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ADAPTATION OF COMMON CARP TO SALINITY

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ABSTRACT

This study was conducted to investigate the ability of common carp, *Cyprinus carpio*, to adapt to various salinity levels. Two experiments were conducted to study the effects of gradual increase of salinity on weight gain, survival, immunological and osmoregulatory responses and consequent resistance to *Vibrio vulnificus* infections in common carp. In the first experiment, sodium chloride was added to the water by 0.5ppt every two days to reach a final concentration of 4, 6 and 8ppt, and fish were sampled after 14 days. In the second experiment, sodium chloride was added by 0.5ppt every week to reach a final concentration of 6 ppt and fish were sampled after one month. Fish of the first experiment exposed to 8ppt salinity showed significant increase in all serum measurements and detrimental effects on weight gain and survival. In the second adaptation experiment, the unaltered glucose, cortisol and ions levels indicated that salinity up to 6ppt did not produce significant stress in common carp it decrease the mortality rate caused by *V. vulnificus*. The above results suggest that common carp, a freshwater fish, can withstand gradual increases of salinity of up to 6ppt, and thus can be raised in brackish water with little or no detrimental effects on growth and performance.

Key words: *Cyprinus carpio*, water salinity, osmoregulation, immune parameters, stress response, bacterial challenge.

INTRODUCTION

Common carp, *C. carpio* belongs to the family *Cyprinidae* of the order *Cypriniformes* has been used in aquaculture almost throughout human history as freshwater fish and are uncommon in brackish water; it can be considered the world´s most widely distributed fish in North America, Africa, and Eurasia (Nelson, 2006). It is used both in pond and restrictive fisheries in many parts of the world because of its possibility rapid growth in eutrophic waters and ability to tolerate unfavorable environmental conditions (Váradi, 2014).

Although this species is commonly propagated in freshwater, preliminary evidence in our research wet lab suggests that moderate salinities allow better performance, exactly the attainment of stable survival rates under stress of salinity increase.

Salinity is known as the total concentrations of all ions in water. It is not just the sodium chloride concentrations in water. In this experiment, sodium chloride was used instead of seawater because these are the most salts commonly applied to raise salinity during management practices of fish species (Tsuzuki *et al.,* 2000).

Water homeostasis and osmoregulation are primary points of concern to maintain physiological homeostasis. In fish either a live in the hyper-osmotic marine environment or in hypo-osmotic freshwater, the regulations of internal water balance and ion concentration are critical aspects (Sheida *et al.,* 2010).

The successful maintenance of a species in a habitat rely on the adaptability of each developmental stage to salinity and several studies conducted in diverse species have proved that (Charmantier, 1998). Therefore, salt addition has been proved to relieve the consequence of stress during handling, crowding and transportation (Carneiro and Urbinati, 2001; Tsuzukin *et al.,* 2001) and to improve embryonic and larval growth and outgrowth of some freshwater fish (Fashina-Bombata and Busari, 2003). Higher growth and survival rates were observed in some freshwater species during larval husbandry at low salinity levels than in freshwater conditions (Luz *et al.,* 2004).

However, to date no studies in Egypt have analyzed the possible changes in physiology of common carp during exposure to gradual increase of salinity. This scarcity of data and our preliminary experiment on

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acclimation of this species to various salinity levels, allow us to proceed the current experiment. So the main objectives were to elucidate the physiological and behavioral adaptations of fingerlings common carp, *C. carpio* to modulation in water salinity. Our interest focuses on weight gain, survival, some immunological parameters such as total protein, albumin and globulin, stress indicators, such as glucose and cortisol, and osmoregulatory serum ions, potassium, chloride and sodium. Moreover, the progression of *V. vulnificus* in experimentally challenged fish will be studied. So this study let us compare changes in common carp in freshwater and in brackish water, which will help us to understand more about better aquaculture salinity condition for this species.

