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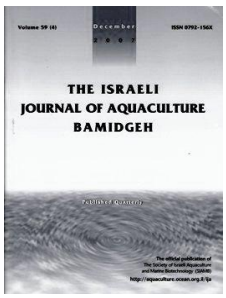

Effects of Dietary Pregelatinized Corn Starch on Growth, Apparent Digestibility, and Digestive Enzyme Activity of Large Yellow Croaker Fingerlings (*Pseudosciaena crocea*)

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Abstract

An 8-week feeding trial in floating seawater cages (1.0 × 1.0 × 1.5 m) was conducted to investigate the effects of dietary carbohydrate level on growth, digestion, and feed utilization of large yellow croaker fingerlings (6.0±0.10 g). Six isonitrogenous (crude protein 41%) and iso-lipid (11%) diets were prepared with pregelatinized corn starch as the carbohydrate source to obtain starch contents of 1.9% (control), 7.3%, 13.6%, 19.4%, 24.8%, or 31.86%. Weight gain, specific growth rate, feed efficiency, and protein efficiency ratio were significantly enhanced ($p<0.05$) by the dietary starch supplementation, and peaked in fish fed the diet containing 19.4% starch. There were no significant differences in survival ($p>0.05$). Dietary starch enhanced the whole body crude lipid but there were no significant differences in crude protein, moisture, or ash. Concentration of liver glycogen, muscle glycogen, and serum glucose positively correlated with the dietary starch level. Dietary starch tended to enhance protease and amylase activity of the liver, intestine, and stomach, while amylase activity of the intestine was significantly improved ($p<0.05$). Apparent digestibility coefficients decreased as the dietary starch increased. Results suggest that the optimum dietary starch level for growth in large yellow croaker is 19.4%.

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Introduction

Large yellow croakers (*Pseudosciaena crocea* R.) are commercially important marine fish in the western Pacific and China. There is increasing demand for adequate cost-effective aquafeeds. Cost effectiveness can be increased by utilizing the protein-sparing effects of carbohydrates in diets (Mohapatra et al., 2003; Stone et al., 2003; Wu et al., 2007; Enes et al., 2008; Ye et al., 2009) or by enhancing the dietary lipid level in feeds (Song et al., 2010). Carbohydrates are the most economical source of dietary energy, compared to protein and lipid, however, the ability of fish to utilize carbohydrate is limited and varies among species (NRC, 1993). No more than 20% digestible carbohydrates appears optimal for marine or coldwater fish although higher levels are utilized by fresh or warm water fish (Wilson, 1994). The utilization of dietary carbohydrate by fish appears to be related to how their digestive and metabolic systems adapt to different aquatic environments and to the dietary carbohydrate level and complexity.

Starch and non-starch polysaccharides (NSP) are the dominant carbohydrate groups in plants used for aquaculture feeds. NSP have a structural role in plants and seeds and are relatively indigestible. Starch comprises primarily glucose and is a potential source of energy (Stone et al., 2003). Hence, much effort has been invested in studying the ability of fish to utilize energy from starch in its raw and processed forms (Wilson, 1994). The processing of raw dietary carbohydrate by gelatinization, cooking, extrusion, or pregelatinization improves digestion and utilization in many fish (Peres and Oliva-Teles, 2002; Stone et al., 2003; Krogdahl et al. 2005).

The purpose of this study was to evaluate the ability of juvenile large yellow croaker to utilize pregelatinized corn starch by determining the effects of different levels on growth performance, whole-body composition, apparent digestibility coefficients, and digestive enzyme activity.

Materials and Methods

Experimental diets. A basal diet was formulated with fishmeal and casein as protein sources, menhaden fish oil as the lipid source, and six levels of pregelatinized corn starch as the carbohydrate source (Table 1). Yttrium oxide was used as an inert tracer to determine the apparent digestibility of the dietary nutrients. The ingredients were ground into a fine powder through a 246-μm mesh and thoroughly mixed with the menhaden fish oil. Water was added to produce a stiff dough that was pelleted with an experimental feed mill (F-26 II, South China University of Technology, China) and dried for about 12 h in a ventilated oven at 45°C. After drying, the diets were broken up, sieved into pellets of 1.5 × 3.0 mm and 2.5 × 4.0 mm, and stored at -20°C until use.

