

Dietary Optimum Phosphorus Level of Juvenile Korean Rockfish (*Sebastes schlegeli*)

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A 10-week feeding experiment was conducted to determine the phosphorus requirement of juvenile Korean rockfish (*Sebastes schlegeli*). Three replicate groups of fish initially averaging 4.2 g were fed the semipurified experimental diets containing graded levels of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ to provide from 0.1% to 1.32% total phosphorus level in a flow-through seawater system. Korean rockfish muscle and casein were used as the protein sources of the basal diet.

Weight gain, feed efficiency and protein retention of fish fed the 0.35% phosphorus were higher than those of fish fed the 0.1% phosphorus, although no significant improvements ($P > 0.01$) were observed above the level. Determined phosphorus requirement using the broken line model was found to be 0.3% for weight gain. Moisture, protein and lipid contents of whole body and muscle were not affected by dietary phosphorus levels ($P > 0.01$). Lipid contents of liver in fish fed the 0.1% phosphorus were lower than those in fish fed the 0.35% and 1.32% phosphorus ($P < 0.01$). Dietary phosphorus increased ash and phosphorus contents of the whole body, while those of bone were not affected ($P > 0.01$). The data obtained in this study indicate that a 0.3% dietary phosphorus level could be recommended for the optimum growth and efficient nutrient utilization of juvenile Korean rockfish.

Key words: phosphorus requirement, rockfish (*Sebastes schlegeli*)

Introduction

Korean rockfish (*Sebastes schlegeli*) is a leading candidate for commercial culture in Korea (Lee and Lee, 1994). This species has several desirable characteristics for aquaculture including high tolerance to both high and low water temperatures, easiness of seedling production due to viviparous reproductive style, and the ability to withstand in high stocking density. Farming of the species has been rapidly developed since 1987, and aquaculture production in 1998 approximated to about 20,000 tons (personal estimation), which is second to olive flounder (*Paralichthys olivaceus*) in mariculture fish production in Korea.

In culturing this species, raw fish or moist feed (a mixture of raw fish and binder meal) has been intensively used. However, the utilization of these feeds not only causes many problems such as introduction of pathogen, occurrence of nutritional

disease, and deterioration of water quality, but it also increases production costs in terms of labor and freeze space. In addition, a stable supply of fresh raw fish has become increasingly difficult. Therefore, for further expansion of Korean rockfish farming, it is essential to employ formulated artificial dry feeds which can support reasonable growth.

Development of nutritionally balanced and cost-effective feeds is dependant primarily upon the knowledge of nutritional requirements for a species. To date, nutrition research for Korean rockfish has identified the protein requirement (Lee et al., 1993 a), essential fatty acids requirement (Lee et al., 1993 b,c,d; 1994; Lee, 1998), vitamin and mineral requirement (Bai and Lee, 1998; Lee et al., 1998a; Lee and Kim, 1996; Lee and Park, 1998; Lee et al., 1998b), digestibility (Lee, 1997a,c), utilization of protein and lipid sources (Lee et al., 1996a,b; Lee and Lee, 1996; Lee and Yoo, 1996) and the feeding schedules (Lee et al., 1996c; Lee, 1997b). Lee and Park (1998) suggested that each of the P, Ca, Zn,

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Mg, Fe, K, Mn or Se was essential for normal growth of Korean rockfish when Korean rockfish muscle and casein were used as protein sources in the basal diet. Lee et al. (1998b) also investigated the influence of the deletion of P, Ca, Zn, Mg, Fe, K, Mn, or Se from mineral premix in the diets containing 40% fish meal on growth performance of juvenile Korean rockfish and showed that each of the Ca, Zn, K, or Se should be added to the diet containing 40% fish meal for normal growth of Korean rockfish. But there is no information available for the minimum requirement on each of these minerals.

