Influence of salinity acclimatization on energy, oxygen consumption rate and glucose levels for *Carassius auratus* (Goldfish)

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Abstract:
This study aimed to investigate the influence of increasing salinity concentrations on some indicators of energy consumption such as dissolved oxygen consumption, the consumed energy, as well as the levels of blood glucose in *Carassius auratus* (Goldfish), because of their direct relation with the metabolism rates when fish are exposed to exertion. Fish with an average weight of 37.5+2.5 g were exposed to four salinity concentrations 0.1, 3, 6, and 9 grams per 1 liter; concentration represented four treatments, and the first one is control. Results showed that there was increase in the rate of oxygen consumption to 92.34, 126.24, and 158.86 mg O_2/kg/hour in the salinity concentration levels 3, 6, and 9 respectively, compared to the control (88.16 mg O2/kg/hour). This increase in oxygen consumption affected the consumption rate of energy, which achieved 0.311, 0.425, and 0.535 kilo calories in the salinity concentrations of 3, 6, and 9 gram/L liter, respectively, compared to the control (0.297 kilo calories/kg/hour). Concerning blood glucose levels rose in the salinity concentrations of 3, 6, and 9 gram/L liter to 68.33, 87.88, and 108.69 mg/100mL, respectively, compared to the control (66.33 mg/100 mL).

Keywords: Energy, Oxygen, Glucose, Salinity, Goldfish

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Introduction

Fish farming aims to provide an ideal and healthy environment for fish to increase their growth to the maximum during the shortest period possible. Salinity is considered one of the most important environmental criteria which affect the growth of fish (Pietri *et al.*, 2013). Fish requirements of energy are fulfilled through carbohydrates, fats, and proteins available in the conservative feed, which is used either for maintenance or for growth, where fish rely on mitochondrial cells to consume this energy (Jobling and Baardvik, 1994). Although glucose is considered a key source of energy used for cell perpetuation and growth, fish may use other sources of energy when exposed to higher and exhausting environmental circumstances such as salinity (Tseng and Hawang, 2008). Most fish adapt within the stable salinity environment, either in fresh or saline water (Whitfield *et al.*, 2012). Therefore, the fish diversity level reaches to the climax in both fresh and saline environments, and this diversity levels decreases whenever this species is stay away from the ideal salinity level for the specified fish species (Kennish, 1986).

Fish living in rivers and estuaries are sometimes exposed to the highest level of salinity change and temperature change, this occurs due to evaporation and solar radiation (Strydom, 2015). Under drought circumstances and lack of fresh water flow, salt concentrations may exceed the normal levels; thus, fish are exposed to very saline environment (Adams *et al.*, 2016), especially when the salinity level rises suddenly and fast, which leads to fish die-off in huge numbers (Alkhshali and Alhilali, 2017). The ability of fish to adapt with the sudden changes in environmental factors may be a decisive factor in survival of the species or its extinction (Potter *et al.*, 2015).

Heterogeneity in time and place of a specific environment is considered a normal issue in the ecosystems (Chang *et al.*, 2007). As for aquatic Animals in general and fish in specific, salinity might be an important environmental factor which affects species distribution and their behavior in the ecosystem (Whitehead *et al.*, 2011). Also, being exposed to salinity concentration levels which are higher than the normal rates may lead to physiological changes in fish and to various responses and reactions to deal with the rise or the decrease in salinity (Kultz, 2015). Fish, behaviorally can respond to change of salinity by avoiding accessing to high or low salinity environments, or unsuitable environments for growth and reproduction (Tietze and Gerald, 2016), or swimming in more suitable environments, this requires the ability of fish to feel the environments and the ability to reach the ideal environment (Kultz, 2015), but if the fish are unable to get such new environments, they must use physiological strategies in order to deal with the change in salinity (Kultz, 2011). Several of changes occur in the gills, gill epithelium, kidneys and digestive system (Hwang and Lin, 2013). One of the physiological changes which fish make in adapting to salinity

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are their ability for osmo-ionoregulation to maintain the internal balance when exposed to different salinity levels (Marshal *et al*., 2006). The osmoregulation process requires spend energy therefore, this reflected in the standard metabolism rate, which represents the rate of oxygen consumption in the state of rest and non-digest in a certain temperature degree (Chabot *et al,* 2016). The energy specified for osmoregulation ranges between 5% to 30% (Ern *et al,* 2014).

