J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1995), 34: 167~178

Effects of Feeding Regime on Biometric Parameters and Hepatic Enzyme Activities of young Red Sea Bream, *Pagrus major*

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Received November 9, 1995

Abstract In order to obtain optimum feeding conditions in young red sea bream *Pagrus major*, fish averaging 5.1 g in body weight were divided into five groups. Three groups were fed to satiation at different frequencies (two, four and six times a day) for 49 days. The other two groups were reared with a fixed daily ration corresponding to two times satiation at different frequencies (four and six times a day). In satiation, increasing feeding frequency elevated the amount of total food intake, intraperitoneal fat body ratio and muscle lipid level. However, feed efficiency and hepatosomatic index were depressed. Fish fed to four satiations daily had the lowest intestinal length. Liver lipogenic and aminotransferase activities were significantly higher in the group fed to four satiations daily. Different feeding frequencies at a fixed ration exhibited only small differences in the parameters concerning lipid accumulation. Reduced feeding frequency significantly extended intestinal length. Frequent feeding tended to enhance the liver enzyme activities associated with gluconeogenesis and glycolysis, and depress lipogenesis. The feed utilization, lipid accumulation, intestinal length and liver enzyme activities showed that two satiations daily would be sufficient in young red sea bream.

Key words: feeding frequency, hepatic enzymes, red sea bream, intestine, lipid metabolism, satiation

INTRODUCTION

Fish can adapt their metabolism to a number of feeding regimes such as feeding frequency and ration size which may lead to improvements in growth performance and carcass composition. In commercial rearing, fish are fed according to widely differing protocols, but optimum feeding regimes with regard to carcass quality, which has recently arisen, are unknown. In addition to optimal growth efficiency, an optimum feeding condition should be established in terms of carcass composition to give the desired lipid level of fish. The increase in reserved lipids actually coincides with increased ration size (ELLIOTT, 1976; HUNG and LUTES, 1987; STOREBAKKEN *et al.*, 1991; KAYANO *et al.*, 1990). Accordingly, high ration size did not always result in high growth (BERGOT, 1979; GRAYTON and BEAMISH, 1977; WEBSTER *et al.*, 1992).

The importance of feeding frequency in intensive fish culture has been described by many authors (ANDREWS and PAGE, 1975; GRAYTON and BEAMISH, 1977; KAYANO *et al.*, 1993; LUQUET *et al.*, 1981; NOESKE-HALLIN *et al.*, 1985; OMAR and GÜNTER, 1987; PALMER *et al.*, 1951; TUNG and SHIAU, 1991; YAO *et al.*, 1994). Enzyme activities are closely related to growth and body composition (WALZEM *et al.*, 1991), but have been examined in only a few studies (TUNG and SHIAU, 1991; HUNG *et al.*, 1993).

Red sea bream *Pagrus major* is widely cultured in Japan, but little attention has been paid to the effect of feeding regime. This study was conducted to elucidate the influences of feeding regimes on biological parameters and activities of selected hepatic enzymes to obtain basic information concerning the optimum feeding regime in red sea bream.

MATERIALS AND METHODS

Fish and rearing conditions

Young red sea bream produced at the Hiroshima Prefecture Fish Farming Center were transported to the Fisheries Laboratory of Hiroshima University. For the experiment, 450 fish averaging 5.1 ± 1.1 g in body weight were divided into three groups and reared for 49 days in one ton tanks ($800m^3$ water volume). The fish were exposed to the natural variation in photoperiod, water temperature and salinity. The temperature and salinity of the water ranged between 18.4–25.8°C and 26.0–33.5 ppt, respectively. The proximate composition of a commercial dry diet was as follows: moisture 2.8%, ash 12.0%, crude protein 52.0% and lipid 15.8%.

After one week acclimation, three groups were fed to satiation with different daily feeding frequencies designated as the following: two times daily at 07:00 and 17:00 (group 1); four times daily at 07:00, 10:00, 13:00 and 17:00 (group 2); six times daily at 07:00, 09:00, 11:00, 13:00, 15:00 and 17:00 (group 3). The fish were fed to satiation for a 15 min period in each feeding. Hand feeding was carried out to encourage the fish to feed at the surface and to keep food wastage to a minimum.