Minerals are an essential nutrient for normal life processes of fish and have important functions such as structural components of hard tissues (bone, exoskeleton, scales, teeth), electron transfer and regulation of acid-base equilibrium, and osmoregulation (NRC, 1993). Phosphorus is an important nutrient to make up for skeletal tissue, to produce energy for cellular reactions and to act as buffers in a variety of physiological metabolism of fish. On the other hand, phosphorus is major source of water pollution. Determination of phosphorus requirement, increment of available dietary phosphorus, improvement of feed efficiency, reduction of uneaten diet in water and development of practical diets with well-balanced nutrients are essential study programs to reduce phosphate pollution in the water. The present study, therefore, was conducted to determine the phosphorus requirement of juvenile Korean rockfish.

Materials and Methods

Experimental diets

Lee et al. (1993e) reported that a purified diet containing casein and gelatin as protein sources was not an adequate nutritional experiment for Korean rockfish because of lower growth and palatability. Therefore, a semi-purified basal diet (Table 1) was formulated to meet the protein requirement of Korean rockfish (Lee et al., 1993a) using rockfish muscle and casein as the dietary protein sources to limit the content of phosphorus contributed by the protein component. Squid live oil (E-Wha Oil & Fat Ind. Co., Pusan, Korea) and α and β starch were added to the diet to meet essential fatty acids and energy requirement of this species (Lee et al., 1994; Lee, 1998; Lee and Lee, 1994).

Table 1. Composition of the basal diet

Ingredients	%
Rockfish muscle ¹	50
Casein, vitamin free ²	8
Starch ³	20
Squid liver oil ⁴	4
Vitamin premix ⁵	3
Mineral premix (phosphorus-free) ⁶	5
Carboxymethyl cellulose ⁷	3
Cellulose ⁷	7
NaH ₂ PO ₄ · 2H ₂ O ⁸	—
Nutrient (% of dry basis)	
Crude protein	49.3
Crude lipid	10.5
Crude ash	6.6
n-3HUFA ⁹	1.2

¹ Prepared by lyophilizing muscle from adult fish cultured in National Fisheries Research and Development Institute, Pusan, Korea

² Serva, Feinbiochemica GmbH & Co. Heidelberg, Germany.

³ α -potato starch and β -potato starch mixture (1 : 1, w/w).

⁴ Provided by E-wha Oil & Fat Ind. Co., Pusan, Korea

⁵ Same as Lee (1997c).

⁶ Mineral premix contained the following ingredients (g/kg mix): MgSO₄ · 7H₂O, 127; KCl, 206; Ferric citrate, 64; ZnSO₄ · 7H₂O, 32; Calcium lactate, 565.69; CuCl, 0.3; AlCl₃ · 6H₂O, 0.2; KI, 0.2; Na₂Se₂O₃, 0.01; MnSO₄ · H₂O, 3; CoCl₂ · 6H₂O, 1.6.

⁷ Sigma Chemical, St. Louis, MO, USA.

⁸ Partially replaced cellulose to achieve graded levels of phosphorus.

⁹ Highly unsaturated fatty acids (C \geq 20).

The basal diet was determined to contain 0.1% total phosphorus. Phosphorus levels (0.1%, 0.35%, 0.69% and 1.32%) in the diets were adjusted by substituting the α -cellulose with monobasic sodium phosphate (NaH₂PO₄ · 2H₂O). The phosphorus availabilities of monobasic sodium phosphate and dietary protein sources were considered to be 100% to Korean rockfish. The experimental diet was mechanically mixed with water (40 g/100 g dried diet mix.) and pressure-pelleted, and stored according to the method described previously (Lee et al., 1993c).

Fish and experimental conditions

Juvenile Korean rockfish (mean weight ca 1.0 g) were obtained from the Puan Hatchery National Fisheries Research and Development Institute Branch (Puan, Korea) in June. Thereafter they were acclimated to our laboratory conditions for 2 months by a feeding diet containing 1.3% phos-

phorus. After that, in order to accustom fish to the experimental conditions, fish were randomly distributed into fifteen 40 ℓ tanks with 100 fish per tank and were fed a pre-feeding diet (basal diet) for 6 days. After the conditioning period, fish were weighed and redistributed into the same tanks with 25 fish (4.2 g initial mean weight) per tank. Fish were fed by hand to satiety two times per day (7 days a week) at 09:00 and 17:00. The experiment lasted for 10 weeks. Filtered sea water was supplied at a flow rate of 3 ℓ/min to each tank. The water was aerated continuously, and water temperature was maintained at $20 \pm 1.0^\circ\text{C}$ (mean \pm S.D.). During the experimental period, photoperiod was left at natural condition. Fish in each tank were weighed as a group on the days of initiation and termination after fish were starved for 24h and anesthetized with MS222 (tricaine methanesulfonate, Sigma Chemical, St. Louis, MO, USA) at 100 ppm.

