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Effects of Carbohydrate and Water Temperature on Nutrient and Energy Digestibility of Juvenile and Grower Rockfish, *Sebastes schlegeli*

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ABSTRACT : A factorial (4×2×2) experimental design was employed to determine apparent digestibilities of dry matter (DM), protein, lipid, energy and nitrogen-free extract (NFE) of the test diets containing either α-potato starch (A-PS), β-potato starch (B-PS), β-corn starch (B-CS) or dextrin (DEX) as dietary carbohydrate energy for juvenile (average weight 30 g) and grower (average weight 300 g) rockfish reared at 13°C and 20°C. Chromic oxide was used as an inert marker. Feces were collected by fecal collectors attached to rearing tanks from triplicate groups of juvenile and grower rockfish. Digestibilities of DM, energy and NFE of the test diets were significantly affected by dietary carbohydrate and water temperature ($p<0.01$), but not by fish size. DM digestibility of the fish fed the A-PS diet was significantly higher than that of fish fed other treatments, except for the DEX diet at 20°C. DM digestibility of rockfish fed the B-CS diet was significantly lower than that of other diets. A similar pattern was observed in apparent digestibility of energy. NFE digestibility of fish fed the test diets was significantly affected by carbohydrate and significantly correlated to DM ($r = 0.97$, $p<0.01$) and energy ($r = 0.99$, $p<0.01$) digestibilities, regardless of water temperature and fish size. NFE digestibility of the fish fed the β-starch diets was relatively lower compared to that of the α-starch diets, and ranged from 35 to 43% and 20 to 27% for B-PS and B-CS, respectively. The present findings indicate that carbohydrate and water temperature significantly affected digestibilities of dry matter, energy and nitrogen-free extract of rockfish. Among dietary carbohydrates, α-potato starch could be effectively used as dietary carbohydrate energy for rockfish at 13°C and 20°C. (**Key Words :** Rockfish, *Sebastes schlegeli*, Carbohydrate, Water Temperature, Digestibility)

INTRODUCTION

Use of dietary carbohydrate by fish relatively varies and depends upon many factors including complexity of carbohydrate, fish species and environmental condition (Cowey, 1988; NRC, 1993; Gaylord and Gatlin III, 1996; McGoogan and Reigh, 1996; Grisdale-Helland and Helland, 1997; Shiau, 1997; Hutchins et al., 1998; Rawles and Gatlin III, 1998). For instance, gilthead sea bream has a limited ability in utilization of dietary carbohydrate and its digestibility was less than 77% regardless of carbohydrate sources (Vergara and Jauncey, 1993; Lupatsch et al., 1997). Starch utilization in salmon appeared to be higher in fresh or brackish water than in salt water and reduced with its dietary concentration increase and consequently resulted in the decrease of digestibility of fat (Storebakken, 2002). Digestibility of carbohydrate in 1 kg Atlantic halibut was reduced when the dietary levels were increased (Grisdale-Helland and Helland, 1998). Morris (1997) suggested that carbohydrate should not be exceeded 15% as dry basis in the fish diets due to the fact that excessive dietary carbohydrate results in growth retardation and reduction of nutrients digestibility in fish. Although no specific carbohydrate requirement has been established for fish, some form of digestible carbohydrate should be supplied in the diet (NRC, 1993; Peragon et al., 1999) to maintain normal growth of fish. Because carbohydrate is the least expensive dietary energy source, the maximum tolerable concentration practically should be incorporated in fish diets with regard to the fish species.

Takeuchi (1991) found that apparent digestibilities of protein and energy from various feed ingredients for common carp were significantly affected by water temperature, but not influenced by the fish sizes ranging from 3 to 295 g. Takeuchi et al. (2002) concluded that the apparent digestibilities of protein and energy in dietary

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ingredients were appeared to be species-specific and temperature-dependent. For rockfish, Lee (2002) demonstrated that apparent digestibilities of dry matter, energy and protein of different dietary ingredients including white fish meal, anchovy meal, meat meal, feather meal, blood meal, corn gluten meal, cottonseed meal, wheat four, and brewer's yeast significantly decreased as nitrogen-free extract (NFE) or fiber contents of ingredients increased, but the effects of water temperature on the digestibility was not evaluated. The aim of the present study therefore was to investigate the effects of dietary carbohydrate and water temperature on digestibities of nutrients and energy of juvenile and grower rockfish.