MATERIALS AND METHODS

1. Fish

Alive common carp, *Cyprinus carpio*, fingerlings with body weight of 8±2g, and length of 6±1cm were transported to the aquatic laboratory at Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Fish were maintained under laboratory conditions (salinity = 0.2ppt) for 2 weeks in a re-circulating system in porcelain aquaria (260 ×65×70cm) according to the protocol of maintaining bioassay fish as was previously described by Ellsaesser and Clem (1986), and fed twice daily on a commercial floating powdered feed containing 45% protein with feeding rate of 3% of their body weight. Experiments were conducted in fiberglass aquaria with dimensions of 60×30×40 cm. Dissolved oxygen level was maintained above 5 mg/L while water temperature was kept at 23°C ±3 and pH value at 7.2 – 7.5.

2. Salinity adaptation

2.1. The first adaptation experiment

Alive acclimated apparently healthy common carp were randomly divided into 6 fiberglass aquaria as 6 experimental groups, with 30 fish each. The experiments were done in 3 replicates. Salinity groups were subjected to gradual increase of sodium chloride by 0.5 ppt every two days to reach a final concentration of either, 4, 6 or 8ppt. These concentrations were chosen after preliminary trial test. The other groups were reared in freshwater and considered as controls for each corresponding salinity group. Water was changed partially every two days to prevent accumulation of ammonia and other toxic metabolites in the tanks, and salinity was checked and adjusted. When the final desired salinity is reached, fish were allowed to acclimate to the new salinities for 2 weeks before any investigations were made.

2.2. The second adaptation experiment

The entire experiment was done in three replicates. It was done the same way as the previous experiment, where common carp were randomly divided into 2 fiberglass aquaria but sodium chloride was added to the water at a rate of 0.5 ppt every week to reach a final concentration of 6ppt. A control group was reared in freshwater. Fish were allowed to acclimate to the new salinity for one month before sampling.

2.3. Clinical examination of fish

Fish were observed daily during the course of experiment for any apparent clinical signs, lesions or mortality. Mortality rate was calculated from the number of dead fish at different salinity levels.

2.4. Fish sampling.

At the end of the acclimation to the new salinity for each group in each experiment the size of the fish were recorded, and blood samples were collected from caudal vein by severing the tail. Non heparinized capillary tube is immediately applied to the vessel until sufficient blood is obtained, and then blood was collected in eppendorf tubes.

The blood sample was allowed to clot overnight at 4°C; serum was obtained by centrifugation at 3,000 rpm for 15 min, and non-hemolysed serum was collected and stored at -80°C until use. Levels of serum total protein and albumin were measured using spectrophotometry and "Total Protein" and "Albumin" kits (Spectrum, Egyptian Company for Biotechnology, Obour City, Cairo, Egypt) according to manufacturer's recommendation. Globulin levels were determined by direct subtracting the values of the albumin from those of the total protein. Glucose concentrations in whole blood samples were determined using the Gluco Dr.auto blood glucose monitoring system and gold plated test strips (All medicus Co., Ltd., Germany) according to the manufacturer's instructions. Samples used for Enzyme-linked immunosorbent assay (ELISA) were stored at -80^{o} c till used. Estimation of serum cortisol level (Silver *et al.*, 1983) was done using Elisa cortisol kit (Diagnostics Biochem Canada Inc.). Chloride (Schales and Schales, 1941), sodium (Trinder, 1951) and potassium (Sunderman, 1958) were colorimetrically measured by spectrophotometer using special Kits (Biodiagnostic Company Egypt). Because of the fish size employed in this study have little blood, it was necessary to pool the serum from all fish in each sampling to obtain sufficient volume for analysis. Because samples were pooled, each value supposedly approximates the true mean of 4 individuals.

2.5. Experimental challenge

2.5.1. Bacterial strain

A *Vibrio vulnificus* strain, isolated from clinical cases of infected fish showing signs of septicemia, was used in the experimental infections. The strain was identified by Gram stain, motility test, and various biochemical characters according to Austin and Austin (2016), preserved in glycerol-Trypticase Soya Broth at -80°C., and were passed three times in

healthy common carp through intraperitoneal injection before using for experimental challenge.

