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Variation in metabolic enzymatic activity in white muscle and liver of blue tilapia, *Oreochromis aureus*, in response to long-term thermal acclimatization*

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Abstract The effects of rearing temperature on white muscle and hepatic phosphofructokinase (PFK), pyruvate kinase (PK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were examined in fingerlings of blue tilapia, *Oreochromis aureus*. The experiment was conducted for 14 weeks at temperatures of 18, 22, 26, 30, and 34°C. The activity of the glycolytic enzymes PFK, PK, and LDH in white muscle increased significantly with increase in water temperature. A reverse trend was observed for these enzymes in the liver, except for LDH, which behaved in the same manner as in white muscle. Cytosolic AST and ALT activity increased in both white muscle and liver in response to warm thermal acclimatization, while a reduction in mitochondrial AST and ALT activity was noticed at high temperatures in comparison with those at a lower temperature.

Keyword: Nile tilapia; thermal acclimatization; metabolism; enzymes

1 INTRODUCTION

The tilapiine fishes (genus *Oreochromis*) are among those that have been reared for food for the longest time. In recent years, however, intensive culture of tilapia under controlled management systems has expanded significantly to meet increasing demand, especially in developing countries. The use of closed culture systems for tilapiids is becoming more common worldwide, particularly in arid areas that face a shortage of freshwater or brackish water, and in areas where salinity and temperature are outside the tolerance range of tilapia (Visser, 1991; Muir et al., 2000).

Knowledge of the effect of temperature on the metabolism and growth of fish is a basic requirement for successful rearing of poikilothermic animals in aquaculture (Berg et al., 1990). The effect of temperature on growth in teleosts has been dealt with in a large number of publications (Jobling, 1996; Person-Le Ruyet et al., 2004; Azaza et al., 2008).

Temperature is one of the most influential factors affecting the physiological and biochemical functions of fish (Woiwodi and Adelman, 1991; Jobling, 1996; Kou and Hsieh, 2006; Couto et al., 2008; Enes et al., 2008a, b; Morerira et al., 2008). These physiological changes have classically been separated into three groups. The first group involves the neuroendocrine system in catecholamine release and activation of the corticotropin-interrenal axis. The second group involves haematological, osmoregulatory, blood enzymatic and metabolic changes. The third group includes growth inhibition, reduced fecundity, increased susceptibility to infection and behavioural changes (Mazeaud et al., 1977). Many physiological and biochemical processes in fishes are fundamentally reorganized during temperature acclimatization; these include changes in metabolic rate, thermal tolerance, optimal temperature, digestive function, food conversion efficiency, growth and development (Brett, 1970). In addition, there are profound alterations in carbohydrate metabolism (Delahunty and Devlaming, 1980), protein metabolism

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(Bennemann, 1977) and lipid metabolism (Hazel, 1979).

The study of the activity of some enzymes acting in particular metabolic pathways during temperature acclimatization is therefore very important (Woo, 1990). Since the body temperature of fish varies with the water temperature, the rate of metabolic reactions is affected accordingly and a thermal compensation occurs to avoid a decrease or increase in metabolic activities in fish acclimatized to cold and warm temperatures respectively. A long-term change in temperature, however, causes fish to display acclimatization responses, which can include enzymatic changes to mitigate the effect of temperature on metabolism (Hazel and Prosser, 1974). Fish will rely on a number of adaptations that are under neural and/or endocrine control to ensure that they meet the energy requirements needed for their physiological compensation and acclimatisation to acute thermal stress (Kuo and Hsieh, 2006). Gelman et al. (1992) reported that tilapia species have certain physiological limits for such temperature adaptations. The goal of the present study, therefore, was to quantify the variation in the metabolic enzymatic activities in the white muscle and liver of blue tilapia, *Oreochromis aureus*, under the stress of long-term thermal acclimatization. In this context, this study investigated the activity of five enzymes, phosphofructokinase (PFK) acting at the beginning of the glycolytic sequence, pyruvate kinase (PK) and lactate dehydrogenase (LDH) acting at the terminal sequence of the glycolytic pathway, and aspartate aminotransferase (AST), that catalyzes an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids), being the most important mechanism for introducing reduction equivalents into mitochondria (Urich, 1994), as well as Alanine aminotransferase (ALT), which predominates in organs with intensive glycogenesis, such as the liver (Urich, 1994; Torre et al., 2000).

