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Influences of dietary lipid and phosphorus levels on retention and excretion of phosphorus and nitrogen in fingerling red sea bream, *Pagrus major*

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Abstract
A laboratory based 2 × 3 factorial experiment was conducted for 12 weeks to investigate the influences of dietary lipid and phosphorus (P) levels on retention and excretion of phosphorus and nitrogen (N) in fingerling red sea bream. Two levels of lipid (210 and 260 g/kg) and three levels of phosphorus (17, 14 and 12 g kg^{-1}) in the dry diets were tested. Duplicate groups of 25 red sea bream (average weight 3.74±0.07 g) per 60L glass tank were fed experimental diets three times a day near to satiation level at 22 to 28°C water temperature. A reduction in dietary fish meal from 500 to 300 g/kg dry diet, corresponding to a supplementation in both dietary lipid and P resulted in significant increase in both P and N retention which resulted in the reduction of their excretion by red sea bream. The overall results of the present study demonstrated that both lipid and phosphorus supplementation are necessary for developing less-polluting feed which in turn, reduce fish meal level in the diet of fingerling red sea bream. Further studies in this regard with different size and age groups of red sea bream are warranted.

Key words: Lipid, Phosphorus, Nitrogen, Excretion, Red sea bream, *Pagrus major*

Introduction

The red sea bream is one of the most popular fin fish species in marine aquaculture through out the world due to its economic feasibility and traditional food habits. The aquaculture production of red sea bream (*Pagrus major*) is the second largest in Japan, followed by yellowtail (*Seriola quinqueradiata*) (Koshio 2000).

Aquaculture effluents contain P and N, which can contribute to excessive algae and macrophyte growth in receiving waters (Pillay 1992). Intensive fish production results in the release of organic wastes and soluble inorganic nutrients such as N and P, which can enrich as well as generate eutrophication in natural ecosystems (GESAMP 1996). As the original source of all aquaculture

waste is the feed fed to the fish, one effective way to reduce the waste load of fish farm effluent is to improve aquaculture diets with the aim of reducing excretion of P, N and total solids relative to fish growth (Lall 1989, Talbot and Hole 1994).

Nutritional strategy to reduce the waste load from aquaculture effluent is to produce high energy diet by reducing protein level and subsequently increasing fat level in the diet. The possibilities for reduced pollution load from fish feed are mostly related to improved feed conversion and reduced protein levels in the feed (Alsted 1991). In high energy diets the energy concentration is increased to improve feed conversion (Cowey and Cho 1991). However, the necessity of phosphorus supplementation in a fish meal-based diet has been reported at growing stage after juvenile (Masumoto 2002). Feed quality improvement involving ways to retain dietary P is one of the main strategies to reduce environmental impact of aquaculture (Lall 1991, Sugiura and Hardy 2000). Phosphorus is an essential dietary nutrient for fish and other animals, and is a major constituent of skeletal tissues, as well as an important component of the nucleic acids, DNA and RNA, energy transport compounds such as adenosine triphosphate (ATP), and phospholipids in cell membranes (Sugiura and Hardy 2000). Factors influencing the ingested P to the animal include the form of ingested P and its availability, the dietary P level, and physiological regulation of intestinal absorption and urinary excretion of P (Lall 1991, Vielma and Lall 1998, Sugiura *et al.*, 2000). Limited information is available on the metabolism, excretion and utilization of dietary P in fish (Lall 1991). Fish meal is the source of most dietary P in fish diets. In fish meal, P combines with Ca and forms the hydroapatite and/or tricalcium phosphate (TCP). Due to the structural complexity, P and Ca from TCP have been reported to be less viable to some fish species (Takamatsu *et al.* 1995, Shitanda *et al.* 1979, Watanabe *et al.* 1980, Hossain and Furuichi 1998). On the other hand, P from water soluble like monosodium/monocalcium phosphate is highly available to all fish. It has been reported that increasing dietary lipid relative to protein has been shown to increase protein retention in salmonids, and to reduce N excretion (Beamish and Medland 1986, Hillestad and Johnsen 1994, Helland and Grisdale-Helland 1998, Medale *et al.* 1995, Sugiura *et al.* 1998). No report is yet available on the effects of dietary lipid and P levels on retention and excretion of P and N by red sea bream. Hence, the present study aimed to investigate the possible effects of dietary lipid and phosphorus levels on retention and excretion of N and P in red sea bream fed fish meal based and alternative protein based diets and to observe whether or not red sea bream need lipid and phosphorus supplementation for optimum growth, feed utilization and utilization of P and N which in turn help in developing environmentally friendly diets.