Sample collection and analyses

Fish samples were selected at the initiation (50 fish) and the termination (25 fish from each tank) of the feeding trial, and stored at -75°C for proximate and/or mineral analyses of whole body, muscle, liver or bone. Protein, lipid, moisture and ash of diet and fish were determined according to AOAC methods (1990). The sample of 7 frozen fish from each tank were homogenized with masscolloider (Masuko Co., Ltd., Japan) for whole body analyses of moisture, crude protein, crude lipid, crude ash, and minerals. Moisture, crude protein and/or crude lipid of muscle and liver, and crude ash and minerals of bone were analysed from the remaining 15 fish/tank. Vertebrate for ash and mineral analyses were separated from the 15 fish/tank removed muscle and viscera, defatted, dried and ground as described by Wilson et al. (1982). Phosphorus was determined by the molybden blue method as described previously (Lee and Park, 1998). K, Ca, Mg, Fe, Zn and Mn were analysed using atomic absorption spectrophotometer as described by Graham et al. (1982). The bone formation of the remaining 3 fish/tank were examined by X-ray. Fatty acids composition of the experimental diet were determined by gas chromatography (HP-5890 II, USA) with capillary column (HP-INNOWax, 30 m \times 0.32 mm \times 0.5 μm , USA) as described previously (Lee, 1997c).

Statistical analyses

One-way analysis of variance and Duncan's

multiple range test (Duncan, 1955) were used to test the significance of the difference among the means of test groups using the SPSS (SPSS Inc., 1997) program. The phosphorus requirement was estimated by the broken line model (Robbins et al., 1979).

Results and Discussion

Survival of juvenile Korean rockfish fed the different phosphorus levels for 10 weeks was 100%. Except for growth performance and body chemical composition, apparent physical phosphorus-deficient signs such as skeletal deformities by the X-ray observation were not showed in any of the groups. Lovell (1978) also reported that channel catfish fed phosphorus-deficient (0.07~0.42% phosphorus) diets did not show any skeletal deformities, whereas bone deformity and spinal curvature were observed in the phosphorus-deficient carp (Ogino and Takeda, 1976).

Weight gain, feed efficiency and protein retention of Korean rockfish fed the diets containing various levels of phosphorus are presented in Table 2. These values were significantly ($P < 0.01$) lower in fish fed the basal diet (0.1% phosphorus) than those in fish fed the diet containing 0.35% phosphorus, and then there were no additional improvements above these levels. Whereas daily feed intake was not influenced by dietary phosphorus levels ($P > 0.01$).

The phosphorus requirement of Korean rockfish was to be 0.3% (Fig. 1) estimated from weight gain using the broken line model (Robbins et al., 1979). Therefore, based on these growth performance, at least 0.3% available phosphorus should be incorporated into a juvenile Korean rockfish diet. Similar depressions in weight gain and feed efficiency were observed in other fishes fed phosphorus-deficient diets (Andrews et al., 1973; Brown et al., 1993; Ketola, 1975; Sakamoto and Yone, 1979; Wilson et al., 1982; Yone and Toshima, 1979). The value of phosphorus requirement for juvenile Korean rockfish in the present study is lower than those of 0.5~0.8% for carp, rainbow trout, red sea bream, sunshine bass (*Morone chrysops*♀ \times *M. saxatilis*♂), and yellowtail (Brown et al., 1993; Ogino and Takeda, 1976; Ketola, 1975; Watanabe et al., 1980). The phosphorus requirement (0.3%) in this species is similar to the requirements (about 0.4%) of eel and channel catfish (Lovell, 1978; Lall, 1989;

