Dietary Effects of Fermented Soybean Meal on Growth Performance, Body Composition and Hematological Characteristics of Silver Perch (*Bidyanus bidyanus*)

Shuenn-Der Yang^{1, 2*}, Tain-Sheng Lin¹, Fu-Guang Liu¹ and Chyng-Hwa Liou²

¹Chupei Station, Freshwater Aquaculture Research Center, Fisheries Research Institute ² Department of Asusculture Metional Teiruan Ocean University Department of Aquaculture, National Taiwan Ocean University

ABSTRACT

An eight-week feeding experiment was conducted in flow-through concrete tanks to evaluate the dietary effects of fermented soybean meal (FSB), at graded levels $(0, 12, 24, 36, 48, 48, 60\%)$, on growth performance, body composition, and hematological characteristics of silver perch with an initial average weight of 48.3 g. The supplementary efficacy of crystalline methionine and lysine added into the 60% FSB diet was also investigated. The results showed that weight gain, feed efficiency and the protein efficiency ratio declined in groups of fish fed higher levels of dietary FSB. The methionine and lysine supplementation significantly improved fish growth performance. While moisture, crude protein and lipid concentrations of the muscle fillet and moisture, crude protein and ash concentrations of the whole fish body were not different ($p > 0.05$) in fish fed diets with different FSB levels, lipid concentration of the whole body in fish was low as high FSB levels given. Lower intraperitoneal fat ratio and viscerosomatic index were also found in the fish fed higher levels of dietary FSB. Hematocrit value, blood hemoglobin, and plasma triacylglycerols and cholesterol were significantly affected by the FSB and crystalline amino acids supplementation. The results of growth trial disclosed that including FSB up to 24% in diet did not have an adverse effect on growth of silver perch. Also, results in body proximate composition, biological measurements and hematological characteristics indicated that the level of dietary FSB affected the lipid metabolism and deposition in the silver perch.

Key words: silver perch, *Bidyanus bidyanus*, fish meal, fermented soybean meal, protein replacement

INTRODUCTION

Fish meal, with a balanced amino acid profile and good digestibility, has traditionally been considered as an important and commonly used protein source in aquaculture. As the industry is dramatically developing throughout the world, the demands for fish meal for aquafeed are increasing.

However, global supplies of fish meal from

conventional sources have peaked and show no signs of increasing (New and Wijkstrom, 2002). Therefore, the limitation of using fish meal in aquaculture associated with price, supply and quality fluctuations has obviously occurred in recent years. In order to reduce the limitation, using alternative protein sources to partially or even entirely substitute fish meal has become an important research field in aquatic animal nutrition (Hasan, 2001). The alternative protein sources in aquafeed typically include fishery and terrestrial animal by-product meals, oilseed proteins and concentrates, aquatic plants, single-cell proteins, legumes and cereal

^{*}Correspondence: 111 Tai-Ho, Chupei, Hsinchu 302, Taiwan. TEL: +886-3-5551190; FAX: +886-3-5554591; E-mail: yangsder@ms45.hinet.net

by-products (Hardy, 1996; El-Sayed, 1999).

Plant protein sources are widely used in feeds, due to global availability and less cost compared to ingredients of animal origin. Among these plant feedstuffs, soybean meal is one of the most utilized in fish diets, because of its high protein content and relatively high digestibility (Tacon, 1994). Nevertheless, like other plant-derived proteins, soybean contains a variety of antinutritional substances that has as negative impact on the growth of cultured fish (Francis *et al.*, 2001). Proper processing methodology, balanced formulation with additives have been reported to improve the nutritive value of soybean meal to certain extent for aquatic animals (Alexis and Nengas, 2001; Dersjant-Li, 2002).

Fermentation has been suggested as one of the processing method to improve the nutritional value of soybean meal for fish feeds. Fermented soybean meal (FSB) has higher protein content and lower antinutrients than the unfermented kind. In addition, phytates and oligosaccharides are eliminated during fermentation and the molecular weight of most proteins in the fermented products decreased (Alexis and Nengas, 2001; Refstie *et al.*, 2005). Chen *et al.* (1988) suggested that a high percentage of FSB might be used to substitute the costly fish meal in Japanese eel (*Anguilla japonica*) feed. The growth of soft-shelled turtle (*Trionyx sinensis*) fed a diet with suitable level of FSB was similar to that of the turtles fed a control fish meal diet (Huang and Lin, 1999). In a yellowtail (*Seriola quinqueradiata*) trial, Shimeno *et al.* (1993) found that the digestibility of FSB was improved and the growth rate of fish fed diets containing FSB was superior to that of fish fed unfermented soybean meal diet.

