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https://doi.org/10.5109/23680

出版情報：九州大学大学院農学研究院紀要. 23 (1/2), pp.79-84, 1978-10. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 23. 79-84 (1978)

Requirement of Red Sea Bream for Dietary Na and K*

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(Received July 5, 1978)

Two experiments were conducted to examine the dietary requirement of red sea bream, *Chrysophrys major*, for sodium and potassium supplementation. The fish were fed on two test diets with and without sodium supplementation (Exp. I), and potassium supplementation (Exp. II), over a 63 day period at 25°C. At the end of the feeding trial, histopathological examination of the tissues, hematological examinations, and chemical analyses of the liver and vertebrae were performed. No significant differences were recognized between the two groups in each experiment in the following determinations : the growth rate, feed efficiency, condition factor, and hepatosomatic index; the hemoglobin content, hematocrit value, red blood cell count, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular diameter, and percentage of immature erythrocytes ; the moisture, lipid, and glycogen content of the liver; and the lipid, ash, calcium, phosphorus, magnesium, sodium, and potassium content of the vertebrae. Furthermore, no pathological change was recognized in the organs of fish fed the diets without the sodium or potassium supplement. From these findings, it appears that sodium and potassium supplementation in the diet for red sea bream is not essential, even if these elements are scarcely contained in the diet.

INTRODUCTION

Many abnormal symptoms were recognized in red sea bream fed on the diet without mineral supplementation. Therefore, a series of studies have been conducted to determine which minerals should be supplemented to the diet for red sea bream. The present study was conducted to examine the dietary requirement of red sea bream for sodium and potassium supplementation.

MATERIALS AND METHODS

Dietary sodium requirement of red sea bream was examined in the experiment I, and potassium in the experiment II. In each experiment, red sea

* Contribution from Fish. Res. Lab., Kyushu Univ., No. 128. Studies on nutrition of red sea bream XIX. This work was orally presented to the Annual Meeting of Japan. Soc. Sci. Fish., at Tokyo in April, 1977.

Table 1. Composition of test diet.

Casein	52.0	L-Lys	0.6
Gelatin	11.0	L-Val	0.7
L-Phe	0.6	Gly	0.4
L-Arg·HCl	1.3	P. L. Oil[1]	9.0
L-cys	0.7	Dextrin	8.0
L-Try	0.2	Minerals[2]	8.0
L-His·HCl·H_2O	0.2	Vitamins[3]	3.0
DL-Ala	1.3	α-Cellulose	2.0
L-Asp	1.0		

[1] Alaska pollack liver oil purified by molecular distillation. [2] Shown in Table 2. [3] Halver's vitamin mixture (1957) + α-cellulose.

Table 2. Composition of mineral mixtures with and without sodium or potassium supplementation.

Experiment	I		II	
Na supplement	Without	With		
K supplement			Without	With
NaCl	— g	11.54 g	— g	— g
KCl	5.18	5.18	—	5.18
Ca $(H_2PO_4)_2 \cdot H_2O$	24.885	24.885	30.805	—
$NaH_2PO_4 \cdot 2H_2O$	1.485	1.485	1.485	30.805
Fe-citrate	68.144	57.204	68.004	1.485
α-Cellulose	9.0 mg	9.0 mg	9.0 mg	62.824
$AlCl_3 \cdot 6H_2O$	178.5	178.5	178.5	9.0 mg
$ZnSO_4 \cdot 7H_2O$	40.0	40.0	40.0	178.5
$MnSO_4 \cdot 4\text{-}6H_2O$			5.5	40.0
CuCl	5.5	5.5	8.5	
KI	52:8.55	52:8.55	52:5	5.5
$CoCl_2 \cdot 6H_2O$				52:8.55
Na (mg/100g diet)	6	367	367	367
K (mg/100g diet)	220	220	4	220

bream were equally divided into two lots based on the average body weight and the number of fish with similar weight. Each lot of 15 fish was maintained in an aquarium of 1501 capacity, which was continuously supplied with sea water (25°C) at a rate of 1501 per hour. The sea water was aerated sufficiently. The fish were fed on four test diets with and without sodium or potassium supplementation over a 63 day period. Each lot of fish was fed continuously as long as the fish accepted the diet. A record was kept on the weight of diet fed to each group. Consequently, the weight of diet fed to each group was assumed to represent the actual amount consumed by that particular group of fish. The composition of test diets is listed in Table 1. All the materials listed in Table 1 except gelatin and pollack liver oil were well mixed. A 11 g portion of gelatin was added to a 200 ml aliquot of water, followed by heating on a steam bath until the temperature rose to 60°C. The gelatin solution was cooled to 40–45°C, and then the previously mixed component and pollack liver oil was added, being thoroughly stirred until a homogeneous mass was obtained. An aliquot of the diet for a 1 week feeding was

