BIOENERGETIC OF OSMOREGULATON IN *Liza abu* JUVENILES DURING SALINITY ACCLIMATION

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ABSTRACT

Several physiological variables related to salinity acclimation in *Liza abu* were examined, using oxygen consumption rate and glucose level as an indication of osmoregulatory energetic. Freshwater acclimated *L. abu* were transferred to two salinity (7 and 15 g/L) oxygen consumption rates, glucose levels and plasma ion concentrations (Na^+, K^+) were measured after three days of transfer to higher salinity. Plasma ion concentrations were elevated after transfer. Blood glucose level increased after transfer to salinity 7 g/L while its level decreased in salinity 15 g/L compared to its level in freshwater. Oxygen consumption rate increased one and half time of that in freshwater but no significant difference were noticed in oxygen consumption rates between fish in both salinities (7 and 15 g/L) .The amount of metabolic energy consumed during salinity acclimation were also determined. The result showed that the physiological changes associated with salinity acclimation impose a significant short – term energetic cost.

INTRODUCTION

Bioenergetic is the study of energy usage by living organisms (Smith, 1982). An energy consumption rate is usually given as kcal/1kg(body weight)/ hr.

Energy consumption in fish is almost always measured indirectly in term of oxygen consumption which has commonly been used as an indicator of metabolism in fish (Lovell, 1989). However, measurement of oxygen consumption rates in different salinities have been imployed in an attempt to determine the energetic cost of osmoregulation (Morgan *et. al.*, 1997), besides it has been reported that oxygen consumption rate by fish is useful in determining carrying capacity of fish culture system (Lovell, 1989). However, it is of great importance to consider species and life stage when interpreting metabolic rate measurement in term of osmoregulatory cost (Morgan and Iwama 1996).

Salinity affect the rate of oxygen consumption in different ways, i. e. metabolic activities in freshwater salmonids (rainbow trout juveniles) increase with increasing salinity;while mullet (*Mugil cephalus)* metabolic activities decrease with increasing salinity (Marias, 1978).

Many local studies were carried out to determine the oxygen consumption rate of fish (Yesser, 1996; Hussein et. al., 2001), but the energetic cost of osmotic regulation has received little attention.

The purpose of the present study was to examine the physiological and respiratory response of *Liza abu* during the acclimation to salt water. Measurement of oxygen consumption rate, plasma ions concentration (Na^+ and K^+), blood glucose level was carried out in *Liza abu* after transfer from freshwater to salt water (7 and 15 g/L).

MATERIALS AND METHODS

Fish: In September 2002 *L .abu* juveniles were obtained from Marine Science Center fish farm / Basrah university. The fish were held in the laboratory in 40 liters polyethylene containers supplied with dechlorinated tap water (salinity 1.2 g/L). The fish were fed a commercial fish pellets (23 % protein) daily. Aeration was used. Acclimation on laboratory condition continued for two weeks.

Salinity exposure: *L. abu* juveniles weighted 5 .26 – 7. 06 g was transferred randomly to three 40-L polyethylene containers at a density of 10 fish per container. One container was supplied with fresh water, the second with salt water (7 g / L) and the third with 15 g / L salt water. These salinity (7 and 15 g / L) were produced by dissolving a known weight of pure sea salt in tap water. Water temperature provided to maintain dissolved oxygen level.

Plasma ions concentration: The concentrations of Na^+ and K^+ in the blood plasma were determined after three days of transfer to higher salinity. Four individuals from each salinity (7 and 15 g / L) besides fresh water were killed, the caudal peduncle was cut and the blood was cut and the blood was collected using micro – capillary tubes. centrifugation was done by micro – centrifuge . Haematocrit values (Hct %) was measured, then the plasma was separated, diluted using deionized distilled water. The diluted plasma was frozen under –12°C for later assay of Na^+ and K^+ using flame photometer (ANA. 10 AL).

