A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Partial replacement of dietary fish meal by soybean meal for juvenile Tiger puffer *Takifugu rubripes*

Gyung-Yong Ko

Department of Marine Life Science

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

2008. 12.

Partial replacement of dietary fish meal by soybean meal for juvenile Tiger puffer *Takifugu rubripes*

Gyung-Yong Ko

(Supervised by Professor Kyeong-Jun Lee)

A thesis submitted in partial fulfillment of the requirement for the degree of
Master of Science

2009. 2.

This thesis has been examined and approved.

Lee Ki Wan

Ki-Wan Lee, Prof. of Marine Life Science

In-Kyu Yeo, Associate Prof. of Marine Life Science

Kyeong Jun Lee

Kyeong-Jun Lee, Assistant Prof. of Marine Life Science

2009. 2
Date

Department of Marine Life Science

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

Contents

국문초록

양어사료에서 주 단백질원으로 사용되는 어분의 가격상승으로 인하여 어분대체 단백질원의 개발은 양식산업의 중요한 사안으로 부각되고 있다. 어분 대체원으로 많이 사용되는 식물성 단백질원인 대두박은 높은 단백질 함량과 비교적 우수한 아미노산 조성으로 양어사료에서 어분을 대체할 수 있는 단백질원으로 많은 연구가 수행되어졌다. 그러나 대두박은 어분과 비교하여 제한 아미노산인 lysine과 methionine이 부족할 뿐만 아니라 phytic acid같은 항영양인자를 포함하고 있어 양어사료에서의 이용이 제한적이다.

이 연구는 최근 새로운 양식 어종으로 각광받는 자주복을 대상으로 사료 내 대두박의 어분대체 가능성 및 그 이용성을 알아보고자 수행되었다.

초기 무게가 20.1g 0.1g인 자주복 치어를 이용하여 15주 동안 급이실험을 하여 성장과 먹이효율 및 일간 성장률, 그리고 단백질 전환효율을 바탕으로 검토한 결과 어분의 30%를 대두박으로 대체할 수 있었다. 대두박의 함량이 많이 첨가된 SBM 45와 60의 실험구에서 성장 결과가 낮게 나타난 이유는 대두박에 함유된 항영양인자의 영향과 phytate의 영향으로 보여진다.

혈장 내 인의 함량은 성장 결과와 유사하게 사료 내 대두박의 함량이 증가할수록 낮은 값을 나타냈다. 이러한 결과는 대두박 함량이 많은 사료 내 인의 함량이 자주복 치어에 있어서 최적의 성장을 위해 사용되지 못함을 의미한다. 이러한 결과가 나타나는 이유는 자주복의 장 내 phytase의 부족현상 때문이라 판단된다.

위의 결과들을 종합하면, 자주복 사료에 대두박을 어분의 30%까지 대체하였을 때, 어류의 건강 상태에 지장 없이 성장할 수 있는 것으로 판단된다. 식물성 단백질원인 대두박을 사료 내 첨가하였을 때, 어류에서 중성지방과 콜레스테

롤 축적이 낮아진 이 연구의 결과는 향후 기능성 어류 생산을 위한 기초 자료로 활용될 수 있을 것으로 판단된다.

List of Figures

List of Tables

REFERENCE REVIEW

Fish meal (FM) replacement in aquaculture industry has been an important issue because of increasing demand, limited supply of FM and its dramatic price increase with the expansion of aquaculture (FAO, 2003). For this reason, studies have been carried out to find candidates for the FM replacement in diets for most cultured fish species (Boonyaratpalrin et al., 1998; Kikuchi, 1999; Refstie et al., 2001; Lim et al., 2004; Lee et al., 2006; Yoshitomi et al., 2007; Lim and Lee, 2008).

Soybean meal (SBM) has been the most frequently studied ingredient as a FM replacer in diets for many fish species, because it has high protein content, relatively well-balanced amino acid profiles, reasonable price and steady supply (Storebakken et al., 2000). A number of studies have shown that SBM protein alone or in combination with other protein sources can successfully replace FM protein from 20% up to 90% in diets for many fish species without growth depression (Shimeno et al., 1993; Gomes et al., McGoogan and Gatlin III, 1997; 1995; Boonyaratpalin et al., 1998; Kikuchi, 1999; Elangovan and Shim, 2000; Chou et al., 2004; Lim et al., 2004; Hernandez et al., 2007; Pham et al., 2007; Lim and Lee, 2008).