prepared at one time, and was stored in a freezer (-20°C). The diet was defrosted in a refrigerator before using, and then was cut into cubes of about 3-4mm size. The composition of mineral mixtures and the contents of sodium and potassium in the diets are listed in Table 2. Sodium and potassium are scarcely contained in the diet without their respective supplementation. At the end of the feeding trial, ten fish from each group were selected, and the histopathological examination of tissues, the hematological examinations, and the chemical analyses of liver and vertebrae were performed. Dorsal muscle tissue, liver, heart, spleen, kidney, and small intestine were fixed with 10% formalin. The fixed tissues were embedded in paraffin and sectioned at 7 micra as a rule. Sections were routinely stained with Delafield's hematoxylin and eosin (Kaneko, 1971). Also, Kossa's silver nitrate method (Kaneko, 1971) for calcium was applied. A blood sample was taken from each fish by a cardiac puncture for the hematological examinations. Hemoglobin content and hematocrit value 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 constants (MCH, MCV and MCHC) were calculated. A mean corpuscular diameter of 1000 cells from each group was obtained by measuring the longest diameter of each cell with a micrometer on a smeared preparation stained with May-Giemsa (Yuki, 1963). Immature erythrocytes are classified in accordance with Nakanoin's method (Nakanoin, 1920) and Yuki's method (Yuki, 1963). The analytical samples of the liver and vertebrae were taken from each fish in the same amount, and were mixed thoroughly from each group. Moisture contents of the liver and vertebrae were determined by a 24 hour drying in an electric oven at 105°C. The Soxhlet extractor and ethyl ether were used to extract lipids of the liver, and lipids of vertebrae were extracted with methyl alcohol for 24 hours followed by a 16 hour extraction with ethyl ether. Glycogen content of the liver was determined by Carroll's method (Carroll *et al.*, 1956). The calcium, phosphorus, and magnesium content of vertebrae were quantified by orthocresolphthalein complexone (Conerty and Briggs, 1966), molybdenum blue (Tausky and Shorr, 1953), and titan yellow method (Kunkel et *al.*, 1947), respectively, after dry ashing with an electric furnace at 450°C for 48 hours. The contents of sodium and potassium in the test diets and vertebrae were quantified by atomic absorption spectrometry after wet ashing with nitric acid and perchloric acid.

RESULTS AND DISCUSSION

No significant differences were recognized between the two groups receiving the diets with and without the sodium or the potassium supplement in the growth rate, feed efficiency, condition factor, and hepatosomatic index (Table 3). Also, the examination of stained sections of tissues of the fish fed the diet without supplemental sodium or potassium revealed no histopath-

Table 3. Effects of dietary sodium and potassium on growth rate, feed efficiency, condition factor, and hepatosomatic index of red sea bream.

Experiment	I		II	
Na supplement	Without	With		
K supplement			Without	With
No. of fish				
at start	15	15	15	15
after 63 days	15	15	15	15
Average body weight (g)				
at start	63.0 ±3.9[1]	63.0± 4.4	72.3± 4.8	73.7± 4.6
after 63 days	121.0±19.0	122.2±20.7	156.8 ±19.5	156.5±15.8
"t" test (5%)	NS[2]	—	NS	—
Feed efficiency (%)	68	67	77	74
Condition factor[3]	2.3310.09	2.38±0.17	2.48±0.16	2.41±0.06
"t" test (5 %)	NS	—		—
Hepatosomatic index (%)[4]	1.68±0.18	1.73±0.46	1.84% 29	1.66±0.26
"t" test (5 %)	NS	—	NS	—

[1] Standard deviation. [2] Non-significant. [3] Body weight(g) x 100 / Fork length(cm)³.
[4] Liver weight(g) x 100 / Body weight(g).

Table 4. Effects of dietary sodium and potassium on the hematological characteristics of red sea bream.