Table 2. Performance of Korean rockfish fed the diets containing different phosphorus levels after 10 weeks of experimental period¹

Dietary P level (%) ²	Weight gain (%) ³	Feed efficiency (%) ⁴	Daily feed intake (%) ⁵	Protein retention (%) ⁶
0.10	117.9 ± 3.95 ^a	68.3 ± 1.94 ^a	1.811 ± 0.0460 ^{ns}	21.8 ± 1.89 ^a
0.35	178.0 ± 10.73 ^b	86.7 ± 2.45 ^b	1.810 ± 0.0098	28.5 ± 2.70 ^b
0.69	192.5 ± 12.35 ^b	90.7 ± 2.38 ^b	1.802 ± 0.0367	28.9 ± 1.37 ^b
1.32	175.7 ± 11.22 ^b	88.0 ± 3.51 ^b	1.756 ± 0.0254	27.3 ± 2.48 ^b

¹ Values (mean ± s.d. of three replications) in the same column not sharing a common superscript are significantly different ($P < 0.01$).

² The apparent availability of dietary phosphorus was considered 100%. Diets contained 0.62% calcium.

³ (Final fish weight - initial fish weight) × 100 / initial fish weight.

⁴ (Fish weight gain × 100) / feed intake (dry matter).

⁵ [Feed intake (dry matter) × 100] / [(initial fish weight + final fish weight) / 2] × days fed.

⁶ (Protein gain × 100) / protein intake.

^{ns} Not significant.

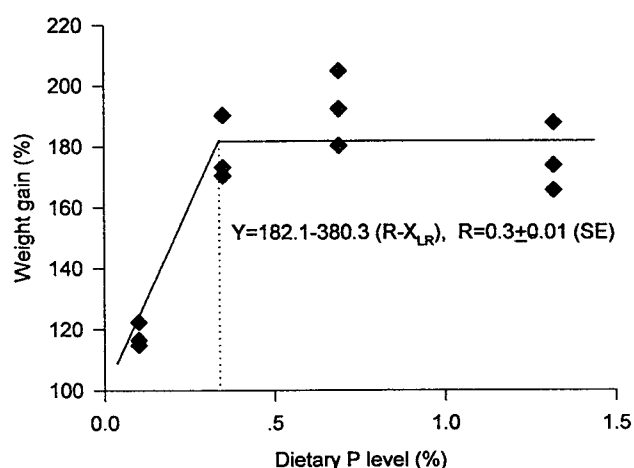


Fig. 1. Broken line analysis of weight gain to dietary phosphorus (P) levels.

Wilson et al., 1982).

We assumed the apparent availability of dietary phosphorus in this study to be 100%, because there is no information about phosphorus availability in this species. Monobasic phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) used in this study had higher availability than those of dibasic or tribasic phosphate for other fish species (Lovell, 1978; Ogino et al., 1979). Rainbow trout (Ogino et al., 1979) were able to absorb 98% of monobasic phosphates such as KH_2PO_4 . Carnivorous fish can digest monobasic, dibasic or tribasic phosphate better than herbivorous fish (Kim et al., 1996; Lovell, 1978; Watanabe et al., 1988; Yone and Toshima, 1979). Korean rockfish is strong carnivores (Hatanaka and Iizuka, 1962).

Phosphorus retention (phosphorus gain × 100 / phosphorus intake) of Korean rockfish fed the diets containing 0.1~1.32% phosphorus decreased from

155% to 50% according to dietary phosphorus level increased ($P < 0.01$). Similar trends of phosphorus retention have also been reported in other fishes (Brown et al., 1993; Ketola et al., 1991; Kim et al., 1996). But Korean rockfish fed the diet containing 0.1% showed 155%, which was an unexpectedly high value. The phosphorus requirement have been reported mainly for fresh water fish (NRC, 1993), and the retention values in the fresh water fish ranged from 26% to 87% (Brown et al., 1993; Ketola et al., 1991; Kim et al., 1996). This different response of the high phosphorus retention value in the phosphorus-deficient group as compared to other fishes is not clear, but it may be due to differences in fish species, feeding trial periods, dietary phosphorus ranges, interactions with other nutrient, or culture surroundings such as phosphorus concentration in water.

