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Acute hypoxia reveals diverse adaptation strategies to fully substituted plant-based diet in isogenic lines of the carnivorous rainbow trout

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ABSTRACT

Rainbow trout—a carnivorous fish—are increasingly fed oil and meal from plant origin for environmental purposes but full substitution of fish meal leads to pathological responses and deterioration of growth performances. Fish may also be more sensitive to environmental perturbations. In this study we investigated whether genetic determinism linked to the ability to growth under PB diet was paired to robustness to environmental perturbation. In this aim we performed an hypoxic challenge on three heterozygous isogenic lines of rainbow trout, fed a marine (M) or a fully substituted plant-based (PB) diet. In addition, we considered two feeding status, fed or starved. Isogenic line responsiveness, i.e. time elapsed between water arrival cut-off and loss of equilibrium, was evaluated using a Cox proportional hazard model. We showed that feeding status, diet and host genotype had a significant impact on trout tolerance to hypoxia. One isogenic line, A22h, exhibited higher tolerance to hypoxia under PB diet when fed. In contrast R23h and AB1h lines, behaved indifferently regardless the nutritional history, highlighting their robustness to diet substitution. Under the M diet, the three lines behaved similarly when starved whereas, when fed, only R23h and AB1h responded similarly. At the opposite, the three lines behaved differently under PB diet regardless the feeding status. These results indicate that response and robustness were related to hypoxia resistance. Thus hypoxic challenge per se is a promising test to assess trout adaptive capacity in response to diet replacement. It may help to identify robust individuals, i.e. presenting minimal responsiveness to fully substituted diets, and implement breeding programmes for better adapted fish.

Statement of relevance: Rainbow trout—a carnivorous fish—are increasingly fed oil and meal from plant origin for environmental purposes but substitution leads to pathological responses, deterioration of growth performances and increased sensitivity to environmental perturbations.

This study responds to the necessity of implementing fish-selective programs with indicators that steer toward the identification of individuals highly tolerant to fully substituted alternative diets.

1. Introduction

Global aquaculture output has increased at an annual rate of 10% since 1984 (FAO, 2014) in response to a growing demand and to ensure sufficient fish production for human consumption. In intensive fish farming, carnivorous fish that require feeds with high levels of protein and oil (NRC, 2011) have traditionally been fed diets elaborated from wild fish. However, the capture from wild fisheries is currently insufficient to provide for the growing demand. Consequently, plant ingredients have increasingly been incorporated into the diet as an alternative (Naylor et al., 2009) to maintain the sustainable production of carnivorous fish. Although novel diets are formulated to meet all known requirements and are processed to reduce the portion of anti-nutritional factors from plant origin, the rainbow trout (*Onchorynchus mykiss*, Walbaum 1792) remains reluctant to consuming such plant-based (PB) diets (Geurden et al., 2013). The main deleterious effects observed when replacing marine proteins or marine oil individually are: decreased feed intake (Geurden et al., 2013), lower feed efficiency (Glencross et al., 2006) and digestibility (Santigosa et al., 2011), altered gut integrity (Glencross et al., 2006) and metabolic pathways (Gómez-

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Requeni et al., 2004; Torstensen et al., 2011). In this context, selective breeding of robust fish, i.e. that would present minimal responsiveness and constant phenotype (Queitsch et al., 2012) to alternate diets is one of the strategies adopted to maintain aquaculture production of carnivorous fish while preserving wild resources (Le Boucher et al., 2012).

Isogenic lines constitute a valiable experimental model (Ihssen et al., 1990; Komen and Thorgaard, 2007; Dupont-Nivet et al., 2009) to investigate the determinism of complex traits. Specifically, they could help to seek the genetic determinism, mechanisms and pathways that could contribute to the trout's ability to maintain growth under a PB diet fully devoid of fishmeal. Data gain from their study may therefore be useful to implement selective programs. Thus, the availability of such genetic material in rainbow trout (Quillet et al., 2007) was used to investigate the genetic variability of isogenic lines for growth performance and feed efficiency in response to the nature of the diet: a marine diet (M) based on fish meal (FM) and fish oil (FO) versus a PB diet (Quillet et al., 2007). In addition, isogenic lines chosen for their phenotypic divergences in response to a PB diet were also characterized through their behaviour (activity, stereotypies, aggressiveness) and their response to confinement as a stress factor (emotional reactivity and plasma cortisol levels) when fed a PB diet (Sadoul et al., 2015; Sadoul et al., 2016). Consequentially, the study highlighted the strong effects of the PB diets on physiological and behavioural welfare parameters in rainbow trout but failed to distinguish a more robust genotype capable of utilizing all the nutrient of a PB diet despite their divergence for growth and survival (Sadoul et al., 2015; Sadoul et al., 2016). It is thus interesting to further characterize the same isogenic lines and investigate if they could diverge in their response to other types of stress factors involving other physiological mechanisms. Indeed, the genotype conservation of lines by way of reproduction between breeders from well-established homozygous clonal lines enables the comparison among different trials performed on different individuals belonging to the same lines (Dupont-Nivet et al., 2009). Moreover it is essential to estimate the ability of the fish to face potentially life-threatening challenges (Sadoul et al., 2015), while considering the nature of the diet they consumed, a PB diet or a M diet.