MATERIALS AND METHODS

Experimental design and diet preparation

A 4×2×2 factorial feeding experiment with three replicates was employed to investigate the effects of dietary carbohydrate, water temperature and fish size on nutrient and energy digestibilities of juvenile and grower rockfish. The reference diet (Table 1) was formulated to meet the nutrients requirement of rockfish using white fish meal and squid liver oil as protein and lipid sources, respectively. Chromic oxide at a concentration of 0.5% dry matter was added as an inert marker. Four test diets were prepared by mixing 70% the reference diet and 30% of each test carbohydrate based on air-dry basis according to Cho and Slinger (1979). The experimental carbohydrate sources were α-potato starch (A-PS), β-potato starch (B-PS), β-corn starch (B-CS) and dextrin (DEX). Proximate composition of the experimental diets is shown in Table 2. The diets were thoroughly mixed with distilled water (400 g/kg diet), pelleted by a wet pelleting machine, dried at room temperature for 24 h and stored at -25°C until used.

Fish and experimental condition

Juvenile rockfish obtained from National Fisheries Research and Development Institute (Busan, Korea) were acclimated to the laboratory condition for 2 months (juvenile) and 10 months (grower), respectively. Then the fish were randomly distributed into 200 L cylindrical fiberglass tanks (containing 100 L of water each) of a flow through aquarium system as designed by Lee (2002) and hand-fed the reference diet (chromic oxide free) to visual satiety twice daily (juvenile) and once a day (grower) for 10 wk. Water temperature was adjusted at 13°C and 20°C using a water temperature control system. After the acclimation period, the fish at initial body weight of 30±0.5 g (juvenile) and 300±4.2 g (grower) were randomly selected and redistributed into the same tanks with 50 and 8 fish per tank, respectively. Filtered seawater was supplied at a flow rate of 3 L/min to each tank. Photoperiod was applied as natural condition during experimental period.

Fecal collection

Three replicate groups of fish were carefully hand-fed the reference and test diets to visual satiety once a day at 1500 h by the same person during experimental period. Two h after feeding, the tanks and collection columns were

Table 1. Ingredients composition of the reference diet

Ingredients	%
White fish meal[1]	62.0
Wheat flour	21.5
Squid liver oil[2]	4.0
Vitamin premix[3]	3.0
Mineral premix[4]	3.0
α-Cellulose[5]	3.0
Carboxymethyl cellulose[5]	3.0
Cr_2O_3	0.5

[1] Produced by steam dry method.
[2] Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[3] Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.
[4] Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[5] Sigma Chemical, St. Louis, MO, USA.

Table 2. Proximate composition (% dry matter basis) of the test diets

Diets	Crude protein	Crude lipid	Crude fiber	Ash	NFE[1]	Energy (MJ/kg)
Reference diet (R)	48.2	8.1	4.2	16.6	22.9	19.9
Test diets (70% R+30% carbohydrate)						
A-PS: α-potato starch	34.1	4.8	2.7	11.9	46.5	19.2
B-PS: β-potato starch	34.9	4.8	2.9	11.8	45.6	19.2
B-CS: β-corn starch	34.3	5.1	3.2	11.6	45.8	19.1
DEX: dextrin	33.9	4.7	3.1	11.6	46.7	19.1

[1] Nitrogen free extract = 100-(crude protein+crude lipid+crude fiber+crude ash).

cleaned by sponges, and uneaten feed and fecal residues were removed. Feces were collected from the collection columns at 800 h and 1300 h next day for 12 d. The feces were immediately filtered with filter papers (Whatman #1) for 60 min at 4°C and stored at -75°C for chemical analyses. Fecal samples from each tank were pooled at the end of the experiment.

