DOES THE WATER TEMPERATURE INFLUENCE THE FATTY ACID METABOLISM OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) FED A VEGETABLE DIET?

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INTRODUCTION

Due to an over-exploitation of wild fishery stocks, the consumers demand for aquaculture products has largely increased during the last decades (FAO, 2014). Climate change issues may interfere with this production. The aquaculture production has notably to deal with the increased temperature of the supply water induced by the global warming (Ficke et al., 2007). Fish being ectothermic animals, a temperature increase beyond their specific optimal range can affect their physiology and metabolism and therefore the aquaculture productivity. The second challenge facing the aquaculture is to provide a fish rich in omega-3 long chain polyunsaturated fatty acids (lcPUFA n-3, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) while reducing the use of unsustainable fish oil-based feed, which tend to become rare and expensive. Consequently, several research projects have evaluated the efficacy of plant-derived oils in fish feed (Corraze and Kaushik, 2009). Unfortunately, only few of these oils contain a significant amount of omega-3 polyunsaturated fatty acids, in the form of α-linolenic acid (ALA); while none of them contains lcPUFA n-3. It becomes therefore of considerable interest to determine the capacity of bioconversion of ALA in lcPUFA n-3, especially in a high commercially cultured fish, such as the rainbow trout (Oncorhynchus mykiss), and to evaluate the influence of an increased water temperature on this capacity. This work investigated the impact of the replacement of fish oil by linseed oil, associated to an increased temperature, on the fatty acid (FA) metabolism of rainbow trout.

MATERIAL AND METHODS

Rainbow trout fed two iso-nitrogenous and iso-lipidic experimental diets: a vegetable diet (100% linseed oil) or a control diet (100% cod liver oil) over 60 days in the Marcel Huet platform facilities (Université catholique de Louvain, Belgium). The vegetable diet FA profile contained 46% ALA and was deprived in lcPUFA n-3, in contrast to the control diet which was rich in these FA (9% EPA and 13% DHA) and contained only 1.5% ALA. The diets were tested at two growth temperatures: an optimal temperature of 15°C and an increased temperature of 19°C. The growth performance was assessed by the feed efficiency (FE, in g/g of dry feed) determined as follows: (final weight – initial weight) /voluntary feed intake, where voluntary feed intake (VFI, in g of dry feed/fish) = $\sum_{i=0}^{t}$ (ingested diet on day i / number of fish on day i) and t = number of days. The estimation of the apparent in vivo FA metabolism was calculated via the implementation of the whole body FA mass balance method (Turchini et al., 2007), with subsequent developments (Turchini et al., 2008; Turchini and Francis, 2009). The fads2 and elovl5 expressions in liver and intestine were estimated by the method described by Geay et al. (2010). The results were normalised by using the housekeeping control genes Elongation factor 1 α and β-actin. The Δ6-desaturase enzymatic activity was

measured in intestinal microsomes, following the method described by Rodriguez et al. (2002).

RESULTS

Fish growth

The mortality was influenced neither by the diet nor by the temperature. The increased temperature had no influence on the growth of fish fed the control diet. The vegetable diet had no effect on fish growth at 15°C but induced a lower growth at 19°C. The FE was indeed significantly ($P<0.01$) lower at 19°C (1.07 ± 0.01 g/g of dry feed) as compared to 15°C (1.18 ± 0.02 g/g of dry feed) even if the VFI values stayed stable.

Apparent enzymatic activity of Δ5-desaturase, Δ6-desaturase and elongases

At the optimal growth temperature of 15°C, the control diet induced similar apparent enzymatic activities of Δ5-, Δ6-desaturases and elongases in the n-6 pathway (0; 19 ± 1 and 33 ± 1 nmol/(g of fish x day), respectively) and the n-3 pathway (0; 13 ± 1 and 24 ± 1 nmol/(g of fish x day), respectively). The apparent enzymatic activities of Δ5- and Δ6-desaturases and elongases were significantly ($P<0.01$) higher for the fish raised on the vegetable diet, as compared to the control one. This was particularly obvious for the n-3 pathway (176 ± 7; 473 ± 10 and 561 ± 14 nmol/(g of fish x day), respectively) but also visible for the n-6 pathway (17 ± 2.; 70 ± 3 and 87 ± 5 nmol/(g of fish x day), respectively).