2 MATERIAL AND METHOD

2.1 Experimental design

Fifteen glass aquaria (100 cm×40 cm×50 cm), each containing 120 L of water, were used for rearing the fish. Each aquarium was equipped with a biological filter containing a high porosity filter sponge that was washed thoroughly every two days. Dissolved oxygen was added with diffused air at the top of the biological filter. The water temperature in the aquaria was maintained by means of a thermostatically controlled heating system, and the water was exchanged periodically using dechlorinated water, which had previously been heated to the desired temperature. One hundred and fifty fingerlings of blue tilapia, *O. aureus*, with an average weight of 11.65±0.229 g were reared in the 15 aquaria, which were divided into five different groups according to their water temperature (18, 22, 26, 30 and 34°C, respectively) with three replicates for each group. The water temperature was maintained to the nearest ±0.5°C, the stocking density to 50 fish/m³, the water was exchanged weekly, the photoperiod was 12 h:12 h, the dietary protein was 35% and the daily food ration was 3% of fish weight.

2.2 Enzyme assays (Bergmeyer, 1981)

The experiment was conducted for 14 weeks, with the fish being taken and used for enzyme assays at the end of the experimental period.

2.2.1 Tissue sampling

The fish were quickly killed by a blow on their head, an incision was made along one side of the dorsal fin and the skin was carefully and quickly peeled down, avoiding tissue squeezing. A sample of white muscle was removed from the dorso-lateral side just behind the head and taken for enzymatic estimation. Only the right lobe of the liver was taken for enzymatic estimation because the left lobe of the liver in tilapian fishes is combined with the pancreas, which is thus called the hepatopancreas.

2.2.2 Glycolytic enzymes

The tissue samples (muscle or liver) were quickly weighed and homogenized in 10 volumes of ice-cold buffer (I) using a polytrone PT-1200 (Kinematica AG, Switzerland). Buffer (I) contained 20 mmol/L N-2-hydroxyethyl-piperazine-N-2-ethane sulphonate (HEPES), 1 mmol/L EDTA and 30 mmol/L β-mercaptoethanol pH 7.6 (adjusted by NaOH). The homogenates were centrifuged at 12 000×*g* for 15 min in a cooled centrifuge at 4°C. The supernatant was carefully drawn from beneath the lipid layer, when present at the top of centrifuged sample, and called fraction (I). Fraction (I) was used for measuring the activity of the glycolytic enzymes phosphofructokinase, pyruvate kinase and lactate dehydrogenase.

2.2.3 Mitochondria separation

The intact mitochondria were isolated from cytosol essentially as described by Pedersen et al. (1978) in a mannitol-sucrose buffered medium (buffer II). Buffer (II) contained 20 mmol/L HEPES, 1 mmol/L EDTA, 30 mmol/L β-mercaptoethanol, 225 mmol/L mannitol, 50 mmol/L sucrose pH 7.6 (adjusted by NaOH). Fish tissues were manually homogenized in cold buffer (II) (1:5W/V) using a manual homogenizer immersed in ice. The homogenate was centrifuged at 700×*g* for 15 min in a cooled centrifuge at 4°C (the pellet contained unbroken cells and cell debris). The supernatant was carefully decanted, to prevent lipid contamination, and centrifuged at 12 000×*g* for 15 min. in order to yield a "high speed" supernatant (cytosolic fraction II) and the pellet (primarily mitochondria). The activity of the cytosolic enzymes, aspartate, aminotransferase and alanine aminotransferase were measured in the cytosolic fraction (II). The pellet was resuspended and washed twice in buffer II. The final resuspension buffer contained no mannitol or sucrose (buffer 1). Mitochondria were ruptured by repeated freezing and thawing. The ruptured mitochondria suspension was then centrifuged at 12 000×*g* for 15 min. The supernatant (fraction III) was saved. The activity of the mitochondrial enzymes, aspartate aminotransferase and alanine aminotransferase were measured in the mitochondrial fraction III.

