

QUANTITATIVE CHANGES OF GLYCOGEN AND LACTATE IN MUSCLE, BLOOD AND LIVER TISSUES OF *OREOCHROMIS MOSSAMBICUS* UNDER HYPOXIA AND RECOVERY

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ABSTRACT

The anaerobic metabolism and its link with lactate and glycogen/glucose levels of muscles, liver and blood were quantitatively analyzed in tilapia, O. mossambicus under four different oxygen concentrations 5.20 ± 0.65 (control), 3.66 ± 0.55 , 2.53 ± 0.69 and 1.58 ± 0.41 and recovery after 2 hrs (3.78 ± 0.46). The glycogen levels of muscle and liver reduced drastically at the oxygen level of 3.66 ± 0.55 mg/l and reduced further towards the exposure of fish to hypoxic condition at the oxygen level of 1.58 ± 0.41 mg/l. However, the muscle and blood lactate showed a reverse trend to the glycogen levels. The total glycogen utilization at the oxygen level of 3.66 mg/l was 1000.3 mg/kg/hr (5.01 m.mol/hr), while at the oxygen concentration of 2.53 and 1.58 mg/l it was 323.1 mg/kg/hr (1.61 m.mols / hr) and 184.85 mg/kg/hr (0.925 m.mols/hr) respectively. Out of the total glycogen utilized by the fish, about 14.77, 17.70 and 26.81% of glycogen was utilized for lactate production at the oxygen concentration of 3.66, 2.53 and 1.58 mg/l. Hence, only a meager percentage (14.7 – 26.8%) of glycogen utilization has been accounted for the production of lactate. The remaining amount of over 75% has supposed to be utilized for oxidation or for the production of end products other than lactate.

Key words: anaerobic, hypoxia, gluconeogenesis, pyruvate, lactate

INTRODUCTION

Metabolism and growth of fishes are dependent on the availability of ambient oxygen (Kutty, 1981). Fish like other vertebrates and most invertebrates are basically aerobic animals deriving their energy from oxidative metabolism with a concomitant requirement for oxygen as the electron acceptor. When muscular activity exceeds the ability of the circulatory system to transport oxygen to the active tissues, anaerobic metabolism supplements the aerobic metabolism and an oxygen debt is acquired (Heath and Pritchard, 1962; Brett, 1972). Very low oxygen concentrations may occur in intensive fish

farming, especially when fishes are stocked at high densities. Most of the earlier studies showed that all fishes are obligate aerobes and a measure of oxygen consumption is always a measure of metabolic rate (Kutty, 1968). Recently many studies have been carried out on the metabolic changes occurring in fish muscle during recovery from high intensive exercise (Milligan and McDonald, 1988; Pearson *et al.*, 1990; Randall and Brauer, 1991). These studies are all confined to temperate fish like trout and salmon. So, there is need for studying the effect of ambient oxygen on anaerobic metabolism coupled with the quantitative changes on lactate and glucose in muscle, liver and blood tissues under

hypoxia and after recovery conditions to simulate the conditions in culture ponds. The results obtained will have direct application on the growth of fish, as the change in ambient oxygen mostly decides the production of fishes in culture ponds.

MATERIALS AND METHODS

The cichlid fish *Oreochromis mossambicus* (average length 14.22 ± 0.54 ; average weight 42.93 ± 3.15 g) were collected from ponds in Fisheries College and Research Institute, Thoothukudi and held in 20 m² acclimation tanks, supplied with continuously flowing (1.5l/min) well water having $30 \pm 1^{\circ}$ C temperature pH 8.0 – 8.5 under 12 hr : 12 hr photoperiod. The fishes were fed once daily with a formulated diet having groundnut oil cake, rice bran and fish meal in the ratio of 1:1:1. The fishes were starved for 24 hours before the beginning of the experiment to avoid faecal contamination in the experimental set up. A modified Fry's respirometer device by Kutty *et al.* (1971) was used for the present study. The capacity of the respirometer was 4000 ml.

