University of Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 220-233

https://iasj.rdd.edu.iq/journals/journal/issue/18682 https://doi.org/10.54174/utjagr.v13i1.323

Effect of different levels of dietary taurine on physiological characteristicsof grass carp fingerlings during Salinity tolerance

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I. Abstract:

This study was conducted to evaluate the effect of different levels of dietary taurine (0, 1, 2 and 3%) in grass carp *Ctenopharyngodon idella* fingerlings during Salinity tolerance. The average weight of fish was 3.16± 0.14 gm and were fed of diet to satiety twice daily for 10 weeks by four treatments, at the end of the feeding experiment, the fish were exposed to a salinity of 10 practical salinity units (PSU) for 14 days. Physiological traits were studied on the first, seventh and fourteenth days of transfer to salinity, it included Packed Cell Volume (PCV), concentration of Alkaline Phosphatase (ALP) in the blood serum, Aspartate Transaminase (AST) and Alanine Transaminase (ALT) enzyme in the blood. The results show that there were no significant differences between the treatments on the first and seventh days of transfusion on PCV of blood, while there were a significant differences ($P<0.05$) in the treatments on the fourteenth day of transportation. There was a fluctuation in the level of alkaline phosphatase, it was increase on the first and seventh days of transportation, while it decreased on the fourteenth day. There was a fluctuation in the levels AST and ALT enzymes on the first and seventh days of transportation, while their levels decreased on the fourteenth day of transportation. It was concluded from the study that adding taurine at level 1, 2, and 3%, it has very little effect on the physiological traits, did not help grass carp fingerlings to tolerate salt, to resist the stress stage.

Keywords: PCV, liver enzymes, *Ctenopharyngodon idella*.

II. Introduction

Changes in salinity levels in the aquatic environment are among the causes of stress for fish, it is one of the environmental factors that plays an important role in the re-distribution of animals, especially fish with limited salt tolerance, because it has a limited ability to move between fresh and salt water, transmission is only possible if the ionic and organic concentrations of body fluids can be kept constant within the living environment. The process of osmoregulation (controlling the amount of water and salts inside the body in order to maintain internal stability) is one of the most prominent means for fish to overcome stress (Eddy, 1982).

Most freshwater fish are classified as stenohaline, because it does not tolerate high salinities, it causes major damage when transported to salt water, due to the occurrence of what is called osmotic shock (Jackson, (1981). Many studies have proven the possibility of overcoming this problem and increasing the salt tolerance of fish through the use of salt feeding technology, includes introducing certain percentages of salt into the diet of acclimatized fish or through

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Gradient Transporting, the fish were transferred to solutions whose salinity gradually increases (Alkhshali, 2011; Alkhshali & Alhilali, 2020).

The PCV is one of the physiological measurements used in measuring osmotic pressure, which fish encounter in the intracellular space as a result of variation in the salinity of the external environment (Prodocimo & Freire, 2006). PCV can be defined as the volume percentage, occupied by red blood cells, it is used as a means of determining whether the red blood cell count is high, low, or normal (Zubieta-Calleja *et al*., 2007). Enzymes are protein catalysts for life's reactions, it is a bio-indicator of disease states in fish or environmental changes. The aminotransferase enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT), they are important in the assimilation of amino acids within the processes of protein metabolism, an elevated level of these two enzymes in the blood serum may indicate damage to the liver cells or the presence of a disease (Yousafzai & Shakoori, 2011; Dorcas & Solomon, 2014). The alkaline phosphatase enzyme may be used as a biological indicator to indicate the success of the salt acclimation process (Ahmed *et al*., 2004).

The grass carp *Ctenopharyngodon idella*, belongs to the family Xenocypridinae (Tan & Arbrumster, 2018). It is one of the most important types of aquaculture from an economic standpoint, the numbers of this species are increasingly declining in many rivers. Seawater leakage is one of the most important causes of fatalities (Zhang *et al*., 2021), these fish are exposed to high or above normal levels of salinity, it can cause major economic losses, especially in a country like Iraq, suffers from clear climate fluctuations and low water levels, leads to a difference in salinity levels in inland waters, causing a threat to this important economic aspect (Al-Khshali, 2011).

Studies indicate that some amino acids, such as glycine, taurine, b-alanine, and arginine, play an important role during fish acclimatization to salinity and in the process of osmoregulation, as energy sources or as substances that contribute to the homeostasis and regulation of cell size (Aragặo *et al*., 2010; Li *et al*., 2009). Taurine (C2H7NO3S) plays an important role in many physiological functions, including membrane stability, osmoregulation, antioxidants, and detoxification, (Onsri & Srisawat, 2016).

The current study aimed to determine the role of the amino acid taurine as a nutritional processor, to combat salt stress problems, explaining the extent of its effect on the process of osmoregulation in the fish of the current study, by studying some physiological parameters during a Salinity tolerance experiment.

