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Protein-sparing effect of carbohydrate in diets for juvenile turbot *Scophthalmus maximus* reared at different salinities*

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Abstract The aim of the present study was to investigate the protein-sparing effect of carbohydrate in diets for juvenile turbot (*Scophthalmus maximus*) reared at five salinities (12, 18, 24, 30, and 36). The fish were fed three isocaloric and isolipidic diets for 60 days. The results show that specific growth rate (SGR) and feed conversion efficiency (FCE) were higher in fish reared at salinities of 18 and 36, but lower at 12. Fish fed with diet C25P40 (25% carbohydrate and 40% protein) had lower SGR and FCE values compared with those fed with the C5P52 (5% carbohydrate and 52% protein) and C15P46 (15% carbohydrate and 46% protein) diets; however, there was no statistical difference between diet C5P52 and C15P46. SGR and FCE values were unaffected by diet composition in fish reared at salinity 36. Hepatic lipogenic enzyme activities were higher in fish reared at 18 and 36, but lower at 12, while glucokinase (GK) activity was higher in fish reared at 12, and lower at 18 and 36. Dietary starch enhanced GK activity while depressing lipogenic enzyme activity. However, lipogenic enzyme activity increased with increasing dietary starch in fish reared at 36. It is recommended that salinity should be maintained >12 in the farming of juvenile turbot. In addition, an increase in gelatinized starch from 5% to 15% could spare 6% dietary protein in fish reared at salinities of 18–30, while higher salinity (36) could improve dietary carbohydrate use and enhance the protein-sparing effect, which is linked with the induction of lipogenic capacities.

Keyword: *Scophthalmus maximus*; carbohydrate; salinity; protein-sparing effect; hepatic glycometabolism

1 INTRODUCTION

Over the past decade, finding alternatives to fishmeal has become an international goal for the sustainable development of the aquaculture feed industry and reducing nitrogen emissions (Hardy, 2010). To minimize protein use, carbohydrate-rich diets, with the advantages of cheap dietary energy, a relatively constant chemical composition and excellent availability on the world market, have been extensively used in aquaculture feed manufacturing (Gatlin et al., 2007). However, fish, especially carnivorous species, are generally considered "glucose intolerant" (Moon, 2001) and have a relatively limited ability to use carbohydrates (Stone, 2003). The principal reason for this is that endogenous glucose production (gluconeogenesis) does not seem to be regulated by carbohydrate intake level (glycolysis), resulting in poor regulation of glucose homeostasis in fish (Enes et al., 2009; Polakof et al., 2012).

Turbot (*Scophthalmus maximus*), a carnivorous fish, were introduced into China from Europe by the second author of this paper in 1992. It has since become a major commercial mariculture species in northern China and, currently, turbot farming is expanding into other coastal regions of China.

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Table 1 Ingredient and proximate composition of experimental diets (% DM)

Ingredient (%)	Diet		
	C5P52	C15P46	C25P40
Fish meal[a]	63.50	53.50	44.00
Casein[b]	11.00	11.00	11.00
Fish oil	6.00	6.50	7.00
Gelatinized starch[c]	5.00	15.00	25.00
Microcrystalline cellulose	9.00	8.50	7.50
Mineral premix[d]	1.50	1.50	1.50
Vitamin premix[e]	1.00	1.00	1.00
Soy lecithin	2.00	2.00	2.00
Binder	1.00	1.00	1.00
Proximate composition			
Dry matter (DM) (%)	93.02	92.43	92.27
Crude protein (% DM)	52.21	45.73	39.55
Crude lipid (% DM)	10.17	10.11	10.08
Starch (% DM)	4.91	14.14	23.30
Energy (kJ/g DM)	17.22	17.18	17.25

[a] Fish meal, produced in Peru: crude protein 66.10% DM, crude lipid 5.98% DM, carbohydrate 0.44% DM; [b] casein, produced by Sigma Chemical (St. Louis, MO, USA): crude protein 93.6% DM; [c] gelatinized starch, produced by Jixian Starch Co. Ltd. (Xiamen, China): dry matter 91.9%, crude protein 0.4% DM, crude lipid 0.5% DM, starch 92.6% DM; [d] mineral premix (mg/kg diet): $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MgSO_4 \cdot 7H_2O$, 1 200; $Ca(H_2PO_3)_2 \cdot H_2O$, 5 000; $Ca(IO_3)_2$ (1%), 60; $MnSO_4 \cdot H_2O$, 45; $CoCl_3$ (1%), 50; Na_2SeO_3 (1%), 20; Zoelite, 8485; [e] vitamin premix (mg or IU if mentioned /kg diet): retinyl acetate, 16 000 IU; thiamin, 24.5; pyridoxine, 19.8; vitamin B12, 0.1; cholecalciferol, 25 000 IU; all-rac-α-tocopheryl acetate, 200; menadione sodium bisulfate, 5.1; calcium propionate, 1 000; ascorbic acidpolyphosphate, 1 000; choline chloride, 2 500; myoinositol, 784; ethoxyquin, 500; niacin, 198; d-calcium pantothenate, 58.8; riboflavin, 36; folic acid, 19.6; biotin, 1.2.

However, environmental factors suitable for aquaculture of this species in different regions can vary, particularly salinity, and variations in salinities within aquatic habitats disrupt normal functioning of cellular and physiological processes (Evans, 2008). Teleost fish require large amounts of energy to achieve internal osmotic and ionic homeostasis through osmoregulation (Imsland et al., 2003; Tseng and Hwang, 2008), which results in adverse effects on growth, survival, feed conversion efficiency, disease resistance, etc. (Gaumet et al., 1995; Imsland et al., 2001; Zhang et al., 2011). Previous studies have demonstrated that carbohydrate metabolism play a critical role in the energy supply for osmoregulation (Tseng and Hwang, 2008), and carbohydrate metabolic pathways, including glycolysis, glycogenolysis and the pentose phosphate cycle, are affected by salinity (Soengas et al., 1993; Sangiao-Alvarellos et al., 2003; Krogdahl et al., 2004; Sangiao-Alvarellos et al., 2005). Therefore, we hypothesize that salinity may influence the protein-sparing effect of dietary carbohydrates.

