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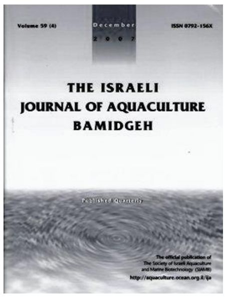
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_66.2014.1029, 8 pages



Effects of Dietary Squid Soluble Fractions on Growth Performance and Feed Utilization in Juvenile Snakehead (*Ophiocephalus argus*) Fed Practical Diets

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(Received 10.10.13, Accepted 26.12.13)

Keywords: snakehead; squid soluble fraction; practical diets; growth

Abstract

An 8-week growth trial was conducted to investigate the effects of dietary squid soluble fractions (SSF) supplementation on growth performance and feed utilization of juvenile snakehead (*Ophiocephalus argus*). A fish meal diet (diet 1) with 36% fish meal (FM) was formulated. This diet was used as a control. The second diet (diet 2) was formulated by replacing 22% of the fish meal protein with poultry by-product meal based on amino acid profile of whole body of this fish. The other three diets were supplemented with 0.4% (diet 3), 0.8% (diet 4) and 1.2% (diet 5) SSF, respectively. Diets 1(control) 2, 3, 4, and 5 were isonitrogenous and isolipidic. No significant differences were observed in survival, feed intake (FI), weight gain rate (WGR), feed efficiency rate (FER) and protein productive value (PPV) between fish fed diets 1 and 2 (P>0.05). Furthermore, compared with fish fed the diet without SSF (diet 2), fish fed diets with 0.8 and 1.2% SSF (diet 4 and 5) demonstrated significantly enhanced WGR, FER, and PPV. These results suggest that 0.8% of dietary SSF is optimal for juvenile snakehead growth enhancement.

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Introduction

Widely farmed snakehead (*Ophiocephalus argus*), is an important commercial carnivorous fish in China and south-east Asian countries (Yu et al., 2012). It is valued for its flesh texture and taste, rapid growth, and extreme tolerance to inferior water quality. Current diets used in snakehead farming are based on high-quality fish meal (FM) as the main protein source. High demands and limited supply have led to high costs for FM. Replacing some FM with animal or plant protein ingredients has become a focal issue (Gatlin et al., 2007; Tacon and Metian, 2008; Gümüş et al., 2013). Terrestrial animal by-products, such as poultry by-product meal (PBM), and meat and bone meal (MBM) are considered to be to be potentially alternative protein sources in aquatic animal feeds due to their high protein content, low carbohydrate content, optimal amino acid profiles, and lack of anti-nutritional factors (Tacon, 1993; Aydin and Gümüş, 2013). These products have also been investigated for a wide range of carnivorous fish species, such as rainbow trout (Bureau et al., 2000), hybrid striped bass (Gaylord and Rawles, 2005), cuneate drum (Wang et al., 2006), Malabar grouper (Wang et al., 2008) and Siberian sturgeon (Zhu et al., 2011; Xue et al., 2012).

Squid soluble fraction is one of the water soluble fractions with low molecular weight proteins, peptides, free amino acids, and nitrogenous compounds (Li et al., 2009; Kousoulaki et al., 2012). Dietary addition of water soluble fractions significantly improved weight gain rate (WGR) in Japanese seabass (Liang et al., 2006), rainbow trout (Aksnes et al., 2006), and Atlantic salmon (Kousoulaki et al., 2009). The small peptides of fish protein hydrolysate, with <100 Da (29.6%) and 1,000-100 Da (18.4%) induced a significant increase in feed consumption in Atlantic salmon and increased fish growth ($P<0.05$) (Kousoulaki et al., 2a012).

There have been no reports regarding the dietary effect of SSF on growth performance and feed utilization of juvenile snakehead. The objective of the present study is to investigate the effects of dietary SSF supplementation on growth performance and feed utilization of juvenile snakehead fed a practical diet.

