The Journal of Nutritional Biochemistry
January 2000; 11(1) : 22-29
http://dx.doi.org/10.1016/S0955-2863(99)00067-4

Lack of significant long-term effect of dietary carbohydrates on hepatic glucose-6-phosphatase expression in rainbow trout (Oncorhynchus mykiss)

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Abstract: Hepatic glucose-6-phosphatase (G6Pase) plays an important role in glucose metabolism because it catalyzes the release of glucose to the circulatory system in the processes of glycogenolysis and gluconeogenesis. The present study was initiated to analyze the regulation of hepatic G6Pase expression by dietary carbohydrates in rainbow trout. The first step in our study was the identification of a partial G6Pase cDNA in rainbow trout that was highly homologous to that of mammals. Hepatic G6Pase activities and mRNA levels were measured in trout fed one of the experimental diets, with or without carbohydrates. We found no significant effect of intake of dietary carbohydrates on G6Pase expression (mRNA and activity) 6 hours and 24 hours after feeding. These results suggest that there is no control of G6Pase synthesis by dietary carbohydrates in rainbow trout and that the lack of regulation of gluconeogenesis by dietary carbohydrates could at least partially explain the postprandial hyperglycemia and the low dietary glucose utilization observed in this species.

Keywords: glucose-6-phosphatase expression; fish nutrition; rainbow trout; dietary carbohydrates

INTRODUCTION

Glucose plays a key role in mammalian energetics but its importance as a metabolic fuel in fish is not fully understood.[1] Even though most of the enzymes involved in glucose metabolism have been detected in fish, the regulation of carbohydrate metabolism differs from that of mammals[2] : (i) glucose contributes minimally to the oxidative fuel demands of locomotory muscle in rainbow trout (*Oncorhynchus mykiss*) and the glucose turn-over rates are below the levels detected in mammals[3,4], (ii) amino acids seem to be more potent stimulators of insulin secretion than glucose[5] (under in vitro conditions or when administred at supra-physiological levels), (iii) glucose appears to be a poor substrate for glycogen synthesis in isolated trout hepatocytes[6], (iv) there is no apparent effect of glucose on hepatocyte gluconeogenesis[7] and (v) low capacity of glucose utilisation as energy source in fish muscle is suspected to be due to low levels of insulin receptors[8], possible lack of glucose transporters[9] or supposedly low levels of hexokinase enzymes.[10] All of these characteristics can explain that oral administration of glucose and high levels of dietary carbohydrates cause persistent postprandial hyperglycaemia[11,12] associated with low dietary glucose utilisation in rainbow trout.[10] Our objective is the understanding of the nutritional regulation of the glucose metabolism in fish because improvement of dietary carbohydrate utilisation is an important challenge in aquaculture.[10]

Liver is known to play a central role in glucose homeostasis by extracting the absorbed dietary glucose and by reducing the release of endogenous glucose (gluconeogenesis).[13] Salmonids are carnivorous in nature and rely on hepatic gluconeogenesis for glucose production.[14] Thus, we hypothesised that lack of such a regulation of endogenous

hepatic glucose production by dietary carbohydrate can, at least partially, explain the postprandial hyperglycemia observed in salmonids as suggested previously.[7] In the whole animal, this would mean that newly synthesized glucose can be added to the one resulting from absorption and already in circulation with a consequent hyperglycemia. To our knowledge, the nutritional regulation of enzymes involved in hepatic glucose production in fish is poorly documented, especially at a molecular level.

One key step in hepatic glucose production is catalysed by the microsomal glucose-6-phosphatase system because this enzyme catalyzes the hydrolysis of glucose-6-phosphate (coming from gluconeogenesis and glycogenolysis) in glucose.[15] There is now convincing molecular evidence in favour of the substrate model of glucose-6-phosphatase in mammals *ie* a protein complex involving a phosphohydrolase (catalytic subunit protein, G6Pase) (E.C.3.1.3.9) (object of the present study) with its active site oriented towards the lumen of the endoplasmic reticulum, and transporter proteins for glucose-6-phosphate (T1), phosphate (T2) and glucose (T3).[16] The expression of the G6Pase in the liver is thought to play an important role in glucose homeostasis[15], as illustrated by an increased G6Pase activity and the corresponding mRNA levels in type II non insulin-dependent diabetes mellitus.[17,18,19,20] Regulation of G6Pase mRNA abundance is a major control of G6Pase activity : it has long been recognised that hepatic G6Pase synthesis is markedly regulated by changes in hormones (G6Pase gene transcription is stimulated by glucagon and glucocorticoids and inhibited by insulin) and nutritional status (starvation-refeeding).[21,22,23,24,25,26] In fish, little is known about the regulation of this enzyme by dietary factors. In some non salmonids such as the « omnivorous » common carp *Cyprinus carpio*, the existence of G6Pase activity in liver

and kidney has been clearly shown[27,28,29,30,31], but the regulation of its expression remains unclear.

