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Dietary antioxidants, food deprivation and growth affect differently oxidative status of blood and brain in juvenile European seabass (*Dicentrarchus labrax*)

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ABSTRACT

Compensatory growth may increase molecular oxidative damage, which may be mitigated through the intake of dietary antioxidants. However, dietary antioxidants may also reduce concentration of antioxidant enzymes, which have a key role in regulating the oxidative status. Here we investigated whether feeding on a diet rich in antioxidants (vitamin E) enables juvenile European seabass (*Dicentrarchus labrax*) to catch up after a period of food deprivation with negligible effects on the oxidative stress to blood and brain as compared to fish feeding on a normal diet (i.e., not enriched in antioxidants). The results show that a higher intake in antioxidants favoured compensatory growth, but this came at a cost in terms of increased oxidative damage. Increased intake of antioxidants also resulted in changes in the activity of enzymatic antioxidant defences and increased protein oxidative damage in both brain and blood. In addition, food deprivation caused increased protein oxidative damage in brain. Our findings show that the beneficial effects of dietary antioxidants on growth may be offset by hidden detrimental effects and that different early life events affect different components of oxidative status of a given tissue.

1. Introduction

Positive selection of individuals capable of reaching an adult size quickly should be expected to sift out slowly growing individuals. Achieving an adult size as soon as possible should carry potential benefits to the individual, such as earlier time to sexual maturity or reduced predation risk (Dmitriew, 2011). Conversely to this prediction, animals do not grow at the maximum rate and much variation in growth strategies persists in both wild and laboratory animals, even when trophic resources are abundant (Blanckenhorn, 2000). The reason for this might lie with the costs of growing fast (Metcalfe and Monaghan, 2001). For example, rapid growth can increase metabolic demands (Criscuolo et al., 2008; Careau et al., 2013) and daily energy expenditure (Careau et al., 2013), or reduce body performance and lifespan (Lee et al., 2016). An additional mechanism through which growth may relate to self-maintenance is that increased cellular activity due to fast growth increases the production of reactive species, such as free radicals (Costantini, 2014; Smith et al., 2016). These unstable molecules, if not counteracted by antioxidants, oxidise proteins, lipids and nucleic acids, causing damage, and hence a state of oxidative stress (Halliwell and Gutteridge, 2015). Recent correlative, experimental and meta-analytical work has suggested that oxidative stress might play a key role as a constraint on, and cost of, growth (Alonso-Alvarez et al., 2007; Nussey et al., 2009; Smith et al., 2016).

Oxidative costs of a rapid growth are particularly relevant when individuals accelerate growth to achieve the body size that is supposed to be normal for their life stage (known also as catch-up growth; Metcalfe and Monaghan, 2001). For example, abrupt reductions in food availability - common in wild populations - might lead young to slow down growth. When food availability becomes adequate again to meet the nutritional requirements of a growing individual, juveniles will compensate by accelerating their growth. Although there are benefits in doing so, an acceleration of growth may also carry costs, such as a reduction in motor skills, sexual ornamentation, cognitive abilities or

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longevity (Metcalfe and Monaghan, 2001; Lee et al., 2016), but also in increased molecular oxidative damage (Smith et al., 2016).

Organisms may mitigate oxidative stress through an increased intake of antioxidants from diet. Animals normally ingest a cocktail of antioxidants and distribute them differently across tissues, depending on the chemical properties (lipophilic vs. hydrophilic) of the antioxidant and the specific requirements of a certain tissue (Surai, 2002; Halliwell and Gutteridge, 2015). Dietary antioxidants may be particularly important for growing individuals as the endogenous antioxidant machinery takes time to become fully mature (Surai, 2002; Costantini, 2014). In many vertebrate species, there is a greater reliance upon non-enzymatic antioxidants at earlier stages of development, such as those acquired from diet or from the mother (Surai, 2002; Costantini, 2014). It would therefore be expected that if an individual catches up while feeding on a diet rich in antioxidants, it might mitigate the oxidative costs of a fast growth. This simple prediction, however, does not consider that increases in the intake of a given antioxidant may also result in no net reduction in oxidative damage because any potential beneficial effects might be offset by compensatory reductions in other antioxidants. For example, long-term intake of a dietary antioxidant (vitamin C) in laboratory mice reduced expression of several genes linked to free radical scavenging, with no net effect on various oxidative damage metrics (Selman et al., 2006). A recent meta-analysis also showed that the effects of antioxidant supplementation on growth are highly variable (from positive to negative), implying that effects of dietary antioxidants on oxidative status might be difficult to predict (Smith et al., 2016).

Another aspect that has received little attention is the impact that accelerated growth has on brain oxidative status and protein oxidative damage (Smith et al., 2016). This is surprising given that acceleration of growth may reduce cognitive abilities (Metcalfe and Monaghan, 2001), brain is a tissue particularly sensitive to oxidative stress (Barja, 2004) and protein turnover is altered during fast growth (Samuels and Baracos, 1995).