Materials and Methods

Feed Ingredients and Diet Formulation. Squid soluble fractions (SSF) was obtained from commercial sources (Haid group Co., Ltd, Guangdong, China). FM, MBM, PBM, and soybean meal (SBM) were used as the primary protein sources (Table 1).

Table 1. Peptide molecular size distribution (% protein) of the squid soluble fractions

Peptide size (Da)	*Squid soluble protein*
>70,000	6.00
70,000-50,000	12.80
50,000-20,000	8.40
20,000-10,000	6.90
10,000-5,000	7.10
5,000-1,000	3.80
1,000-100	20.40
<100	34.60

Fish oil and soybean lecithin were used as lipid sources. Wheat flour was used as the carbohydrate source. Lysine-HCl and DL-methionine were supplemented to meet the essential amino acid (EAA) requirements of juvenile snakehead. These were based on the whole body amino acid profile (Table 2 and 3). A fish meal diet (diet 1) with 36% FM was formulated and used as the control. The second diet (diet 2) was formulated by replacing 22% of the fish meal protein with poultry by-product meal with an amino acid profile similar to that of the whole body of snakehead. The other three diets were supplemented with 0.4% (diet 3), 0.8% (diet 4) and 1.2% SSF (diet 5), respectively. They were isonitrogenous and isolipidic.

Table 2. Formulation & proximate composition of experimental diets (%)

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Fish meal[1]	36.0	28.0	27.6	27.2	26.8
Meat and bone meal[2]	4.0	4.0	4.0	4.0	4.0
Poultry by-product meal[3]		8.0	8.0	8.0	8.0
Squid soluble fractions			0.4	0.8	1.2
Corn gluten meal	4.0	4.0	4.0	4.0	4.0
Soybean meal	24.0	24.0	24.0	24.0	24.0
Wheat flour	22.0	22.0	22.0	22.0	22.0
Fish oil	4.5	4.3	4.3	4.2	4.2
Soybean lecithin	1.0	1.0	1.0	1.0	1.0
$Ca(H_2PO_3)_2$	2.0	2.0	2.0	2.0	2.0
NaCl	0.3	0.3	0.3	0.3	0.3
Vitamin premix[5]	0.85	0.85	0.85	0.85	0.85
Mineral premix[6]	0.5	0.5	0.5	0.5	0.5
Ethoxyquin (60%)	0.01	0.01	0.01	0.01	0.01
Lys-HCl		0.40	0.40	0.40	0.40
DL-Methionine		0.20	0.20	0.20	0.20
Rice bran	0.69	0.44	0.44	0.54	0.54
Analyzed nutrients compositions (dry matter basis)					
Dry matter	93.6	93.5	93.7	93.4	93.2
Crude protein	47.9	47.9	47.4	47.6	47.4
Crude lipid	11.5	11.7	11.7	11.7	11.7
Ash	11.6	11.3	11.2	11.2	11.1

(Diet no.: Diet 1–Diet 5)

[1] Fishmeal: steam dried fishmeal, (COPENCA Group. Lima, Peru, dry matter basis), with crude protein: 70.1%, crude lipid: 9.6%;
[2] Meat and bone meal composition (dry matter basis): crude protein, 70.3%; crude lipid, 13%;
[3] Poultry by-product meal composition (dry matter basis): crude protein, 70 %; crude lipid, 12%;
[4] Squid soluble fractions (dry matter %): crude protein, 72 %; crude lipid, 14%;
[5] Vitamin premix supplied the diet with (mg kg^{-1} diet) the following compounds: retinyl acetate, 32; vitamin D_3, 5; DL-α-tocopherol acetate, 240; vitamin K_3, 10; thiamin, 25; riboflavin (80%), 45; pyridoxine hydrochloride, 20; vitaminB_{12} (1%), 10; L-ascorbyl-2-monophosphate-Na (35%), 2000; calcium Pantothenate, 60; nicotinic acid, 200; inositol, 800; biotin (2%), 60; folic acid, 20; choline chloride (50%), 2500; cellulose, 2473.
[6] Mineral premix consisted of (mg kg^{-1} diet) the following ingredients: $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $CuSO_4 \cdot 5H_2O$, 10; $MnSO_4 \cdot H_2O$, 45; KI, 60; $CoCl_2 \cdot 6H_2O$ (1%), 50; Na_2SeO_3 (1%), 20; $MgSO_4 \cdot 7H_2O$, 1200; calcium propionate, 1000; zoelite, 2485.