In the present study, juvenile turbot were reared at five salinities (12, 18, 24, 30, and 36) and fed three isocaloric (17.20 kJ/g dry matter, DM) and isolipidic (10% crude lipids) diets containing three levels of carbohydrates (5%, 15%, and 25%) and three levels of crude protein (52%, 46%, and 40%) for 60 days. The effects of salinity and dietary composition on growth performance, biochemical indices and hepatic glycometabolic enzyme activities were analyzed. The objective of the study was to examine the influence of salinity on the protein-sparing effect of dietary carbohydrates in the expectation that this will contribute to further understanding the potential use of carbohydrates as energy sources in juvenile turbot reared at different salinities.

2 MATERIAL AND METHOD

2.1 Diet

The ingredients and approximate composition of the experimental diets are listed in Table 1. The experimental diets were isocaloric (17.20 kJ/g DM) with three levels of carbohydrate: 5%, 15%, and 25%. The more efficiently used gelatinized starch was used as the carbohydrate source (Lee et al., 2003b). The optimum protein and lipid levels required for juvenile turbot are ~50% and 10%, respectively (Caceres-Martinez et al., 1984; Lee et al., 2003a). To determine the protein-sparing effect of dietary carbohydrates, the amount of gelatinized starch in the experimental diets was increased at the expense of protein and microcrystalline cellulose. The three diets were designated C5P52 (C5=5% carbohydrate, P52=52% protein), C15P46 (C15=15% carbohydrate, P46=46% protein), and C25P40 (C25=25% carbohydrate, P40=40% protein). All ingredients were supplied by Qingdao Great Seven Bio-Tech Co. Ltd. (National Oceanographic Center, Qingdao, China) and were finely ground, thoroughly mixed and pelletized through a hand pelletizer into 2.0- or 3.0-mm pellets. The experimental diets were stored in airtight containers at -20°C until use.

2.2 Fish and experimental design

Juvenile turbot with an initial weight of 7.16±0.70 g

(mean±SD) were obtained from Tianyuan Aquatic Co. Ltd. (Yantai, China). Fish were randomly held in 240-L fiberglass tanks (45 tanks) containing natural seawater with salinity of 30 for 2 weeks. Then, the fish were acclimated to the target ambient salinities (12, 18, 24, and 36) with one to three salinity changes per day. The five salinity levels were obtained by adding either dechlorinated tap water or marine salt to natural seawater. Fish were acclimated to the salinities for 1 week and fed a commercial formulated feed (Forever International Trading Co. Ltd. Qingdao, China) before being fed with the three experimental diets (Table 1) for 60 days (26 turbot per tank with three replicates). Fish were fed twice daily (08:00–09:00 and 17:00–18:00) slightly in excess of satiation during the experimental period. The fish was fed the 2-mm pellet diet in the first month; in the second month, the 3-mm pellet diet was used. Residual food was removed daily with a siphon tube to estimate feed intake. The water was prepared at least 1 day before changing, aerated continuously to maintain dissolved oxygen near saturation levels, and 100% of the water volume was renewed daily. Water quality parameters during the experimental period were pH 7.67–8.04, temperature 14.2–19.9°C (average 17.4±1.9°C), dissolved oxygen 6.55–8.61 mg/L and total ammonia nitrogen <0.17 mg/L. Fish were exposed to natural photoperiod.

2.3 Sample

At the end of the 60-day feeding trial, the turbot were counted and weighted under moderate anaesthesia (benzocaine at 100 mg/L) after fasting for 24 h. Twelve fish were randomly sampled from each tank. Gill, liver and muscle tissue from three fish were taken for estimating Na^+, K^+-ATPase (NKA) activity, liver and muscle glycogen content, respectively. Total length (0.01 cm) of the other nine fish was measured, and whole fish, liver and visceral mass (0.01 g) were weighed for determination of condition factor (CF), hepatosomatic index (HSI), and visceral index (VSI), respectively. The livers were equally divided into two portions for measurement of hepatic glycolytic, gluconeogenic and fatty acid synthase enzyme activities. All samples were immediately frozen in liquid nitrogen and kept at -80°C until analyzed.

To minimize stress caused by handling, the remaining fish were fed for 4 more days. Nine fish per group were anesthetized (benzocaine at 100 mg/L) after fasting for 24 h. Blood was removed from the caudal vein using a 1-mL sterile plastic syringe and in under 1 min at each sampling time to avoid any blood metabolite response induced by handling stress. The blood was allowed to clot overnight at 4°C and the serum was obtained after centrifugation (5 000×*g* for 10 min at 4°C). All samples were immediately frozen in liquid nitrogen and kept at -80°C until analyzed.

2.4 Analytical method

2.4.1 Feed composition analyze

The chemical composition of the experimental diets was determined following standard AOAC methods (2000). Dry matter was determined by drying in an oven at 105°C to a constant weight; ash content was measured by incinerating in a muffle furnace at 600°C for 6 h; crude protein (*N*×6.25) was estimated by the Kjeldahl method after an acid digestion using an Auto Kjeldahl System (Buchi, Flawil, Switzerland); crude lipid was measured by petroleum ether extraction in a Soxtec Auto Extraction Unit 2050 (Foss Tecator, Hoganas, Sweden); gross energy was analyzed in an Oxygen Bomb Calorimeter Model 6100 (PARR, Moline, IL, USA); starch content was determined according to Thivend et al. (1972).

2.4.2 Enzyme activity

2.4.2.1 Glycolytic enzymes

To measure glucokinase (GK, EC 2.7.1.2) and pyruvate kinase (PK, EC 2.7.1.40) activities, liver samples were homogenized in 10-fold volumes of ice-cold buffer (80 mmol Tris, 2 mmol DTT, 5 mmol EDTA, 1 mmol 4-(2-aminoethyl)benzenesulfonyl fluoride, 1 mmol benzamidine, pH 7.6) and centrifuged at 900×*g* for 10 min at 4°C. The resultant supernatant was separated into two fractions for the measurement of GK and PK activities. GK activity was measured at 37°C and was estimated as previously described by Tranulis et al. (1996). To measure PK activity, the supernatant was centrifuged at 10 000×*g* for 20 min at 4°C and the resultant cytosolic fraction was used for enzyme activity measurement according to Foster and Moon (1986).

2.4.2.2 Gluconeogenic enzymes

To measure glucose-6-phosphatase (G6Pase, EC 3.1.3.9) activity, microsomes were obtained from liver samples as previously described by Mol et al. (1998). The microsomes were then suspended in buffer (100 mmol NaH_2PO_4, 2 mmol EDTA, 25 mmol Na_2HPO_4, 1 mmol DTT, pH 7), without further

treatment. The procedure of evaluating G6Pase activity of each sample, as previously described by Alegre et al. (1988), involved monitoring the increase in absorbance at 340 nm (NADH) using purified glucose dehydrogenase (Sigma, Sintra, Portugal) in excess as the coupling enzyme.

