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Effects of Petroleum Microorganisms, as Diet, upon the Growth Rate and Blood Properties of Sea Bream, *Chrysophrys major* TEMMINCK & SCHLEGEL.

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(Fig. 1; Tables 1-4)

Petroleum microorganisms, one of the by-products of petroleum industries, have been observed to be a new type of nutritive resources, especially protein resources, for various animals. Some fisheries researchers, recently, have reported that the introduction of petroleum microorgnisms in the diet kept the growth rates of cultivated fresh water fishes unaltered, especially in the cases of eel[1], carp[2], and rainbow trout[2]. Their reports hint that such a by-product may be a good substitute for fish meal.

The present investigations have, therefore, been carried out with a view to see if this by-product stands equally good in the case of cultivated marine fishes too. Studies have been made by comparing growth rates and blood properties of one of the commercially most important marine fishes, popularly known as 'Madai' or sea bream (*Chrysophrys major*), under the two types of feeding i.e. one when fed on a diet containing petroleum microorganisms and the other when fed on a diet of fish meal alone.

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MATERIAL AND METHODS

The material for the study was sea bream bred in the tanks of the Hiroshima Prefectural Fisheries Experimental Station in May, 1968. This stock was divided into two groups on the 3rd July, 1970. Each group consisting of 130 fish was kept separately in floating nets ($3 \times 3 \times 3$m) made up of synthetic fibers. The two nets were set up at the shore adjacent to the experimental station. Two groups were initially fed on a fish meal diet[3], the composition of which is shown in Table 1. This diet was continued until the beginning of the experiment which was carried out from 23rd July, 1970 through 12th November, 1970. During this period, one group, hereafter referred to as test group, was constantly fed on a test diet in which some part of fish meal was substituted by petroleum microorganisms. The composition of

this test diet is presented in Table 1. Another group, on the other hand, was fed on the regular fish meal diet and was maintained as control group.

Table 1: Composition of the two types of diets offered to sea bream.

	Fish meal %	Pertroleum* microorganisms %	Starch %	** Mixed vitamins %	*** Minerals %	Gluten %	Feed oil %	Water %	Crude protein %
Control diet	70	—	28	1.5	0.5	5	5	45	50.2
Test diet	50	30	18	1.5	0.5	5	5	45	51.5

* Dainippon Ink and Chemicals, Inc.
** 1/5 concentration of HALVER's.
*** McColum No. 185.

Thirty fish sampled at random out of each group were used for the measurements of their standard length and body weight individually. This sampling was performed on the 23rd July, 27th August, 5th October, and 5th November. After taking the measurements, at each sampling, 10 fish from each group were sacrificed for blood sampling. Before sampling, fish were anaesthetized by Sandoz MS-222 (tricaine methanesulfonate) at a dose of 1: 20,000. At this concentration, fish of both groups were usually anaesthetized within 8 minutes period. Blood was collected invariably from the Cuvierian duct by using a syringe equipped with human hypodermic needle, rinsed in advance with 'Anticlot' to prevent blood coagulation. Each blood sample was divided into two sub-samples, one for determinations of hemoglobin concentration and hematocrit value and the other one was centrifuged for 10 minutes at 1500 rpm in order to obtain the plasma. Plasma, thus obtained, was used for determinations of protein concentration, glucose concentration, total cholesterol concentration, and activity of alkaline phosphatase.

