マダイ稚魚用飼料の適正タンパク質･脂質含量

誌名	日本水産學會誌
ISSN	00215392
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巻/号	57巻2号
掲載ページ	p. 293-299
発行年月	1991年2月

Nippon Suisan Gakkaishi 57(2), 293-299 (1991)

Suitable Protein and Lipid Levels in Diet for Fingerlings of Red Sea Bream *Pagrus major*

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(Received May 22, 1990)

Two feeding experiments were conducted to determine the suitable dietary protein and lipid levels for fingerlings of red sea bream by feeding them with various white fish meal-casein diets containing different levels of protein (CP: 37–52%) and lipid (CL: 5–20%).

In Experiment I, the growth rate and feed efficiency increased with increasing CP content in diet, both values reaching a maximum at the diet level of 52%. In Experiment II, the growth rate and feed efficiency were higher for diets containing 52% CP, compared to 42% CP groups, irrespective of the lipid levels. On the other hand, with an increase in the amount of dietary n-3HUFA, the values for growth rate, feed efficiency, and protein efficiency ratio increased, recording high in diets combining 52% CP with 15 and 20% lipids.

This result indicated that the growth of red sea bream fingerlings was influenced by not only dietary protein and lipid levels but also n-3HUFA contents in diet. It is also suggested that the suitable CP and CL levels in diet for red sea bream fingerlings may be around 52% and 15%, respectively.

Of late, the net cage culture of red sea bream has been replacing that of yellowtail. The figerlings of red sea bream are generally fed raw fish such as minced sardine, while dry pellets are in limited use. However, pollution with raw fish has become a serious problem. Since the feed type of the fry and fingerlings will have profound impact on their preferences in the later periods, there is an urgent need to develop dry pellets for them.

The nutritional requirements of red sea bream have already been worked out. Yone *et al.* has published a series of papers,[1-3] wherein they have concluded that the optimum content of protein and lipid in a diet should be 55 and 10%, respectively.[2,3] However, these studies were conducted with semi-purified moist pellet (MP; water content ≧30%) and it might be inappropriate to apply their results directly for dry pellet manufacture. Furthermore, only limited knowledge is available concerning the nutritional requirements of the fingerlings. More information should be gathered to develop a dry feed for fingerlings of red sea bream.

This experiment was conducted to determine the suitable dietary protein and lipid levels for fingerlings of red sea bream by feeding them with various white fish meal-casein diets containing different levels of protein (37–52%) and lipid (5–20%).

Materials and Methods

Experimental Condition

Two feeding experments were conducted in the Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries. Before starting the experiments, red sea bream *Pagrus major* fingerlings were fed for two weeks on minced fish and a commercial red sea bream diet in Expt. I and the commercial diet alone in Expt. II. Then, the fingerlings were randomly divided into four groups of 50 fish each in Expt. I (initial average weight: 1.6 g) and into eleven groups of 30 fish each in Expt. II (initial average weight: 7.5 g). Fish were fed five times a day in Expt. I and four times a day in Expt. II, each time to satiation. Each group of fish was held in a 100 *l* tank with areation (1.0–1.5 *l*/min), and sea water was supplied at a rate of 2.0–2.5 *l*/min. Fish were fed experimental diets for 35 days in Expt. I and for 42 days in Expt. II. The water temperature

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ranged from 23 to 27°C in Expt. I and 22 to 26°C in Expt. II.

Experimental Diets

The composition of the experimental diets used in Expts. I and II are shown in Tables 1 and 2, respectively. In both experiments, white fish meal containing 65% crude protein (CP) and vitamin free casein were used as the main protein sources. α-Starch was the carbohydrate source and pollock liver oil and beef tallow (Riken Vitamin Co. Ltd.) were the lipid sources. The melting point of beef tallow was below 40°C. In order to adjust the level of n-3 highly unsaturated fatty acids (n-3HUFA) in diet, Ester 85 (purity of n-3HUFA: 85%) obtained from Oriental Yeast Co. Ltd. was used in Expt. I. Experiment I: Diets 1 to 3 were arranged at low-, medium- and high-protein levels containing 37, 45 and 52% CP. The contents of crude lipid (CL) and n-3HUFA in diets 1 to 3 were 15% and 3%, respectively. Diet 4 had a low lipid level (10% CL) with 3% n-3HUFA.

