J. of Aquaculture
Vol. 16(2) : 104-109, 2003

한국양식학회지
Journal of Aquaculture

Protein and Phosphorus Availabilities of Five Different Dietary Protein Sources in Juvenile Olive flounder (*Paralichthys olivaceus*) as Determined by Growth Performance and Phosphorus Retention

Se-Min Choi, Kang-Woong Kim[1], Xiaojie Wang[2], Kyung-Min Han[2] and Sungchul C. Bai*

Department of Aquaculture, Pukyung National University, Busan 608-737, Korea
[1]East Sea Fisheries Research Institute, NFRDI, Pohang 791-802, Korea
[2]Feeds & Foods Nutrition Reseach Center, Pukyong National University, Busan 608-737, Korea

The present study aims to evaluate protein and phosphorus availabilities of five different dietary protein sources during the 6-week feeding trial in juvenile olive flounder, *Paralichthys olivaceus* as determined by growth performance and phosphorus retention. Five diets containing blood meal (BM), poultry by-product (PBP), squid liver powder (SLP), feather meal (FM) and soybean meal (SM) were prepared by mixing a basal diet (BD) with one of five test ingredients at the ratio of 7 to 3. As a reference diet, BD contains three different protein sources such as white fish meal, casein and gelatin. After 2 weeks of the conditioning period, fish initially averaging 2.7±0.02g (mean±SD) were randomly distributed into each aquarium as a group of 30 fish reared in the recirculating system. Fish of triplicate groups were fed one of six experimental diets (BD+five test diet). After 6-week feeding trial, protein efficency ratio (PER) of fish fed BM diet was the lowest in experiment groups. While fish fed PBP diet showed a significant higher PER as compared to the FM diet, and fish fed SLP diet and BD were a higher PER than did fish fed PBP diet. However, there was no significant difference in PER among fish fed SLP diet, BD and SM diet, and between SM diet and PBP diet. Phosphorus retention efficiency of bone (PRE_b) of fish fed BM diet was the lowest in all the diets, and fish fed FM diet showed a higher PER_b than fish fed BD and SM diet. However, there was no significant difference in PER_b among fish fed FM diet, SLP diet and PBP diet, and among SLP diet, PBP diet, SM diet and BD. These results indicate that SLP could be a suitable protein source for low pollution diets of olive flounder in the future fish feeds market. Furthermore, PBP and SM are available protein source to reduce P waste in the oliver flounder aquaculture with the use of proper mixture of other protein sources and more processing to improve protein availibility of these.

Keywords: Protein sources, Growth performance, Phosphorus retention, Olive flounder, Water pollution

Introduction

Environmental and sustainable aquaculture relies on reduction of water pollution loads without negative effect on growth performance. The ultimate source of nitrogen and phosphorus in aquaculture effluent is feeds (Cho et al., 1991). In order to improve the quality of aquaculture effluents, the dietary considerations such as nitrogen and phosphorus concentrations in feeds, availability of the selective ingredients, and feeding practices must be considered.

Digestibility is one of the most important aspects in evaluating the suitability of feed stuffs. Studies on the digestibility of nutrients in feedstuffs have revealed differences in efficiency of energy utilization among the fish species, due to differences in digestive physiology, thus it is necessary to evaluate digestibility species by species. Recently, the most rapid and economical methods of protein availavility assessment involve in vitro protein digestibility determinations, including multienzyme pH-stat (Anderson et al., 1993; Dimes and Haard, 1994) and pepsin digestibility (Anderson et al., 1993; Miyazono and Inoue, 1990). Multienzyme pH-stat method correlated with apparent protein digestibility (APD) had been reported in many fish species (Anderson et al., 1993; Dimes and Haard, 1994). Multienzyme pH-stat was found to correlate well with rat protein efficiency ratio (PER) data as growth performance for both plant and animal proteins (Satterlee et al., 1979). PER correlated with APD ($r^2 = 0.82$, $P<0.05$) had been reported in Korean rockfish (Bai et al., 2001). Bai et al. (2001) has proposed that PER could predict APD indirectly in Korean rockfish, and PER as growth

*Corresponding author: scbai@pknu.ac.kr

performance is also one of the important parameters in protein quality studies as well as ADP. Many researchers have been studied new methods to determine phosphorus availability such as phosphorus concentrations in the vertebrae, scales and blood in fish (Lovell, 1978; Nordrum et al., 1997). Total body elemental analysis at different growth stages allows a fairly accurate estimation of retention (Pfeffer and Piper, 1979). Bone mineralization and weight gain were used as an alternative method to determine phosphorus availability of feedstuffs for Channel catfish, *Ictalurus punctaus* (Li and Robinson, 1996). The dietary phosphorus retention was determined to evaluate availability of phosphorus in fish bone meal and inorganic salts in Atantic salmon, *salmo salar* (Nordrum et al., 1997).