Materials and methods

Six practical diets were formulated to contain two levels of lipid (210 and 260 g/kg) and three levels of phosphorus (17, 14 and 12 g/kg) in the dry diets

respectively. Increasing lipid level was achieved by addition of soybean oil in the diets. Changes in dietary P content from 17 to 12 g/kg was achieved by partial substitution of dietary fish meal with a combination of defatted soybean meal, corn gluten meal, feather meal and blood meal with the supplementation of monosodium phosphate. The diets were labeled according to factors (L and P) and levels (1-3) and they were designated as L1P1, L1P2, L1P3, L2P1, L2P2 and L2P3, respectively. The compositions of the experimental diets are presented in Table 1. Dietary protein of the experimental diets, in a range of from 471 to 454 g/kg dry diet and gross energy were from 23 to 25 MJ/kg. The carbohydrate sources and binders were wheat flour and pregelatinized starch while the lipid sources were pollock liver oil and soybean oil.

Table 1. Composition of the experimental diets (g kg^{-1} dry diet)

Ingredients	Diet code					
	L1P1	L1P2	L1P3	L2P1	L2P2	L2P3
Jack mackeral meal	500.0	300.0	300.0	500.0	300.0	300.0
Defatted soybean meal	50.0	150.0	150.0	50.0	150.0	150.0
Corn gluten meal	50.0	100.0	100.0	50.0	100.0	100.0
Feather meal	0.0	40.0	40.0	0.0	40.0	40.0
Meat flour	159.0	100.0	100.0	109.0	54.0	64.0
Blood meal	0.0	40.0	40.0	0.0	40.0	40.0
Pregelatinized starch	50.0	50.0	50.0	50.0	50.0	50.0
Pollok liver oil	135.0	150.0	150.0	135.0	150.0	150.0
Soybean oil	0.0	0.0	0.0	50.0	50.0	50.0
P-free mineral mixture[a]	10.0	10.0	10.0	10.0	10.0	10.0
NaH_2PO_4	10.0	20.0	10.0	10.0	20.0	10.0
Vitamin premixture[b]	30.0	30.0	30.0	30.0	30.0	30.0
Choline chloride	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin E (50%)	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	0.0	4.0	14.0	0.0	0.0	0.0

[a] P-free mineral mixture composition (%) : NaCl, 5 0; manganese sulfate, 74.5; iron (III) citrate n-hydrate, 12.5; trace element mix,a*5.0; cellulose, 3.0. a* The trace element mixture had the following components (%): zinc sulphate heptahydrate, 35.3; manganese sulphate, 162; copper (II) sulfate pentahydrate, 3.1; aluminium chloride hexahydrate, 1.0; cobalt chloride, 03; potassium iodate, 0.1; cellulose, 44.0.

[b] The vitamin mix had the following components (mg 100 g^{-1})-Thiamine hydrochloride 6; riboflavin 10; pyridoxine hydrochloride 4; cyanocobalamin 0 .01; ascorbic acid 500; niacin 40; Ca-pantothenate. 10; inositol 200; biotin 0.6; folic acid 1.5; p-aminobenzoic acid 5; vitamin K_3 5; vitamin A acetate 4000 IU; vitamin D_3 4000 IU.

The diets were pelleted using the laboratory pelletizer, dried a vacuum freeze-drier (RI-E-206, Kyowa Vacuum Tech., Saitama, Japan) and stored at 4^0C until used. The minimum level of dietary P was estimated to be about 11.8 g kg^{-1} dry diets. The experiment was designed as a 2×3 factorial arrangement with the factors 'dietary lipid level' and 'phosphorus level'.

Fingerling red sea bream *Pagrus major* were obtained from Seiho Suisan Co. Ltd. (Mie, Japan) and fed commercial red sea bream feed prior to the start of the experiment. Twenty five fish (average weight 3.74 ± 0.07 g) were randomly distributed in each well-aerated 60-L glass tanks with two replications. The feeding trial was conducted in re-circulated artificial seawater (Sea Life®, Tokyo, Japan) at a flow rate of 700-800 ml/min. The water renewal rate in the system was 50% in every week. Important water quality parameters such as temperature, pH and salinity were monitored daily and dissolved oxygen was measured fortnightly. All the parameters were observed to be within the acceptable limits for fish culture. Water temperature ranged from 22 to 28^{0}C. The fish were fed four times a day until near satiation for 12 weeks.

Initial weight data were obtained at the start of the experiment and growth of fish was measured every 3 weeks subsequently. Upon termination of the experiment, five fish from each tank were randomly selected for the chemical analyses of the whole body. Whole body samples were pooled from 5 fish and minced by a centrifugal mill (Retsch ZM 1. Germany) fitted with a 0.25 mm screen. The homogenate was collected and kept at -20°C until analysis. Proximate composition and chemical analyses of the diets and fish whole body samples were made in three replicates as follows: moisture contents was measured gravimetrically, crude ash contents was determined by incinerating a known amount of sample in an electric muffle furnace (Yamato, FA-21) at 600 °C for 8 hours, crude protein was analyzed using the Kjeltec Auto Sampler System 1035/38 (Netherland), and crude lipid was measured by following the method of Folch *et al.* (1957) (Table 2). Samples for P analysis were digested in nitric acid using the MLS-1200 Mega Microwave Digestion System (Italy). Phosphorus contents were analyzed by a visible light spectrophotometry (Shimadzu, UV 265 FW, Kyoto, Japan) at 750 nm.

