Proximate and Fatty Acid Compositions of Liver, Hematological Characteristics, and Chemical Components in Plasma of Red Sea Bream Fed on Scrap Meals Fermented with Molds^{*1}

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The percentage of organ weight to body weight, proximate and fatty acid compositions of liver, hematological characteristics and chemical components in plasma of red sea bream *Chrysophrys major* reared for 10 weeks on the fermented-resteamed scrap meal (AT, AO, AF, PG, PC, and FS) diets were compared with those of the nonfermented scrap meal (NF) and the white fish meal (W) diet groups. The condition factor, % of liver and intestine weights, and lipid, glycogen and ω 3 HUFA contents in liver of the NF group were low, and the moisture content in liver was high as compared with the W group. Furthermore, in the NF group the Hb content, RBC count, M. C. H. C., and the contents of protein and cholesterol in blood plasma were lower, but the M. C. V. and the values of GOT, GPT, γ -GTP and TTT were higher than those of the W group. In contrast with the above findings, no significant differences were recognized between the W group and the fermented-resteamed scrap meal diet groups in all of the investigated parameters, except the condition factor, % of liver and heart weights in the PC group, and the % of liver weight of the AF group.

In the previous paper,¹⁾ the fermented-resteamed scrap meals with molds isolated from smoked-semidried skipjack showed the remarkably lower POV and TBA value and slightly lower VBN value than those of the nonfermented scrap meal. Furthermore, the growth and feed efficiency of red sea bream fed on the fermented-resteamed scrap meal diets were markedly superior to those of the nonfermented scrap meal diet group.²⁾ From these findings, it is presumed that the improvement of the growth and feed efficiency are mainly caused by the removal of toxic oxidized lipids by the fermentation. On the other hand, it has been reported that fishes fed on the diets containing oxidized lipids exhibit poor growth, high mortality, muscular dystrophy, fatty degeneration of liver, fatty liver and anemia.³⁻⁸⁾ Also, Sakaguchi and Hamaguchi⁹⁾ showed that the administration of oxidized lipids caused the low contents of liver glycogen and plasma cholesterol and high activity of plasma GOT in red sea bream. In this paper, therefore, in order to prove positively the above presumption, the proximate compositions of liver and muscle, fatty acid composition in liver, hematological characteristics, chemical compon-

ents in blood plasma of fish fed on the fermented-resteamed scrap meal diets were compared with those of the nonfermented scrap meal and white fish meal diet groups after the 10 week feeding trial, which was reported in the previous paper.²⁾

Materials and Methods

Fish

The fish are red sea bream which were reared for 10 weeks on the diets containing the fermented-resteamed scrap meals with *Aspergillus terreus* (AT), *Asp. oryzae* (AO), *Asp. flavus* (AF), *Penicillium citrinum* (PG), *Peni. citrinum* (PC), and the combined microbe (FS), and the nonfermented scrap meal (NF) and the white fish meal (W). The details were reported in the previous paper.²⁾

Blood Analysis

At the end of feeding trials, 5 fish from each group were sampled at random. The blood sampling, biological analysis of erythrocyte (number of red blood cell, hematocrit and hemoglobin values) and biochemical analysis of plasma (contents of total protein, total bilirubin and total

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cholesterol, and activities of GOT, GPT, ALP and γ -GTP, and the values of ZTT and TTT) were carried out by the same methods as described in the previous paper.¹⁰⁾ Furthermore, mean corpuscular constants (mean corpuscular hemoglobin, M. C. H., mean corpuscular volume, M. C. V., and mean corpuscular hemoglobin concentration, M. C. H. C.) were calculated from the values of red blood cell, hematocrit and hemoglobin.

Fatty Acid Analysis

At the end of feeding trial, other 5 fish from each group were sampled at random. The same amount of liver was taken from each fish and the liver samples from 5 fish were pooled separately for each group and homogenized. The total lipid extraction and fatty acid analysis were done by the methods described previously.¹¹⁾

Determination of Condition Factor, Percentages of Organs, and Proximate Compositions of Liver and Muscle