Salinity change may lead to variances in energy consumption and using additional energy for osmoregulation, and can be seen clearly through calculating the standard metabolism rate. In addition, energy used by fish to complete the osmoregulation relies on fish activity, where the increase in movement and activity leads to increasing the oxygen demand, and then, leads to the increase in water and salts diffusion in gills, which requires an increase in energy cost (Chabot *et al.*, 2016) .

Glucose is considered the key prop in the process of metabolism, and blood glucose in fish is not subject to change only among different fish species, but also among fish with same species (Hemre *et al,* 2002). This is supported by many proofs which indicate that glucose in fish increases when fish are exposed to exertion (West *et al,* 1993).

This study aimed to determine the negative effects of salinity rise through measuring some indicators of energy consumption as physiological responses of *Carassius auratus* (goldfish) when exposed to different salinity concentrations during the gradual salinity acclimation.

Materials and methods

Fish acclimation

This experiment was designed to know the influence of gradual increase of salinity on consuming some energy indicators for *Carassius auratus* (goldfish). Twelve (12) glass tankswere used with 40 liters of water, salt concentrations previously had been prepared through dissolving a specific weight of sea salt in 1 liter of tap water Salt concentrations used were 3, 6, and 9 gram/L liter with three replications for each salt concentration, as five fish/ replicate. After acclimatizing of fish with the laboratory conditions using tap water (0.1 gram/L liter - control), fish with weights ranging between 35 – 40 grams were gradually exposed to the mentioned salt concentrations, as the fish were exposed to the new concentration at the end of the fourth day of exposure to the lower concentration. Fish were fed on a diet with a protein content of 28% at a rate of 3% of body weight with consideration of starting feeding after 24 hours from exposing fish to the new salt concentration. Samples were taken every four days before exposing fish to the new salt concentration with consideration of stopping feeding 24 hours before taking the samples (Olufayo, 2009).

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Measuring of consumed oxygen

The measurement method is based on the decreased value in the quantity of oxygen dissolved in water using the closed bottles method (Nordlie and Leffer, 1975). A single fish with determined weight is placed in a dark conical glass bottle of 1 liter capacity filled with oxygen-saturated water through pumping oxygen into water till saturation level. The gradually-acclimated fish were taken to the salt concentrations: Tap water (0.1) 3, 6, and 9 gram/L liter, four days for each salt concentration in the bottles designed for measure the consumed oxygen. Fish were left in the bottles for 24 hours to acclimatize them to detention. After, ventilation was stopped; all pots were closed firmly to prevent oxygen leakage into the bottles to start the experiment. Oxygen quantity was measured in close intervals (every 30 minutes) until noticing the decrease of the oxygen dissolved in water by 60% from the saturation level. Oxygen meter type WTW-OXI 91 device was used to measure the concentration of oxygen with the unit O_2/kg/hour. The consumed oxygen quantity is transformed to the energy according to Brett (1972) by the following equation:

Each 1mg O_2/kg/hour equals 0.00337 kilo calories/kg/hour.

Measuring of blood glucose

Samples were taken at the end of the fourth day of the gradual moving of each salt concentration used in the experiment (tap water, 3, 6, and 9 gram/liter) to measure the level of glucose in blood. The device ACCU-CHECK ACTIVE 68305 was used. In this process, fish is temporarily anesthetized with a hit on the head, and then the peduncle cut with a sharp scalpel. After that, fish is positioned in a leaning-downward position with its head up to make the blood flow continuously. Then, a blood drop is placed on the device tape, which is fixed in its specified spot. Result of blood glucose level (mg/liter) is read immediately through the number that appears on the screen of the device.

Results and Discussion

Environmental factors

Table 1 shows the environmental factors during the first experiment, including temperature (°C), dissolved oxygen (mg/liter), and the degree of acidity (pH) in the different salt concentrations. All the mentioned environmental factors are within the range of the safe levels of fish life and the survival of *Carassius auratus* (goldfish) (Newell, 2013)

Salinity influence on the consumption of oxygen and energy

Table 2 shows the rise in the rates of oxygen and energy consumption for *Carassius auratus* to 92.34, 126.24, and 158.86mg O2/kg/hour for the salinity levels 3, 6, and 9, respectively, compared to the control (88.16mg O2/kg/hour). As for energy consumption, it also rose to 0.311, 0.425, and 0.535 kilo calories/kg/hour, compared to the control (0.297 kilo calories/kg/hour). Results of statistical analytics indicated that there were

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significant differences at ($p \leq 0.05$) in the rate of oxygen and energy consumption between the control and the salt concentrations 6 and 9 gram/liter, whereas no significant differences were found between the control and the second experimental group (3 gram/liter); while the experimental groups three and four have significant differences with each other and with the experimental group two.