Analytical methods

Freeze-dried diets and feces were finely ground using a grinder. Fish scales contaminating in the fecal samples were removed using a 300 μm sieve before analyses. Crude protein was determined by Kjeldahl method using Auto Kjeldahl System (Buchi B-324/435/412; Flawil, Switzerland). Crude lipid was analyzed with ether extraction in a soxhlet extractor, crude fiber was determined by an automatic analyzer (Fibertec, Tecator, Sweden), and moisture was determined by a dry oven at 105°C for 12 h. Ash was determined after combustion of the samples at 550°C for 4 h in a muffle furnace. Nitrogen free extract (NFE) was calculated by difference. Gross energy was analyzed by a bomb calorimeter (Parr, USA). Chromic oxide content in the experimental diets and fecal samples was determined by a wet-digestion method (Furukawa and Tsukahara, 1966). All chemical analyses from each tank were performed in triplicates.

Apparent digestibility coefficients (ADCs) for dry matter, nutrient and energy of the experimental diets were computerized using the following equations:

ADC of dry matter (%)

$$= (100-(\text{dietary } Cr_2O_3/\text{fecal } Cr_2O_3)\times 100)$$

ADC of nutrients or energy (%)

$$= \left(1-\frac{\text{dietary } Cr_2O_3}{\text{fecal } Cr_2O_3}\times\frac{\text{fecal nutrient or energy}}{\text{dietary nutrient or energy}}\right)\times 100$$

Statistical analysis

All data were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test (Duncan, 1955) at a significance level of 0.05. Correlation of nutrients and energy was assessed using Pearson Regression. All statistical analyses were carried out using SPSS version 14.0 (SPSS, Michigan Avenue, Chicago, IL, USA).

RESULTS

Apparent digestibilities of nutrients and energy of rockfish fed the reference diet were not influenced by dietary carbohydrate source, fish size and water temperature.

Apparent digestibility coefficient (ADC) of dry matter (DM) in juvenile and grower rockfish fed the test diets at different water temperature is presented in Figure 1. Overall, DM digestibility of the fish was significantly affected by dietary carbohydrate and water temperature. Higher DM digestibility was obtained in both juvenile and grower rockfish fed the diets containing A-PS at 13°C and 20°C, and DEX at 20°C. Lower value was observed in the fish fed

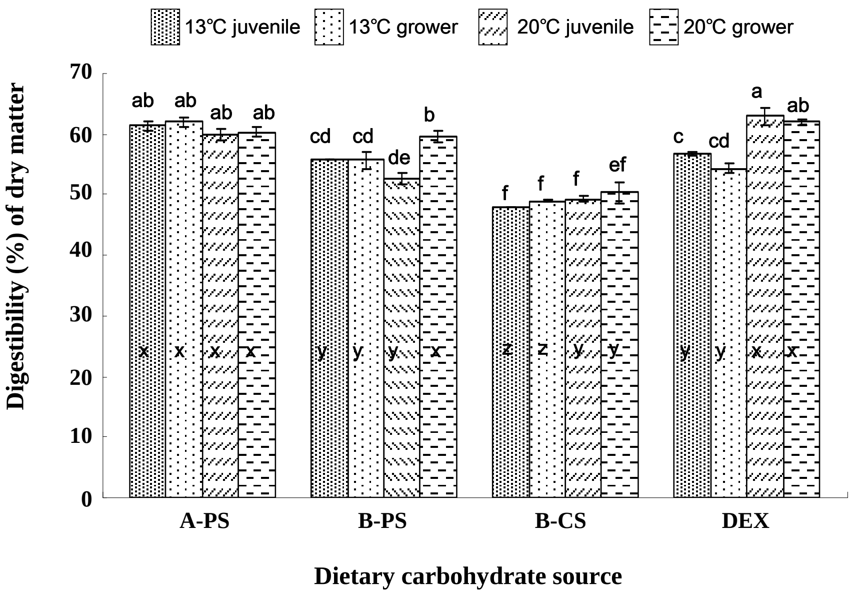

Figure 1. Apparent digestibility coefficient of dry matter in the test diets containing different carbohydrate sources for juvenile and grower rockfish at different water temperatures. Bars having different superscripts (a-f) are significantly different (p<0.05) among groups. Bars with same pattern bearing different letters (x-z) are significantly different (p<0.05) at the same fish size and water temperature. A-PS = Alpha potato starch, B-PS = Beta potato starch, B-CS = Beta corn starch, and DEX = Dextrin.

