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Effect of Starvation on Hematological Characteristics, and the Contents of Chemical Components and Activities of Enzymes in Blood Serum of Red Sea Bream *

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This study was conducted to determine the effect of starvation on the hematological characteristics, and the contents of chemical components and activities of enzymes in blood serum of red sea bream, *Ckrysopkrys major*. Wild fish were starved after the net fishing for 90 days at 13318°C. At each fixed day (0, 1. 2, 4. 9, 15, 30, 70 and 90th day), ten fish were presented for the hematological examinations and the biochemical analyses of blood serum. Hemoglobin content, hematocrit value, red blood cell count, and specific gravity of the whole blood increased early in the starving period and then decreased, and the fish exhibited a serious anemia. From the mean corpuscular constants (MCH. MCV and MCHC), the anemia was categorized as the hyperchromic microcytic type. Furthermore, it should be noted that the anemia was accompanied by the disappearance of immature erythrocytes. The contents of total protein and total cholesterol, and the activity of LAP in serum, also increased early in the starving period and greatly decreased later. BUN in serum was stable at a high level for some time after the abrupt increase, and decreased thereafter. The activities of GOT, GPT, and LDH in serum did not change till the 30th day, before the remarkable increase set in. Though the changes of the contents of glucose and total bilirubin, and the ALP activity in serum were recognized during the starvation, those levels were within the normal range.

INTRODUCTION

Numerous studies have reported that the majority of fishes suffering from nutritional and microbiological diseases lose their appetite, and these fishes may be easily assumed to have been in a malnutritional state. Accordingly, to more positively analyze the symptoms recognized in the diseased fishes, the effect of starvation on the hematological characteristics and the levels of chemical components and activities of enzymes in blood serum of red sea bream was examined in the present study.

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* Contribution from Fish. Res. Lab., Kyushu Univ., No. 126. This work was orally presented to the Annual Meeting of Japan. Soc. Sci. Fish., at Tokyo in April, 1976.

MATERIALS AND METHODS

Wild red sea bream (fork length: 11.6–16.1 cm) were used immediately after the net fishing. Fish were starved for 90 days in 1501 aquaria with a flow rate of approximately 1501 per hour. The water temperature dropped from 18°C to 13°C during the starving period. At each fixed day (0, 1, 2, 4, 9, 15, 30, 70 and 90th day), ten fish were selected, and the blood was sampled from each fish by a cardiac puncture. A portion of the whole blood was subjected to the determination of hemoglobin content (Hb), hematocrit value (Ht), red blood cell count (RBC), specific gravity, and percentage of immature erythrocytes. Serum from the remaining blood was applied to determine the contents of total protein, total bilirubin, total cholesterol, urea-N (BUN), and glucose, and the activities of glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and leucine aminopeptidase (LAP). Hb and Ht were determined by the cyanmethemoglobin method (Wintrobe, 1956) and microhematocrit method (Hesser, 1960), respectively. Erythrocytes were counted on a hemocytometer (improved Neubauer type) after dilution in a Thoma pipette with the physiological salt solution for marine teleostei (Hesser, 1960). From these values, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated. Specific gravity was determined by the copper sulfate method (Phillips et ***al.,*** 1950). Immature erythrocytes were counted on a smeared preparation stained with May-Giemsa solution (Yuki, 1963; Enomoto, 1969). The contents of total protein, total bilirubin. total cholesterol, BUN, and glucose, and the activities of GOT, GPT, ALP, LDH, and LAP were determined with Rapid Blood Analyzer 3010 and Unikit (Chugai Pharmaceutical Co.).

RESULTS AND DISCUSSION

Hb, Ht, RBC, and specific gravity of the whole blood temporarily increased early in the starving period, and then decreased (Table 1 and Figs. 1, 2). At the 70th and 90th day, the fish showed a serious anemia. MCH was stable during the starvation. However, MCV decreased and MCHC increased gradually (Table 1 and Fig. 3). From these results, the anemia recognized in starved red sea bream is categorized as the hyperchromic microcytic type, which was observed in fasted rainbow trout, ***Salmo*** *gairdneri* (Kawatsu, 1966; Kawatsu, 1974). Immature erythrocytes immediately decreased and disappeared at the 30th day (Table 1 and Fig. 4). The disappearance of immature erythrocytes indicates the disruption of hematopoiesis. The poor hematopoiesis may be caused by the non-administration of dietary protein, which is required to synthesize the hemoglobin. And the poor hematopoiesis and the destruction of erythrocytes may cause a serious anemia recognized during the prolonged starvation. The contents of total protein and total cholesterol, and the activity of LAP in serum, also increased early in the starving period and then decreased (Table 2 and Fig. 5). Total protein in serum could decrease

Table 1. Effect of starvation on hematological characteristics of red sea bream.