To measure phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) activity, liver samples were homogenized in 10-fold volumes of ice-cold buffer (250 mmol saccharose, 10 mmol *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethansulfonic acid], 1 mmol DTT). The homogenate was centrifuged at 900×*g* for 10 min at 4°C and the resultant supernatant was centrifuged at 10 000×*g* for 20 min at 4°C. The cytosolic (supernatant) and mitochondrial (1-mL pellet suspended per 500 mg liver buffer of homogenization) portions were separately frozen at -20°C before enzymatic assays. The mitochondrial fractions were sonicated at 150 W for 1 min (1 pulse/s, amplitude 50%) using a Scientz-II D ultrasonic cell crusher (Ningbo Scientz Biotechnology, Co. Ltd., Ningbo, China). The procedure of evaluating cytosolic and mitochondrial PEPCK activity of each sample, as previously described by Scholz et al. (1998), involved monitoring the decrease in absorbance (β-NAD) using purified malate dehydrogenase (Sigma, Portugal) as the coupling enzyme.

2.4.2.3 Lipogenic enzymes

To measure glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and fatty acid synthase (FAS, EC 2.3.1.38) activities, liver samples were homogenized in 5-fold volumes of ice-cold buffer (250 mmol sucrose, 2 mmol EDTA, 100 mmol NaF, 20 mmol Tris-HCl, 10 mmol β-mercaptoethanol, 0.5 mmol phenylmethyl sulphonyl fluoride, pH 7.4) and centrifuged at 30 000×*g* for 20 min at 4°C. The resulting supernatant was separated in two fractions for G6PD and FAS activity measurement. G6PD was evaluated at 30°C, as previously described by Bautista et al. (1988). FAS measured at 37°C following the method of Chakrabarty and Leveille (1969).

2.4.2.4 Specific enzyme activities

All enzyme activities were expressed per mg of hepatic soluble protein (specific activity). Protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as standard (Bradford protein assay kit, Tiangen Biotech, Beijing, China). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate per min at assay temperature.

2.4.3 Glycogen content and Na^+, K^+-ATPase (NKA) activity

Tissue glycogen content was measured by an enzymatic method using amyloglucosidase, as described by Keppler and Decker (1974). Gill Na^+, K^+-ATPase (NKA) activity was measured using a commercial kit: NKA (No. A016) from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.4.4 Serum metabolite assays

Serum glucose (GLU), cholesterol (CHOL) and triacylglycerol (TAG) levels were measured on a Hitachi Model 7600-11 auto-biochemistry machine (Hitachi High-Technologies Corporation, Tokyo, Japan). Serum growth hormone and cortisol levels were measured by radioimmunoassay. The antibodies were salmon/trout growth hormone antiserum (rabbit) (PAN1 from GroPep) and anti-cortisol antibody for fish (rabbit) (No. C8409 from Sigma). Salmon/trout growth hormone antiserum has been validated for flounder (Zang et al., 2013). Total plasma proteins were measured by the Bradford method, as mentioned above.

2.5 Calculations and statistical analysis

Specific growth rate (SGR) was calculated according to the formula of Ricker (1979): $SGR=(e^g-1)\times100$, where $g=(\ln(W_2)-\ln(W_1))/(t_2-t_1)$, W_2, and W_1 are mean weights on days t_2 and t_1, respectively. Daily feeding rate (F%) was calculated as $100C_T/(W_1+W_2)/2$ per day, where C_T is mean total food consumption calculated as (total feed supplied minus total remained feed) (dry weight). Feed conversion efficiency (FCE) was calculated as biomass gain per unit weight of food consumed (dry weight). Protein efficiency ratio (PER) was calculated as biomass gain per unit weight of protein consumed (dry weight). Condition factor (CF) was calculated as $100\ W/L^3$, where W is the weight of the fish and L the corresponding total length. Hepatosomatic index (HSI, %) was calculated as (liver weight/fish weight)×100%, and viscerasomatic index (VSI, %) was calculated as (viscera weight/fish weight)×100%.

Results were subjected to two-way analysis of variance (ANOVA), followed by a Duncan's test to delineate significance among fish groups using SPSS

Table 2 Initial body weight (g), final body weight (g), survival rate (%), specific growth rates (SGR, %/day), daily feeding rate (*F*, %), feed conversion efficiency (FCE, g/g) and protein efficiency ratio (PER, g/g) of juvenile turbot reared at five salinities (12, 18, 24, 30, and 36) and fed with three diets (C5P52, C15P46, and C25P40) (means±SD, *n*=3)