Enzymatic activity was assayed with an UV/VIS LKB (ultrospect II) 4050 Spectrophotometer using the molar extinction of NADP or NADH at 340 nm (Ex=6.22 cm^2/μmol), with appearance or disappearance being monitored. All reactions were linear with respect to the reaction time and to the enzyme concentration over the period for the calculation of enzyme activity. The concentration of the extracted protein "enzyme extract" was adjusted to provide a linearly detectable decrease or increase in the extinction for at least 4 min. Conditions for the enzyme assays were optimized with respect to substrate conditions. Control assays without substrate were made. The enzyme activities were expressed as μmol/(L·min·g) wet weight tissue (cytosolic enzymes) or nmol/(L·min·mg) mitochondrial protein (mitochondrial enzymes). The total protein in the mitochondrial suspension was determined by using the Biuret Method (Plummer, 1978). All enzyme activities were performed at 37°C in 80 mmol/L of imidazol-Hcl buffer PH 7.4 in a final volume of 1 mL as follows:

Phosphofructokinase (PFK, EC 2. 7. 1. 11):

Reaction mixture: 6 mmol/L fructose 6-phosphate, 0.15 mmol/L NADH, 2mmol/L ATP, 100 mmol/L KC1, 10 mmol/L $MgCl_2$ and excess triose phosphate isomerase (TPI, EC 5.3.1.1), glycerol 3-phosphate dehydrogenase (G3PDH EC, 1.1.1.8) and aldolase (Ald, EC 4.1.2.7).

Pyruvate kinase (PK, EC 2.7.1.40):

Reaction mixture: 2.5 mmol/L phosphoenol-pyruvate, 0.15 mmol/L NADH, 3 mmol/L ADP, 10 mmol/L $MgCl_2$, 50 mmol/L KC1 and excess lactate dehydrogenase (LDH, EC 1.1.1.27).

Lactate dehydrogenase (LDH, EC 1.1.1.27):

Reaction mixture: 1 mmol/L pyruvate and 0.15 mmol/L NADH.

Aspartate aminotransferase (AST, EC 2.6. 1.1):

Reaction mixture: 40 mmol/L L-aspartate, 7 mmol/L α-ketoglutarate 0.15 mmol/L NADH, 0.025 mmol/L pyridoxal phosphate and excess malate dehydrogenase (MDH, EC 1.1.1.37).

Alanine aminotransferase (ALT EC 2.6.1.2):

Reaction mixture: 200 mmol/L L-alanine, 10.5 mmol/L α-ketoglutarate 0.15 mmol/L NADH, 0.025 mmol/L pyridoxal phosphate and excess lactate dehydrogenase (LDH, EC 1.1.1.27).

2.3 Statistical analysis

The collected data was subjected to statistical analysis using one way Analysis of Variance (ANOVA). The average values (means±standard deviation) were compared by using Fisher's Least Significant Differences test (LSD-test) as described by Snedcor and Cochran (1989).

3 RESULT

3.1 White muscle (Table 1)

3.1.1 Cytosolic enzymes (μmol/(L·min·g) wet weight tissue)

Phosphofructokinase (PFK, EC 2.7.1.11)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 34°C, (7.96±0.699), 30°C (6.14±0.229), 26°C (5.85±0.401), 22°C (5.64±0.438), 18°C (4.82±0.310). The difference between the enzyme activity assayed in fish acclimatized at 34°C was statistically significant (P<0.05) in comparison to fish acclimatized at all other water temperatures.

Pyruvate kinase (PK, EC 2.7.1.40)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 34°C, (101.24±4.906), 30°C (98.72±5.444), 26°C

Table 1 Effect of long-term thermal acclimatisation (14 weeks) on enzyme activity (cytosolic: µmol/(L·min·g)wet weight tissue and mitochondrial: nmol/(L·min·mg) mitochondrial protein) of white muscle of *O. aureus*