At the end of the feeding trial, whole body ash and phosphorus contents (Table 3) of juvenile Korean rockfish fed phosphorus-deficient diet were significantly ($P < 0.01$) lower than those of fish fed the diets containing 0.69% and/or 1.32% phosphorus. Ca, Zn, Mg, Fe and K of the whole body were not affected by dietary phosphorus levels ($P > 0.01$). Similar change trends of phosphorus content in the whole body have been reported for carp (Ogino and Takeda, 1976), channel catfish (Wilson et al., 1982) and red drum (Davis and Robinson, 1987). Ash and minerals including P, Ca, Zn, Mg and K in the bone of Korean rockfish were not influenced by dietary phosphorus levels ($P > 0.01$). Shim and Ho (1989) have reported that phosphorus and calcium of bone were not changed ($P > 0.05$) by dietary phosphorus levels (0.05~1.23%) in guppy (*Poecilia reticulata*). On the other

Table 3. Ash and mineral contents of whole body after 10 weeks of experimental period¹

Dietary P level (%) ²	Ash (% of wet wt.)	P (% of dry wt.)	Ca (% of dry wt.)	Zn (mg/100 g dry wt.)	Mg (% of dry wt.)	Fe (mg/kg dry wt.)	K (% of dry wt.)
0.10	3.62 ± 0.095 ^a	1.92 ± 0.155 ^a	3.78 ± 0.498 ^{ns}	14.5 ± 0.20 ^{ns}	0.23 ± 0.017 ^{ns}	58.0 ± 50.47 ^{ns}	1.52 ± 0.102 ^{ns}
0.35	3.95 ± 0.306 ^{ab}	2.21 ± 0.089 ^{ab}	4.50 ± 0.594	13.6 ± 0.75	0.23 ± 0.015	87.7 ± 28.74	1.41 ± 0.030
0.69	4.25 ± 0.181 ^b	2.27 ± 0.070 ^{ab}	4.03 ± 0.751	10.4 ± 3.21	0.26 ± 0.015	80.3 ± 26.65	1.42 ± 0.100
1.32	4.41 ± 0.311 ^b	2.60 ± 0.340 ^b	4.68 ± 0.020	10.0 ± 0.70	0.23 ± 0.030	76.3 ± 66.01	1.48 ± 0.095

¹ Values (mean ± s.d. of three replications) in the same column not sharing a common superscript are significantly different (P<0.01).

² The apparent availability of dietary phosphorus was considered 100%. Diets contained 0.62% calcium.
^{ns} Not significant.

Table 4. Chemical composition (%) of whole body, dorsal muscle and liver after 10 weeks of experimental period¹

Dietary P level (%) ²	Moisture		Crude protein		Crude lipid		
	Whole body	Muscle	Whole body	Muscle	Whole body	Muscle	Liver
0.10	75.0 ± 0.43 ^{ns}	79.0 ± 1.00 ^{ns}	15.5 ± 0.50 ^{ns}	18.2 ± 0.17 ^{ns}	4.5 ± 0.17 ^{ns}	1.4 ± 0.43 ^{ns}	11.6 ± 2.52 ^a
0.35	73.5 ± 0.95	78.7 ± 0.70	16.1 ± 0.75	18.7 ± 0.57	5.9 ± 0.34	1.1 ± 0.40	16.9 ± 1.85 ^b
0.69	74.4 ± 0.75	78.4 ± 0.20	15.6 ± 0.25	18.6 ± 0.37	5.2 ± 0.55	1.2 ± 0.23	14.0 ± 1.59 ^{ab}
1.32	73.0 ± 0.85	79.2 ± 0.32	15.9 ± 0.55	18.4 ± 0.40	5.9 ± 0.87	1.0 ± 0.45	16.6 ± 0.81 ^b

¹ Values (mean ± s.d. of three replications) in the same column not sharing a common superscript are significantly different (P<0.01).