Respirometry:

After three days in salinity treatments, oxygen consumption rates of *L. abu* juveniles were measured in closed, opaque flask (1000 ml) as in Nordllie and Leffler (1975). A single fish that had been acclimated to the initial test salinity and temperature was placed in a sealed test flask containing air – saturated water that maintained by using artificial aeration. Prior to each Respirometry trail, the fish were allowed to acclimate in the sealed flask for 24 h. the flask was covered with black plastic cover throughout acclimation and testing to shed the fish from visual disturbance. After acclimation, the flask was sealed and artificial aeration was stopped. Subsequent samples were taken at 30 min. intervals for 120 min. Oxygen concentration in the water samples were determined using micro –Winkler method (Sumish *et. al.,* 1996) then determination of oxygen concentration was done using spectrophotometer (spectronic- 20) on wave length 450nm using a standard fixed oxygen solutions.

Following metabolic determination, the fish was removed and weighted. The mean weight of fish run at each salinity were maintained at between 5.2 – 7.06 g so that salinity effects and metabolic rate would not be confounded with body size effect.

Water temperature during the trials was kept similar to the holding tanks (22 – 24° C).

Dissolved oxygen decreased at a constant rate and about 20 – 30 % of the initial oxygen was consumed during each trial.

Oxygen consumption rates were estimated and expressed as milligram of oxygen per hour per kilogram of fish (mg O_2 / kg / hr).

Blood glucose levels:

After determining oxygen consumption rates, the fish were removed from the flask, killed by a blow on head and blood were collected from the caudal vein or artery using heparinized capillary tubes. After centrifuging, the plasma removed and used for glucose determination. Glucose concentration in blood was determined by enzymatic – calorimetric method by using Enzymatic – calorimetric (GOD – PAP) kit (Biomaghreb, UK). This method is based on oxidation of glucose by glucose oxidase to gluconate and H_2O_2 .The optical density was measured by spectrophotometer on wavelength 505 nm.

Statistical analysis:
Data are presented as means SD. when analyzed of variance (ANOVA) indicated significant different treatment – means ($P < 0.05$)

RESULTS

Plasma ion concentration:
The transfer of fish from fresh water to salt water resulted in a significant increase ($P < 0.01$) in plasma sodium and potassium levels, these values were over freshwater values. The mean sodium levels in 7 and 15 g / L salinity were 125±4 .5 and 150 ± 7.5 mmol / L respectively, comparing with 98 ± 3.4 mmol / L in freshwater acclimated fish (Fig 1).The same pattern was true for potassium. The mean potassium levels in 7 and 15g / L salinity were 10 ±1.2 and 14 ± 1.5 mmol / L respectively, comparing with 7 ±0.9 mmol/L in freshwater adapted fish (Fig 2). The results showed no significant differences in packed cell volume (PCV %) between the three groups of fish (fig 3)

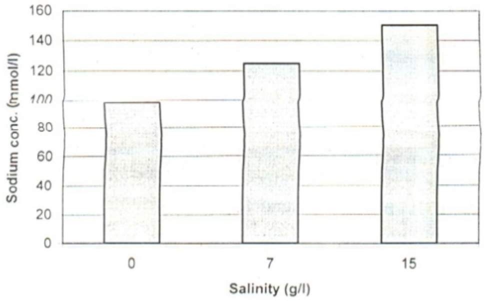

Fig.1: Sodium levels in the blood of L.abu during salinity acclimation.

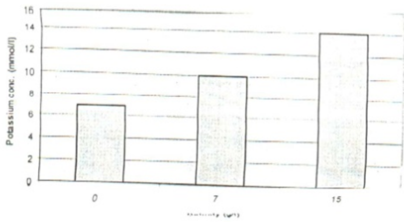

Fig.2: Potassium level (mmol/l) in the blood of *L.abu* during salinity acclimation.

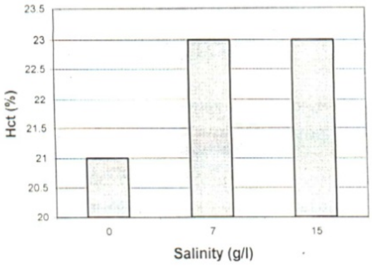

Fig.3: Haematocrit values (%) of *L.abu* during salinity acclimation.

