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Effect of Amino Acid Taurine on Grass Carp *Ctenopharyngodon idella* Fingerlings during Salinity Stress

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Abstract: The present study was carried out to evaluate the effect of different levels of dietary taurine (0%, 1%, 2% and 3%) on grass carp *Ctenopharyngodon idella* fingerlings during salinity stress. Fishes (1.73-8.57 g) were fed a diet up to satiation twice daily for ten weeks in four treatments: T1, T2, T3 and T4. At the end of the feeding trial, fishes were stressed by exposure to 10 PSU salinity for 14 days during which fish survival rates, total protein, glucose, oxygen and energy consumption were observed. The results showed that there were no significant differences ($P>0.5$) in the survival rates between treatments, which ranged from 73.3% to 93.3%. Total protein and glucose were significantly ($P\leq0.05$) improved by a dietary taurine diet after 14 days of salinity stress in T2 and T3. Taurine supplementation significantly ($P\leq 0.05$) influenced oxygen consumption with the highest value observed for T1 (255.8 mg O_2/kg/hr) and T2 (213.6 mg O_2/kg/hr), while the lowest value was in T3 (131.5 mg O_2/kg/hr) and T4 (112.4 mg O_2/kg/hr) after 14 days of salinity stress. Energy consumption rates were significant ($P\leq0.05$) different between the treatments on the 14th day, T1 (0.85 kcal/kg/hr) significantly exceeded the other treatments, followed by T2 (0.72 kcal/kg/hr), T3 (0.44 kcal/kg/hr) and T4 (0.38 kcal/kg/hr). It appeared that taurine could be used as a feed supplement to confirm better energy consumption and blood biochemical parameters during salinity exposure of grass carp fingerlings with the optimal level of a 2% and 3% diet.

Keywords: Fingerlings, Grass carp, Glucose, Oxygen consumed, Total protein, Salinity stress

Introduction

In regions where fresh water is scarce, fish farming in brackish or salt water can provide a source of extra income (DiMaggio et al., 2009; Marengoni et al., 2010). Water salinity stress is one of the most common stresses that occasionally occurs in freshwater fishes and, if prolonged, can reduce production efficiency or lead to death. In many studies, freshwater fishes, exposed to high salinities, were able to change the internal osmolarity of the plasma, allowing an overflow of water. Carps and most other bony fishes can regulate a narrow range of ionic concentrations in their internal body fluids (Luz et al., 2008; Lawson & Alake, 2011; Alkhshali &

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Alhilali, 2020). In some parts of Basrah province, the water salinity of fish ponds may be higher than usual in some months of the year. Therefore, grass carps as freshwater fishes may be exposed to salinities higher than the normal range and are considered to have a limited ability to withstand changes in salinity. They are exposed to high levels of salinity or above normal, which can cause significant economic losses in the field of fish farming, especially in countries like Iraq, which suffers from clear climatic variability and a decrease in water levels, which leads to a difference in salinity levels in inland waters, causing a threat to this important economic aspect (Al-khshali, 2011).

The amino acids that play a vital role in the ability of fishes to adapt to salinity include arginine, aspartic acid, glycine, serine and taurine. In addition, many studies indicated that non-essential amino acids are more important than the essential amino acids in the process of osmotic regulation of fishes (Hosoi et al., 2008; Li et al., 2009; Aragặo et al., 2010; Salze & Davis, 2015). Taurine synthesis varies greatly between fish species and has been shown to play a significant role in the aquaculture feeding of freshwater and marine fishes. Animal proteins are rich in taurine, whereas, plant proteins are deficient in taurine (Sundararajan et al., 2014). So, it may require a plant-based protein food, fish feeder exogenous taurine to maintain physiological functions (El-Sayed, 2014). Taurine represents 30-50% of the total amino acids, depending on the species of animal. It is concentrated mainly in the heart, retina, skeletal muscles, brain, large intestine, plasma and blood cells in mammals (Jacobsen & Smith, 1968; Huxtable, 1992; Schuller-Levis & Park, 2003).

There were many local studies on grass carp. Taher et al. (2022) studied the effect of some food additives (thebax and vitamin C) on the growth parameters of grass carp fingerlings. Laboratory experiments were also conducted for the cultivation of grass carp, which indicated that the best protein level in the prepared diets was 25% (Taher, 2017). Al-khshali (2011) showed a high rate of oxygen consumption and energy with the high salinity in both grass carp and goldfish *Carassius auratus*. The current study aims to determine the effect of taurine on survival rates and energy consumption parameters of grass carp fingerlings during salinity stress.