Experiment	I		II	
Na supplement	Without	with		
K supplement			Without	with
Hb (g/dl)	7.4±0.3	6.9±0.5	7.8±0.7	7.1±0.4
"t" test (5 %)	NS	—	NS	—
Ht (%)	32.5±1.6	30.6±3.0	34.8±3.4	31.3±2.9
"t" test (5 %)	NS	—	NS	—
RBC (x 10^6/mm³)	3.20±0.42	3.26±0.12	3.85±0.12	3.38±0.33
"t" test (5 %)	NS			—
MCH (rr)[1]	23.47±2 55	21.7321.33	20.2% 35	21.12±1.36
"t" test (5 %)	NS'	—	NS	—
MCV (μm^3)[2]	100.61± 12.52	98.28±7.07	90.40±7.78	92.68±2.65
"t" test (5 %)	NS	—	NS	—
MCHC (%)[3]	22.85 ± 0.56	22.14 ± 0.86	22.46±1.48	22.78±0.99
"t" test (5 %)	NS			
MCD (μm)	9.44	9.43	9 %	9.40
Immature' erythrocytes (%)	4.2	4.6	4.0	4.2

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

ological change. Calcium deposition was not observed in all tissues of each group. As shown in Table 4, no differences were recognized between both groups with and without sodium or potassium supplementation in the hemoglobin content (Hb), hematocrit value (Ht), red blood cell count (RBC), Wintrobe's mean corpuscular constants (MCH, MCV and MCHC), mean corpuscular diameter (MCD), and percentage of immature erythrocytes. Also, in both experiments, Price-Jones curves of the groups with and without supplementation

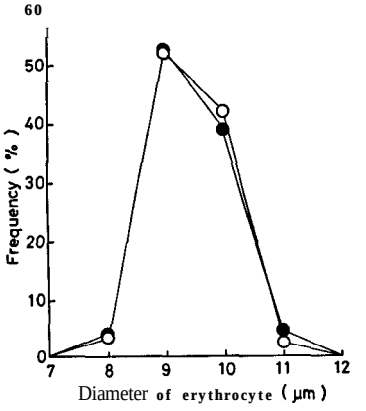

Fig. 1. Price-Jones curve of red sea bream fed the diets with and without sodium supplementation. ○: Without, ●: With.

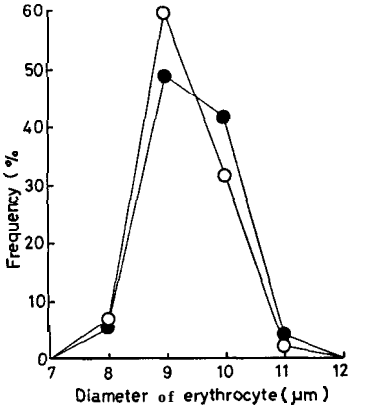

Fig. 2. Price-Jones curve of red sea bream fed the diets with and without potassium supplementation. ○: Without, ●: With.

were similar, as shown in Fig. I and Fig. 2. Furthermore, morphological abnormality of erythrocytes and leucocytes was not observed on the smeared preparation stained with May-Giemsa in all groups. A noteworthy difference could not be found between both groups in the moisture, lipid, and glycogen content of the liver, and in the lipid, ash, calcium, phosphorus, magnesium, sodium, and potassium content of the vertebrae in the *two* experiments (Table 5). Sodium deficiency symptoms and potassium deficiency symptoms have been well established with terrestrial animals (Sato, 1972). However, in the present study, red sea bream reared on the diets without sodium and potassium supplementation did not manifest any deficiency symptoms. From these findings, it is presumed that red sea bream satisfy the requirement of sodium and potassium by absorption from sea water.

Table 5. Effects of dietary sodium and potassium on contents of chemical components in the liver and vertebrae of red sea bream.

Experiment		I		II	
Na supplement		Without	With		
K supplement				Without	With
Liver	Moisture (%)	68.1	68.2	67.3	67. 6
	Lipid (% db)*	30.9	31. 2	36.0	35. 2
	Glycogen (% db)	15. 7	15.1	11.0	11.2
Vertebrae	Lipid (% db)	20.8	21.7	26.0	25.1
	Ash (% db)	51.1	52.4	48.8	49. 0
	Ca (mg/g ash)	361	362	361	362
	P (mg/g ash)	185	186	185	187
	Ca/P ratio	1.95	1.95	1.95	1.94
	Mg (mg/g ash)	12. 5	12. 4	12. 3	12.2
	Na (mg/g ash)	2.04	2. 02	2. 02	2. 10
	K (mg/g ash)	7.29	7. 50	6.83	6.93

* % of dry weight basis.

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