In Taiwan, silver perch are commonly fed commercial sea bass feeds which contain higher animal protein levels than the diets formulated for omnivorous species. Although studies have been conducted to evaluate the effects of replacing fish meal with plant protein sources on silver perch aquaculture (Allan and Booth, 2004; Rowland *et al.,* 2007), the effects of FSB on the fish have yet been studied. Therefore, the current study was undertaken to investigate the effects of partial substitution of fish meal with FSB in diets on growth performance, body composition and certain hematological characteristics of silver perch. Moreover, the efficacy of lysine and methionine supplement to the diet containing the highest inclusion level of FSB was also examined.

MATERIALS AND METHODS

1. Experimental diets

Compositions of the seven isonitrogenous and isocaloric diets are shown in Table 1. Diets from FSB0 to FSB60 were formulated to give 12% increments of FSB from 0 to 60%, whereas FSB60A was diet FSB60 supplemented with crystalline L-lysine and L-methionine so that the diet had similar quantity of these two essential amino acids as FSB0. The FSB was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The product was fermented from defatted soybean meal by *Aspergillus* spp. and *Lactobacillus* spp. using the solid fermentation method.

The ground ingredients were thoroughly blended in a food mixer and then homogenized after fish oil and soybean oil were added. Distilled water was added to achieve dough; and the mixture was further homogenized and extruded through a food processor with 4 mm die and a cutter. The pellets were air-dried at 20 °C and then stored at -20 °C for use. The proximate composition and essential amino acid profile of experimental diets are shown in Table 2.

2. Experimental design

Fingerlings of silver perch were obtained from Chupei Station of Fisheries Research Institute. The fish were first conditioned with FSB60 diet for 2 weeks. Then, the fish were randomly grouped into 21 tanks at a density of 60 fish per tank. Every three tanks were randomly assigned to each treatment as replication. Those outdoor concrete tanks (2.4×1.4) \times 0.7 m) were provided with ground water at a flow

				Diet			
	FSB ₀	FSB12	FSB ₂₄	FSB36	FSB48	FSB60	FSB60A
Fish meal ¹	43.44	35.79	28.14	20.49	12.84	5.19	5.19
Fermented Soybean meal ²		12.00	24.00	36.00	48.00	60.00	60.00
Squid visceral meal ³	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Wheat gluten	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil	3.37	3.87	4.37	4.87	5.37	5.87	5.87
Soybean oil	3.37	3.05	2.74	2.42	2.10	1.79	1.79
Gelatinized cassava starch	29.08	25.13	21.18	17.23	13.29	9.34	9.34
Lysine ⁴	٠					$\overline{}$	0.40
Methionine ⁴	$\overline{}$					÷	0.38
Glutamic acid ⁴	1.18	1.18	1.18	1.18	1.18	1.18	÷
Vitamin premix ⁵	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Mineral premix ⁵	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	1.50	1.50	1.50	1.50	1.50	1.50	1.50
α -Cellulose	3.06	2.48	1.89	1.30	0.72	0.13	0.53

Table 1 Formulation of the experimental diets (as g/ 100g)

¹ Chile super prime meal contains (as % of dry matter): crude protein, 77.96; lipid, 6.55.

² Fermented soybean meal contains (as % of dry matter): crude protein, 49.73; lipid, 2.64.

3 Korean squid offal meal contains (as % of dry matter): crude protein, 49.41; lipid, 12.60.

4 Ajinomoto Co., Inc., Japan.

5 Same as Moon and Gatlin (1991).

rate of about 220 l/hr with sufficient aeration. The ambient temperature ranged from 23.4 to 26.3 °C. Dissolved oxygen and pH of tank water were monitored weekly and the means were 5.52 mg/l and 7.44 respectively throughout the trial period. Fish in each tank were fed one of the seven experimental diets to apparent satiation twice daily. This trial was conducted for 8 weeks, and every two weeks fish in each tank were bulk-weighed.