Name of Enzymes	Enzyme activity at different water temperatures				
	18°C	22°C	26°C	30°C	34°C
PFK	4.82±0.310 ^{c}	5.64±0.438 ^{b}	5.85±0.401 ^{b}	6.14±0.229 ^{b}	7.96±0.699 ^{a}
PK	46.33±4.071 ^{e}	80.56±5.427 ^{d}	93.85±6.138 ^{c}	98.72±5.444 ^{b}	101.24±4.906 ^{a}
LDH	164.79±8.235 ^{e}	194.83±7.313 ^{d}	259.67±6.301 ^{c}	287.94±14.49 ^{b}	296.55±14.275 ^{a}
AST (Cyto)	2.13±0.433 ^{d}	2.45±0.433 ^{c}	3.38±0.365 ^{b}	4.23±0.429 ^{a}	4.41±0.340 ^{a}
AST (Mito)	13.49±1.657 ^{a}	11.37±1.134 ^{b}	9.52±0.919 ^{c}	7.64±0.774 ^{d}	7.25±0.723 ^{d}
ALT (Cyto)	3.64±0.393 ^{d}	4.83±0.522 ^{c}	6.33±0.448 ^{b}	7.59±0.364 ^{a}	8.12±0.439 ^{a}
ALT (Mito)	2.76±0.360 ^{a}	2.10±0.331 ^{b}	1.55±0.252 ^{c}	0.99±0.131 ^{d}	0.87±0.102 ^{d}

Cyto=Cytosolic; Mito=Mitochondrial. Each reading represents mean±SD of n=5. Means with different superscript in the same row are significantly different at (P<0.05).

(93.85±6.138), 22°C (80.56±5.427), 18°C (46.33±4.071). The difference between enzyme activities was statistically significant at (P<0.05).

Lactate dehydrogenase (LDH, EC 1.1.1.27)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 34°C, (296.55±14.275), 30°C (287.94±14.49), 26°C (259.67±6.301), 22°C (194.83±7.313), 18°C (164.79±8.235). The difference between enzyme activities was statistically significant at (P<0.05).

Aspartate aminotransferase (AST, EC 2.6.1.1)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 34°C, (4.41±0.340), 30°C (4.23±0.429), 26°C (3.38±0.365), 22°C (2.45±0.433), 18°C (2.13±0.433). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

Alanine aminotransferase (ALT, EC 2.6.1.2)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 34°C, (8.12±0.439), 30°C (7.59±0.364), 26°C (6.33±0.448), 22°C (4.83±0.522), 18°C (3.64±0.393). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

3.1.2 Mitochondrial enzymes (nmol/(L·min·mg) mitochondrial protein)

Aspartate aminotransferase (AST, EC 2.6.1.1)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 18°C, (13.49±1.657), 22°C (11.37±1.134), 26°C (9.52±0.919), 30°C (7.64±0.774), 34°C (7.25±0.723). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

Alanine aminotransferase (ALT, EC 2.6.1.2)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 18°C, (2.76±0.360), 22°C (2.10±0.331), 26°C (1.55±0.252), 30°C (0.99±0.131), 34°C (0.87±0.102). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

3.2 Liver enzymes (Table 2)

3.2.1 Cytosolic enzymes (µmol/(L·min·g) wet weight tissue)

Phosphofructokinase (PFK, EC 2.7.1.11)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 18°C, (1.62±0.045), 22°C (0.817±0.070), 26°C (0.466±0.079), 30°C (0.225±0.026), 34°C (0.198±0.019). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

Pyruvate kinase (PK, EC 2.7.1.40)

The order of enzyme activity assayed in fish acclimatized to the different temperatures was, at 18°C, (1.87±0.052), 22°C (1.51±0.140), 26°C (1.43±0.052), 30°C (1.37±0.170), 34°C (1.28±0.178). The difference between the enzyme activity assayed in fish acclimatized at 18°C was statistically significant (P<0.05) in comparison to fish acclimatized at all other water temperatures.

Table 2 Effect of long-term thermal acclimatisation (14 weeks) on enzyme activity (cytosolic: μmol/(L·min·g) wet weight tissue and mitochondrial: nmol/(L·min·mg) mitochondrial protein) of liver of *O. aureus*