Therefore, the purpose of this study is to evaluate protein and phosphorus availabilities of five different dietary protein sources in juvenile olive flounder, *Paralichthys olivaceus.* as determined by growth performance and phosphorus retention.

Materials and Methods

Experimental diets

The basal diet (BD) was formulated to contain 52.8% crude protein (CP), 13.6% lipid and 1.66% phosphorus (P) with an estimated energy value of 4.3 kcal g^{-1} (Table 1). As a reference diet, BD contains three different protein sources such as white fish meal, casein and gelatin. Feed ingredients tested for protein and phosphorus availibilities were blood meal (BM; CP: 88.5%, P: 0.69%), poultry by-product (PBP; CP: 71.2%, P: 0.32%), squid liver powder (SLP; CP: 51.2%, P: 0.73), feather meal (FM; CP: 88.7%, P: 0.63%) and soybean meal (SM; CP: 48.7%, P: 0.91%). Test diets were prepared by mixing BD with one of the test ingredients at the ratio of 7 to 3 (Cho and Slinger, 1979). The composition of test ingredients and diets is shown in Table 2. Procedures for diet preparation and storage were as previously described by Bai & Kim (1997). The experimental diets were stored at -20°C until used.

Experimental fish and feeding trial

Juvenile olive flounder, *Paralichthys olivaceus* were produced at Keo-je seedling hatchery in Korea. Prior to the start of feeding trial, fish were fed BD for 2 weeks to acclimate to the semi-purified diet and standardized conditions. The feeding trial was conducted in the newly developed recirculating system with 60 L aquarium receiving filtered seawater at a

Table 1. Composition of the basal diet (% of dry matter).[1]

Ingredient	%
White fish meal[2]	57.00
Casein[3]	5.00
Gelatin[3]	5.00
Dextrin[3]	10.00
Wheat flour[4]	9.55
Squid liver oil[5]	7.30
EPA & DHA[5]	0.60
Vitamin premix[6]	1.00
Mineral premix[7]	2.00
Vitamin C	0.05
Cr_2O_3	0.50
Carboxymethylcelluose	2.00
Proximate analysis	
Crude Protein (%, DM)	52.8
Crude Fat (%, DM)	13.6
Crude ash (%, DM)	11.0
Phosphorus (%, DM)	1.66

[1]Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.
[2]Kum Sung Feed Co., Busan, Korea.
[3]United States Biochemical, Cleveland, Ohio 44122.
[4]Young Nam Flour Mills Co., Busan, Korea.
[5]Ewha Oil Company, Busan, Korea.
[6]Vitamin premix (mg/100g feed unless indicated otherwise): vit.A,375 IU; vit.D_3, 125 IU; vit.E, 2; menadione sodium bisulfate, 0.05; vit. B1-HCl, 2; vit.B_2, 0.75; vit.B_6, 0.75; vit. B_6-HCl, 0.87; vit.B_{12}, 0.0005; vit.C, 5; calcium pantothenate, 10; nicotin amide, 4; inositol, 0.5; d-biotin, 0.0025; choline chloride, 50; pancreatin, 1.25.
[7]Mineral premix (g/Kg feed): $MnSO_4$, 0.7; $ZnSO_4$, 3; $FeSO_4$, 5; $CuSO_4$, 0.5; $CaCo_3$, 211.9; $MgSO_4$, 17.25; K_2SO_4, 212.24; NaCl, 51.88; K_2HPO_4, 136.09; $NaSeO_3$, 0.013; KI, 0.15.