Table 1: Environmental factors in the different salinity levels during experiment.

Salt concentration (mg/liter)	Temperature (°C)	Dissolved oxygen	Acidity pH
Liquifying water (0.1)	20 -22	5.6 -6.1	6.8 -7
3	20 -22	5.6 -6.0	7.2 -7.5
6	20 -22	5.2 -5.4	7.4 -7.6
9	20 -22	4.8 -5.0	7.6 -7.8

Table 2: Levels of consumed oxygen and energy in the different salinity levels by *Carassius auratus* (value represents rates±standard deviation–different letters denote the existence of significant differences).

Salt concentration (gram/liter)	Oxygen consumption (mg oxygen/ kg/ hour)	Energy consumption (Kilo calories/kg/hour)
0.1	88.16^{c}	0.297^{c}
3	92.34^{c}	0.311^{c}
6	126.24^{b}	0.425^{b}
9	158.86^{a}	0.535

Salinity influence on of glucose in the blood of Carassius auratus

Table 3 shows the rise of glucose in the blood of *Carassius auratus* in parallel with the rise of salt concentrations of 3, 6, and 9 gram/liter to 68.33, 87.88, and 108.69mg/100mL, respectively, compared to the control (66.67mg/100mL). The statistical results showed that there were significant differences ($p \leq 0.05$) in the rate of glucose between the third and fourth treatments, (6 and 9 g/L), with the control (the first), while both of third and fourth treatments had significant differences between each other, and with the first and the second treatments.

Table 3: Glucose levels in the blood of *Carassius auratus* as exposed to different salt concentrations.

Salinity concentration gram/liter	Glucose concentration in plasma mg/100mL
0.1	66.33 c
3	68.33 c
6	87.88 b
9	108.69 a

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Discussion

The metabolism rate represents the measurement of the energy consumed by fish to perform some important physiological operations to survive, including the osmoregulation and the main respiratory functions (Eren *et al*., 2014). When salinity concentrations are far from the normal rates which fish are accustomed to, this negatively influences the metabolism rate, and fish will be exposed to exertion (AL-Khshali and Al-Hilali, 2019). Thus, fish to adapt and overcome the new situation, they have to show certain physiological responses through performing some physiological operations to achieve a state of internal balance (homeostasis). The oxygen rate has been used as an indicator of the metabolism for all animals since the beginning of the 20th century (Svendsen, 2016). When freshwater fish are exposed to high salinity concentration, they work on performing the operation of osmotic and ionic balance through allowing ions and salts to enter their bodies by drinking water (Evans *et al*., 2005); they also work on absorbing important ions, such as chloride, sodium, and calcium, via mitochondria rich cells called chloride cells, specialized in taking and disposing ions with ATPase enzymes, where this process needs energy consumption (Eren *et al.*, 2014). Oxygen consumption is considered an indicator to measure the consumed energy used to complete the process of osmoregulation, where the change in the breathing rate of fish is usually used as an indicator to estimate the change occurred in the metabolism rate and consumption under environmental imbalance circumstances (Dube and Hosetti, 2010).

The gills are thought to be the most energy-consuming organ during the acclimatization in high-salinity concentration environments, and that the energy needs in gills are secured through oxidation of glucose and lactate resulting from blood circulation, in addition to the liver which represents the main place of glucose for fish (Perry and Walsh, 1989). Results of various previous studies about different species of fish demonstrated that there was increase in the rate of oxygen consumption in line with the increase of the environment salinity, and these results are consistent with results of this current study. De Boeck *et al*. (2000) mentioned that there was increase in oxygen consumption for common carp when exposed to the salinity concentration of 0 – 10 gram/liter, while Al-Khshali (2011) mentioned that the oxygen consumption rate for grass carp reached 132.11, 197.08, and 241.77mg O_2/kg/hour in the salt concentrations of 4, 8, and 12gram/liter, respectively, compared to the control (102.72mg O_2/kg/hour), while the rate of the consumed energy reached 0.44, 0.66, and 0.81 kilo calories/kg/hour for the salt concentrations of 4, 8, and 12 gram/liter, respectively, compared to the control (0.34 kilo calories/kg/hour). Results of this study were also agreed with the results of the study of Zhao *et al.* (2011) and Swanson (1998) who