Salinity	Diet	Initial body weight (g)	Final body weight (g)	Survival rate (%)	SGR (%/day)	*F* (%)	FCE (g/g)	PER (g/g)
12	C5P52	7.17±0.08	17.05±1.11^{b}	80.77±3.85^{b}	1.45±0.10^{b}	1.19±0.04	1.12±0.03^{b}	2.14±0.06^{c}
	C15P46	7.19±0.05	19.58±2.93^{a}	97.44±2.22^{a}	1.68±0.08^{a}	1.24±0.03	1.22±0.01^{a}	2.67±0.02^{b}
	C25P40	7.12±0.03	19.03±2.86^{a}	93.59±2.22^{a}	1.70±0.08^{a}	1.21±0.03	1.22±0.02^{a}	3.13±0.02^{a}
18	C5P52	7.13±0.08	22.99±3.04^{a}	100	1.97±0.06^{a}	1.25±0.01	1.37±0.01^{a}	2.63±0.02^{c}
	C15P46	7.15±0.07	23.02±3.66^{a}	100	1.94±0.05^{a}	1.28±0.04	1.34±0.04^{a}	2.95±0.08^{b}
	C25P40	7.14±0.11	20.24±2.89^{b}	100	1.75±0.05^{b}	1.23±0.01	1.26±0.03^{b}	3.20±0.07^{a}
24	C5P52	7.18±0.08	21.76±1.90^{a}	100	1.86±0.07^{a}	1.21±0.05	1.36±0.02^{a}	2.60±0.04^{c}
	C15P46	7.19±0.08	20.98±2.25^{a}	100	1.80±0.03^{a}	1.24±0.01	1.29±0.01^{ab}	2.84±0.02^{b}
	C25P40	7.19±0.05	19.56±1.53^{b}	100	1.68±0.05^{b}	1.24±0.01	1.22±0.03^{b}	3.08±0.07^{a}
30	C5P52	7.13±0.02	21.57±1.07^{a}	100	1.86±0.09^{a}	1.28±0.03	1.28±0.01^{a}	2.45±0.02^{c}
	C15P46	7.18±0.08	20.06±1.64^{ab}	100	1.73±0.04^{ab}	1.26±0.03	1.22±0.04^{b}	2.68±0.09^{b}
	C25P40	7.15±0.01	19.15±1.90^{b}	100	1.65±0.08^{b}	1.24±0.01	1.20±0.04^{b}	3.04±0.11^{a}
36	C5P52	7.07±0.06	23.25±2.38	100	2.00±0.10	1.25±0.05	1.39±0.01	2.66±0.03^{c}
	C15P46	7.16±0.13	22.21±1.56	100	1.85±0.09	1.23±0.02	1.35±0.02	2.97±0.04^{b}
	C25P40	7.18±0.06	21.99±2.10	100	1.80±0.07	1.22±0.03	1.35±0.05	3.42±0.14^{a}
12	All diet combined	7.16±0.06	18.56±1.51^{c}	90.60±8.73^{b}	1.61±0.14^{c}	1.21±0.02^{b}	1.19±0.04^{d}	2.65±0.44^{d}
18		7.14±0.08	22.08±1.58^{a}	100^{a}	1.89±0.12^{a}	1.25±0.02^{a}	1.33±0.06^{ab}	2.93±0.26^{ab}
24		7.19±0.06	20.77±1.11^{b}	100^{a}	1.78±0.09^{b}	1.23±0.01^{ab}	1.29±0.03^{b}	2.84±0.22^{b}
30		7.15±0.05	20.26±1.31^{b}	100^{a}	1.75±0.10^{b}	1.26±0.02^{a}	1.23±0.02^{c}	2.72±0.27^{c}
36		7.14 ±0.09	22.45±1.55^{a}	100^{a}	1.88±0.10^{a}	1.24±0.01^{ab}	1.36±0.03^{a}	3.01±0.34^{a}
All salinity combined	C5P52	7.14±0.08	21.32±2.50^{a}	96.15±8.60^{b}	1.83±0.22^{a}	1.24±0.04	1.30±0.12^{a}	2.49±0.22^{c}
	C15P46	7.15±0.08	21.17±1.47^{a}	99.49±1.15^{a}	1.80±0.10^{a}	1.25±0.02	1.29±0.05^{a}	2.82±0.14^{b}
	C25P40	7.17±0.06	19.98±1.03^{b}	98.72±2.87^{a}	1.72±0.06^{b}	1.23±0.01	1.25±0.04^{b}	3.17±0.16^{a}
Interaction		ns	***	***	***	ns	**	**

Note: ns: non-significant; **P*<0.05; ***P*<0.01; ****P*<0.001. Different letters denote significant differences (*P*<0.05) between dietary treatments within each salinity regime or between different salinities independent of dietary treatments.

16.0 software (SPSS Inc., Chicago, IL, USA). For all analyzes, significant levels were set at $P<0.05$ unless otherwise stated. All data are represented as means±SD.

3 RESULT

3.1 Survival rate, growth performance, feed use and Na^+, K^+-ATPase activity

The initial mean weight±SD was 7.16±0.07 g with no significant difference among all groups ($P>0.05$) (Table 2). The survival rates of fish were 100% except those reared at a salinity of 12, which was significantly lower than other salinity groups ($P<0.05$), and the survival rate of fish fed with the lowest starch and highest protein diet C5P52 was significantly lower than those fed diet C15P46 and C25P40 ($P<0.05$) when they adapted to 12 (Table 2). Irrespective of diet composition, specific growth rate (SGR) and feed conversion efficiency (FCE) were significantly higher in fish maintained at 18 and 36, and lower at 12 ($P<0.05$). Overall, SGR and FCE of fish fed with the highest starch and lowest protein diet C25P40 were significantly lower than those fed with the other two diets ($P<0.05$). It is worth noting that SGR and FCE of fish reared at 36 were not affected by diet composition ($P>0.05$), and fish fed with the lowest starch and highest protein diet C5P52 had lower SGR and FCE than those fed with diet C15P46 or C25P40 when maintained at a salinity of 12 ($P<0.05$) (Table 2). The feed intake (*F*, %) of fish maintained at 12 was significantly lower than those reared at 30

Table 3 Condition factor (CF, % g/cm^3), hepatosomatic index (HSI, %), viscerasomatic index (VSI, %), liver glycogen (mg/g), muscle glycogen (mg/g) and gill Na^+, K^+-ATPase (NKA, μmol ADP/mg protein) of juvenile turbot reared at five salinities (12, 18, 24, 30, and 36) and fed with three diets (C5P52, C15P46, and C25P40) (means±SD)