Oxygen Consumption Rates:

The average oxygen consumption rate of *L. abu* three days after transfer was significantly ($P < 0.01$) higher in 7 and 15g/L salinity than in freshwater adapted fish (Fig 4). Oxygen consumption rates in 7 and 15g/L salinity were 256.5 ± 8.5 mg / kg / hr and 262 ± 10. 0 mg / kg / hr respectively, comparing with the freshwater adapted fish (151±12.4 mg / kg / hr). There were no significant differences ($P > 0.05$) in oxygen consumption rates between fish transfer to 7 and 15 g / L salinity.

The percentage of increase in oxygen consumption rate was 70 % in 7 g/L salinity and 73 % in 15g/L salinity.

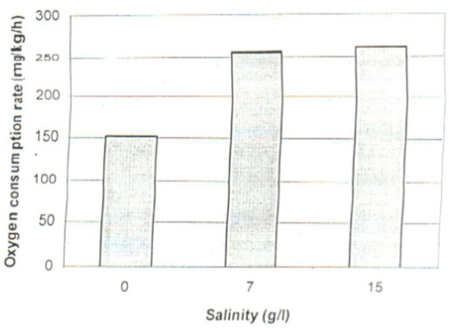

Fig.4: Oxygen consumption rate during salinity acclimation of *L.abu*

Blood Glucose Level:

The transfer of fish from fresh water to 7g/L salinity resulted in increase in plasma glucose to 171.17 mg/100 ml (Fig 5). In contrast, plasma glucose level decreased to 108. 18mg/100 ml after three days of transfer to 15g/L salinity (fig 5). The level in freshwater acclimated fish was 121.62 mg/100 ml.

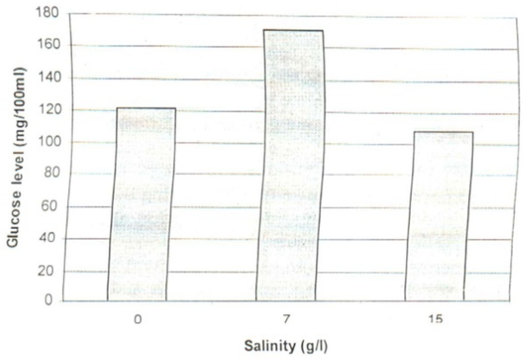

Fig.5: Glucose level (mg/100ml) in the blood of L.abu during salinity acclimation.

Metabolic Cost:

According to Brett (1972): The conversion from oxygen consumption (mg/kg/hr) to kilocalories is that: 1 mg O_2/kg/hr = 0.00337 kcal/kg/hr = 297 mg O_2/kg/hr. The results showed that the metabolic rate increased to 0.84 and 0.88 kcal/kg/hr in salt water (7 and 15 g/L respectively) compared with 0.508 kcal/kg/hr in freshwater adapted fish (Fig.6).

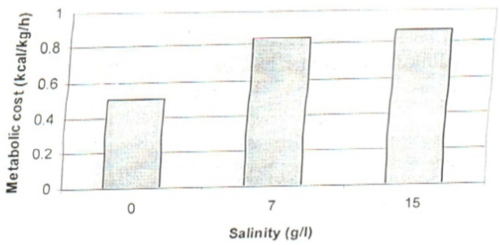

Fig.6: Metabolic cost (kcal/kg/h) of *L.abu* during salinity acclimation.

DISCUSSION

Oxygen consumption was measured in *L.abu* as a metabolic response to salinity acclimation, and before reaching the homeostasis state which achieved after ten days of transfer to higher salinity (Ahmed, 1996). This study showed that the oxygen consumption rate increased significantly after three days of transfer from fresh water to salt water (7 and 15 g/l). This reflects the increase in metabolic rate in more saline water which was one and halftime that in fresh water. This finding is in general agreement with the conclusion of Morgan et.al.,(1997); Nordlie and Leffler(1975);Muir and Niimi(1972). While Yesser (1996) found that oxygen consumption rate of *Liza carinata* juveniles increase with increasing or decreasing salinity beyond 15g/L. Moreover, it has been found that fasted *L. abu* do not osmoregulate perfectly when compared with fed fish during salinity acclimation (Sultan, 2001), this give an indication that salinity acclimation needs energy.