Interestingly, hypoxic challenges regroup all of these considerations: by impairing the oxygen cascade, hypoxia leads to a large range of physiological responses in reaction to the O_2-depleted environment (Chabot and Claireaux, 2008). The ability to adapt to a reduced oxygen concentration environment has already been studied in several species of fish (Speers-Roesch et al., 2012; Vanderplancke et al., 2014) and trout (Van Raaij et al., 1996; Eliason et al., 2007; Dombkowski et al., 2011). Succinctly, it has been demonstrated that the fish's ability to survive in tanks with reduced oxygen availability depends mainly on their capacity i) to uptake O_2 from the O_2-depleted environment (Richards, 2011), ii) to improve the efficiency of their heart contractions (Rytter and Gesser, 2007), iii) to reduce and shift their metabolism from aerobic to anaerobic (Ishibashi et al., 2007; McKenzie et al., 2008; Almeida-Val et al., 2011), iv) to redistribute their blood flow toward priority organs (Skovgaard and Olson, 2012), and v) to set an acute adrenergic stress response implicating release (Reid et al., 1998; Van Heeswijk et al., 2006; Perry et al., 2009; Thomas and Gilmour, 2012). The hypoxic challenge will therefore stimulate a large range of physiological responses that, when occurring concomitantly to diet replacement, could be impaired. In addition, hypoxia is recognized as a major concern in aquaculture because fish are reared at a high stocking density requiring costly aeration energy systems and finally selection may affect fish ability to resist to low oxygen concentration (Wang et al., 2009; Fetherman et al., 2016). The evaluation of how isogenic lines face such life-threatening challenges when fed PB diet thus deserves attention in the context of fish farming and implementation of breeding programs.

The primary goal of this study was thus to investigate the responsiveness of various rainbow trout isogenic lines—that diverge in their capacity to grow under a PB diet—when submitted to an acute hypoxic challenge test, and to compare their robustness., i.e. if they presented a constant phenotype despite perturbation (Queitsch et al., 2012), based on the dietary treatment they received (starved, M or PB diet). Morgan and Iwama (1996) have demonstrated that corticoid release enhances oxygen consumption in fish. We therefore suspected that isogenic lines already known to diverge on their corticosteroid response during confinement (Sadoul et al., 2015; Sadoul et al., 2016) would also diverge on their tolerance to hypoxia. Furthermore, based on energy trade-off theories (Zera and Harshman, 2001), it was posited that the isogenic lines when fed the PB diet would dedicate a strong energy allocation to maintain an appropriate growth while reducing other energy expenditures (Sadoul et al., 2016). Consequently, we surmised that some lines may demonstrate a greater robustness to hypoxia. Finally, opposing observations have been made about fish's ability to tolerate hypoxia according to their size: Nilsson and Sara (2008) and Roze et al. (2013) have reported that larger fish are more resistant to hypoxia, while Zambonino-Infante et al. (2013) noted that they are more sensitive to hypoxia. We thus hypothesised that the trout fed the marine diet, that had displayed a greater feed intake and a faster growth rate than the trout fed the PB diet, would exhibit marked differences in their tolerance to hypoxia.

We tested our hypotheses by performing hypoxic challenges on rainbow trout isogenic lines fed a fully substituted diet (PB) from their first meal, as opposed to trout fed a diet composed of fishmeal and fish oil (M). We further considered two feeding statuses to assess the contribution of meal digestion and feed intake to hypoxia resistance and tolerance. We first performed the challenges on starved trout —the basal metabolism of which is referred to as the standard metabolic rate (SMR) (Wang et al., 2009)—and then on fed trout. It was important to proceed this way because the post-prandial status requires an additional adaptation due to an increase in oxygen demand (Eliason and Farrell, 2014) and in energy expenditure to ensure digestion, absorption and utilization of the food nutriments (Cho et al., 1982). This concomitant increase in metabolic rate following meal ingestion is referred to as the specific dynamic action (SDA) (Eliason et al., 2007) which subsequently leads to an increased blood flow toward the gastro-intestinal organs (Seth et al., 2010). This study was performed on the two isogenic lines previously investigated for their response to diet replacement by Sadoul et al. (2015, 2016) and Geurden et al. (2013) —A22h and R23h respectively—and on a third divergent isogenic line, AB1h, that has not much been the object of research (Geurden et al., 2013).

2. Methods

2.1. Ethics statement

The experiment was conducted in strict compliance with the guiding principles for the use and care of laboratory animals and in compliance with French and European regulations on animal welfare (Decree No. 2001–464 of 29 May 2001 and Directive 2010/63/EU, respectively).

2.2. Isogenic lines

Three experimental families of rainbow trout previously characterized for their divergent ability to cope with plant-based diets, A22h, R23h (Geurden et al., 2013; Sadoul et al., 2016) and AB1h (Geurden et al., 2013; unpublished data), were used for this experiment. Heterozygous isogenic line R23h (Sadoul et al., 2016; unpublished data) was shown to be particularly performant in terms of growth when fed a fully substituted plant based diet from first feeding. The three lines were obtained by crossing parents from the homozygous isogenic trout lines established at INRA and that were used as broodstock (Quillet et al., 2007). To summarise, lines were obtained after two generations of gynogenesis and further maintained by within-line pair mating using homozygous sex-reversed XX males. To produce the experimental families, eggs from a single maternal homozygous line were collected and