However, the use of SBM in fish feeds is still limited because of the presence of some antinutritional factors, such as polysaccharide, high fiber content and phytate (NRC, 1993; Storebakken et al., 2000; Francis et al., 2001; Hendricks, 2002). Phytate (inositol hexaphosphate) is one of major obstacle in using plant protein for fish diets and approximately two-thirds of total phosphorus is bound as phytate (Lall, 1991) which is not efficiently utilized by fish (NRC, 1993). In addition, the deficiency of some essential amino acids in the SBM such as methionine and lysine reduces the inclusion level of this material in fish diets (NRC, 1993). Therefore, many fish nutritionists have tried to supplement phosphorus and/or methionine and lysine when SBM was used in fish diets. Shiau et al. (1988) reported that the dietary supplementation of methionine could improve growth

performance in milkfish. Albrektsen et al. (2006) also showed that phosphorus supplementation in diets containing vegetable protein could replace 50% FM protein in Atlantic cod without growth impairment. In contract, inclusion of phosphorus in SBM containing diets for gilthead sea bream did not improve the growth performance (Robaina et al., 1998).

Tiger puffer, *Takifugu rubripes,* is carnivorous species and has been regarded as emerging aquaculture species in Japan and South Korea because of its advantages of high economic value, excellent meat quality and strong resistance to diseases. In field condition, this specie was fed diets at four to six times a day (Kumamoto Prefectural Fisheries Research Center, 2001), because puffer fish do not have a stomach. The puffer fish grow very slowly and reach 1 kg body weight by 17–18 months. Little is known about their nutritional information, especially the replacement of FM. Therefore, the present study was conducted to investigate the use of SBM as fish meal replacer with phosphorus, lysine and methionine supplementations in diets for juvenile tiger puffer.

ABSTRACT

This study was conducted to investigate the effect of partial replacement of dietary fish meal by soybean meal (SBM) on growth performance of juvenile tiger puffer, *Takifugu rubripes*. Isonitrogenous (45% crude protein) and isocaloric (16.0 MJ/kg) diets were formulated to replace fish meal by 0%, 15%, 30%, 45% and 60% SBM (designated as FM, SBM15, SBM30, SBM45 and SBM60, respectively). Juvenile tiger puffer with an initial average size of 20.1 ± 0.1g (mean ± SD) were randomly distributed into 15 groups (15 fish per tank). Triplicate groups of fish were fed one of the five experimental diets for 15 weeks. At the end of feeding trial, weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio of fish fed diets 15% and 30% SBM were not significantly ($P>0.05$) different compared to that of fish fed the control diet. Condition factor, hemoglobin and hematocrit showed the same trend in growth performance. Hepatosomatic index was significantly decreased as the inclusion level of the SBM increased. Phosphorus concentration in plasma was decreased with increasing dietary SBM. The present results suggest that juvenile tiger puffer could accept the diets containing 30% SBM for fish meal replacement.

MATERIALS AND METHODS

Experimental diets

Five experimental diets were formulated to replace FM protein by SBM at 0, 15, 30, 45, 60% (designated as FM, SBM15, SBM30, SBM45 and SBM60, respectively). The SBM containing diets were supplemented by L-methionine, L-lysine and monocalcium phosphate to meet their estimated dietary requirement of fish (NRC, 1993). All the experimental diets were formulated to be isonitrogenous (45% crude protein) and isocaloric (16.0 MJ/kg diet). The formulation and proximate composition of the diets are presented in Table 1. All the dry materials were thoroughly mixed with 30% double distilled water, extruded through a meat chopper machine (SMC-12, Kuposlice, Busan, Korea) at 5 mm in diameter, freeze-dried at -40 ^{0}C for 24 hours and stored at -20 ^{0}C until use.

Feeding trial and sample collection

Juvenile tiger puffer were transported from a private hatchery (at Cheju Island, Korea, to Marine and Environmental Research Institute, Jeju National University, Jeju, Korea. The fish were fed with a commercial diet (Suhyupfeed, co., Ltd, GyeongNam, Korea) for 4 weeks to be acclimated to experimental conditions and to be recovered from the stress of transportation. After the acclimation, the fish (initial body weight 20.1±0.1g) were randomly distributed into fifteen 30 L polyvinyl conical tanks. The tanks were supplied with filtered seawater at a flow rate of 2.5 l/min and aeration to maintain proper level of the dissolved oxygen. The triplicate groups of fish were fed the experimental diets to apparent satiation (twice a day, 9:00 and 17:30 h) for 15 weeks. Uneaten feeds were collected 30 min after feeding and reweighed to calculate feed utilization accurately. The growth of fish was measured every 3 weeks. Feeding was stopped 24 h prior to weighing to minimize stress of the fish.

At the end of feeding trial, three fish per tank (9 fish per dietary treatment) were randomly selected and anaesthetized with 2-phenoxyethanol (100 ppm) for blood analyses. The blood samples were taken from caudal vein with heparinized syringes. Three fish from each tank (nine fish per treatment) were sampled for determination of hepatosomatic index and condition factor. Liver samples from three fish per tank (nine fish per treatment) were taken and stored at -80 ^{0}C for analysis of lipid concentration.