Feeding trial. Large yellow croaker (*Pseudosciaena crocea*) juveniles were obtained from a commercial farm in Ningbo, China, stocked into sea cages (3.0 × 3.0 × 3.0 m) and conditioned for two weeks during which they were fed the basal diet twice daily to satiation. At the start of the experiment, the fish were fasted for 24 h, anesthetized with tricaine methane-sulfonate (MS-222, Shanghai Reagent Corporation, China; 1:10000), and weighed. Fish (6.0±0.10 g) were randomly distributed into 18 floating seawater cages (1.0 × 1.0 × 1.5 m) at 60 fish per cage. Each diet was randomly assigned to three cages. Fish were hand-fed to apparent satiation twice daily (05:30, 17:30) for eight weeks. During the experimental period, rearing temperature was 26-32°C, salinity was 22-28, and dissolved oxygen was 6-7 mg/l.

Sampling. At the end of the feeding trial, the fish were fasted for 24 h and harvested. All fish from each cage were collectively weighed to obtain final biomass. Five fish per cage were collected and stored frozen at -20°C to determine proximate composition. Another three fish from each cage were anesthetized with eugenol, and blood samples were collected from the caudal vasculature with a 1-ml syringe and 27-gauge needle. The blood was allowed to clot at room temperature for 4 h, the serum was removed by centrifugation (836 × *g*, 10 min, 4°C) and frozen at -80°C until assayed, and the liver, stomach, and intestinal tract contents were obtained. Chyme was removed from the gut and stomach using distilled water and stored frozen at -80°C for analysis.

Table 1. Formulation and proximate composition of diets for large yellow croaker containing different levels of starch.

	Pregelatinized corn starch (% in diet)					
	0 (control)	*6.5*	*13*	*19.5*	*26*	*32.5*
Ingredient						
Fishmeal[1]	34.5	34.5	34.5	34.5	34.5	34.5
Casein[2]	20.0	20.0	20.0	20.0	20.0	20.0
Microcrystalline cellulose[3]	32.6	26.1	19.6	13.1	6.6	0.1
Pregelatinized corn starch[4]	0.0	6.5	13.0	19.5	26.0	32.5
Fish oil	7.0	7.0	7.0	7.0	7.0	7.0
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[5]	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix[6]	1.5	1.5	1.5	1.5	1.5	1.5
Sodium alginate	0.5	0.5	0.5	0.5	0.5	0.5
Glycin and lycine	0.3	0.3	0.3	0.3	0.3	0.3
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05
Y_2O_3[7]	0.04	0.04	0.04	0.04	0.04	0.04
Proximate analysis (% dry weight basis)						
Crude protein	40.7	41.2	40.7	40.7	40.7	40.5
Crude lipid	11.0	11.0	10.4	10.8	11.1	11.4
Starch	1.9	7.3	13.6	19.4	24.8	31.8
Ash	9.2	9.1	9.1	9.3	9.1	9.1
Energy (kJ/g)[8]	14.2	15.3	16.0	17.2	18.2	19.5
P:E (mg/kJ)	28.7	26.9	25.4	23.7	22.4	20.8

[1] dry-matter basis: 67.5% crude protein, 7.1% crude lipid; Evergreen Group, Guangzhou, China
[2] dry-matter basis: 96.4% crude protein, 1.2% crude lipid, Sigma Chemicals, St. Louis, MO, USA
[3] Qufu Tianli Medical Supplements, Shandong, China
[4] Modistar, Jilin, China; gelatinization grade >90%
[5] per kg diet: NaF 2 mg; KI 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%) 50 mg; $CuSO_4 \cdot 5H_2O$ 10 mg; $FeSO_4 \cdot H_2O$ 80 mg; $ZnSO_4 \cdot H_2O$ 50 mg; $MnSO_4 \cdot H_2O$ 60 mg; $MgSO4 \cdot 7H_2O$ 1200 mg; $Ca(H_2PO_3)_2 \cdot H_2O$ 3000 mg; NaCl 100 mg; Zoelite 5.447 g.
[6] per kg diet: thiamin 25 mg; riboflavin 45 mg; pyridoxine HCl 20 mg; vitamin B_{12} 0.1 mg; vitamin K_3 10 mg; inositol 800 mg; pantothenic acid 60 mg; niacin acid 200 mg; folic acid 20 mg; biotin 1.20mg; retinol acetate 32 mg; cholecalciferol 5 mg; alpha-tocopherol 120 mg; ascorbic acid 2000 mg; choline chloride 2500 mg ethoxyquin 150 mg microcrystalline cellulose 9.011 g.
[7] Fluka Chemicals
[8] calculated as protein 24 kJ/g, fat 38 kJ/g, starch 17 kJ/g

To determine apparent digestibility coefficients (ADC) of protein, lipid, and starch, the remaining fish were anesthetized with eugenol and manually stripped of feces 6 h after feeding. Feces were collected twice a week until sufficient dried feces was collected for analysis. Pooled feces from each group of fish were freeze-dried prior to analysis for yttrium oxide, crude protein, lipid, and starch content. Yttrium oxide in the diet and feces was determined by inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, VISTA-MPX, Varian, USA) after perchloric acid digestion.