3. Sample collection and analysis

At the end of the experimental period, all groups of fish were fed their respective feeding trial diets for a further week. After 24 hrs fasting, six fishes from each tank were randomly selected and anesthetized with MS-222. They were individually weighed, total length measured and blood sample withdrawn from caudal vein with heparinized syringes. The hematocrit (Hct) value was measured using standard heparinized hematocrit tubes. Blood hemoglobin (Hb) was determined spectrophotometrically using a commercial diagnostic kit (Randox Laboratories Ltd., UK). The whole blood was centrifuged at $3000 \times g$ to separate the plasma and frozen at -80 °C until measurement. Plasma samples were analyzed using a clinical analyzer (1011 Metertech Inc., Taiwan) with diagnostic kits (Spinreact Co., Spain) for triacylglycerols (TAG) and total cholesterol (CHL) concentrations.

The liver, intraperitoneal fat and viscera of each fish were weighed after blood withdrawal. The filleted muscle and dissected animals from each tank were stored at -80°C for subsequent measurement of proximate composition. Crude protein of fish muscle,

	FSB ₀	FSB12	FSB ₂₄	FSB36	FSB48	FSB60	FSB60A	
Proximate (% dry matter)								
Crude protein	39.41	39.72	39.78	40.23	40.99	39.77	40.50	
Lipid	9.37	9.11	9.25	9.38	9.89	9.39	9.75	
Ash	11.00	10.58	10.12	9.65	9.26	8.79	8.77	
Gross energy(MJ/kg)	20.10	20.12	20.84	20.86	20.97	20.65	20.94	
Essential amino acid contents (g/ 100g diet)								
Histidine	0.79	0.81	0.83	0.90	0.92	0.92	0.93	
Arginine	2.57	2.36	2.57	2.68	2.59	2.59	2.61	
Threonine	1.64	1.57	1.55	1.54	1.51	1.55	1.55	
Valine	1.86	1.87	1.76	1.79	1.82	1.81	1.77	
Methionine	1.13	1.03	0.95	0.86	0.77	0.66	1.10	
Lysine	2.22	2.13	2.05	1.97	1.90	1.73	2.18	
Isoleucine	1.60	1.64	1.61	1.65	1.71	1.73	1.69	
Leucine	2.94	3.02	3.01	3.00	3.07	3.04	3.07	
Phenylalanine	1.56	1.56	1.61	1.72	1.78	2.07	1.93	

Table 2 Proximate and essential amino acid compositions of experimental diets

whole fish and experimental diets were determined in a Kjeltec semiautoanalyzer model 2100 (Tecator, Sweden) with semi-microkiedahl method. Lipids were extracted by chloroform-methanol method (Folch *et al.*, 1957). Moisture and ash were measured by the AOAC methods (1998). Gross energy of experimental diets was determined by calorimetry (Parr Calorimeter 6200).

Experimental diets were hydrolyzed in 6N HCl for 24 h at 110°C for determination of amino acid contents. Acid hydrolysis for amino acids and performic acid oxidation for methionine and cystin followed AOAC methods (1998). The hydrolysates were filtered and diluted, then were analyzed by reversed-phase high-pressure liquid chromatography by AQC method (Cohen, 2000), where norleucine served as an internal standard.

4. Calculations

Percent weight gain (PWG) was used as an indicator for growth performance. Feed efficiency (FE) and protein efficiency ratio (PER) were used as indicators for feed utilization. These parameters were calculated as the following: PWG $(\%)$ = (final weight – initial weight) / initial weight \times 100; FE (%) $=$ (final weight – initial weight) / dry feed intake \times 100; PER $(g/g) = (final weight - initial weight)$ protein intake.

The relative weights of liver, intraperitoneal fat and viscera are expressed in terms of the hepatosomatic index (HSI), intraperitoneal fat (IPF) and viscerosomatic index (VSI) that are calculated as follows: HSI (%) = (liver weight/fish wet weight) \times 100; IPF $(\frac{9}{6})$ = (intraperitoneal fat weight / fish wet weight) \times 100; VSI (%) = (viscera weight / fish wet weight) \times 100. On the other hand, the ratio of length to weight in fish is expressed as the condition factor (CF), where CF=wet body weight / (total body length)³ \times 100.