Two groups with a fixed daily ration at different feeding frequencies were designated. The same daily ration which was prescribed as ration fed to group 1 was administrated four times daily (group 4) and six times daily (group 5).

Body parameters

At the end of the experiments, starved fish for 48 hours were sampled at random from each group for morphological and anatomical measurements. The coefficient variation (CV) of body weight, condition factor, muscle ratio, hepatosomatic index, intraperitoneal fat body (IPF) ratio, relative length of intestine (RLI) and relative weight of stomach (RWS) were calculated from the following equations:

$$\text{CV (\%)} = (\text{standard deviation} / \text{mean}) \times 100$$

$$\text{Condition factor} = (\text{body weight} / \text{body length}^3) \times 10^5$$

$$\text{Muscle ratio (\%)} = (\text{muscle weight} / \text{body weight}) \times 100$$

$$\text{Hepatosomatic index (\%)} = (\text{liver weight} / \text{body weight}) \times 100$$

$$\text{IPF ratio (\%)} = (\text{IPF weight} / \text{body weight}) \times 100$$

$$\text{RLI} = \text{intestine length} / \text{body length}$$

$$\text{RWS} = (\text{stomach weight} / \text{body weight}) \times 100$$

Biochemical analyses

Five fish taken from each group were frozen immediately after sacrifice by stubbing medulla and kept at −20°C until muscle lipid analysis. The muscle taken from five to eight fish were pooled and the lipid was extracted from muscle according to BLIGH and DYER (1959). The livers stored under liquid nitrogen were subjected to analysis of enzyme activities. The pooled livers obtained from five to nine fish were homogenized with nine volumes of cold water for one min. After centrifugation at 5,000 rpm for 10 min at 0−4°C, the supernatant was immediately submitted to enzyme analysis. Enzyme reaction was initiated by the addition of substrate. Protein of the enzyme solution was measured by the Lowry method (LOWRY *et al.*, 1951).

Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (PGDH, EC 1.1.1.44) were determined by reduction of NADP according to the method of GLOCK and MCLEAN (1953). Glucose-6-phosphatase (G6Pase, EC 3.1.3.9) and fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11) were determined by measureing produced inorganic phosphate according to the method of SHIMENO (1982). Phosphofructokinase (PFK, EC 2.7.1.11) and pyruvate kinase (PK, EC 2.7.1.40) were determined by measuring ADP and ATP respectively according to the method of MOON and JOHNSTON (1980). Aspartate aminotransferase (ASA, EC 2.6.1.1) and alanine aminotransferase (ALA, EC 2.6.1.2) were determined by the method of KARMAN (1955). Reaction products were measured by spectophotometer.

Enzyme activities were expressed as units per 100 g body weight, where one unit was defined as the amount of enzyme required to convert 1.0 μmol of substrate or coenzyme per min at 30°C. The activities are shown as means of triplicate analyses.

Statistical analysis

All data were subjected to a two-way analysis of variance. Differences among the group means were analyzed for significance by Duncan's multiple range test. Probabilities 0.05 or less were considered to indicate statistical significance.

RESULTS

Table 1 shows data of growth performance and biological characteristics. When the fish were fed to satiation, the amount of food was increased with increased feeding frequency. However, increasing the amount of food intake were not accompanied by increasing weight gain and feed efficiency. Mean body weight and body length were slightly different among the groups. The coefficient variation of body weight is shown in Fig. 1. When the coefficient of variation of body weight was used to express variability in inter individual differences in growth, the enhanced feeding frequency did not reduce size variation in the fish. The size variation in body weight could be minimized by four satiations daily (group 2). When the fish were fed an equal amount of food (group 1, 4 and 5), weight gain and feed efficiency were better in the fish fed to satiation twice daily (group 1).

