Science of the Total Environment 612 (2018) 276–285

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# A reassessment of the carnivorous status of salmonids: Hepatic glucokinase is expressed in wild fish in Kerguelen Islands

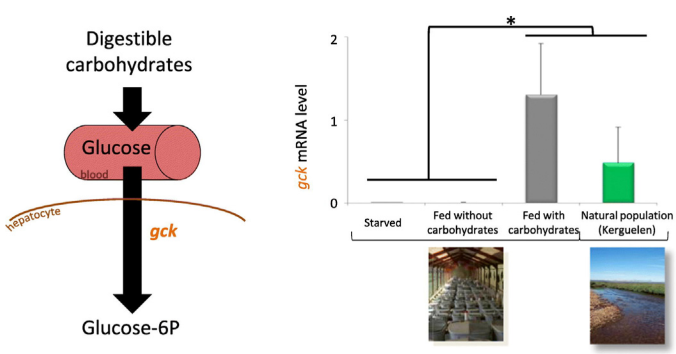
Lucie Marandel [a,*], Philippe Gaudin [b], François Guéraud [b], Stéphane Glise [b], Alexandre Herman [a], Elisabeth Plagnes-Juan [a], Vincent Véron [a], Stéphane Panserat [a], Jacques Labonne [b]

[a] INRA, Univ Pau & Pays Adour, UMR 1419, Nutrition, Metabolism and Aquaculture, Saint Pée sur Nivelle F-64310, France
[b] INRA, Univ Pau & Pays Adour, UMR 1224, ECOBIOP, Saint-Pée sur Nivelle F-64310, France'

## HIGHLIGHTS

- Kerguelen trout are mainly fed on preys containing digestible carbohydrates.
- Glycaemia and *gck* mRNA level demonstrate that they metabolise dietary glucose.
- We conclude that trout in natural environment consume dietary carbohydrates.
- This may explain the evolutionary conservation of the *gck* nutritional regulation.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 26 June 2017
Received in revised form 23 August 2017
Accepted 24 August 2017
Available online xxxx

Editor: D. Barcelo

*Keywords:*
Gluconeogenesis
Glucose metabolism
Environment
Carbohydrates
Polar

## ABSTRACT

Salmonids belong to a high trophic level and are thus considered as strictly carnivorous species, metabolically adapted for high catabolism of proteins and low utilisation of dietary carbohydrates. However they conserved a “mammalian-type” nutritional regulation of glucokinase encoding gene and its enzymatic activity by dietary carbohydrates which remains puzzling regarding their dietary regime. The present study investigates the hypothesis that this conservation could be linked to a real consumption by trout of this nutrient in their natural habitat. To do so, brown trout were sampled in the sub-Antarctic Kerguelen Islands, a site presenting oligotrophic hydrosystems and no local freshwater fish fauna prior the introduction of salmonids fifty years ago. Qualitative and quantitative analysis of carbohydrate content within Kerguelen trout stomachs demonstrate that these animals are fed on food resources containing digestible carbohydrates. Additionally, glycaemia and more particularly *gck* mRNA level and gck enzymatic activity prove that Kerguelen trout digest and metabolise dietary carbohydrates. Physiological and molecular analyses performed in the present study thus strongly evidence for consumption of dietary carbohydrates by wild trout in natural environments. Investigating differences between Kerguelen individuals, we found that smaller individuals presented higher glycaemia, as well as higher carbohydrates contents in stomach. However no relationship between scaled mass index and any physiological indicator was found. Thus it appears that Kerguelen trout do not turn to carbohydrate diet because of a different condition index, or that the consumption of carbohydrates does not lead to a generally degraded physiological status.
As a conclusion, our findings may explain the evolutionary conservation of a “mammalian-type” nutritional regulation of gck by dietary carbohydrates in these carnivorous fish.