Table 1. Formulation and proximate composition of experimental diets (% dry matter).

Ingredients	Experimental diets				
	FM	SBM15	SBM30	SBM45	SBM60
Fish meal	45.0	38.3	31.5	24.8	18.0
Soybean meal	0.0	10.0	20.2	30.2	40.2
Corn gluten meal	8.0	8.5	8.7	8.8	9.2
Wheat flour	37.0	32.1	27.1	22.5	17.6
Yeast	2.0	2.0	2.0	2.0	2.0
Mineral Mix[1]	1.0	1.0	1.0	1.0	1.0
Vitamin Mix[2]	1.0	1.0	1.0	1.0	1.0
Squid liver oil	5.0	5.3	5.7	6.1	6.4
CMC	1.0	1.0	1.0	1.0	1.0
Lysine	0.0	0.2	0.4	0.6	0.8
Methionine	0.0	0.2	0.4	0.6	0.8
Monocalcium phosphate	0.0	0.5	1.0	1.5	2.0
Proximate composition					
Dry matter (%)	95.9	95.3	95.8	97.2	95.0
Protein (%, DM)	44.9	45.4	45.4	46.2	46.7
Lipid (%, DM)	9.4	9.7	9.4	8.5	8.9
Ash (%, DM)	8.2	8.2	8.1	8.3	8.1
Gross energy, MJ/kg DM[3]	16.0	16.0	16.0	16.0	16.0

[1] Mineral premix (g/kg of mixture): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370; KCl, 130.0; Ferric citrite, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5;CuCl2, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0

[2] Vitamin premix (g/kg of mixture): L-ascorbic acid, 121.2; DL- α tocopheryl acetate, 18.8;thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

[3] Gross energy of experimental diets was calculated according to gross energy values 5.64 kcal/g protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g fat respectively (NRC, 1993).

Analyses

At the end of the feeding trial, all fish in each tank were weighed and counted to compute the weight gain, feed conversion ratio, specific growth rate, protein efficiency ratio, feed intake and survival. Hematocrit was determined for three individual fish per tank by microhematocrit technique (Brown, 1980). Hemoglobin, plasma tryglycerides, total cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in the same three fish by using the automated blood analyzer (SLIM, SEAC Inc, Florence, Italy). Analyses of crude protein, moisture and ash in the experimental diets were performed by the standard procedures (AOAC, 1995). Dietary lipid was determined according to the method described by Folch et al. (1957).

Phosphorus concentration in diets and plasma were measured using spectrophotometric method described by Nahapetian and Bassiri (1975). The plasma and dry diets were digested with 10 mL of a mixture of concentrated H_2SO_4 and HNO3 (1:1;v:v) in Kjeldahl flasks. The digested mixture was volumed up to 100 mL with distilled water and used for total phosphorus measurement. Inorganic phosphorus of the experimental diets was extracted with 12.3% trichloroacetic acid solution using a shaker (WiseCube, DAIHAN Scientific, Co., Ltd., Seoul, Korea) for 12 h at room temperature. After centrifugation at 2000 × g for 20 min, the supernatant was used for measurement of inorganic phosphorus. Phosphorus retention was calculated as following equation described by Nordrum et al. (1997); Dietary phosphorus retention (%) = 100 × (final body phosphorus – initial body phosphorus) / dietary phosphorus consumed.

Statistical analysis

All experimental diets were assigned by a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, the differences in mean values were made with Duncan's multiple range test. Statistical significance was determined by setting the aggregate type I error at 5% ($P<0.05$) for each set of comparisons. Data are presented as mean±SD. Percentage data were arcsine transformed before statistical analysis. The correlation between weight gain and plasma phosphorus was estimated using MICROSOFT EXCEL regression.

Table 2. Growth performance of juvenile tiger puffer fed the experimental diets for 15 weeks[1].