Satiation feeding (group 1, 2 and 3)

When the fish were fed to satiation, the condition factor was increased with increased ration. Hepatosomatic index was decreased with increasing food intake. Muscle ratio was significantly highest in the group satiated four times daily (group 2). The IPF ratio and

Table 1. Effects of feeding regime on biological parameters of young red sea bream

	Initial	Experimental group				
		1	2	3	4	5
Feeding frequency (times/day)		2	4	6	4	6
Total amount of feed (g)		1629	2199	2335	1629	1629
Biomass increased (g)		1599	1870	1627	1310	1403
Survival (%)		76.7	79.3	74.0	76.0	78.0
Feed efficiency (%)		98.2	85.0	69.7	80.4	86.1
Mean body weight (g)	5.1 ± 1.1	20.4 ± 3.1^{ab}	22.0 ± 2.5^{b}	21.4 ± 3.3^{b}	18.2 ± 2.5^{a}	18.4 ± 4.1^{a}
Mean body length (mm)	55 ± 4	83 ± 4^{ab}	84 ± 3^{b}	83 ± 4^{ab}	80 ± 4^{ac}	80 ± 5^{c}
Condition factor	2.94 ± 0.16	3.49 ± 0.15^{a}	3.69 ± 0.18^{b}	3.69 ± 0.39^{b}	3.50 ± 0.18^{a}	3.58 ± 0.19^{ab}
Hepatosomatic index (%)	1.21 ± 0.26	2.33 ± 0.54^{a}	2.25 ± 0.41^{a}	1.88 ± 0.34^{b}	2.21 ± 0.49^{a}	2.39 ± 0.30^{a}
Muscle ratio (%)	32.4 ± 3.6	44.1 ± 1.5^{a}	45.8 ± 1.7^{b}	44.7 ± 1.6^{a}	44.5 ± 1.1^{a}	44.3 ± 2.6^{a}
IPF ratio (%)*1	1.27 ± 0.35	2.91 ± 0.92^{ab}	3.26 ± 0.69^{ab}	3.48 ± 0.96^{b}	2.65 ± 0.48^{a}	2.76 ± 0.82^{a}
RLI*2	1.34 ± 0.20	1.90 ± 0.28^{a}	1.93 ± 0.30^{a}	1.68 ± 0.24^{b}	1.73 ± 0.29^{ab}	1.68 ± 0.24^{b}
RWS (%)*3		0.73 ± 0.11	0.80 ± 0.09	0.75 ± 0.06	0.73 ± 0.15	0.68 ± 0.11
Muscle lipid (%)*4	1.33 ± 0.08	2.54 ± 0.08^{a}	3.10 ± 0.37^{b}	3.30 ± 0.16^{b}	2.33 ± 0.22^{a}	2.30 ± 0.16^{a}

Mean ± SD (n=15). Values in each row with different superscripts are significantly different ($p<0.05$).
*1 Intraperitoneal fat body ratio (IPF weight / body weight × 100).
*2 Relative length of intestine (intestine length / body length).
*3 Relative weight of stomach (stomach weight / body weight × 100).
*4 The values are means of triplicate analyses (pooled sample of 5–8 fish each).

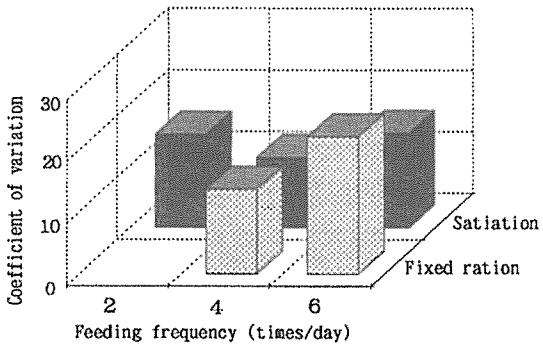

Fig. 1. Coefficient of variation in mean body weight (CV) of fish fed at different regimes.

muscle lipid content, which are indices to express lipid accumulation were significantly increased with increasing food intake. The RLI increased during the rearing period for 5 weeks and reached a plateau thereafter. While the RLI showed a tendency to be higher in the group satiated two and four times daily, there was no observable difference in stomach weight.