Table 1. Composition of the experimental diets for red sea bream in Expt. I (%)

Ingredient	Diet no.			
	1	2	3	4
CP	37	45	52	52
CL	15	15	15	10
n-3HUFA in diet	3.0	3.0	3.0	3.0
White fish meal	55	67	57	57
Casein	0	0	15	15
α-Starch	10	10	5	10
Mineral mix.[4]	5	5	5	5
Vitamin mix.[5]	2	2	2	2
Choline chloride	0.9	0.9	0.9	0.9
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	17	6	5	5
Pollock liver oil	10	9	10	0
Beef tallow	0	0	0	2.6
Ester 85*	0	0	0	2.4
	Analytical result			
Crude protein	38.3	46.9	53.0	53.4
Crude lipid	16.1	15.3	15.9	10.7
n-3HUFA in diet	3.0	3.1	3.1	3.2

* Purity of n-3HUFA: 85%.

Table 2. Composition of the experimental diets*1 for red sea bream in Expt. II (%)

Ingredient	Diet no										
	1	2	3	4	5	6	7	8	9	10	11
CP	42	42	42	42	52	52	52	52	52	52	52
CL	5	10	15	20	5	10	15	15	20	20	20
n-3HUFA in diet	1.0	2.2	2.2	2.2	1.2	2.2	2.2	3.2	2.2	3.2	4.2
White fish meal	46	46	46	46	57	57	57	57	57	57	57
Casein	12	12	12	12	15	15	15	15	15	15	15
α-Starch	18	18	18	18	10	10	10	10	5	5	5
Mineral mix.[4]	5	5	5	5	5	5	5	5	5	5	5
Vitamin mix.[5]	2	2	2	2	2	2	2	2	2	2	2
Choline chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Vitamin E (50%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cellulose	15.6	10.6	5.6	0.6	10	5	0	0	0	0	0
Pollock liver oil	0.4	5	5	5	0	5	5	10	5	10	15
Beef tallow	0	0.4	5.4	10.4	0	0	5	0	10	5	0
	Analytical result										
Crude protein	41.2	42.2	42.3	42.2	52.8	54.0	53.8	54.6	54.1	53.3	53.7
Crude lipid	4.7	10.7	15.2	21.7	6.2	11.1	16.2	15.4	20.9	20.7	21.0
Crude starch	19.2	20.0	20.3	18.0	10.0	10.0	8.5	11.5	7.0	6.8	8.2
Moisture	8.0	6.3	5.0	4.4	6.7	4.6	5.2	4.5	3.1	4.5	4.0
Calorie*2 (kcal/100 g)	277	332	369	414	315	360	396	401	430	425	433
C/P*3	67	79	87	98	60	67	74	73	80	80	81
n-3HUFA in diet	1.1	2.1	2.0	1.8	1.6	2.2	2.1	2.7	1.8	2.9	4.0

*1 All diets include 0.01% ethoxyquin of 50% purity.

*2 Calculated calorie: protein, 4.5 kcal; lipid, 8 kcal; starch, 2.8 kcal/g.[9]

*3 Calorie to protein ratio (kcal/kg/% protein).

Table 3. Results of a 35-day feeding trial with red sea bream*1

Diet no.	Final average body weight (g)	Growth rate (%)*2	Feed efficiency (%) (g gain/g feed)	PER*3	Daily feed intake (g/100 g b.w.)
Initial	1.60±0.45	—	—	—	—
1	5.08±3.21	218	54.9	1.4	5.9
2	5.59±3.35	249	58.8	1.3	5.9
3	6.78±3.02	324	77.5	1.4	5.0
4	5.80±4.97	463	66.0	1.2	4.9

*1 Number of fish: 50.

*2 $\frac{\text{Final body wt. (g)} - \text{Initial body wt. (g)}}{\text{Initial body wt. (g)}} \times 100$

*3 Protein efficiency ratio.