² The apparent availability of dietary phosphorus was considered 100%. Diets contained 0.62% calcium.
^{ns} Not significant.

hand, other fishes had lower bone ash and phosphorus content in the phosphorus-deficient groups (Andrews et al., 1973; Ketola, 1975; Lovell, 1978; Ogino and Takeda, 1976; Wilson et al., 1982). These differences may depend on species, dietary phosphorus levels, feeding periods, or seawater characteristics. Further study is needed to investigate distinctive differences.

Moisture, crude protein and crude lipid contents of whole body, muscle and/or liver after the 10 weeks feeding trial are shown in Table 4. These values of whole body and muscle were not affected by dietary phosphorus levels (P>0.01). Korean rockfish fed 0.35% and 1.32% phosphorus diets had higher crude lipid contents of liver (P<0.01) than fish fed the 0.1% phosphorus diet. Higher lipid contents have been reported for other fishes fed low dietary phosphorus (Sakamoto and Yone, 1973; Ogino et al., 1979; Shim and Ho, 1989; Takeuchi and Nakazoe, 1981; Watanabe et al., 1980). They considered that the lipid increase of fish fed the phosphorus-deficient diets could be due to inhibited lipid metabolism and promoted the conversion carbohydrate to lipid. However, this trend of lipid change was not found in our study. Previous studies with juvenile Korean rockfish have reported that lipid content was higher in fish fed the nutrient-sufficient diets than that in fish fed

the nutrient-deficient diets (Lee et al., 1993c; Lee et al., 1994; Lee et al., 1996b; Lee and Park, 1998). These results suggest that lower growth with lower lipid content of Korean rockfish fed the phosphorus-deficient diet may be due to inhibition of energy metabolism for growth and energy storage.

In conclusion, the present study suggests that the optimum phosphorus requirement is 0.3% for normal growth of juvenile Korean rockfish. This information will be useful to design dietary formulation of Korean rockfish for lower water pollution.

References

- Andrews, J.W., T. Murai and C. Campbell. 1973. Effect of dietary calcium and phosphorus on growth, food efficiency, bone ash and hematocrit levels of catfish. *J. Nutr.*, 103, 766~771.
- AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists. Arlington, Virginia. 1298pp.
- Bai, S.C. and K.J. Lee. 1998. Different levels of dietary DL- α -tocopheryl acetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. *Aquaculture*, 161, 405~414.
- Brown, M.L., F. Jaramillo Jr and D.M. Gatlin III. 1993. Dietary phosphorus requirement of juvenile sunshine bass, *Morone chrysops* ♀ × *M. saxatilis* ♂. *Aquaculture*, 113, 355~363.
- Davis, D.A. and E.H. Robinson. 1987. Dietary phosphorus