Table 3 Analyzed amino acid composition of the experimental diets (% dry weight)

Essential amino acid	*Diet no.*					
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*47% whole body protein*
Thr	1.96	1.89	1.95	1.91	1.91	1.82
Val	2.09	2.09	2.07	2.02	2.03	1.82
Met	1.01	1.04	1.03	1.04	1.04	1.05
Ile	1.92	1.84	1.87	1.82	1.81	1.61
Leu	3.45	3.35	3.43	3.34	3.30	2.95
Phe	2.08	2.07	2.10	2.02	2.02	1.67
Lys	3.21	3.30	3.37	3.28	3.33	3.23
His	1.33	1.25	1.27	1.23	1.25	0.87
Arg	2.73	2.69	2.74	2.66	2.71	2.59

Ingredients were ground into fine powder and sieved through 246 μm mesh. All the ingredients were then thoroughly mixed with menhaden fish oil, and water was added to produce stiff dough. The dough was then pelleted using an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for about 12h in a ventilated oven at 45°C, and then kept frozen at -20°C.

Fish, Experimental Conditions and Samples Collection. Juvenile snakehead was obtained from the Haid fish farm (Panyu, Guangdong, China). Fish were acclimated to the system and fed diet 1 (control) for 2 weeks before the commencement of the trials. Juvenile snakehead (initial body weight: 22.3±0.1 g) were randomly distributed into 15 flat bottomed tanks, filled with 3200 l freshwater which was continuously pumped from a pond adjacent to the experiment station, and sieved through separate sand filters into each tank at approximately 1.5 l/min. Three replicate tanks were randomly assigned to each diet group and 120 fish were bulk weighed and stocked in each tank. During the 8 week feeding period, fish were fed the experimental diets to apparent satiation twice daily at 0700 and 1700 h respectively. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70°C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying, and reweighing.

Before the experiment, 10 fish from the same population were randomly selected for determination of initial whole-body proximate composition. At the end of the experiment, 6 fish weighing approximately the same as fish in the experimental groups were sampled and stored frozen at -20°C for whole body composition analysis. During the 8-week feeding period, water temperature ranged from 26 to 30°C, pH 7.5-8.0, ammonia nitrogen was lower than 0.1 mg/l, nitrite was lower than 0.1 mg/l, and dissolved oxygen was higher than 6.0 mg/l.

Chemical analyses. Fish samples, ingredients, and experimental diets were analyzed for dry matter, crude protein, crude lipid, and ash. (AOAC, 1995).

Dry matter was analyzed by drying the samples to constant weight at 105°C. Crude protein was determined using the Kjeldahl method and estimated by multiplying nitrogen by 6.25. Crude lipid was measured after diethyl ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550°C for 16 h. Duplicate analyses were conducted for each sample.

The SSF of the experimental diets was extracted with boiling water, the extract was then filtered through Black ribbon filter paper and the crude protein content in the water phase was determined using the Kjeldahl method. Size distribution of peptides was analyzed by HPLC size exclusion chromatography using a TSK G2000 column and detection at 220 nm according to Aksnes and Asbjørnsen (2003).

Calculations and Statistical Methods. Growth parameters were calculated as follows:
Weight gain rate (WGR) (%) = 100 × [(final body weight − initial body weight) / initial body weight]
Feed intake (FI) (%/d) = 100 × total amount of the feed consumed (g) / [(initial body weight + final body weight) / 2] / days
Feed efficiency rate (FER) = wet weight gain (g) / total amount of the feed consumed (g)
Protein productive value (PPV) (%) = 100× body wet protein gain (g)/ protein intake (g)
Survival rate (SR) (%) = 100 × (final fish number / initial fish number).