In the light of the mammalian data and in order to understand the mechanisms of the low dietary glucose utilisation by teleost in general and by salmonids in particular, the objective of the present paper was to analyse the nutritional regulation of G6Pase expression (mRNA and activity) in livers of rainbow trout. The first step of our work was to characterise the G6Pase at a molecular level in rainbow trout. Then, an attempt was made to analyse the control of G6Pase expression (mRNA and activity) by dietary levels of carbohydrates.

MATERIAL AND METHODS

Fish and diets.

Triplicate groups of juvenile immature rainbow trout were reared in our experimental fish farm at 18°C with the respective diets for 10 weeks during spring under natural photoperiod. Fish were fed twice a day to near satiation, one of the experimental diets containing 8%, 12%, 20% digestible starch (supplied as dehulled extruded peas or extruded wheat) or without starch (Table 1). At the end of 10 weeks, after a 24h fast, fish (weighing about 150 g) were fed once and then 9 fish from each group were sacrificed 6 and 24 h after the meal. Whole liver, kidney, heart, intestine, brain and a small piece of dorsal muscle tissue were sampled clamp frozen in liquid nitrogen and stored at -80°C.

RNA isolation and reverse transcription.

Total RNA was extracted from rainbow trout tissues as described by Chomczinski and Sacchi.[32] cDNA was obtained by annealing 2 µg of total RNA with 1 µg of random primers and incubating with AMV reverse transcriptase (Boehringer, Roche Molecular Biochemicals, Germany) for 1h at 42°C.

Cloning of partial G6Pase cDNA.

Glucose-6-phosphatase sequences from human (Genbank accession number UO1120), rat (Genbank accession number RNU07993) and a cichlid fish (*Haplochromis nubilus)* (Genbank accession number AF008945) were compared using the Clustal-W multiple alignment algorithm.[33] Primers were chosen corresponding to the most conserved coding regions of G6Pase at positions 426-445 and 1043-1062 of the human gene. The

sequence of the upstream primer was (5'-CTTCAGGTCATGCCATGGGC-3') and that of the downstream degenerate primer was (5'-CMRAARGAMARGRYRTAGAA-3'), where Y=C/T ; M=A/T ; R=A/G. cDNA (1µl) was amplified by polymerase chain reaction (PCR) using 100 pmol of the degenerate primers, in a reaction mixture containing 2 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl, 0.25 mM dNTP, and 2.5U of Taq polymerase (Boehringer, Roche Molecular Biochemicals, Germany). Thirty five cycles of denaturation for 1 min at 94°C, annealing at 50°C for 40s, and extension at 72°C for 1 min were performed. PCR products were subjected to electrophoresis in 1% agarose gels and fragments of the expected size range were purified (Micropure System, Amicon, USA). The purified DNA fragments were inserted into the pCR™II plasmid and used for transformation of One Shot™ competent cells (Invitrogen, Carlsbad, CA, USA). Inserts were detected by *Eco*RI digestion of the extracted plasmid DNA. Clones with inserts were sequenced (Sequenase-2 sequencing kit, Amersham, England).

Sequence analysis.

Nucleotide sequences (excluding the primer sequences) were compared with DNA sequences from the Genbank database with the basic local alignment search tool (BLAST) algorithm.[34] Sequence alignments and percentage of amino acid conservation were assessed with the Clustal-W multiple alignment algorithm[31] using the cloned fish sequence and other G6Pase sequences corresponding to the amplified regions from databases.

Northern analysis.

20 µg of extracted total RNA samples were electrophoresed in 1% agarose gels containing 5% formaldehyde and capillary transferred onto nylon membrane (Hybond-

N^+, Amersham, England). Membranes were hybridized with [^{32}P]-DNA probes labeled by random priming (Stratagene, USA) recognizing rainbow trout G6Pase cDNA. Membranes were also hybridized with a common carp 16 S ribosomal RNA probe (the 3021-3100 bp fragment Genbank accession number MICCCG) to confirm equivalency of loading and specificity of response. After stringent washing, the membranes were exposed to X-ray film and the resulting images were quantitated using Visio-Mic II software (Genomic, France).

RT-PCR analysis.

cDNAs were amplified by PCR using specific primers chosen in the partial rainbow trout G6Pase cDNA sequence : (5'-TCAGTGGCGACAGAAAGGCG-3') and (5'-CAGCAGGTCCAGGCCTATAG-3'). The PCR reaction was carried out in a final volume of 25 μl containing 1.5 mM $MgCl_2$ and 4 pmol of each primer, 2 μl cDNA and 1 U of Taq polymerase (Boehringer, Roche Molecular Biochemicals, Germany). Number of cycles was 35 composed of 20 s for hybridization (at 59°C), 20 s for elongation (at 72°C) and 20s for denaturation (at 94°C). The PCR products were characterized by hybridization with the labeled [^{32}P]-DNA rainbow trout G6Pase probe.