Salinity	Diet	CF (% g/cm^3) n=9	HSI (%) n=9	VSI (%) n=9	Liver glycogen (mg/g) n=6	Muscle glycogen (mg/g) n=6	NKA (μmol ADP / mg protein) n=9
12	C5P52	3.30±0.23	1.19±0.11	5.07±0.45	42.29±4.28	0.89±0.06	3.48±0.27
	C15P46	3.29±0.10	1.18±0.09	5.20±0.16	41.52±4.77	0.94±0.07	3.37±0.15
	C25P40	3.33±0.18	1.27±0.10	5.27±0.55	44.97±4.18	0.93±0.06	3.16±0.28
18	C5P52	3.29±0.14	1.04±0.08^b	4.84±0.19	40.65±3.49	0.94±0.08	6.93±0.20
	C15P46	3.24±0.05	1.16±0.11^{ab}	5.18±0.27	41.06±3.19	0.96±0.06	7.03±0.19
	C25P40	3.29±0.12	1.25±0.05^a	5.33±0.58	43.00±3.46	1.02±0.07	6.83±0.12
24	C5P52	3.23±0.17	1.17±0.09	5.13±0.34	41.16±2.66	0.93±0.06	7.26±0.22
	C15P46	3.31±0.28	1.20±0.03	5.30±0.34	42.05±2.69	0.98±0.11	7.16±0.28
	C25P40	3.32±0.18	1.29±0.05	5.27±0.07	43.01±3.19	0.99±0.09	7.03±0.26
30	C5P52	3.37±0.10	1.18±0.16	5.48±0.37	38.88±4.33	0.92±0.08	8.16±0.43
	C15P46	3.32±0.21	1.19±0.19	5.28±0.07	42.00±2.67	0.94±0.12	7.75±0.14
	C25P40	3.33±0.11	1.28±0.09	5.38±0.04	42.83±4.15	0.96±0.07	7.53±0.35
36	C5P52	3.42±0.05	1.20±0.14	5.45±0.11	39.09±3.36	0.97±0.13	8.30±0.11
	C15P46	3.37±0.20	1.22±0.12	5.31±0.07	43.81±3.47	1.02±0.06	8.23±0.47
	C25P40	3.38±0.19	1.33±0.07	5.37±0.14	43.64±3.69	1.07±0.10	8.17±0.24
12	All diet combined	3.31±0.23	1.21±0.05	5.18±0.10	42.93±1.81	0.92±0.03^b	3.34±0.16^c
18		3.27±0.15	1.15±0.10	5.11±0.25	41.57±1.26	0.97±0.04^{ab}	6.93±0.10^b
24		3.29±0.20	1.22±0.06	5.23±0.10	42.07±0.92	0.96±0.03^{ab}	7.15±0.11^b
30		3.34±0.18	1.21±0.06	5.38±0.10	41.24±2.08	0.94±0.02^b	7.81±0.32^a
36		3.39±0.19	1.25±0.07	5.38±0.07	42.18±2.67	1.02±0.06^a	8.23±0.06^a
All salinity combined	C5P52	3.32±0.15	1.16±0.06^b	5.19±0.27	40.41±1.43^b	0.93±0.03^b	6.82±1.96
	C15P46	3.31±0.23	1.19±0.02^{ab}	5.25±0.06	42.09±1.04^{ab}	0.97±0.04^{ab}	6.71±1.93
	C25P40	3.33±0.19	1.28±0.03^a	5.32±0.05	43.49±0.88^a	0.99±0.05^a	6.54±1.96
Interaction		ns	ns	ns	ns	**	***

Note: ns: non-significant; *P<0.05; **P<0.01; ***P<0.001. Different letters denote significant differences (P<0.05) between dietary treatments within each salinity regime or between different salinities independent of dietary treatments.

(P<0.05), but not affected by diet composition (P>0.05) (Table 2). Protein efficiency ratio (PER) was significantly higher in fish maintained at 18 and 36, and lower at 12 (P<0.05). PER increased with the increase in dietary carbohydrates at each water salinity (Table 2).

3.2 Condition factor, hepatosomatic index, viscerasomatic index, liver glycogen, muscle glycogen and gill Na^+, K^+-ATPase

Hepatosomatic index (HSI) and liver glycogen content increased with the increase in dietary carbohydrate, but was unaffected by salinity (Table 3). Muscle glycogen content increased with the increase in dietary carbohydrate and was significantly higher in fish reared at 36 than those maintained at 30 (P<0.05) (Table 3). Condition factor (CF) and viscerasomatic index (VSI) were not affected by diet composition or water salinity (P>0.05) (Table 3). Gill Na^+, K^+-ATPase (NKA) activity increased with the increase in salinity, but was unaffected by dietary treatments (Table 3).

3.3 Serum glucose, triacylglycerol, cholesterol, growth hormone and cortisol

Serum glucose (GLU) levels were higher in fish reared at a salinity of 12, and lower at 18 and 36 (P<0.05), but unaffected by dietary treatments (Table 4). Triacylglycerol (TAG) and cholesterol (CHOL) levels were not affected by diet composition or water salinity (P>0.05) (Table 4). Growth hormone (GH) levels were higher in fish reared in salinities of

Table 4 Serum glucose (GLU, mmol/L), triacylglycerol (TAG, mmol/L), cholesterol (CHOL, mmol/L), growth hormone (GH, ng/mL) and cortisol (COR, ng/mL) levels in juvenile turbot reared at five salinities (12, 18, 24, 30, and 36) and fed with three diets (C5P52, C15P46, and C25P40) (means±SD, *n*=6)

Salinity	Diet	GLU (mmol/L)	TAG (mmol/L)	CHOL (mmol/L)	GH (ng/mL)	COR (ng/mL)
12	C5P52	1.87±0.09^{a}	1.88±0.14	3.01±0.27	0.41±0.05^{c}	76.41±5.55
	C15P46	1.54±0.13^{b}	1.89±0.08	2.99±0.24	1.00±0.07^{b}	69.33±2.34
	C25P40	1.70±0.10^{ab}	1.95±0.07	3.09±0.21	1.13±0.05^{a}	69.93±3.90
18	C5P52	1.34±0.05	1.74±0.10	2.86±0.14	1.45±0.04^{a}	53.06±4.24
	C15P46	1.40±0.10	1.79±0.14	2.87±0.18	1.38±0.03^{b}	53.76±2.89
	C25P40	1.47±0.09	1.82±0.05	3.01±0.11	1.29±0.04^{c}	55.03±1.05
24	C5P52	1.46±0.05	1.77±0.08	2.89±0.16	1.39±0.05^{a}	57.12±2.76
	C15P46	1.51±0.10	1.81±0.07	2.94±0.09	1.36±0.04^{a}	56.29±4.07
	C25P40	1.52±0.11	1.85±0.11	3.07±0.17	1.18±0.02^{b}	62.01±1.79
30	C5P52	1.56±007	1.81±0.08	2.93±0.08	1.28±0.07	59.65±3.23
	C15P46	1.61±0.09	1.89±0.13	2.99±0.09	1.22±0.08	61.44±4.05
	C25P40	1.63±0.03	1.88±0.10	3.03±0.15	1.23±0.05	64.13±3.80
36	C5P52	1.33±0.10^{a}	1.84±0.05	2.85±0.14	1.66±0.04^{a}	37.04±1.87
	C15P46	1.29±0.06^{a}	1.83±0.15	2.88±0.24	1.34±0.06^{b}	36.51±1.60
	C25P40	1.10±0.07^{b}	1.86±0.08	2.89±0.14	1.26±0.03^{b}	41.06±2.27
12	All diet combined	1.70±0.16^{a}	1.91±0.03	3.03±0.06	0.85±0.38^{d}	71.89±3.93^{a}
18		1.40±0.07^{d}	1.78±0.04	2.91±0.08	1.37±0.08^{a}	53.95±1.00^{d}
24		1.51±0.03^{c}	1.81±0.02	2.97±0.09	1.31±0.11^{b}	58.47±3.09^{c}
30		1.60±0.03^{b}	1.86±0.02	2.99±0.05	1.24±0.03^{c}	61.74±2.26^{b}
36		1.24±0.12^{e}	1.84±0.05	2.87±0.02	1.42±0.21^{a}	38.20±2.49^{e}
All salinity combined	C5P52	1.51±0.20	1.81±0.06	2.91±0.07	1.24±0.48^{ab}	56.66±14.12^{ab}
	C15P46	1.47±0.11	1.84±0.05	2.93±0.06	1.26±0.16^{a}	55.47±12.15^{b}
	C25P40	1.48±0.21	1.87±0.05	3.02±0.08	1.22±0.07^{b}	58.43±11.08^{a}
Interaction		***	ns	ns	***	**

Note: ns: non-significant; *P<0.05; **P<0.01; ***P<0.001. Different letters denote significant differences (P<0.05) between dietary treatments within each salinity regime or between different salinities independent of dietary treatments.

