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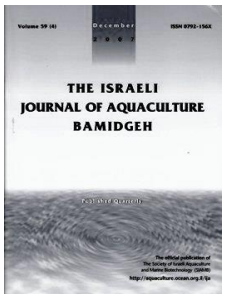

Effects of Dietary Carbohydrates with Different Molecular Complexity on Growth Performance, Feed Utilization, and Metabolic Responses of Juvenile Turbot *Scophthalmus maximus*

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Keywords: turbot; carbohydrate; nutrition; growth; feed utilization

Abstract

A 9 week study was conducted to evaluate the ability of juvenile turbot *Scophthalmus maximus* (initial body weight: 8.06 ± 0.08 g) to utilize carbohydrates of different molecular complexity (glucose, sucrose and dextrin) diets. Triplicate groups of fish were hand-fed each of the diets in a re-circulated water system. Results showed that weight gain rate and feed efficiency of fish fed dietary dextrin and the control diets were higher than those fed dietary glucose and sucrose diets ($P<0.05$). Fish fed dietary sucrose had significantly higher daily feed intake than those fed the other three diets ($P<0.05$). The apparent digestibility coefficients of carbohydrates were glucose > dextrin > sucrose with the lowest occurring in fish fed dietary sucrose. Lipid content in muscle and liver was significantly higher in fish fed the control diet, and muscle glycogen was significantly highest ($P<0.05$) in fish fed dietary glucose. The order of the liver glycogen was glucose > sucrose > dextrin > control. Insulin was significantly highest ($P<0.05$) in fish fed dietary dextrin plasma. In fish fed the control diet total cholesterol in plasma was highest ($P<0.05$), and triacylglycerols in plasma of fish fed the control and dietary dextrin diets were significantly highest ($P<0.05$). In conclusion, the present study suggests that turbot can utilize dextrin more efficiently than glucose and sucrose.

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Introduction

Protein is the most expensive single nutrient in formulated diets. Excess dietary protein levels may lead to consumption of protein for energy purposes, which increases nitrogenous losses. Therefore, both from an economical and environmental perspective, it is important to provide adequate energy from lipids and carbohydrates in fish diets to minimize the use of protein.

Carbohydrate is the least expensive energy source, and its inclusion in diets reduces feed costs. However, unlike most mammals, fish have a limited capacity to utilize dietary carbohydrates (Wilson, 1994; Krogdahl et al., 2005). Excess levels of carbohydrate can reduce growth rate in fish, and are often accompanied by poor feed utilization. The efficiency of carbohydrate utilization is not only associated with the particular strain of fish (Peres et al., 1999), but with other factors such as culture strategy, diet composition, treatment of carbohydrates especially the molecular complexity, and level of carbohydrate (Stone, 2003). Certain species, such as rainbow trout *Oncorhynchus mykiss* (Hung and Storebakken, 1994), grouper *Epinephelus malabaricus* (Shiau and Lin, 2001) and grass carp Ctenopharyngodon idella (Tian et al., 2004), have been shown to utilize mono- and di-saccharides as well as, or better than polysaccharides and oligosaccharides. Species such as sunshine bass *Morone chrysops* female × *M. saxatilis* male (Hutchins et al., 1998), flounder *Paralichthys olivaceus* (Lee et al., 2003), southern catfish *Silurus meridionalis* (Fu, 2005), European sea bass *Dicentrarchus labrax* (Enes et al., 2006), gilthead sea bream *Sparus aurata* (Enes et al., 2008) and cobia *Rachycentron canadum* (Cui et al., 2010) are unable to efficiently utilize simple carbohydrates as an energy source.

Due to tolerance of salinity, crowded environments, and rapid growth, turbot *Scophthalmus maximus* adapts well to in-door industrialized culture and is a commercially important marine flatfish cultured in China, especially in north. Turbot are able to metabolize dietary carbohydrates (Nijhof and Bult, 1994). After being injected with glucose, turbot were able to adjust carbohydrate metabolism efficiently within 24 h (Garcia-Riera and Hemre, 1996). Limited research has been conducted on carbohydrate utilization in turbot. The aim of the present study was to evaluate the effects of carbohydrates with different molecular complexity (glucose, sucrose, and dextrin) on growth performance, feed utilization, and metabolic responses in turbot. Excessive dietary carbohydrate may result in prolonged hyperglycemia (Hatlen et al., 2005), hepatic anti-oxidative damage (Azaza et al., 2013), and high fat deposition in whole body and liver (Hemre et al., 2002). Some hematological parameters related to carbohydrate metabolism were also analyzed in this study.