The Software SPSS, 11.5 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when $P<0.05$. Data are expressed as means ± standard error.

Results

All experimental diets were well accepted by the fish. No pathological signs were observed during the 8-week feeding period.

Squid Soluble Fractions Composition. The peptide size distribution of SSFs are given in Table 1.

Survival Rates and Growth Performance. Survival rate was higher than 95% in all treatments and no significant differences ($P>0.05$) were found between the treatments (Table 4).

Table 4 Growth performance and survival rate of snakehead fed the experimental diets (Means ± SE, n = 3)*

	Diet no.				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
IBW[1]	22.3±0.1	22.3±0.1	22.3±0.1	22.3±0.1	22.3±0.1
FBW[2]	115.6±3.1[a]	117.8±2.6[a]	120.0±1.9[ab]	124.7±3.1[b]	125±1.0[b]
WGR[3]	419.5±14.1[a]	429.3±11.7[a]	439.3±8.5[ab]	460.3±13.7[b]	461.8±4.5[b]
FI[4]	2.39±0.02	2.38±0.02	2.32±0.08	2.19±0.06	2.22±0.02
FER[5]	0.94±0.02[b]	0.95±0.02[b]	0.99±0.03[ab]	1.06±0.03[a]	1.05±0.01[a]
SR[6]	98.3±0.8	99.2±0.8	95.0±2.5	99.2±0.8	97.5±1.4

[1] IBW: initial body weight; [2] FBW: final body weight
[3] Weight gain rate (WGR) (%) = 100 × [(FBW − IBW) / IBW]
[4] Feed intake (FI) (%/d) = 100 × total amount of the feed consumed / [(IBW + FBW) / 2] /days
[5] Feed efficiency rate (FER) = wet weight gain (g) / total amount of the feed consumed (g)
[6] Survival rate (%) = 100 × (final fish number / initial fish number)
*****Values in the same row with different superscripts are significantly different ($P<0.05$)

The feed intake (FI) of fish ranged from 2.19%/day to 2.39%/day (*P*>0.05), without any significant differences between dietary treatments. There were also no significant differences in weight gain rate (WGR), feed efficiency rate (FER) and protein productive value (PPV) between diet 1 and diet 2 (*P*>0.05). Furthermore, compared with fish fed the diet without SSF (diet 2), and those fed diets with higher SSF (diets 4 and 5) had significantly enhanced WGR, FER, and PPV (*P*<0.05) Fig 1.

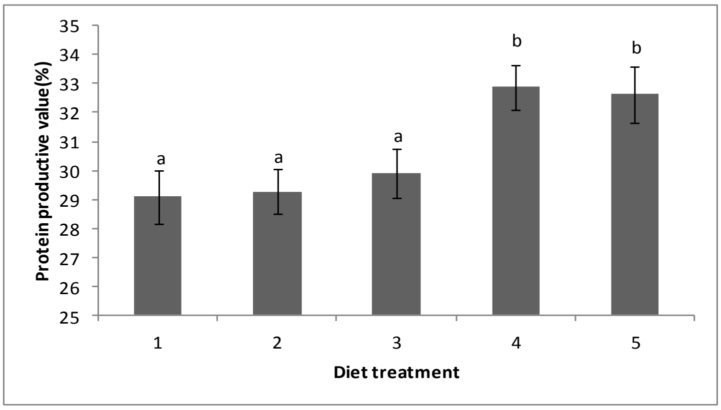

Fig 1. Protein productive value (PPV) of snakehead fed the experimental diets. Data of the five experimental treatments are presented as mean ± S.E. (n=3). Different letters above the bars denote significant differences among treatments at the *P*<0.05 level.

Body Composition. No significant differences (*P*>0.05) were detected between dietary treatments with respect to whole-body moisture (73.8-75.4%), crude protein (14.6-15.0%), crude lipid (7.7-8.2%), and ash (3.5-3.8%) contents of fish (Table 5).

