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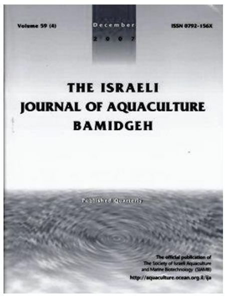


# Effect of Dietary Cornstarch Level on The Growth Performance of Japanese Seabass (*Lateolabrax japonicus*) in Grow-Out Phase

**Keke Zheng, Bingshuai Dou, Houguo Xu, Mengqing Liang***

*Key Laboratory of Chinese Ministry of Agriculture for Sustainable Utilization of Marine Fisheries Resources, Yellow Sea Fisheries Research Institute, Qingdao, 266071, China*

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Key words: Japanese seabass, cornstarch level, grow-out phase, growth

**Abstract**

A 10-week growth trial was conducted to investigate the effect of dietary cornstarch levels on the growth performance, body composition, liver/muscle glycogen, and selected serum biomarkers, of Japanese seabass in grow-out phase. Six isonitrogenous and isolipidic diets containing graded levels (0%, 6%, 12%, 18%, 24%, 30%) of cornstarch were fed to Japanese seabass (initial weight 343g). Weight gain (WG) significantly increased up to 18% with increasing dietary cornstarch, and then reached a plateau. There was no significant difference in WG between fish fed 18%, 24%, or 30% cornstarch ($P>0.05$). Based on WG, the optimal dietary cornstarch level of Japanese seabass estimated by second-order polynomial model was 27.0%. Body protein and lipid content as well as total cholesterol and triglycerides in serum showed similar trends, i.e. increasing at first and then reaching a plateau despite increasing levels of dietary cornstarch. Ash content decreased at first and then reached a plateau with increasing dietary cornstarch. Hepatosomatic index and liver glycogen significantly increased with increasing dietary cornstarch ($P<0.05$) while muscle glycogen, the condition factor was not influenced by dietary treatments. Serum glucose significantly increased with increasing dietary cornstarch and then decreased ($P<0.05$). Results of this study suggest the provision of adequate dietary cornstarch (>18%) could improve the growth performance of Japanese seabass and the optimal dietary cornstarch level is 27.0% during grow out phase.

* Corresponding author. E-mail: liangmq@ysfri.ac.cn

## Introduction

Carbohydrates are excellent sources of energy and carbon, and they are the least expensive dietary energy sources. Although it is generally accepted that fish do not have a specific dietary carbohydrate requirements, the provision of an appropriate amount of digestible carbohydrate in aqua-feed is important to reduce the use of more expensive lipids and protein as sources of energy (NRC, 2011). Incorporation of carbohydrates may be beneficial to pellet quality, waste production and also to fish growth performance (Meriac, et al., 2014; Wilson, 1994). Carbohydrates in fish feed can help to meet environmental regulation objectives in addition to economic objectives. In addition, dietary carbohydrate utilization by fish deserves attention because of efforts to effectively replace fish meal with plant protein sources. Starch is the major polysaccharide stored as an energy reserve in many plants. The ability of fish to use dietary carbohydrates for growth varies greatly among species and basically corresponds to the feeding habits of the species. Herbivorous and omnivorous species generally digest and utilize carbohydrates more effectively than carnivorous species (Hemre et al., 2002; Moon, 2001; NRC, 2011). Based on the reported findings for fish, the maximum recommended levels of digestible starch inclusion in feed ranges between 15-25% in salmonids and other marine fish, and up to 50% in omnivorous species (NRC, 2011).

Japanese seabass, *Lateolabrax japonicus*, is a carnivorous species widely cultured in China because of its delicious meat and rapid growth. It is one of the most commercially valuable aquaculture species in Asia, where 138,800 tons were produced in 2013. Japanese seabass is recognized as a potential candidate for worldwide aquaculture because of its good taste, tolerance to high stocking density, and adaptability to a wide range of environmental factors such as salinity and temperature. There have been some studies on the nutrition of Japanese seabass (Mai et al., 2006; Li et al., 2010; Xu et al., 2010; Ai et al., 2004a, b) however most of them have focused on early juveniles. To our knowledge, information on optimal dietary carbohydrate level of the species is still very limited, and no information is available on the grow-out phase. The present study investigated the effect of dietary cornstarch on growth performance, body composition, glycogen level and serum biomarkers of Japanese seabass in the grow-out phase.