18 and 36, and lower at 12 (P<0.05); GH levels of fish fed with the highest starch and lowest protein diet (C25P40) were significantly lower than those fed with the other two diets (P<0.05) (Table 4). Cortisol (COR) levels were higher in fish reared at 12, and lower at 18 and 36 (P<0.05); COR levels of fish fed with the highest starch and lowest protein diet (C25P40) were significantly higher than those fed with the other two diets (P<0.05) (Table 4). Although GH and COR levels varied among dietary treatments and salinity groups, GH levels were positively correlated while COR levels were negatively correlated with SGR. Linear regression analysis of the relationship between SGR and GH was expressed as y=1.403 1x–1.270 6 (R^2=0.663), and between SGR and COR as y=-55.688x+156.51 (R^2=0.561 8).

3.4 Hepatic glycolytic (GK, PK), gluconeogenic (G6Pase, PEPCK) and lipogenic (G6PD, FAS) enzyme activities

Hepatic glycolytic glucokinase (GK) activity was higher in fish reared at a salinity of 12, and lower at 18 and 36 (P<0.05); hepatic lipogenic glucose-6-phosphate dehydrogenase (G6PD) and fatty acid synthase (FAS) activities were higher in fish reared at 18 and 36, and lower at 12 (P<0.05) (Table 5). GK activity of fish fed with the highest starch and lowest protein diet (C25P40) was significantly higher than those fed with the other two diets (P<0.05). G6PD activity of fish fed with the highest starch and lowest protein diet (C25P40) was significantly lower than those fed with the other two diets (P<0.05); FAS

Table 5 Hepatic glycolytic (GK, PK), gluconeogenic (G6Pase, PEPCK) and lipogenic (G6PD, FAS) enzymes specific activities (mIU/mg protein) in juvenile turbot reared at five salinities (12, 18, 24, 30, and 36) and fed with three diets (C5P52, C15P46, and C25P40) (means±SD, *n*=6)

Salinity	Diet	GK (mIU/ mg protein)	PK (mIU/ mg protein)	G6Pase (mIU/ mg protein)	PEPCK (mIU/ mg protein)	G6PD (mIU/ mg protein)	FAS (mIU/ mg protein)
12	C5P52	1.22±0.06^{a}	14.98±1.36	6.40±1.39	0.49±0.07	3.93±0.17^{b}	0.47±0.09^{b}
	C15P46	0.92±0.05^{b}	14.69±1.08	5.92±2.06	0.52±0.12	4.27±0.12^{a}	0.75±0.12^{a}
	C25P40	1.05±0.11^{b}	15.32±1.23	7.51±1.15	0.44±0.12	4.03±0.13^{ab}	0.73±0.11^{a}
18	C5P52	0.31±0.02^{c}	13.54±0.57	6.04±1.85	0.55±0.11	5.13±0.07^{a}	1.32±0.19^{a}
	C15P46	0.42±0.04^{b}	13.76±1.26	6.96±1.24	0.53±0.10	4.97±0.11^{a}	1.14±0.11^{ab}
	C25P40	0.60±0.02^{a}	14.27±0.57	8.25±1.87	0.51±0.13	4.40±0.19^{b}	0.99±0.07^{b}
24	C5P52	0.37±0.03^{c}	13.90±1.88	6.79±0.95	0.53±0.07	4.69±0.07^{a}	1.13±0.19^{a}
	C15P46	0.47±0.04^{b}	14.17±1.05	6.69±1.39	0.49±0.10	4.54±0.19^{ab}	0.96±0.12^{ab}
	C25P40	0.66±0.03^{a}	13.71±0.50	5.72±2.13	0.51±0.02	4.27±0.19^{b}	0.81±0.08^{b}
30	C5P52	0.64±007^{b}	14.54±1.06	8.10±1.31	0.54±0.12	4.44±0.13^{a}	0.91±0.09
	C15P46	0.71±0.08^{b}	14.71±2.32	5.98±2.23	0.50±0.10	4.33±0.06^{ab}	0.82±0.08
	C25P40	0.86±0.06^{a}	15.28±1.03	5.37±0.72	0.46±0.07	4.20±0.14^{b}	0.76±0.11
36	C5P52	0.52±0.02^{b}	13.67±1.27	7.66±1.08	0.63±0.09	4.24±0.15^{b}	1.02±0.18^{c}
	C15P46	0.58±0.04^{ab}	14.03±1.40	7.35±1.00	0.59±0.05	4.73±0.08^{a}	1.37±0.10^{b}
	C25P40	0.60±0.05^{a}	14.78±0.72	5.36±1.26	0.52±0.07	4.85±0.21^{a}	1.67±0.09^{a}
12	All diet combined	1.06±0.15^{a}	15.00±0.32	6.61±0.81	0.48±0.03	4.11±0.21^{d}	0.65±0.16^{e}
18		0.45±0.14^{e}	13.86±0.37	7.08±1.11	0.53±0.01	4.84±0.30^{a}	1.15±0.11^{b}
24		0.50±0.17^{d}	13.93±0.23	6.40±0.59	0.51±0.02	4.50±0.14^{b}	0.97±0.18^{c}
30		0.74±0.11^{b}	14.84±0.38	6.49±1.43	0.50±0.03	4.32±0.05^{c}	0.83±0.07^{d}
36		0.57±0.04^{c}	14.16±0.56	6.79±1.25	0.58±0.02	4.61±0.21^{b}	1.35±0.33^{a}
All salinity combined	C5P52	0.61±0.36^{b}	14.13±0.61	7.00±0.86	0.55±0.05	4.49±0.45^{a}	0.96±0.32
	C15P46	0.62±0.20^{b}	14.27±0.42	6.58±0.62	0.53±0.04	4.57±0.29^{a}	1.01±0.25
	C25P40	0.75±0.20^{a}	14.67±0.69	6.44±1.35	0.48±0.04	4.37±0.0.2^{b}	0.99±0.39
Interaction		***	ns	ns	ns	***	***

Note: ns: non-significant; **P*<0.05; ***P*<0.01; ****P*<0.001. Different letters denote significant differences (*P*<0.05) between dietary treatments within each salinity regime or between different salinities independent of dietary treatments.

activity was unaffected by dietary treatments. It is worth noting that GK, G6PD, and FAS activities of fish fed with the highest starch and lowest protein diet (C25P40) were all significantly higher than those fed with diet C5P52 (*P*<0.05) when reared at 36 (Table 5). Hepatic glycolytic pyruvate kinase (PK), gluconeogenic glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) activities were not affected by dietary composition or water salinity (Table 5).