Compensatory growth is particularly common in fish at almost every stage of their life cycles because of their indeterminate growth patterns (Ali et al., 2004). Despite the expansion of the field of ecological oxidative stress, relatively little is known about the role of oxidative stress in mediating key life-history stages of fish (Birnie-Gauvin et al., 2017), such as those fish go through during growth and development.

In this study, we tested experimentally whether feeding on a diet rich in antioxidants enables young European seabass (*Dicentrarchus labrax*) to catch up after a period of food deprivation with negligible effects on the oxidative stress as compared to fish feeding on a normal diet (i.e., not enriched in antioxidants). To this end, we have measured metrics of oxidative damage and of antioxidant enzyme activity (i.e., proxy of enzyme concentration) in both blood and brain in juvenile fish that underwent a food deprivation period and then a refeeding protocol either with or without an enriched diet.

2. Materials and methods

2.1. Ethical note

All experiments complied with the Guidelines of the European Union Council and were approved by the Ethical Committee of the Tuscia University for the use of live animals (D.R. n. 677/16 and D.R. 644/17).

At the end of the food deprivation period, the loss of body mass in fish that were food-deprived was 11.4 and 12.6% for the two experimental groups (mean ± se: prior food deprivation, 59.7 ± 5.1 g and 59.1 ± 5.1 g; end of food deprivation, 52.8 ± 5.2 g and 51.6 ± 5.2 g), respectively. Hence, it was below the threshold of 15% recommended by the UK Home Office and the Canadian Council for Animal Care. The mortality rates calculated using sample sizes at the beginning of Phase 1 were similar among groups at the end of the experiment: 12.5% (number of fish dead were 3), 25.0% (number of fish dead were 7), 14.3% (number of fish dead were 4) and 14.3% (number of fish dead were 4) for no food deprivation/standard diet, no food deprivation/enriched diet, food deprivation/standard diet, and food deprivation/enriched diet, respectively.

2.2. Animals and husbandry

Juvenile European seabass (n = 112, one year of age) were obtained from a commercial hatchery (Cooperativa Orbetello Pesca Lagunare, Santa Liberata, Orbetello, Italy) located on the coast at about 40 km from the facility where the study took place. The fish were transported to the facility and randomly allocated into four fiberglass tanks (80 × 80 × 403 cm, all equipped with aeration system) in groups of similar size (26, 29, 29 and 28 individuals). Before being released into the tanks, each fish was measured (mean ± SD, body mass: 64.5 ± 22.7 g; body length 18.9 ± 2.0 cm; body width 4.2 ± 0.5 cm, see below for method details). In each tank, fish were maintained in a volume of 1290 l on an open circuit system with flow of 30 l/min (replicating the conditions of the supplier hatchery) on natural light dark cycle and illumination, as ample windows were present in the building. Being an open circuit meant that each tank was subject to natural seasonal variations of chemical/physical parameters: pH ranged between 8.0 and 8.2, temperature varied from 15 °C in January to 23 °C in August, while salinity ranged between 35 and 38 PSU; ammonia and nitrite were below detectable levels, while nitrate was about 5 mg/l. The European seabass is an euryhaline coastal species known to tolerate (at both juvenile and adult stage) a large range of chemical-physical water parameters, including salinity and temperature (in the order of 5–28 °C) extremes (e.g. Dülger et al., 2012).

Tanks were cleaned daily to avoid accumulation of food remains. Fish were fed by hand ad libitum once per day on the same commercial standard feed (company name undisclosed) used by the supplier for 12 acclimation days. In order to make sure the ration was ad libitum, we provided food until fish did not eat pellets anymore. The residual pellets that deposited at the bottom of the tank were removed within around 60 min in order to minimise any potential confounding effects arising from very bold/dominant individuals monopolising the access to the food. The feed composition was as follows: crude proteins 48.0%; crude fats and oils 22.0%; crude fibre 3.0%; crude ash 6.0%; vitamin E 150 mg/Kg.

2.3. Experimental procedure and sampling

Phase 1, food deprivation: after the acclimation period, the fish of two tanks started a food deprivation protocol that lasted 81 days, while the control fish in the other two tanks continued to be fed daily on the same commercial feed as previously (Fig. 1). All fish were measured on the day Phase 1 was started; the sample size was 108 because four fish died before the start of Phase 1.

Phase 2, re-feeding and enriched diet: as the food deprivation protocol stopped, the pool of animals was re-fed as follows: two groups were fed daily on the standard feed, and the other two groups were fed by hand ad libitum once per day on a feed enriched with vitamin E (feed composition: crude proteins 48.0%; crude fats and oils 20.0%; crude fibre 2.0%; crude ash 6.0%; vitamin E 600 mg/Kg). In this way any effect of the enriched diet on growth and oxidative status could be tested on both the food restricted and the control fish (Fig. 1). This phase lasted until the final sampling, which occurred 180 days later.

In order to quantify the growth, fish were caught with hand nets by two operators, massed (Sartorius TE 612) and measured with a caliper for length and body width (excluding dorsal and ventral fins) by a third operator once at the end of phase 1 and three times during phase 2 (Fig. 1). At the final sampling, the animals were bled after a mild anesthesia and were straightaway euthanized with an overdose of 2-