein and A/G ratio of fish fed on anchovy decreased with the progress of the disease. Those decreases both in total plasma protein concentration and A/G ratio indicated a decrease of globulin fraction. A/G ratio of control group, however, decreased more noticeably.

v) Cellulose acetate electrophoresis separated the plasma protein of yellow tail into six major fractions. The electrophoretic pattern varied especially on plasma fraction III.

vi) The main lipoprotein fraction agreed to the plasma fraction IV. Fish fed on anchovy freqnently lacked a lipoprotein corresponding to plasma fraction IV.

vii) The considerable decrease of plasma lipid of the fish fed on anchovy was judged to be due to a lower degree of staining intensity for lipid and to the decrement of total plasma cholesterol concentration.

viii) In the lipid classes of fish fed on anchovy, low proportions of triglycerides and phospholipids and high proportions of wax esters were noticed.

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養殖ハマチの血液性状

I カタクチイワシ投餌の影響

難波憲二・中川平介・村地四郎
鹿山 光・中村達夫

養殖ハマチの主たる餌料であるカタクチイワシに起因する食餌性疾病の本態を究明するために，200尾のハマチにカタクチイワシを単一投与し，これを試験群とし，同時にイカナゴを単一投与した200尾のハマチを対照群として，50日間の飼育を行い，試験群に生ずる食餌性疾病の影響を血液性状，成長，肝蔵重量等について調べた．得られた結果を以下に示す．

1）試験群では実験開始10日後には摂餌の不活発，成長の遅れ，軽度の肝萎縮等が認められ，20日後には食餌性疾病による斃死が始まり，実験終了時までに試験群の約50%が斃死した．斃死魚に見られる特徴は，体色の変化（主として黒変），肝萎縮，眼球突出などであった．

2）試験群は血漿蛋白質量の減少及び低い血漿総コレステロール量を示したが，ヘモグロビン量及びヘマトクリット値については，斃死が盛んな時期においても，対照群に比較して有意な差を示さなかった．

3）試験群においては，血漿蛋白質量の減少及びA/G比の変化から，グロブリン画分の減少が推測された．

4）ハマチの血漿蛋白質には電気泳動的に，6成分が認められ，両群においてⅢ画分の組成比の変化が認められた．

5）リポ蛋白質画分は対照群ではⅣ画分に相当したが，試験群では一定せず，Ⅲ，Ⅳ，Ⅴ，Ⅵ画分にわたり不明瞭な脂質の染色が認められた．

6）試験群においては，セルローズアセテート膜上の，リポ蛋白質の染色が不明瞭である事，及び血漿総コレステロール量の減少から血漿脂質の減少が考えられた．

7）試験群の脂質のＴＬＣから，トリグリセライドの著しい減少と，それに相対的なワックスエステルの増加が認められた．