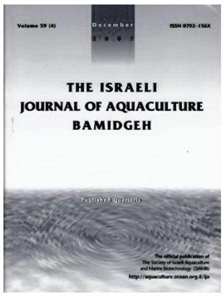

Evaluation of Dietary Anti-nutritional Factors and Amino Acids Supplementation on Growth, Feed Efficiency Ratio, and Apparent Digestibility of Japanese Flounder (*Paralichthys olivaceus*) at Equal Feed Intake

Wei Chen, Kangsen Mai, Yanjiao Zhang *, Qinghui Ai, Rantao Zuo, Houguo Xu

Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture), and the Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao, P.R. China

Keywords: anti-nutritional factor; crystalline amino acid; force feeding; Japanese flounder.

Abstract

A 30-day feeding trial was conducted to evaluate the anti-nutritional factors in fish meal-based diets, and limiting amino acids in soybean meal-based diets on growth, feed efficiency ratio (FER), and nutrient apparent digestibility (AD) of Japanese flounder (*Paralichthys olivaceus*) at equal feed intake. Four isonitrogenous and isoenergetic diets were formulated: Diet 1 contained fish meal as the main dietary protein source; Diet 2 was based on Diet 1 and supplemented with anti-nutritional factors; Diet 3 contained both fish meal and soybean meal as main protein source; Diet 4 was based on Diet 3 and supplemented with crystalline amino acids. All diets were assigned to triplicate groups of 5 fish per aquarium. Results from Diet 1 showed that force-feeding was a possible method to rear Japanese flounder with high FER and 100% survival rate. In the first three periods (4 days/period), weight gain (WG) and FER of fish fed Diets 2, 3 & 4 were significantly lower ($P<0.05$) than in fish fed Diet 1. After 30 days of the feeding trial, WG and FER of fish fed Diets 3 & 4 were significantly lower than in fish fed Diet 1 ($P<0.05$). No significant difference in WG and FER was observed either between fish fed Diets 1 & 2, or between fish fed Diets 3 & 4. The AD of dry matter and crude lipid of fish fed Diets 3 & 4 were significantly lower than those of fish fed Diet 1 ($P<0.05$). Results indicated that supplementation of anti-nutritional factors in a fish meal-based diet, or supplementation of crystalline amino acids in a soybean meal-based diet did not significantly affect growth performance of Japanese flounder by force feeding.

* Corresponding author. Fax: +86 532 8203 1627; Email: yanjiaozhang@ouc.edu.cn

Introduction

Aquaculture is one of the fastest growing food-producing sectors, supplying approximately 50% of the world's fish food (FAO, 2014). Due to the limitation of fish meal, substitution of fish meal by plant-derived protein sources has been the main objective of the aqua-feed industry for many years (Rahman et al., 2015). Among all plant proteins, soybean meal is regarded as an economical and nutritious alternative due to its relatively low cost, high crude protein content, and reasonably balanced amino acid profile. However, high inclusion of soybean protein has been reported to reduce the feed intake, utilization, and ultimately inhibits growth performance of carnivorous fish such as turbot (Yun et al., 2015; Day and Plascencia-GonzÁlez, 2000) and Japanese flounder (Kikuchi, 1999; Saitoh et al., 2003; Deng et al., 2006).

Amino acid imbalance, anti-nutritional factors, and poor palatability are responsible for the deficient utilization of vegetable proteins by marine fish according to NRC (2011). However, few studies have been conducted under equal feed intake to investigate the effects of amino acid supplementation and anti-nutritional factors. Thus, it is not clear whether the amino acid balance or the anti-nutritional factor is the limiting factor under circumstances of equal feed intake. Little information has been available about the effects of anti-nutritional factor supplementation on growth, feed conversion, and nutrients apparent digestibility of fish, especially at equal feed intake.

Japanese flounder *Paralichthys olivaceus* is an economically important marine fish, widely cultured in the north of China. Previous studies have shown that dietary fish meal supplementation must be above 40% (Kikuchi, 1999). This increases feed costs and limits development of this industry. The aim of this study was to evaluate the effects of anti-nutritional factor supplementation in fish meal based diets, as well as on soybean based diets supplemented with crystalline methionine and lysine, on survival, growth, feed efficiency, and nutrients apparent digestibility in Japanese flounder at equal feed intake based on a force feeding model.

Materials and Methods

Experimental diets and feeding procedure. Four diets were formulated to contain 50% crude protein and 20.9MJ/kg energy (Table 1). The fish meal-based diet (Diet 1) that served as control included 70% fish meal. Diet 2 was based on Diet 1 with the addition of 1.5% anti-nutritional mixture [equal in amount with anti-nutritional factors contained in 41% dietary soybean meal, 0.2% soybean trypsin inhibitor (Sigma, USA), 0.2% soybean saponin (North China Pharmaceutical, China), 0.2% soy isoflavones (Zhejiang Xinxin, China), 0.4% sodium phytate (Sigma, United States) and 0.5% raffinose (Amresco, USA)]; Diet 3 included 39.5% fish meal and 41% soybean meal; Diet 4 was based on Diet 3 supplemented with 0.25% methionine and 0.75% lysine.