After the sampling for blood and fatty acid analyses, the remaining 12–14 fish in each group were killed instantly by stabbing a sharp knife to the brain and then dissected. The fork length, and the weight of entire body, liver, heart, intestine and spleen were measured individually. Also, the condition factor was calculated from the body weight and the fork length. The same amount of liver and dorsal muscle were taken from 11–14 fish and the samples were pooled separately for each group and homogenized. The homogenized samples were subjected to the determination of moisture, protein, lipid, glycogen and ash contents. The methods of determination were identical to those in the previous paper.¹⁰⁾

Results

Condition Factor and Percentages of Liver, Heart, Intestine and Spleen Weight

As shown in Table 1, no significant differences in these values were recognized between the AT, AO, AF, PG or FS diet group and the white fish meal diet group (W), except a significantly low percentage of liver weight in the AF diet group. On the other hand, the condition factor and percentage of liver weight to body weight were significantly low in both nonfermented scrap meal (NF) and PC diet groups. A significantly low percentage of intestine weight in the NF diet

Table 1. Effects of nonfermented scrap meal (NF), fermented-resteamed scrap meals (AT, AO, AF, PG, PC, FS), and white fish meal (W) on condition factor, percentages of liver, intestine, heart, and spleen weight to body weight

Diets	NF	AT	AO	AF	PG	PC	FS	W
Condition factor* ¹	2.29±0.15	2.49±0.18	2.44±0.23	2.42±0.15	2.47±0.12	2.25±0.28	2.52±0.17	2.50±0.13
"t" test (5%)	S*	NS*	NS	NS	NS	S	NS	—
Liver wt./B. wt. (%)	0.99±0.17	1.54±0.40	1.50±0.30	1.32±0.34	1.49±0.32	1.18±0.30	1.65±0.35	1.78±0.45
"t" test (5%)	S	NS	NS	S	NS	S	NS	—
Intestine wt./B. wt. (%)	1.37±0.27	1.68±0.39	1.81±0.35	1.84±0.37	1.63±0.23	1.72±0.42	1.88±0.32	1.85±0.30
"t" test (5%)	S	NS	NS	NS	NS	NS	NS	—
Heart wt./B. wt. (%)	0.87±0.12	0.83±0.10	0.96±0.22	0.85±0.14	0.79±0.11	0.99±0.21	0.99±0.26	0.84±0.14
"t" test (5%)	NS	NS	NS	NS	NS	S	NS	—
Spleen wt./B. wt. (%)	0.55±0.10	0.53±0.10	0.52±0.10	0.61±0.10	0.52±0.13	0.55±0.16	0.56±0.18	0.58±0.20
"t" test (5%)	NS	NS	NS	NS	NS	NS	NS	—

*¹ Body wt. (g) × 100/[Body length (cm)]³. *² S: significant, *³ NS: nonsignificant.

Table 2. Effects of NF, AT, AO, AF, PG, PC, FS and W meals on proximate compositions of liver and muscle

Diets		NF	AT	AO	AF	PG	PC	FS	W
(Liver)									
Moisture	(%)	73.8	69.4	69.2	71.1	71.1	70.7	69.9	65.2
Protein	(% d.m.)	49.2	49.0	49.7	48.4	49.5	42.7	48.5	44.3
Lipid	(% d.m.)	23.7	26.1	32.5	27.9	25.6	27.6	29.9	35.9
Glycogen	(% d.m.)	7.6	14.5	14.0	13.4	14.2	6.9	13.1	13.1
Ash	(% d.m.)	5.3	5.2	4.4	5.2	4.8	4.9	4.6	4.0
(Muscle)									
Moisture	(%)	76.8	74.6	75.1	75.6	75.3	75.3	74.7	74.8
Protein	(% d.m.)	75.9	78.7	84.3	75.8	83.4	72.5	77.1	80.6
Lipid	(% d.m.)	5.8	5.6	6.2	5.6	5.7	5.3	5.5	6.0
Glycogen	(% d.m.)	0.6	1.1	0.9	0.9	1.1	0.6	0.6	1.0
Ash	(% d.m.)	7.8	7.1	6.4	6.6	7.3	6.9	6.3	7.1

Table 3. Effects of NF, AT, AO, AF, PG, PC, FS and W meals on fatty acid compositions of liver lipid