Device used for creating hypoxia

The apparatus used for the present study to reduce the ambient oxygen was a PVC tube (1 m length, 7.5 cm diameter) which was fitted laterally on a wall. Both ends were covered with PVC caps fitted with tubes. The one at the bottom was connected with a nitrogen cylinder. The column of the tube was filled with uniform size gravel (1-1.5 cm). Nitrogen gas was passed through the bottom hole and water was allowed to trickle from above. The nitrogen gas stripped of oxygen present in the water as it passed down through the gravel in the tube. The required level of oxygen in water was maintained by adjusting the flow of nitrogen. The fish taken from acclimation tank was introduced into the respirometer. Four sets of experiments were carried out at four different oxygen concentrations

(5.20 ± 0.65 (for control), 3.66 ± 0.55 , 2.53 ± 0.69 and 1.58 ± 0.41) and two hours after recovery. In each experiment a separate fish was used. For each set of experiment, 8 replicates have been done in a separate fish. After exposing the fish to required level of oxygen for a period of 2 hr, blood sample was collected in a micro-syringe which was already smeared with heparin solution (4 mg of heparin/1 ml of distilled water) to avoid clotting. The blood sample was collected from dorsal peduncle. Then the fish was kept in an anesthetic solution of benzocaine for 10 min. Once ventilation ceased, immediately muscle and liver samples were collected for lactate and glucose estimation. Glycogen and blood glucose were assayed by Anthrone method described by Carroll *et al.* (1956). Lactate was estimated by the method of Barker and Summerson (1941). From the measured values of total glycogen, the amount of glycogen accounted for the production of lactate and for glucose oxidation values were calculated. The calculation is based on the assumption that one molecule of glucose will produce two molecules of lactate (Sukumaran and Kutty, 1986). The results obtained were statistically analyzed using ANOVA technique.

RESULTS AND DISCUSSION

Glycogen

The levels of glycogen in liver were decreased significantly ($P < 0.01$) with decrease in oxygen concentrations and reached very low value from 4201.68 mg/l to 1483.97 mg% at 1.58 ppm of oxygen level and regained 3127.91 mg % after recovery. The liver glycogen in tilapia showed the same decreasing trend with decrease in ambient oxygen as observed in the case of freshwater mullet (Sukumaran and Kutty 1986). The blood glucose concentration went up from 247.73 mg % at 3.66 ppm of oxygen level and slightly decreased to 218.88 mg % at 2.53 ppm and maintained the same

level at hypoxic condition. The concentration of glucose in blood is found to be higher in tilapia than that of *C. mrigala* (Padmavathy *et al.* 2002). Blood glucose level of fishes is said to be related to their habits and active fishes are said to have high blood sugar content than the sluggish one (Khanna and Singh, 1971).

Experiments of Johnston and Goldspink (1973) on their glycogen showed that even at the highest speed studied, no significant change occurred in the lower glycogen content of fish swimming for three minutes. In contrast to this, in the present study, liver glycogen decreased as observed in cut throat trout (*Salmo clarki*) and blue gill sun fish (*Lepomis macrochirus*) under similar conditions (Heath and Pritchard, 1965). It was also reported that liver glycogen deposits were used during hypoxia in many animals as a source of glucose for catabolism (Scarabello *et al.*, 1992; Schulte *et al.*, 1992). Increase in the blood glucose level under hypoxia, in *O. mossambicus* may be due to mobilization of liver glycogen into blood as in blue gill sun fish, *L. macrochirus* (Heath and Pritchard, 1965).

Lactate

The lactate levels showed opposite trend of glycogen. As the level of ambient oxygen decreased, the lactate level increased in all tissues. At 3.66 ppm of oxygen level both muscle and blood lactate showed steep increase (significant at $P < 0.05\%$ level), whereas liver lactate slightly increased from 23.52 mg % to 24.92 mg %. The trend of muscle and blood lactate subsequently showed a gradual increase to a maximum value at 1.58 ppm of oxygen (Table 1).

The concentration of muscle lactate is higher than that present in liver and blood. The increase in the concentration of lactic acid in muscle is marked through out the period as the depletion

of oxygen level proceeds. Lactate is shown to be a substrate for gluconeogenesis in some fishes (Renaud and Moon, 1980; Cowey and Walton, 1989). The rising level of blood lactate after the stress may be attributed to the movement of lactic acid from the muscle tissue into the blood stream. This is supported by findings of Black *et al.* (1966) who have shown a very rapid diffusion of lactate from the muscles into the blood stream of exercised trout.

The increase in concentration of liver lactate in *O. mossambicus* may be due to anaerobic lactic acid production within the liver itself, when adequate oxygen is not supplied and energy is in high demand. This was also shown by Dando (1969) in trawl caught bass, cod and plaice.

The lactate concentration under hypoxic stress increases to a maximum followed by a sudden decrease even close to the value of control fish during recovery phase. This is due to the oxidation of lactate when oxygen is plenty. Improved ability to recover from exhaustive exercise has been shown in a number of long term endurance type training studies (Hochachka, 1961; Lackner *et al.*, 1988) as well as after prolonged sprint training (Pearson *et al.*, 1990).