Days of starvation	0	1	2	4	9
Hb (g/dl)	6.7 ±0.7[1]	6.8 ±0.7	7.3 ±0.7	7.4 ±0.7	7.3 ±0.9
Ht (%)	28.2 t2.8	28.6 ±3.0	29.3 ±2.5	28.6 ±3.1	29.4 t3.2
RBC (x 10^6/mm³)	3.03±0.15	3.11±0.28	3.35±0.30	3.27±0.29	3.44±0.40
MCH (γγ)[2]	21.70±1.22	22.01±1.23	21.71±1.61	23.05±1.25	22.06±2.57
MCV (μm³)[3]	91.32±5.03	92.28±5.69	87.50±4.62	86.01±4.57	86.50±6.10
MCHC (%)[4]	23.78±0.10	23.93±1.93	24.82±1.53	26.10±1.21	24.93±1.38
Specific gravity (x-l. 0) x 1000	41-t-2	42±2	43±2	43±3	44±3
Immature erythrocytes (%)	8.1	7.3	3.4	3.9	0.7

Days of starvation	15	30	70	90
Hb (g/dl)	6.9 ±0.6	7.1± 0.7	6.3 10.7	6.6 ±1.1
Ht (%)	27.8 ±2.9	26.1 ± 2.9	21.5 ±2.5	22.7 14.4
RBC (x 10^6/mm³)	3.22 ±0.31	3.21± 0.34	2.67±0.34	2.87±0.48
MCH (γγ)	22.06±1.74	21.92± 3.28	24.05±2.12	23.1310.88
MCV (μm³)	86.33±4.05	80.41±13.14	81.95±6. 5 7	78.98±4.98
MCHC (%)	25.35-t1.87	27.18± 1.22	29.31±1.38	29.34±1.51
Specific gravity (x-l. 0) x 1000	43±3	40±3	37±3	38±5
Immature erythrocytes (%)	0.2	<o.1	<0.1	<0.1

[1] Standard deviation. [2] Mean corpuscular hemoglobin : Hb(g/dl) x 10 / RBC(x 10^6/mm³). [3] Mean corpuscular volume : Ht(%) x 10 / RBC(x 10^6/mm³). [4] Mean corpuscular hemoglobin concentration : Hb(g/dl) x 100 / Ht(%).

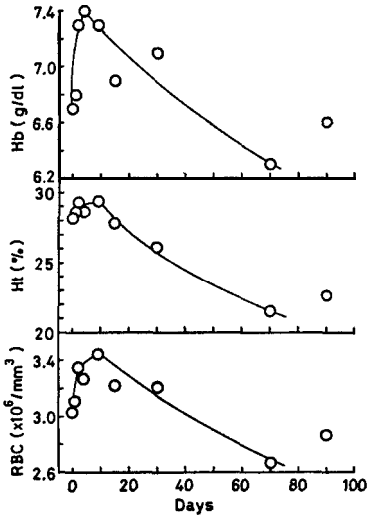

Fig. 1. Change of the hemoglobin content. hematocrit value, and red blood cell count of red sea bream during the starving period.

by the deficiency of amino acids required to compose the protein. Also, the serum cholesterol could decrease by the lack of sterol absorption through the

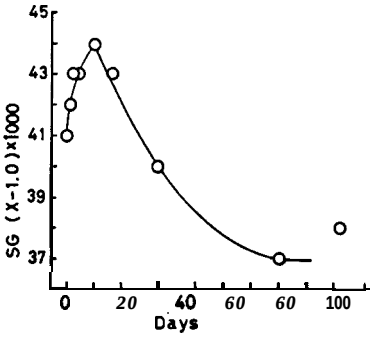

Fig. 2. Change of the specific gravity of whole blood of red sea bream during the starving period.

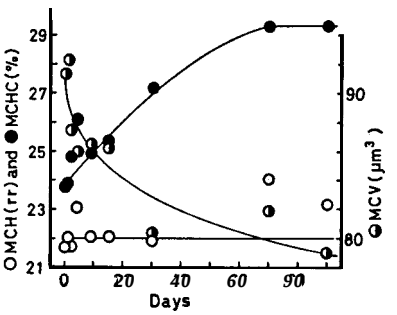

Fig. 3. Change of the Wintrobe's mean corpuscular constants of red sea bream during the starving period.