## Materials and Methods

*Experimental diets.* Using fish meal and casein as protein sources, fish oil as the lipid source, corn starch as the carbohydrate source, six semi-purified diets were formulated with graded levels (0%, 6%, 12%, 18%, 24%, 30%) of dietary carbohydrate (Table 1).

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

| *Ingredients (g 100 g/dry diet)* | *Dietary cornstarch level* | | | | | |
|---|---|---|---|---|---|---|
| | *0%* | *6%* | *12%* | *18%* | *24%* | *30%* |
| Fish meal | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 |
| Casein | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 |
| Fish oil | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Corn starch | 0 | 6.0 | 12.0 | 18.0 | 24.0 | 30.0 |
| Soy lecithin | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin premix1 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix2 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Carboxymethyl cellulose | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Microcrystalline cellulose | 30.5 | 24.5 | 18.5 | 12.5 | 6.5 | 0.5 |
| Chemical composition (in dry matter) | | | | | | |
| Crude protein | 43.73 | 44.13 | 44.26 | 44.40 | 44.42 | 44.78 |
| Crude lipid | 10.49 | 10.53 | 11.00 | 10.65 | 11.28 | 10.61 |
| Carbohydrate | 1.59 | 6.78 | 12.52 | 17.90 | 22.91 | 29.32 |
| Ash | 6.65 | 7.15 | 7.11 | 7.25 | 7.34 | 7.32 |
| Gross energy(KJ/g) | 22.06 | 21.29 | 21.34 | 21.96 | 22.46 | 23.47 |

[1] Vitamin Premix (mg or g/kg diet)：Riboflavin, 45mg; Thiamine, 25mg; Menadione, 10mg; Inositol, 800mg; Pyridoxine, 20mg; Vitamin B12, 0.1mg; Pantothenate, 60mg; Biotin, 1.2mg; Vitamin A, 32mg; Vitamin D, 5mg; Tocopherol acetate, 200mg; Folic acid, 20mg; Vitamin E, 120mg; Wheat flour l8.67g.

[2] Mineral Premix (mg or g/kg diet); KI, 0.8mg; NaF, 2mg; FeSO4·7H2O, 80mg; ZnSO4·7H2O, 50mg; CoCl2·6H2O, 50mg; CuSO4·5H2O, 10mg; MnSO4·4H2O, 1200mg; NaC1, l00mg; Ca(H2PO4)2·H2O, 3000mg; Mordenzeo, 15.51g.

The protein and lipid contents in all diets were designed to be about 44% and 11%, respectively. These appear to be sufficient to support optimal growth of Japanese seabass (Ai et al., 2004a, b). Ingredients were ground into fine powder through 200 μm mesh. All ingredients were thoroughly mixed with the oils, and water was added to produce stiff dough. The dough was then pelletized (3 mm in dimension) with a laboratory scale, single screw extruder (SLP-45, Fishery Machinery and Instrument Research Institute, Shanghai, China). All the diets were dried in a ventilated oven at 50°C and stored at -20 °C.

*Experimental fish and procedure.* Japanese seabass were obtained from a local fish farm in Xiangshan, Ningbo City. Prior to the onset of the experiment, fish were reared in floating sea cages (3.0×3.0×3.0 m) for 2 weeks to acclimate to the experimental conditions. Fish were fed twice daily with the basal diet (0% dietary cornstarch) to satiation during this period. At the start of the feeding trial, Japanese seabass were fasted for 24 h and weighed after being anesthetized with eugenol (1:10,000) (Shanghai Reagent, Shanghai, China). Fish of similar sizes (343.3±10.0g) were randomly distributed into 18 floating sea cages (1.5×1.5×2.0 m), and each cage was stocked with 12 fish. Each diet was randomly assigned to triplicate cages. Fish were hand-fed to apparent satiation twice daily (06:30 and 17:00) for 10 weeks. During the experimental period, the temperature ranged from 19-24.5°C, salinity from 29‰-33‰ and the dissolved oxygen was approximately 7 mg/L.