2.5.2. Bacterial challenge suspension and counts
Colony forming units (cfu) counts in bacterial suspensions were determined using spectrophometery optical density values at wavelength of 600nm and plate drop method with ten-fold serial dilutions (Miles *et al.*, 1938).

2.5.3. Experimental challenge
The challenge groups were challenged by intraperitoneal (I/P) injection of 0.2ml of 1.3×10^5 cfu/ml of pathogenic strain of *V. vulnificus*. While sham controls were injected with 0.2ml of sterile saline solution. The clinical signs, post mortem lesions and mortalities were recorded daily for up to 3 weeks. Re-isolation and identification of bacteria was done from freshly dead fish as mentioned above.

2.5.4. The first challenge experiment
Fish were divided into 6 experimental groups in fiberglass aquaria with 9 fish each, and there were 3 replicates for each group. The freshwater control group was maintained in freshwater and remained un-injected, while the freshwater sham control was maintained in freshwater and I/P injected with normal saline. The salinity control group was exposed to gradual increase in salinity with 0.5ppt every two days to reach a final concentration of 6ppt, while the salinity sham control was treated in the same way and I/P injected with normal saline. The salinity challenge group was treated as the salinity sham control group and then I/P challenged with *V. vulnificus*, while the freshwater challenge group was maintained in freshwater and then I/P challenged with *V. vulnificus*. Fish exposed to gradual increase of salinity were allowed to acclimate to the new salinity level for 14 days before challenge or saline injection. All challenges and injections were done at the same time.

2.5.5. The second challenge experiment
It was done in the same way as the first challenge experiment but fish in the salinity groups were exposed to gradual increase in salinity 0.5ppt every week to reach a final concentration of 6ppt. All challenges and injections were done after an acclimation period of one month in the new salinity level.

2.6. Statistical analysis
Results were analyzed using (Prism 5, version 5.01, *Graph pad software Inc*.). The differences were considered significant if the (P- value < 0.05) by using unpaired t- test or one-way ANOVA test (tukey's compare all pairs of column) or two way ANOVA test.

RESULTS

1. The first adaptation experiment
Fish exposed to either 4 or 6ppt salinity looked and acted normal which presented by normal swimming and they did not show any external signs of stress or mortalities. The total weight gain of fish exposed to 4 or 6 ppt salinity insignificantly increased than the control group. All measured serum parameters of fish exposed to 4 or 6 ppt salinity are presented in tables 1 and 2, respectively.

Fish exposed to 8ppt salinity showed marked reduction in swimming activities (easily caught) and mortality rate of 13.3%. The body weight gain was significantly decreased than that of the control group. The total protein and globulin values showed a significant decline with increasing salinity to 8ppt. Glucose, cortisol, potassium, sodium and chloride values were significantly higher than those of control (table 3). Comparison data of the 3 salinity groups are presented in table (4).

2. The second adaptation experiment
Fish exposed to 6ppt salinity, looked and acted normal and did not show any external signs of stress or mortalities. The body weight gain significantly increased in fish kept at 6ppt for one month (table 5). All measured serum parameters did not change significantly except for serum total protein, albumin and globulin that were significantly changed than control (table 5).

3. The challenge experiment
In the first challenge experiment, clinical signs started one day post challenge with signs of shallow skin ulcers. A similar mortality rate of 66.7% was noticed in the freshwater challenge and salinity challenge groups.

In the second challenge experiment, clinical signs started one day post challenge with signs of shallow skin ulcers. Mortality rate was 58.3% in freshwater challenge group, while the salinity challenge group showed a significant reduction in mortalities that were only 33.3%.

Post mortem findings of dead fish showed septicemia, enlarged friable liver and body cavity filled with bloody ascetic fluids.

Table 1: Effects of adapting of common carp, *C. carpio* to 4ppt salinity for two weeks on weight gain and serum parameters.