Experiment II: Diets 1 to 4 and diets 5 to 11 were formulated as medium- and high-protein, containing 42% and 52% CP respectively. At both protein levels, the lipid content in diet was elevated from 5 to 20%. Furthermore, n-3HUFA levels were also adjusted to 1 or 1.2% in 5%CL diets and to 2.2% in 10–20%CL diets containing both beef tallow and pollock liver oil. In order to clarify the effect of dietary lipid levels on n-3HUFA requirements in diet, high n-3HUFA levels, 3.2% (diets 8 and 10) and 4.2% (diet 11), were incorporated in 15 and 20%CL diets. The vitamin and mineral mixtures employed were the same as those described in previous papers.[4,5] These experimental diets were provided for the fish in a dry pellet form.

Analytical Methods

The contents of CP, CL and n-3HUFA in the experimental diets were determined by the methods of Kjeldahl, Folch *et al.*[6] and GLC analysis, respectively. Crude starch was determined by the procedure of Somogi-Nelson.[7] Fatty acids were methyl esterified as described earier.[8] The calorie content of diets was calculated using the following values: protein, 4.5 kcal; lipid, 8 kcal; starch, 2.8 kcal/g.[9]

Whole body samples obtained from both Expts. I and II were completely homogenized[10] and subjected to the analytical procedures mentioned above.

Results

Experiment I

Results of a 35-day feeding in Expt. I are shown in Table 3. The daily feed intake ranged from 4.9 to 5.9% among the experimental groups; the highest in diets 1 and 2 containing 37% and 45%CP (analytical value of crude protein is 38.3% and 46.9%, respectively). This result agreed with that usually observed that feed intake is high in fish fed low protein diets in order to satisfy the daily requirement of nitrogen. The growth rate and feed efficiency were improved as the dietary protein levels increased, when the lipid level was fixed at 15%, reaching a maximum with the 52%CP diet. At the level of 52%CP in diets, these values of the fish fed the diet containing 10% lipid were inferior to those of the fish fed 15% lipid. The proximate composition of the whole body is shown in Table 4. The final fish, after 35 days of feeding showed low moisture, high protein, lipid and ash in the body, in comparison with the initial fish. The whole body protein of fish fed on diets high in protein levels (52%CP; diets 3 and 4) was higher than that on low and medium protein diets (37%CP, 45%CP; diets 1 and 2). On the other hand, the whole body lipid was high in fish fed diets 1 and 2.

Experiment II

Results of a 42-day feeding in Expt. II are shown in Table 5 and Fig. 1. The daily feed intake did not vary much among the groups, the value ranging from 2.7 to 3.3. However, the intake recorded in this experiment was lower than in Expt. I, probably due to the size of fish. The growth rate and feed efficiency improved as dietary lipid levels increased, reaching a maximum

Table 4. Proximate composition of the whole bodies of red sea bream in Expt. I (%)

Diet no.	Crude protein	Crude lipid	Crude ash	Moisture
Initial	15.4	2.4	4.2	78.8
1	16.9	8.1	4.5	72.4
2	16.2	8.5	4.6	72.2
3	17.7	6.2	5.1	73.6
4	18.2	7.6	5.2	71.6

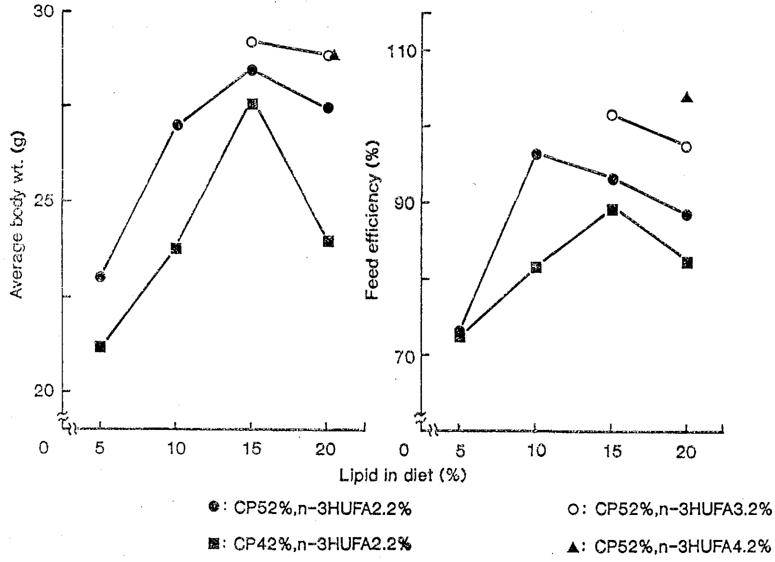

Fig. 1. Effect of dietary protein and lipid levels on growth and feed efficiency of red sea bream.

