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Glucose, Lactate and Pyruvate Metabolism in *Labeo rohita* with Reference to Ambient Oxygen

P. PADMAVATHY, N. RAMANATHAN* and T. FRANCIS

Fisheries College and Research Institute
Tamil Nadu Veterinary and Animal Sciences University
Thoothukkudi-628 008, Tamil Nadu
India

Abstract

Anaerobic metabolism and its link in energy utilization such as glycogen utilization, lactate production and pyruvate utilization were quantitatively analyzed in *Labeo rohita* under four different oxygen concentrations (5.20-control, 3.52, 2.46 and 0.48 ppm oxygen levels) and recovery after two hours. The glycogen levels in the muscle and liver decreased significantly with reduction in oxygen levels, but the blood glucose level increased from 48.90 to 68.49 mg% at 3.52 ppm oxygen level but slightly decreased to 60.99 mg% at 2.46 ppm. The lactate level increased as the level of ambient oxygen decreased in all tissues. Muscle tissue showed the highest accumulation of lactate (48.93 mg%) under hypoxia and it was reduced to 13.50 mg% during recovery period. The level of residual pyruvate decreased from 9.12 mg% (at 5.20 ppm oxygen level) to 2.08 mg% under hypoxia. The amount of glycogen accounted for pyruvate production, lactate production and oxidative phosphorylation was calculated under anaerobic condition and discussed.

Introduction

Metabolism and growth of fishes are dependent on the availability of ambient oxygen (Kutty 1981). Hence changes in dissolved oxygen, lowering of oxygen and resultant hypoxia and diel flux of oxygen can affect fish production in ponds. Such changes in oxygen can be evident in fertilized ponds, where phytoplankton development is prominent (Boyd 1979). There are evidence that the diel flux of oxygen reduce growth in fishes, for example, in *Micropterus salmonides* (Stewart et al. 1967) and carps (Itazawa 1967). The effect of anaerobic metabolism and its energy utilization has been studied earlier in most of the teleosts (Johnston 1975; Sukumaran and Kutty 1986). Unfortunately such information on Indian major carps is not available. Hence, there is a need to study the effect of ambient oxygen and anaerobic metabolism on glycogen utilization, lactate production and the level of residual

**Corresponding author*

pyruvate in tissues of Indian major carps, especially rohu (*Labeo rohita* Cyprinidae) to understand the physiological mechanisms involved in these teleosts.

Materials and Methods

A modified Fry's respirometer devised by Kutty et al. (1971) was used for the present study. The seeds of Indian major carp *L. rohita* were collected from the State Fish Seed Farm, Manimuthar and acclimated in freshwater at 30 ±1°C for two weeks before the experiment. The fishes were fed with a formulated diet daily. Before feeding the fish, fecal matter was removed from the tank and water was changed daily. The fishes were starved for 24 h before the start of the experiments (Beamish 1964) to avoid fecal contamination in the experimental set up.

For each set, 20 *L. rohita* (total length 17.6±1.4 cm and average weight 42.2±5.2 g) were used. The fishes taken from the acclimation tank were introduced into the respirometer. Four sets of experiments were carried out at four different oxygen concentrations (5.20 for control, 3.52, 2.46 and 0.48 mg l^{-1}). The fish was exposed to the required level of oxygen for two hours. The required level of oxygen was maintained by passing nitrogen gas into the water. Finally the experimental fish was sacrificed and blood sample was taken. The lactate, glycogen/glucose levels of muscle, blood and liver and residual pyruvate levels of muscle were estimated under different oxygen levels. Glycogen and blood glucose were assayed using the Anthrone method of Carroll et al. (1956). Pyruvate, an intermediate product was estimated following the method of Oser (1965). Under experimental conditions, some amount may be utilized for lactate production and some for oxidative phosphorylation. Thus, estimated pyruvate is a residual pyruvate. A set of experiment was also conducted at recovery after two hours.