Parameters	Control 1 (mean±SE)	4ppt (mean±SE)
Total weight gain (g)	0.667 ± 0.14 [a]	0.75 ± 0.13 [a]
Total protein (g/dl)	6.56 ± 0.44 [a]	5.87 ± 0.32 [a]
Albumin (g/dl)	2.85 ± 0.30 [a]	2.22 ± 0.18 [a]
Glogulin (g/dl)	3.70 ± 0.19 [a]	3.65 ± 0.19 [a]
Glucose (mg/dl)	37 ± 3.78 [a]	38 ± 3.51 [a]
Cortisol (µg/dl)	18.95± 1.16 [a]	19.55± 0.79 [a]
Sodium (mmol/l)	42.67 ± 2.03 [a]	45.68 ± 1.22 [a]
Chloride (mmol/l)	121.57 ± 6.41 [a]	122.13 ± 7.23 [a]
Potassium (mmol/l)	1.17 ± 0.13 [a]	1.49 ± 0.23 [a]

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

Table 2: Effects of adapting of common carp, *C. carpio* to 6ppt salinity for two weeks on weight gain and serum parameters.

Parameters	Control 2 (mean±SE)	6ppt (mean±SE)
Total weight gain (g)	0.667 ± 0.14 [a]	0.67 ± 0.14 [a]
Total protein (g/dl)	5.85 ± 0.57[a]	5.73 ± 0.27 [a]
Albumin (g/dl)	2.87 ±0.4 [a]	3.47 ± 0.18 [a]
Glogulin (g/dl)	2.98 ± 0.68 [a]	2.26 ± 0.17 [a]
Glucose (mg/dl)	35.22 ±1.9 [a]	46.90 ± 2.9 [b]
Cortisol (µg/dl)	18.26 ±0.65 [a]	23.19 ±0.85 [b]
Sodium (mmol/l)	46.83 ±3.8 [a]	71.67 ± 1.8 [b]
Chloride (mmol/l)	127.30 ±2.2 [a]	149.85 ± 5.9 [b]
Potassium (mmol/l)	1.42 ±0.25 [a]	2.25 ± 0.28 [b]

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

Table 3: Effects of adapting of common carp, *C. carpio* to 8ppt salinity for two weeks on weight gain and serum parameters.

Parameters	Control 3 (mean±SE)	8ppt (mean±SE)
Total weight gain (g)	0.917±0.15 [a]	0.25 ± 0.22 [b]
Total protein (g/dl)	6.55 ±0.42 [a]	4.05 ± 0.53 [b]
Albumin (g/dl)	3.12 ±0.2 [a]	2.53 ± 0.28 [a]
Glogulin (g/dl)	3.43 ±0.37 [a]	1.52 ± 0.31 [b]
Glucose (mg/dl)	36.33 ±1.85 [a]	56.58 ± 1.99 [b]
Cortisol (µg/dl)	17.33 ±1.2 [a]	26.87 ±1.33 [b]
Sodium (mmol/l)	41.76 ±1.66 [a]	95.13 ± 3.32 [b]
Chloride (mmol/l)	130.08 ± 4.05[a]	230.33 ± 13.5 [b]
Potassium (mmol/l)	1.30 ± 0.04[a]	2.37 ± 0.17 [b]

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

Table 4: Effects of exposure gradually to 3 different salinity levels used for acclimation of common carp, *C. carpio* for two weeks on weight gain and some serum parameters.