Table 5. Results of a 42-day feeding trial with red sea bream*1

Diet no.	1	2	3	4	5	6	7	8	9	10	11
CP	42	42	42	42	52	52	52	52	52	52	52
CL	5	10	15	20	5	10	15	15	20	20	20
n-3HUFA in diet	1.0	2.2	2.2	2.2	1.2	2.2	2.2	3.2	2.2	3.2	4.2
Av. body wt. (g)											
Initial	7.5 ±0.9	7.5 ±1.0	7.5 ±1.3	7.5 ±1.1	7.5 ±1.2	7.5 ±1.1	7.5 ±1.2	7.5 ±1.1	7.5 ±1.0	7.5 ±0.9	7.5 ±0.9
Final	21.2 ±4.1	23.8 ±3.6	27.6 ±4.4	24.0 ±3.4	23.0 ±3.6	27.0 ±4.1	28.5 ±3.8	29.2 ±4.5	27.5 ±3.5	28.9 ±4.2	28.9 ±3.0
Daily feed intake (g/100 g b.w.)	3.1	3.0	3.0	3.0	3.3	2.8	3.0	2.8	3.0	2.9	2.7
Growth rate*2 (%)	183	217	268	220	207	260	280	289	267	285	285
FE (%)*3	72.9	81.7	89.9	82.5	73.1	96.6	93.3	101.6	88.6	97.4	103.6
PER*4	1.8	1.9	2.1	2.0	1.4	1.8	1.7	1.9	1.6	1.8	1.9
Hepatosomatic index (%)*5	2.1 ±0.3	2.7 ±0.6	3.5 ±0.8	4.0 ±0.5	2.2 ±0.4	2.5 ±0.6	3.0 ±0.6	2.6 ±0.5	2.3 ±0.3	1.9 ±0.3	2.2 ±0.3

*1 Number of fish: 30.
*2 See the footnote of Table 3.
*3 Feed efficiency (g gain/g feed).
*4 Protein efficiency ratio.
*5 Mean±SD, n=13.

when 15% lipid was added at a fixed protein level of 42% and n-3HUFA level of 2.2%. A similar tendency was noted in fish fed 52% protein diets. A low growth rate was observed in the fish fed low lipid diets (5%CL), regardless of the dietary protein levels. On the other hand, with an increase in the amount of dietary n-3HUFA, the values for both the growth rate and feed efficiency increased, recording high in diets combining 52%CP with 15 and 20% lipids.

Protein efficiency ratio (PER) was almost inversely proportional to the protein content in diet (Fig. 2). However in the 52% protein diets, PER increased with an increase in the amount of dietary n-3HUFA. Hepatosomatic index was greater in fish fed the diet containing more dietary lipid, especially 20% lipid with 42%CP (diet 4). On the other hand, low hepatosomatic indices were observed in the fish fed diets of 52%CP and 20%CL supplemented with 2.2 to 4.2% n-3HUFA.

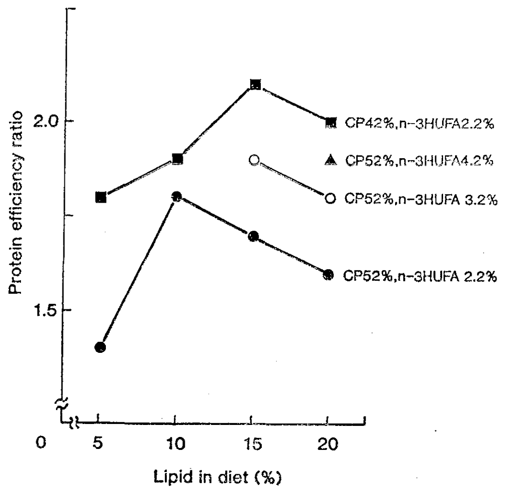

Fig. 2. Effect of dietary protein and lipid levels on protein efficiency ratio in red sea bream.