From the values of total glycogen utilized, the amount of glycogen that accounted for the production of pyruvate, lactate and oxidative phosphorylation was calculated based on the assumption that one molecule of glucose will produce two molecules of lactate or pyruvate (Sukumaran and Kutty 1986). The results obtained were statistically analyzed using ANOVA technique.

Results

Glycogen lactate and pyruvate levels

The mean values of glycogen/glucose and lactate levels in the muscle, liver and blood under different oxygen concentrations and subsequent recovery after two hours are given in table 1. At 3.52 ppm oxygen level, there was a sudden drop of glycogen in the muscle and liver tissues, whereas blood showed an increase in the glucose level. The blood glucose concentration increased from 48.90 to 68.49 mg% at 3.52 ppm oxygen level but slightly decreased to 60.99 mg% at 2.46 ppm. The levels of glycogen in liver decreased

significantly ($P<0.01$) with a decrease in oxygen concentrations; it reached a very low value from 3115.04 to 1047.03 mg% at 0.48 ppm oxygen level and regained at 2636.46 mg% after recovery.

The lactate levels showed an opposite trend of glycogen. As the level of ambient oxygen decreased, the lactate level slightly decreased in all tissues. During recovery period the lactate level reduced in all tissues such as muscle, blood and liver (Table 1).

The level of residual pyruvate decreased significantly ($P<0.01$) with a decrease in oxygen concentration (Table 2). In control fish, the level was 7.21 mg%. At 3.52 ppm of oxygen, this was reduced to 6.38 mg%. It was further reduced to 1.33 mg% at 0.48 ppm oxygen concentration. At the time of recovery the level was 3.55 mg%.

Glycogen utilization and lactate production

Glycogen utilization and lactate production by the whole fish was estimated and the values are given in table 3. Glycogen utilization by whole fish

Table 1. Glycogen/glucose and lactate levels in muscle, blood and liver of *L. Rohita*

Oxygen concentration (mg l^{-1})	Tissues	Glycogen level (mg %)	Lactate level (mg %)
5.20 (control)	Muscle	82.69 ± 2.38*	16.11 ± 1.78
	Blood	48.90 ± 2.27	14.37 ± 2.07**
	Liver	3115.04 ± 172.65*	8.95 ± 1.60
3.52	Muscle	64.04 ± 2.78*	19.11 ± 1.78
	Blood	68.49 ± 3.14	16.09 ± 1.67**
	Liver	2559.46 ± 218.09*	9.28 ± 1.27
2.46	Muscle	53.55 ± 2.28*	27.16 ± 1.25
	Blood	60.99 ± 2.52	20.94 ± 1.15**
	Liver	1998.64 ± 151.85*	14.95 ± 0.91
0.48	Muscle	40.08 ± 1.86*	48.93 ± 1.61
	Blood	68.40 ± 3.07	27.01 ± 1.64**
	Liver	1047.03 ± 109.52*	26.34 ± 1.37
Recovery after 2 h	Muscle	101.41 ± 2.72*	61.89 ± 2.17
	Blood	2636.46 ± 172.68*	13.50 ± 1.00
	Liver	5.11 ± 0.80**	7.67 ± 1.06

Each value is presented as the mean ± S.D from 8 replicates and all are observed values. Values in column 3 for blood represents glucose level.
*Significant at 1% level
** Significant at 5% level

Table 2. Residual pyruvate in muscle tissue of *L. rohita* exposed to different oxygen concentrations.

Oxygen concentration mg l^{-1}	Residual pyruvate mg% *
5.20	7.21 ± 0.78
3.52	6.38 ± 0.80
2.46	4.63 ± 0.53
0.48	1.33 ± 0.29
Recovery after 2 h	3.55 ± 0.51

Each value is presented as the mean ± S.D from 8 replicates and all are observed values.
*Significant at 1% level

was 0.330 mmols·kg^{-1}·h^{-1} at 3.52 ppm of oxygen level, while at 0.48 ppm, it was 0.930 mmols· kg^{-1}· h^{-1}. The lactate production in *L. rohita* was 0.093 mmols·kg^{-1}·h^{-1} at 3.52 ppm oxygen in which 0.088 mmols·kg^{-1} of lactate was produced by muscle tissue alone.