Materials and Methods

Experimental diets. Ingredients and proximate composition of four isonitrogenous and isoenergetic experimental diets are given in Table 1. There was no supplementation of carbohydrates in the control diet (C0). Based on the control, three experimental diets were prepared with the supplementation of glucose (G15), sucrose (S15), and dextrin (D15) at the level of 15%, respectively. Yttrium oxide (0.1%) was added as an indicator for digestibility measurements. All ingredients were ground into fine powder with a mini-type mill (YQ50, Shanghai Saishan Powder Machinery Manufacturing CO., LTD, China) through 246-μm mesh, and then thoroughly mixed with fish oil. Water was then gradually added to produce stiff dough which was pelleted using an experimental feed mill (F-26(II), South China University of Technology, China). The pellets (1.5 × 3.0 mm) were dried for about 12 h in a ventilated oven at 50°C and then sealed in a sample bag and stored at -20°C.

Table 1. Ingredients and proximate compositions of the experimental diets

Ingredients	*C0*	*G15*	*S15*	*D15*
white fish meal	360.00	360.00	360.00	360.00
casein	172.00	172.00	172.00	172.00
gelatin	43.00	43.00	43.00	43.00
carboxymethyl cellulose	10.00	10.00	10.00	10.00
glucose		150.00		
sucrose			150.00	
dextrin				150.00
microcrystalline cellulose (CMC)	225.00	135.00	135.00	135.00
soybean lecithin	20.00	20.00	20.00	20.00
fish oil	140.00	80.00	80.00	80.00
vitamin premix [1]	5.00	5.00	5.00	5.00
mineral premix [2]	10.00	10.00	10.00	10.00
Attractants[3]	5.00	5.00	5.00	5.00
choline chlorine	2.50	2.50	2.50	2.50
ethoxyquin	0.50	0.50	0.50	0.50
calcium propionate	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	5.00	5.00	5.00	5.00
yttrium oxide	1.00	1.00	1.00	1.00
proximate analysis (% dry matter)				
dry matter	90.45	90.08	91.01	90.87
crude protein	44.12	45.34	44.59	45.62
Crude lipid	16.48	10.93	10.36	10.78
N-free extract[4]	2.05	15.48	17.00	15.61
Ash	14.85	14.75	14.55	14.49
Gross energy (kJ/ g)	21.71	20.59	20.03	20.61
Digestible energy (kJ/g)	17.52	17.11	12.48	16.50

[1]vitamin premix (mg/kg diet): vitamin A, 32 mg; vitamin D, 5 mg; vitamin E, 240 mg; vitamin K, 10 mg; vitamin B_1, 25 mg; vitamin B_2, 45 mg; nicotinic acid, 200 mg; vitamin B_6, 20 mg; biotin, 60 mg; inositol, 800 mg; calcium pantothenate, 60 mg; folic acid, 20 mg; vitamin B_{12}, 10 mg; vitamin C, 2000 mg; microcrystlline cellulose, 4292.54 mg.

[2]mineral premix (mg/kg diet): $CuSO_4 \cdot 5H_2O$, 10 mg; Na_2SeO_3, 20 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $Ca(IO_3)_2$, 60 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; Zeolite Powder, 18485 mg.

[3]attractants: taurine/glycine/betaine = 1/3/3.

[4]calculated by difference (100-protein-lipid-CMC-ash)

Experimental animals and procedure. Juvenile turbots were obtained from a commercial hatchery in Jiaonan, Shandong, China. Fish were acclimated to the re-circulating water system for 2 weeks and fed a commercial diet (Qingdao Great Seven Bio-Tech Co. Ltd., Qingdao, China). At the start of the 9 week feeding trial, fish (initial body weight: 8.06 ± 0.08 g) were not fed for 24 h and were then randomly distributed into 12 cylindrical fiberglass tanks. There were three replicates of each treatment in 500L tanks stocked with 28 turbots each. Three tanks were randomly assigned to each diet. Fish were hand-fed to apparent satiation twice daily at 07:00 and 18:00. Feed consumption and mortalities were recorded every day. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70°C and reweighed. Fecal samples were collected each morning for 15 days. During the 9-week feeding trial, animals were held under natural photoperiod. The water temperature was 19 ± 1 °C, pH 7.7 ± 0.1, salinity 29.2 ± 1 gm/L, dissolved oxygen concentration not less than 7.0 mg/L. Levels of free ammonia and nitrite were negligible.