Enzyme assays.

Microsomes were obtained from rainbow trout livers, as described by Mol et al.[35] The final preparation which was stored at -80°C averaged 3-6 mg protein/ml and was used in the spectrophotometric assays. Microsomes were suspended in the buffer (NaH_2PO_4 100mM, Na_2HPO_4 25mM, EDTA 2mM, DTT 1mM pH=7), without further treatment. The standard procedure followed was as described by Alegre et al.[36], monitoring the increase in absorbance (NADH production) using glucose dehydrogenase (Sigma, USA)

in excess as coupling enzyme. One unit of G6Pase activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of glucose-6-phosphate per minute under the specified conditions (30°C). The latency (percentage of the activity of fully disrupted microsomes that is not expressed in microsomes not treated with detergents) was 33% in rat microsomes[36] and can be estimated to be relatively similar in trout microsomes.

Data analysis.

The results are expressed as the means ± standard deviation (SD). Statistical analysis between two series of data was determined using an unpaired two-tailed Student's t-test (Statview software). Differences were considered significant with $p<0.05$.

RESULTS

The available G6Pase cDNA sequences were aligned and highly conserved regions from cichlid fish (*Haplochromis nubilus*) (Genbank accession number AF008945) to human (Genbank accession number UO1120) were identified (data not shown). A set of primers (the reverse primer was degenerated) was designed and made it possible to amplify a fragment of about 630 bp in size. RT-PCR was performed on hepatic total RNA extracted from fish fed without carbohydrates. PCR conditions were optimized and a major amplification product of the expected size was obtained (Figure 1a). The fragments were purified, cloned and sequenced. The cDNA sequence of 625 bp was very similar to those of genes from other G6Pase genes (Blast algorithm, $p=10^{-77}$ to 10^{-33}). The corresponding amino acid sequences were deduced from the cDNA sequences showing an open reading frame of 207 codons (Figure 1b) highly homologous to G6Pase (Blast algorithm, $p=10^{-63}$ to 10^{-43}) (Figures 2 a and b). The highest level of homology (73% for the nucleotide sequence and 67% for the deduced amino acid sequence) was observed with the G6Pase sequence of the cichlid fish (*Haplochromis nubilus).*

Growth rates of rainbow trout fed with (20%) or without carbohydrates were comparable : daily growth coefficients were 3.31±0.09 and 3.36±0.06 respectively. As all fish were fed nutritionally adequate diets, comparative analysis on the effect of dietary carbohydrates on the regulation of G6Pase expression between fish groups fed different carbohydrate levels were possible. At 6h after meal, glycemia of rainbow trout fed with (20%) and without carbohydrates were significantly different ($p<0.01$), 10.5±2.5 and 4.3±0.6 mM respectively. Hepatic G6Pase activities measured in

microsomal samples were similar (Table 2) between fish groups fed with and without carbohydrates at 6h and 24h after feeding.

G6Pase gene expression was analyzed in fish livers by Northern blotting (Figure 3). A unique G6Pase mRNA of about 2.6 kb of size was found. Our results showed a high and constant G6Pase gene expression in the fish livers fed with and without carbohydrates, indicating that hepatic G6Pase gene expression was not dependent on the presence of dietary carbohydrates (Figure 3a). G6Pase gene expression did not depend also upon the levels of dietary carbohydrates (8% to 20% of digestible starch) (Figure 3b) and the time interval after feed intake, 6h *versus* 24h (Figure 3c). By using RT-PCR performed with specific rainbow trout G6Pase primers, G6Pase mRNA could be detected in liver and kidney (the gluconeogenic tissues) but not in muscle and heart, irrespective of the composition of the diet (Figure 4). By contrast, G6Pase gene expression was observed in the intestine and brain only in fish fed without carbohydrates (Figure 4).

DISCUSSION

G6Pase belongs to a family of enzymes, such as L-type pyruvate kinase (E.C.2.7.1.40), phosphoenolpyruvate carboxykinase (E.C.4.1.1.32) and fatty acid synthetase (E.C.2.3.1.85), whose expression has been shown to be regulated by dietary carbohydrates in mammals.[37] In fish, literature data in this area is scarce. Although a formal proof will await the cloning of the full-length cDNA sequence, the high similarity (up to 73%) between the cDNA sequence of G6Pase in rainbow trout and the G6Pase sequences previously characterized in other vertebrates strongly suggest that this sequence corresponds to a functional enzyme.