- requirement of juvenile red drum *Sciaenops ocellatus*. J. World Aquacult. Soc., 18, 129~136.
- Duncan, D.B. 1955. Multiple-range and multiple F tests. Biometrics, 11, 1~42
- Graham, P.P., J. Bittel, K.P. Board, A. Lope and H.L. Williams. 1982. Mineral element composition of bovine spleen and separated spleen compounds. J. Food Sci., 47, 720~722.
- Hatanaka, M. and K. Iizuka. 1962. Trophic order in a fish group living outside of the zostera area. Bull. Jap. Soc. Sci. Fish., 28 (2), 155~161.
- Ketola, H.G. 1975. Requirement of Atlantic salmon for dietary phosphorus. Trans. Am. fish. Soc., 104, 548~551.
- Ketola, H.G., H. Westers, W. Houghton and C. Pecor. 1991. Effect of diet on growth and survival of coho salmon and on phosphorus discharges from a fish hatchery. Am. Fish. Soc. Symp., 10, 402~409.
- Kim, J.D., K.S. Kim, J.S. Song, M.W. Kang, S.B. Lee, K.Y. Yoon and K.S. Jeong. 1996. Dietary phosphorus requirement of juvenile Israeli carp (*Cyprinus carpio*) of 34G fed practical diets. Kor. Anim. Nutr. Feed., 20, 489~496.
- Lall, S.P. 1989. The minerals. In: Fish nutrition, edited by J.E. Halver. Acad. Press, New York & London. pp. 219~257.
- Lee, J.Y., Y.J. Kang, S.M. Lee and I.B. Kim. 1993a. Protein requirements of the Korean rockfish *Sebastes schlegeli*. J. Aquaculture, 6, 13~27.
- Lee, S. M., J. Y. Lee, Y.J. Kang, H. D. Yoon and S. B. Hur. 1993b. n-3 highly unsaturated fatty acid requirement of the Korean rockfish *Sebastes schlegeli*. Bull. Korean Fish. Soc., 26, 477~492.
- Lee, S.M., J.Y. Lee, Y.J. Kang and S.B. Hur, 1993c. Effects of n-3 highly unsaturated fatty acids on growth and biochemical changes in the Korean rockfish *Sebastes schlegeli* I. growth and body composition. J. Aquaculture, 6 (2), 89~105.
- Lee, S.M., J.Y. Lee, Y.J. Kang and S.B. Hur, 1993d. Effects of n-3 highly unsaturated fatty acids on growth and biochemical changes in the Korean rockfish *Sebastes schlegeli* II. changes of blood chemistry and properties of liver cells. J. Aquaculture, 6 (2), 107~123.
- Lee, J.Y., Y.J. Kang, S.M. Lee and Y.J. Park. 1993e. Evaluation of protein source for Korean rockfish (*Sebastes schlegeli*) test diets. Bull. Nat'l. Fish. Res. Dev. Agency, Korea, 48, 97~105.
- Lee, S.M., J.Y. Lee and S.B. Hur. 1994. Essentiality of dietary eicosapentaenoic acid and docosahexaenoic acid in Korean rockfish, *Sebastes schlegeli*. Bull. Korean Fish. Soc., 27, 721~726.
- Lee, J.Y. and S.M. Lee. 1994. Nutritional studies and feed development for Korea rockfish (*Sebastes schlegeli*). Proceedings of FOID, '94 The Third International Conference on Fisheries and Ocean Industrial Development for Productivity Enhancement of the Coastal Waters. pp. 75~92.
- Lee, S.M. and S.M. Kim. 1996. Evaluation of supplemental vitamin premix in a test diet containing fish meal as protein source for juvenile Korean rockfish (*Sebastes schlegeli*). J. Aquaculture, 9, 159~166.
- Lee, S.M. and J.H. Yoo. 1996. Evaluation of cotton seed meal or rapeseed meal as a partial substitute for fish meal in formulated diets for Korean rockfish (*Sebastes schlegeli*). Kor. J. Anim. Nutr. Feed., 20, 128~135.
- Lee, S.M. and J.Y. Lee. 1996. Substitution of plant and animal proteins for fish meal in the practical formulated feeds for juvenile Korean rockfish (*Sebastes schlegeli*). Kor. J. Anim. Nutr. Feed., 20, 409~418.
- Lee, S.M., J.H. Yoo and J.Y. Lee. 1996a. The use of soybean meal, corn gluten meal, meat meal, meat and bone meal, or blood meal as a dietary protein source replacing fish meal in Korean rockfish (*Sebastes schlegeli*). Kor. J. Anim. Nutr. Feed., 20, 21~30.
- Lee, S.M., I.G. Jeon, C.K. Lee, C.W. Im, T.J. Kim and J. G. Min. 1996b. Evaluation of economical feed formulations for Korean rockfish (*Sebastes schlegeli*). J. Aquaculture, 9, 255~264.
- Lee, S.M., S.H. Kim, I.G. Jeon, S.M. Kim and Y.J. Chang. 1996c. Effects of feeding frequency on growth, feed efficiency and body composition of juvenile Korean rockfish (*Sebastes schlegeli*). J. Aquaculture, 9, 385~394.
- Lee, S.M. 1997a. Evaluation of the nutrient digestibilities by different fecal collection methods in juvenile and adult Korean rockfish (*Sebastes schlegeli*). J. Korean Fish. Soc., 30 (1), 62~71.
- Lee, S.M. 1997b. Effects of feeding rates on growth, feed efficiency and body composition of the juvenile Korean rockfish (*Sebastes schlegeli*). Kor. J. Anim. Nutr. Feed., 21, 327~334.
- Lee, S.M. 1997c. Effects of dietary lipid source and water temperature on nutrient digestibilities in juvenile and adult Korean rockfish (*Sebastes schlegeli*). Kor. J. Anim. Nutr. Feed., 21, 381~390.
- Lee, S.M. and S.R. Park. 1998. Influence of P, Ca, Zn, Mg, Fe, K, Mn, or Se in the dietary mineral premix on growth and body composition of Korean rockfish (*Sebastes schlegeli*). J. Korean Fish. Soc., 31 (2), 245~251.
- Lee, K.J. K.W. Kim and S.C. Bai. 1998a. Effects of different dietary levels of L-ascorbic acid on growth and tissue vitamin C concentration in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Research, 29, 237~244.
- Lee, S.M., S.R. Park, T.J. Kim, J.I. Myeong and Y.J. Chang. 1998b. Effects of deletion of P, Ca, Zn, Mg, Fe, K, Mn, or Se from mineral premix in the diets containing 40% fish meal on growth performance of juvenile Korean rockfish (*Sebastes schlegeli*). J. Korean Fish. Soc., 31 (2), 252~258.