Table 2 shows hepatic enzyme activities of the fish fed to satiation at different feeding frequencies after 7 week rearing. The activities of G6PDH and PGDH were increased through the rearing period. The four satiations daily (group 2) significantly elevated these activities. The activity of G6Pase being high in the beginning of the experiment decreased with the progress of the rearing period, but the activity was not significantly different among the groups at the end of the experiment. The activity of FBPase increased during

Table 2. Effects of feeding regime on hepatic enzyme activities (unit/100 body weight) in young red sea bream

Enzyme	Initial	Experimental group				
		1	2	3	4	5
G6PDH	13.4±1.1	52.3±6.9^{ac}	73.9±7.2^{b}	59.2±1.8^{c}	44.5±3.8^{a}	43.5±5.8^{a}
PGDH	6.8±0.8	29.2±3.8^{a}	38.1±2.6^{b}	29.3±0.9^{a}	25.0±2.2^{ac}	21.8±1.4^{c}
G6Pase	11.2±1.4	6.5±0.2^{ab}	6.9±0.4^{ab}	5.6±0.8^{b}	6.9±0.2^{a}	7.2±1.2^{a}
FBPase	4.2±0.7	9.0±1.4^{ab}	10.5±1.1^{b}	8.5±1.2^{a}	8.0±0.5^{a}	9.1±0.6^{ab}
PFK	6.0±1.0	14.0±2.4	15.0±1.0	13.6±1.1	14.1±1.1	14.2±0.7
PK	28.7±1.3	28.0±6.5	32.7±3.6	34.5±2.6	32.5±9.9	35.5±3.6
ASA	133±8	281±36^{a}	351±21^{b}	251±48^{a}	226±30^{a}	254±6^{a}
ALA	nd*	205±15^{ab}	227±25^{b}	190±16^{ac}	208±25^{ab}	164±8^{c}

* not determined. The values are means of triplicate analyses (pooled sample of 5 to 8 livers).
Values in each row with different superscripts are significantly different ($p<0.05$).
G6PDH; glucose-6-phosphate dehydrogenase, PGDH; phosphogluconate dehydrogenase,
G6Pase; glucose-6-phosphatase, FBPase; fructose-1, 6-bisphosphatase,
PFK; 6-phosphofructokinase, PK; pyruvate kinase, ASA; aspartate aminotransferase,
ALA; alanine aminotransferase

rearing, but was relatively high in the group satiated four times daily (group 2). The activities of PFK and PK were independent of ration size with different feeding frequencies. In addition, the activity of PK was kept constant during the experimental period. The activities of ASA and ALA of the group satiated four times daily (group 2) were higher than the other groups.

Feeding frequency with a fixed ration (group 1, 4 and 5)

Table 1 shows the effect of feeding frequency with the same amount of food on biological parameters. There were no significant differences ($p>0.05$) in the hepatosomatic index, muscle ratio, IPF ratio or muscle lipid among the three groups. The RLI was extended with decreasing feeding frequency, but stomach weight was not influenced.

The enhanced feeding frequency depressed the activities of G6PDH and PGDH (Table 2). The activity of G6Pase was not influenced by the feeding frequency. The activities of FBPase and PFK were independent of feeding frequency, as well. Frequent feeding tended to enhance PK activity, but not significantly ($p>0.05$). The feeding frequency did not affect ASA activity, but the ALA activity was significantly lower in the fish fed six times daily (group 5).

All experiments combined

Tables 3 and 4 show probability from the analysis of variance in biological and biochemical parameters. The fish size, IPF and muscle lipid were affected by ration size. Feeding frequency accounted for intestinal length alone. Combined effects of ration size and feeding frequency were seen in hepatosomatic index and muscle lipid level. On hepatic enzyme activities, ration size and feeding frequency showed combined effects on those relating to lipogenesis and protein turnover.