* Corresponding author.
*E-mail addresses:* lucie.marandel@inra.fr (L. Marandel), philippe.gaudin@inra.fr (P. Gaudin), francois.gueraud@inra.fr (F. Guéraud), stephane.glise@inra.fr (S. Glise), alexandre.herman@inra.fr (A. Herman), elisabeth.plagnes-juan@inra.fr (E. Plagnes-Juan), vincent.veron@inra.fr (V. Véron), stephane.panserat@inra.fr (S. Panserat), jacques.labonne@inra.fr (J. Labonne).

http://dx.doi.org/10.1016/j.scitotenv.2017.08.247

## 1. Introduction

In order to improve the sustainability of salmonid aquaculture it is now essential to reduce dietary levels of fish meal (FM) by inclusion of alternative terrestrial plant products, and particularly digestible carbohydrates, in aquafeed formula (Naylor et al., 2009). Indeed, optimal inclusion of carbohydrates in diet can lead to a "sparing effect" for protein, meanwhile reduce the nitrogen waste in water and spare protein for growth (Naylor et al., 2009). However, salmonids, including brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*), belong to a high trophic level and is thus considered as a strict carnivorous species (Jonsson and Gravem, 1985). Indeed, when FM is substituted at more than 20% by digestible carbohydrates, rainbow trout (*Oncorhynchus mykiss*) displays a persistent postprandial hyperglycaemia defining it as a glucose-intolerant species (Bergot, 1979; Moon, 2001; Polakof et al., 2011, 2012; Seiliez et al., 2011; Skiba-Cassy et al., 2013). At the end of the 90s, one hypothesis put forward to explain this persistent hyperglycaemia was the absence of an inducible hepatic glucokinase (gck) activity (Vandercammen and Van Schaftingen, 1993) and thus the inability of these fish to convert efficiently the intracellular glucose into glucose-6-phosphate (Cowey and Walton, 1989; Wilson, 1994). Indeed, in vertebrates, glucokinase from hepatic tissue plays an important role in controlling the rate of glucose utilisation as well as in glucose homeostasis (Wilson, 1995). Finally, in 2000 the full-length cDNA sequence encoding for a gck was cloned in rainbow trout (Panserat et al., 2000a). Based on this discovery, additional investigations revealed that hepatic *gck* expression is mainly regulated by dietary carbohydrates in fish (Panserat et al., 2014) and that this regulation works like an ON/OFF system linked to the presence of digestible carbohydrates in the diet (Marandel et al., 2015). Moreover, induction of gck activity has been shown to be closely linked to the increase of *gck* mRNA level both in the context of long-term feeding or just after one meal with a high carbohydrate diet (Panserat et al., 2001; Seiliez et al., 2011; Skiba-Cassy et al., 2013). A second gene encoding for gck was recently identified in rainbow trout thanks to the newly sequencing of its genome (Berthelot et al., 2014; Marandel et al., 2015) and was shown to display the same regulation by dietary carbohydrates than the gene previously discovered. Together with the fact that the level of gck activities in fish fed a high carbohydrate diet is equivalent to that found in mammals (Panserat et al., 2000b; Polakof et al., 2007), all these findings demonstrate that induction of gck is not a limiting factor to the use of dietary carbohydrates in carnivorous fish. Nevertheless, the conservation of a "mammalian-type" nutritional regulation of *gck* by dietary carbohydrates remains puzzling if we consider that carnivorous fish have, by definition, a proteins- and lipids-based diet without carbohydrates. Panserat et al. (2014) previously hypothesised that such a conservation can suggest that gck in these animals can play major roles in life probably not only linked to dietary carbohydrates (Panserat et al., 2014). However we cannot also exclude that the conservation of this specific regulation of *gck* by dietary carbohydrates in trout, mainly monitored in farmed fish assays (i.e. fish fed an experimental controlled diet), could be linked to a real consumption of this nutrient in their natural habitat. Indeed, several studies reported that wild trout consumed invertebrates and more particularly molluscs and insects (Ball, 1961; Lesel et al., 1971; Ogrady, 1983) which main energy storage, together with lipids, is done as glycogen (Arrese and Soulages, 2010; Sminia, 1972). Indeed, in contrast to healthy vertebrates (Ceperuelo-Mallafre et al., 2016), glycogen storage occurs in adipose tissue of invertebrates (Azeez et al., 2014) and constitutes a significant fraction of tissue weight (Hochachka, 1991). This kind of consumption may be sufficient to require an efficient *gck* regulatory system to metabolise glucose resulting from the hydrolysis of glycogen.