- Lee, S.M. 1998. Lipid nutrition of Korea rockfish (*Sebastes schlegeli*). Proceedings of the Fourth International Symposium on the Efficient Application and Preservation of Marine Biological Resources. pp. 95~118.
- Lovell, R.T. 1978. Dietary phosphorus requirement of channel catfish (*Ictalurus punctatus*). Trans. Am. Fish. Soc., 107, 617~621.
- NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Acad. Press, Washington, D.C. 114pp.
- Ogino, C., and H. Takeda. 1976. Mineral requirements in fish. 3. Calcium and phosphorus requirements of carp. Bull. Jpn. Soc. Sci. Fish., 42, 793~799.
- Ogino, C., L. Takeuchi, H. Takeda, and T. Watanabe. 1979. Availability of dietary phosphorus in carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish., 45, 1527~1532.
- Robbins, K.R., H.R. Norton and D.H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr., 109, 1710~1714.
- Sakamoto, S. and Y. Yone. 1973. Effect of dietary calcium/phosphorus ratio upon growth, feed efficiency and blood serum Ca and P level in red sea bream. Bull. Jap. Soc. Sci. Fish., 39, 343~348.
- Sakamoto, S. and Y. Yone. 1979. Availabilities of phosphorus compounds as dietary phosphorus sources for red sea bream. J. Fac. Agric. Kyushu Univ., 23, 177~184.
- Shim, K.F. and C.S. Ho. 1989. Calcium and phosphorus requirements of guppy *Poecilia reticulata*. Nippon Suisan Gakkaishi, 55, 1947~1953.
- SPSS Inc. 1997. SPSS Base 7.5 for Window, SPSS Inc., 444N. Michigan Avenue, Chicago, IL, 60611.
- Takeuchi, M. and J. Nakazoe. 1981. Effect of dietary phosphorus on lipid content and its composition in carp. Bull. Jap. Soc. Sci. Fish., 47, 347~352.
- Watanabe, T., A. Murakami, T. Takeuchi, T. Nose, and C. Ogino. 1980. Requirement of chum salmon held in freshwater for dietary phosphorus. Bull. Jpn. Soc. Sci. Fish., 46, 361~367.
- Watanabe, T., S. Satoh and T. Takeuchi. 1988. Availability of minerals in fish meal to fish. Asian Fisheries Science, 1, 175~195.
- Wilson, R.P., E.H. Robinson, D.M. Gatlin III and W.E. Poe. 1982. Dietary phosphorus requirement of channel catfish. J. Nutr., 112, 1197~1202.
- Yone, Y. and N. Toshima. 1979. The utilization of phosphorus in fish meal by carp and black sea bream. Bull. Japan. Soc. Sci. Fish., 45, 753~756.