4 DISCUSSION

Gill Na^+, K^+-ATPase (NKA) plays a critical role in maintaining ion homeostasis in fish (Evans et al., 2005). NKA activity is strongly regulated by water salinity and is usually minimal when fish are maintained in isosmotic surroundings (Evans, 2008). In the present study, NKA activity increased with the increase in water salinity, indicating that less energy was expended to maintain osmotic homeostasis when fish were reared at lower salinity. Similar findings on turbot were also recorded by Gaumet et al. (1995) and Imsland et al. (2003). However, NKA activity was not affected by dietary treatments and, thus, the mechanisms of osmoregulation in fish via diet composition will require further study.

There was no mortality in the experimental groups, except in fish reared at salinity of 12. The main reason may be that juvenile fish are sensitive to stressors (Wendelaar, 2011) and physiological functions, particularly non-specific immunity, are adversely affected when juvenile turbot are maintained in a low-

salt environment (salinity of 12 in the present study) (Zhang et al., 2011). This contention is further supported by the fact that festering of the gills and body surface were observed only in fish adapted to a salinity of 12 during the feeding trial. Therefore, juvenile turbot should be reared at salinities greater than 12. Interestingly, fish fed with higher starch and lower protein diets (C15P46 and C25P40) had significantly higher survival rates than those fed with diet C5P52, when reared at 12. The main reason is that, compared with proteins, carbohydrate metabolites (mainly glucose) can more rapidly generate ATP to meet a fish's energy requirements (Tseng and Hwang, 2008), indicating that fish fed with carbohydrate-rich feed could have improved survival rates when under severe stress.

Growth performance and FCR may be improved when fish are reared in a low-salinity environment, since they expend less energy to meet the metabolic demands of osmoregulation (Tseng and Hwang, 2008). Previous studies have demonstrated that the best growth performance and FCE were observed at 19 in juvenile turbot reared at water salinities ranging from 10 to 35 (Gaumet et al., 1995; Imsland et al., 2001). As expected, our data show that, independent of diet composition, improvements in SGR and FCE were observed in fish maintained at 18 compared with those reared at 24 or 30. However, better SGR and FCE values were obtained in fish adapted to a salinity of 36. Previous studies on turbot have demonstrated that the highest growth rates are observed at a salinity of 33.5, compared with 15 or 25, in fish reared at 18°C, while growth rates decreased with increased water salinity in fish reared at 22°C (Imsland et al., 2001). The water temperature was mainly below 18°C throughout the experimental period in the present study. This may also be the main reason why SGR and FCE values were greatly improved in fish reared at 36. Although fish maintained at 12 required minimal energy costs to meet osmoregulation, they had to expend considerable amounts of energy for other important physiological functions such as immunity improvement, which results in serious adverse effects on SGR and FCE.

Fish, especially carnivorous species, display inadequate use of dietary carbohydrates and, therefore, the protein-sparing effect is relatively limited (Stone, 2003). Previous studies have shown that the maximum amount of dietary carbohydrate is generally lower than 20% for marine fish species (Wilson, 1994). In the present study, irrespective of water salinity, SGR, and FCE showed no statistically significant difference in fish fed with two different starch level diets (C5P52 and C15P46). The results indicate that good growth performance and FCE could be obtained even at low dietary protein levels with sufficient carbohydrates in the diet, which may reduce cost on fish feed and, in turn, nitrogen emissions into the environment resulting in eutrophication. There was also a trend towards improved PER with increased dietary starch content, suggesting that carbohydrates can spare some protein when dietary protein is low. However, extra high levels of starch (25% in the present study) significantly decreased SGR and FCE. This may be ascribed to the poor use of high levels of dietary starch, resulting in carbohydrate energy being wasted as heat and a reduction in energy retention in fish (Lovell, 1989; Bureau et al., 1998). It is worth noting that SGR and FCE were unaffected by dietary starch when fish were maintained at 36, but those fed with higher starch and lower protein diets (C15P46 and C25P40) had higher SGR and FCE than those fed with diet C5P52 when fish were adapted to 12, at which disease-resistance in fish is impaired. The results are consistent with data in rainbow trout (*Oncorhynchus mykiss*) where swimming exercise greatly improved the use of gelatinized starch (Felip et al., 2012; Friedrichsen et al., 2013), indicating that some stressors can improve carbohydrate use and enhance the protein-sparing effect. The principal reason is that carbohydrates serve as major energy substrates in meeting the metabolic demand of these stressors, which has been confirmed by the oxygen:nitrogen (O:N) atomic ratio (Mayzaud and Conover, 1988).

Irrespective of dietary treatments, *F*% was not affected by salinity in fish reared at salinities ranging from 18 to 36; a similar result was previously reported in turbot, i.e., that food consumption is mainly related to temperature not salinity (Imsland et al., 2001). Lower *F*% in fish maintained at 12 may be attributed to a deterioration in non-specific immunity and hyperglycemia (Polakof et al., 2011). Independent of salinity, *F*% was not affected by diet composition and, in turbot, *F*% may be mainly regulated by the dietary energy content (Boujard and Médale, 1994; Paspatis and Boujard, 1996).