Hemoglobin concentration was determined by using Spencer's A.O. Hb-meter (American Optical Cooperation). Although this instrument is ordinarily used to determine hemoglobin concentration in the human blood, MATSUZATO[4] found it applicable in the case of fish too. He verified the applicability by determining the hemoglobin concentration of sea bream by this instrument by correlating the results with those obtained analytically for the same fish. This test showed both the values to be linearly related. For the determination of the hematocrit value, a part of blood sample was transferred from the syringe to a heparinized capillary tube (Trident) which was then centrifuged for 5 minutes at 12,000 rpm in Microhematocrit Centrifuger (Kokusan H25-D). The ratio was then measured by using a special type of scale exclusively designed for the measurement of hematocrit value. The protein concentration was determined by the commonly used Biuret's method. Glucose concentration and total cholesterol concentration were determined by Sasaki's method[5] and Zak-Henly's method[6][7] respectively. Activity of alkaline phosphatase in plasma was measured by Kind-King's method[8]. This method is actually used for

the determination of the activity of alkaline phosphatase in human plasma. In the case of human beings, plasma samples are kept at a temperature of about 37°C, the same as the body temperature of man (one of homoiothermal animals). In the present study, however, plasma sample of sea bream was kept at a temperature of 20°C during the entire course of experiment, for the environmental temperature of sea bream where they were caged, averaged to 20°C during that season.

RESULTS AND DISCUSSION

Details regarding the administration of petroleum microorganisms in the diet of sea bream and its effect on the growth have been precisely reported in the progress report of the Hiroshima Prefectural Fisheries Experimental Station[3]. The results obtained on the growth rates of sea bream under two types of feeding are presented in Table 2 and the growth curves of the two groups in Fig. 1. It can be seen in these presentations that the body size and the growth of fish did not differ significantly from group to group during the course of experiment. As shown in Table 2, the quantitative amount of two types of diets were kept at more or less equal level throughout the experiment with a view to preclude out the possibilities of their influence upon the growth rate of the fish. Thus, it clearly points out a fact that the test diet was almost equally efficient as the control diet for the growth of sea bream. The conversion efficiencies of the test diet and the control diet were calculated to be as 45.7% and 43.5% respectively. These two types of diets kept the mortality rates of the fish at an appreciably low level, the test group showing 0.7% mortality and the control group 2.2%, within 105 days of experiment. Anatomical observations neither did reveal any change in the morphological structure of visceral organs.

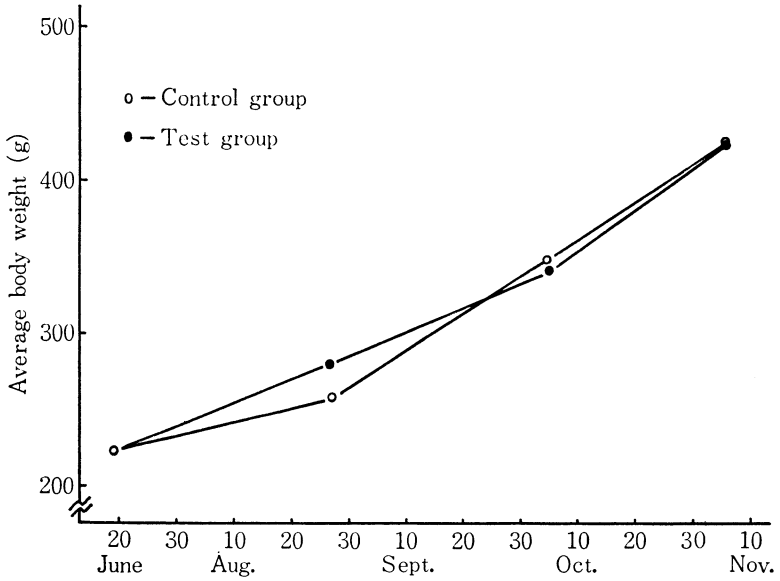

Fig. 1: Growth patterns of sea bream in control and test groups during the course of experiment.

Table 2: Growth rates of sea bream in two groups during experiment.

Diet	Average Body Weight (g)		Percentage gain	Percentage conversion efficiency	Percentage mortality	Feeding period in days
	Initial	Final				
Control	222	422	90.1	43.5	2.2	105
Test	222	423	90.5	45.7	0.7	105

All these results, thus, allow us to consider that both diets are good enough for maintenance of sea bream under culture conditions and equally efficient for the growth of this fish.