The enzyme activities and muscle lipid content were plotted and their interactions were monitored. Fig. 2 shows the relationship among hepatic enzyme activities. The activities of PGDH and G6PDH, which are relating to lipogenesis, were well correlated (n=48,

Table 3. Probability from the analysis of variance in biological and biochemical parameters

Source	Body weight	Body length	Condition factor	HSI*1	Muscle ratio	IPF ratio*2	RLI*3	RWS*4	Muscle lipid
Ration	p<0.01	p<0.05	p<0.05	NS	NS	p<0.05	NS	NS	p<0.01
Frequency	NS	NS	NS	NS	NS	NS	P<0.01	NS	NS
Interaction	NS	NS	NS	p<0.05	NS	NS	NS	NS	p<0.05

*1 Hepatosomatic index. *3 Relative length of intestine. NS = Not significant (p>0.05).
*2 Intraperitoneal fat body ratio. *4 Relative weight of stomach.

Table 4. Probability from the analysis of variance in hepatic enzymes

Source	G6PDH	PDGH	G6Pase	FBPase	PFK	PK	ASA	ALA
Ration	p<0.01	p<0.01	NS	NS	NS	NS	p<0.05	NS
Frequency	NS	p<0.05	NS	NS	NS	NS	NS	p<0.05
Interaction	p<0.05	p<0.05	NS	NS	NS	NS	p<0.05	NS

Abbreviations of enzymes, see Table 2. NS = Not significant (p>0.05).

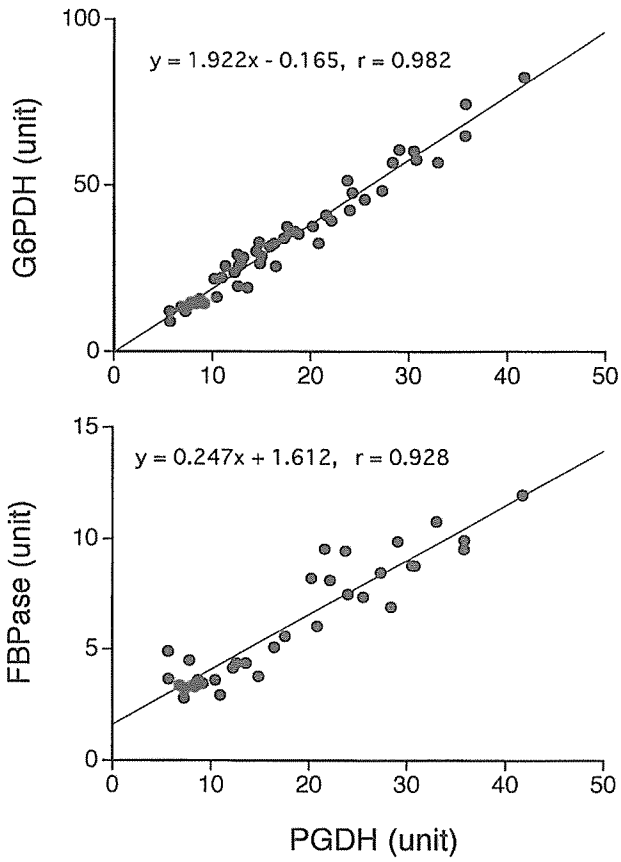

Fig. 2. Relationship among hepatic enzyme activities (units/100g body weight) of red sea bream.
PGDH: phosphogluconate dehydrogenase
G6PDH: glucose-6-phosphate dehydrogenase
FBP: fructose bisphosphatase

r=0.982). FBPase, which is related to glycogenesis, was well correlated with lipogenic enzyme PGDH (n=33, r=0.927), as well. Fig. 3 shows the relationship between lipogenic enzymes and muscle lipid content. The muscle lipid content was correlated with the activities of G6PDH (n=48, r=0.741) and PGDH (n=48, r=0.782).