Parameters	4ppt (mean±SE)	6ppt (mean±SE)	8ppt (mean±SE)
Total weight gain (g)	0.75 ± 0.13 [a]	0.67 ± 0.14 [ab]	0.25 ± 0.22 [b]
Total protein (g/dl)	5.87 ± 0.32 [a]	5.73 ± 0.27 [ab]	4.05 ± 0.53 [b]
Albumin (g/dl)	2.22 ± 0.18 [a]	3.47 ± 0.18 [b]	2.53 ± 0.28 [a]
Glogulin (g/dl)	3.65 ± 0.19 [a]	2.26 ± 0.17 [b]	1.52 ± 0.31 [c]
Glucose (mg/dl)	38 ± 3.51 [a]	46.90 ± 2.9 [a]	56.58 ± 1.99 [b]
Cortisol (µg/dl)	19.55± 0.79 [a]	23.19 ±0.85 [b]	26.87 ±1.33 [c]
Sodium (mmol/l)	45.68 ± 1.22 [a]	71.67 ± 1.8 [b]	95.13 ± 3.32 [c]
Chloride (mmol/l)	122.13 ± 7.23 [a]	149.85 ± 5.9 [b]	230.33 ± 13.5 [c]
Potassium (mmol/l)	1.49 ± 0.23 [a]	2.25 ± 0.28 [b]	2.37 ± 0.17 [b]

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error).

Table 5: Effects of adapting of common carp, *C. carpio* to 6ppt salinity for one month on weight gain and serum parameters.

Parameter	Control 4 (mean±SE)	6ppt (mean±SE)
Total weight gain (g)	1.083 ± 0.08 [a]	1.833 ± 0.24 [b]
Total protein (g/dl)	5.72 ± 0.34 [a]	6.14 ± 0.26 [b]
Albumin (g/dl)	3.52 ± 0.31 [a]	2.87 ± 0.13 [b]
Glogulin (g/dl)	2.20 ± 0.50 [a]	3.27 ± 0.37 [b]
Glucose (mg/dl)	38.33 ± 1.2 [a]	35.33 ± 1.8 [a]
Cortisol (µg/dl)	18.48± 0.78 [a]	20.42± 1.33 [a]
Sodium (mmol/l)	36.61 ± 1.2 [a]	38.39 ± 1.4 [a]
Chloride (mmol/l)	147.58 ± 20.04 [a]	165.49 ± 7.6 [a]
Potassium (mmol/l)	1.47 ± 0.19 [a]	1.66 ± 0.21 [a]

Means of the same raw with different letters are significantly different ($p<0.05$). Data was presented as (mean ± standard error)

DISCUSSION

With increasing scarcity of freshwater based aquaculture due to increasing requests on the need of freshwater for agricultural, industrial and domestic targets, the use of marine and brackish environments for aquaculture become a vital alternative (Suresh and Lin, 1992). Thus, there is an increasing need for growing freshwater fish that can tolerate high salinity. Consequently, a precise salt acclimation schemes for freshwater fishes are instantaneous practical and effective mode to enhance salt tolerance.

This study describes a process of adapting of common carp to water that contains higher salt levels than those in freshwater. To investigate the highest salinity level that allow normal growth and other physiological functions, two gradual increase regimes of salt were used with the possibility of raising common carp in brackish water. The present study had the dual purpose of obtaining information about the salinity tolerance of common carp fingerling and obtaining preliminary information on their osmoregulatory and compensatory stress responses under different sodium chloride concentrations.

Carp are widely distributed in the wild as well as in aquaculture; this fish has been considered to be a good candidate for cultivation owing to its resistance to diseases, satisfactory growth rate and suitability for human consumption. Among carps, the common carp is highly tolerant to salinity; therefore it is a suitable model for studies on osmoregulatory mechanisms and salinity tolerance.

Although the stress of salinity transfer can be fatal for trout following transfer to full strength seawater (Johnston and Cheverie, 1985), longer acclimation