Table 2. Nutrient contents of the experimental diets (g kg^{-1} dry diet)

Nutrients	Diet code					
	L1P1	L1P2	L1P3	L2P1	L2P2	L2P3
Crude ash	94.0	79.0	72.0	92.0	75.0	71.0
Crude protein	468.0	471.0	466.0	455.0	454.0	460.0
Crude lipid	216.0	217.0	200.0	264.0	266.0	262.0
GE* (MJ kg^{-1})	22.6	23.4	23.0	24.3	24.7	24.7
Protein/GE ratio (g MJ^{-1})	20.7	20.1	20.2	18.7	18.4	18.6
Total P	17.6	14.8	12.1	16.6	14.2	11.8

* GE, Gross energy

Results were analyzed using one-way and two-way ANOVA (Systat 8.0, SPSS, Chicago, USA). Differences between treatments were compared by Tukey's test. Values were considered significant at $P<0.05$.

Results and discussion

The results of overall growth performance and feed utilization in fish feeding on the experimental diets are presented in Table 3. Dietary lipid level did not show significant differences on weight gain, specific growth rate, thermal-unit growth coefficient, and feed conversion ratio (FCR) through out the rearing period. Reduction of FM with lipid and P supplementation (L1P2 and L2P2) had no significant effect on WG, SGR, and TGC compared to the control diet, L1P1 whereas, FCR was significantly improved. These results indicate that reduction of FM level (500-300 g/kg) has no remarkable influence on growth performance and feed utilization. Similar results were reported indicating no significant difference for growth in rainbow trout fed diets with different P levels (Green *et al.* 2002). Studies conducted with different species of fish have reported that fish fed monocalcium phosphate supplemented diets showed a significant improvement in weight gain and feed utilization, compared to fish fed diets without monocalcium phosphate (Kim *et al.* 1998).

Table 3. Growth and feed performance in red sea bream cultured for 12 weeks*

Diet code	Weight gain (g)	SGR[1] (% day^{-1})	TGC[2] × 1000	FCR[3]
L1P1	52.53 ± 1.31^{ab}	3.24 ± 0.00^{a}	0.939 ± 0.01^{ab}	1.14 ± 0.03^{ab}
L1P2	50.31 ± 2.06^{ab}	3.17 ± 0.06^{abc}	0.915 ± 0.02^{abc}	1.02 ± 0.01^{c}
L1P3	43.54 ± 1.18^{b}	3.01 ± 0.02^{c}	0.847 ± 0.01^{c}	1.16 ± 0.02^{a}
L2P1	53.78 ± 2.51^{a}	3.25 ± 0.06^{a}	0.948 ± 0.02^{a}	1.06 ± 0.01^{bc}
L2P2	52.66 ± 2..15^{ab}	3.21 ± 0.04^{ab}	0.936 ± 0.02^{ab}	1.01 ± 0.01^{c}
L2P3	44.19 ± 0.38^{ab}	3.05 ± 0.02^{bc}	0.857 ± 0.00^{bc}	1.09 ± 0.02^{abc}

*Values (means ± S.D.) in the same column not sharing the common superscript letters are significantly different ($P<0.05$). [1]Specific growth rate (SGR) = 100 × (ln final body weight - ln initial body weight)/days. [2]Thermal-unit growth coefficient (TGC) = (Final body weight$^{1/3}$ - Initial body weight$^{1/3}$)/ (water temperature °C × days). [3]Feed conversion ratio (FCR) = Feed consumption/weight gain.

Proximate composition and P content of the whole body of red sea bream for the initial groups and at the end of the experiment fed different experimental diets are presented in Table 4. Increased crude ash contents and increased lipid contents were obtained for the final groups in contrast to the initial group. Whole body lipid content of the lipid supplemental group was higher than that of the group fed with the other diets. Highest body lipid content was achieved in the diet group L2P2 (127 g/kg). Whole body total P increased with reduction of FM and P supplementation, while low level P containing diets showed the lowest growth and feed performance among the treatments. Increasing dietary lipid level resulted in increased final whole body lipid content. These results indicate that the improved FCR is associated with increased dietary lipid content which might have increased the deposition of lipid in the body, because of a protein sparing effect of dietary lipid

in the red sea bream.