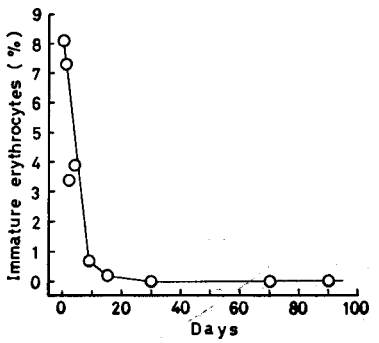

Fig. 4. Change of the count of immature erythrocytes of red sea bream during the starving period,

intestinal lumen and the lowering of sterol-biosynthesis in fish body. BUN in serum was stable at a high level for some time after the abrupt increase, and decreased thereafter (Table 2 and Fig. 6). From this finding, it is presumed that a large quantity of body protein was being decomposed as an energy source while BUN was at a high level. The change of glucose level in serum

Table 2. Effect of starvation on the contents of chemical components and activities of enzymes in blood serum of red sea bream.

Days of starvation	0	1	2	4	9	15	30	70	90
Total protein (g/dl)	3.5	4.0	4.2	4.1	4.4	4.2	3.4	1.7	1.5
Total bilirubin (mg/dl)	0.52	0.44	0.43	0.67	0.60	0.40	0.37	—	—
Total cholesterol (mg/dl)	307	287	337	354	315	365	270	115	—
BUN (mg/dl)	5.2	6.0	6.0	7.6	3.8	7.6	7.4	3.9	—
Glucose (mg/dl)	60	47	66	89	68	57	56	—	—
GOT (K. U.)[1]	51	59	37	66	94	78	74	124	—
GPT (K. U.)[1]	11	10	2.5	8	16	11	12	33	—
ALP (K.-A. U.)[2]	2.4	4.0		4.3	2.4	2.4	2.0	—	—
LDH (W. U.)[3]	783	723	631'	742'	895'	831'	783'	1208	—
LAP (G.-R. U.)[4]	655	583	587	679	527	616	531	400	—

[1] Karmen Unit. [2] King-Armstrong Unit. [3] Wroblewski Unit. [4] Goldberg-Ruteinburg Unit.

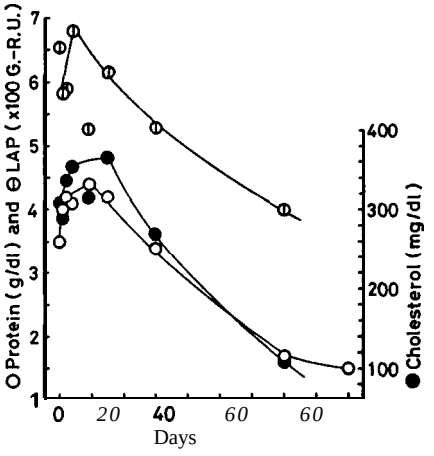

Fig. 5. Change of the contents of protein and cholesterol, and the activity of LAP in serum of red sea bream during the starving period.

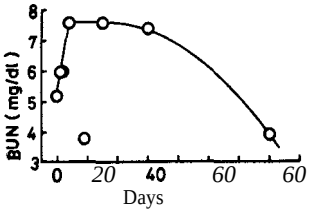

Fig. 6. Change of the content of urea-N in serum of red sea bream during the starving period.

was recognized during the starvation, but the levels were within the normal range (Table 2). During the starvation, the gluconeogenesis in liver may control the blood sugar within the normal range to maintain the functions of brain. spinal cord, peripheral nerve, erythrocytes and leucocytes *et al.*, which

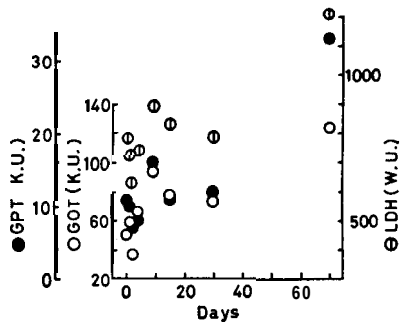

Fig. 7. Change of the activities of GOT, GPT. and LDH in serum of red sea bream during the starving period.

require glucose as the energy source. The activities of GOT, GPT, and LDH in serum did not change till the 30th day, but increased remarkably at the 70th day (Table 2 and Fig. 7). The promoted gluconeogenesis in liver of mammals was accompanied by the increased activities of GOT, GPT, and LDH during the starvation (Rosen *et al.*, 1959; Lardy *et al., 1965).* Also, the same phenomena were recognized in the eel, *Anguilla japonica*, (Inui and Yokote, 1974) and yellow-tail, *Seriola quinqueradiate* (Sakaguchi, 1976). Therefore, the remarkable increase of GOT, GPT, and LDH activities in serum of red sea bream, also may be related with the promoted gluconeogenesis in the liver, and may be caused by the destruction of liver tissue. The changes of total bilirubin content and ALP activity in serum were low during the starvation (Table 2). Increase of many constituents of blood early in the starving period may be caused by the decrease of water content of whole blood, because erythrocyte count and protein content also increased with other constituents at the same period.

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