time decreases this stress (Fuentes *et al.,* 1997) and this supports the findings of the current study. The more gradual process of increasing salinity could be less stressful to fish as was indicated by the finding that common carp subjected to every two days increase in salinity can adapt only up to 4ppt salinity but start to stress at 6ppt, while when common carp subjected to per week increase of salinity it can survive and acclimate to 6ppt without any stress. This cleared that, pre-acclimation of common carp at low salinity and gradual transfer to higher salinities may diminish the magnitude of stress response. While (Sheida *et al.,* 2010) data indicate that in culture conditions, adult common carp can survive successfully in brackish water with salinity of up to 9ppt, this difference attributed to, that the age and body size have been supposed as determining factors of the salinity tolerance of fish (Mc Enroe and Cech, 1985; García-Gallego *et al.,* 1998). Also on the contrary, in natural environments, juvenile ship sturgeon, *A. nudiventris* in migratory populations could be able to migrate and adjust successfully into brackish water with a salinity of up to 8ppt without any short-term hematological stress responses (Erfan *et al.,* 2015). The different rhythms in acclimation recorded between these researches and our study may be explained by differences in osomoregulatory ability among different species and even populations of the same species. Although the present study dealt with different concentrations of sodium chloride instead of seawater, the results suggest a tolerance to a wide range of salinities, (Tsuzuki *et al.,* 2000). Also, differences may be due to different fish species, size and environmental conditions.

Mortality starts to appear at salinity more than 7ppt in the first adaptation experiment, this may be due to inability of fish to adjust osmoregulation, as the mortality among fish transferred to brackish water is inversely related to their ability to adjust osmo-regulation and depending on fish size, and such transfer often results in high mortality rates (Cataldi *et al.,* 1999).

Development and growth in fish are controlled by internal factors including CNS, endocrinological and neuroendocrinological systems. Among vertebrates, they are also highly dependent on environmental conditions. Among other factors, many studies have reported an influence of water salinity on fish development and growth. Data are also available in terms of food intake and stimulation of food conversion, which are both dependent on the environmental salinity (Gilles and Patrick, 2001). In fish, almost always a better growth rate is observed in intermediary salinity conditions, i.e. in brackish water (Gilles and Patrick, 2001).

Several studies indicated that oligohaline water for freshwater fish cause more rapid growth (Overton *et al.,* 2008). In isosmotic water, food uptake and growth rate increases. Fish converts more feed to energy and uses less energy for standard metabolic rate, including osmoregulation. A lot of the remaining energy is saved for growth. This explains the significant increase in weight gain in experiment two. In experiment one, common carp had good growth rate at 0 to 6ppt and it began to decrease in at 8ppt salinity. The significant decrease in total weight gain in fish exposed to 8ppt salinity may be due to that freshwater fish generally grow well in both fresh water and low salinity environments. If salinity level increases more, growth rate starts declining (Semra, 2013). Wang *et al.* (1997) showed that food consumption rate decreased by increasing salinities in common carp and results cleared that specific growth rate was high in common carp at 0 to 2.5ppt. It began to decrease at ≥4.5ppt. While (Overton *et al.,* 2008) observed that Eurasian perch had higher growth rate at 0 to 8ppt and it began to reduce at 10ppt. (Altinok and Grizzle, 2001) indicated that ≥9ppt of salinity negatively affected goldfish growth. (Luz *et al.,* 2008) indicated that high growth rate was observed in goldfish adapted to 0 to 2ppt at about 1.2% / day. But growth rate was low in 0.4% / day and 0.2 % / day at 8 and 10ppt, respectively.

Osmoregulation is a function of salinity and is reflected in the concentrations of sodium, chloride and potassium ions in the blood serum. Therefore, these ions serve as a general measure of osmoregulatory dysfunction (Robertson *et al.,* 1987). In the present study, electrolytes of the treatment groups in the first adaptation experiment had a marked deviation from the control values; however, values did not vary between the experimental group and control in the second adaptation experiment. Results in the first adaptation experiment showed an increase in water salinity more than 4ppt can have a significant increase on blood serum ions. These changes have been more severe especially in the higher salinity of 8ppt, which results from the osmoregulatory mechanisms that stimulate removal of water from fish and the uptake of ions from the hyperosmotic environment into the fish (Hwang *et al.,* 1989). Therefore, these changes can be effective in the pathogenesis of salinity stress condition, osmoregulatory imbalance as the gills may be more difficulty excreting Na^+ and Cl^- absorption by the epithelium (Grosell *et al.,* 2009a), and this explain fish death at this salinity level. Furthermore, the opposite direction occurs in the control group, lower ions concentration than salinity groups results from the osmoregulatory mechanisms that induced entry of water into fish and the excretion of ions (Alderdice, 1988). Results of Ala *et al.* (2013) showed an increase in water salinity can have a significant impact on blood serum minerals especially in the higher salinity of 8ppt. Concentrations of serum ions in mature kutum from brackish water were significantly higher than in specimens from fresh water (Zahra *et al.,* 2014). As well as that increases in