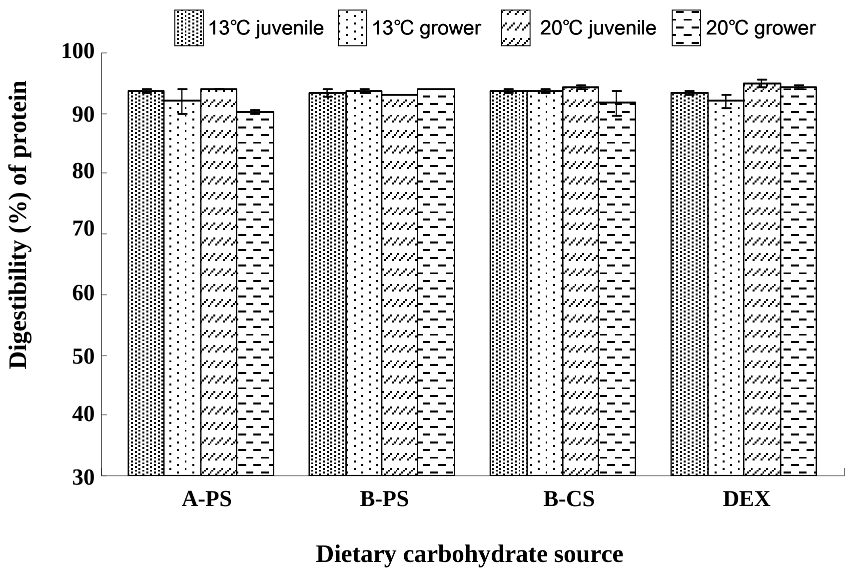

Figure 2. Apparent digestibility coefficient of protein in the test diets containing different carbohydrate sources for juvenile and grower rockfish at different water temperatures. A-PS = Alpha potato starch, B-PS = Beta potato starch, B-CS = Beta corn starch, and DEX = Dextrin.

the B-CS diet, regardless of water temperature and fish size. Within fish groups fed the DEX diet, DM digestibility of rockfish reared at 20°C was significantly higher than that of fish reared at 13°C, regardless of fish size.

Protein and lipid digestibilities of juvenile and grower rockfish fed the test diets containing different carbohydrates at 13°C and 20°C are presented in Figure 2 and 3, respectively. ADCs of protein and lipid in the test diets for rockfish ranged from 90% to 95% and from 87% to 94%, respectively and were not affected by both dietary carbohydrate and water temperature.

Digestibility of energy in the test diets containing different carbohydrates for juvenile and grower rockfish reared at 13°C and 20°C is presented in Figure 4. Digestibility of energy relatively reflected the differences in DM digestibility. Energy digestibility of rockfish was