Material and Methods

Experimental Fishes

Fingerlings of grass carp *C. idella* (4.27±1.28 g) were obtained from a local fish farm located in Al-Musharrah District, Maysan Province. All handling and treatment procedures were conducted according to the ethical requirements. Upon arrival at the laboratories of the Department of Fisheries and Marine Resources, College of Agriculture, the fishes were reared in glass tanks with circulating fresh water for ten days. The dissolved oxygen was maintained above 7 mg/l by continuous air pumping. During acclimatization, the fishes were fed to satiation once daily at 09:00 with a commercial diet (25.35% protein). A total of 180 fishes were sterilized with saturated saline (NaCl) to get rid of pathogens and parasites (Herwig, 1979).

Diet Formation

The diet was prepared according to Lovell (1989). The proximate chemical composition of the experiment diets includes 25.35% protein, 3.54% lipids, 11.63% ash, 2.96% fibre, 44.99% carbohydrates and 11.26% moisture. Four experimental diets, using a fish meal and soya meal as the main protein source, were prepared: a basal diet (T1) and three diets (T2, T3 and T4) supplemented with 1%, 2% and 3% of taurine. The dietary ingredients were mixed in a feed mixer and moistened with the addition of 50% (w/v) water (75 ºC) and then converted to pellets. Then the diet was formed by using a 50 ml plastic syringe, dried in the laboratory for two days, placed in plastic bags and stored at 5 ºC until used.

Experiment Design

The experiment was carried out in the laboratories of the Department of Fisheries and Marine Resources, College of Agriculture. One hundred and eighty grass carp fingerlings (4.27±1.28 g) were randomly divided into four groups. Fishes were fed supplementary diets with different levels of taurine (0%, 1%, 2% and 3%) for ten weeks. At the end of the feeding trial, fishes were stressed by exposure to 10 PSU salinity for 14 days in 12 glass tanks (30 × 40 × 60 cm). Samples of fishes were taken after the 1st day, 7th day and 14th day for physiological measurements (glucose and total protein).

Survival Rate

The survival rate was expressed as a percentage of total fishes tested and calculated according to the following equation:

$$\text{Survival rate} = \frac{\text{Number of fishes at the end of the experiment}}{\text{Number of fishes at the beginning of the experiment}} \times 100$$

Blood Collection

After the 1st day, 7th day and 14th day, the fishes were anesthetized and blood samples were collected from the fishes. For blood collecting, bleeding was done from the caudal peduncle vein (Svobodová et al., 1991). Blood samples were immediately transferred to sterile tubes and the serum was separated by centrifugation (3000 rpm for 2-3 minutes).

Serum Glucose Content

The China Bene Check devices were used to measure the level of glucose in the blood. A drop of blood was placed on the tape of the device, which was installed in its proper location, and the result was read directly. The glucose level was measured in g/dl.

Serum Total Protein Content

The attached instructions, along with the used kit from the French company Biolabo, were followed to estimate the total proteins in the serum. The samples were read by using a spectrophotometer at a wavelength of 550 nm, according to the method of work attached by the company, as shown in the following equation:

$$\text{Protein concentrate (g/dl)} = \frac{\text{Sample}}{\text{Standard}} \times 6/100\text{ml}$$

Oxygen and Energy Consumption

Oxygen consumption rates of grass carp fingerlings were determined according to Nordlie & Leffer (1975) on the 1st day and 14th day. Individual fishes were placed in a 1 L closed opaque chamber for 24 hours to acclimate them to their new conditions. Compressor air was added to maintain oxygen-saturated conditions. Following chamber acclimation, the chamber was sealed. Oxygen concentrations from water samples were determined by utilizing an oxygen meter electrode. Additional water samples were collected from each chamber and oxygen concentration was determined at half-hour intervals. Chamber oxygen was not allowed to drop below 3.3 mg/l. Following oxygen concentration measurements, fishes were removed and weighted to determine oxygen consumption rates. The oxygen consumed was estimated at mg O_2/kg/hr. According to Brett (1972), the consumed oxygen was converted into energy according to the following equation:
1 mg O_2/kg/hr is equivalent to 0.00337 kcal/kg/hr.