*Sampling and analytical methods.* For both feeding trials, at the conclusion of the trial, fish were fasted for 24 h before harvest. Fish were weighed individually and three fish per tank/cage were collected for determination of proximate body composition. Samples of liver and serum were also collected from 5 fish per tank/cage.

At the end of the experiment, fish were fasted for 24 h and batch weighed. Total number of fish in each cage was measured. Two fish were randomly sampled from each cage and frozen for analyses of proximate composition. Four fish from each cage were randomly sampled and euthanized with an overdose of tricaine methanesulfonate (1g/L, Argent, Redmond, WA, USA). Blood samples were taken from the caudal vessels using syringes and allowed to clot for 5 h at 4°C. Then the blood samples were centrifuged (2000×g at 4°C for 10 min) and serum obtained and frozen at -80°C until further analysis. Tissue samples including liver, muscle were also obtained and kept at -80°C until analysis.

Glycogen in liver and muscle was determined using the Liver / Muscle glycogen assay kit (#043, Jiancheng, Nanjing, China) and the procedure conducted by closely following the manufacturer's protocol. Serum glucose, triglyceride and total cholesterol were analyzed using biochemistry analyzer.

The fish samples were autoclaved at 120°C, homogenized, and oven-dried at 70°C. Crude protein, crude lipid, ash and gross energy were analyzed for fish and diets. Chemical analyses were conducted following standard laboratory procedures (AOAC 1997): dry matter by drying at 105°C to constant weight; crude protein (N*6.25) by the Kjeldhal method (UDK142 automatic distillation unit, VELP,); crude lipid by petroleum ether extraction in a SoxtecTM System (SoxtecTM 2050 system, Foss Tecator, Hoganas, Sweden); ash by incineration in a muffle furnace at 550°C; gross energy by combustion in a microbomb calorimeter (6100 Compensated Jacket Calorimeter, Parr Instrument Company, Moline, Illinois, USA). Carbohydrate was estimated using the 3'5-dinitrosalicylic acid method (Yu et al. 1998).

*Calculations and statistical methods.* The following variables were calculated:

Weight gain (WG, %) =100×(FBW-IBW)/IBW

Hepatosomatic index (HSI, %) =100×liver weight/body weight

Viscerosomatic index (VSI, %) =100× (Weight of viscera/total fish weight)

Condition factor (CF, %g/cm$^3$) =100× body weight(in grams)/total length$^3$(in cm)

Where FBW and IBW were final and initial fish weight respectively.

The results were presented as means ± SD. SPSS 16.0 for Windows was used for statistical analysis. Data were compared by one-way ANOVA followed by Duncan's multiple range tests. The homogeneity of variances was first checked using the Levene's test. Differences between treatments were considered significant at the P<0.05 level.

According to the coefficient of determination ($r^2$) among the models tested, second-order polynomial model was used to estimate the optimum dietary cornstarch level of experimental fish based on weight gain.

## Results

Survival was not affected by dietary cornstarch levels (*P*>0.05) see Table 2.

**Table 2.** Growth performance and feed utilization of *Lateolabrax japonicus* fed experimental diets containing different carbohydrate levels (mean±SD) *