In rainbow trout, the main gluconeogenic tissues (liver and kidney) express a higher level of G6Pase mRNAs than other tissues, as observed in mammals.[15] G6Pase gene expression in the intestine of fish fed without carbohydrates is comparable to what has been found in mammals.[16] G6Pase expression in the brain of rainbow trout is more intriguing[15] and needs further studies to be confirmed. Our data are also in accordance with the observation of Shimeno and Ikeda[28] showing an absence of G6Pase activities in the muscle and heart and high level of G6Pase activity in the liver.

The low hepatic G6Pase activity observed in this study (around 20mU/mg protein) compared to mammalian data (around 400mU/mg protein)[36] can be explained by the low glucose turnover rates in fish[3,4] : thus, the relatively low needs for glucose as an energy source, necessitating a low level of endogenous glucose production in fish compared to mammals, have been previously observed.[3,4,10] A comparison with

published data on carp[29,30,31] is rather difficult to make since the methods of measurement of G6Pase activity were quite different.

With regard to the regulation of G6Pase by dietary factors in rainbow trout, our data show that G6Pase expression (mRNA and activity) is neither affected by the dietary carbohydrate levels tested here nor it is apparently modified between 6 and 24h after feeding. This is in contrast with our own data (from the same samples) on glucokinase (E.C.2.7.1.1) expression in rainbow trout livers : glucokinase which catalyzes the phosphorylation of glucose to glucose-6-phosphate is significantly induced by dietary carbohydrates.[38 and unpublished data a] Studies of nutritional regulation of G6Pase activity have been previously realised in common carp : dietary supplementation with starch, glucose or fructose seems to depress the activities of G6Pase (and the other gluconeogenic enzymes) in common carp[29,30,31], as in mammals.[15,39] Thus, there is an apparent absence of nutritional control of G6Pase activity and synthesis in rainbow trout in contrast to common carp. Further studies are needed to definitely conclude about the absence of nutritional control of G6Pase synthesis, using ex-vivo analysis on hepatocytes and checking the existence of putative allosteric controls (not tested in this paper) involved in G6Pase expression in fish. In mammals, some products of lipid metabolism such as long-chain fatty acyl CoA are potent inhibitors of G6Pase[15,40,41] without modifying G6Pase production. Knowledge of potential endocrine control (insulin and/or glucagon) on G6Pase is also lacking in fish. In order to understand the possible physiological significance of the absence of nutritional regulation of G6Pase expression in rainbow trout, comparative studies on molecular regulation of G6Pase gene expression in species such as common carp are required.

The absence of regulation of G6Pase enzyme in salmonids such as rainbow trout is probably not surprising given that salmonids are carnivorous and that glucose is mainly produced through gluconeogenesis even in the fed state because of the low glucose availability from the natural preys.[14] Glucose in constant demand is provided mainly, if not exclusively, by the liver in rainbow trout.[14] This means that the need for a rapidly responding system to transient periods of high plasma glucose is reduced, as they do not occur naturally. It is, therefore, apparent that trout liver is not adapted to respond with a rapid change in gluconeogenic rates when challenged with unusually high glycemic conditions. Supported data are also provided by the studies on trout hepatocytes that show a weak regulation of glycogen metabolism in response to glucose and insulin.[6] However, control of hepatic glucose production can be realized through other metabolic reactions than the one catalyzed by G6Pase[13] : gluconeogenesis seems to be affected by the nutritional status with an increase in the activities of the gluconeogenic enzymes (fructose 1,6-biphosphatase (E.C.3.1.3.11) and phosphoenolpyruvate carboxykinase) in fish fed a low carbohydrate diet compared to those fed a carbohydrate rich diet.[42]

In conclusion, our data strongly suggest a lack of control of hepatic G6Pase expression by dietary carbohydrates in rainbow trout. Besides possible abnormal hormonal (insulin/glucagon ratio) response to dietary carbohydrates[5] and tissue resistance to insulin[8,9,10], poor carbohydrate utilization by rainbow trout is also possibly linked to a poor regulation of hepatic gluconeogenesis. Inter-specific comparative studies are warranted to fully elucidate differences in dietary carbohydrate utilisation between species.

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Acknowledgments :

We are grateful to Dr F. L'Horset for helpful discussions and encouragement. We thank F Sandres, F Terrier and Y Hontang for their assistance during the growth trials in the experimental fish farms. We acknowledge also C. Vachot and O. Lapoyade-Deschamp for their technical assistance.

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LEGENDS

Figure 1 : **Partial cloning of the G6Pase gene in rainbow trout. a** : RT-PCR on hepatic total RNA using degenerated primers chosen in the conserved region of known G6Pase. M : molecular weight marker X (Boerhinger, Roche Molecular Biochemicals, Germany). **b** : nucleotide and deduced amino acid sequences of the rainbow trout G6Pase clone. Underlined letters correspond to the primer sequences.

