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Short communication

Ontogenesis of hexokinase I and hexokinase IV (glucokinase) gene expressions in common carp (*Cyprinus carpio*) related to diet

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The expressions of hexokinase IV (glucokinase, GK) and hexokinase (HK)-I genes were analysed during early ontogenesis of common carp (*Cyprinus carpio*). Unlike HK-I gene, which was expressed during all the stages of the development, GK was only induced by the first feeding with high levels of dextrin as a source of carbohydrate. This study confirms the high capacity of common carp to use glucose even at the very early stages of development.

Glucose phosphorylation: Larvae nutrition: Carbohydrate: Carp

The prey organisms encountered by fish larvae under natural conditions (phytoplankton and zooplankton) contain little carbohydrate except for some structural polysaccharides (Whyte & Nagata, 1990; Brown *et al.* 1997). Under aquaculture conditions, development of complete dry diets for larvae is a major issue (Charlon & Bergot, 1984; Watanabe & Kiron, 1994; Cahu *et al.* 1998). Such diets necessarily contain relatively high amounts of carbohydrate compared with live prey. In addition, differences in carbohydrate utilisation between fish species are known to exist: the common carp (*Cyprinus carpio*) is able to use higher levels of dietary carbohydrate than the rainbow trout (*Oncorhynchus mykiss*) (Wilson, 1994).

One of the hypotheses to explain the low glucose utilisation by those species reported to be glucose intolerant (Palmer & Ryman, 1972) was a low capacity for glucose phosphorylation, with rainbow trout having lower activities compared with common carp (Cowey & Walton, 1989; Moon & Foster, 1995). Glucose phosphorylation is catalysed by the hexokinase (HK) enzymes composed of four isozymes in mammals, including the ubiquitous HK-I which catalyses the first step of glucose utilisation for energy in cells and the hepatic–β-pancreatic hexokinase IV (commonly named as glucokinase, GK), which converts excess glucose to lipid and glycogen in liver (Wilson, 1995).

Having cloned hepatic HK-I and GK cDNA in common carp and GK cDNA in rainbow trout (Blin *et al.* 2000, Panserat *et al.* 2000*a*), we recently showed that: (1) hepatic GK activity and RNA levels increased in juvenile common carp and rainbow trout by high levels of dietary carbohydrate; (2) activity of the low-K_m HK (including the HK-I enzyme) was independent of the nutritional status (Panserat *et al.* 2000*b*). The importance of glucose during embryonic or larval development of fish is yet not entirely clear since lipids and amino acids constitute the main energy sources in the eggs (Wiegand, 1996; Ronnestad *et al.* 1999). During embryonic development of common carp, protein indeed appears to be a major source of energy (Kaushik *et al.* 1982). Given our limited knowledge on dietary carbohydrate utilisation by first-feeding fish larvae, the aim of the present work was to describe the HK-I and GK gene expressions during early development of common carp and in larvae fed with different levels of dietary carbohydrate.

First-feeding carp larvae were obtained by induced spawning from one female and two males as described previously (Fontagné *et al.* 1999). The day following hatching, larvae were randomly distributed into tanks in a semi-circulating system (400 larvae per 6-litre tank) as described previously by Charlon & Bergot (1984). Once yolk nutrients are no longer sufficient to support the metabolic demand of the larvae, they must initiate exogenous feeding (2 d after hatching when larvae exhibited an inflated swim-bladder). Carp larvae were fed with the formulated diets described in Table 1: the main

Abbreviations: GK, glucokinase; HK, hexokinase; RT–PCR, reverse transcriptase–polymerase chain reaction.

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Table 1. Formulation and chemical composition of the experimental diets

	LC	MC	HC
Ingredients (g/kg)			
Casein basis*	300	564	564
Vitamin mixture†	80	94	94
Mineral mixture‡	50	47	47
Dextrin§	100	175	235
Peanut oil	–	10	10
Soyabean lecithin	70	20	20
Triolein	100	90	30
Soluble fish protein concentrate‖	300	–	–
Analytical composition			
DM (g/kg)	930	940	940
Crude protein (g/kg DM)	560	550	550
Crude lipid (g/kg DM)	170	110	60
Digestible starch (g/kg DM)	70	120	160
Gross energy (MJ/kg DM)	23	23	21

LC, low-carbohydrate diet; MC, medium-carbohydrate diet; HC, high-carbohydrate diet.

