Effect of Fermented and Fermented-Resteamed Scrap Meals on Growth and Feed Efficiency of Red Sea Bream^{*1}

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Two attempts were conducted to examine the effect of fermentation on proximate composition, POV, TBA, and VBN values of fish waste, and the effects of fermented and fermented-resteamed scrap meals on the growth of red sea bream and feed efficiency. Fermentation by a combination of *Aspergillus oryzae*, *Aspergillus sojae*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* decreased the lipid content, POV, and TBA in fish waste, and increased VBN. However, VBN decreased by resteaming after fermentation. The growth and feed efficiency of fish fed on fermented scrap meal diet was superior to those of the nonfermented scrap meal diet group. However, both groups were significantly inferior to white fish meal diet group. On the other hand, no significant difference was recognized between fermented-resteamed scrap meal diet group and white fish meal diet group in the growth and feed efficiency. These findings indicate that the removal of both toxic oxidized lipids and protein degenerates is essential in the utilization of fish waste as a dietary protein source and that fermentation and steaming are effective for the removal.

The fishes are generally reared on high protein diets, which contains mainly fish meal from pollack, sardine *etc.* Accordingly, it is necessary to search for protein sources which could be utilized in place of fish meal, because the fish population may decline in the near future.

At the present time, fish product plants are producing a large amount of fish waste in Japan. However, scrap meal from the waste could not be utilized as a protein source for fishes, due to the high levels of toxic oxidized lipids and protein degenerates existed.

On the other hand, Tomiyama¹⁾ reported that rainbow trout fed on silk-worm pupae fermented with *Aspergillus* sp. showed higher growth and survival rate than the group fed on pupae without fermentation. On the basis of this result and the established facts that the lipid in silk-worm pupae¹⁾ and skipjack²⁾ decreased by fermentation with *Asp.* sp., Yone assumed that the decrease in oxidized lipids by the fermentation is a cause of the improvement. Recently, based on this assumption, microorganisms which decrease oxidized lipids were selected, and it was found that *Asp. sojae* K, *Asp. oryzae*, and *Saccharomyces cerevisiae*

IFO 2114 are more active than other tested.³⁾

In the present study, therefore, the effects of the fermentation by a combination of the microorganisms and the resteaming after fermentation on the proximate composition, POV, TBA, and VBN values of fish waste were determined. Furthermore, the effects of diets containing fermented and fermented-resteamed scrap meals on the growth and feed efficiency were compared with those of the diets with white fish meal and nonfermented scrap meal by a feeding trial with red sea bream *Chrysophrys major*.

Materials and Methods

Microorganism Seeds

Asp. oryzae, *Asp. sojae*, *Sac. cerevisiae*, and *Bacillus subtilis* were inoculated into 10 ml medium in a 40 ml test tube and cultured at 28–30°C for 48, 48, 36, and 24 h, respectively, with shaking on a reciprocal shaker. The media are MY for fungus, yeast and bouillon for bacteria. Each cultured fungus, furthermore, was inoculated on 25 g sterilized wheat bran and cultured in a 500 ml flask at 30°C for 3–4 days. The seeds were prepared by mixing each 5 g of fungus cultured on

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Table 1. Composition of nonfermented (NF), fermented (F), fermented-resteamed (FS) scrap meal diets and white fish meal diet (W)

Diets	NF	F	FS	W
Meals	Scrap meals			White fish meal
	Non-fermented	Fermented	Fermented-resteamed	
White fish meal				68.4
Scrap meals	82.9	85.3	85.4	
α -Starch	5.0	3.0	3.0	5.0
Vitamin mixture* ¹	3.0	3.0	3.0	3.0
NaH ₂ PO ₄ ·2H ₂ O	3.43	3.43	3.43	3.43
Fe-citrate	0.11	0.11	0.11	0.11
HUFA concentrate* ²		1.2	1.2	1.2
Pollack liver oil				6.3
L-Aspartic acid-Na	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	1.96	1.96	1.96	1.96
α -Cellulose	2.6	1.0	0.9	9.2

*¹ Halver's mixture (1957).*² ω 3 highly unsaturated fatty acids content: 85.3% (Ester-85).

wheat bran and each 5 ml of yeast and bacteria culture with a 25 g sterilized wheat bran.

Scrap Meal

Raw waste from a salted and dried mackerel manufacturing plant was minced with a meat chopper, steamed for 30–40 min, and pressed to remove water and oil as much as possible. A part of the pressed waste was dried in an air oven at 60–70°C for 5–6 h. Then, the dried waste was milled and sieved through 210 μ mesh, after being crushed with a mixer and sieved through a 1 mm mesh.

Fermented and Fermented-Resteamed Scrap Meals

The remainder of the pressed waste was formed into pellet of about 1 cm in length and 1 cm diameter with a pelleter, and then was sterilized. A 500 g of the pellet was mixed with seeds and incubated at 30°C for 18 h on an aluminium foil in steel container. The cover of the container was slightly opened during the incubation period and the pellet was stirred with a spoon at intervals of 6 h to aerate. A part of the fermented pellet was steamed again. Finally, the remainder of the fermented pellet and the fermented-resteamed pellet were dried at 80–90°C for 4–5 h, and then were ground and sieved by the same processes as those in the preparation of scrap meal, respectively.