Diets	FM	SBM15	SBM30	SBM45	SBM60
Weight gain (WG)[1]	458 ± 23.4^{a}	485 ± 20.2^{a}	476 ± 19.7^{a}	337 ± 57.7^{b}	244 ± 19.1^{c}
Feed intake, g (FI)[2]	1.07 ± 0.18^{a}	1.14 ± 0.04^{ab}	0.96 ± 0.23^{a}	1.24 ± 0.08^{ab}	1.39 ± 0.11^{b}
Feed conversion ratio (FCR)[3]	1.34 ± 0.22^{ab}	1.40 ± 0.06^{ab}	1.20 ± 0.29^{a}	1.73 ± 0.16^{b}	2.17 ± 0.29^{c}
Specific growth rate (SGR)[4]	1.68 ± 0.04^{a}	1.68 ± 0.03^{a}	1.67 ± 0.03^{a}	1.40 ± 0.13^{b}	1.18 ± 0.05^{c}
Protein efficiency ratio (PER)[5]	1.69 ± 0.31^{ab}	1.57 ± 0.06^{ab}	1.92 ± 0.53^{a}	1.26 ± 0.12^{b}	1.00 ± 0.14^{c}
Condition factor [6]	4.03 ± 1.07^{a}	3.74 ± 0.34^{ab}	4.08 ± 0.32^{a}	3.53 ± 0.42^{b}	3.50 ± 0.28^{b}
Survival (%)	77.8 ± 7.7	86.7 ± 6.7	84.4 ± 10.2	75.6 ± 13.9	71.1 ± 3.8

Means of triplicate groups, values are presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

[1] Weight gain (%) =100×(final mean body weight-initial mean body weight)/initial mean body weight

[2] Feed Intake (g/g BW) = dry feed consumed/body weight

[3] Feed conversion ratio = dry feed fed/wet weight gain

[4] Specific growth rate (%) = 100×[(ln final mean body weight-ln initial mean body weight)/days]

[5] Protein efficiency ratio = wet weight gain (g)/total protein given (g)

[6] Condition factor = 100×(body weight/body length^{3})

RESULTS

At the end of 15 weeks of feeding trial, negative effects on growth performance were observed when over 45% of FM protein was replaced by SBM protein (Table 2). The values of weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio were significantly reduced when the replacement level for FM protein was increased from 30% to 45%. Groups of fish fed SBM60 diet had significantly lower feed intake values than groups of fish fed the FM diet. Survival was not affected by dietary inclusion levels of SBM. These results showed that 30% of FM protein could be replaced by SBM protein with phosphorus and limited amino acids (methionine and lysine) supplementations for juvenile tiger puffer without reduction of the growth performances.

Hematocrit and hemoglobin were significantly influenced by dietary SBM levels (Table 3). However, groups of fish fed the SBM15 and SBM30 diets were not significantly different compared to those of fish fed the FM diet. Plasma total cholesterol and triglycerides concentrations were decreased as dietary SBM inclusion levels increased (Table 3). The lowest plasma total cholesterol and triglycerides concentrations were observed in groups of fish fed the SBM60 diet (the highest incorporation of SBM). There were no significant differences in alanine aminotransferase and aspatate aminotransferase among all the dietary treatments (Table 3).

Dietary total and inorganic phosphorus and plasma total phosphorus concentration of fish fed experimental diets are given in Table 4. Dietary inorganic phosphorus and plasma phosphorus concentration were decreased as dietary SBM increased and had a strong positively relationship.

Hepatosomatic index (HSI) of fish fed the experimental diets was significantly influenced by dietary SBM levels (Table 5). HSI was significantly decreased as dietary SBM inclusion levels increased and the lowest value was observed in fish fed SBM60 diet. Lipid concentration in liver of the fish fed the experimental diets was not significantly different among all the dietary treatments (Table 5).

Table 3. Blood parameters of juvenile tiger puffer fed the experimental diets for 15 weeks.

Diets	FM	SBM15	SBM30	SBM45	SBM60
Hematocrit (%)	25.6 ± 3.9[a]	23.5 ± 2.4[a]	24.4 ± 2.2[a]	20.3 ± 1.2[b]	19.5 ± 1.3[b]
Hemoglobin (g/dL)	6.9 ± 1.46[a]	6.8 ± 0.98[a]	6.8 ± 1.19[a]	5.2 ± 0.90[b]	5.3 ± 0.70[b]
ALT (U/L)	13.5 ± 9.2	12.2 ± 4.5	13.9 ± 5.3	10.2 ± 2.7	16.8 ± 5.9
AST (U/L)	28.9 ± 16.4	30.1 ± 11.3	21.4 ± 4.6	19.1 ± 11.8	31.8 ± 13.2
Cholesterol (mg/dL)	135 ± 4.0[a]	123 ± 16.5[ab]	125 ± 20.6[ab]	112 ± 9.9[ab]	102 ± 6.4[b]
Triglyceride (mg/dL)	134 ± 37.6[a]	122 ± 32.9[ab]	94 ± 7.1[ab]	76 ± 22.9[b]	80 ± 14.7[b]

Mean values of triplicate groups, values are presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

Table 4. Dietary total and inorganic phosphorus and plasma total phosphorus of fish fed the experimental diets for 15 weeks.