* Prolabo, Fontenay-sous-bois, France.

† Vitamin mix (g/kg): retinyl acetate 1, cholecalciferol 2·5, DL-α-tocopherol acetate 5, menadione 1, thiamin-HCl 0·1, riboflavin 0·4, D-calcium panthothenate 2, pyridoxine-HCl 0·3, cyanocobalamin 1, niacin 1, choline 200, ascorbic acid 5, folic acid 0·1, D-biotin 1, meso-inositol 30. All ingredients were diluted with α-cellulose.

‡ Mineral mix (per kg). KCl 90 g, KI, 40 mg, $CaHPO_4.2H_2O$ 500 g, NaCl 40 g, $CuSO_4.5H_2O$ 3 g, $ZnSO_4.7H_2O$ 4 g, $CoSO_4$ 20 mg, $FeSO_4.7H_2O$ 20 g, $MnSO_4$ H_2O 3 g, $CaCO_3$ 215 g, MgOH 124 g, Na_2SeO_3 30 mg, NaF 1 g.

§ Sigma, Saint-Quentin Fallavier, France.

‖ C.P.S.P. 90; Sopropêche, Boulogne-sur-mer, France.

difference between the diets was the carbohydrate:lipid ratio, which varied between 0·5 and 2·5. Food particles were delivered continuously (five times every 10 min) and in excess throughout the 16 h artificial light period (06.00–22.00 hours) using automatic feed dispensers (Charlon & Bergot, 1986).

About 100 eggs and fish at different developmental stages were sampled (at 12.00 hours for the fed larvae) and frozen at −80°C until RNA extraction. Total RNA was extracted from eggs and entire fish as described previously (Panserat *et al.* 2000*b*). GK and HK-I mRNA were analysed by reverse transcriptase-polymerase chain reaction (RT–PCR) using specific primers chosen in the cDNA sequences from carp HK-I gene 5′-GATGCTTTTGGTA-AAGATTC-3′ and 5′-TTCTTCATCCCCATATAGTC-3′, and from carp GK gene 5′-AGTGATGCTGGTCA-AAGTGG-3′ and 5′-GCTTCTTATGTTTCAGATTA-3′ as described previously (Blin *et al.* 2000; Panserat *et al.* 2000*a*) (Genbank accession numbers are AF119837 and AF053333 for the HK-I and GK sequences respectively). The RT–PCR conditions were the same as previously described (Blin *et al.* 2000; Panserat *et al.* 2000*a*). The RT–PCR assay conducted in the present study did not include any competition during the amplification; thus the outcome of the assay can only be taken as an indication of the presence of an mRNA template in the assay.

Data on GK and HK-I gene expression during carp development are shown in Fig. 1. The HK-I gene