Statistical Analysis

Complete Random Design (CRD) was used to study the effect of different treatments on the studied traits by analysis of variance (ANOVA). The significant differences between the means were compared with LSD under the significance level of 0.05. The program SPSS (Version 26) was used in the statistical analysis.

Results

Survival Rate

There were no significant (P>0.5) differences between treatments when fishes were transferred to a saline concentration of 10 PSU throughout the salinity stress period. T1 and T4 had the lowest survival rate on the 14th day of the transfer (Figure 1).

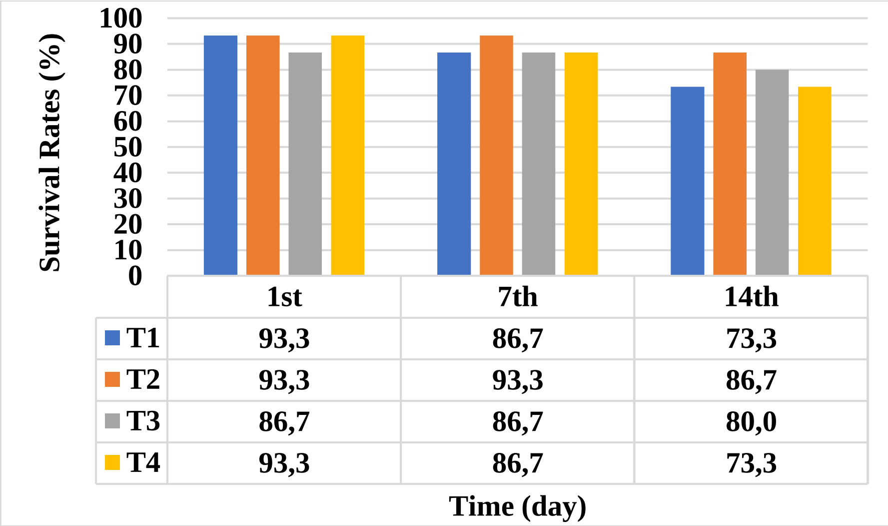

Figure 1: Survival rates of grass carp fingerlings in different treatments during the salinity stress period.

Serum Glucose Content

Significant differences (P≤0.05) were observed between treatments on the 1st day of salinity stress. T1 is higher than T2 and T4, but there were no significant differences (P>0.5) between T1 and T3. T1 was significantly (P≤0.05) higher on the 7th and 14th days after salinity stress compared to the other treatments, followed by T4. On the 14th day of salinity stress, there were no significant differences (P>0.5) between T2 and T3 on the 14th day (Figure 2).

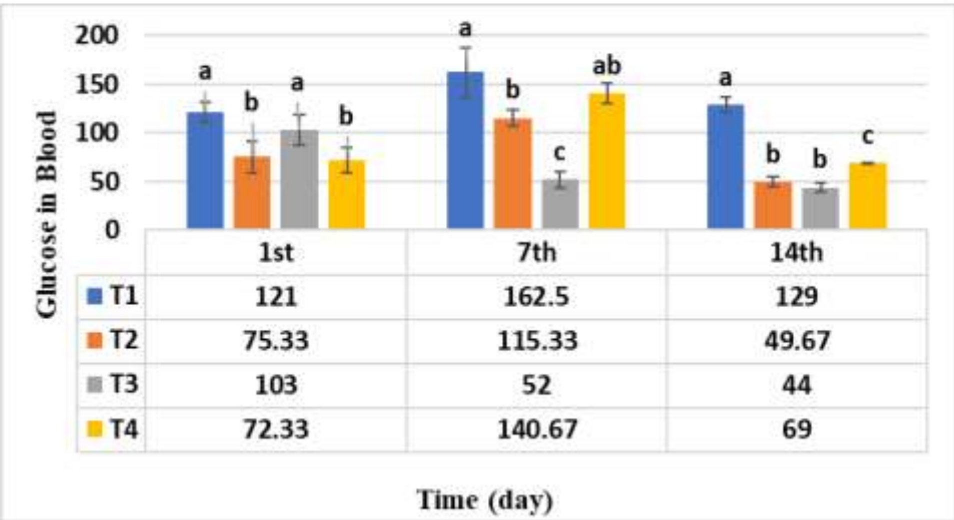

Figure 2: Blood glucose content (g/dl) of grass carp fingerlings in various treatments during the salinity stress period.