Name of Enzymes	Enzyme activity at different water temperatures				
	18°C	22°C	26°C	30°C	34°C
PFK	1.62±0.045 [a]	0.817±0.070 [b]	0.466±0.079 [c]	0.225±0.026 [d]	0.198±0.019 [d]
PK	1.87±0.052 [a]	1.51±0.140 [b]	1.43±0.052 [b]	1.37±0.170 [b]	1.28±1.178 [b]
LDH	13.56±0.948 [c]	14.27±1.108 [c]	29.74±1.756 [b]	32.68±1.695 [a]	33.92±1.744 [a]
AST (Cyto)	12.78±1.295 [d]	18.55±1.308 [c]	22.69±1.348 [b]	26.40±2.541 [a]	26.83±2.737 [a]
AST (Mito)	39.75±4.148 [a]	27.59±4.805 [b]	20.88±2.765 [c]	15.92±1.801 [d]	15.23±1.524 [d]
ALT (Cyto)	4.38±0.312 [d]	6.69±0.290 [c]	20.37±1.160 [b]	26.78±1.950 [a]	27.54±2.510 [a]
ALT (Mito)	9.13±1.251 [a]	6.34±1.026 [b]	4.47±0.799 [c]	2.53±0.748 [d]	1.98±0.498 [d]

Cyto=Cytosolic; Mito=Mitochondrial. Each reading represents mean± SD of n=5. Means with different superscript in the same row are significantly different at (P<0.05).

Lactate dehydrogenase (LDH, EC 1.1.1.27)

The order of enzyme activity assayed in fish acclimatized to the different temperature was, at 34°C, (33.92±1,744), 30°C (32.68±1.695), 26°C (29.74±1.756), 22°C (14.27±1.108), 18°C (13.56± 0.948). The difference between enzyme activity assayed in fish acclimatised at 30 and 34°C was statistically significant (P<0.05) in comparison to fish acclimatized at 18, 22 and 26°C.

Aspartate aminotransferase (AST, EC 2.6.1.1)

The order of enzyme activity assayed in fish acclimatized to the different temperature was, at 34°C, (26.83±2.737), 30°C (26.40±2.541), 26°C (22.69±1.348), 22°C (18.55±1.308), 18°C (12.78± 1.295). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

Alanine aminotransferase (ALT, EC 2.6.1.2)

The order of enzyme activity assayed in fish acclimatized to the different temperature was, at 34°C, (27.54±2.510), 30°C (26.78±1.950), 26°C (20.37±1.160), 22°C (6.69±0.290), 18°C (4.38± 0.312). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

3.2.2 Mitochondrial enzymes (nmol/(L·min·mg) mitochondrial protein)

Aspartate aminotransferase (AST, EC 2.6.1.1)

The order of enzyme activity assayed in fish acclimatized to the different temperature was, at 18°C, (39.75±4.148), 22°C (27.59±4.805), 26°C (20.88±2.765), 30°C (15.92±1.801), 34°C (15.23± 1.524). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

Alanine aminotransferase (ALT, EC 2.6.1.2)

The order of enzyme activity assayed in fish acclimatized to the different temperature was, at 18°C, (9.13±1.251), 22°C (6.34±1.026), 26°C (4.47± 0.799), 30°C (2.53±0.748), 34°C (1.98±0.498). The difference between enzyme activities was statistically significant (P<0.05) except at the two highest temperatures of 30 and 34°C, where there is no significant difference.

4 DISCUSSION

4.1 White muscles

The activity of the three glycolytic enzymes, PFK, PK and LDH, was abruptly decreased in white muscle of fish acclimatised at the lower temperatures. A decrease in PK and LDH activity with cold acclimatisation has previously been reported in the swimming muscles of green sunfish, goldfish, striped bass and chain pickerel (Shaklee et al., 1977; Sidell, 1980; Jones and Sidell, 1982; Kleckner and Sidell, 1985; Blier and Guderley, 1988; Guderley and Foley, 1990). The activity levels of LDH in white muscles isolated from fish kept at 26.5±0.5°C was significantly higher than that of the same muscles isolated from fish kept at 20.5±0.3°C (Mawangangi and Mutungi, 1994).

Blier and Guderley (1988) reported a similar observation with regard to the two enzymes PK and in LDH in Lake Whitefish acclimatised at low temperatures. They suggested that this might be related to the fact that the two enzymes are at higher activity levels in comparison with PFK. I am inclined

to agree with this opinion, especially since the reported thermal response to cold acclimatisation in PFK activity in Lake Whitefish is qualitatively similar to that in *O. aureus* in the present study. Generally, the activity levels revealed by the three glycolytic enzymes, PFK, PK and LDH, studied show the same profile at the five tested temperatures, albeit a profile which did not change in a coordinated fashion. Nonetheless, it is striking that the three glycolytic enzymes show their lowest activity at the lowest temperature, indicating an abrupt decrease in the mechanical power efficiency of white muscle at this temperature. The outcomes of the present study for the activity of the three glycolytic enzymes, PFK, PK and LDH, indicate a low metabolic rate at cold acclimatization and these results are consistent with the findings stated above.