Analysis of diets and fish body composition. Samples of the diets and fish body were dried to a constant weight at 105°C to determine the dry matter content. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method, lipid by ether extraction using Soxhlet (B-801, Switzerland), ash by combustion at 550°C, and starch according to Thivend et al. (1972).

Glycogen concentration. Glycogen concentrations in the liver and muscle were determined according to the method described by Hassid and Abraham (1957). Hydrolyzed glucose was assayed using a commercial glucose reagent (Jiancheng Bio-engineering Institute, Nanjing, China). Serum glucose concentration was measured by the glucoseoxidase method (Barham and Trinder, 1972) using a commercial diagnostics glucose kit (Huili Biological Science & Tech. Co., Ltd. Changchun, China).

Digestive enzyme assay. Liver, stomach, and intestinal tract contents were removed from five fish per cage, weighed, homogenized in ice-cold distilled water at 1:5 (w/v), and centrifuged (1800 × *g*, 30 min, 4°C). The supernatants were removed and kept at 4°C for analysis within 24 h. Protease activity of the liver, stomach, and intestine was analyzed following the method of Natalia et al. (2004). Activity was assessed using different buffers to test pH ranges (Chong et al., 2002). Protease activity of the stomach was analyzed in pH 2.2 and of the liver and intestine in pH 7.5. Amylase activity of the supernatants was measured according to Worthington (1993) using specific analytical procedures and commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). A total of 0.5 ml substrate solution was added to 0.01 ml enzyme preparation followed by 7.5 min incubation at 37°C. This was followed by addition of 0.5 ml dinitrosalicylic acid and 3.0 ml distilled water. Absorbance at 660 nm was recorded.

Calculations and statistical methods. The following variables were calculated: weight gain (%) = 100(Wt - W0)/W0, where Wt is the final average weight and W0 is the initial

average weight of the fish, specific growth rate (SGR) = 100(Ln Wt - Ln W0)/t, where t is the duration of experimental days, feed efficiency (FE) = wet wt gain/dry diet fed, survival = 100(final no. fish/initial no. fish), protein efficiency ratio (PER) = 100(wt gain/protein intake). Apparent digestibility coefficients (ADC) of the dry matter and nutrients were determined using the equation: ADC of nutrient or energy = 100(1 - [dietary Y_2O_3/fecal Y_2O_3] × [fecal nutrient or energy/dietary nutrient or energy]). Data were subjected to analysis of variance and Tukey's multiple range test using SPSS 11.0 for Windows with significance at $p<0.05$.

Results

The dietary starch level did not significantly influence survival but there were significant differences between treatments in weight gain, specific growth rate, feed efficiency, and protein efficiency ratio (Table 2). There were no significant differences in whole-body protein, moisture, or ash but crude lipid significantly increased with increasing dietary starch levels. The dietary starch levels did not significantly affect protease activity but amylase activity in the intestine significantly increased when pregelatinized corn starch was included in the diet. ADC of protein, lipid, and starch decreased when pregelatinized corn starch was included in the diets. The starch supplement significantly improved concentrations of liver glycogen, muscle glycogen, and serum glucose (Fig. 1).

Table 2. Growth, feed utilization, survival, whole body composition, enzyme activity, and nutrient digestibility in large yellow croaker fed diets containing different amounts of starch (mean±SE, n = 3).