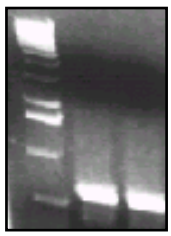
Figure 2 : **Alignments of the partial G6Pase cDNA clone of the rainbow trout with human** (Genbank accession number: UO1120) **and cichlid fish *(Haplochromis nubilus)*** (Genbank accession number: AF008945). Underlined letters correspond to the primer sequences. **a :** Alignment of nucleotide sequences. **b** : Alignment of amino acid sequences.

Figure 3 : **G6Pase gene expression in livers of fish. a:** fish fed with 20% of carbohydrates (+) or without (-) carbohydrates (Northern blotting) 6h after feeding. Each band is from a different fish. The 16S rRNA served as internal control of sample loading. An analysis by densitometry of G6Pase mRNA levels for 9 fish from each treatment group weighted by 16S rRNA values was performed (Visio-Mic II software). NS : non significant (Student t-test). **b**: fish fed with two intermediary levels of dietary carbohydrates (8%, 12%) at 6h after feeding. **c :** fish fed with carbohydrates at 6h and 24h after feeding.

Figure 4 : Tissue-specificity of G6Pase gene expression in rainbow trout fed with 20% of carbohydrates or without carbohydrates (at 6h after feeding). Analysis by

RT-PCR (n= 2 fish per treatment). X : Molecular Weight Marker phiX174 DNA/HaeIII (Promega, USA). L : Liver, M : Muscle, K : Kidney, I : Intestine, H : Heart, B : Brain, - : negative controls (RT-PCR reactions performed without RNA and without reverse transcriptase). The exact length of the G6Pase fragment (300 bp) is determined following the known G6Pase gene sequence.

a



625 bp

M Trout

b

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    S   G   H   A   M   G   S   S   G   V   W   Y   V   M   I   T   A     17
CT TCA GGT CAT GCC ATG GGC TCG TCT GGG GTG TGG TAC GTG ATG ATA ACA GCT    53

 V   F   S   V   A   T   E   R   R   F   P   P   L   L   Y   R   F   L     35
GTC TTC TCA GTG GCG ACA GAA AGG CGG TTC CCC CCT CTC CTG TAC AGG TTC TTG   107

 Q   V   G   L   W   M   L   L   C   T   V   E   L   L   V   C   M   S     53
CAG GTG GGG CTC TGG ATG CTG CTG TGT ACA GTA GAG CTG TTG GTG TGC ATG TCC   161

 R   V   Y   M   A   A   H   F   P   H   Q   V   I   S   G   V   I   T     71
AGA GTC TAC ATG GCT GCC CAC TTC CCA CAC CAG GTC ATC AGT GGG GTC ATC ACA   215

 G   I   M   V   A   E   A   F   S   R   V   Q   W   I   Y   G   A   S     89
GGT ATC ATG GTG GCT GAG GCC TTC TCC AGA GTG CAG TGG ATC TAT GGA GCC AGT   269

 L   K   K   Y   F   Y   T   T   L   F   L   L   S   F   A   V   G   F    107
CTG AAG AAG TAC TTC TAC ACC ACC CTC TTT CTG CTC TCC TTT GCT GTG GGC TTC   323

 Y   E   L   L   K   A   I   G   V   D   L   L   W   S   L   E   K   A    125
TAC GAG CTA CTG AAA GCT ATA GGC GTG GAC CTG CTG TGG TCC CTG GAG AAA GCC   377

 Q   K   W   C   V   R   A   E   W   V   Y   M   D   S   T   P   F   A    143
CAG AAG TGG TGT GTG AGA GCC GAG TGG GTC TAC ATG GAC TCC ACT CCT TTC GCC   431

 I   L   L   R   N   M   G   T   L   F   G   L   G   L   G   L   H   S    161
ATC CTC CTG CGC AAC ATG GGC ACC CTG TTT GGC CTG GGC CTG GGC CTG CAC TCA   485

 P   L   Y   T   E   N   K   N   S   S   I   P   F   R   V   G   C   I    179
CCC CTC TAC ACC GAG AAC AAG AAC AGC AGC ATC CCC TTC AGG GTG GGG TGT ATC   539

 T   V   S   L   L   L   L   Q   I   L   D   G   L   T   F   S   S   R    197
ACT GTC TCT TTA TTG TTG CTA CAG ATT TTG GAT GGC TTG ACG TTC TCC TCG AGA   593

 D   Q   A   M   F   Y   T   L   S   F                                    207
GAC CAG GCA ATG TTC TAC ACC CTT TCC TTT GG                               625
```

Figure 1

a