Table 2. Proximate composition of test ingredients and experimental diets (% of dry matter)[1]

	Protein	Lipids	Ash	P
Ingredients				
White fish meal (WFM)[2]	70.4	8.9	16.4	3.12
Blood meal (BM)[2]	88.5	0.2	6.6	0.69
Poultry by-products (PBP)[2]	71.2	10.7	12.5	0.32
Squid liver powder (SLP)[2]	51.2	19.1	7.9	0.73
Feather meal (FM)[2]	88.7	8.2	3.5	0.63
Soybean meal (SM)[2]	48.7	2.3	7.6	0.91
Diet[3]				
BD	52.8	13.6	10.9	1.66
BMD	63.8	8.2	9.6	1.37
PBPD	54.6	14.1	11.0	1.33
SLPD	51.2	14.6	10.0	1.47
FMD	57.3	10.9	9.7	1.23
SMD	50.9	10.4	10.0	1.42

[1]Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently. Test ingredients and diets were analyzed in triplicate.
[2]Kum Sung Feed Co., Busan, Korea.
[3]BD=basal diet, BMD=BD+BM, PBPD=BD+PBP, SLPD=BD+SLP, FMD=BD+FM, SMD=BD+SM.

rate of 1 L/min (Park, 2000). Supplemental aeration was provided to maintain dissolved oxygen near saturation. Water temperature was kept at 19±1°C using the cooler, and salinity was maintained at 31±1 ppt during the experimental period. Fish averaging 2.7±0.02 g (mean±S.D.) were distributed to each aquarium as a group of 30 fish and fed one of six experimental diets (BD+5 test diets) in triplicate at a rate of 3% to 5% of wet body weight per day for 6 weeks. Total fish weight per aquarium was determined every 3 weeks, and the amount of diet fed was adjusted accordingly.

Fish performance and analysis

Percent weight gain (WG), feed efficiency ratio (FER), specific growth rate (SGR), protein efficiency ratio (PER), hepatosomatic index (HSI), condition factor (CF), haematocrit (PCV) and hemoglobin (Hb) were calculated as described by Lee et al. (1998)

Analyses of crude protein, moisture and crude ash were performed by the standard procedure of AOAC (1995). Crude fat was determined using the Soxtec system 1046 (Tecator AB, Sweden) after freeze-drying samples for 12 hours. Phosphorus contents were determined by the vanado-molybdate method (Kim et al., 1998).

Phosphorus retention determination

Retention of phosphorus in the feeds and retention of supplemental phosphorus sources were calculated as described by Nordrum et al. (1997).

Phosphorus retention efficiency of whole body (PRE_{wb})
= 100×{(final fish wt ×P% whole body) - (initial fish wt ×P% whole body)}/P intake

Phosphorus retention efficiency of bone (PER_b)
= 100×{(final fish wt ×P% bone) - (initial fish wt ×P% bone)}/P intake

Statistical analysis

All data were subjected to ANOVA test using Computer Program Statistix 3.1 (Analytical Software, St. Paul, MN. USA). When a significant treatment effect was observed, a Least Significant Different (LSD) test was used to compare means. Treatment effects were considered significant at $P<0.05$.

Results

Growth performances

Growth performances to evaluate protein and phosphorus availibilities could be useful in our experiment because all diets were formulated to contain proper protein and phosphorus concentration for optimum growth of juvenile olive flounder, and was shown in Table 3. After 6 week feeding trial, weight gain (WG) of fish fed blood meal diet (BMD, CP: 63.8%) containing higher dietary protein content was the lowest of all the diets. Fish fed feather meal diet (FMD, CP: 57.3%) also showed a significant lower WG as compared to fish fed soybean meal diet (SMD) ($P < 0.05$). However, fish fed squid liver power diet (SLPD, CP: 51.2%) having similar dietary protein content to SMD (CP: 50.9%) showed a significant higher WG than fish fed SMD ($P < 0.05$), and fish fed BD (CP: 52.8%) also showed a significant higher WG than fish fed poultry by-produt diet (PBD, CP: 54.6%) ($P < 0.05$). There were no significnat differences in WG between BD and SLPD, between SLPD and PBPD, and between PBPD and SMD. Similar to WG results, specific growth rate (SGR)

Table 3. Effects of a basal diet and five test diets on growth performances in olive flounder