This study also showed no significant differences in oxygen consumption between fish in both salinity (7 and 15 g/L). In spite of increasing the osmotic gradient between the blood and the outer medium, suggesting that acclimation of *L. abu* to higher salinity does not increase the energetic demand of this species. Farmer and Beamish (1969) suggested that the consumed energy for osmoregulation increase with increasing the osmotic gradient between blood and external medium. While Morgan and Iwama (1998) suggested that isosmotic salinity did not lower metabolic rate although the osmotic gradient is in its lower range and they again suggested that the lowest metabolic rate will be found in the environment that is natural for a particular species and life history stage.

Blood glucose increased after transfer to salinity 7 g/L, while it decreased in fish transfer to 15-g/L salinity. This increase is accompanied with increasing cortisol level in the blood (Morgan *et. al.*, 1997) during salt water acclimation while Smith (1982) referred that glucagon is responsible for increasing in blood glucose through glycogenolysis. Assem and Hanke (1979) found that blood glucose in tilapia increased during transfer from freshwater to salt water and they explain this increase as a transfer stress. The lowest glucose level in fish in salinity 15g/L may reflect the high uptake of glucose by metabolizing cells (Morgan *et.al.*,1997). However, Morgan and Iwama (1998) noticed that glucose is used as an indication of energy requirement for ion transport.

This study showed that Haematocrit values (Hct%) did not significantly change with increasing salinity, this may lead us to say that the high level of blood glucose is not a result of changing in blood volume.

The increasing levels of plasma ions (Na^+ and K^+) of *L. abu* as a result of increasing the ambient salinity was recorded also by Ahmed (1996) and Sultan (2001).

In conclusion, the salt-water acclimation process in *L. abu* juveniles involves several osmoregulatory adjustments in order to establish ionic homeostasis. The present study demonstrate that these physiological changes represent a significant energetic cost, elevating metabolic rate by about 64% and 72% after three days of transfer to salinity 7 and 15g/L respectively.

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استهلاك الطاقة للتنظيم الازموزي في صغار اسماك الخشني(*Liza abu*) أثناء الأقلمة الملحية

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الخلاصة

تم دراسة العديد من المتغيرات الفسلجيه خلال الأقلمة الملحية لصغار اسماك الخشني *Liza abu* وقد استخدم معدل استهلاك الأوكسجين ومستوى الكلوكوز كمؤشرات للطاقة المستهلكة أثناء التنظيم الازموزي .

نقلت اسماك الخشني المؤقلمه على الماء العذب إلى مياه مالحة (ملوحة ٧ و ١٥ غم / لتر). تم قياس استهلاك الأوكسجين ومستوى الكلوكوز في الدم وتركيز أيونات الدم (الصوديوم والبوتاسيوم) وذلك بعد ثلاثة أيام من النقل إلى الملوحات العالية . لقد سبب النقل حصول ارتفاع في أيونات بلازما الدم ، أما مستوى الكلوكوز في الدم فقد لوحظ ارتفاعه عند النقل إلى ملوحة ٧ غم / لتر ، بينما انخفض مستواه في ملوحة ١٥ غم / لتر وذلك مقارنه مع مستواه في المياه العذبة . وقد ازداد معدل الأوكسجين المستهلك بمقدار ١,٥ مره عند النقل إلى الملوحات العالية ولكن لم يلاحظ وجود اختلافات معنوية في مستوى الأوكسجين المستهلك بين الأسماك في كلا الملوحتين . كذلك تم تقدير كميه الطاقة المستهلكة خلال الأقلمة الملحية .

النتائج بينت ان التغيرات الفسلجيه المرافقة للأقلمة الملحية في صغار اسماك الخشني تمثل استهلاك معنوي قصير الأمد للطاقة .

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