```
         10         20         30         40         50         60
          |          |          |          |          |          |
CTTCAGGTCATGCCATGGGCTCGTCTGGGGTGTGGTACGTGATGATAACAGCT-GTCTTC
CTTCAGGTCATGCCATGGGCGCAGCTGGTGTCTGGTATGTCATGGTAACAGCA-CTGCTC
CCTCTGGCCATGCCATGGGCACAGCAGGTGTATACTACGTGATGGTCACATCTACTCTTT
* **  ** ************ *  * **  ** *  ** ** *** * *** *   *  *

         70         80         90         100        110        120
          |          |          |          |          |          |
TCAGTGGCGACAGAAAGGCGGTTCCCCCCTCTCCTGTACAGGTT------CTTGCAGGTG
TCTATTGCAAGAGAAAAACAGTGCCCCCCATTGCTATACAGATT------TTTGTATATA
CCATCTTTCAGGGAAAGATAAAGCCGACC-------TACAGATTTCGGTGCTTGAATGTC
 *       *  ****       **  **       ***** **       *** *  *

         130        140        150        160        170        180
          |          |          |          |          |          |
GGGCTCTGGATGCTGCTGTGTACAGTAGAGCTGTTGGTGTGCATGTCCAGAGTCTACATG
GGCCTGTGGATGCTAATGGGCCTGGTCGAGCTGGTGGTATGCATTTCCAGGGTCTACATG
ATTTTGTGGTTGGGATTCTGGGCTGTGCAGCTGAATGTCTGTCTGTCACGAATCTACCTT
    * *** **    *  *    **  *****   ** **  * **  *  ***** *

         190        200        210        220        230        240
          |          |          |          |          |          |
GCTGCCCACTTCCCACACCAGGTCATCAGTGGGGTCATCACAGGTATCATGGTGGCTGAG
GCTGCTCACTTCCCACACCAGGTTATTGCAGGAATCATTACAGGCACACTGGTAGCTGAA
GCTGCTCATTTTCCTCATCAAGTTGTTGCTGGAGTCCTGTCAGGCATTGCTGTTACAGAA
***** ** ** ** ** ** **  *    **  ** *  **** *     **  * **

         250        260        270        280        290        300
          |          |          |          |          |          |
GCCTTCTCCAGAGTGCAGTGGATCTATGGAGCCAGTCTGAAGAAGTACTTCTACACCACC
GTTGTTTCCAAGGAGAAATGGATCTACAGCGCAAGCCTGAAGAAGTACTTCTTAATTACC
ACTTTCAGCCACATCCACAGCATCTATAATGCCAGCCTCAAGAAATATTTTCTCATTACC
    *   *       *  * *****    ** ** ** ***** ** **    *  ***

         310        320        330        340        350        360
          |          |          |          |          |          |
CTCTTTCTGCTCTCCTTTGCTGTGGGCTTCTACGAGCTACTGAAAGCTATAGGCGTGGAC
CTCTTCCTCACCTCCTTTGCTGTTGGCTTTTATGTGCTCCTTAAAGCTCTGGATGTGGAC
TTCTTCCTGTTCAGCTTCGCCATCGGATTTTATCTGCTGCTCAAGGGACTGGGTGTAGAC
 **** **   *  *** **  * ** ** **   *** ** ** *   * *  ** ***

         370        380        390        400        410        420
          |          |          |          |          |          |
CTGCTGTGGTCCCTGGAGAAAGCCCAGAAGTGGTGTGTGAGAGCCGAGTGGGTCTACATG
CTGCTGTGGACCATGGAGAAAGCCCAGAAGTGGTGCATCAGGCCAGAGTGGGTTCACCTA
CTCCTGTGGACTCTGGAGAAAGCCCAGAGGTGGTGCGAGCAGCCAGAATGGGTCCACATT
** ****** *  *************** ******        * ** *****  ** *

         430        440        450        460        470        480
          |          |          |          |          |          |
GACTCCACTCCTTTCGCCATCCTCCTGCGCAACATGGGCACCCTGTTTGGCCTGGGCCTG
 GACTCTGCCCCCTTTGCTAGCCTCCTGCGGAACATGGGTAGCCTGTTTGGTCTGGGCCTC
GACACCACACCCTTTGCCAGCCTCCTCAAGAACCTGGGCACGCTCTTTGGCCTGGGGCTG
*** *  * ** ** ** * ******    *** **** *  ** ***** ***** **