Sample collection and chemical analysis. At the end of the feeding trial, fish were starved for 24 h. after which they were anesthetized with MS-222 and weighed. Blood was drawn from the caudal vein of 10 randomly chosen turbot juveniles using a heparinized syringe. Plasma was obtained by centrifuging blood at 2000 rpm for 10 min. These fish were then dissected to obtain liver and muscle, and then stored at -80°C.

Growth performance and feed utilization are expressed as follows:

Weight gain rate, WGR (%) = 100×[(final body weight–initial body weight)/initial body weight]

Daily feed intake, DFI (%) = 100×feed intake in dry matter/[(initial body weight+final body weight + the dead fish weight/2]/days;

Feed efficiency FE = wet weight gain/ feed intake in dry matter

Apparent digestibility coefficients, ADC (%) = 100×[1-(F×Dy)/(D/Fy)]

where F = nutrient or energy contents in feces, D = nutrient or energy contents in diet, Dy = Yttrium concentration in diet and Fy = Yttrium concentration in feces

Chemical composition of dietary ingredients, experimental diets, muscle and liver of fish were determined by methods of the Association of Official Analytical Chemist (AOAC, 1995). 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 24 h. Gross energy was analyzed using the Parr 6100 Automatic Bomb Calorimeter (Parr, Moline, IL, USA). Content of glycogen in muscle and liver was measured according to Plummer (Plummer, 1987). Yttrium concentration in diets and feces was determined according to Refstie, Helland and Storebakken (Refstie et al., 1997). Plasma glucose was determined using the glucose oxidase method (Sigma kit No. 510; Sigma Chemicals, St. Louis, MO). Plasma insulin was analyzed according to the method described by Plisetskaya, Dickhoff, Paquette and Gorb-man (Plisetskaya et al., 1986). Total cholesterol (CHO) and triacylglycerols (TAGs) were measured by the colorimetric enzymatic method (Trinder, 1969).

Statistical analysis. All statistical analyses were performed using the SPSS 13.0 for Windows. Results are presented as mean±S.E.M. All data were subjected to one-way ANOVA. When overall differences were significant at less than 5% level, Tukey's test was used to compare the mean values between individual treatments.

Results

Survival rate, growth performance and feed utilization. The results of survival rate, growth performance, and feed utilization of juvenile turbot fed experimental diets are shown in Table 2. There was no significant difference in survival rate between all the treatments (*P>0.05*). Survival rates ranged from 96.43%-100%.

Table 2. Survival, growth performance and feed utilization of juvenile *Scophthalmus maximus*

Diets	*Initial weight*	*Survival rate (%)*	*Final weight (g)*	*WGR (%)*	*DFI (%)*	*FE*
C0	8.06±0.08g	98.81±1.19	33.14±0.49b	278.58±3.31c	1.43±0.01a	1.24±0.03c
G15	8.06±0.08g	100.00±0.00	28.78±0.28a	223.67±3.57b	1.44±0.01a	1.17±0.02ab
S15	8.06±0.08g	96.43±2.06	27.17±0.46a	178.84±2.45a	1.66±0.02b	1.07±0.01a
D15	8.06±0.08g	97.62±1.19	33.05±0.80b	282.89±2.83c	1.48±0.03a	1.27±0.02c

Values (mean±S.E) of three replications in the same row with different superscripts are significantly different (*P*<0.05).
WGR: weight gain rate; DFI: daily feed intake; FE: feed efficiency

WGR of fish fed dietary dextrin (282.89±2.83%) was significantly higher (*P<0.05*) than in fish fed dietary glucose (223.67±3.57), and sucrose (178.84±2.45). There was no significant difference (*P>0.05*) in WGR between the control group and fish fed dextrin diets. WGR in fish fed dietary sucrose (178.84± 2.45%) was lowest.

Dietary treatments significantly affected DFI and FE of turbot (*P<0.05*). DFI (1.66 ± 0.06%) was significantly higher (*P<0.05*) in fish fed dietary sucrose than all other treatments. There was no significant difference in FE between the control and dextrin diet groups. However, FE in both groups was significantly higher *(P<0.05)* than in the glucose or sucrose diet groups.

