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A screening of lipophilic antioxidants in muscle tissue of cage reared bluefin tuna (*Thunnus thynnus thynnus* L.): A potential tool to assess oxidative stress

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SUMMARY – Oxidative stress research in aquaculture is particularly important in the assessment of both the health of farmed fish and the quality of sea food. Lipophilic antioxidant levels in relation to fatty acid patterns of total lipids in muscle tissue of *Thunnus thynnus* were investigated. Significant differences in ubiquinol/ubiquinone and α-tocopherol content are well characterising white and red muscle, in relation to their different energetic metabolism. Variations in lipophilic antioxidant levels may represent a specific oxidative stress marker in relation to alteration in swimming behaviour.

Key words: Ubiquinol/ubiquinone, vitamin E, molecular markers, oxidative stress, confinement, bluefin tuna.

RESUME – *"Examen des antioxydants lipophiles dans les tissus de muscle de thons rouges (*Thunnus thynnus *L.) maintenus en cages : Un outil potentiel pour évaluer le stress oxydatif". Les recherches sur le stress oxydatif en aquaculture sont particulièrement importantes pour l'évaluation de la santé des animaux d'élevage et la qualité des produits de la mer. Des études ont été menées sur les niveaux d'antioxydants lipophiles en liaison avec les tendances des acides gras des lipides totaux dans le tissu musculaire de* Thunnus thynnus*. Des différences significatives sur les teneurs en ubiquinol/ubiquinone et α-tocophérol caractérisent bien les muscles blancs et rouges, en liaison avec leur différent métabolisme énergétique. Les variations de niveaux d'antioxydants lipophiles peuvent représenter un marqueur spécifique du stress oxydatif en liaison avec une altération du comportement natatoire.*

Mot-clés : *Ubiquinol/ubiquinone, Vitamine E, marqueurs moléculaires, stress oxydatif, confinement, thon rouge.*

Introduction

Oxidative stress research in aquaculture is particularly important in the assessment of both the health of farmed fish and the quality of sea food. Moreover stressed fish have been demonstrated to be more vulnerable to diseases due to an impairment of antioxidant defence systems (Sakai *et al.*, 1998). Oxidative stress is characterized by an overload of oxidant species and/or a significant depletion of antioxidants (Gutteridge and Halliwell, 1994).

As in mammalians, Vitamin E (d-RRR α–tocopherol) and reduced and oxydized forms of ubiquinones (CoQ_nH_2 –CoQ_n) are the main lipophilic antioxidants in fish tissues and constitute powerful defence tools against ω-3 HUFA oxidation (Filho, 1996; Di Marco *et al.*, 2001). Ubiquinone are ubiquitous and essential for life, so they exist in all body cells and support cellular energy production by helping in the generation of adenosin triphosphate (ATP). It is well known that CoQ_n, in addition to its function as an electron and proton carrier in mitochondria, acts as a powerful antioxidant in its reduced form ubiquinol (CoQ_nH_2), by preventing both the initiation and the propagation steps of lipoperoxidation in biological membranes. Furthermore, it is able to sustain the chain breaking antioxidant capacity of Vitamin E efficiently, by regenerating it from the α-tocopheryl radical.

In this study lipophilic antioxidant (ubiquinol/ubiquinone and α-tocopherol) levels in relation to fatty acid patterns of total lipids in muscle tissue of *Thunnus thynnus* were investigated. The aim was to evaluate if these antioxidant compounds may be useful tool to assess oxidative stress in Bluefin Tuna (BFT) under rearing conditions.

Materials and methods

White and red muscle portions of 14 wild BFT specimens (TL = 137.4±11.2 cm; BW = 32.1±6.0 kg), which were reared in sea cages (Favignana island, TP, Italy) for about 4 months, were analysed. Samples were collected, 6 hours after fish death, and stored at –80°C.

Analyses were performed by liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) techniques (Passi *et al.,* 1998; De Luca *et al.*, 1999).

Statistical analysis of the antioxidant levels were performed by the Student Test; differences between groups were considered statistically significant at $p<0.05$.

Results

BFT has $CoQ_{10}H_2/CoQ_{10}$ as coenzyme Q. From HPLC analyses it resulted that $CoQ_{10}H_2$ is fully oxidised and the values obtained should be considered as the total content of ubiquinone. Though, ubiquinol depletion may only partially be related to the oxidation process that took place during the time between fish death and sampling. Our previous work (Di Marco *et al.*, 2001), on ω-3 PUFA degradation in muscle tissue of Sea Bass during a storage period of 7 days at 4°C, indicated that ubiquinol was oxidised *post mortem*; however it was fully oxidised to ubiquinone within 3 days. In the same way, Vitamin E values are probably underestimated, because this compound also degrades.

Lipophilic antioxidant levels were significantly different ($p<0.01$): in red muscle there are higher levels of both ubiquinone (13.66±6.46 μg/g) and α-tocopherol (15.87±13.18 μg/g), in spite of 8.01±2.57 μg/g and 4.14±1.25 μg/g respectively in white muscle. These significant differences may be related to the different contractile and metabolic activity of white and red muscle tissue. The former is involved in short and fast contractions, the second in the long ones and for resistance in swimming activity. Thus, a higher energetic metabolism is needed for red muscle functions (red muscle is in fact characterised by a high number of mitochondria). Consequently, high ubiquinol/ubiquinone and α-tocopherol levels are required for the ATP production and the antioxidant activity.

The total lipid content was very low and similar in white and red muscle tissue, 2.36% and 2.94% respectively. These values may be related to the long period of starvation during rearing confinement.

A high level of polyunsaturated fatty acid (PUFA) (40-60%), in particular of eicosapentaenoic acid (C20:5 ω-3) (7.9-5.9%) and docosahexaenoic acid (C22:6 ω-3) (40.8-27.7%), in white and red muscle tissue was measured, confirming the high nutritional value of this species. The saturated and monounsaturated fatty acids percentages were higher in red (33.4%; 25.0%) than in white muscle (23.0%; 17.3%). Given the oxidation of a saturated or a monounsaturated fatty acid produces more energy, i.e. a higher number of ATP molecules, in spite of the same polyunsaturated fatty acid oxidation, this result would seem to depend on the stronger energetic activity of red muscle.

Conclusions

In literature there is a lack of reference values on lipophilic antioxidants in tuna fish, thus our results can not be used presently to quantify the oxidative status of reared tuna compared to wild ones. However, as the complete degradation from ubiquinol to ubiquinone, measured in white and red muscle tissue, is only partially related to the oxidation *post mortem*, our results may indicate an oxidative stress status in BFT, probably related to rearing conditions.

Significant differences in ubiquinol/ubiquinone and α-tocopherol content are well characterising white and red muscle, in relation to their different energetic metabolism. Since cage rearing may induce some chronic stress responses in pelagic fish, e.g. alteration in swimming behaviour, variations in lipophilic antioxidant levels may represent a specific oxidative stress marker.

On applying aspects, lipophilic antioxidants such as ubiquinols, ubiquinones and Vitamin E represent powerful defence tools against ω–3 PUFA oxidation in fish and their degradation can be considered to be a reliable marker of sea food quality.

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