         490        500        510        520        530        540
          |          |          |          |          |          |
GGCCTGCACTCACCCCTCTACA------CCGAGAACAAGAACAGCAGCA------TCCCC
GGTCTGCACTCACCGTTCTACAAGACAACCAAGATGAGGATTATGAGCG------CCCCT
GCTCTCAACTCCAGCATGTACAGGGAGAGCTGCAAGGGGAAACTCAGCAAGTGGCTCCCA
*  **  ****     * ****       *   *    **     ***        ***

         550        560        570        580        590        600
          |          |          |          |          |          |
TTCAGGGTGGGGTGTATCACTGTCTCTTTATTGTTGCTACAGATTTTGGATGGCTTGACG
TTGAGGATTGGATGTATTGTCATCTCTGTATCCCTGCTTCACCTGTTAGATGGATGGACA
TTCCGCCTCAGCTCTATTGTAGCCTCCCTCGTCCTCCTGCACGTCTTTGACTCCTTGAAA
**  *  *  * * ***      ***  *     * ** **  * ** **    * **

         610        620        630        640
          |          |          |          |
TTCTCCTCGAGAGACCAGGCAATGTTCTACACCCTTTCCTTTGG
TTTTCCCCTGAAAACCATATGACTTTCTATGCCCTTTCCTTTGG
CCCCCATCCCAAGTCGAGCTGGTCTTCTACGTCTTGTCCTTCTG
    *  *   *  * *       *****   * * *****  *
```

b

```
                10        20        30        40        50        60
                 |         |         |         |         |         |
Trout       SGHAMGSSGVWYVMITAVFSVATERRFPPLLYRFLQVGLWMLLCTVELLVCMSRVYMAAH
Cichlid     SGHAMGAAGVWYVMVTALLSIARBKQCPPLLYRFLYIGLWMLMGLVELVVCISRVYMAAH
Human       SGHAMGTAGVYYVMVTSTLSIFQGKIKPTYRFRCLNVILWLGFWAVQLNVCLSRIYLAAH
            ******  ** *** *   *       *   * *   **    * * ** ** * ***

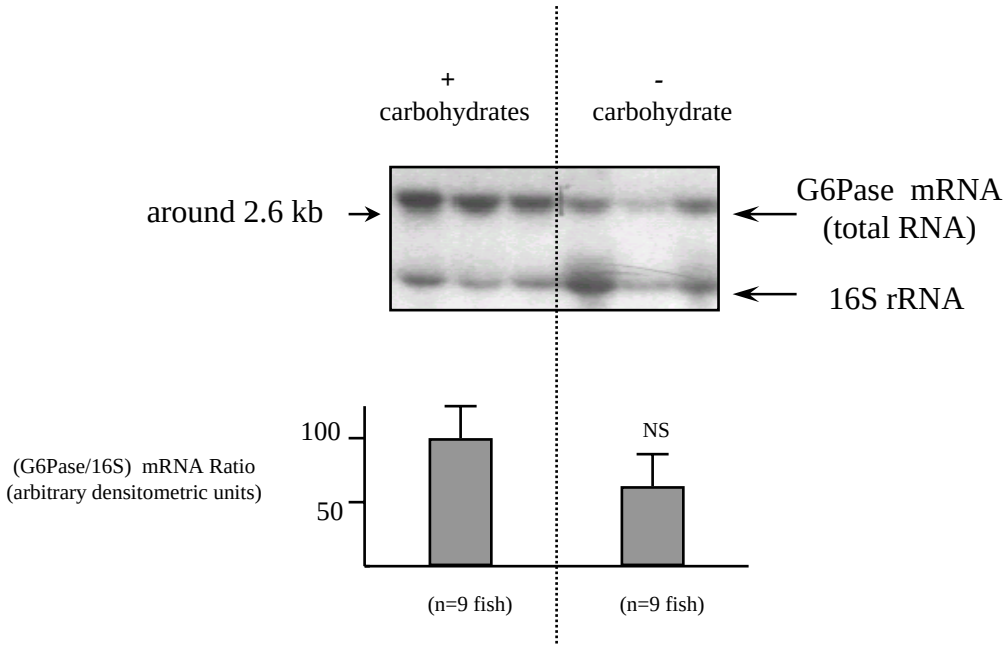
                70        80        90        100       110       120
                 |         |         |         |         |         |
Trout       FPHQVISGVITGIMVAEAFSRVQWIYGASLKKYFYTTLFLLSFAVGFYELLKAIGVDLLW
Cichlid     FPHQVIAGIITGTLVAEVVSKEKWIYSASLKKYFLITLFLTSFAVGFYVLLKALDVDLLW
Human       FPHQVVAGVLSGIAVTETFSHIHSIYNASLKKYFLITFFLFSFAIGFYLLLKGLGVDLLW
            *****  *   *  * *  *   ** *******  * ** *** *** ***   *****