5. Statistical methods

All data were analyzed by one-way analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS version 8.02). The means were then compared using Duncan's new multiple range tests to resolve differences among

	FSB ₀	FSB ₁₂	FSB ₂₄	FSB ₃₆	FSB48	FSB60	FSB60A	Pooled se
$W_i(g)$	48.24	48.51	48.32	48.27	48.18	48.47	48.52	0.93
$W_f(g)$	104.84^{cd}	105.93^d	103.84^{cd}	96.00^{bc}	90.51^{ab}	85.64^{a}	98.09bcd	2.97
PWG (%)	117.18 ^d	118.09 ^d	115.08^{cd}	99.05^{bc}	87.92^{ab}	76.88 ^a	102.16^{bcd}	5.14
FE (%)	60.64^c	60.30 ^c	59.55^{bc}	56.74^{bc}	52.83^{ab}	49.36 ^a	57.13^{bc}	2.09
PER (q/q)	1.54^c	1.52 ^c	1.49 ^c	1.41^{bc}	1.29^{ab}	1.24 ^a	1.41^{bc}	0.05
Survival (%)	95.56	96.67	92.78	95.00	93.33	93.89	93.33	-

Table 3 Growth performance and feed utilization of silver perch fed the experimental diets for 8 weeks¹

¹ Means with different superscripts in the same row are significantly different ($p < 0.05$).

 W_i : initial fish weight; W_f : final fish weight.

treatment means. Statistical significance was examined at $p < 0.05$.

RESULTS

The growth performance, feed utilization and survival of silver perch fed different diets are shown in Table 3. Higher dietary FSB inclusion levels $($ 36%) resulted in decreasd final weight and percent weight gain of the fish. Significant reductions in growth were found in the fish fed diets containing FSB levels higher than 36% (FSB36). Feed efficiency showed the similar trend as percent weight gain with the 60% FSB inclusion diet resulted the poorest feed efficiency. Protein utilization expressed as PER, was significantly affected by the dietary treatment (Table 3). Low PER value was observed for silver perch fed diet FSB60, while no significant differences in PER were found within fish fed diets containing FSB levels less than 36%. Lysine and methionine supplementation in FSB60A diet obtained better growth performance than FSB60 diet without the amino acid supplementation. A similar tendency was also observed in FE and PER.

The proximate composition of whole body and muscle of the fish are shown in Table 4. The whole body moisture, protein and ash contents in the fish were not different among all treatments. Lipid concentration of the whole body in fish was low as high FSB levels given, while lysine and methionine supplementation significantly increased body lipid

(diets FSB60, FSB60A). Almost no significant differences were found among the treatments for the muscle proximate composition, except that the ash content of FSB24 group was significantly higher than those of groups fed diets FSB48, FSB60 and FSB60A.

The morphological measurements and hematological characteristics of fish are shown in Table 5. Both CF and HSI were not affected by dietary treatments, while VSI and IPF were generally lower in the fish fed higher FSB inclusion diets. Regarding the serological parameters, the Hct value and blood Hb concentration were significantly lower in fish fed diets containing higher levels of FSB. In plasma constituents, TAG and CHL were significantly affected by dietary treatments. In general, both levels decreased with increasing levels of FSB; however, the levels in fish fed FSB60 were significantly higher than those of fish fed other diets.

DISCUSSION

Our results demonstrated that FSB could be used as an alternative protein source for partial substitution of fish meal protein up to 24% in diet without an adverse effect on growth of silver perch (Table. 3). Our findings were in line with Luo *et al.* (2004) in which grouper feed with FSB added diets did not affect the nutritive utilization for grouper. It has also been suggested that FSB could be added to replace fish meal partially in diets for Japanese eel, yellowtail, soft-shelled turtle, Atlantic salmon (*Salmo salar*) and channel catfish (*Ictalurus punctatus*) without adverse effects on growth performance (Chen *et al.*, 1988; Shimeno *et al.*, 1993; Huang and Lin, 1999; Refstie *et al.*, 2005; Li *et al.*, 2007). According to the aforementioned reports, FSB level ranging from 10% to 43% can be included in fish diets without compromising growth.