	Diets[1]						Pooled SEM[2]
	BD	BMD	PBPD	SLPD	FMD	SMD	
WG(%)[3]	240^{a}	36^{e}	207^{bc}	229^{ab}	165^{d}	199^{c}	16.8
SGR[4]	1.26^{a}	0.32^{d}	1.16^{ab}	1.23^{ab}	1.01^{c}	1.13^{b}	0.07
PER[5]	2.42^{a}	0.41^{d}	2.11^{b}	2.46^{a}	1.65^{c}	2.18^{ab}	0.17
FER(%)[6]	110^{a}	26^{c}	97.3^{ab}	102.7^{a}	89.3^{b}	92.2^{b}	6.67
Hematocrit[7]	21.8	19.2	24.0	22.5	18.2	21.0	0.9
Survival (%)	95.6^{ab}	94.4^{ab}	96.7^{ab}	98.9^{a}	90.0^{b}	97.8^{a}	1.04

[1]BD=basal diet, BMD=BD+BM, PBPD=BD+PBP, SLPD=BD+SLP, FMD=BD+FM, SMD=BD+SM.
[2]Pooled standard error of mean.
[3]Weight gain (%): [(final weight - initial weight)x100]/initial weight.
[4]Specific growth rate: [($\log_e$ final weight - $\log_e$ initial weight)x100]/days.
[5]Protein efficiency ratio: body weight gain/protein intake.
[6]Feed efficiency ratio (%): (body weight gainx100)/dry feed intake.
[7]PCV (%)=Hematocrit.

Table 4. Phosphorus retention efficiency of whole body (PRE_{wb}) and bone (PRE_b) of flounder fed experimental diets for 6 weeks (%, dry mater basis)

	Diets[1]						Pooled SEM[2]
	BD	BMD	PBPD	SLPD	FMD	SMD	
PRE_{wb} (Whole body, %)[3]	15.02^{c}	14.87^{c}	17.31^{bc}	21.86^{a}	16.81^{bc}	17.94^{b}	2.72
PRE_b (Bone, %)[4]	28.76^{b}	28.82^{c}	31.52^{ab}	32.10^{ab}	34.15^{a}	30.51^{b}	0.82

[1]BD=basal diet, BMD=BD+BM, PBPD=BD+PBP, SLPD=BD+SLP, FMD=BD+FM, SMD=BD+SM.
[2]Pooled standard error of mean.
[3]Phosphorus retention efficiency=100×{(final fish weight×% phosphorus in whole body) - (initial fish weight×% Phosphorus in whole body)}/Phosphorus intake.
[4]Phosphorus retention efficiency=100×{(final fish weight×% phosphorus in bone) - (initial fish weight×% phosphorus in bone)}/P intake.
[3,4]Phosphorus retention values were calculated as previously described by Sigve et al. (1997).

of fish fed BMD was the lowest of all the diets ($P < 0.05$). Fish fed SMD showed a significant higher SGR than fish fed FMD, and fish fed BD showed a significant higher SGR than fish fed SMD ($P < 0.05$). However, there were no significant differences in SGR among BD, SLPD and PBPD, and among SLPD, PBPD and SMD. Protein efficency ratio (PER) of fish fed BMD was the lowest in all the diets ($P < 0.05$). Fish fed PBPD showed a significant higher PER than fish fed FMD, and fish fed SLPD and BD showed higher PER than fish fed PBPD ($P < 0.05$). However, there were no significant differences in PER among SLPD, BD and SMD, and between SMD and PBPD. Feed efficiency ratio (FER) of fish fed BMD was the lowest in all the diets, and fish fed BD and SLPD showed a significant higher FER than did fish fed SMD and FMD ($P < 0.05$). However, there were no significant differencs in FER among BD, SLPD and PBPD, and between PBPD and SMD.

There was no significant difference in hematocrit (PCV) among all the dietary treatments.

Phosphorus retention

Phosphorus retention efficiency in whole body and bone is shown in Table 4. Phosphorus retention efficiency in whole body (PRE_{wb}) of fish fed BMD and BD were significantly lower than that of fish fed SLPD and SMD, and fish fed SLPD showed the highest PRE_{wb} of all the diets ($P < 0.05$). However, there were no significant differences in PRE_{wb} among SMD, PBPD and FMD, and among PBPD, FMD, BD and BMD.

Phosphorus retention efficiency of bone (PRE_b) of fish fed BMD was the lowest in all the diets, and fish fed FMD showed a higher PER_b than fish fed BD and SMD ($P < 0.05$). However, there were no significant differences in PER_b among FMD, SLPD and PBPD, and among SLPD, PBPD, SMD and BD.