Table 4. Proximate composition of red sea bream at initial and end of the experiment*

Diet code	Moisture (g/kg)	Crude ash (g/kg)	Crude protein (g/kg)	Crude lipid (g/kg)	Phosphorus (g/kg)
L1P1	670 ± 4^{a}	39.50 ± 0.5^{b}	175 ± 1^{ab}	111 ± 1^{d}	7.04 ± 0.1^{b}
L1P2	676 ± 6^{a}	45.80 ± 0.2^{a}	173 ± 2^{bc}	116 ± 1^{cd}	8.59 ± 0.1^{a}
L1P3	682 ± 4^{a}	42.00 ± 0.2^{b}	173 ± 0^{abc}	106 ± 1^{e}	7.05 ± 0.1^{b}
L2P1	667 ± 2^{a}	40.75 ± 0.1^{b}	178 ± 1^{a}	124 ± 1^{ab}	7.21 ± 0.0^{b}
L2P2	669 ± 1^{a}	42.95 ± 0.0^{b}	174 ± 0^{abc}	127 ± 0^{a}	8.17 ± 0.1^{a}
L2P3	675 ± 6^{a}	37.00 ± 0.7^{c}	171 ± 1^{c}	120 ± 2^{bc}	7.00 ± 0.1^{b}
Initial	754	45.5	155	47	7.42

*Values presented as means ± S.D. (n= pooled samples of 5 fish/tank) in a column not sharing the same superscript letters are significantly different ($P<0.05$).

Retention and excretion of P and N by red sea bream after 12 weeks feeding are presented in Table 5 and Table 6. Increasing lipid level significantly increased both P and N retention which in turn reduced their excretion. Reduction of FM with P supplementation in the diet significantly increased both P and N retention which in turn resulted in reduction of their excretion. Increasing dietary lipid slightly increased N retention (%), while significantly decreased N excretion (kg/ton) in this experiment. The results emphasized that retention efficiencies of dietary nutrients such as P and nitrogen are important for the evaluation of fish feed quality and might change according to fish weight, temperature, amount of feed consumed and feed composition (Lall 1991, Cho 1994).

Table 5. Retention and excretion of phosphorus in red sea bream fed diet supplemented with lipid and phosphorus for 12 weeks*

Diet code	P retention (%)[1]	P excretion (kg/ton)[2]
L1P1	35.46 ± 1.4^{d}	12.88 ± 0.7^{a}
L1P2	56.91 ± 0.4^{a}	6.46 ± 0.0^{e}
L1P3	49.52 ± 0.2^{b}	7.06 ± 0.0^{e}
L2P1	40.93 ± 0.3^{c}	10.40 ± 0.2^{b}
L2P2	56.74 ± 1.1^{a}	622 ± 02^{e}
L2P3	54.09 ± 0.1^{a}	5 85 ± 0.1^{e}
Lipid (L) & Phosphorus (P) level	<0.05	<0.05
L × P	<0.05	<0.05

*Values are presented as means ± S.D. (n = pooled samples of 5 fish/tank). Means in a column not sharing the same superscript letters are significantly different ($P<0.05$).

[1]Retention (%) = {(Final nutrient content - initial nutrient content)/nutrient intake} × 100.

[2]Excretion (kg t^{-1}) = [{FCR nutrient in diet (g) - nutrient retained in fish (g)/production (t)] × 1000.

Table 6. Retention and excretion of nitrogen in red sea bream fed diet supplemented with lipid and phosphorus for 12 weeks*

Diet code	N retention (%)[1]	N excretion (kg ton^{-1})[2]
L1P1	33.38 ± 1.3^{bc}	56.67 ± 2.8^{a}
L1P2	35.83 ± 0.3^{ab}	49.11 ± 0.0^{b}
L1P3	32.05 ± 0.3^{c}	58.80 ± 0.7^{a}
L2P1	37.26 ± 0.1^{a}	48.38 ± 0.5^{b}
L2P2	38.12 ± 0.4^{a}	45.38 ± 0.7^{b}
L2P3	33.94 ± 0.3^{bc}	52.76 ± 1.0^{ab}
Lipid (L) level	<0.05	<0.05
P level	<0.05	<0.05
L × P	NS	NS

*Values are presented as means ± S.D. (n = pooled samples of 5 fish/tank). Means in a column not sharing the same superscript letters are significantly different (P<0.05); NS: Not significant.

[1]Retention (%) = {(Final nutrient content - initial nutrient content) /nutrient intake} × 100.

[2]Excretion (kg t^{-1}) = [{FCR nutrient in diet (g) - nutrient retained in fish (g)}/production (t)] × 1000.

Therefore, the overall results of the present study demonstrated that both dietary lipid and P supplementation are needed in the diet of fingerling red sea bream for developing less-polluting feed. Hence, further study is necessary with different sized and aged red sea bream to determine the possible effect of dietary lipid and phosphorus supplementation for formulating environmentally clean diets.

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