Anaerobic oxidation is based on the ability of muscle to produce lactate, which enables energy stores (glycogen), that are depleted in the course of exercise, ready to be quickly replenished. The excess lactate formed in the white muscles of fish can be used as a readily accessible source of carbon and energy in certain oxidative tissues, such as the heart (Driedzic et al., 1985), or red muscle fibres (Wieser et al., 1986). This ability of tissue to form lactate under fully aerobic conditions, thus providing a carbon source for other tissues (heart, gills and liver), has been called the lactate shuttle (Brooks, 1986). This shuttle acts to transport the reducing equivalents from the anaerobic environment in white muscle to other tissue, which has a powerful oxidative capacity. Hence, the decrease in the anaerobic capacity of the swimming muscle may indicate its use as a carbon source for other tissues. This would be reflected in a decrease in the metabolic rate of these tissues at cold temperatures and this supports my report about the low metabolic rate of white muscles at cold acclimatization.

Studies related to oxygen consumption as an indicator for general metabolic rate have reported that fish that undergo winter dormancy with decreased metabolic rates. The American eel, *Anguilla rostrata*, for example, displays compensatory adjustments in its metabolic rate at temperatures between 10 and 20°C, and acclimatisation to 5°C leads to a marked decrease in metabolic rate (Walsh et al., 1983). Sunfish, *Lepomis gibbosus*, and crucian carp, *Carassius carassius*, also exhibit metabolic depression at low temperatures (Guderley, 1990). Other species, such as the brown bullhead, *Ictalurus nebulosus*, and largemouth bass, *Micropterus salmoides*, simply use cold temperatures as a signal to become quiescent without showing any increase in the thermal sensitivity of metabolic rate at low temperatures (Crawshaw et al., 1982). The present study, therefore, shows that the depression in activity of the three glycolytic enzymes, PFK, PK and LDH, of *O. aureus* at low temperatures has been reflected in a decreased metabolic rate as in other fish species that become inactive when cold.

AST and ALT in white muscle cytosol (present study) show a significant increase in their activity as the temperature of acclimatisation increased, except at the two highest temperatures of 30 and 34°C, where there is no significant increase in the enzyme activity. However, the reverse was found to be true for the enzyme activity in mitochondria. The enzymatic activity of AST and ALT, involved in amino acid metabolism in white muscle of the Nile tilapia, is in the normal range for teleost white muscle (Gaudet et al., 1975; Hochachka et al., 1978; Mommsen et al., 1980; Moon, 1993).

The white muscles of fish do not have the capacity for complete amino acid oxidation in situ. There is some evidence that fish mobilize amino acids from white muscle tissue during migration (Mommsen et al., 1980) or winter dormancy (Walsh et al., 1983), both of which lead to anorexia. In this situation, amino acids in white muscle can be converted to alanine and transported into the blood to be metabolized on other aerobic oxidative tissues. Alanine has often been identified as a carrier of amino acid nitrogen and carbon from fish skeletal muscle (Driedzic and Hochachka, 1978; Leech et al., 1979). Generally, ALT enzyme seems to be the key enzyme in this process. In the present study, ALT activity in white muscle cytosol has been shown to be lowest in fish acclimatised to lower temperatures (18°C) but it increases with an increase in temperature up to 30°C. Above this level, however, there is no significant increase in the enzyme activity. It is evident that amino acid transport is augmented at higher acclimatisation temperatures to counterbalance the increased energy requirements of *O. aureus*.

4.2 Liver enzymes

The role of glycolysis in the liver of fish is probably to supply precursors for biosynthetic processes rather than to provide pyruvate for oxidation. In fish, the oxidation of keto acid derived from amino acid catabolism via the TCA cycle may be the main means

of energy production in the liver of trout, with some contributions from β-oxidation of fatty acids. In muscle, however, the conversion of glycogen to lactase is a major source of energy (Walton and Cowey, 1982).