                130       140       150       160       170       180
                 |         |         |         |         |         |
Trout       SLEKAQKWCVRAEWVYMDSTPFAILLRNMGTLFGLGLGLHSPLYTENKNS----SIPFRV
Cichlid     TMEKAQKWCIRPEWVHLDSAPFASLLRNMGSLFGLGLGLHSPFYKTTKMRIM--SAPLRI
Human       TLEKAQRWCEQPEWVHIDTTPFASLLKNLGTLFGLGLALNSSMYRESCKGKLSKWLPFRL
              **** **   ***  *  *** ** * * ****** * *  *           * *

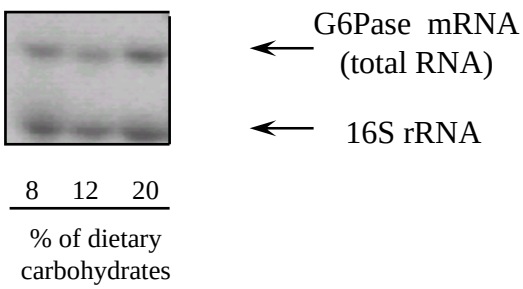
                190       200       210
                 |         |         |
Trout       GCITVSLLLLQILDGLTFSSRDQAMFYTLSF
Cichlid     GCIVISVSLLHLLLDGWTFSPENHMTFYALSF
Human       SSIVASLVLLHVFDSLKPPSQVELVFYVLSF
              *  *  **   *          ** ***
```

ure 2

a


+
carbohydrates
-
carbohydrate
around 2.6 kb
G6Pase mRNA
(total RNA)
16S rRNA
(G6Pase/16S) mRNA Ratio
(arbitrary densitometric units)
100
50
NS
(n=9 fish)
(n=9 fish)

b


G6Pase mRNA
(total RNA)
16S rRNA
8 12 20
% of dietary
carbohydrates

c

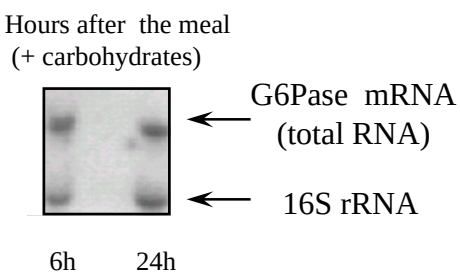

Hours after the meal
(+ carbohydrates)
G6Pase mRNA
(total RNA)
16S rRNA
6h
24h

Figure 3

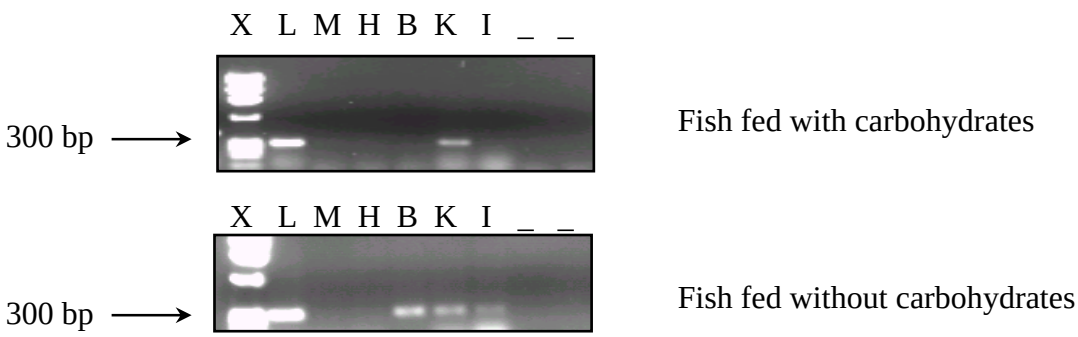

X L M H B K I _ _
300 bp
Fish fed with carbohydrates
X L M H B K I _ _
300 bp
Fish fed without carbohydrates

Figure 4

Table 1. Formulation and chemical composition of the experimental diets for rainbow trout

DIETS	20% (+) carbohydrates	(-) carbohydrate	8% carbohydrates	12% carbohydrates
Analytical composition				
Dry matter (DM, %)	85.5	92.7	83.6	84.3
Crude Protein (CP), %DM	39.5	54.8	43.1	42.2
Crude Lipid , %DM	16.6	18.3	18.0	17.8
Digestible Starch , %DM	20.4	<0.2	8.0	11.7
Gross Energy, kJ/gDM	22.1	23.1	22.1	22.3

Table 2. G6Pase activities in livers of rainbow trout fed with 20% of carbohydrates or without carbohydrates

	G6Pase activities (mU/mg protein)	
Diets	6h	24h
+ carbohydrates*	22±6	20±1
- carbohydrate	15±5	18±3

Data (means ± SD) are from 2 pools of microsomes (1 pool = 3 fish)
*: 20% of digestible starch