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

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*
Fish meal+	70.0	70.0	39.5	39.5
Soybean meal	0.0	0.0	41.0	41.0
Wheat meal	20.0	18.5	7.5	6.5
Menhaden fish oil	3.5	3.5	5.5	5.5
Soy lecithin	2.5	2.5	2.5	2.5
Vitamin premix-	2.0	2.0	2.0	2.0
Mineral premix§	2.0	2.0	2.0	2.0
ANFs premix*	0.0	1.5	0.0	0.0
Amino acid pemix**	0.0	0.0	0.0	1.0
Yttrium oxide	0.02	0.02	0.02	0.02
Nutritional composition				
Crude protein (%)	50.0	50.0	50.2	51.0
Crude lipid (%)	12.2	12.2	12.3	12.3
Energy (MJ/kg)	20.9	20.9	20.7	20.5

+ Supplied by Liuhe Feed Co., Ltd. (Shandong, China), crude protein is 69%.

- Vitamin premix (mg/kg diet): retinol acetate, 32.0; cholecalciferol, 12.9; alpha-tocopherol, 200.0; thiamin, 110.0; riboflavin, 360.0; pyridoxine HCl, 86.0; pantothenic acid, 359.0; niacin acid, 1026.0; biotin, 10.0; folic acid, 20.0; vitamin B12, 1.2; inositol, 4000.0; ascorbic acid, 500.0; choline chloride , 10000.0.

§ Mineral premix (mg/kg diet) : $MgSO_4 \bullet 7H_2O$, 5066.9; KCl, 3020.0; $KAl(SO_4)_2$, 12.7; $CoCl_2$, 40.0; $ZnSO_4 \bullet 7H_2O$, 253.0; $CuSO_4 \bullet 5H_2O$, 10.0; KI, 8.0; $MnSO_4 \bullet 4H_2O$, 73.2; Na_2SeO_3, 2.5; $C_6H_5O_7Fe \bullet 5H_2O$, 1632.0; NaCl, 100.0; NaF, 4.0; KH_2PO_4, 10000.0.

* Anti-nutritional factors (ANFs) premix (g/kg diet): soybean trypsin inhibitor, 2.0; soybean saponins, 2.0; soybean isoflavones, 2.0; sodium phytate, 4.0; raffinose, 5.0.

** Amino acid pemix (g/kg^{-1} diet): methionine, 2.5; lysine, 7.5.

All ingredients were ground into fine powder and passed through a 320 μm mesh sieve. They were then mixed thoroughly with menhaden fish oil and soy lecithin. The ingredients were then mixed in a drum-type vertical mixer (MuYang- SYTH0.1, Yangzhou, China) and stored at -20℃ until use.

Feeding was performed according to the force-feeding method described by Ng (1996) with some modifications. One fish at a time was removed randomly from each tank and placed on a wet cloth. The assigned diet was mixed with distilled water (3:2, g/g) and introduced into the digestive tract through a plastic straw attached to a syringe. The straw was gently pushed into the mouth and then into the esophagus until little resistance was felt when pushing the straw back and forth indicating it had reached the stomach. The assigned diet was then inserted gently through the straw into the stomach. The entire force-feeding procedure lasted 30 seconds per fish, and no regurgitation occurred.

Experimental fish and rearing regime. Japanese flounder were obtained from Maoyu Aquaculture Co. Ltd., Jiaonan, China. Fish were reared in an indoor sea water recirculating system and fed a commercial feed (Nisshin Flour Milling Co., Ltd, Japan) to acclimatize them to experimental conditions for 2 weeks before the feeding trials. At the initiation of the experiment, fish were fasted for 24 h. Their initial average weight was 60.0 ± 0.66 g. They were distributed into 12 culture tanks (60L), with 5 fish in each tank. Each diet was randomly assigned to triplicate groups. All fish were force fed once a day. During the feeding period, water temperature ranged from 20-22°C, salinity 30-33‰, pH 7.7-7.9, and dissolved oxygen levels were at or near air-saturation.

Sample collection and chemical analysis. At the end of each experiment period (4 days/period, six times sampling in total), all fish were weighed and counted after being fasted for 24h. The feces collection method was conducted according to Kaushik et al. (2004).