Table 5. Proximate composition in whole body of snakehead fed the experimental diets (% diet in wet basis; means ± SE, n = 3)*

	Diet no.				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Moisture	75.0±0.4	74.9±0.8	73.8±1.0	74.6±1.1	75.4±0.8
Crude protein	14.7±0.2	14.7±0.2	15.0±0.9	14.7±0.6	14.6±0.6
Crude lipid	7.7±0.5	7.9±0.6	8.2±0.8	7.9±0.4	7.7±0.2
Ash	3.8±0.2	3.5±0.4	3.6±0.4	3.8±0.2	3.8±0.2

*****Values in the same row with different superscripts are significantly different (*P*<0.05).

Discussion

Limited supply, increasing demand, and high prices of FM are challenges to the sustainable development of the fish culture industry, notably carnivorous fish which are frequently fed feeds with high FM levels (Wang et al., 2006; Glencross et al., 2007). Cost effectiveness of feed could be improved by replacing FM with more economical protein sources. Numerous studies have focused on assessing the potential to reduce FM level in fish feeds. The present study shows that there was no significant difference in the growth of snakehead with an initial body weight of 22.3 g when fish meal protein was replaced by 22% poultry by-product meal. An optimal EAA profile is a prerequisite for fish growth and nitrogen retention (Luo et al., 2006; Peres and Oliva-Teles, 2009). The content of the EAA, especially the Lysine (Lys), Methionine (Met), and Threonine (Thr) content, is generally the limiting amino acid content in economical alternative protein sources. A deficiency in one EAA will lead to poor utilization of the provided dietary protein (Wilson, 2002). In the present study, Lysine-HCl and DL-methionine (Crystalline amino acids) were supplemented so that the EAA requirements of juvenile snakehead were met based on the whole-body amino acid profiles. The dietary FM levels were reduced to 28% by incorporating poultry by-product meal based on whole-body amino acids profile without negative effects on growth performance and feed utilization.

In the present study, the growth rate of fish fed diets with 0.8% or 1.2% SSF was significantly better (*P*<0.05) compared with that of fish fed the diet without SSF (diet 2). Similar results have been observed in Atlantic salmon (Espe et al., 1999), rainbow trout (Aksnes et al., 2006) and Japanese seabass (Liang et al., 2006) fed the small peptide fraction of fish protein hydrolysate. In this study, the inclusion of SSF enhanced the effect of bioactive nitrogenous compounds such as small peptides present in high amounts in the soluble part of marine raw materials (Table 1). Significant effects of fish hydrolysate fraction have been observed on the spatial expression of peptide transporter in the digestive tract of Atlantic cod (Bakke et al. 2010). This may also suggest that the growth promoting effect of SSF may be partly attributed to the modification in the nutrient absorption patterns in fish. However, a diet with excessive SSF may retard growth in larvae or juvenile fish, such as in larvae of sea bass (Cahu et al., 1999), turbot juveniles (Oliva-Teles et al., 1999), larvae of cobia (Han et al., 2010), and larvae of tongue sole (Liu et al., 2010). Some of these detrimental effects may be linked to a fast flow of short peptide and free amino acids through the gut wall, a flow that the larvae cannot handle. As a result, most of these metabolites could be flushed out of the digestive system (Espe and Njaa, 1991; Kolkovski and Tandler, 2000).

In the present study, no significant differences were detected between dietary treatments with respect to whole-body moisture, crude protein, crude lipid, and ash content of fish (P>0.05). These results are in agreement with previous studies on Atlantic salmon (Kousoulaki et al., 2009, 2012) and Japanese flounder (Zheng et al., 2011).

In conclusion, results of the present study show that: dietary FM levels can be reduced to 28% by incorporating poultry by-product without negative effects on growth performance and feed utilization; and 0.8% of dietary SSF seems optimal for significantly better growth rate and feed utilization of juvenile snakehead.

Acknowledgements

This research was supported by the Guangdong Marine economy development demonstration area special project (GD2012—B01-010).

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