serum ions have been reported in tilapia that have adapted from freshwater to seawater (Lea Master *et al.,* 1990). This observation indicates that a difference exists in the ion levels between fish acclimated to brackish water and freshwater. These differences might be due to species-specific physiological mechanisms for salinity adaptation and tolerance.

The insignificant increase in serum ions in experiment two can be attributed to the change in the water content in the blood, caused by the change in environmental salinity (Plaut, 1998). Thus, ability of the fish to osmoregulate at this salinity level would be able to return serum ions to initial values as a result of the osmoregulatory mechanisms, which act to re-establish the extracellular volume (Martínez-Álvarez *et al.,* 2002).

The decline in serum proteins during increase of salinity to 8ppt in experiment one could be accounted for the high osmoregulatory energy demand. This, together with a reduced appetite of the fish at higher salinity (Plaut, 1998), would regard for this reduction. Huang *et al.* (2006) noted that as environmental salinity increased, fish consumed more energy, while glucose and lipids provided the energy required for metabolism. Therefore, when the available food source lacks sufficient energy, protein in the feed would be utilized as energy source (Lin, 1999). The reverse of all that explain the significant rise in serum total protein in experiment two as previously mentioned before, when common carp subjected to per week increase of salinity it can survive and osmoregulate at 6ppt without any stress. Significant decrease in Albumin in experiment two consequently lead to significant increase in globulin, as globulin obtained by direct subtracting the values of the albumin from those of the total protein.

The increase of serum cortisol levels is considered to be a primary indicator of stress response (McDonald and Milligan, 1992; Cataldi *et al.,* 1998) which leads to osmotic imbalances in fish subjected to hypertonic and hypotonic environments (Pickering and Pottinger, 1995). The trend for the cortisol level to rise in response to increase of environmental salinity should, like a hyperglycemia-causing hormone, elevate the plasma glucose value. Consequently, hyperglycemia is an expected result of stress in fishes (Hrubec *et al.,* 1997). Similarly, we found such rise in our results thus; in the present study increases observed in the group exposed to 6 and 8ppt salinity may be the result of elevated corticol, which facilitate gluconeogenesis (Barton and Iwama, 1991). This appears to be a high glucose demand in order to supply the energy by osmoregulatory mechanisms (Krumschnabel and Lackner, 1993; Plaut, 1998). Previous studies of this issue are contradictory, showing both a rise (Bashamohideen and Parvatheswararao, 1972; Assem and Hanke, 1979) and a fall (Soengas *et al.,* 1991; Krumschnabel and Lackner, 1993) in glucose value during high salinity adaptation.

It is known that the degree of hyperglycemia may change depending on the type of stress and the sampling times (Rotllant *et al.,* 1997). Therefore, in the fish exposed to 4ppt salinity slight increase in plasma glucose level is possibly related to relatively lower salinity.

High salinity is one of the stress-causing factors, affects fish in three dimensions. In the primary response, sympathetic nervous system is stimulated for release of catecholamines and plasma cortisol. In the secondary response, these hormones activate the release of glucose into the blood for energy production for heart rate, gill blood flow and metabolic rate. In the tertiary response, those changes in blood physiology cause reduction in growth, survival and disease resistance (Overton *et al.,* 2008). This could illustrate the decrease in body weight, mortality recorded and high glucose and cortisol values at salinity more than 6ppt in experiment one.