| *Dietary carbohydrate level (%)* | *Survival (%)* | *Final body weight (g)* | *Weight gain (%)* | *Hepatosomatic index (%)* | *Viscerosomatic index (%)* | *Condition factor (%g/cm³)⁶* |
|---|---|---|---|---|---|---|
| 0 | 95.00±4.81 | 423.0±9.0$^{a}$ | 23.26±3.65$^{a}$ | 0.75±0.15$^{a}$ | 7.19±1.17$^{ab}$ | 1.38±0.11 |
| 6 | 89.44±4.81 | 455.7±14.5$^{ab}$ | 32.70±5.95$^{a}$ | 0.84±0.01$^{ab}$ | 8.18±0.35$^{ab}$ | 1.44±0.04 |
| 12 | 89.44±4.81 | 506.7±12.7$^{bc}$ | 47.56±3.64$^{b}$ | 0.85±0.08$^{ab}$ | 6.77±0.55$^{a}$ | 1.39±0.07 |
| 18 | 94.45±4.81 | 568.0±4.4$^{d}$ | 64.94±1.54$^{c}$ | 1.01±0.16$^{ab}$ | 7.13±0.18$^{ab}$ | 1.39±0.08 |
| 24 | 91.67±8.33 | 594.7±28.1$^{d}$ | 73.20±8.13$^{c}$ | 1.20±0.26$^{bc}$ | 8.05±0.22$^{ab}$ | 1.47±0.03 |
| 30 | 94.45±4.81 | 563.0±36.0$^{cd}$ | 63.99±10.53$^{c}$ | 1.57±0.14$^{c}$ | 8.58±0.82$^{b}$ | 1.46±0.02 |

*Means in the same column with different superscripts are significantly different (*P*<0.05). Initial body weight of Japanese seabass was 343.3±10.0g

Final body weight and weight gain showed similar trends, increasing at first and then decreasing with increasing dietary cornstarch. Weight gain of fish fed with 0-12% cornstarch was significantly lower than fish fed with 18-30% cornstarch (*P*<0.05). There was no significant difference in weight gain between fish fed with 18%, 24% and 30% cornstarch (*P*>0.05). Based on WG, the optimal dietary cornstarch level of Japanese seabass estimated by second-order polynomial model was 27.0% (Fig. 1).

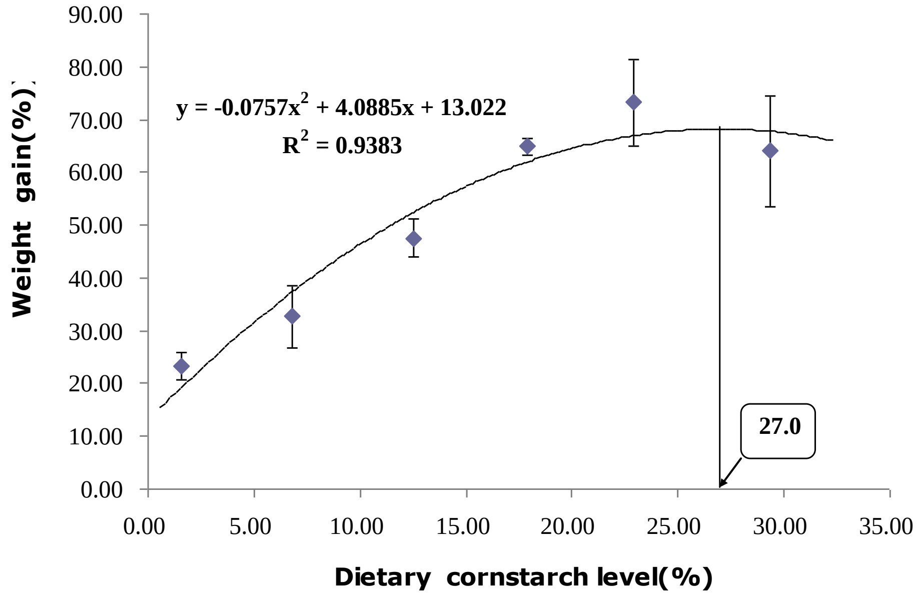


**Fig. 1.** Requirement of dietary cornstarch level based on weight gain of Japanese seabass, *Lateolabrax japonicus*.

Hepatosomatic index and viscerosomatic index significantly increased with increasing dietary cornstarch (*P*<0.05) while their condition factor was not significantly influenced by dietary treatments (*P*>0.05). Fish fed with 24% dietary cornstarch showed the best growth performance. Fish body composition was significantly influenced by dietary treatment (Table 3).