	Pregelatinized corn starch (% dry diet)						*Anova*	
	1.9	*7.3*	*13.6*	*19.4*	*24.8*	*31.8*	*F*	*p*
Wt gain (%)	154.0±9.54^{d}	210.1±9.56^{c}	328.0±11.55^{b}	383.2±7.06^{a}	305.8±11.90^{b}	308.1±6.68^{b}	78.525	<0.001
SGR (%/d)	1.64±0.07^{d}	2.02±0.06^{c}	2.60±0.05^{ab}	2.81±0.03^{a}	2.50±0.05^{b}	2.51±0.03^{b}	78.999	<0.001
Feed efficiency	0.50±0.01^{d}	0.75±0.01^{c}	0.93±0.01^{a}	0.95±0.04^{a}	0.88±0.02^{ab}	0.81±0.02^{bc}	58.052	<0.001
PER	1.23±0.03^{d}	1.82±0.03^{cd}	2.28±0.04^{a}	2.33±0.10^{a}	2.16±0.05^{ab}	2.00±0.05^{bc}	59.102	<0.001
Survival (%)	100.0±0.00	100.0±0.00	99.4±0.56	100.0±0.00	99.4±0.56	100.0±0.00	0.800	0.571
Whole body composition (% wet wt)								
Moisture	75.8±0.86	76.0±0.12	75.9±0.27	75.6±0.22	75.9±0.84	76.5±0.09	0.311	0.897
Crude protein	14.6±0.15	14.4±0.23	14.5±0.09	14.4±0.06	14.4±0.09	14.0±0.06	2.051	0.143
Crude lipid	4.2±0.15^{c}	4.3±0.21^{c}	4.4±0.21^{bc}	4.4±0.16^{bc}	5.1±0.19^{ab}	5.7±0.08^{a}	11.657	<0.001
Ash	4.0±0.09	3.9±0.12	3.9±0.02	3.8±0.06	3.8±0.05	4.1±0.05	1.699	0.209
Enzyme activity (U/mg protein)								
Liver protease	0.17±0.020	0.18±0.044	0.22±0.024	0.26±0.019	0.25±0.015	0.26±0.023	2.607	0.081
Intestine protease	0.40±0.04	0.51±0.07	0.62±0.05	0.66±0.09	0.77±0.09	0.72±0.11	2.985	0.056
Stomach protease	12.99±0.96	19.12±2.84	19.59±1.64	20.57±1.37	20.53±1. 00	18.06±2.48	2.389	0.101
Liver amylase	0.23±0.01	0.24±0.03	0.25±0.01	0.25±0.03	0.28±0.04	0.30±0.04	0.880	0.523
Intestine amylase	0.34±0.06^{b}	0.58±0.06^{ab}	0.58±0.04^{ab}	0.51±0.06^{ab}	0.59±0.04^{a}	0.48±0.03^{ab}	3.567	0.033
Stomach amylase	0.07±0.012	0.08±0.015	0.09±0.009	0.09±0.003	0.08±0.009	0.09±0.009	0.533	0.747
Nutrient digestibility (%)								
ADC of protein	90.2±0.76^{a}	89.4±0.64^{a}	88.2±0.42^{a}	87.8±0.41^{a}	84.6±0.55^{b}	84.7±0.56^{b}	17.129	<0.001
ADC of lipid	92.2±0.61^{a}	90.4±0.58^{ab}	89.1±0.39^{b}	88.1±0.41^{bc}	85.5±0.52^{d}	86.0±0.51^{cd}	25.621	<0.001
ADC of starch	-	88.0±0.72^{a}	87.5±0.45^{a}	89.7±0.35^{a}	81.2±0.68^{b}	74.3±0.93^{c}	94.444	<0.001

Means in a row with different superscripts significantly differ ($p<0.05$).
SGR = specific growth rate, PER = protein efficiency ratio, ADC = apparent digestibility coefficient.

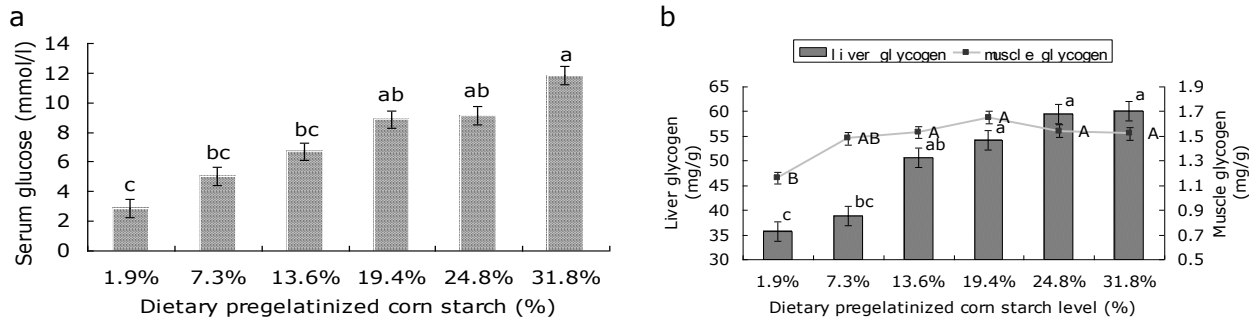

Fig. 1. Levels of (a) serum glucose and (b) glycogen concentration in liver and muscle of large yellow croaker fed diets containing pregelatinized corn starch for 8 weeks.