IIDA *et al.* (1970)[2] stated that the carp fed on a diet containing fish meal and petroleum microorganisms for 55 days, grew at a faster rate than those fed on the diet not containing the latter at all. In both these diets, the amount of total crude protein was kept at the same level. They also stated that the fish grew at a slower rate when fed on a diet of petroleum microoganisms alone. They further reported that an increased amount of petroleum microorganisms added to the diet resulted in the increased growth rates of rainbow trout. Such observations provide ample reasons to conclude that the changes in the growth rates and blood properties of a fish are dependent upon the composition of the diet i.e. the rate at which petroleum microorganisms are mixed in the diet of the fish and also the species of fish which is used.

Table 3: Blood properties of sea bream fed on test diet.

	Body weight (g)	Body length (cm)	Hb g/*dl*	Ht %	Glucose mg/*dl*	Cholesterol mg/*dl*	Protein g/*dl*	Alkaline * phosphatase mg/*dl*
Maximum	444	23.9	9.2	40.3	113	305	4.6	2.7
Minimum	202	17.7	7.0	28.0	50	162	3.2	0.6
Mean	311.9	20.35	7.93±0.67	34.69±4.08	81.4±17.2	233.2±45.7	3.86±0.49	1.35±0.47

* Unit of activity is expressed as concentration of phenol liberated from the substrate.

Table 4: Blood properties of sea bream fed on control diet.

	Body weight (g)	Body length (cm)	Hb g/*dl*	Ht %	Glucose mg/*dl*	Cholesterol mg/*dl*	Protein g/*dl*	Alkaline * phosphatase
Maximum	578	25.3	9.1	45.3	82	434	4.5	5.2
Minimum	126	15.4	6.2	31.0	59	149	2.0	1.2
Mean	329.2	20.66	8.28±0.87	38.08±4.57	68.7±7.6	324.8±93.5	3.66±0.51	2.73±0.68

* Unit of activity is expressed as concentration of phenol liberated from the substrate.

Results of the determinations on blood properties of the test group are shown in Table 3 and the control group in Table 4. Observed differences in the blood properties of the two groups are as follows:

Hemoglobin concentration and hematocrit values of the test group are slightly lower than those of the control group, while the reverse is true for plasma protein concentrations. Plasma glucose concentrations of the test group are higher than those of the control group, a difference of 12.8 mg/*dl* on an average. Many factors could be responsible for such fluctuations in glucose concentration. Black *et al.* (1960)[9] reported that glucose concentration in the blood of rainbow trout increased very much with the increase of their muscular activity. SATO *et al.* (1966)[10] observed that the glucose concentration in the blood of yellow tail varied with different sampling made at the different hours after feeding. However, the present results in relation to significantly higher plasma glucose concentration in the blood of sea bream of the test group are not expected to be due to the greater behavioral activity supposedly caused by the excessive handling of fish in this group and less in another. In all possibilities, a reverse situation could be true on the basis of results obtained for hemoglobin concentrations. Test group showed a lower concentration of hemoglobin than that of the control group, indicating a sign of lesser behavioral activity as per report of BLACK *et al.* (1966)[11] who found out such relations in the case of rainbow trout. Time-dependent-cause of SATO *et al.* for high glucose concentration in the test group can also be eliminated on the ground that the blood sampling were done randomly from each group. The observed differences in the present study are, therefore, not due to the differences in the handling of the fish.