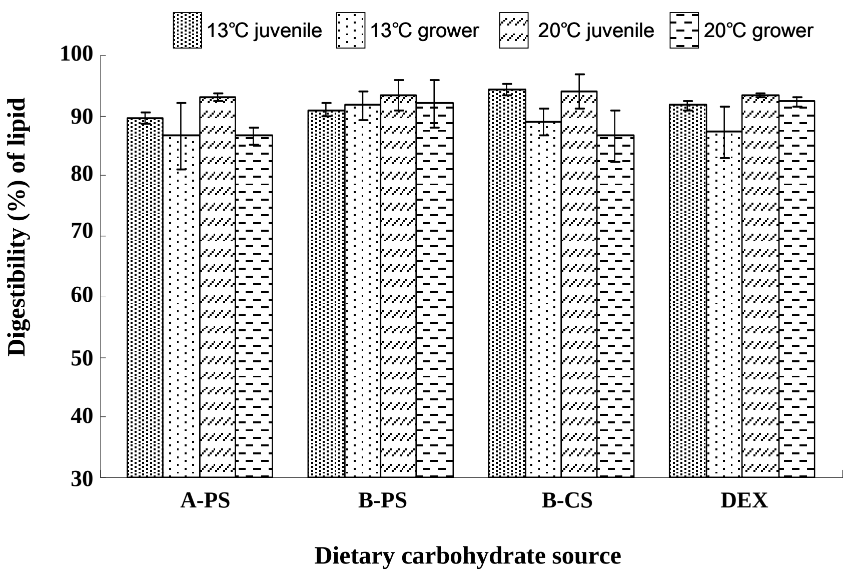

Figure 3. Apparent digestibility coefficient of lipid in the test diets containing different carbohydrate sources for juvenile and grower rockfish at different water temperatures. A-PS = Alpha potato starch, B-PS = Beta potato starch, B-CS = Beta corn starch, and DEX = Dextrin.

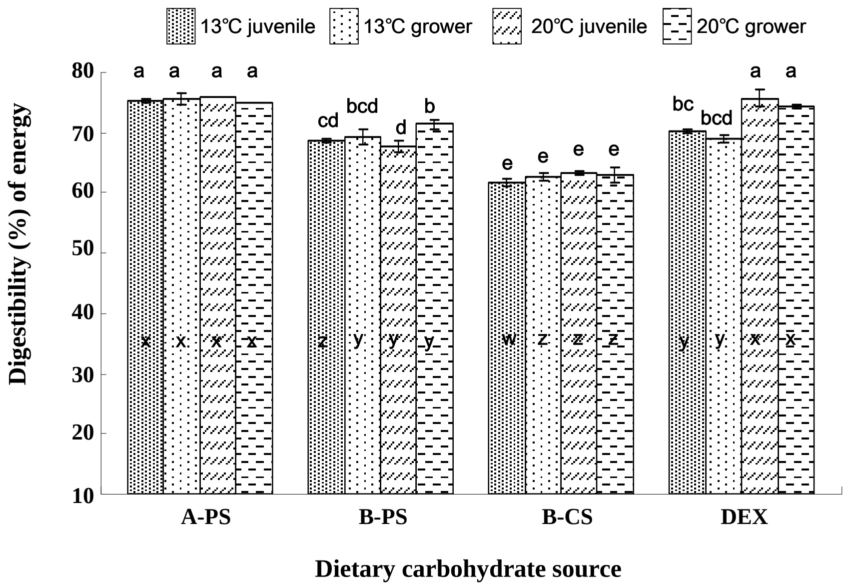

Figure 4. Apparent digestibility coefficient of energy in the test diets containing different carbohydrate sources for juvenile and grower rockfish at different water temperatures. Bars having different superscripts (a-e) are significantly different (p<0.05) among groups. Bars with same pattern bearing different letters (w-z) are significantly different (p<0.05) at the same fish size and water temperature. A-PS = Alpha potato starch, B-PS = Beta potato starch, B-CS = Beta corn starch, and DEX = Dextrin.

significantly affected by dietary carbohydrate and water temperature, regardless of fish size. Higher ADC of energy was obtained in fish fed the A-PS diet at 13°C and 20°C, and the DEX diet at 20°C. The lowest value was found in fish fed the diet containing B-CS. Within fish fed the DEX diet, energy digestibility was significantly higher in fish reared at 20°C compared to that of fish reared at 13°C.

Apparent digestibility of NFE in the test diets containing different carbohydrates for rockfish was significantly affected by both dietary carbohydrate and water temperature (Figure 5). Fish fed the B-CS diet was registered for the lowest value. NFE digestibility in the test