Serum Total Protein Content

The total protein content in blood serum on the 1st, 7th and 14th days of salinity stress is shown in Figure 3. The T4 was significantly ($P \leq 0.05$) superior to the other treatments on the 1st day, while there were no significant differences ($P > 0.5$) between the treatments on the 7th day. There were significant differences ($P \leq 0.05$) between T1 (0.93 g/dl) and T2 (1.81 g/dl) on the 14th day of the salinity stress period, and there were no significant differences ($P > 0.5$) between T2 (1.81g/dl), T3 (1.7 g/dl) and T4 (1.15 g/dl). The highest value was obtained in T2, while the lowest value was observed in T1.

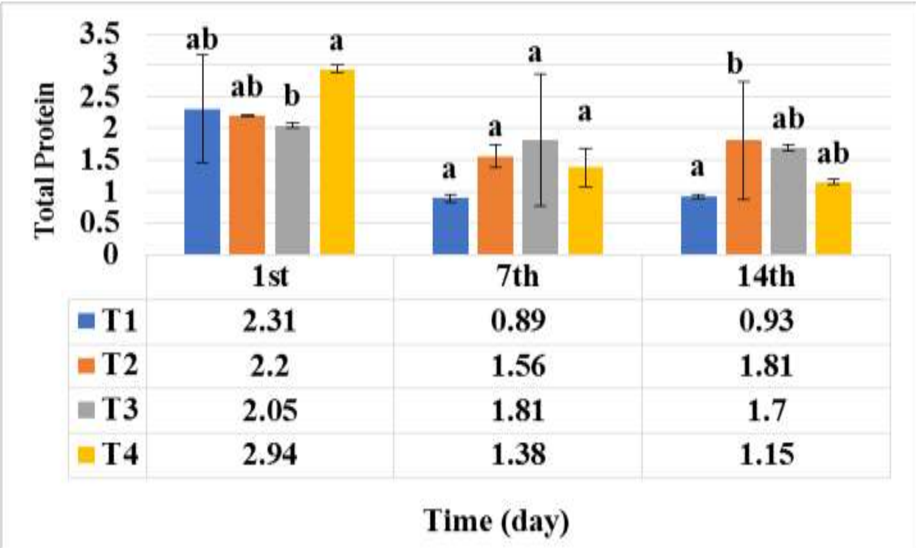

Figure 3: Total protein content (g/dl) of grass carp fingerlings in different treatments during the salinity stress.

Oxygen Consumption Rate

Oxygen consumption of grass carp fingerling (mg O_2/kg/hr) increased during the salinity stress period (Figure 4), Salinity exposure increases oxygen consumption in T1 from 144.5 mg O_2/kg/hr on the 1st day of exposure to 250.8 mg O_2/kg/hr on the 14th day and in T2 from 95.7 mg O_2/kg/hr to 213.6 mg O_2/kg/hr on the 14th day of exposure. While the changes in oxygen consumption rate were less in T3 and T4 during the 1st and 14th days of exposure, which were 99.4 to 131.5 mg O_2/kg/hr (T3) and 116.4 to 112.4 mg O_2/kg/hr (T4).

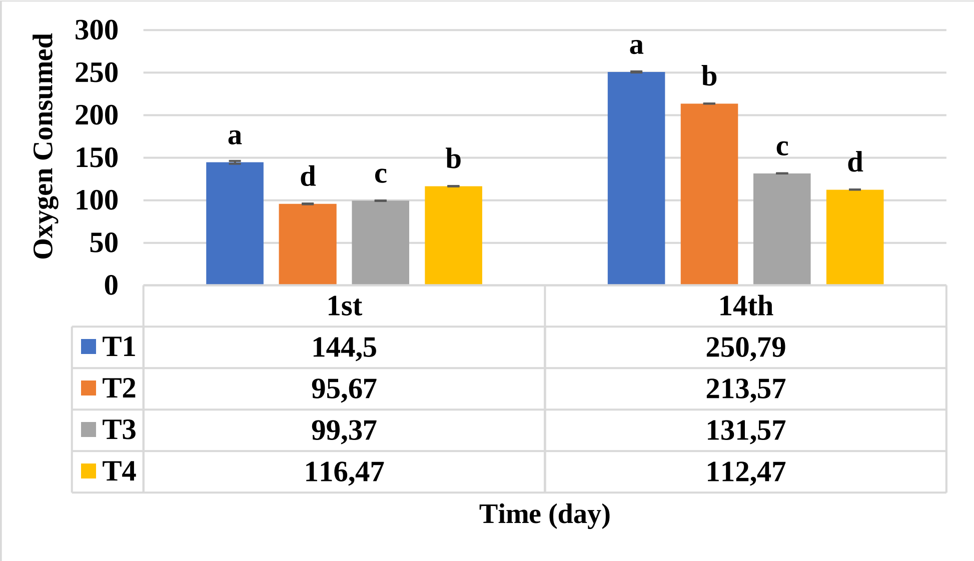

Figure 4: Oxygen consumption rate (mg O_2/kg/hr) of grass carp fingerlings in different treatments during salinity stress.