Diets

Two test diets containing the fermented scrap meals with or without restearing and two control diets with nonfermented scrap meal or white fish meal were prepared (Table 1). The ingredients of

Table 2. Proximate composition of diets used

Diets	NF	F	FS	W
Moisture (%)	12.7	17.4	20.2	7.7
Protein (% d.m.)	44.9	45.5	46.1	45.2
Lipid (% d.m.)	16.0	14.6	14.1	11.2
Carbohydrate* (% d.m.)	5.3	6.8	6.9	5.4
Ash (% d.m.)	18.3	17.8	17.6	16.2

* As glucose.

each diet were mixed thoroughly, and an aliquot of water was added to the mixture. After pelleting, the diets were dried in an air oven at 60°C. Proximate composition of the diets are shown in Table 2.

Fish

Red sea bream, which grew at a comparable rate during a 2 months pre-feeding period with control diet containing white fish meal, were used.

Rearing and Feeding

Fish averaging 39.5 g in body weight were divided into 4 groups with 25 fish each, and kept in a 150 l aquarium. Aquaria were continuously supplied with sand and cotton filtered sea water at a rate of 150 l/h. Water temperature was maintained within the range of 23°C to 25°C. The diet was fed to fish twice a day for 8 weeks, slowly and continuously, as long as fish accepted the diet. The weight of diet fed to each group was recorded and was assumed to be the actual amount consumed by fish. Fish were individually weighed after anesthetization with MS 222 (100 ppm) every 2 weeks. After weighing, fish were bathed in 1 ppm sodium nifurstyrenate for 1 h.

Table 3. Proximate composition, POV, TBA, and VBN of nonfermented, fermented, and fermented-resteamed scarp meals and white fish meal

Meals		Scarp meals			White fish meal
		Non-fermented	Fermented	Fermented-resteamed	
Moisture	(%)	3.9	5.3	5.6	7.9
Lipid	(% d.m)	19.5	16.3	15.8	5.9
Protein	(% d.m.)	55.3	54.5	54.6	69.8
Carbohydrate* ¹	(% d.m.)	—	3.9	3.8	—
Ash	(% d.m.)	19.0	18.9	18.8	18.5
VBN* ²	(mg%)	39.8	92.2	36.8	32.8
POV* ³	(meq/kg)	225.1	29.8	28.7	18.8
TBA* ⁴	(Number)	68.0	31.0	30.0	25.2

*¹ As glucose, *² Volatile basic nitrogen content, *³ Peroxide value, *⁴ Thiobarbituric acid value.

Table 4. Effect of nonfermented (NF), fermented (F), and fermented-resteamed (FS) scarp meals and white fish meal (W) on growth of red sea bream and feed efficiency

Diets		NF	F	FS	W
No. of fish	at start	25	25	25	25
	after 8 weeks	20	23	22	22
Av. body wt (g)	at start	39.5±4.8* ²	39.6±4.9	39.5±4.9	39.5±4.3
	after 8 weeks	48.4±6.3	63.3±6.9	70.8±14.2	79.1±13.4
"t" test (5%)* ¹		S	S	NS	—
Gain/fish (g)		8.9	23.7	31.3	39.6
Feed efficiency (%)		13.5	33.1	39.2	40.9

*¹ S: significant, NS: nonsignificant. *² Standard deviation.

Analytical Methods

Proximate composition of diets, white fish meal, scarp meal, and fermented and fermented-resteamed scarp meals were analyzed. Moisture was determined by a 16 h drying in an electric oven at 100–110°C. Protein and carbohydrate were quantified by the methods of Kjeldahl and of Hodge and Hofreiter,⁴⁾ respectively. Lipid was extracted with ethyl ether and the Soxhlet extractor. Peroxide (POV), thiobarbituric acid (TBA), and volatile basic nitrogen (VBN) values of meals were also determined before and after fermentation. POV and TBA were quantified by the modified methods of Lea⁵⁾ and of Sinnhuber and Yu,⁶⁾ and VBN by the Tomiyama *et al.* method.⁷⁾

Results

Changes in Proximate Composition, POV, TBA, and VBN Values of Fish Waste by Fermentation

As shown in Table 3, crude protein and ash contents scarcely changed by the fermentation, but lipid content, POV, and TBA decreased. Especially, the decrease of POV and TBA were remarkable. On the other hand, VBN increased considerably, but it decreased to the similar value

to that of white fish meal by resteaming after fermentation.

Growth and Feed Efficiency

The growth and feed efficiency of fish fed on fermented scarp meal diet were markedly superior to those of scarp meal diet group (S), but were inferior to white fish meal diet group (W), as shown in Table 4. However, the improvements in growth and feed efficiency were recognized by the resteaming. Namely, fermented-resteamed scarp meal diet group (FS) showed the similar feed efficiency to that of white fish meal diet group (W). Furthermore, the differences in average body weight at the end of this feeding experiment between white fish meal diet group (W) and scarp meal diet group (S) or fermented scarp meal diet group (F) were significant at a 5% level, but that between white fish meal diet group (W) and fermented-resteamed scarp meal diet group (FS) was insignificant.

Discussion

In the present study, fermented scarp meal, whose POV and TBA decreased, improved the

growth and feed efficiency. This seems to be caused by the decline of toxic oxidized lipids by fermentation. However, protein degenerates produced during the fermenting period, which is found with the increased VBN, appears to suppress the improvement of growth and feed efficiency. That is supposed by the comparison with the higher growth and feed efficiency in fish fed on fermented-resteamed scrap meal with decreased VBN. Furthermore, these findings indicate that the removal of both toxic oxidized lipids and protein degenerates is essential in the utilization of fish waste as a dietary protein source, and that fermentation and steaming are effective for the removal.

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