Diets	FM	SBM15	SBM30	SBM45	SBM60
Diet Pt (%)[1]	0.65±0.1	0.67±0.1	0.65±0.2	0.67±0.1	0.68±0.1
Diet Pi (%)[2]	0.52±0.1	0.54±0.1	0.52±0.1	0.49±0.1	0.47±0.1
Plasma Pt (mg/dL)[3]	4.55±0.6^{a}	4.26±0.4^{ab}	4.42±0.6^{a}	3.69±0.5^{b}	3.61±0.4^{b}

Mean values of triplicate groups, values are presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).
1 Dietary total phosphorus.
2 Dietary inorganic phosphorus.
3 Plasma total phosphorus.

Table 5. Hepatosomatic index (HSI) and lipid concentration in liver of juvenile tiger Puffer fed the experimental diets for 15 weeks.

Diets	FM	SBM15	SBM30	SBM45	SBM60
HSI	13.3 ± 1.5^{a}	11.4 ± 0.8^{b}	11.2 ± 0.5^{b}	8.9 ± 1.0^{c}	7.5 ± 0.6^{d}
Lipid	58.9±1.7	58.9±2.4	61.5±1.8	59.6±1.3	56.0±3.2

Mean values of triplicate groups, values are presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

The present study demonstrated that the SBM with phosphorus, lysine and methionine supplementations could replace dietary FM protein up to 30% without negative effects on growth performances, feed utilizations and survival of juvenile (20-120 g) tiger puffer. The result of the present study is significant because, to our knowledge, it is the first SBM used dietary formulation for juvenile tiger puffer.

In the present study, fish fed the diets containing over 30% SBM protein produced lower growth performance and feed utilization. The finding that FM replacement by SBM resulted in lower growth performance comparable to the findings in other species (Pongmaneerat and Watanabe, 1992; Rumsey et al., 1994; Stickney et al., 1996; Yoo et al., 2005). The suppression in overall growth performance in fish fed SBM 45 and SBM 60 diets may be attributed to the presence of antinutrional factors in soybean meal (Kakade et al., 1973; Vaintraub and Bulmaga, 1991; Liener, 1994) and an adverse effect of phytate (Spinelli et al., 1983; Richardson et al., 1985; Satoh et al., 1989).

After 15 weeks of feeding trial, a significantly lower hematocrits and hemoglobin values were observed in fish fed the SBM-rich diets (SBM45-60) compared to fish fed FM diet. In previous studies, reduced blood hematocrit and hemoglobin were found in fish fed plant protein-rich diets (Blom et al., 2001; Lee et al., 2002; Yildirim et al., 2003; Pham et al., 2007; Lim and Lee, 2008). Many researchers have found that hematocrits and hemoglobin varies according to the deficiency of essential nutrients, environmental conditions and growth status (Garrido et al., 1990). In this study, therefore, the reason for the significantly lower blood hematocrits and hemoglobin in SBM-rich diet groups (SBM45-60) than those in FM based diet groups might be attributed to the deficiency of essential nutrients and antinutritional factors in SBM.

The present study clearly demonstrated that dietary supplementation of SBM significantly reduces levels of plasma triglycerides and total cholesterol. Cholesterol lowering effect of soybean and other plant proteins has been intensively investigated on vertebrate animal, including fish (Golgberg et al., 1982; Sugiyama et al., 1996; Kaushik et al., 1995; 1998; Ali et al., 2004; Gilbert and Thompson, 2005; Chisholm et al., 2005; Dias et al., 2005; Batta et al., 2006; Venou et al., 2006). Kaushik et al. (1995) reported that plasma cholesterol levels were decreased in rainbow trout fed soybean protein diets in comparison to those fed 100% FM diet. Likewise, Venou et al. (2006) reported that plasma cholesterol was decreased with inclusion of SBM in diet for gilthead seabream. In the present study, the dietary polyphenolic compound contents were increased at each incremental level of dietary SBM protein (data were not shown). SBM is source of important polyphenols such as

flavonoids, isoflavones, glycitein, genistein and daidzein that exert a strong antioxidant action (Andlauer et al., 1999; Fritz et al., 2003). In present study, the plasma cholesterol and triglycerides lowering effects could be attributed by the higher dietary polyphenol contents. Up to date, cholesterol metabolism has not well understood in fish (Este'vez et al., 1996). Further studies on plant protein-rich diets are needed to investigate the pathway of lipid metabolism in fish

In the present study, dietary inorganic phosphorus and plasma phosphorus concentration were decreased as dietary SBM increased and had a strong positively relationship. In addition, plasma phosphorus concentration showed a high correlation with weight gain of tiger puffer (Fig 1). The lower plasma total phosphorus concentrations in fish fed SBM45 and SBM60 diets compared to fish fed FM diet might be attributed to the presence of phytate in SBM. In general, dietary phosphorus requirement for marine fish ranges from 0.5 to 1.0% (Kim et al., 1998; Borlongan and Satoh, 2001; Roy and Lall, 2003; Oliva-Teles and Pimentel-Rodrigues, 2004). The dietary total phosphorus levels of this study were approximately 0.7% indicating that the levels seemed to meet its requirement for experimental fish. In plant protein sources, however, approximately 70% total phosphorus is present as phytate phosphorus which cannot be absorbed and utilized by monogastric animals including fish (Lall, 1991). Therefore, the dietary available phosphorus levels in SBM-rich diets (SBM45-60) could not be enough to meet its requirement for optimal growth in the juvenile tiger puffer resulting in the impairment of growth performances due to the absence of intestinal phytase (Jackson et al., 1996).