Energy Consumption

Figure 5 shows that the energy consumption rate of grass carp fingerlings was increased on the 14th day of salinity exposure compared to the 1st day in T1, T2 and T3, while it was decreased in T4. There were significant ($P \leq 0.05$) differences between the treatments on the 14th day. T1 (0.85 kcal/kg/hr) significantly exceeded the other treatments, followed by T2 (0.72 kcal/kg/hr), T3 (0.44 kcal/kg/hr) and T4 (0.38 kcal/kg/hr).

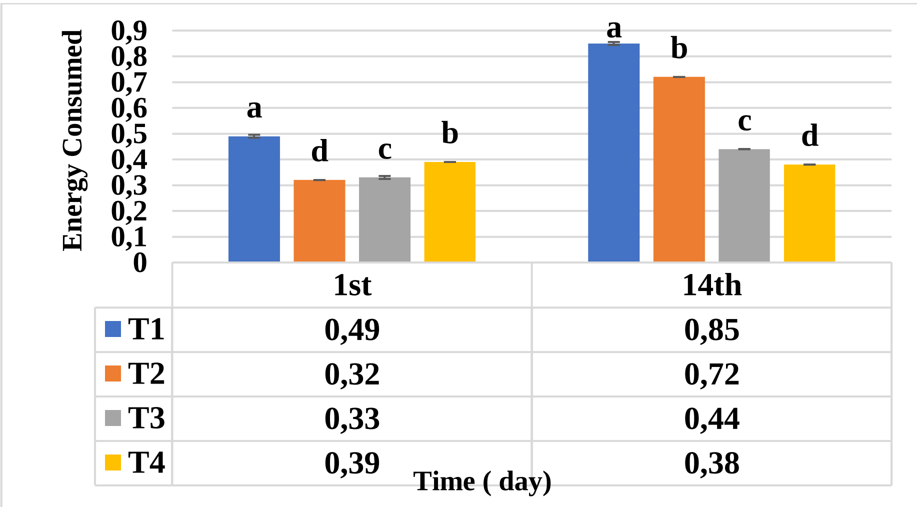

Figure 5: The rate of energy consumed (kcal/kg/hr) of grass carp fingerlings in different treatments during salinity stress.

Discussion

Survival Rate

The current study results indicated that there were no significant differences ($P>0.5$) between the treatments. These results are consistent with those of Abdel-Tawwab & Monier (2018), who observed no significant difference in the survival rate of the common carp fed taurine-supplemented or control diet. However, the current study did not agree with Jaaffar (2010) findings that grass carps have a limited tolerance to sudden increases in salinity, with a survival rate of 0% recorded at a salinity of 10 PSU. Furthermore, Saoud & Al-Shami (2006) demonstrated that a sudden salinity increase to 9 PSU was lethal to grass carp within 48 hours, whereas the same species was able to maintain a concentration of 8 PSU during a 168-hour experiment.

Serum Glucose Content

Serum biochemical parameters are important indicators of fish physiological stress and can be used to promote fish health management (Lermen et al., 2004; Jian et al., 2003). Environmental stress exposure in fish necessitates energy supply, and glucose is an energy source derived from metabolism (Cho et al., 2015; Liu et al., 2020). The current results showed that in grass carp fingerlings serum glucose content significantly decreased after the 14^{th} day of salinity exposure. This indicates a stronger energy demand in grass carp adaptation to a hypertonic environment. T2 and T3 were lower than in the other treatments, which may imply that the fishes in those treatments were more stressed, and had a greater energy demand. This is consistent with Magalhães et al. (2019), who demonstrated that taurine can lower blood glucose levels by interacting with insulin receptors. They also found that taurine supplementation causes a decrease in the level of glucose in the blood of white seabream *Diplodus sargus*. However, the results of Rodrigues et al. (2020) are not agreeable with the results of the current study, as they reported that taurine supplementation did not affect the physiological parameters of the blood, including glucose level in the pirarucu *Arapaima gigas*. The high blood sugar level in T1 can be attributed to the production of glucose after exposure to salt stress, which contributes to stimulating the process of building glucose from non-sugar materials (glyconeogenesis in the liver), in addition to taking glucose from the body (Nelson & Cox, 2005).