Proximate compositions of the whole body are given in Table 6. The terminal group of fish fed each diet for 42 days showed the same ash content in their bodies, compared with the initial fish. On the contrary, dietary lipid levels reflected the lipid and moisture contents. The highest estimate of body lipid was in the fish fed diet 4 containing 42%CP and 20%CL. Moreover, the protein content of this group of fish was lower than that of other terminal group of fish.

Discussion

Yone *et al.*[3] has already investigated the requirement of red sea bream for essential amino acids and proteins and reported that the feed efficiency for red sea bream increased linearly, until it became constant at a CP level of 55%. These results are in agreement with those of this experiment. In Expt. I, the growth rate and feed efficiency increased with increasing CP content in diet, both values reaching a maximum at the CP level of 52%. In Expt. II, assuming that the suitable CP level in diet for fingerlings of red sea bream is around 50%, two series of diets were prepared with a CP level of either 42 or 52% and lipid supplements ranging between 5 and 20%. The results demonstrated that the growth and feed

Table 6. Proximate composition of the whole bodies of red sea bream in Expt. II (%)

Diet no.	Initial	1	2	3	4	5	6	7	8	9	10	11
CP		42	42	42	42	52	52	52	52	52	52	52
CL		5	10	15	20	5	10	15	15	20	20	20
n-3HUFA in diet		1.0	2.2	2.2	2.2	1.2	2.2	2.2	3.2	2.2	3.2	4.2
Whole body												
Crude protein	17.6	18.6	18.2	18.2	17.6	18.8	19.2	18.1	18.4	18.3	18.3	18.1
Crude lipid	5.9	8.9	10.9	14.3	16.1	8.4	10.6	14.1	14.0	14.0	14.6	14.5
Crude ash	5.4	5.7	6.1	5.5	5.2	5.3	5.5	5.3	5.4	5.1	5.0	5.5
Moisture	72.5	68.7	68.1	64.5	64.5	70.0	66.5	63.9	64.6	63.9	62.4	62.4

Table 7. Protein and energy required for 100 g b.w. gain and daily nitrogen budget in red sea bream

Diet no.	Calorie in diet (kcal/100 g)	Total diet consumed (g)	Nutrient required for 100 g b.w. gain		Daily intake		Daily N increase (mg/100 g b.w.)
			Protein (g)	Energy (kcal)	N (mg/100 g b.w.)	Energy (kcal/100 g b.w.)	
1	277	568.7	57.0	383	207.3	8.7	69.6
2	332	600.1	51.7	406	205.3	10.1	73.4
3	369	669.4	47.0	410	205.0	11.2	80.3
4	414	601.4	51.0	502	204.4	12.5	70.3
5	315	634.4	72.2	431	279.4	10.4	74.9
6	360	597.4	55.9	372	238.0	10.0	84.5
7	396	665.9	57.7	424	253.8	11.8	80.6
8	401	641.6	53.7	394	242.3	11.1	84.2
9	430	679.2	61.1	485	261.8	13.5	79.6
10	425	658.7	54.7	436	244.9	12.2	83.0
11	433	620.4	51.8	417	232.2	11.7	81.8

efficiency were higher for diets containing 52%CP, when compared with 42%CP groups, irrespective of the lipid levels. Some diets containing 52%CP showed more than 100% feed efficiency. Furthermore, the daily nitrogen intake was also higher in the diet containing 52%CP (Table 7). Though Expts. I and II do not include the diets containing more than 52%CP, it is actually difficult to increase the CP levels above 55%, because practical feed contains fish meal as a major protein source. Therefore, why the present study did not consider CP levels above 55% is justified.

Most commercial feeds currently available for growing red sea bream have a CP level of about 45%.[11] Watanabe *et al.*[12] has reported that cuttlefish meal was better than white fish meal as a protein source for brood stock red sea bream, with the optimum CP content being around 45%. Comparison of our results with those found in literature suggests that the suitable dietary CP level for fingerlings of red sea bream was 7% higher than that for adult and brood stock fish, although dietary protein is utilized for reproduction in brood fish. Thus, the fingerlings appear to require more protein than do adult fish.