**Table 3.** Effect of dietary carbohydrate level on body composition of *Lateolabrax japonicus* (% in wet weight) (Mean±SD) *

| *Dietary carbohydrate level (%)* | *Moisture* | *Crude protein* | *Crude lipid* | *Ash* |
|---|---|---|---|---|
| 0 | 71.26±0.40 | 14.01±0.56[a] | 8.19±0.51[a] | 5.25±0.43[bc] |
| 6 | 70.55±1.58 | 15.72±0.54[ab] | 8.45±0.97[a] | 6.02±0.87[c] |
| 12 | 69.74±0.25 | 16.48±1.07[b] | 12.98±0.11[c] | 3.71±0.08[a] |
| 18 | 69.91±0.94 | 17.47±0.52[b] | 10.51±0.45[b] | 3.75±0.21[a] |
| 24 | 69.56±1.16 | 17.51±0.93[b] | 10.56±0.52[b] | 3.57±0.08[a] |
| 30 | 69.37±0.93 | 17.42±0.72[b] | 10.37±0.07[b] | 3.22±0.02[a] |

*Means in the same column with different superscripts are significantly different ($P< 0.05$).

Crude protein and lipid first showed an increase with increasing dietary cornstarch and then reached a plateau. Fish fed with 12%-30% dietary cornstarch had significantly higher body protein and lipids than fish fed with 0%, 6% cornstarch ($P<0.05$). The dietary effects on ash content were reversed. Fish fed 12%-30% dietary cornstarch had significantly lower ash content than that fed with 0%, 6% cornstarch ($P<0.05$). There was no significant difference in moisture of whole body between diet treatments ($P>0.05$).

Muscle glycogen was relatively low (0.63-0.72mg/g) while liver glycogen was much higher (14.39-39.95mg/g) (Table 4). There was no significant difference in muscle glycogen while liver glycogen increased significantly with increasing dietary cornstarch ($P<0.05$). Serum glucose increased significantly with increasing dietary cornstarch and then decreased ($P<0.05$).

Total cholesterol and triglyceride in serum increased first with increasing dietary cornstarch and then reached a plateau. Total cholesterol of fish fed 0% cornstarch was significantly lower than fish fed 24%-30% cornstarch ($P<0.05$). Triglycerides of fish fed 0% cornstarch was significantly lower than fish fed 12-24% cornstarch ($P<0.05$).

**Table 4.** Effect of dietary carbohydrate level on glycogen and serum biomarker of *Lateolabrax japonicus* (Mean±SD) *

| *Dietary carbohydrate level (%)* | *Liver glycogen (mg/g)* | *Muscle glycogen (mg/g)* | *Serum glucose (mmol/L)* | *Total cholesterol (mmol/L)* | *Triglyceride (mmol/L)* |
|---|---|---|---|---|---|
| 0 | 14.39±0.39[a] | 0.71±0.05 | 6.48±0.18[a] | 5.94±0.63[a] | 4.68±0.41[a] |
| 6 | 19.65±0.23[b] | 0.64±0.14 | 6.91±0.10[b] | 7.14±1.21[ab] | 5.72±0.35[ab] |
| 12 | 21.04±1.26[b] | 0.72±0.04 | 7.47±0.12[c] | 7.71±0.65[ab] | 6.38±0.46[b] |
| 18 | 24.30±0.77[c] | 0.71±0.03 | 8.36±0.14[d] | 7.80±0.43[ab] | 6.18±0.10[b] |
| 24 | 38.71±1.76[d] | 0.63±0.02 | 8.50±0.36[d] | 8.25±0.69[b] | 6.74±0.54[b] |
| 30 | 39.95±1.55[d] | 0.72±0.04 | 7.79±0.18[c] | 8.08±0.79[b] | 5.70±0.69[ab] |

*Means in the same column with different superscripts are significantly different ($P< 0.05$).