Serum Total Protein Content

External stressors cause physiological responses in fishes, such as changes in blood properties or indicators of energy consumption. Measuring the level of protein in the blood is one of the important indicators which can reflect the level of salt stress to which fishes are exposed (Martínez-Porchas et al., 2009).

After salinity exposure, the total protein content of grass carp fingerlings in T1 and T4 decreased and was significantly lower than in T2 and T3. It is possible that the protein biosynthesis in the T1 and T4 was inhibited, or the biosynthesized proteins were utilized for metabolic purposes, for example energy supply

(McDonald & Milligan, 1992; Priya et al., 2012). The current study results suggest that a decrease in serum total protein levels with an increasing period of salinity exposure may be due to a decrease in fish appetite and a lack of food intake during salinity exposure, which negatively affects the rate of protein assimilation in the body (Plaut, 1998). High salinity leads to an increase in the metabolic rate, to fill the high demand for energy to maintain the ionic and osmotic balance in the new environment (Morgan & Iwama, 1991).

The decrease which occurred to a greater degree in the T1 (0% taurine) compared to the other treatments is supported by the fact that taurine has a positive effect on protein levels. This is consistent with Huang et al. (2021) who found that the level of total protein in taurine-free treatment was lower than in other taurine-supported treatments.

The results of the current study did not agree with Rodrigues et al. (2020), who showed that taurine supplementation did not affect the level of total protein and other physiological parameters in the pirarucu *A. gigas*.

Oxygen Consumption and Energy Consumption Rate

The current study found that the rate of oxygen and energy consumption in the T1 is higher than in other taurine-supported treatments. This indicates that the addition of taurine has a positive effect on grass carp fingerlings in reducing the rate of oxygen consumption and the amount of energy when exposed to salt stress. This is in agreement with Yang et al. (2013), who showed that dietary taurine can improve the tolerance of hypoxia in grass carp juveniles.

The change in salinity level is one of the most important factors affecting the life of fishes, because of its direct effect on metabolic rate, oxygen consumption, growth and survival rate (Jian et al., 2003). According to Dube & Hosetti (2010), one of the physiological responses to salt stress in fishes is a change in the respiratory rate. This was evident from the change in the rate of oxygen consumption, which was often used to estimate the metabolic rate under conditions of environmental imbalance. The difference in the rate of oxygen consumption that occurs as a result of a change in salinity is due to increased activity in the active transport of ions, which increases energy requirements to complete the process of osmotic regulation (Sangiao-Alvarellos et al., 2003).

The active metabolism of oxygen consumption during stress to salinity, needs additional energy to modify and stimulate the ion transport mechanisms in fishes. A sufficient supply of energy at the right time is required to operate the mechanisms of osmotic and ionic regulation in fishes where metabolic reorganization and alterations in the intermediate metabolic pathways occur to meet the growing demand for associated energy, adapt to the new environmental salinity (Mayzaud & Conover, 1988; Gracia-López et al., 2006; Rocha et al., 2007; Tseng & Hwang, 2008).

The gills are assumed to be the most energy-consuming organs in fishes exposed to high salt concentrations. The energy needs are met by the oxidation of glucose and lactate obtained from the blood circulation. The liver is the main site of glucose processing in fishes (Perry & Walsh, 1989). Elevated liver metabolism in saltwater acclimatized fishes leads to the preparation of the base material for the energy necessary for metabolic processes in the gills (Nakano et al., 1997).

The results of the current study are consistent with those of Al-khshali (2011), who found that increasing salinity had increased the rate of oxygen and energy consumption in grass carp and goldfish. According to Jaaffar (2010), increasing salinity levels resulted in a significant increase in the rate of oxygen consumption, which was accompanied by a significant increase in the amount of energy expended in both grass carp and common carp.

Conclusions

The current findings indicated that taurine has no effect on the survival rates of grass carp fingerlings when exposed to a saline concentration of 10 PSU, but it does contribute to an increase in total protein concentration, lowers the level of glucose in the blood serum and slows the rate of oxygen and energy consumption when grass carp fingerlings are exposed to salt stress.

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