After the feeding trial in this study, HSI was significantly decreased as dietary SBM inclusion levels increased and the lowest value was observed in fish fed SBM60 diet whereas lipid concentration in liver was not significantly different in all the dietary treatments. Likewise, Venou et al. (2006) reported that HSI was decreased with inclusion of SBM in diet for gilthead seabream. In contract, sharpsnout seabream (Hernandez et al., 2007) and red seabream (Biswas et al., 2007) fed SBM were not significantly different in HSI compared to fish meal based control diet. Lower HSI of fish fed the SBM-rich diets (SBM45-60) in the present study could be attributed to the reduced growth of these fish. The lower HSI of fish fed the SBM45-60 diets is responsible for the highest feed intake of the fish, because feed intake of fish is regulated by their body lipid storage.

According to the results of the present study, it is concluded that juvenile tiger puffer could accept the diets containing 30% soybean meal with phosphorus, lysine and methionine supplementation for fish meal replacement.

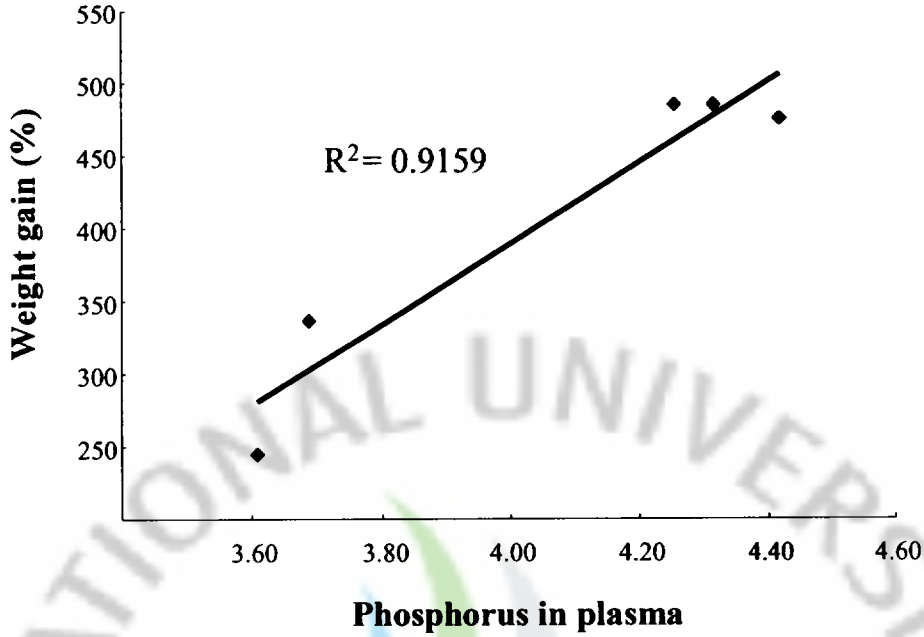

Figure. 1. Correlation between weight gain (WG) and plasma phosphorus concentration of tiger Puffer *Takifugu rubripes* fed the experimental diets for 15 weeks. Each dot represents the average of three groups of fish.

SUMMARY

Dietary replacement of fish meal (FM) have been an important issue in aquaculture industry due to a limited supply of FM and its dramatic price increase in recent years. Feed costs account for over 50% of total production costs in most marine fish species, because of the use of the expensive FM with a large dietary proportion. Plant origin byproducts have been promising candidates for the FM replacement and successfully used in many fish species. Soybean meal (SBM) has been the most frequently studied ingredient as a FM replacer in diets for many fish species, because it has high protein content, relatively well-balanced amino acid profiles, reasonable price and steady supply.

Isonitrogenous (45% crude protein) and isocaloric (16.0 MJ/kg) diets were formulated to replace fish meal by 0%, 15%, 30%, 45% and 60% SBM (designated as FM, SBM15, SBM30, SBM45 and SBM60, respectively). Juvenile tiger puffer with an initial average size of 20.1 ± 0.1g (mean ± SD) were randomly distributed into 15 groups (15 fish per tank). Triplicate groups of fish were fed one of the five experimental diets for 15 weeks.