From the values of total glycogen utilization, the amount of glycogen that accounted for residual pyruvate, lactate production and oxidative phosphorylation were calculated and the values are given in table 4. In *L. rohita*, about 17.96, 18.54 and 85.54% of glycogen were utilized for lactate production at 3.52, 2.46 and 0.48 ppm oxygen levels, respectively.

Discussion

Glycogen

The concentrations of glucose in blood and lactate in muscle are found to be similar to those as previously investigated by Tandon and Joshi (1975). Blood glucose level of fishes are said to be related to their habitats and active fishes are said to have high blood sugar content than the sluggish ones (Mackay and Beatty 1968; Kanna and Singh 1971). The level of blood glucose was found to be lower than that of *Tilapia mossambica* and *Clarius* sp.

Table 3. Glycogen utilization and lactate production in *L. rohita* under different oxygen concentrations

Oxygen concentration (mg l^{-1})	Total glycogen utilized (mmols·kg^{-1}·h^{-1})		Total lactate produced (mmols·kg^{-1}·h^{-1})	
	By fish	By muscle tissue	By fish	By muscle tissue
3.52	0.330	0.245	0.093	0.088
2.46	0.580	0.383	0.162	0.141
0.48	0.930	0.560	1.000	0.957
Recovery after 2 h	0.320	0.246	0.102	0.077

Each value is presented as the mean ± S.D from 8 replicates and all are calculated values from observed values of table 1.

Table 4. Substrate utilization in *L.rohita* under different oxygen concentrations.

Oxygen concentration (mg l^{-1})	Total glycogen utilized in muscle (mmols·kg^{-1})	Total lactate produced in muscle (mmols·kg^{-1})	Residual pyruvate utilized in muscle (mmols·kg^{-1})	Glycogen accounted for pyruvate production (mmols·kg^{-1})	Glycogen accounted for lactate production (mmols·kg^{-1})	Glycogen accounted for oxidative phosphorylation (mmols·kg^{-1})
3.52	0.245	0.088	0.025	0.013 (5.31%)	0.044 (17.96%)	0.189 (77.14%)
2.46	0.383	0.141	0.077	0.039 (10.18%)	0.071 (18.54%)	0.274 (71.54%)
0.48	0.560	0.957	0.175	0.088 (15.71%)	0.479 (85.54%)	-0.006
Recovery after 2 h	0.246	0.077	0.109	0.055 (22.36%)	0.039 (15.85%)	0.153 (62.20%)

Each value is presented as the mean ± S.D from 8 replicates and all are calculated values from observed values of tables 1 and 2.

The earlier observations were based on the quantitative changes occurring in muscle and liver glycogen reserves during sustained swimming ignoring the changes of blood glucose (Johnston and Goldspink 1973). In the present study, glycogen utilization was estimated considering the blood glucose level also. Glycogen utilization was less during fish exposure to initial phase of lower oxygen levels and maximum under hypoxic conditions as observed by Heath et al. (1980). This suggests greater energy demand under hypoxic stress.

The earlier observations showed no significant change in liver glycogen with reference to swimming (Black et al. 1962; Johnston and Goldspink 1973). In contrast, in the present study, there was reduction in liver glycogen and an increase in the blood glucose was observed in the cutthroat trout (*Salmo clarki*) and in blue gill sun fish (*Lepomis macrochirus*) under similar conditions (Heath and Pritchard 1965). This may be due to mobilization of liver glycogen into blood.