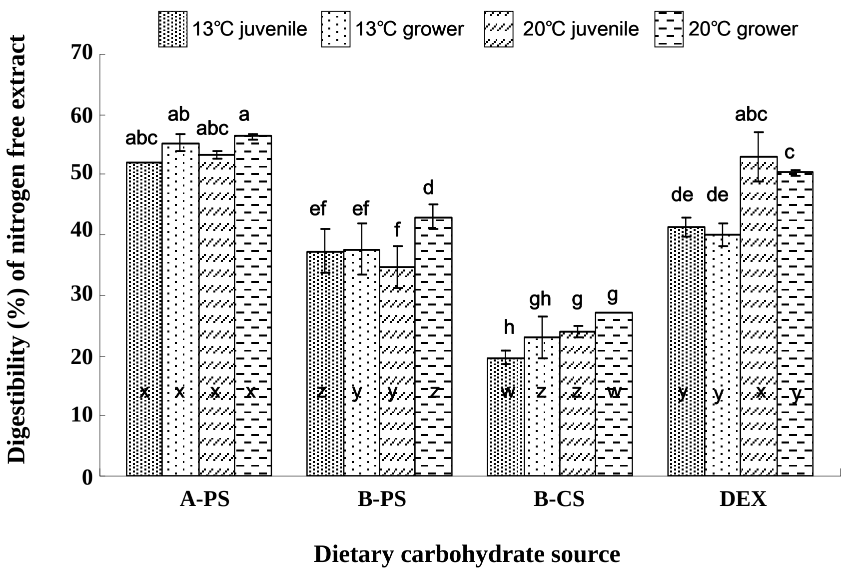

Figure 5. Apparent digestibility coefficient of nitrogen free extract in the test diets containing different carbohydrate sources for juvenile and grower rockfish at different water temperatures. Bars having different superscripts (a-h) are significantly different (p<0.05) among groups. Bars with same pattern bearing different letters (w-z) are significantly different (p<0.05) at the same fish size and water temperature. A-PS = Alpha potato starch, B-PS = Beta potato starch, B-CS = Beta corn starch, and DEX = Dextrin.

diets ranged from 52% to 56%, from 40% to 53%, from 35% to 43%, and from 20% to 27% for the A-PS, DEX, B-PS, and B-CS diets, respectively. Within the fish fed the A-PS diet, no significant differences were observed in NFE digestibility at different water temperatures and fish sizes. Whereas higher value was observed in the fish fed DEX fed at 20°C compared to that of fish at 13°C, regardless of fish size. Interaction between water temperature and fish size was found in apparent digestibility of NFE in rockfish ($p<0.05$). ADC of NFE was significantly correlated to the dry matter in the test diets ($r = 0.97$, $p<0.01$).

DISCUSSION

Overall, dry matter (DM) digestibility in the present study ranging from 48% to 63% was much lower than that in studies for rainbow trout (from 73% to 81%), tilapia (from 81% to 85%), and silver perch (from 83% to 90%) (Podoskina et al., 1997; Stone et al., 2003; Amirkolaie et al., 2006). Lee (2002) reported that DM digestibility of rockfish fed the diets containing different ingredients appeared to relate to the quantity and chemical composition of carbohydrate used. It is well demonstrated that complexity of carbohydrate significantly influences its utilization in the fish diet, and simple carbohydrate often tends to have higher DM digestibility compared to that of the complex ones (Jobling, 2001; Stone et al., 2003). The effects of water temperature on digestibility of dietary carbohydrate have been reported for rainbow trout. Medale et al. (1991) reported that digestibility of maize starch by rainbow trout was significantly improved by the elevation of water temperature from 8°C to 18°C.