Independent of salinity, serum glucose levels showed no significant difference among diet groups. Previous studies on turbot have demonstrated that glucose is relatively rapidly metabolized and returns to basal values within 24 h (Garcia-Riera and Hemre,

1996). Blood was sampled after fish were fasting for 24 h and this is the main reason why glucose was unchanged. HSI rose significantly with the increase in dietary starch. Glucose from carbohydrate-rich diets could enhance liver glycogen content and consequently increase HSI. Additionally, feeding high starch diets leads to increase hepatic lipogenesis and results in increased liver lipid content (Fernandez et al., 2007). Considering that hepatic FAS and G6PD activities were affected by dietary carbohydrate levels, the higher HSI in fish mainly resulted from increased liver glycogen and lipid levels. VSI was unaffected by dietary starch levels; the main reason being that only negligible amounts of glycogen and lipid accumulated in the liver compared with the amount of absorbed glucose and lipid. Muscle glycogen increased with the increase in dietary carbohydrate levels, which may be strongly related to converted dietary carbohydrates.

Both GH and COR are generally considered as seawater-adapting hormone (McCormick and Bradshaw 2006; Almeida et al., 2013). However, recent studies have shown that COR plays a critical role in regulating ion uptake; thus COR is considered to have a dual osmoregulatory function (McCormick et al., 2008). In addition, COR has been used as an important stress indicator (Mommsen et al., 1999; Barton, 2002). In the present study, independent of diet composition, GH levels displayed a significant positive correlation with SGR, but had an ambiguous relationship with salinity. Previous studies on turbot have showed that IGF-I levels are positively correlated with growth rate, but unrelated to environmental salinity (Imsland et al., 2008). Since IGF-I levels are strongly regulated by GH in fish (Björnsson et al., 2002), our data are in agreement with this result. COR levels showed a significant negative correlation with SGR, but were unrelated to water salinity. These findings are consistent with those reported in Japanese flounder (*P. olivaceus*) (Bolasina et al., 2007).

The endocrine system is regulated by nutritional and metabolic factors (Fuentes et al., 2012; Nagel et al., 2012), but the relationship is complex and has not been clarified. For example, a contradictory result has been observed in European whitefish (*Coregonus lavaretus*) fed with high carbohydrate diets that promoted (Ruohonen et al., 2007) or reduced (Vielma et al., 2003) serum cortisol levels. In the present study, fish fed with the highest starch and lowest protein diet C25P40 had lower GH and higher COR than those fed with the other two diets. The results indicated that excess levels of starch (25%) cause stress in fish, resulting in a significant reduction in growth rate and feed use.

As the principal organ of glucose homeostasis, the liver plays a critical role in regulating intermediary metabolism in response to nutritional status in addition to supplying carbohydrate metabolites to the osmoregulatory organs (Klover and Mooney, 2004; Tseng and Hwang, 2008). To evaluate the response of physiological and biochemical processes to dietary starch levels, the activities of a number of key carbohydrate metabolic enzyme, including hepatic glycolytic (GK and PK), gluconeogenic (G6Pase and PEPCK) and lipid synthase (G6PD and FAS), were examined. Irrespective of salinity, GK activity increased with increasing dietary starch levels. Elevated GK activity could lead to an increase in glucose uptake from circulation into the liver and offset any possible hyperglycemic response, which may further result in an increase in liver glycogen content and HSI (Table 3). Independent of diet composition, although hepatic GK activity varied among the salinity treatment groups, GK activity increased with decreasing SGR. Hepatic glycogen content was unaffected by salinity. Elevated GK activity could generate ATP, which is used for other important physiological function, such as osmoregulation and immunity enhancement, resulting in an adverse effect on growth performance. PK activity appears to be unregulated by either dietary carbohydrate levels or water salinity. The response of GK is generally stronger than that of PK, suggesting that GK may play a more important role in adaptation to carbohydrate diets than PK.

Hepatic gluconeogenic G6Pase activity was not regulated by diet composition or water salinity in juvenile turbot. Although PEPCK activity may be closely related to plasma glucose levels (Elo et al., 2007), which were shown to be strongly affected by salinity in the present study, hepatic PEPCK activity was not significantly different among groups. The absence of change in gluconeogenic activities indicates inadequate regulation of glucose homeostasis (Polakof et al., 2012) and at least partly explains that turbot have a relatively limited capacity to metabolize dietary glucose.

Usually, the pentose phosphate pathway, together with glycolysis, is enhanced in fish fed with high carbohydrate and low protein diets (Meton et al., 1999), indicating their carbohydrate use ability and the protein-sparing effect. As a key enzyme in the

pentose phosphate pathway, G6PD can produce NADPH for biosynthesis, including lipid synthesis. In the present study, irrespective of water salinity, hepatic G6PD activity was significantly reduced in fish fed with the highest starch and lowest protein diet (C25P40) compared with those fed diets C5P52 and C15P46, while GK activity increased with increasing dietary carbohydrate levels. This partly explains the reason that fish fed with diet C25P40 had an inferior growth performance than those fed with diets C5P52 and C15P46. It is worth noting that G6PD, FAS and GK activities all increased with increasing dietary starch levels in fish reared at a salinity of 36, which may be the principal reason that the SGR and FCE of these fish was unaffected by dietary carbohydrate levels. To date, the effects of water salinity on lipogenic enzyme activities have not been studied. The increased lipogenic enzyme activities, but unchanged gluconeogenesis, in fish reared at higher salinities was consistent with data from exercised rainbow trout (*O. mykiss*) in juvenile sea bream (*Sparus aurata*) showing greatly improved the use of carbohydrates (Felip et al., 2012; Friedrichsen et al., 2013). In turbot, lipogenesis was enhanced by higher salinities, suggesting a potential role of water salinity in the use of dietary glucose. The mechanisms for the regulation of lipogenic enzymes in fish reared at different salinities require further study and clarification.

The pentose phosphate pathway is also regulated by water salinity, but the relationship has not been identified. For example, a contradictory result was observed in sea bream (*S. auratus*) reared in low-salt environments that promote (Sangiao-Alvarellos et al., 2005) or reduce (Sangiao-Alvarellos et al., 2003) gill G6PD activity. In the present study, although hepatic G6PD and FAS activities varied among salinity treatment groups, G6PD and FAS increased with increasing SGR. The principal reason is that the increase in G6PD and FAS activities enhanced biosynthesis, such as fatty acid synthesis from glucose, to meet the metabolic demands of growth.

5 CONCLUSION

Juvenile turbot should be reared at salinities of above 12 to achieve good growth. Compared with diet C5P52, feeding diet C15P46 had no adverse effect on growth performance or feed use in fish reared at salinities ranging from 18 to 30, suggesting that an increase of 10% in gelatinized starch can spare 6% protein in the diet. Growth performance and feed use were unaffected by dietary starch levels in fish reared at 36, indicating that higher salinity could boost carbohydrate use and enhance the protein-sparing effect.

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