In the present work, the key enzyme data of PFK indicates that the metabolic potential of the liver remains relatively active during cold temperatures. PK in fish liver (unlike muscle) has a regulatory role in glycolysis and gluconeogenesis. LDH also has a different metabolic role in fish liver. Whereas in skeletal muscle, the enzyme catalyses the reduction of pyruvate in tissue through anaerobic metabolism, and therefore does not utilize lactate but rather produces it (Nadal and Markert, 1975). In the liver, where aerobic metabolism is predominant, lactate is utilized in this process (Everse and Kaplan, 1975). Lactate is therefore consumed in the liver for oxidation *in situ* or for gluconeogenesis, and this may account for the higher lactate dehydrogenase activities reported here in comparison to the other glycolytic enzymes in the liver of *O. aureus*. Kuo and Hsieh (2006), however, supposed that the increase in liver LDH is the result of lactate clearing through the Cori cycling and, in most fish, the liver isoform of LDH functions best in the lactate to pyruvate direction. It is possible, therefore, that a high temperature of 34°C may inhibit hepatic glucose metabolic enzymes or specific glucose metabolic pathways in Nile tilapia (Qiang et al., 2014).

The activities of AST and ALT in the liver of *O. aureus* examined in this study significantly increased in cytosol as the temperature of acclimatisation increased, except at the highest temperature of 34°C, where there was no significant increase in enzyme activity. A reverse trend was recorded for the activity of these enzymes in mitochondria. The mitochondrial aspartate system acts in aspartate-malate inter-conversions (the malate-oxaloacatate shuttle) for the transport of reducing equivalents and carbon compounds across mitochondrial membranes (Lardy et al., 1965; Hayashi and Ooshiro, 1979). The operation of a vigorous TCA cycle is a feature of this oxidative tissue and the maintenance of oxaloacetate at an appropriate level would be necessary for this (Cornish et al., 1978). The liver of *O. aureus* seems to have a highly active malate-oxaloacetate shuttle; however, the activity of enzymes in the shuttle is depressed in the low temperature ranges. This may indicate that at cold temperatures liver tissue relies on lipids as an energy source.

The present study has shown that the ability of the liver of *O. aureus* to perform amino acid catabolism was at its lowest at lower temperatures but increased at higher temperature ranges. The decrease in the activity of amino acid metabolizing enzymes in the liver of *O. aureus* seems to be necessary to preserve amino acids under the severe stress of lower temperatures, at which fish become inactive. The highest rate observed at the optimum preference temperature for *O. aureus* (30°C) reflects a high ability for amino acid catabolism. The energy requirement of fish at this temperature seems to be high. Generally, the activities of AST and ALT in the liver of *O. aureus* show that they have a moderate potential for amino acid catabolism. Ahmad et al. (2011), however, suggested that the increase in AST and ALT in common carp might be due to hepatic pathological alterations in thermally stressed fishes.

Overall, compensation for temperature changes occurred in blue tilapia through rate limiting enzymes (through an increase in enzyme concentration and hence activity) resulting in biochemical adjustments to minimize the effect of low temperature on the catalytic processes. The temperature range over which a metabolic profile remains functional is important for biochemical design. Optimal design would allow an organism to maintain metabolic systems that function well throughout the range of temperatures the organism might encounter. The quantitative adjustment of the metabolism organization in muscle demands a considerable energetic investment. Such metabolic organization could be costly for a cold-inactive species such as blue tilapia, unlike for cold-active species.

5 CONCLUSION

This study has emphasized that blue tilapia are not able perfectly to compensate for cold temperature in white muscle so as to function optimally. This metabolic saving may be needed for the decreased metabolic maintenance costs associated with lower temperatures, which are accompanied by a period of fasting. While this conclusion may satisfy the observation in white muscles, the metabolic profile in liver shows a noticeably more active maintenance, especially in the glycolytic flux and the oxidative enzymes in mitochondria. These alterations in enzymatic patterns may be related to the nature of each tissue. Generally, the metabolic profile of the blue tilapia *O. aureus* is at its highest rate at a temperature of 30°C.

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