DISCUSSION

The amount of food consumed by the fish fed to satiation was raised by enhancing feeding frequency. Although the experiment did not statistically assess the influence on growth and feed efficiency, body weight did not correlate to food intake. The lowest size variation was found in the group fed to satiation four times daily (group 2). Enhancement of feeding frequency improves growth and feed efficiency, possibly due to the reduction of hierarchy formation (JOBLING, 1983). MCCARTHY *et al.*, (1992) suggested that the strength of feeding hierarchy and variability in individual consumption decreased with increasing food availability. In contrast, the present study did not show that frequent feeding reduced

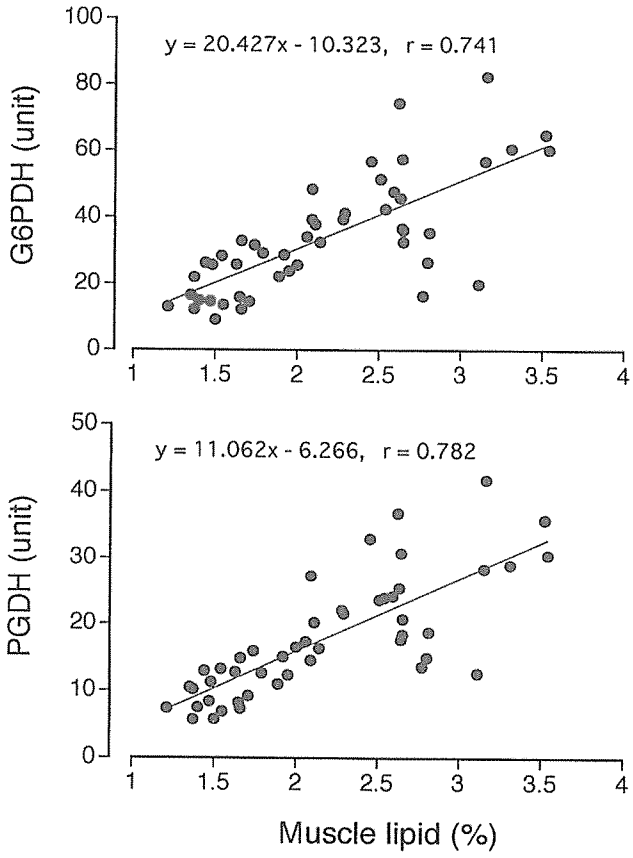

Fig. 3 Relationship between muscle lipid and hepatic enzyme activities (units/100g body weight) of red sea bream.
G6Pase: glucose-6-phosphate dehydrogenase
PGDH: phosphogluconate dehydrogenase

size variation. The variability in body weight might be attributed to individual differences in appetite rather than hierarchical behavior.

Since the liver is highly sensitive to nutritional status, the increase in hepatosomatic index is explained by high nutritional status. Nevertheless, the present study showed that the hepatosomatic index was decreased with the increase of food given. The fish could not adapt their metabolisms to satiation at high feeding frequency. The results might be a phenomenon caused by overfeeding.

Morphological adaptation of the digestive tract, including extended intestine and enlarged intestinal surface, could be the result of a need for effective utilization of restricted food (HOFER, 1988). KONO and NOSE (1971) concluded that the frequency of feeding requirements is related to stomach size. The present study showed the reduction of intestinal length by high feeding frequency. The red sea bream has a relatively large stomach, so the fish might consume sufficient food with at least two feedings daily. The short intestine caused by high ration would be due to the unnecessary increase in storage capacity and evacuation time of food. YAMASHITA *et al.* (1995) found shorter intestinal length in artifi-

cially produced black sea bream, *Acanthopagrus schlegeli*, than in wild ones. Intestinal length is a phenotypic characteristic, but it would be variable in response to feeding regime (DABRWSKI, 1993; STROBAND, 1977).