Plant protein generally contains low levels of one or more essential amino acids, and can not meet the fish requirement (Ambardekar and Reigh, 2007). Lacking suboptimal essential amino acid balance in diets containing plant protein is one of the important limiting factors and results in poor growth and feed utilization in fish. Therefore, adding proper amounts of essential amino acids, especially methionine and/or lysine, to the soybean products containing diets could significantly improve the growth of fish (Takagi et al., 2001; Pham et al., 2007). In this study, the effect of FSB60A on the growth performance and PER of the fish were significantly higher than those from FSB60 (Table 3). Thus, methionine and lysine supplementation improved the growth performance and PER when fish meal protein was mostly replaced by FSB in the diet for silver perch. This is inconsistent with our previous study (Yang *et al.*, 2001), which reported that methionine or lysine supplementation is unnecessary when up to 30% fish meal protein was replaced by soybean meal or lupin meal in black carp (*Mylopharyngodon piceus*) diets. In yellowtail, Shimeno *et al*. (1992) also illustrated that adding lysine and methionine in the diet in which fish meal was partially replaced with soybean meal, did not improve the growth performance. Regarding this concern, Ambardekar and Reigh (2007) reviewed that the level and source of dietary plant protein influence the growth of fish and that the utilization of synthetic amino acids in these diets could vary from species to species.

Except for amino acid pattern, the palatability and pelletability of the diets might be considered as the possible causes for the poor performance in higher replacement levels of fish meal by FSB. Wm Kissil *et al.* (2000) suggested that palatability of plant proteins could be a limiting factor in their use.

Also, it was suggested that solvent-extracted, extruded full-fat or steamed full-fat soybean meals could be used as alternative protein sources in Asian seabass (*Lates calcarifer*) diets, if palatability of the diet could be improved (Boonyaratpalin *et al*., 1998). On the other hand, Shimeno *et al.* (1994) found that inclusion of FSB decreased the pelletability of the diet and caused the pellets to crumble. The diets containing higher FSB levels in our study were more easily dispersed in water than the lower FSB inclusion diets, which increased the time span needed to properly feed the fish and minimize the feed loss. Further study on improving the pellet stability at higher FSB inclusion levels is needed.

In the present study, lower body lipid content was observed in the fish fed higher FSB included diets, while muscle lipid content was not significantly different among treatments (Table 4). Furthermore, the FSB60-fish had the lowest values of IPF ratio and VSI than other fish fed less levels of dietary FSB (Table 5). In fish, lipid is stored mainly in perivisceral adipose tissue, liver and muscle or even subcutaneous tissue, with specific difference in the localization of lipid deposits (Corraze, 2001). Our observations indicate that perivisceral adipose tissue is the primary lipid storage site in silver perch due to the content of whole body lipid coinciding generally with IPF ratio but not with the muscle lipid content. Additionally, several studies have similarly found that body lipid content decreased in fish fed diets with high inclusion level of plant protein sources (Noble *et al.*, 1998; Wm Kissil *et al.*, 2000; Opstvedt *et al.*, 2003; Tibaldi *et al.*, 2006). In contrast, the body lipid content of fish was reported to be increased or not affected by diets containing various levels of plant protein sources (Yang *et al.*, 2001; Kaushik *et al.*, 2004; Dias *et al.*, 2005; Zhou *et al.*, 2005). It is difficult to give a simple but comprehensive explanation for the effect of dietary plant protein on lipid deposition of fish. Differences among these studies in species and fish size (Corraze, 2001), levels of dietary intake (Wm Kissil *et al.*, 2000; Gómez-Requeni *et al.*, 2004), plant protein sources selected (Dias *et al.*, 2005), dietary available energy (Opstvedt *et al.*, 2003), amino acid pattern

	FSB ₀	FSB ₁₂	FSB ₂₄	FSB ₃₆	FSB48	FSB60	FSB60A	Pooled se
Whole body								
Moisture	59.92	59.20	59.79	60.07	60.23	60.82	59.80	0.50
Protein	16.05	15.93	16.00	16.38	16.32	15.92	16.05	0.30
Lipid	19.36 abc	20.15 ^c	19.93c	18.44ab	18.60^{ab}	18.17 ^a	19.65^{bc}	0.39
Ash	4.54	4.61	4.74	4.86	4.73	5.01	4.59	0.16
Muscle								
Moisture	71.83	71.60	71.10	72.02	70.96	71.18	70.93	0.48
Protein	20.24	20.45	20.76	20.57	20.28	20.02	20.08	0.26
Lipid	6.01	6.35	6.19	5.61	6.61	6.44	6.50	0.43
Ash	1.24^{ab}	1.28^{ab}	1.37 ^b	1.26^{ab}	1.14 ^a	1.17 ^a	1.15 ^a	0.05

Table 4 Whole body and muscle proximate composition (% in wet basis) of silver perch fed the experimental diets for 8 weeks 1

¹ Means with different superscripts in the same row are significantly different ($p < 0.05$).

