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# The metabolic response in fish to mildly elevated water temperature relates to species-dependent muscular concentrations of imidazole compounds and free amino acids

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ABSTRACT

Fish species show distinct differences in their muscular concentrations of imidazoles and free amino acids (FAA). This study was conducted to investigate whether metabolic response to mildly elevated water temperature (MEWT) relates to species-dependent muscular concentrations of imidazoles and FAA. Thirteen carp and 17 Nile tilapia, housed one per aquarium, were randomly assigned to either acclimation (25 °C) or MEWT (30 °C) for 14 days. Main muscular concentrations were histidine (HIS; $P < 0.001$) in carp versus *N*-α-acetylhistidine (NAH; $P < 0.001$) and taurine (TAU; $P=0.001$) in tilapia. Although the sum of imidazole (HIS +NAH) and TAU in muscle remained constant over species and temperatures ($P > 0.05$), (NAH+HIS)/TAU ratio was markedly higher in carp versus tilapia, and decreased with MEWT only in carp ($P < 0.05$). Many of the muscular FAA concentrations were higher in carp than in tilapia ($P < 0.05$). Plasma acylcarnitine profile suggested a higher use of AA and fatty acids in carp metabolism ($P < 0.05$). On the contrary, the concentration of 3-hydroxyisovalerylcarnitine, a sink of leucine catabolism, ($P=0.009$) pointed to avoidance of leucine use in tilapia metabolism. Despite a further increase of plasma longer-chain acylcarnitines in tilapia at MEWT ($P=0.009$), their corresponding beta-oxidation products (3-hydroxy-longer-chain acylcarnitines) remained constant. Together with higher plasma non-esterified fatty acids (NEFA) in carp ($P=0.001$), the latter shows that carp, being a fatter fish, more readily mobilises fat than tilapia at MEWT, which coincides with more intensive muscular mobilization of imidazoles. This study demonstrates that fish species differ in their metabolic response to MEWT, which is associated with species-dependent changes in muscle imidazole to taurine ratio.

## 1. Introduction

Exposure of fish to elevations in water temperature can occur due to global warming (Ficke et al., 2007) or because aquaculture is increasingly established in warm countries (FAO, 2014). Fish are poikilothermic ectotherms, whose metabolism and growth is influenced by the water temperature and also farming conditions such as food availability, uptake and utilization (Jobling, 1994). Elevations in water temperature increase basal metabolism that results in a higher energy demand for maintenance and less energy available for growth (Jobling, 1994). Previous studies have shown that elevated water temperature can lead to changes in nutrient metabolism; for example, it can result in an increased breakdown of amino acids (AA) in fish (Geda et al., 2012). However, there is uncertainty about whether these AA are derived from the diet or from free AA (FAA) concentrated in muscle.

Fish tissues, like other animals, contain FAA and non-protein nitrogenous compounds (imidazole compounds, taurine, trimethylamine oxide and other methylamine compounds) (Van Waarde, 1988). The imidazole compounds are the major non-protein nitrogenous constituents present in skeletal muscles of vertebrates (Crush, 1970; Abe, 1983a, 1983b). There are five imidazole compounds in skeletal muscle of fish: histidine (HIS), carnosine (β-alanyl-L-histidine), anser-

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ine (β-alanyl-L-methyl-L-histidine), ophidine (balenine; β-alanyl-3-methyl-L-histidine), and *N*-α-acetylhistidine (NAH) (Van Waarde, 1988; Yamada et al., 2009). The imidazole compounds have a number of biological roles such as $H^+$ buffer (Sewell et al., 1992; Abe, 2000), neurotransmitter (Petroff et al., 2001), non-enzymatic free-radical scavenger (Guiotto et al., 2005), antioxidant (Boldyrev et al., 2004) and blood glucose regulator (Sauerhofer et al., 2007).

The distribution and abundance of imidazole compounds vary among fish species. For instance, skeletal muscle of carp (*Cyprinus carpio*) contains higher levels of HIS (Van Waarde, 1988) whereas that of Nile tilapia (*Oreochromis niloticus*) has higher levels of NAH (Yamada et al., 1992). It is not fully elucidated why animals store high concentrations of the FAA and imidazole compounds in their muscles (Shiau et al., 1997). The reason for the difference in the distribution patterns of the imidazole compounds in the skeletal muscle among fish species is also not yet understood. To our knowledge, it has not been investigated whether the different muscular concentrations of FAA and imidazole compounds would determine the use of nutrients in metabolism of fish at elevated temperature.

When fish cannot store high amounts of body fat (e.g., carp store more fat than tilapia: Abdelghany and Ahmad, 2002), as in many terrestrial animals, readily available muscular concentrations of FAA and imidazole compounds might serve as a rapid source of energy. The species difference in muscular concentration of imidazole compounds and their metabolic response to elevated water temperature would relate to the difference in body fat concentration between species. Therefore, due to the aforementioned reasons, carp and Nile tilapia were used as model fish in the present study to investigate the metabolic response of fish to a mildly elevated water temperature (MEWT).