## Discussion

Carbohydrate utilization within species is affected by a number of factors, including carbohydrate source, other dietary ingredients, fish size, physical state and environmental factors etc. (El-Sayed, 2006; NRC, 2011). Complex carbohydrates such as starches are more efficiently utilized than disaccharides and monosaccharides by most fish species (Shiau et al., 2002). Numerous studies have demonstrated that carbohydrate must be supplied in available forms in the diets, and the positive effect of starch gelatinization on digestion efficiency was demonstrated (NRC, 2011; Cheng et al. 2013, Yun, 2014). In our study, we set dietary protein (44%) and lipid (11%) levels to be optimal for Japanese seabass and chose cornstarch as the carbohydrate source. We achieved starch gelatinization by heat treatment and extrusion of the whole feed during feed processing. High cornstarch levels (18-30%) significantly improved the growth of Japanese seabass. Growth promotion and protein-sparing effects of digestible starch has been described in many fish species; this may be related to the fact that glucose is the preferred oxidative substrate for nervous tissue and blood cells, and carbohydrate in fish diets can depress gluconeogenic activity, thus diverting amino acids away from oxidative pathways (Hemre et al., 2002; Sánchez-Muros et al., 1996; Stone, 2003).

Nutrient absorption and utilization can change during the different stages of growth of fish, mainly due to the different rates of growth and metabolism throughout the life cycle (Einen and Roem, 1997; Azevedo et al., 2004). Studies on tilapia, Atlantic salmon, and Atlantic halibut, have shown that early juvenile fish require a diet higher in protein and lipids, and lower in carbohydrates. Sub-adult fish require more energy from lipids and carbohydrates for metabolism and a lower proportion of protein for growth. Adult fish require even less dietary protein for growth and can utilize even higher levels of carbohydrates as a source of energy (Einen and Roem, 1997; Storebakken, 2002; Hamre et al., 2003). Our previous study, using the same diet evaluated the effect of dietary cornstarch levels on growth performance of juvenile Japanese seabass (initial body weight: 34g) (Dou, et al., 2014). Growth of juvenile Japanese seabass increased first with increasing dietary cornstarch and then decreased when dietary cornstarch levels were above 18%. Based on the specific growth rate, the optimal cornstarch level for juvenile Japanese seabass is 17.7%. In the present study, we raised the fish from 343g to market size (500-600g). In the grow-out phase, Japanese seabass are capable of utilizing high levels of cornstarch of between 18-30 percent of the diet. Larger fish can utilize cornstarch better than small fish.

Several studies with different fish species have shown that body lipid deposition increased with the levels of digestible carbohydrates in the diet (Hemre et al., 2002; NRC, 2011). Lipid content of Japanese seabass fed 0-6% corn starch was lower than those fed 12-30% corn starch, while body lipids did not increase with increasing dietary carbohydrate from 12%-30%. The observations made with radiolabelled glucose shows that the amount of de novo synthesis of lipids from carbohydrate is quite limited (Hemre & Kahrs, 1997). Carbohydrate in the diet is needed to stimulate lipid biosynthesis not so much through delivery of carbon backbones, but rather via increased availability of cytosolic reducing equivalents (Hemre et al. 2002). When fish are fed adequate carbohydrate, glucose could reduce the contribution of lipid to oxidative metabolism. This could explain the increased lipid deposition under these conditions. A similar situation was also found in body protein content of Japanese seabass in this study.

An increase of HSI with an increasing dietary cornstarch has been observed, which may be related to the increased glycogen deposition in the liver of Japanese seabass fed high cornstarch diets and an increase of lipid deposition. Studies of European seabass also showed an increase of deposition of both glycogen and fat in the liver with the increase of available dietary carbohydrates intake (Peres and Oliva-Teles, 2002; Moreira et al. 2008). The liver is the major site of glycogen storage in fish while glycogen reserves in white muscle are very low regardless of species (NRC, 2011). In our study, muscle glycogen was low and not affected by dietary cornstarch level while liver glycogen was significantly increased with increasing dietary cornstarch level. Deng et al. (2005) also reported similar results in white sturgeon fed increasing digestible carbohydrate.

Results of this study provided useful information for better diet formulation for this emerging commercial species. The provision of adequate dietary cornstarch (>18%) could improve the growth performance of Japanese seabass (weight from 343g to 594g). Based on WG, the optimal dietary cornstarch level of Japanese seabass in grow-out phase estimated by second-order polynomial model was 27.0%.

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