Beneficial effects of salinity have been reported in the decrease occurrence of some diseases in juvenile and adult fish (Altinok and Grizzle, 2001a). Results of experimental challenge in experiment two, showed decrease in the mortality rate by increasing salinity; this could be explained by that *V. vulnificus* abundance was to be inversely correlated with salinity. However, this relationship depends on the salinity level (Wright *et al.,* 1996; Mark *et al.,* 2004). In the current study, levels of the serum immunoglobulin of fish significantly increased by gradual acclimation to 6ppt salinity for one month. This increase in serum antibodies provides immediate and broad protection against bacterial diseases which also explained decrease in mortality rate in experiment two. This cleared that 6 ppt salinity in common carp is optimum in decreasing the mortality caused by diseases. Previous studies that analyzed the relationship between salinity and *V. vulnificus* abundance have been contradictory (Motes *et al.,* 1998; Lipp *et al.,* 2001). The lack of consensus among the various studies may be due to the fact that the studies were conducted at sites with different salinity regimes and temperature and the effects of salinity on *V. vulnificus* may be interdependent, as has been proposed by Kaspar and Tamplin (1993). While we have no mechanistic explanation for these observations, the results illustrate the complexity of physicochemical interactions on *V. vulnificus* population dynamics in the environment.

According to our knowledge, this is the first study that clearly shows the adaptability and tolerance of common carp, *C. carpio* to varied salinity levels in Egypt, and it clearly concluded that, Salinities up to and including 6ppt did not affect any changes in the results of survival, osmoregulatory, immunological

and stress responses in common carp subjected to per week increase of salinity as it could remain at this level of salinity for one month with no mortality in comparison to the corresponding groups subjected to every two days increase of salinity as these fish showed variations in plasma cortisol, glucose and no mortality following disturbances in water at 6ppt salinity which indicate only a partial tolerance to brackish water. These circumstances, induced to conclude that a salinity of 6ppt represents the upper tolerance limit in common carp. Therefore, for this species to be cultured in low salinity water, it need to be acclimated gradually to low salinities, so as to minimize the impact of sudden change in the environment on the physiology of the fish and enhances its performance in the water medium.

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تأقلم المبروك العادى مع الملوحة

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أجريت هذه الدراسة لمعرفة قدرة المبروك العادى ، *Cyprinus carpio* ، على التكيف مع مستويات الملوحة المختلفة. تم إجراء تجربتين لدراسة آثار الزيادة التدريجية في الملوحة على زيادة الوزن والبقاء والاستجابات المناعية والأسموزية والمقاومة المترتبة على الأصابة *Vibrio vulnificus* في المبروك العادى. في التجربة الأولى ، تم إضافة كلوريد الصوديوم إلى الماء بمقدار ٠.٥ جزء في البليون كل يومين للوصول إلى التركيز النهائي من ٤ و ٦ و ٨ جزء من البليون ، وتم تجميع عينات من الأسماك بعد ١٤ يوما. في التجربة الثانية ، تم إضافة كلوريد الصوديوم بمقدار ٠.٥ جزء في البليون كل أسبوع للوصول إلى تركيز نهائي قدره ٦ جزء في البليون وتم أخذ عينات من الأسماك بعد شهر واحد. أظهرت الأسماك من التجربة الأولى التي تعرضت لملوحة ٨ جزء من البليون زيادة كبيرة في جميع قياسات المصل وتاثيرات عكسية على زيادة الوزن والبقاء على قيد الحياة. في تجربة التكيف الثانية ، أشارت مستويات الجلوكوز والكورتيزول والأيونات غير المتغيرة إلى أن الملوحة التي تصل إلى ٦ جزء في البليون لا تنتج اجهادا كبيرًا في المبروك العادى ، فهي تقلل من معدل الوفيات الناجم عن *V. vulnificus*. تشير النتائج المذكورة أعلاه إلى أن المبروك العادى ، وهو من أسماك المياه العذبة ، يمكن أن يتحمل الزيادات التدريجية في الملوحة التي تصل إلى ٦ جزء من البليون ، وبالتالي يمكن أن تربى في المياه المالحة مع تأثيرات ضئيلة أو معدومة على النمو والأداء.