Table 5 Biological measurements and hematological characteristics of silver perch fed the experimental diets for 8 weeks¹

	FSB ₀	FSB ₁₂	FSB ₂₄	FSB ₃₆	FSB48	FSB60	FSB60A	Pooled se
CF	1.23	1.27	1.20	1.22	1.18	1.17	1.16	0.03
VSI (%)	15.07^{ab}	15.58^{b}	14.60^{ab}	14.77^{ab}	14.27^{ab}	13.78^{a}	14.31^{ab}	0.40
HSI (%)	1.41	1.45	1.26	1.46	1.27	1.42	1.51	0.15
IPF $(%)$	6.81^{ab}	7.82 ^c	7.72 ^c	7.09^{bc}	7.41^{bc}	5.93 ^a	6.71^{ab}	0.29
Hct (%)	48.40°	47.05^{bc}	46.84^{b}	46.89^{b}	46.22^{ab}	45.39^{a}	47.28^{bc}	0.43
Hb (g/dl)	9.25^{d}	9.06 ^{cd}	8.77 ^{bcd}	8.42^{bc}	8.24^{ab}	7.49 ^a	8.23^{ab}	0.25
TAG (mg/dl)	599.84 ^{bc}	571.84abc	529.28 ^{ab}	440.46°	464.45^{ab}	871.14 ^d	675.28^{c}	41.43

¹ Means with different superscripts in the same row are significantly different ($p < 0.05$).

(Kaushik *et al.*, 1995) and specific botanical component (Dias *et al.*, 2005) as well as rearing conditions and the duration of feeding experiments (Noble *et al.*, 1998) may in part account for the discrepancies in lipid deposition.

The use of FSB as major feed protein sources led to hematological variations. The hematological characteristics from our experiment revealed that values of Hct and Hb were significantly decreased in silver perch when fed diets containing higher FSB

inclusion levels (Table 5). Similar results were found in red sea bream (*Pagrus major*) and cobia (*Rachycentron canadum*) when soy protein concentrate and defatted soybean meal were used as dietary alternative protein for fish meal, respectively (Takagi *et al.*, 2001; Zhou *et al.*, 2005). Imbalanced amino acid pattern in diet containing high level of soy products is one of the most important factors in the reductions of Hct and Hb levels. Takagi et al. (2001) speculated that an amino acid balance poor diet containing soy protein concentrate as the main protein source induced a decline in the iron transport and iron supply to reticulocytes, due to a decrease of plasma protein content accompanied by a decrease of the plasma transferrin content. In the current study, Hct and Hb levels were higher in the FSB60A-group than the group fed FSB60 diet that had a lower level of lysine and methionine. The results indicated that essential amino acid supplementation increased levels of Hct and Hb in silver perch fed diet containing higher FSB inclusion level.

Values of plasma TAG and CHL observed in this study were significantly affected by levels of dietary FSB (Table 5). It has reported that values of TAG and CHL are significantly reduced in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) fed plant protein, soy products especially rich diets when compared those fish fed the fish meal based diet (Gómez-Requeni *et al.*, 2004; Kaushik *et al.*, 2004; Dias *et al.*, 2005). In an earlier report, a negative linear relation between dietary soy protein inclusion level and plasma CHL was also found in rainbow trout (Kaushik *et al.*, 1995). In general, data from the present study showed a similar tendency on cholesterol-lowering effect of FSB. Values of plasma TAG and CHL decreased in silver perch fed with increasing dietary FSB levels, but the fish fed FSB60 diet led to the highest of both values and differed remarkably from those of other fish-fed diets. Interestingly, FSB60A diet containing higher levels of lysine and methionine than FSB60 diet significantly reduced values of TAG and CHL of fish plasma.

From the present study it can be concluded that up to 24% of fish meal can be replaced by FSB without causing significant reduction in growth of silver perch. The overall results of body proximate composition, biological measurements and hematological characteristics show that the level of dietary FSB affects the lipid metabolism and deposition of fish. Meanwhile, the supplementation of methionine and lysine to balance the amino acid pattern is benefitial if FSB is used to provide large amounts of dietary protein. Several reports have suggested that fish grow better with a combination of

plant proteins as compared to single plant protein or even fish meal alone (Kaushik *et al.*, 2004; Wm Kissil and Lupatsch, 2004). The combination is considered basically to compensate for the essential amino acid pattern and improve the energy digestibility of plant protein diets (Wm Kissil and Lupatsch, 2004). Further investigation is needed to explore the possibility of using FSB and other plant proteins combinations as alternative sources for fish meal in silver perch feeds.