Calculations and statistical analysis. The following variables were calculated:

Weight gain (WG) = $W_t - W_0$

Feed efficiency ratio (FER) = Wet weight gain in g/dry feed fed in g

Survival rate = $100\times (N_t / N_0)$

Apparent digestibility coefficient of nutrients and energy (%) = (1− (dietary Y_2O_3 / fecal Y_2O_3) × (fecal nutrient or energy/dietary nutrient or energy) ×100)

Where W_t and W_0 are final and initial body weight, respectively, t is duration of experimental days; N_t and N_0 are final and initial number of fish, respectively.

Statistical analysis was performed by using SPSS 16.0 for Windows. All data were subjected to one-way analysis of variance (ANOVA) and differences between the means were tested by Tukey's multiple range test. The level of significance was chosen at $P<0.05$ and the results are presented as means ± S.E.M. (standard error of means).

Results

Survival and growth. No mortality occurred during the whole experimental period. The WG and FER of fish fed Diets 2, 3 & 4 during periods 1, 2 and 3 were significantly lower ($P<0.05$) than fish fed the control diet, Diet 1. The WG and FER of fish fed Diets 3 & 4 during last three periods were significantly lower than fish fed Diets 1 & 2 ($P<0.05$). No significant difference in WG and FER was observed either between fish fed Diets 1 & 2, or between fish fed Diets 3 & 4. (Table 2).

Table 2. Feed intake, Weight gain and feed efficiency ratio (FER) of Japanese flounder fed experimental diets (six periods: P-1 to P-6, 4 days/period) [*]

		Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Pooled S.E.M.* [**]	*ANOVA* [***]	
							F value	*P value*
Feed intake(g	P-1	2.14	2.14	2.14	2.14	0	—	—
	P-2	4.28	4.28	4.28	4.28	0	—	—
	P-3	6.42	6.42	6.42	6.42	0	—	—
	P-4	8.56	8.56	8.56	8.56	0	—	—
	P-5	10.7	10.7	10.7	10.7	0	—	—
	P-6	12.84	12.84	12.84	12.84	0	—	—
Weight gain(g	P-1	2.87^a	1.43^b	0.93^c	1.20^{bc}	0.39	128.66	＜0.001
	P-2	4.95^a	4.40^b	2.50^c	2.43^c	0.29	119.24	＜0.001
	P-3	8.32^a	7.20^b	4.07^c	4.17^c	0.48	190.88	＜0.001
	P-4	10.30^a	9.77^a	5.42^b	5.70^b	0.59	148.61	＜0.001
	P-5	12.70^a	11.65^a	6.63^b	6.73^b	0.73	80.11	＜0.001
	P-6	15.68^a	14.85^a	7.73^b	8.27^b	0.97	62.11	＜0.001
Feed efficien ratio	P-1	1.34^a	0.66^b	0.43^c	0.56^{bc}	0.092	128.66	＜0.001
	P-2	1.16^a	1.03^b	0.58^c	0.57^c	0.069	119.24	＜0.001
	P-3	1.30^a	1.12^b	0.63^c	0.65^c	0.076	190.88	＜0.001
	P-4	1.20^a	1.14^a	0.63^b	0.67^b	0.069	148.61	＜0.001
	P-5	1.18^a	1.08^a	0.62^b	0.63^b	0.069	80.11	＜0.001
	P-6	1.22^a	1.15^a	0.60^b	0.64^b	0.076	62.11	＜0.001

[*] Values are presented as means of triples. Values in the same column with different superscripts are significantly different from each other determined by Tukey's test ($P < 0.05$); [**]S.E.M., Standard Error of Mean; [***]*ANOVA*, one-way analysis of variance.

Apparent digestibility. No significant difference ($P>0.05$) of apparent digestibility (AD) of crude protein was observed among all treatments. However, the AD of dry matter and crude lipid of fish fed Diets 3 & 4 was significantly lower ($P<0.05$) than in fish fed Diet 1. (Table 3)

Table 3. Apparent digestibility coefficients (%) of dry matter, crude protein and crude lipid in Japanese flounder fed experimental diets*

Diets	*Dry matter*	*Crude protein*	*Crude lipid*
Diet 1	66.0[a]	90.3	74.1[a]
Diet 2	64.4[ab]	88.6	74.6[a]
Diet 3	63.1[b]	88.3	63.0[c]
Diet 4	64.2[b]	90.3	67.8[b]
Pooled S.E.M.**	0.35	0.35	1.46
*ANOVA****			
F value	10.68	5.08	148.67
P value	0.004	0.092	< 0.001

* Values are presented as means of triples. Values in the same column with different superscripts are significantly different from each other determined by Tukey's test ($P<0.05$); * * S.E.M., Standard Error of Means; ****ANOVA*, one-way analysis of variance.