III. Materials and Methods

Experimental Fishes:

Fingerlings of grass carp *C. idella* (The average weight was 3.16± 0.14 gm) were obtained from a local fish farm located in Al-Musharrah District, Maysan Province. All handling and

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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, Fish were fed to satiation twice daily 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 (3.16± 0.14gm) 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 (Packed Cell Volume (PCV), concentration of Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT) enzyme) in the blood.

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).

Packed Cell Volume Percentage (PCV%):

Packed Cell Volume Percentage (PCV) was estimated using the Microhaematocrit method based on Svobodova *et al*. (1991), involves anesthetizing the fish and then cutting the caudal peduncle. The fish was held in such a way that the head is facing upward to help the blood flow into the capillary tubes that contain an anticoagulant. The tube is filled 90% with blood, then one end of the tube was closed with artificial clay. The capillary tubes were placed in a

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Hematocrit Centrifu SH120 Microcenterfuge for 2-3 minutes at a speed of 3000 rpm, to separate the serum from the blood cells, then PCV% was measured, using a special measuring ruler Microhaematocrit reader. Blood plasma is drawn from capillary tubes using a microsyringe. Samples were stored in 1 ml plastic bottles under freezing (-12°C) until laboratory testing was performed.

Alkaline phosphatase (ALP) enzyme:

Alkaline phosphatase (ALP), for the purpose of calculating the level of alkaline phosphatase enzyme in blood serum. The instructions included with the kit manufactured by the German company Spectrum were followed. The samples were read using a spectrophotometer at a wavelength of 510 nm according to the following equation:

$$Alkaline\ phosphatase = \frac{\text{Sample absorbance} - \text{Absorbance of the zeroing sample}}{\text{Absorbance of standard solution}}$$

Aminotransferase enzymes (AST and ALT):

Aspartate transaminase (AST) assay:

The enzyme concentration in blood serum was measured using a ready-made test kit from the French company Biolabo. The samples were read using a spectrophotometer at a wavelength of 505 nm to read the samples. Concentration was calculated by projecting the data onto a curve.

Alanine Transaminase (ALT) assay:

Enzyme concentrations in blood serum were measured using ready-made solutions (Kit from a French company, Biolabo). Samples were read using a spectrophotometer at a wavelength of 505 nm according to the manufacturer's instructions. Concentration is calculated by projecting data onto a graph.

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.

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IV. Results

Packed Cell Volume Percentage (PCV%):

Figure (1) show that there were no significant differences (P>0.05) in PCV among treatments on the first and seventh days of transfer to the PSU10 saline concentration. On the fourteenth day of transfer, significant differences (P<0.05) appeared between the treatments. T1 recorded the highest value (36.59%), compared to T2, T3 and T4 (28.32, 26.8 and 30.16)%, respectively. There was an increase in PCV in T1 on the fourteenth day compared to the first day of transfer, while T2, T3 and T4 on the fourteenth day of transfusion resulted in a decrease in PCV.

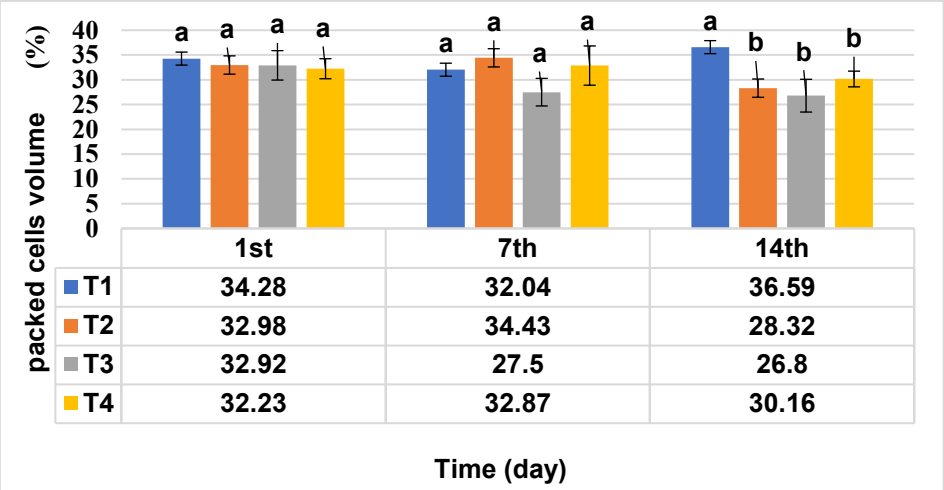

Figure (1) Packed Cells Volume of grass carp fingerlings in different treatments in 10PSU salt concentration.

Alkaline phosphatase enzyme:

Figure (2) shows that the concentration of Alkaline phosphatase enzyme in blood serum on the first, seventh, and fourteenth days of transfer to the saline concentration PSU10, on the first day of transfer, T4 (19.75 IU/L) decreased significantly (P<0.05) compared to the other treatments, while there were no significant differences (P>0.05) between T1 (57.67 IU/L), T2 (48.32 IU/L), and T3 (50.25 IU/L), it became clear on the fourteenth day of transfer that T1 (24.33 IU/L) increased significantly (P<0.05) compare to the other treatments, while there were no significant differences (P>0.05) between T2 (10.32 IU/L), T3 (11.76 IU/L) and T4 (9.39 IU/L) treatments.