## 2. Materials and methods

### 2.1. Animals and experimental design

Thirteen carp *(Cyprinus carpio) and* 17 Nile tilapia *(Oreochromis niloticus)* were acclimated to laboratory conditions, in two batches (batch 1: 6 carp and 6 tilapia; batch 2: 7 carp and 11 tilapia) for two weeks. The fish were individually and randomly allocated in 63L-glass aquaria of 60×30×36 cm (JUWEL Aquarium, Rotenburg, Germany). The acclimated fish were fasted for 24 h, weighed (average initial body weight: 119 ± 4 g tilapia, 123 ± 9 g carp), distributed into the aquaria, and randomly assigned to two groups, a control ("T25") and treatment ("T30"). This experiment was set up using two species of fish under two levels of temperature, in a 2×2 factorial design. All experimental methods and procedures used in this study were approved by the ethics committee of animal experiments at Faculty of Veterinary Medicine, Ghent University. The work described has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

### 2.2. Feed, feeding protocol and elevated temperature

The fish were hand-fed (feed: Benelux NV, Wielsbeke - Ooigem, Belgium) (Table 1) for 14 days at a feeding rate of 1.5% of average wet body weight per fish per day twice at 10:00 and 15:00. The experimental diet was analyzed for proximate chemical analysis of dry matter (DM), moisture (M), crude protein (CP), crude fat (diethyl ether-extract, EE), crude fibre (CF) and ash (Table 1). The DM and M contents were determined by drying feed samples in freeze dryer for 24 h and heating in a forced air oven at 103 °C to a constant weight. The ash content was determined by combustion of the feed at 550 °C. The EE was analyzed with the Soxhlet method (ISO 1443, 1973). The CF was determined using the Association of Official Analytical methods (Method 962.09 and 985.29, 1995). The Kjeldahl method (ISO 5983–1, 2005) was used to determine CP (6.25× N). The percentage of nitrogen-free extract (NFE) was calculated as: NFE=100–(M+Ash+CP+EE+CF). The T25 group was managed at an acclimation temperature of 25 °C and the T30 group was managed at a constant MEWT of 30 °C, set-up at a rate of 5 °C per 60 h. All the aquaria were maintained at 12:12 h light-dark photoperiod using fluorescent lights controlled by timers.

**Table 1**
Chemical composition of the experimental diet[a] (on as fed basis).

| | |
|---|---|
| Proximate composition (g/kg) | |
| Dry matter | 922.00 |
| Moisture | 78.00 |
| Crude protein | 326.20 |
| Crude ash | 54.40 |
| Crude fat | 55.80 |
| Crude fibre | 31.30 |
| NFE[b] | 454.30 |
| Amino acid composition (g/kg) | |
| Alanine | 19.98 |
| Arginine | 19.90 |
| Aspartic acid+Asparagine | 31.62 |
| Cysteine | 5.46 |
| Glutamic acid+Glutamine | 67.55 |
| Glycine | 13.98 |
| Histidine | 8.11 |
| Isoleucine | 15.44 |
| Leucine | 34.21 |
| Lysine | 16.85 |
| Methionine | 6.28 |
| Phenylalanine | 18.46 |
| Proline | 21.18 |
| Serine | 16.51 |
| Threonine | 13.23 |
| Tryptophan | 3.56 |
| Tyrosine | 12.52 |
| Valine | 17.06 |

Vitamin and mineral premix added per kg feed (as given in the commercial feed technical sheet): retinol, 3 mg; vitamin C, 100 mg; cholecalciferol, 0.025 mg; vitamin E, 33 mg; calcium, 6 g; phosphorus, 6 g; sodium, 0.8 g; iron, 100 mg; copper, 2.5 mg; manganese, 15 mg; zinc, 50 mg; selenium, 0.25 mg.
List of ingredients reported in Aqua-KI in decreasing order of inclusion: Fish products, products and by-products of oil seeds, vegetal products and by-products, algae, AA, vitamins, minerals, calcium propionate, antioxidants, oils and fats.
[a] Aqua-KI feed, manufactured by Benelux NV, Wielsbeke – Ooigem, Belgium.
[b] NFE =100–(M+Ash+CP+EE+CF).

Daily feed intake was determined by removing any uneaten feed after 60 min from each aquarium, drying and weighing as previously described (Geda et al., 2015). All fish were fasted for 24 h during measurements of their initial and final body weights. Average daily measured water quality parameters were pH (8.2) (Merck KGaA, Darmstadt, Germany), dissolved oxygen (5.7 mg/L for T25, 5.3 mg/L for T30) (Hanna Instruments Srl, Nufalau, Romania), ammonium ( < 0.10 mg/L) and nitrite (0.13 mg/L) (JBL GmbH and Co KG, Neuhofen/Pfalz, Germany).

### 2.3. Blood sampling, plasma collection and analysis

All fish were fasted for 24 h and were euthanized using an overdose of a benzocaine (ethyl 4-aminobenzoate) solution (1g/10 mL acetone) and subjected to blood sampling. Blood samples were collected from the heart (cardiac puncture) using a 1 mL syringe (Becton Dickinson S.A., Madrid, Spain) and a 26G needle (Becton Dickinson, Drogheda, Ireland) rinsed with heparin (LEO Pharma, Ballerup, Denmark). The blood in the heparinized plasma tubes was centrifuged at 1200×*g* and 5 °C for 10 min. The plasma samples were collected and stored at –20 °C until analyzed. Acylcarnitine profile of the plasma samples was determined using quantitative electrospray tandem mass spectrometry (Zytkovicz et al., 2001). For determination of non-esterified fatty acids (NEFA), the plasma samples were analyzed spectrophotometrically (EZ Read 400 Microplate Reader, Biochrom Ltd., Cambridge, United