ADC of nutrients is presented in Table 3. There was no significant difference (*P>0.05*) in ADC of protein and lipid between diets. However, ADC of carbohydrate and energy in diets was significantly affected (*P<0.05*) by different carbohydrate complexity. The order of the ADC of carbohydrates was glucose > dextrin > sucrose. ADC of energy was significantly lower in fish fed dietary sucrose; there was no significant difference (*P>0.05*) among the other three treatments.

Table 3. Apparent digestibility coefficients (ADC, %) of nutrients in juvenile *S. maximus*

Diets	*protein*	*lipid*	*carbohydrate*	*energy*
C0	93.10±0.34	92.49±0.69	N D	80.71±0.68^{b}
G15	92.68±0.27	90.98±0.66	85.77±0.76^{c}	83.09±0.71^{b}
S15	91.24±0.53	89.86±0.28	72.40±1.27^{a}	62.31±0.79^{a}
D15	92.89±0.31	91.28±0.79	80.84±0.36^{b}	80.08±0.44^{b}

Values (mean±S.E of three replications) in the same row with different superscripts are significantly different (P<0.05)

Muscle and liver proximate composition. Composition of muscle and liver are shown in Table 4. Dietary treatments did not significantly affect moisture, crude protein and ash content in muscle and liver (*P>0.05*). Crude lipid and glycogen content in muscle and liver were significantly affected by dietary treatments (*P<0.05*). Crude lipid content was significantly highest in muscle and liver of fish fed the control diet. Differences in crude lipid content in muscle were not significant among the three experimental treatments. However, crude lipid content in liver of fish fed dietary dextrin was significantly higher than dietary glucose or sucrose treatment groups. Glycogen content in both muscle and liver was significantly highest (*P<0.05*) in the glucose supplemented diet group. Difference was not significant in muscle glycogen contents between the control, sucrose or dextrin diets. The order of the liver glycogen content was glucose > sucrose > dextrin > control.

Table 4. Proximate chemical composition of muscle and liver in juvenile *S. maximus*

Diets	*C0*	*G15*	*S15*	*D15*
muscle				
moisture (%)	76.95±0.44	77.67±0.41	77.05±0.49	77.18±0.42
crude protein (%)	20.77±0.45	20.20±0.43	20.61±0.39	20.77±0.33
crude lipid (%)	1.54±0.03^{b}	1.16±0.05^{a}	1.27±0.06^{a}	1.26±0.03^{a}
ash (%)	0.14±0.00	0.14±0.00	0.14±0.00	0.14±0.00
glycogen	0.63±0.05^{a}	0.87±0.00^{b}	0.70±0.01^{a}	0.69±0.01^{a}
Liver moisture (%)	63.04±0.70	63.31±0.39	63.60±0.70	63.62±0.67
crude protein (%)	10.02±0.43	9.29±0.57	9.51±0.52	9.64±0.42
crude lipid (%)	21.05±0.69^{c}	13.40±0.64^{a}	14.57±0.68^{a}	18.46±0.31^{b}
ash (%)	N. D.	N. D.	N. D.	N. D.
glycogen	1.30±0.11^{a}	10.58±0.28^{d}	7.51±0.55^{c}	4.40±0.36^{b}

Values (mean±S.E) of three replications in the same row with different superscripts are significantly different (P<0.05);
Values of crude protein, crude lipid and ash were expressed on a wet weight basis.
N. D.: not detected.

Hematological parameters. The hematological parameters are presented in Table 5. There was no significant difference (*P>0.05*) in the concentration of plasma glucose among the all treatments. Insulin concentration in plasma of fish fed dietary dextrin was significantly higher (*P<0.05*) than the other three experimental diet groups. Plasma insulin concentrations in dietary glucose and sucrose groups were higher than the control group. CHO concentration in dietary glucose or sucrose supplementation groups were significantly lower (*P<0.05*) than the control. The order of CHO concentrations in plasma was control > dextrin > sucrose > glucose. Concentrations of plasma TAGs in the control group or dextrin-supplemented group were significantly higher (*P<0.05*) than the dietary glucose or sucrose groups.