Discussion

An appropriate amount of dietary carbohydrate can speed the growth of fish (Mohapatra et al., 2003; Wu et al., 2007). In the present study, fish fed diets with higher levels of energy had higher PER. To some extent, the dietary starch supplied energy in a protein-sparing manner, in accordance with Stone et al. (2003). Weight gain and SGR significantly increased with the dietary starch level from 1.9% to 19.4% and then slightly decreased, suggesting that incorporation of dietary corn starch increases growth performance in the large yellow croaker in comparison to a diet without corn starch and that 19.4% inclusion is optimal for large yellow croakers. Similar results were found in Pacific salmon (Hardy, 1991) and cobia (Ren et al., 2011). Better growth was found in striped bass and sunshine bass fed a diet containing 25% dietary polysaccharide (Rawles and Gatlin, 1998), European sea bass fed a diet containing 12.5% raw+12.5% gelatinized starch (Peres and Oliva-Teles, 2002), and *Labeo rohita* fry fed a diet containing 45% gelatinized carbohydrate (Mohapatra et al., 2003). Yellowfin seabream utilize raw corn, tapioca, and potato starch better than pre-gelatinized corn starch at a level of 20% (Wu et al., 2007). The differences between fishes could be due to species variations, processing conditions, source of carbohydrate, or experimental conditions.

In the present study, whole-body lipid contents significantly increased with the increasing dietary starch level, similar to results in silver perch (Stone et al., 2003) and gilthead sea bream (Enes et al., 2008). High levels of dietary carbohydrate resulted in increased liver glycogen, muscle glycogen, and serum glucose, indicating that excess dietary carbohydrate was deposited as lipid and glycogen in large yellow croaker.

Amylase activity in the intestine increased with the increasing dietary starch, evidence of the adaption of amylase to the dietary level of carbohydrate. Similar results were reported in juvenile cobia (Ren et al., 2011) and *L. rohita* fry (Mohapatra et al., 2003). However, improved amylase activity did not enhance the ADC of carbohydrate. A significant decrease was observed in ADC of starch in fish fed the diets containing 24.82% or 31.76% starch, similar to the reduced ADC of starch that acompanied increased dietary starch in silver perch (Stone et al., 2003). This results, perhaps, from an overload on the digestive carbohydrases (α-amylase, β-glucanases, β-xylanases) in the digestive tract. Carbohydrases can improve the energy digestibility of fish by releasing previously unavailable glucose, galactose, and xylose (Kumar et al., 2006). Unavailable glucose, galactose, and xylose increases with increasing dietary starch as there is a threshold at which the enzyme system is saturated by substrates and no further digestion can occur (Stone et al., 2003).

The ADC of protein decreased with the increasing dietary starch level but there were no significant differences among groups, similar to findings of Mohapatra et al. (2003). Dextrin and glucose have a negative affect on protein digestibility (Storebakken et al., 1998). Monosaccharides inhibit amino acid transport in the intestine (Vinardell, 1990). Increased levels of glucose production at higher starch levels could therefore be one reason for the decrease in protein digestion. In this study, protein digestibility was significantly reduced as the dietary starch level rose, similar to protease activity in European sea bass (Enes et al., 2006) and gilthead sea bream (Enes et al., 2008). Lipid digestibility significantly dropped with the increasing starch level and reached its lowest value in fish fed the diets containing 24.8% or 31.8% starch. A similar effect of carbohydrate on lipid digestibility was shown in rainbow trout (Storebakken et al., 1998) and gilthead sea bream (Venou et al., 2003), indicating a negative influence of undigested starch and/or an excessive intake of lipid. Gelatinization of starch can enhance the digestibility of starch, compared to the low digestibility of native starch (Peres and Oliva-Teles, 2002; Mohapatra et al., 2003). Results of the present study indicate that juvenile large yellow croaker can digest gelatinized starch well (ADC starch ranged 87.5-89.7%) when fed diets containing 1.9-19.4% starch. However, ADC of starch significantly dropped in fish fed higher amounts of starch, similar to findings in cobia (Ren et al., 2011).

In conclusion, results from this study demonstrate that diets containing 19.4% starch, primarily from pregelatinized corn starch, are well utilized by large yellow croaker

as the highest weight gain, SGR, and feed efficiency were obtained with this level. These observations may be useful in the development of relatively low-cost aquafeeds in China.

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