Carbohydrate is considered as the least expensive energy source for fish however its utilization relatively varies and depends on several factors such as fish species and fish size, carbohydrate sources and their complexity, and environmental condition (Bergot, 1993; Catacutan and Coloso, 1998; Hutchins et al., 1998; Guillaume et al., 2001; Peres and Oliva-Teles, 2002; Stone et al., 2003). In the present study, energy digestibility of juvenile and grower rockfish fed the test diets at different water temperatures was followed the same pattern in dry matter digestibility and affected by dietary carbohydrate. Higher ADC of energy was obtained in fish fed the A-PS diet at 13°C and 20°C, and the DEX diet at 20°C, and the lowest value was observed in the fish fed the test diet containing B-CS, regardless of fish size. ADC of energy appeared to have a positive relationship with that of DM ($r = 0.98$, $p<0.01$) and NFE ($r = 0.99$, $p<0.01$) in the test diets, regardless of fish size. The present results are in agreement with the previous studies (Storebakken et al., 1998; Lee, 2002; Stone et al., 2003). Stone et al. (2003) reported that energy ADC of the dietary carbohydrates including dextrin, gelatinized wheat starch, glucose, maltose, raw wheat starch and raw pea starch was significantly affected by carbohydrate kind. Dextrin was reported to be a good carbohydrate energy source and efficiently digested by fish (Lee et al., 2003; Stone et al., 2003). McGoogan and Reigh (1996) reported that DM and energy digestibilites of red drum were negatively affected by crude fiber content, and differences in energy digestibility reflected difference in DM digestibility. The inversely effects of dietary crude fiber on dry matter and energy digestibilities have been observed in the studies for rainbow trout, common carp, red drum and rockfish (Hilton et al., 1983; Kirchgessner et al., 1986; McGoogan and Reigh, 1996; Lee, 2002). In the previous study, DM and energy digestibilities of rockfish (grower and juvenile) were significantly correlated with dietary fiber and nitrogen-free extract contents (Lee, 2002). The present results suggest that energy digestibility of rockfish was significantly influenced by both dietary carbohydrate source and water temperature, but not fish size. Among carbohydrates tested, α-potato starch is appeared to be effectively digested by rockfish as carbohydrate energy source at 13°C and 20°C, and dextrin at 20°C, respectively.

Differences in NFE digestibility of the test diets containing different carbohydrates might be due to its indigestible polysaccharides content which consequently resulted in the differences in digestibilities of dry matter and energy in rockfish. NFE digestibility exhibited a significant correlation to DM ($r = 0.97$, $p<0.01$) and energy ($r = 0.99$, $p<0.01$) digestibilities of the test diets. Although the mechanism being responsible for digestion of different carbohydrates by fishes has not been well determined (NRC, 1993), inefficient digestion of carbohydrate was strongly suggested to relate to the environmental condition which they evolved (McGoogan and Reigh, 1996). The present results indicate that NFE digestibility of the carbohydrate was strongly associated to its properties and water temperature and that was in agreement with the finding of Kaushik and Medale (1994).

Regarding of carbohydrate kinds, lower NFE digestibility of β form starch compared to α form starch indicates that digestibility of carbohydrates might be associated to its solubility and water temperature. Generally monogastric animals including fish have relatively low ability in digestion of β-starch compared to that of α-starch (Jobling, 2001) due to its specific carbohydrase enzyme. Effects of solubility and of α-amylase on digestibility of different carbohydrates have been reported in perch (Stone et al., 2003). NFE digestibility of juvenile and grower rockfish fed the dextrin diet was lower at 13°C compared to that of fish reared at 20°C. This suggests that low water temperature might have reduced digestive carbohydrase activities and led to reduction of NFE digestibility. Like other poikilothermes, digestion and metabolism of fish heavily depend upon environmental condition, particularly water temperature. Starch is predominantly digested in the anterior segment of digestive tract of fish (Fange and Grove, 1979; Lovell, 1989) and depends on its solubility in fluid of digestive tract. Low water temperature could reduce temperature and solubility of starch in digestive fluid, and consequently resulted in lower digestibility of starch. In an *in vitro* study, Munilla-Moran and Saborido-Rey (1996) demonstrated that α-amylase activity in sea bream and turbot appeared to depend on temperature and showed the breakpoints at temperatures (19.9°C and 23.6°C, respectively) to close to those of their physiological activities. There is no available information on digestive enzymes of rockfish to date. Hence, studies on the topic are necessary to elaborate the carbohydrate utilization of rockfish.

In conclusion, the findings of the present study indicate that both source and physical property of carbohydrate appeared to be an important factor that affects digestibilities of dry matter, energy and nitrogen-free extract of rockfish, followed by water temperature. Among carbohydrates tested, α-potato starch could be effectively used as dietary carbohydrate energy for rockfish at 13°C and 20°C, and dextrin showed to be a good energy source at 20°C.

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