ACKNOWLEDGEMENTS

The authors wish to extend their sincerest appreciation to Dr. C. C. Chen of the Food Industry Research and Development Institute for providing the fermented soybean meal and all the relevant information. We thank the staffs of the Chupei Station, Freshwater Aquaculture Research Center of Fisheries Research Institute for their help in many ways. This experiment was supported by a grant from the Council of Agriculture of the Republic of China, grant number 92AS-9.2.1-AI-A1.

REFERENCES

- Alexis, M. N. and I. Nengas (2001) Current state of knowledge concerning the use of soy products in diets for feeding sea bass and sea bream. Needs for future research. American Soybean Association, Athens, Greece, 32 pp.
- Allan, G. L. and M. A. Booth (2004) Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch *Bidyanus bidyanus* (Mitchell) diets. Aquacult. Res., 35: 981-991.
- Ambardekar, A. A. and R. C. Reigh (2007) Sources and utilization of amino acids in channel catfish diets: a review. North Am. J. Aquacult., 69: 174-179.
- Association of Official Analytical Chemists (AOAC) (1998) Official Methods of Analysis of the AOAC International, 16th edition, Washington, D.C.
- Boonyaratpalin, M., P. Suraneiranatand T. Tunpibal (1998) Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. Aquaculture, 161: 67-78.
- Chen, J. Y., H. Y. Tsen, H. G. Tang and S. C. Liou (1988) Effect of partial substitute of fish meal with fermented soybean meal in feed on the growth and the flesh composition of eel. J. Chinese Agric. Chem. Soc., 26: 179-188. (in Chinese with English abstract)
- Cohen, S. A. (2000) Amino acid analysis using precolumn derivatization with 6 aminoquinolyl-N-hydroxysuccinimidyl carbamate. Methods Mol. Biol., 159: 39-47.
- Corraze, G. (2001) Lipid nutrition. *In* Nutrition and Feeding of Fish and Crustaceans (J. Guillaume, S.J. Kaushik, P. Bergot and R. Métailler eds.), Praxis Publishing, Chichester, UK, 111-130.
- Dersjant-Li, Y. (2002) The use of soy protein in aquafeeds. *In* Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola (L. E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M. G. Gaxiola-Cortés and N. Simoes eds.), 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México, 541—558.
- Dias, J., M. J. Alvarez, J. Arzel, G. Corraze, A. Diez, J. M. Bautista and S. J. Kaushik (2005) Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol., 142(A): 19-31.
- El-Sayed, A. F. M. (1999) Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture, 179: 149-168.
- Folch, J., M. Lees and G. H. S. Stanely (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
- Francis, G., H. P. S. Makkar and K. Becker (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197-227.
- Gómez-Requeni, P., M. Mingarro, J. A. Calduch-Giner, F. Médale, S .A. M. Martin, D. F. Houlihan, S. Kaushik and J. Pérez-Sánchez (2004) Protein growth performance, amino acid utilization and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture, 232: 493-510.
- Hardy, R. W. (1996) Alternate protein sources for salmon and trout diets. Anim. Feed Sci. Tech., 59: 71-80.
- Hasan, M. R., (2001) Nutrition and feeding for sustainable aquaculture development in the third

millennium. *In* Aquaculture in the Third Millennium, Technical Proceedings of the Conference on Aquaculture in the Third Millennium (R. P. Subasinghe, P. Bueno, M. J. Phillips, C. Hough, S. E. McGladdery and J. R. Arthur eds.), Bangkok, Thailand, 20-25 February 2000, NACA, Bangkok and FAO, Rome, 193-219.