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observed a evident relation between the increase of salinity and the increase of oxygen consumption. Kinne (1967), in turn, affirmed that the rate of oxygen consumption increased for four fish species which were exposed to differences in salt concentrations. Additionally, Wang *et al*. (1997) showed that the rate of oxygen consumption for *Cyprinus carpio* in fresh water reached 134.1mg O_2/kg/hour, but it decreased to 123.8mg O_2/kg/hour in salinity level of 2.5 g/L, compared to higher level 8.5 and 10.5 of salinity g/L, where the consumption level reached 175.2 and 183.0mg O_2/kg/hour, respectively. Another study conducted by McKenzie, (2001) on *Acipenser naccarii* showed that there was increase in oxygen consumption rate with the increase in salinity within a range of 0-11gram/liter; the oxygen consumption rate rose from 112 to 146.4mg O_2/kg/hour, then it rose to 216mg O_2/kg/hour during 70 days. Other studies signaled the rise in metabolism rate when fish are exposed to high salinity concentrations which result in an increase in oxygen consumption rate above average (Fischer, 2000; Keddy, 2001).

However, results of some other studies were not agreed with the results of this current study. Grøtan *et al.* (2012) showed that there was no change in oxygen consumption rate and metabolism rate for yellowfin seabream *Acanthopagrus latus* when exposed to three different environments (fresh water, brackish water, and saline water) through fast exposing to high or low salinity concentrations when moving them from saline water to fresh water or from saline water to brackish water. The reason for that no-change is that this species of fish possesses genes which are responsible for retaining the energy when there is change in salinity concentration during a short time as a part of adaptation to salinity concentration (McCairns and Bernatchez, 2010). Awal *et al.* (2012) also mentioned that there was a decrease in oxygen consumption rate for Tilapia to 2.14, 0.71, 1.43, and 1.42 mg/ kg/hour when exposed to salt concentrations of 0, 10, 20, and 30 gram/liter, respectively; the researchers attributed that decrease in oxygen consumption rate to the decrease in fish activity as a result of salinity increase, which resulted, in turn, in fish exertion. Also, Al-Hilali and Al-Khshali (2016) indicated that there was a rise in the rate of oxygen and energy consumption for *Cyprinus carpio* when salinity was raised to 5, 10, and 15 gram/liter compared to the control. In addition, Christensen *et al.* (2018) showed that there was a rise in oxygen consumption when salinity was raised to 0%-15% with different temperature degrees 5, 10, and 15°C for shiner Perch *Cymatogaster aggregata*; Christensen *et al.* (2018) also mentioned that there was rise in oxygen consumption rate, and estimated the used energy quantity at 20% as a difference between the salinity concentration 12% and 30% for shiner Perch *Cymatogaster aggregata*. Noor *et al.* (2019), through a study conducted on the hybrid *Grouper Epinephelus*, found

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that, when fish were exposed to different salt concentrations of 10, 15, 20, 25, and 30, oxygen consumption rate was less for the salinity concentration 15 gram/liter, but it increased for the salinity concentration rates 25 and 30, and this reflects the rise of metabolism rate when salinity concentration is high

The influence of salinity rises in blood glucose levels

Glucose estimation is considered one of the signs of fish exertion (Sultan, 2007). This is because the rise of glucose may be attributed to various causes, including, as the most important ones, the rise of noradrenaline and adrenaline hormones in blood (Tsui *et al.*, 2012). The rise of salinity concentration leads to the rise in the percentage of catecholamine hormones, which include dopamine, norepinephrine and adrenaline hormones, where the adrenal gland sends catecholamines to the blood when fish are under effect of exertion (Wendelaar Bonga,1997).

It was observed that exertion and the rise of glucose level in blood for *Cyprinus carpio* occurred after 20 minutes from being exposed to cold in what is termed cold shock response (Tanck *et al*., 2000). Also, it was recorded that there was increase in the levels of glucose for channel catfish *Ictalurus punctatus* when exposed to a high percentage of ammonia or when there was decrease in oxygen level, and these are other sources for stress (Small, 2004). Moreover, glucose level increase was also noticed in the blood of *Oreochromis mossambicus* Tilapia in a stable manner after four hours from adaptation in fresh water, water salinity 10%, and water salinity 30% (Cataldi *et al*., 2005), The changes in energy amount and its consumption can also lead to the rise in the rate of oxygen consumption, which leads, in turn, to the rise of glucose level in blood (Wendelaar Bonga, 1997). The rise of glucose in fish blood is considered a proof of exertion, and it represents an indicator of secondary responses which fish do as a primary response to resist exertion after the rise of the cortisol (Al-khshali and Al-hilali, 2016). Zhao *et al.* (2011) also observed the rise of glucose in blood of Chinese sturgeon (*Acipenser sinensis*) at the beginning of moving to high salinity, and then the decrease occurs in levels of glucose after that. Also, Gamperl *et al.* (1994) mentioned that glucose is considered an indicator which is used to estimate exertion volume, which leads to changes in metabolism as a result of glucose rise, which motivates the emission of catecholamines and corticoids. The increase in blood glucose which usually accompanies salinity rise is attributed to mobilizing of liver glycogen (Sangiao–Alvarellos *et al*., 2003), where adrenalin works on raising sugar level in blood through the glycogenolysis process in cases of exertion; and this process is important for making a sufficient level of sugar in blood (Xu, *et al*., 2013) as to face the increasing demand for energy in the organs of osmotic balance during the salinity acclimatization (DeBoeck *et al*., 2000). The production of glucose