Glycogen utilization

The glycogen utilization and lactate production by the whole fish was estimated and the values are given in Table 2. The glycogen utilization by whole fish was 5.01 m. mols/kg/hr at 3.66 ppm of oxygen level while at 2.53 ppm it was 1.61 m.mols/kg/hr. Total lactate production in tilapia was 1.48 m.mols / kg/hr, 1.14 and 0.496 m.mols/kg/hr at 3.66, 2.53 and 1.58 ppm of oxygen concentrations.

From the values of total glycogen utilization, the amount of glycogen accounted for lactate production and oxidation were calculated and the values are given in Table-2. In tilapia, about 14.77 and 17.70 and 26.81 percent of glycogen was utilized

for lactate production at 3.66, 2.53 and 1.58 ppm of oxygen concentration respectively.

The result indicates that only a meager percentage (14.70 – 26.80%) of glycogen has been accounted for the production lactate. The remaining amount of over 75% supposed to be utilized for oxidation. Under hypoxic condition, there is not much possibility of oxidative metabolism of glucose and quite a portion of glycogen would be utilized for the production of some other end products other than acetate such as pyruvate, succinate and alanine (Driedzic and Hochachka, 1975; Sukumaran, 1976; Padmavathy, 2003). Kutty and Peer Mohamed (1975) suggested that in hypoxic mullet (*R. corsula*), anaerobic energy is derived under hypoxia through pathways other than that of conventional glycolysis. The lesser utilization of glycogen at 2.53 and 1.58 ppm of oxygen concentration may be due to the non availability of adequate amount of glycogen as

substrate (Sukumaran, 1976).

In conclusion, it appears that tilapia has high anaerobic capacity when compared to mrigal and rohu (Padmavathy *et al.*, 2002 and 2003), as it is evident from more amount of lactic acid accumulation in muscle. It confirms that tilapia is less sensitive towards oxygen than mrigal and rohu. Metabolic adaptations of tilapia subjected to hypoxia contribute to their hardiness and success in spreading in the warm waters of the world.

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Table 1.
Glycogen/ glucose and lactate levels in muscle, blood and liver of tilapia, *O. mossambicus* under different oxygen levels (each value is presented as the mean ± S.D. from 8 replicates and all are observed values)

Oxygen level (mg/l)	Tissue	Glycogen level (mg/l)	Lactate level (mg/l)
5.20 ± 0.65 (control)	Muscle	435.41 ± 3.48**	41.18 ± 2.33*
	Blood	196.43 ± 3.68 (glucose)	14.70 ± 1.42*
	Liver	4201.68 ± 75.46*	23.52 ± 3.18
3.66 ± 0.55	Muscle	270.98 ± 6.86**	62.77 ± 1.71*
	Blood	247.73 ± 7.91 (glucose)	42.51 ± 1.47*
	Liver	2067.85 ± 53.83*	24.92 ± 1.21
2.53 ± 0.69	Muscle	220.09 ± 7.23**	81.84 ± 0.79*
	Blood	218.88 ± 4.57 (glucose)	52.34 ± 0.80*
	Liver	1553.38 ± 30.84*	71.10 ± 1.63
1.58 ± 0.41	Muscle	149.09 ± 9.24**	98.09 ± 2.69*
	Blood	227.90 ± 7.67 (glucose)	64.60 ± 3.08*
	Liver	1483.97 ± 32.99*	42.14 ± 1.02
3.78 ± 0.46 (Recovery after 2 hours)	Muscle	291.60 ± 3.25**	23.71 ± 1.81*
	Blood	215.83 ± 5.37 (glucose)	7.12 ± 1.13*
	Liver	3127.91 ± 103.54*	15.61 ± 1.01

* significant at 1% level ** significant at 5% level

Table 2.
Glycogen utilization and lactate production in tilapia, *O.mossambicus*
under different oxygen concentration (each value is presented as the
mean \pm S.D. from 8 replicates and all are observed values)

Oxygen concentration (mg/l)	Total glycogen (glucose) utilized by the fish (m.mol.hr)	Total lactate produced by the fish (m.mol.hr)	Glycogen (glucose) utilized for lactate production (m.mol/hr)	Glycogen utilized for possible oxidation (m.mol/hr)
3.66 \pm 0.55	5.01 (1000.30mg/kg/hr)	1.48	0.74 (14.77%)	4.27 (85.23%)
2.53 \pm 0.69	1.61 (323.10mg/kg/hr)	1.14	0.285 (17.70%)	1.33 (82.61%)
1.58 \pm 0.51	0.925 (184.85mg/kg/hr)	0.496	0.248 (26.81%)	0.68 (73.51%)

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