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Original Research Article

Gluconeogenesis during starvation and refeeding phase is affected by previous dietary carbohydrates levels and a glucose stimuli during early life in Siberian sturgeon (*Acipenser baerii*)

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ABSTRACT

Gluconeogenesis responses was assessed during a short starvation period and subsequent refeeding in Siberian sturgeon (*Acipenser baerii*) previously fed different dietary carbohydrates levels and experienced to a glucose stimuli during early life. The sturgeon larvae were previously fed either a high glucose diet (G) or a low glucose diet (F) from the first feeding to yolk absorption (8 to 12 d post-hatching [dph]). Each group of fish was sub-divided into 2 treatments at 13 dph and was fed either a high-carbohydrate diet (H) or a low carbohydrate diet (L) until 20 wk. In the current study, the fish in 4 groups (GL, FL, GH and FH) were experienced to starvation for 21 d following by re-feeding of their corresponding diets for 21 d. Fish were sampled at postprandial 6 and 24 h before starvation (P6h and P24h), starvation 7, 14 and 21 d (S7, S14 and S21) and 1, 7, 14 and 21 d during refeeding (R1, R7, R14 and R21). Plasma samples during refeeding were taken at P6h at each time point. Glycaemia levels, liver and muscle glycogen contents, activities and mRNA levels of hepatic gluconeogenic enzymes were examined. We found that both dietary carbohydrate levels and early glucose stimuli significantly affected the metabolic responses to starvation and refeeding in Siberian sturgeon ($P < 0.05$). During prolonged starvation, Siberian sturgeon firstly mobilized the liver glycogen and then improved gluconeogenesis when the dietary carbohydrates were abundant, whereas preserved the liver glycogen stores at a stable level and more effectively promoted gluconeogenesis when the dietary carbohydrates are absent to maintain glucose homoeostasis. During refeeding, as most teleostean, Siberian sturgeon failed controlling the activities and mRNA levels of phosphoenolpyruvate carboxykinase cytosolic forms (*PEPCK-C*), fructose-1,6-bisphosphatase (*FBPase*), but particularly controlled phosphoenolpyruvate carboxykinase mitochondrial forms (*PEPCK-M*) activities and mRNA expression of glucose-6-phosphatase (*G6Pase*, except in GL group). Siberian sturgeon has a full compensatory ability on growth, but this ability would be obstructed by early glucose stimuli when refeeding the low carbohydrate diet after S21.

1. Introduction

Both in aquaculture and in natural water, fish could experience periods of food deprivation or starvation, which are caused by seasonal fluctuations, reproductive process, or imposed by routine aquaculture procedures. Fish in northern latitudes experience low winter water temperatures often accompanied with limited food availability and low apatite controlled by endocrine system

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(Pottinger et al., 2003; Brodersen et al., 2011). Other factors may induce long-term fasting includes the abnormal increased ocean water temperature by changing climate, salinity changes and reproduction, etc. (Polakof et al., 2006; Brodersen et al., 2011). To face these nutritional stresses, fish mobilize their endogenous reserves to obtain the energy to maintain vital processes, which impose metabolic adjustments that are species dependent (Wang et al., 2006). Intraspecific adjustments to these conditions also depend on different factors such as fish age or nutritional status (Navarro and Gutiérrez, 1995).

In most species, liver glycogen is generally the first substrate used as an energy source, and mobilized to maintain the glucose homoeostasis or normoglycaemia during the first stages of starvation (Figueiredo-Garutti et al., 2002; Metón et al., 2003; Furné et al., 2012). Together with glycogen mobilization, reserved lipids are used to obtain energy, and the protein, mainly from skeletal muscle would be mobilized subsequently (Navarro and Gutiérrez, 1995; Metón et al., 2003). The muscle glycogen may be depleted during starvation or maintained stable through the continuous generation of glucose in the liver (Navarro and Gutiérrez, 1995). In contrast to this pre-established dogma, some species try to preserve liver glycogen stores by degrading protein for gluconeogenesis and use lipid and/or protein as energy substrates (Sheridan and Mommsen, 1991; Gillis and Ballantyne, 1996). The different responses of fish refed after a starvation period may depend on species, environmental conditions, starvation period, and the feeding history prior to starvation (Navarro and Gutiérrez, 1995). In most species, the metabolic profiles would return to pre-starvation levels after a short refeeding period with a fast weight recovery known as compensatory growth (Metón et al., 2003; Furné et al., 2012; Morshedi et al., 2013).