Fatty acids (%)	NF	AT	AO	AF	PG	PC	FS	W
12:0	0.9	0.6	0.9	0.6	0.5	0.7	0.4	0.5
13:0	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.4
14:0	5.7	4.2	4.2	4.9	4.2	2.8	3.6	3.4
15:0	1.3	1.2	1.0	0.9	0.5	0.8	1.1	0.7
16:0	26.1	21.7	20.8	18.7	18.5	23.5	20.6	16.1
16:1 ω 7	4.9	4.6	4.1	4.5	3.8	4.4	4.8	3.5
18:0	7.5	5.9	6.5	6.6	6.0	9.2	7.6	7.2
18:1 ω 9	25.7	18.4	21.7	18.7	19.5	26.0	22.3	19.7
18:2 ω 9	1.8	3.0	2.3	4.1	1.6	1.9	2.3	3.3
18:2 ω 6	0.6	1.0	0.6	0.9	0.7	0.4	0.7	1.5
18:3 ω 3	0.6	0.9	1.0	0.7	0.7	0.4	1.0	1.3
20:1 ω 9	5.6	9.1	5.6	10.0	9.8	5.4	5.8	9.7
20:1 ω 7	0.2	0.3	1.3	0.6	1.1	0.4	0.8	0.7
20:2 ω 9	1.1	2.1	2.0	1.7	2.6	1.1	1.0	1.9
20:4 ω 6	1.0	1.8	2.4	1.1	1.9	2.2	2.5	1.8
22:1	2.1	4.1	5.4	5.5	5.0	4.8	5.2	4.8
20:5 ω 3	1.6	3.5	3.9	3.4	3.5	4.0	4.9	3.9
22:4 ω 6	0.2	0.5	0.7	0.6	0.6	—	0.3	0.5
22:5 ω 6	1.2	2.4	2.4	2.6	2.3	0.9	1.3	2.6
22:5 ω 3	0.8	1.6	1.6	2.3	2.6	1.8	1.8	2.4
22:6 ω 3	5.6	8.4	7.6	6.9	10.7	5.9	7.5	10.4
$\Sigma\omega$ 3 HUFA	8.0	13.5	13.1	12.6	16.8	11.7	14.2	16.7

group and a significantly high percentage of heart weight in the PC diet group were also recognized.

Proximate Compositions of Liver and Muscle

Table 2 shows the proximate compositions of liver and muscle. The moisture, protein and ash contents of liver were slightly higher and the lipid content of liver was considerably lower in the nonfermented scrap meal (NF) and fermented-resteamed scrap meal (AT, AO, AF, PG, PC and FS) diet groups than those of the white fish meal

diet group (W). Furthermore, the NF and PC diet groups showed the appreciably low content of liver glycogen. On the other hand, the proximate compositions of muscle were similar among all the groups.

Fatty Acid Compositions of Liver Lipid

The ω 3 HUFA percent in total fatty acids of liver lipid of the fermented-resteamed scrap meal diet groups except the PC group were considerably high as compared with that of the NF diet

Table 4. Effects of NF, AT, AO, AF, PG, PC, FS and W meals on hematological characteristics

Diet	NF	AT	AO	AF	PG	PC	FS	W
Hb	7.3±0.6	8.7±1.0	8.3±0.4	7.8±0.6	8.0±0.4	7.9±0.7	8.2±0.3	8.3±0.5
"t" test	S	NS	NS	NS	NS	NS	NS	—
Ht	32.3±1.6	35.1±2.3	33.1±2.2	34.0±1.2	34.8±0.6	33.1±1.9	33.9±1.6	34.1±1.7
"t" test	NS	NS	NS	NS	NS	NS	NS	—
RBC	3.0±0.4	3.7±0.5	3.5±0.2	3.4±0.6	3.5±0.1	3.3±0.5	3.8±0.5	3.7±0.5
"t" test	S	NS	NS	NS	NS	NS	NS	—
MCV	107.8±12.0	94.5±8.7	94.8±8.1	101.3±14.5	100.9±3.3	104.0±16.3	90.3±9.9	93.1±7.6
"t" test	S	NS	NS	NS	NS	NS	NS	—
MCH	24.3±1.8	23.4±2.4	23.6±1.5	23.0±2.1	23.3±1.4	24.7±3.5	21.9±2.9	22.6±2.5
"t" test	NS	NS	NS	NS	NS	NS	NS	—
MCHC	22.6±1.5	24.8±1.6	25.0±2.2	22.9±1.6	23.1±1.2	23.8±1.6	24.2±0.9	24.2±1.4
"t" test	NS	NS	NS	NS	NS	NS	NS	—

group and were similar to that of the W diet group (Table 3).