Plasma cholesterol concentrations of fish in the test group were observed to be lower than those in the control group (Table 3 & 4). There was a difference of 91.6 mg/*dl* on an average. Cholesterol concentration in animal blood, in general, has been considered to be rather stable. In the case of human blood, it has been observed that the concentration of cholesterol does not fluctuate within a day. In other words, it is independent of the factor like feeding times. It does not fluctuate significantly from season to season either. In man, the highest value of cholesterol concentration has been observed during spring and summer months which is higher than the autumn and winter values by just about 25 mg/*dl*, a very minor difference. SHIMIZU *et al.* (1963)[12] have reported that total cholesterol concentration in blood serum of yellow tail has very little seasonal fluctuations ranging from 157.5 ± 18.3 mg/*dl* in September to 118.6 ± 12.4 mg/*dl* in November, the maximum difference being less than 40 mg/*dl*. In the present study the total cholesterol concentration in plasma of sea bream of the test group is at a lower level than that of the control group. IIDA *et al.* (1970)[2] stated that the administration of diet containing higher percentage of petroleum microorganisms causes decrement in the amount of crude fat in the carp body but increment in the phosphorus-concentration in their total lipid. It can be construed that when phospholipid concentration in a fish body increases, other lipid components decrease probably in a similar manner. Therefore, it can be suggested that the significant decrease in cholesterol concentration in plasma of sea bream might

be due to an increase in the amount of other phospholipid concentration in their body due to the administration of petroleum microorganisms in their diet.

Activities of plasma alkaline phosphatase of fish in the test group were significantly lower than those of the control group. Former was calculated to be almost half of the latter. In the case of yellow tail, according to SHIMIZU *et al.* (1963)[12], activity of alkaline phosphatase of the serum fluctuates seasonally, highest value in summer being five times higher than that in the winter. However, they also stated that inter-seasonal variations in the activities were not so large. Variations observed in the present study are really significant.

In conclusion it can be said that the diet containing petoroleum microorganisms appeared not to differ from the normal fish meal diet in their conversion efficiency and probably in their nutritional values within 112 days of experiment. However, introduction of petroleum microorganisms in the diet did affect the blood properties, particularly plasma cholesterol concentration and activity of alkaline phosphatase in plasma. Physiological meanings of such differences in the blood properties yet remain to be understood. It is quite likely, however, that the differences were caused as a result of two types of feeding. It is also likely that the magnitude of such differences may increase manyfold with a longer use of such diet. These assumptions lead us to believe that the use of petroleum microorganisms in the diet of the fish, in the long run, may worsen the conversion efficiency and affect adversely the health of the fish. At the moment, these are mere assumptions. Investigations upon the physiological mechanisms responsible for such differences are needed to confirm such assumptions.

SUMMARY

Investgations have been carried out with a view to see the effects of the introduction of petroleum microorganisms in the diet of cultivated marine fishes. Sea bream, *Chrysophrys major*, was used as the experimental fish. Studies were made by comparing the growth rates and blood properties of fish under two types of feeding i.e. one when fed on a diet containing petroleum microorganisms and the other when fed on a diet of fish meal alone. Results can be summarized as follows:

i) The mortality rate remains almost the same.
ii) The body size and growth rate do not differ significantly.
iii) Anatomical observations do not reveal any change in the morphological structure of the visceral organs.
iv) Hemoglobin concentration, hematocrit values, and plasma glucose concentration remain almost the same.
v) Cholesterol concentration and alkaline phosphatase activity in plasma are lowered by the introduction of petroleum microorganisms in the diet. This lowering is significant especially in the latter case.

Physiological mechanisms causing such differences in the blood properties, yet remain to be understood.

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石油酵母添加飼料のマダイの成長および血液性状に及ぼす影響

難波憲二・村地四郎・北島　力・松里寿彦

石油酵母30％，フィッシュミール50％を含む飼料（試験飼料）とフィッシュミール70％を含む飼料（対照飼料）とを，マダイに投与して，105日間の飼育結果を比較した．成長ならびに餌料効果の面では，両者に差異が認められなかったが，飼育試験終了時における試験飼料投与群の血液性状は対照飼料投与群にくらべ，一般に血漿グルコース濃度はやや高く，血色素量およびヘマトクリット値は僅かに低く，さらに血漿中アルカリ性フォスファターゼ活性及び血漿総コレステロール濃度の著しい低下が認められた．