Discussion

In the present study, no mortality and regurgitation was observed in any of the experimental groups during the force-feeding process. FER was around 1.16-1.30 at different periods in the control group. This indicated the method was applicable and reasonable in this study. Force-feeding enabled feed intake to be equalized, and allowed feed intake to be set above or below the voluntary intake. This method is used in studies on fish nutrition, even at the larval stage (Rust et al., 1993; Deng et al., 2000). Previous studies have investigated metabolic processes of several nutrients such as carbohydrates (Wilson and Poe, 1987; Hung, 1991), fatty acids (Denstadli et al., 2004) and amino acids (Ng et al., 1996; Schuhmacher et al., 1997) by force-feeding.

The present study showed that WG, FER, AD of dry matter and crude lipid significantly decreased in the soybean meal groups compared with the fishmeal groups. This is consistent with findings of previous studies on carnivorous fish which showed that high levels of fish meal replaced by soybean meal may depress growth performance of fish.

According to NRC (2011), imbalance in amino acid profiles, anti-nutritional factors, and poor palatability were responsible for the deficient utilization of plant protein sources. Few studies have examined the effects of a mixture of several anti-nutritional factors on fish growth and survival. The present results showed the supplementation of anti-nutritional factors significantly decreased WG and FER compared with the control group when fish were fed the same amount of diet in the first three experimental periods. However, this trend gradually weakend and no significant difference was observed between the anti-nutritional factors supplemented diet group and the control group from the fourth period on. Feed intake and growth of Japanese flounder were not significantly influenced by adding 0.8% phytic acid, 0.32% soybean saponin, 0.8% stachyose, 0.4% soy isoflavones, 1.5% raffinose and 10% soy oligosaccharides to diets after 8 weeks feeding (Cai, 2006; Deng, 2006; Chen, 2009; Mai et al., 2012). Our results indicated that these anti-nutritional factors may not be the main factors inhibiting fish growth, given the limits of the present concentrations. The absence of other anti-nutritional factors, such as the allergenic protein in soybean which could reduce growth due to its strong immunoreactivity (Amigo-Benavent et al., 2011), may also be a reason. The high levels of fish meal (70%) used in the diets may also counteract the negative effects due to the attractant factors in fish meal, such as essential amino acids (Zhou et al., 2004) and taurine (Gaylord et al., 2006; Takagi et al., 2008). In this study, with the use of force feeding, we were unable to determine whether poor palatability caused by anti-nutritional factors would account for reduced growth performance of flounder. Further research is needed in this area.

Crystalline amino acids have been successfully used in amino acid deficient or unbalanced diets to improve growth and feed utilization efficiency in fish (Espe et al., 2006). However, no significant improvement in WG, FER, and nutrients AD of flounder was observed in this study after adding crystalline methionine and lysine to the soybean meal. This is in accordance with findings in previous studies (Rodehutscord et al., 1995, 1997; Zarate et al., 1999). In our study, the leaching of dietary crystalline-amino acid was eliminated because of force-feeding. This could be due to the differences in amino acid absorption rate or the amino acid availability in the amino acid pool consequently affecting protein synthesis (Cowey, 1995; Ambardekar et al., 2009). In several species of fish, more rapid absorption of crystalline amino acids compared to protein-bound-amino acids has been demonstrated (Zarate et al., 1999), resulting in rapid increase and earlier peak levels of plasma amino acid concentration (Schuhmacher et al., 1997).

Compared with fish meal, soybean meal supplies less digestible energy due to ingredients that cannot be used by fish, such as non-starch polysaccharide and soybean oligosaccharides. In this study, the AD of dry matter and lipid of fish fed soybean meal was significantly lower than in fish fed fish meal, while no significant difference of AD of protein was observed among all groups. This may indicate that absorption of protein of the fish fed soybean meal was used for energy rather than growth. Thus, it is more appropriate to use isoproteic and isoenergetic, rather than isoproteic and isolipidic formulae (Kaushik et al., 2004).

In conclusion, high levels of soybean meal (41%) in diets could reduce growth and FER of Japanese flounder, however, neither anti-nutritional factors in fish meal based diets nor supplementation of crystalline-amino acids in soybean meal based diets had any significant influence on WG and FER of Japanese flounder at equal feed intake. These results may indicate that, to some extent, anti-nutritional factors and balance of amino acids with crystalline-amino acids were not the main causes of growth inhibiting effects when replacing fish meal with soybean meal in diets of Japanese flounder. Other factors accounting for growth reducing effects of soybean meal need further investigation.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 30371120, 31202006), National Program on Key Basic Research Project (973 Program, 2014CB138600) and the Promotive Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (No. BS2013SW007). The authors thank Mingchun Ren, Chunfeng Yao, Shuyan Chi and Fangyi Meng for their help in feeding, sampling and analysis. Thanks are also due to Jinghua Chen, Youqing Miao and Xiaoning Li for their assistance in the study.

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