Such hypothesis, if proven, would dramatically alter the paradigm around carnivory in salmonids, and subsequently impact several areas of knowledge. First and as previously explained, tremendous scientific and societal efforts are undertaken in carnivorous fish farming to replace animal-based food by plant-based food, in order to reduce environmental impacts, which directly implies to ingest significant amounts of carbohydrates (Kamalam et al., 2017). Second, such paradigm conditions our view of their ecological niche, and therefore of their ability to invade or to maintain into ecosystems under global change (Sax et al., 2007). Third, it may provide a missing causal link usually absent when retracing the long term evolution of glucose regulatory system such as glucokinase (Irwin and Tan, 2014). In this context, full knowledge of the natural abilities of a species will promote acceptance of plant based diet for consumers. In parallel, steps have been undertaken to rely on selection in farmed stocks to achieve acceptable yields (Le Boucher et al., 2012), and a better definition of the extent of available genetic resources could act as a catalyst for such approaches.

The aim of the present study was thus to investigate the hypothesis that wild salmonids make use of their *gck* regulatory system following ingestion of carbohydrates through natural feeding. To do so, the sub-Antarctic Kerguelen Islands seem to be a site of choice to explore in particular from an historical point of view with the recent introduction of salmonids. Indeed, several species of salmonids, including brown trout, have been introduced since the 50′s in these oligotrophic freshwater ecosystems which contained no other native fish species (Labonne et al., 2013), limiting the usual trophic resources to terrestrial or aquatic invertebrates (Lesel et al., 1971). Moreover, previous studies (Lecomte et al., 2013) showed that in some habitats, such as the upstream part of the Château river (where first natural spawning occurred in 1962 after the initial introduction), a significant fraction of the population is resident, meaning that these individuals do not migrate to the marine environment for their growth phase. Such a behavior thus limits the diet of these fish to invertebrates, at least until a given body size is reached (i.e., before piscivory through cannibalism is possible). Interestingly, these resident brown trout were also shown to frequently display hepatic alterations similar to steatosis such as lipid vacuolization. Such symptoms were hypothesised to be due to pollution in the Kerguelen Islands (Jaffal et al., 2015) but this pollution remains nevertheless moderate and its statistical correlation with symptoms was either non-existent or weak. However ingestion of dietary carbohydrates was also shown to increase hepatic lipid depositions through lipogenesis (Brauge et al., 1994) in trout. In this context, hepatic phenotypes noticed in the Kerguelen resident brown trout may also be due to carbohydrates ingestion through the consumption of invertebrates. Resident wild brown trout (called "Kerguelen trout" thereafter) were thus sampled in the Château river in the Kerguelen Islands to elucidate the possible consumption and use of carbohydrates by wild brown trout. To that end, physiological and molecular markers related to ingestion and metabolism of dietary carbohydrates were investigated and compared to those obtained in farmed rainbow trout (called "farmed trout" thereafter) after fasting (F) or after being fed a no-(NC) or a high carbohydrate (HC) diet. We also assumed that such consumption could vary among wild individuals, so we investigated whether these physiological markers related to dietary carbohydrates could correlate with either body size or condition index in Kerguelen trout.

## 2. Methods

### 2.1. Fish and sampling

Forty five brown trout (*Salmo trutta*, L., group Ker) were caught by electrofishing in the upstream part of the Château river (latitude: 49°17′55.90″S, longitude: 70°10′12.61″E) on Kerguelen Islands (Fig. 1A). All fish were sampled within the same day, between 10 and 16 pm. The daily temperature varied from 7 to 11 °C from the beginning to the end of the sampling, however, we found no correlation between any of our indicators and the timing of fish sampling during the experiment. Immediately after fishing, each fish was first anesthetized by bathing in phenoxy-ethanol (at 0.3 mL/L, namely 7.2 mg/L), then killed by terminal anaesthetization (at 0.6 mL/L, namely 14.4 mg/L). Death