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happens usually due to the rise of cortisol in blood when moving to high salinity concentration. This is because glucose gets liberated from the liver and muscles toward the blood stream, and then enters into the cells because of insulin secretion (Nelson and Cox, 2005).

Results of current study show consistency with the results of various previous studies which indicated that there was a rise in the level of glucose for fish as a result of the rise in the levels of salinity above its normal levels. In this context, DeBoeck *et al*., (2000) showed that there was a rise in the level of glucose for *Cyprinus caprio* exposed to the salinity of 10 gram/liter for 28 days. Ahmed *et al*., (2004) observed a rise in glucose for *Liza abu* when moved to the salt concentration of7 g/L. In addition, Karsi and Yildiz (2005) demonstrated that there was a significant rise in blood glucose for *Oreochromis niloticus* when exposed to the salt concentration of 18 g/L. Sultan (2007) also concluded that a rise in blood glucose occurred for Juvenile *of Acanthopagrus latus* when exposed to the salt concentrations of 7 and 15 g/L; however, it decreased for fish which were moved to higher salinity of 23 and 30 g/L. Also, glucose level decreased for *Rainbow trout* when moved from fresh to saline water (Soengas *et al*., 1995), and for Black seabream which were moved to higher salinity concentrations (Kelly *et al*., 1999). This decrease in glucose level in higher salinity concentrations might be attributed to the high need and the frequent taking of glucose to be used in cells metabolism (Morgan *et al*., 1997). Therefore, the frequent use of glucose is very important in providing energy for the osmoregulation organs, which prevents glucose deposition in cells (Martinez–Alvarez *et al*., 2002). Other studies in this respect, such as McLeay (1977), Barton *et al.* (1988), and Wedemeyer *et al*. (1990) mentioned that there are factors which affect indirectly the glucose response level in blood; thus, changes including nutritional status, year seasons, and age may affect the response to exertion and the level of glucose because they can influence glycogen volume stored in the liver. Results of current study are consistent with various studies which confirmed the occurrence of rise in blood glucose for fish as a result of the rise in salinity levels above the normal levels in the environments where fish live. This supports Al- Alkhshali (2011) who concluded that there was rise in blood glucose for grass carp (*Ctenopharyngodon idella*) when the salinity was increased to 4, 8, and 12 g/L. Tsui *et al.* (2012) also mentioned the rise of glucose concentration when *Grouper epinphelus* were moved from 29% to 34% of salinity for a period of 10 – 30 minutes as an indication of the first exertion factors. In addition, Al-Khshali and Al-Hilali (2015) showed that there was rise in blood glucose level for *Cyprinus carpio* when salt concentrations were increased to 5, 10, and 15 compared to the control. This is attributed to the increase in demand for

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energy, thus, secretion of stress hormones, such as adrenalin and norepinephrine, which have a role in raising the percentage of blood glucose, and the body's continuous demand for energy, which, in turn, motivates burning glycogen that exists in the liver and transforming it into glucose to be used as the primary source of energy for fish.

However, the results of current study were not consistent with the results of Farghaly *et al*., (1973) who did not find any significant influence in blood glucose level when *Tilapia zilli* were exposed to four salt concentrations: fresh water, 9, 19, 39g/L, where no significant variances were found. Fast *et al*., (2008) also recorded that there was no blood glucose rise for Atlantic salmon (*Salmo salar*) when exposing to the sudden rise in salinity, but he recorded a rise in blood glucose when the same fish were exposed to saline exertion for a long time.

Conclusion

The rise in salinity led to an increase in oxygen consumption, energy consumption and blood glucose consumption, and this is evidence of the fish's attempt to reach an adaptation process with the rise in salt concentrations in order to reach to "homeostasis"

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