Table 5. Hematological parameters of juvenile *S.maximus*

Diets	*glucose (mM)*	*insulin (mM)*	*CHO (mM)*	*TAGs (mM)*
C0	0.27±0.03	9.29±0.90^{a}	6.63±0.59^{b}	2.40±0.10^{b}
G15	0.33±0.03	12.08±1.61^{a}	4.21±0.36^{a}	1.45±0.13^{a}
S15	0.26±0.06	11.23±1.51^{a}	4.80±0.47^{a}	1.27±0.14^{a}
D15	0.28±0.03	16.61±1.06^{b}	5.31±0.74^{ab}	2.32±0.11^{b}

CHO: total cholesterol; TAGs: triacylglycerols;
Values (mean ± S.E) of three replications in the same row with different superscripts are significantly different (P<0.05)

Discussion

Data on WGR and FE in the present study suggest that dextrin was preferable to glucose and sucrose as a dietary carbohydrate source for turbots. This concurs with results reported for other fish species such as flounder (Lee et al., 2003), cobia (Cui et al., 2010) and gilthead sea bream (Enes et al., 2010). Dextrin was utilized more efficiently than simple carbohydrates by most fish (Wilson, 1994). This is related to absorption and metabolism of carbohydrates. Simple carbohydrates, such as glucose, are absorbed rapidly in the gut in a short time. Insulin secretion lags, and insulin receptors are deficient in fish. These three factors jointly cause a large portion of the absorbed simple carbohydrates to be excreted or converted into glycogen before body cells can utilize them efficiently (Deng et al., 2001). This hypothesis was supported by our data. Grass carp had been shown to utilize glucose and maltose more efficiently than starch and dextrin (Tian, 2004). WGR and FE of grouper were not significantly affected by different complex carbohydrates such as glucose and starch (Shiau and Lin, 2001). The ability of fish to utilize different complex carbohydrates varies according to the digestive physiology of individual species, as well as diet formulation and culture strategy (Wilson, 1994; Stone, 2003; Krogdahl et al., 2005; Luo and Xie, 2010).

Dietary energy, blood glucose, stomach volume, and gastric emptying rates have been recognized as major factors involved in feed intake regulation in fish (Rubio et al., 2009). However, information on the relationship between dietary carbohydrates and maximum feed intake is limited (Tran et al., 2008). High-carbohydrate diets with relative low digestible energy content could lead to increase in feed intake to meet the energy requirement of fish (Rubio et al., 2009).

Fish fed the sucrose-supplemented diet had the lowest glucose concentration in their blood, but significantly higher DFI (Table 2). This may be due to the interaction between digestible dietary energy and blood glucose, but further research is needed.

In the present study, dietary glucose, sucrose, or dextrin was negatively related to crude lipid in muscles compared to the control. This is in agreement with previous studies on other fish species, such as *piracanjuba Brycon orbignyanus* (Borba et al., 2006), European sea bass *Dicentrarchus labrax* (Moreira et al., 2008) and catfish *Rhamdia quelen* (Giovanni et al., 2010). This could be: (1) dietary lipid may be important in affecting carcass lipid in fish; (2) fish were able to digest the carbohydrates, and also move excess carbohydrates into long-term storage as fats in the liver (Peres and Oliva-Teles, 2002). In the present study, fish fed dietary dextrin had significantly higher liver lipid content than those fed dietary glucose or sucrose. At the same time, compared to the glucose- or sucrose-supplemented diets, the dextrin-supplemented diet resulted in significantly lower liver glycogen content. This suggests that turbot are more likely to convert excessive dextrin to lipids, not to glycogen that is stored in the liver. Turbot fed the dextrin-supplemented diet also had significantly higher concentrations of insulin, TAGs, and CHO in plasma than those fed with glucose- or sucrose-supplemented diets (Table 5).

In a glucose tolerance test on turbot *S. maximus*, there was a peak in plasma glucose 3 h post intraperitoneal injection, and then a gradual decrease to basal levels within 24 h (Garcia-Riera and Hemre, 1996). In the present study plasma glucose concentrations after feeding did not differ among all groups. This may be due to the sampling time. Hematological samples were collected from fish that had been starved for 24h. Concentrations of insulin, TAGs, and CHO in plasma and lipid and glycogen content in muscle or liver were significantly influenced by different complex dietary carbohydrates (glucose, sucrose and dextrin). This suggests that turbot have the ability to adjust their glucose metabolism through gluconeogenesis and lipogenesis.

Conclusion

Based on the growth performance and feed utilization data from this study we found that turbot utilize dextrin more efficiently than glucose and sucrose. Further research on the metabolism of carbohydrates and the interaction with other metabolic pathways (e.g., protein and lipid) in turbot is needed.

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