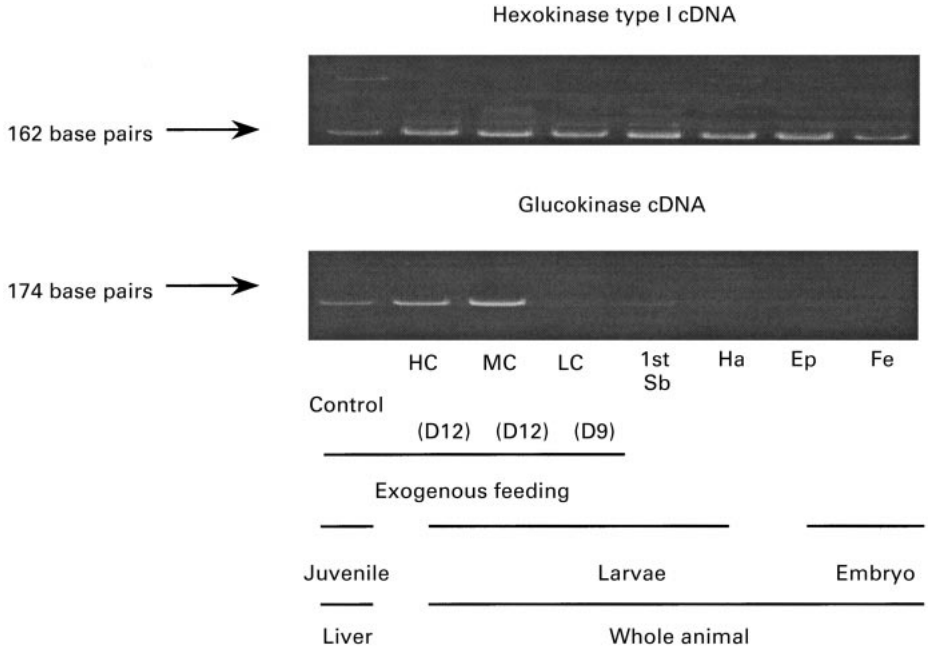

Fig. 1. Expression of glucokinase and hexokinase-I genes during carp development. Analysis by reverse transcriptase–polymerase chain reaction (RT–PCR) on eggs, embryos and larvae corresponding to almost 100 individual fish. HC, high-carbohydrate diet; MC, medium-carbohydrate diet; LC, low-carbohydrate diet; 1st Sb, first swim-bladder (7 d after fecundation); Ha, hatching (6 d after fecundation); Ep, epiboly (1 d after fecundation); Fe, fertilised eggs; D12, D9 represent 12 d and 9 d after the first exogenous feeding respectively. Exogenous feeding began 8 d after fecundation. The control was total RNA extracted 6 h after feeding from the liver of a juvenile fish (120 g) fed on 200 g digestible carbohydrate/kg diet and 120 g lipids/kg diet for 10 weeks as described previously (Panserat *et al.* 2000*b*). The RT–PCR products were electrophoresed on acrylamide:bisacrylamide (29:1) gel.

expression is observed during all the stages of development suggesting the importance of glucose as a source of energy in carp even during embryonic stages. Moreover, the expression of HK-I gene expression is probably ubiquitous; although not verified here, a ubiquitous expression of HK-I gene has been observed in juvenile common carp (Blin *et al.* 2000). This is interesting, since so far, mass-balance studies have shown that the free amino acids and lipids are used as metabolic fuel during fish development (Kaushik *et al.* 1982; Wiegand, 1996; Ronnestad *et al.* 1999).

GK expression is observed only when the larvae are fed with exogenous diet and specifically when they are fed with a diet containing the highest carbohydrate:lipid ratio (12 d after first feeding) (Fig. 1). Even though our present data are based on analysis at a whole-body level and that the size of hepatopancreatic tissue in larvae is relatively small (almost 20 % g/kg body weight) (Durante, 1986), we can speculate that the increase of GK gene expression is specific to the hepato-pancreas since, in juvenile carp, it is expressed only in this tissue (Panserat *et al.* 2000*a*). Our present data also strongly suggest that dietary carbohydrate is the main nutrient controlling the expression of the GK enzyme in tune with earlier findings with juvenile carps (Panserat *et al.* 2000*b*). It is worth mentioning that with the diet used, as the dietary carbohydrate level increases, there is a concurrent drop in dietary fat level. However, information available at present does not indicate any effect of dietary lipid on GK gene expression.

Even though data obtained here were from progeny of just one female and two males, our results show that common carp possess HK-I and GK enzymes involved in glucose phosphorylation even at early developmental stages. Based on these data, we can speculate that: (1) glucose is a potentially efficient energy fuel during ontogenesis due to the presence of HK enzymes involved in glucose phosphorylation; (2) carp larvae are capable of utilising dietary carbohydrate right from first feeding. These results are not surprising because the omnivorous common carp is known to use dietary carbohydrate efficiently (Wilson, 1994). Thus, further studies are now necessary to analyse the developmental pattern of expression of these HK–GK enzymes in those fish known to poorly utilise dietary carbohydrate, such as salmonids or other marine fish (Wilson, 1994).

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