Feeding regime and diet composition influence the metabolic response of fish (TUNG and SHIAU, 1991; HUNG *et al.*, 1993). The fish fed a fixed ration at different frequencies (group 1, 4 and 5) exhibited only small differences in biological parameters and lipid accumulation. Lipogenic enzyme activities proved that the high feeding frequency likely suppressed lipid accumulation. This is in line with observations on rats which showed that high feeding frequency might depress lipogenic activities, and has effects on storage lipids (COHN and JOSEPH, 1960; PHILIPPENS *et al.*, 1977). Four satiations daily (group 2) elevated G6PDH and PGDH, which participate in the conversion of energy into NADPH which is related to lipogenesis (HUNG *et al.*, 1993). The lipogenic enzyme activities were relatively higher in the fish fed to satiation four times daily (group 2). Nutrients provided by two and four satiations daily (group 2 and 3) might be effectively utilized for growth, and the surplus energy was accumulated as reserved lipids. Lipid accumulation coincides with activities of such lipogenic enzymes as G6PDH and PGDH (HUNG *et al.*, 1993). The present results proved the close relationship among these lipogenic enzyme activities and lipid accumulation. However, the highest lipid accumulation in the fish fed to satiation six times daily (group 3) was not accompanied by high lipogenic activities. The results agreed with those of HUNG *et al.* (1993) and WALZEM *et al.* (1990), indicating that high ration accelerated lipid accumulation but depressed lipogenic enzyme activities. The excessive lipid accumulation might be due to active liponeogenesis and/or lowered lipolysis.

While the G6Pase involved a role in the final stage of gluconeogenesis was poorly influenced by feeding frequency, the group fed to satiation six times daily (group 3) showed the lowest activity. The fish fed to satiation four times (group 2) had the highest FBPase activity, suggesting high gluconeogenesis. The activities of PFK and PK slightly increased but not significantly ($p>0.05$) by various feeding regimes. The glycogenetic enzyme might closely relate to lipogenesis. The highest activities of aminotransferic and lipogenic activities in the fish fed to satiation four times daily (group 2) implied high metabolic interaction between protein turnover and lipogenesis. The highest ration resulted in the depression of lipogenesis and protein turnover, and the highest lipid accumulation. However, high feeding frequency at a fixed ration tended to accelerate glycolysis and gluconeogenesis, and inversely to depress lipogenesis and protein turnover.

In conclusion, the results revealed a sensitive metabolic response to feeding regime. Four satiations daily (group 2) extended intestinal length and accelerated lipogenesis, glycolysis, gluconeogenesis and protein turnover. As these parameters likely change with fish size, the results would be applicable to a commercial culture to improve the feeding economy. Enhanced feeding frequency reduced intestinal length. Two satiations daily would be sufficient for maximal physiological condition. The present results coincided with the findings of AZETA *et al.* (1980) which stated that young red sea bream feed two times daily, in the morning and evening, in a natural environment.

Acknowledgements We are grateful to Dr. S. Shimeno of Kochi University for his advice in the

analytical methods of enzyme activities. We wish to thank the Hiroshima Prefecture Fish Farming Association for donating the fish and Dr. O. Ohtsuka and Mr. S. Izumi of the Fisheries Laboratory of Hiroshima University for useful technical support with regard to fish rearing.

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マダイ幼魚の生物学的性状および肝臓酵素活性に及ぼす給餌方法の影響

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体重5.1gのマダイ幼魚を５群に分けて，３つの群は日間給餌頻度を２回，４回，６回とし，49日間飽食させた。他の２群には日間給餌回数２回の飽食量相当分を４回，６回に分けて投与した。飽食給餌では給餌頻度の増加と共に摂取量，脂肪組織，筋肉脂質が上昇した反面，飼料効率，比肝重量は低下した。４回飽食の場合，腸管長は最も短く，肝臓脂質合成酵素，アミノ基転移酵素活性は高かった。一方，２回分の飽食量を４回，６回に分けて給餌した場合，脂質蓄積に変化はなかったが給餌回数の減少と共に腸管は伸張し，肝臓の糖新生関連酵素，解糖酵素活性は低下し，脂質合成関連酵素が向上した。飼料効率，脂質蓄積，腸管長，酵素活性からみて１日２回の飽食給餌が最適であることが判明した。

キーワード：給餌頻度，肝臓酵素，マダイ，腸管，脂質代謝，飽食