Hematological Characteristics

As shown in Table 4, the values of hemoglobin and red blood cell (RBC) of the nonfermented scrap meal diet group (NF) were significantly lower than those of the white fish meal diet group (W) and the mean corpuscular volume (M. C. V.) was significantly higher. These parameters suggest a slight macrocytotic hyperchromemia in the NF diet group. On the other hand, there were no significant differences in hematological characteristics between the fermented-resteamed scrap meal diet groups (AT, AC, AF, PG, PC and FS) and the white fish meal diet group (W).

Chemical Components and Activities of Enzymes in Plasma

The biochemical indexes of blood for the diagnosis of liver function are shown in Table 5. The low contents of bilirubin and cholesterol, low values of ATP and γ -GTP, and high values of GOT, GPT and TTT were recognized in the non-fermented scrap meal diet group (NF), as compared with the white fish meal diet group (W) and the fermented-resteamed scrap meal diet groups (AT, AO, AF, PG, PC and FS). From these findings, the fish, when fed on the nonfermented scrap meal for a long time, appear to get a slight liver malfunction. On the other hand, the fermented-resteamed scrap meal and white fish meal diet groups showed similar values in all of the investigated parameters except low bilirubin content in the PC diet group.

Discussion

In the present study, it was found first that the fermented-resteamed scrap meal diet groups exhibited the higher level of ω 3 HUFA in liver lipid than the nonfermented scrap meal diet group (Table 3). It is sure to be affected by the fatty acid compositions of the scrap meals used.¹⁾

It has been reported that the administration of oxidized lipids causes an anemia in rainbow trout^{8,9)} and channel catfish⁹⁾ and the low contents of liver glycogen and plasma cholesterol and high activity of plasma GOT in red sea bream.⁹⁾ The present study also recognized that red sea bream fed on the nonfermented scrap meal diet with high POV and TBA value¹⁾ showed a slight macrocytotic hyperchromemia and liver malfunction. On the

Table 5. Effects of NF, AT, AO, AF, PG, PC, FS and W meals on levels of chemical components and activities of enzymes in plasma

Diets		NF	AT	AO	AF	PG	PC	FS	W
Protein	(g/dl)	4.2	5.0	5.3	5.2	5.0	4.7	4.8	5.2
Bilirubin	(mg/dl)	0.3	0.6	0.6	0.4	0.6	0.3	0.5	0.6
Cholesterol	(mg/dl)	170	324	335	301	335	302	329	442
GOT	(K. U.)	19.0	7.0	6.0	8.0	9.0	10.0	8.0	8.0
GPT	(K. U.)	10.0	6.0	6.0	6.0	7.0	7.0	7.0	6.0
GOT/GPT		1.9	1.2	1.0	1.3	1.3	1.4	1.1	1.3
ALP	(K-A. U.)	1.7	2.1	2.2	3.0	2.5	2.2	2.4	2.7
γ -GTP	(mu/ml)	17.5	22.4	23.1	18.7	21.2	19.3	21.8	22.4
TTT* ¹	(Mac. U)	1.5	0.8	1.0	1.0	1.1	0.8	0.8	1.1
ZTT* ²	(K. U.)	0	0	0	0	0	0	0	0

*¹ Thymol turbidity test value.*² Zinc sulfate test value.

other hand, the fermented-resteamed scrap meal diet and white fish meal diet groups exhibited the similarly higher values of liver glycogen, hemoglobin, RBC count, plasma cholesterol and bilirubin, and the lower values of M. C. V., plasma GOT, GPT and TTT than those of the non-fermented scrap meal diet group. The same results also were obtained in the previous study.¹⁰⁾ Accordingly, it appears that the symptoms observed in the nonfermented scrap meal diet group probably attributed to the feeding of oxidized lipids and were improved chiefly by the removal of toxic oxidized lipids by fermentation.

The fish fed on the fermented-resteamed scrap meal with *Penicillium citrum* (PC) showed the lower values of liver glycogen, liver protein, growth and feed efficiency and the higher M. C. V. than those of the other fermented-resteamed scrap meal diet groups, in spite of the remarkably decreased POV and TBA value in the scrap meal.¹⁾ Therefore, these symptoms seem to occurred from some causes except the oxidized lipid.

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