The results indicated that SBM with phosphorus, lysine and methionine supplementations could replace dietary FM protein up to 30% without negative effects on growth performances of growing tiger puffer. In the present study, fish fed the diets containing over 30% SBM protein produced lower growth performance and feed utilization. The suppression in overall growth performance in fish fed SBM 45 and SBM 60 diets seemed to be attributed to the presence of antinutritional factors in soybean meal and an adverse effect of phytate.

Phosphorus concentration in plasma was decreased with increasing dietary SBM. The results indicated that the dietary available phosphorus levels in SBM-rich diets (SBM 45-60) were not enough to meet its requirement for optimal growth in the juvenile tiger puffer resulting in the impairment of growth performances due to the absence of intestinal phytase

According to the results of the present study, it is concluded that juvenile tiger puffer could accept the diets containing 30% soybean meal with phosphorus, lysine and methionine supplementation for fish meal replacement.

REFERENCES

Association of Official Analytical Chemists (AOAC) (Ed.), 1995. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Barlow, S., 1989. Fishmeal-world outlook to the year 2000. Fish Farmer, Sept./Oct., pp. 40-43.

Benedito-Palos, L., Saera-Vila, A., Calduch-Giner, J-A., Kaushik, S., Perez-Sanchez, J., 2007. Combined replacement of fish meal and oilin practical diets for fast growing juveniles of gilthead sea bream (*Sparus aurata L.*):Networking of systemic and local components of GH/IGF axis. Aquaculture 267, 119-212.

Cai, X., Luo, L., Xue, M., Wu, X., Zhan, X., 2005. Growth performance, body composition and phosphorus availability of juvenile grass carp (*Ctenopharyngodon idellus*) as affected by diet processing and replacement of fishmeal by detoxified castor bean meal. Aquacult. Nutr. 11. 293-299.

Garrido, L.G., Chapuli, R.M., Andres, A.V., 1990. Serum cholesterol and triglyceride levels in Scyliorhinus canicula (L.) during sexual maturation. J. Fish Biol. 36, 499–509.

Dabrwski, K., Poczyczynski, P., Kock, G., Berger, B., 1989. Effect of partially or totally replacing fishmeal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New in vivo test for exocrine pancreatic secretion. Aquaculture 77, 29-49.

Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Xu, W., Liufu, Z., 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and growthof juvenile Japaneses flounder, Paralichthys olivaceus. Aquaculture 258, 503-513.

Francis, G., Makkar, H.P.S., Backer, K., 2001. Antinutritional factors present in plant derived alternate fish feed ingredients and the effects in fish. Aquaculture 199, 197-227.

Folch, J., Lees, M., Stanley, S.G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. 226, 497-509.

Ghosh, s., Hazra, A.K., Banerjee, S., Mukherjee, B., 2005. Ecological monitoring for ascertaining the bio-safety of liver lipids from some Indian marine puffer fishes. Fisheries science 71, 29-37.

Go, H.B., Rho, S., 1996. Low salinity tolerance of eggs and juvenile of tiger puffer, *takifugu rubripes*. J. Aquacult. 9, 43-55.

Hayashi, K., 1987. Liquid wax esters in liver oils of deep sea teleosts *Laemonema longipes*. Nippon Suisan Gakkaishi 53, 2263-2267.

Hendrick, J.D., 2002. Adventitious toxins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish

Nutrition, 3rd. Academic Press, SanDiego, CA, USA, pp. 601-649.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout *Oncorhynchus mykiss*. Aquaculture 133, 257-274.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost replacement of fish meal by plant protein sources in the diet of a marine teleost, the European sea bass, *Dicentratchus labrax*. Aquaculture 230, 391-404.

Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 179, 3-11.

Kikuchi, K., Furuta, T., Ishizuka, H., Yanagawa, T., 2007. Growth of tiger puffer, Takifugu rubripes, at different salinities. J. World Aquacult. Soc. 38, 427-434.

Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 17, 3-11.

Lie, O., Lied, E., Lambersten, G., 1986. Liver retention of fat and fatty acids in Cod (*Gadus morhua*) fed different oils. Aquaculture 59, 187-196.

Lim, S.R., Choi, S.M., Wang, X.J., Kim, K.W., Shin, I.S., Min, T.S., Bai, S.C., 2004. Effects of dehulled soybean meal as a fish meal replacer in diets for fingerling and growing Korean rockfish *Sebastes schlegeli*, Aquaculture 231, 457-468.

Lovell, R.T., 1984. Use of soybean products in diets for aquaculture species. Animal Nutrition Bulletin. American Soybean Association, February 1984, pp. 1-6.