Sturgeon are the only members of a primitive group of fish, the chondrostean, surviving today. They occupy an intermediate position between elasmobranches and teleosts. Siberian sturgeon distributes in almost all river systems of northern latitudes. They have a very long-life span (up to 100 years), and become sexual maturity at 9 to 15 years for males or 16 to 20 years for females in natural environment. In water recirculation systems, sexual maturity can firstly occur at 5 years (http://www.fishbase.org/ Kottelat and Freyhof, 1972). Owing to its long-life span and the distribution condition, sturgeon encounters regular periods of low food availability, making it a suitable species for studies of gluconeogenesis strategies to face starvation and refeeding. It has been reported that the metabolic responses to some nutritional conditions, including starvation and refeeding on Adriatic sturgeon (*Acipenser naccarii*), are different from rainbow trout (*Oncorhynchus mykiss*) (Furné et al., 2009, 2012). Besides, some studies showed the dietary carbohydrates can be utilized effectively by Siberian sturgeon (*A. baerii*) (Kaushik et al., 1989; Yun et al., 2014; Gong et al., 2015) and other sturgeon species (Lin et al., 1997; Furné et al., 2005). A few studies have reported the responses of compensatory growth, plasma performances and/or body compositions in white sturgeon (*A. transmontanus*), Chinese sturgeon (*A. sinensis*), Persian sturgeon (*A. persicus*) and Siberian sturgeon facing starvation and refeeding respectively (Liu et al., 2011; Yarmohammadi et al., 2012; Morshedi et al., 2013), but there are no reports on the effects of nutritional history, including early programming and latter dietary carbohydrates levels on metabolic responses on any sturgeon species.

Early nutritional programming might be a way to modify metabolic responses during later life. The programming stimulus exerted in early ontogeny stages may have long-term consequences on physiological functions in later life stages (Lucas, 1998). Several studies demonstrated that fish also showed an obvious developmental plasticity by nutritional conditioning during the critical developmental stages early in life, which is just similar to the responses in mammals (Geurden et al., 2007; Vagner et al., 2007, 2009; Fang et al., 2014; Gong et al., 2015). Gong et al. (2015) had found that high glucose intake during start feeding stage disturbed gluconeogenesis regulation in later life of Siberian sturgeon (Gong et al., 2015). However, no any reports on the adaptability to nutritional history of this primitive species in its long-life span. Therefore, the objectives of present study were to evaluate the possible influence of dietary carbohydrates on the metabolic strategy of Siberian sturgeon during a short starvation period and subsequent refeeding, and to determine whether an acute glucose stimulus during start feeding period could modify later gluconeogenesis response.

2. Materials and methods

The experimental protocols used for sturgeon in this study have been approved by Chinese Academy of Agricultural Sciences Animal Care and Use Committee following the principle of the State Council Regulation on Laboratory Animal Administration (July 18, 2013).

2.1. Experimental diets, fish husbandry and sampling

The present study was conducted on the leftover fish of Gong et al. (2015), in which 3 experimental diets were prepared and the formulation and compositions are shown in Table 1. The diet G contained 57% glucose and was used during the first feeding period of larvae as a hyperglucidic stimulus. Glucose was chosen as the carbohydrate source in this diet because glucose may allow the larvae to bypass the carbohydrate digestion step and induce pronounced stimulation (Geurden et al., 2007). Two other isoenergetic (19.6 kJ/g gross energy) diets were fed to fish after the stimulus until 20 wk. One contained a high level of digestible carbohydrates (35% dextrin, H diet), the other was with very low carbohydrates (3.6%, F/L diet). All ingredients were thoroughly mixed and formed into pellets (0.4, 0.6, 1, and 2.5 mm in diameter) with an extrusion-bending roller (Yanggong Machine, Beijing, China). All diets were air-dried and stored at −20 °C throughout the experimental period. Chemical compositions of experimental diets were determined using the methods of AOAC (2006) and the data are shown in Table 1.

The feeding and sampling protocol are shown in Fig. 1. The sturgeon larvae were fed diet G (high glucose stimulation) or diet F (free from stimulation) from the first feeding to yolk absorption (8 to 12 d post-hatching [dph]). At 13 dph, each group of fish was assigned to 2 treatments. One treatment was fed the high-carbohydrate diet (as groups GH and FH), and the other treatment was fed the low-carbohydrate diet (as groups GL and FL) until 20 wk with 6 replicates for each treatment and 30 fish in each tank (diameter: 80 cm; volume: 0.3 m^3). All fish were fed 3 times per day at 08:00, 14:00 and 20:00 (published in Gong et al., 2015). After 20 weeks feeding, all fish were starved for 21 d and then refed for 21 d. During refeeding period, the fish were fed to apparent satiation 3 times daily with the corresponding diet (diet H or diet L) following the feeding protocol before starvation. The water temperature was maintained at 18 to 20 °C, with dissolved oxygen levels of 6.8 to 7.8 mg/L, pH = 8.5 and NH_4—N < 0.5 mg/L. Aeration was supplied to each tank 24 h per day and fluorescent light was separately designed above the tanks and kept on from 08:00 to 21:00 for photoperiod of 13 D: 11 L.

Six fish in each tank from 3 of 6 replicates ($n = 18$) were individually weighed and sampled at postprandial 6 (the glycaemia peak time point, Gong et al., 2015) and 24 h before starvation (P6h and P24h), starvation 7, 14 and 21 d (S7, S14 and S21). Eighteen fish from other 3 tanks were sampled at P6h at refeeding 1, 7, 14 and 21 d (R1, R7, R14 and R21). Body weight and liver weight were

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