Discussion

It is necessary to develop low pollution diets without significantly reducing growth for the future environmental-friendly sustainable aquaculture industry. In order to develop low pollution diet, the first important thing is to evaluate the availability of each nutrients in aquacultured fish. Especially for phosphorus and nitrogen, the major factor in water pollution, such kind of evaluation should be conducted urgently.

In order to select ingredients that could be included in low pollution diets, the present experiment was conducted to evaluate protein and P availability of 5 different protein sources in olive flounder. Growth performances and P retention in bone & whole body was used as criteria to evaluate the protein utilization and P availability of each protein sources.

In this study, although the protein content in BMD was higher than the other diets, the fish fed the BMD had significantly lower growth performance and phosphorus retention than the other dietary treatments. Similar result has been reported in Korean rockfish (Choi, 2001). Also in chinook salmon, the low digestibility of protein (30%) and phosphorus in fish fed BM diet was observed (Hajen et al., 1992). The reason why those fish fed blood meal showed poor growth rate is not well understood, but the possible influence might be fish species. It is necessary to research the utilization of BM in marine fish species in the near future.

Previous studies showed that BM can replace up to 50% of fish meal protein without any negative effects on growth in some freshwater fish species such as rainbow trout (Smith et al., 1980) and channel catfish (NRC, 1993), and the P digestibility of BM was 69% and 74% in rainbow trout and channel catfish, respectively.

Although the P digestibility of BM was 74.6% higher than the other dietary sources in Korean rockfish (Choi, 2001), the present studies showed low growth perfermances because the reason might be the low protein availibility of BMin flounder.

There were no significant differences in FER and SGR between fish fed PBPD and BD. According to the growth data, it might be possible to conclude that PBP could have high digestibility in olive flounder. PBP has high digestibility as 74% in Chinook salmon (Hajen et al., 1992). However, Korean rockfish fed PBP diet showed low growth and protein digestibility (Choi, 2001). There was no significant difference in PRE_b of fish fed SLP and FMD diets.

Although protein content in SLPD was lower than BD, there were no significant differences in WG, FE, SGR and PER between fish fed SLPD and BD. Also in PRE_{wb}, no significant difference was observed between fish fed SLPD and BD. Meanwhile, fish fed SLPD showed significantly higher PRE_b than fish fed BD. Similar result has been reported in Korean rockfish that the fish fed SLP showed high growth performance and P digestibility (Choi, 2001).

In the present experiment, fish fed FMD showed low WG, FE, SGR, and PER than fish fed BD, though FMD had high protein content. Meanwhile, PRE_b of fish fed FMD was higher than that of fish fed BD. It has been reported that FM could replace up to 50% of fish meal in Indian major carp (Hasan et al., 1997), however, only 12 to 25% of fish meal in juvenile Japanese flounder (Kikuchi et al., 1994).

In this experiment, there were no significant differences in PER and PRE_b between fish fed SMD and BD. However, low PRE_b was observed in fish fed SM when compared with those from fish fed FMD. Day and Plascencia Gonzalez (2000) reported that soybean protein concentration could replace up to 25% of fish meal in turbot. Kikuchi (1999) mentioned that SM could replace up to 47% of fish meal in flounder. McGoogan and Gatlin (1997) found that SM could replace fish meal up to 90% in red drum, and could replace even up to 95% of fish meal when supplemented with glycine and fish solubles. However, opposite result was observed in Korean rockfish when growth, protein and P digestibility were used as parameters (Choi, 2001). The availibility of protein source varies according to palatability and specific characteristic of fish species such as digestive physiology, environmental conditions and so on.

Also, compared with the sole use of SM, the combination of several protein sources had improved fish growth (Viyakarn et al., 1992; Kikuchi, 1999). For example, although the methionine and cystine content in SM was low, its content in corn gluten meal and meat & bone meal was similar to that contained in fish meal.

In conclusion, the olive flounder shows preference for SLP. These results indicate that SLP could be a suitable protein source for low pollution diets for olive flounder in the future fish feeds market. Furthermore, PBP and SM are available protein sources to reduce P waste in the oliver flounder aquaculture with the use of proper mixture of other protein sources and more processing to improve protein availibility.

Acknowledgements

This research was supported in the funds of the Ministry of Marine Affairs and Fisheries, and Feeds and foods Nutrition Research Center at Pukyong National University, Busan, Korea.

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Manuscript Received: March 22, 2003
Revision Accepted: April 9, 2003
Responsible Editorial Member: Jeong-Yeol Lee