The increased temperature of 19°C had no influence on the enzymatic activities of interest, when the fishes were grown on the control diet. The same was observed with the vegetable diet when the n-6 pathway was analysed (14 ± 1; 62 ± 3 and 81 ± 5 nmol/(g of fish x day), respectively). In contrast, the apparent enzymatic activities of Δ5-, Δ6-desaturases and elongases tended to decrease on the n-3 pathway (P between 0.05 and 0.1) at 19°C (140 ± 16; 403 ± 31 and 469 ± 42 nmol/(g of fish x day), respectively), as compared to fish fed the vegetable diet at 15°C. Interestingly enough, a significantly lower Δ6-desaturase apparent activity was observed at the increased temperature of 19°C, when the sum of the Δ6-desaturation FA products of both n-6 and n-3 series was considered for fish grown on the vegetable diet (465 ± 34 nmol/(g of fish x day) at 19°C vs 543 ± 9 nmol/(g of fish x day) at 15°C).

Relative gene expression of fads2 and elovl5 in liver and intestine

The dietary treatment had no effect on the fads2 and elovl5 expressions in liver and intestine at 15°C, as well as at 19°C. The increased temperature of 19°C significantly reduced ($P<0.01$) the fads2 and/or elovl5 expressions in the liver when trout were fed the control diet (6- and 1.5-fold less, respectively) or the vegetable diet (4-fold less for fads2). In the intestine, only fads2 expression was significantly reduced by the increased temperature in fish fed the control diet (2-fold less) or the vegetable diet (1.7-fold less).

In-vitro measured Δ6-desaturase enzymatic activity in intestine

The rate of desaturation of [1-14C]18:3n-3 in [1-14C]18:4n-3 could be measured on intestinal microsomes. Whatever the temperature, the values were not influenced by the dietary treatment. In contrast, the increased temperature significantly reduced the rate desaturation (0.15 ± 0.07 vs 0.81 ± 0.40 pmol/(h x mg protein) in the case of the control diet ; 0.14 ± 0.04 vs 0.84 ± 0.18 pmol/(h x mg protein) in the case of the vegetable diet).

DISCUSSION

The present study investigated the impact of the temperature increase on the FA metabolism of rainbow trout fed a vegetable diet. At the optimal temperature of 15°C, the fish growth was not affected by the dietary fish oil replacement, as already observed in the literature (Bell et al., 1994; Bendiksen et al., 2003; Richard et al., 2006). Nevertheless, the dietary treatment had a negative effect on growth at 19°C and the FE was reduced with the increased temperature despite a stable feed intake. Similar observations have been reported for common carp (Cyprinus carpio) raised at two temperatures with a 4°C difference (Desai and Singh, 2009) and with Atlantic salmon (Salmo salar L.) raised at 2 or 8°C (Bendiksen et al., 2003).

The apparent enzymatic activities of Δ5-desaturase, Δ6-desaturase and elongases significantly increased in fish fed the vegetable diet, as already observed for this species (Turchini and Francis, 2009) and the murray cod (Maccullochella peelii peelii) (Francis et al., 2007) for example. Nevertheless, this can be explained neither by an increase of fads2 and elovl5 gene expressions, nor by a higher microsomal Δ6-desaturase activity. Conversely to us, a vegetable diet induced an over-expression of genes involved in lipid biosynthesis in rainbow trout liver (Panserat et al., 2009) and Atlantic salmon liver and intestine (Morais et al., 2012). Moreover, the desaturase activity is generally considered to be under nutritional control (Buzzi et al., 1996) and increased in hepatocytes and intestinal enterocytes of rainbow trout fed a plant-derived oil diet (Tocher et al., 2004). Nevertheless, the same group of investigators also observed a decrease of desaturation activity in intestinal enterocytes of Atlantic salmon fed a linseed oil-based diet (Tocher et al., 2002).

A significantly lower Δ6-desaturase apparent activity was observed at the increased temperature of 19°C, when the sum of the Δ6-desaturation FA products of both n-6 and n-3 series was considered for fish grown on the vegetable diet. In accordance, the expression of the associated gene fads2 was significantly decreased both in liver and intestine. Moreover, the Δ6-desaturase activity that we could measure on intestinal microsomes was also significantly lowered in the condition of increased supply water temperature. The water temperature has already been reported to affect the desaturase activity in fish (De Torrengo and Brenner, 1976; Hagar and Hazel, 1985). Tocher et al. (2004) have also observed a reduction of desaturases and elongases activities in liver and intestine of rainbow trout raised at 15°C compared to 7 and 11°C. Conversely to us, Vagner et al. (2007) showed no effect of the temperature on the Δ6-desaturase expression in sea bass larvae fed a lcPUFA n-3 deficient diet.

CONCLUSION

This work highlights that the increased temperature that fish may face due to the global warming could reduce their capacity to transform dietary ALA in more desaturated lcPUFA n-3. Future studies on the replacement of fish oil by sustainable plant-based oils will have to take this undeniable environmental issue in aquaculture in account.

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