Lovell, R.T., 1991. Replacing fishmeal in channel catfish diets. In: Allan, G.L., Dall, W. (Eds.), The Proceeding of the Aquaculture Nutrition Workshop. Salamander Bay, NSW, Australia, 15-17 April 1991, pp. 118-121.

Lovell, R.T., 1991. Use of soybean products in diets for aquaculture species; revised. In: Proceedings of the Aquaculture Feed Processing and Nutrition Workshop D.M. Akiyama and R.K.H. Tan. American Soybean Association, Singapore. pp. 173-187.

Nahapetian, A., Bassirt, A., 1975. Changes in concentration and interrelationship of phytate, P, Mg, Cu, Zn in wheat during maturation. J. Agr. Food. Chem., 23, 1179-1182.

Nordrum, S., Asgard, T., Shearer, D.K., Arnessen, P., 1997. Availability of phosphorus in fish bone meal and inorganic salts to Atlantic salmon (Salmin salar) as determination retention. Aquaculture 157, 51-61.

NRC (National Research Council), 1993. Nutritional Requirements of fish. National Academy Press, Washington, DC, USA.

Olli, J., Berg-Lea, T., Krogdahl, A., 1989. Effects of soybean trypsin inhibitor activity on

nutrient digestibility in salmonids fed practical diets containing various soybean meals. Proc. Third Int. Symp. on Feeding and Nutrition in Fish. Toba, Japan, 28 Aug.-1 Sep., pp. 263-270.

Pham, M.A., Lee, K.J., Lim, S.J., Park, K.H., 2007. Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder Paralichthys olivaceus. The Japanese Society of fisheries science 73, 760-769.

Pongmaneerat, J., Watanabe, T., 1993. Effect of extrusion processing on the utilization of soybean meal diets for rainbow trout. Nippon Suisan Gakkaishi 59, 1407-1414.

Quartararo, N., Allan, G.L., Bell, J.D., 1991. Fishmeal substitution in a diet for Australian snapper, *Pagrus aurarus*. In: Allan, G.L., Dall, W. (Eds.), Proc. Aquaculture Nutririon Workshop, Salamander Bay, 15-17 April 1991. NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp. 125-126.

Regost, C. Arzel, J. Kaushik, S.J., 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (Psetta maxima). Aquaculture 180, 99-117.

Robinson, E.H., Li, M.H., 1994. Use of plant proteins in catfish feeds. Replacement of soybean meal with cottonseed meal and replacement of fishmeal with soybean meal and cottonseed meal. J. World Aquacult. Soc. 25, 271-276.

Shiau, S.Y., Lin, S.F., Yu, S.L., Lin, A.L., Kwok, C.C., 1990. Defatted and full-fat soybean emal as partial replacements for fishmeal in tilapia (*Oreochromis niloticus* × *O. aureus*) diets at low protein level. Aquaculture 86, 401-407.

Storebakken, T., Refstie, S., Ruyter, B., 2000. Soy products as fat and protein sources in fish diets for intensive aquaculture. In: Drackey, J.K.(Eds.), Soy in Animal Nutrition. Fed. Anim. Sci. Soc., Savoy, IL, 127-170.

Watanabe, T., Viyakarn, Kimura, H., 1992. Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. Nippon Suisan Gakkaishi 58, 1761-1773.

Wee, K.L., Shu, S.W., 1989. The nutrition value of boiled full-fat soybean meal in pelleted feed for Nile tilapia. Aquaculture 81, 303-314.

Wu, C.S., Tsay, Y.J., Liou, H.J., 1980. Studies on the content and hydrogenation condition of squalene from liver oil of deep sea shark. J. Fish. Soc. Taiwan 7, 43-55.

ACKNOWLEDGEMENT

석사과정동안 부족한 저를 이끌어 주신 이경준 교수님께 진심으로 감사드립니다. 그리고 바쁘신 와중에도 제 논문을 위해 신경을 써주신 이기완 교수님과 여인규 교수님께도 감사의 말씀을 드립니다.

실험실 생활을 하는 과정에서 선배로서 조언과 충고를 아끼지 않았던 장계환 선배님과 박영준 선배님, 그리고 실험실 생활하는 점에 있어서 실질적으로 도움을 많이 주신 이봉주 선배님, 임세진 선배님과 김성삼 선배님께도 감사의 마음을 전합니다. 그리고 이제까지 같이 고생한 동기인 지웅이와 저를 이해해주고 배려해 준 대한이와 진우한테도 진심으로 감사의 마음을 전합니다.

저를 끝까지 믿어주시며 묵묵하게 바라보기만 하신 부모님과 동생이 있었기에 지금의 저의 모습이 있을 수 있다고 생각됩니다. 끝으로 같이 공부해왔고 같이 졸업하는 사랑하는 진이에게도 감사의 마음을 전합니다.