Lactate

Lactate is shown to be a substrate for gluconeogenesis in some fish (Cowey and Walton 1989). In this study, the increase in concentration of lactic acid in muscle was marked throughout the period as the depletion of oxygen level proceeded. The level of lactate in muscle tissue of *L. rohita* was less than that of *Cirrhinus mrigala* (Padmavathy 1994). Under hypoxia, lactate production was higher, coinciding with glycogen utilization; which could reflect a greater energy demand at low oxygen concentration. This is well supported by Heath et al. (1980). 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 (Black et al. 1962).

The increase in concentration of liver lactate in *L. rohita* may be due to anaerobic lactic acid production within the liver itself, when adequate oxygen is not supplied and energy is in high demand. There is a possible existence of barrier between blood and liver as far as lactate is concerned. This was also shown by Dando (1969) in trawl caught bass, cod and plaice.

Residual pyruvate

Pyruvate is an important energy source in fish erythrocytes, since extra cellular pyruvate was found to be able to maintain nucleotide triphosphate (NTP) levels in rainbow trout erythrocytes (Houston et al. 1985). Increase in the level of pyruvate was observed by Johnston (1975). In contrast to the above investigations, reduction in residual pyruvate was noticed under hypoxia in the present study. Pyruvate kinase is normally thought to be an irreversible enzyme (Schulte et al. 1992). Fish white muscle lacks phosphoenol pyruvate carboxy kinase (Moon and Johnston 1980). Consequently gluconeogenesis in trout's white muscle may require the reversal of pyruvate kinase, which requires high ATP and pyruvate and low ADP and PEP (Dyson et al. 1975). Still in the present study, the decreased level of residual pyruvate can be used for the reversal of pyruvate kinase. This is also supported by the findings of

Tiihonen and Nikinamaa (1991), who found that carp erythrocytes prefer the use of lactate and pyruvate as a substrate.

Glycogen in lactate and pyruvate production

The decrease in concentration of pyruvate may be due to the conversion of pyruvate to some other end product. At the time of recovery, a small amount of pyruvate has been recovered in *Leuciscus caphalus* and in *S. gairdneri*. An elevation in lactate: pyruvate ratio occurred in rohu at each level of hypoxia studied, indicating a marked increase in anaerobic glycolysis. Under hypoxia, pyruvate utilization was higher but there was no oxidative phosphorylation because of lack of oxygen.

Glycogen in oxidative phosphorylation

The value for oxidative phosphorylation was negative under hypoxic condition, because almost all the glycogen is utilized for lactate production under such condition and there is no chance of oxidative phosphorylation (Hochachka 1986).

In conclusion, it appears that *L. rohita* has poor anaerobic capacity compared to tilapia, mullet and mrigal. Since *L. rohita* accumulated less amount of lactic acid and utilized less quantity of pyruvate compared to *C. mrigala*, rohu has less energy demand and poor anaerobic capacity in relation to ambient oxygen concentration. The reason for this is that rohu occupies a column region in the culture system, while mrigal is a bottom dweller.

From this study it appears that rohu prefer the use of substrates other than glucose as an energy source. They prefer pyruvate as an energy source under hypoxia. It reveals that *L. rohita* are less adapted to tolerate frequent low oxygen levels compared to tilapia, mullet and mrigal since it has poor anaerobic metabolic capacity. This finding may be significant in terms of fish handling practices in hatchery and stocking operations and in understanding the ability of fish to survive under low oxygen conditions. In this study, an attempt was made to derive some general conclusions regarding hypoxia induced anaerobiosis in rohu. The study of energy metabolism and the involvement of anaerobic end products with reference to ambient oxygen concentration in fish muscle is an integrated system responding to a number of controlling factors such as glycogen, lactate, pyruvate, amino acid, succinate, ATP, ADP, SDH, IMP and phosphoenol pyruvate. The effects of interaction of these factors with ambient oxygen will have some influence on the growth, food intake and food conversion – ratio and as a whole the fish production in fishponds.

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