- Huang, C. H. and W. Y. Lin (1999) Effects of substituting fermented soybean meal for fish meal in diets on growth and body composition of soft-shelled turtle, *Triony sinensis*. J. Fish. Soc. Taiwan, 26: 225-232.
- Kaushik, S. J., J. P. Cravedi, J. P. Lalles, J. Sumpter, B. Fauconneau and M. Laroche (1995) Partial or total replacement of fishmeal by soybean protein on growth, protein utilisation, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout. Aquaculture, 133: 257-274.
- Kaushik, S. J., D. Covès, G. Dutto and D. Blanc (2004) Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture, 230: 391-404.
- Li, H., F. Huang, B. Hu, Y. P. Zhou and L. Zhang(2007) Effects of replacement of fish meal with fermented soybean in the diet for channel catfish (*Ictalurus punctatus*) on growth performance and apparent digestibility of feed. Freshwater Fish., 37(5): 41-44. (in Chinese with English abstract)
- Luo, Z., Y. J. Liu, K. S. Mai, L. X. Tian, D. H. Liu and X. Y. Tan (2004) Partial replacement of fish meal by soybean protein in diets for grouper *Epinephelus coioides* juveniles. J. Fish. China, 28: 175-181.
- Moon, H. Y. and D. M. Gatlin III (1991) Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture, 95: 97-106.
- New, M. B. and U. N. Wijkstrom (2002) Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap. *In* FAO Fisheries Circular No. 975. Food and Agriculture Organization of the United Nations, Rome, Italy, 61 pp.
- Noble, E., A. Demaёl, D. Garin, C. Moulin and H. Barré (1998) Effects of an hypoproteic soybean based diet on the energy stores and growth of carp (*Cyprinus carpio* L.). Comp. Biochem. Physiol., 120(A): 157-161.
- Opstvedt, J., A. Aksnes, B. Hope and I. H. Pike (2003) Efficiency of feed utilization in Atlantic salmon (*Salmo salar* L.) fed diets with increasing substitution of fish meal with vegetable proteins. Aquaculture, 221: 365-379.
- Pham, M. A., K. J. Lee, S. J. Lim and K. H. Park (2007) Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder *Paralichthys olivaceus*. Fish. Sci., 73: 760-769.
- Refstiea, S., S. Sahlström, E. Bråthen, G. Baeverfjord and P. Krogedald (2005) Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture, 246: 331-345.
- Rowland, S. J., G. L. Allan, C. Mifsud, P. A. Read, D. Glendenning and B. A. Ingram (2007) Effects of diets with different plant proteins on the performance of silver perch (*Bidyanus bidyanus* Mitchell) and on water quality in earthen ponds. Aquacult. Res., 38: 748-756.
- Shimeno, S., H. Hosokawa, H. Morie, M. Takeda and M. Ukawa (1992) Inclusion of defatted soybean meal to diet for young yellowtail. Suisanzoshoku, 40: 51-56. (in Japanese with English abstract)
- Shimeno, S., T. Mina and O. Yamamoto (1993) Effects of fermented defatted soybean meal in diet on the growth, feed conversion, and body composition of juvenile yellowtail. Nippon Suisan Gakkaishi, 59: 1883-1888. (in Japanese with English abstract)
- Shimeno, S., A. Hashimoto, Y. Ando and K. Hayakawa (1994) Improvement of nutritive value of defatted soybean meal through purification and fermentation for fingerling yellowtail. Suisanzoshoku, 42: 247-252. (in Japanese with English abstract)
- Tacon, A. G. J. (1994) Feed ingredients for carnivorous fish species: alternatives to fish meal and other fishery resources. *In* FAO Fisheries Circular No.

881, Food and Agriculture Organization of the United Nations, Rome, Italy, 35 pp.

- Takagi, S., S. Shimeno, H. Hosokwa and M. Ukawa (2001) Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. Fish. Sci., 67: 1088-1096.
- Tibaldi, E., Y. Hakim, Z. Uni, F. Tulli, M. de Francesco, U. Luzzana and S. Harpaz (2006) Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). Aquaculture, 261: 182-193.
- Wm Kissil, G., I. Lupatsch, D. A. Higgs and R. W. Hardy (2000) Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquacult. Res., 31: 595-601.
- Wm Kissil, G. and I. Lupatch (2004) Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream, *Sparus aurata* L. Israeli J. Aquac.- Bamidgeh, 56: 188-199.
- Yang, S. D., T. S. Lin, C. H. Liou, Y. H. Chien, H. K. Peng and I C. Liao (2001) Partial substitution of white fish meal with soybean meal or lupin meal in diets for fingerling black carp (*Mylopharyngodon piceus*). J. Fish. Soc. Taiwan, 28: 317-328.
- Zhou, Q. C., K. S. Mai, B. P. Tan and Y. J. Liu (2005) Partial replacement of fishmeal by soybean meal in diets for juvenile cobia *Rachycentron canadum*. Aquacult. Nutr., 11: 175-182.

 24%

(Bidyanus bidyanus)