As indicated in Fig. 3, when the n-3HUFA content was constant at 1.2% or 2.2%, addition of lipid to a diet containing 52%CP increased the dietary energy (DE) and the growth rate which reached a maximum at around 400 kcal/100 g diet (15%CL). The growth rate however, was lowered when lipid was added up to 430 kcal/100 g diet. For diets containing 42%CP, on the other hand, the highest growth rate was achieved at 370 kcal/100 g diet (15%CL), but was reduced when the DE was increased to 410 kcal/100 g diet (20%CL). Yong *et al.*[13] have reported that the daily feed consumption of Tilapia depends on the DE content in the diet, the ingestion rate immediately decreasing with an increase of the DE content, leading to a lower growth rate. In our study, the growth rate was found to decrease in diets with DE at more than 400 kcal/100 g, regardless of the protein levels. Nevertheless, the daily feed consumption of these diets was virtually the same as that in other diets and furthermore, the daily energy intake in these diets was larger than in the others (Table 7), indicating that the decrease in growth rate in these diets was not the result of a decrease in daily feed consumption. An analysis suggested that when the n-3HUFA content in diet is kept constant at 2.2%, the addition of 20% lipid leads to a de-

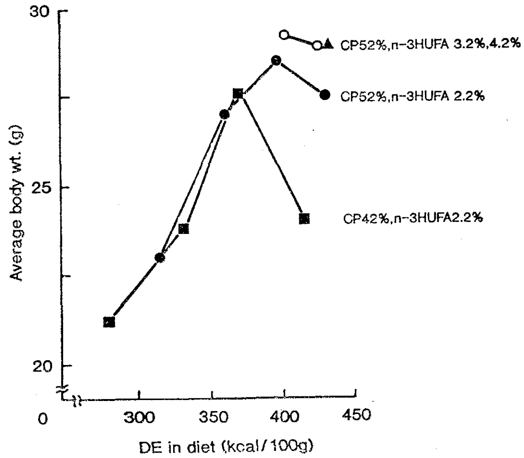

Fig. 3. Relationship between digestible energy (DE) in diet and growth of red sea bream.

ficiency of n-3HUFA, an essential fatty acid (EFA). In fact, an increase in the n-3HUFA content permits an increase in growth rate, and it can be concluded that an increase in the requirement for n-3HUFA is caused by the addition of 20% lipid (descussed in detail later).

These results, coupled with the fact that diets containing 15%CL produced a higher growth rate and feed efficiency regardless of the protein levels, suggest that the suitable CL level in diet for fingerlings of red sea bream is around 15% when the n-3HUFA is kept constant at 2.2%.

In the diets incorporating either 15 or 20%CL with a varying n-3HUFA content in the range from 3.2 to 4.2%, the growth rate, the feed efficiency and PER were higher than in the diets containing the same CL contents but with 2.2% n-3HUFA (Figs. 1 and 2). These results suggest that the growth rate of red sea bream depends on the content of n-3HUFA and the n-3HUFA requirement increase with the CL level. For the rainbow trout, the amount of linolenic acid, an EFA, required to achieve the maximum growth is known to increase with increasing CL level of diet,[14] but no reports have appeared for these relations for red sea bream. It seems inappropriate to discuss the optimum CL level in diet for fingerlings of red sea bream only in terms of the lipid levels. Further investigations are necessary to identify the optimum n-3HUFA content at different CL levels.

Thus, this result indicates that suitable CP and CL levels in the diet for red sea bream fingerlings may be around 52% and 15%, respectively, but further experiments are being performed to study the optimum CL content in relation to the n-3HUFA content in diet. These results will be presented shortly.

Acknowledgements

The authors express here our sincere thanks to Mr. Toshihisa Arakawa, Nagasaki Prefectural Institute of Fisheries, and Mr. Ichiro Kobayashi, Tokyo University of Fisheries, for their techical assistance. The present work was supported in part by a research fund from Marino-Forum 21 and Riken Vitamin Co. Ltd.

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