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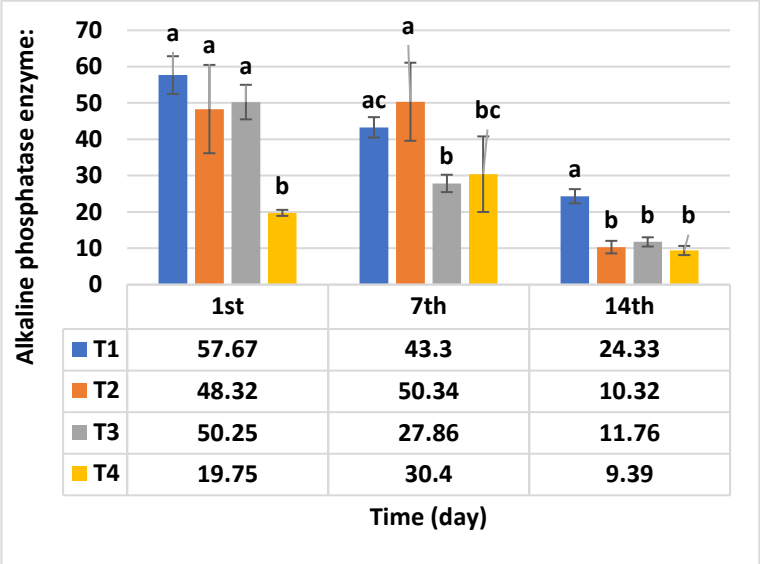

Figure (2) Alkaline phosphatase (ALP) concentration (IU/L) of grass carp fingerlings in different treatments in salt concentration 10PSU

Aminotransferase enzymes (AST and ALT):

Aspartate aminotransferase (AST) concentration

Figure (3) shows the concentration of the AST enzyme on the first, seventh, and fourteenth days after transfer to the PSU10 saline concentration. On the first day of transfer, there were significant differences (P<0.05) between the different treatments. T1 (75.15 IU/L) was significantly superior to the other treatments, while there were no significant differences (P>0.05) between T2 (61.59) and T3 (65.51 IU/L) treatments. T1 (56.04 IU/L) was significantly superior (P<0.05) to the other treatments on the fourteenth day of transfer to the saline concentration PSU10, while no significant differences (P>0.05) appeared among T2 (45.23 IU/L), T3 (42.23 IU/L) and T3 (46.5 IU/L) treatments.

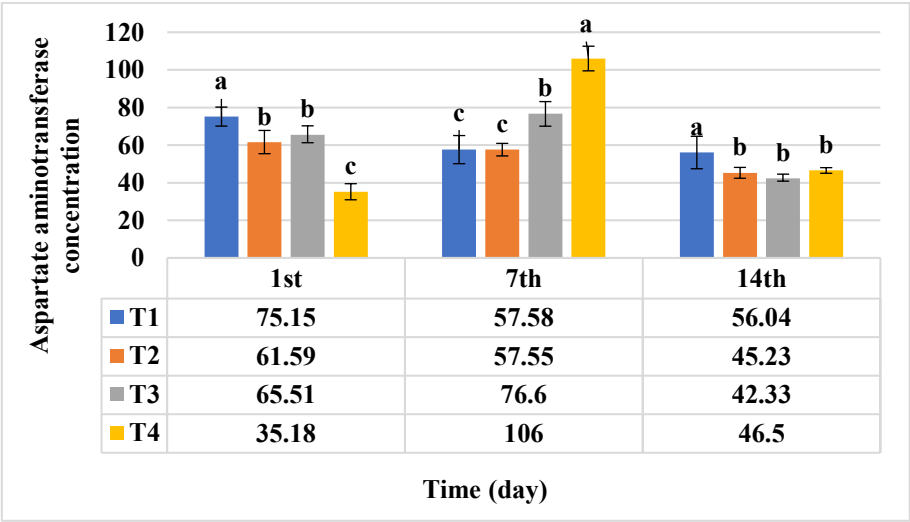

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Figure (3) AST enzyme concentration (IU/L) of grass carp fingerlings in different treatments in salt concentration 10 PSU

Alanine aminotransferase (ALT) concentration:

Figure (4) shows the ALT enzyme concentration on the first, seventh, and fourteenth days after transfer to the PSU10 saline concentration. On the first day, the significant superiority ($P<0.05$) appears for T3 (23.95 IU/L) and T1 (23.5 IU/L), followed by T2 (17.5 IU/L) and T4 (12.26 IU/L), while there were no significant differences ($P>0.05$) between T1 and T3. T1 (21.04 IU/L) was significantly superior ($P<0.05$) to T3 (13.04 IU/L), T4 (10.51 IU/L), then T2 (10.5 IU/L), while there were no significant differences ($P>0.05$) between T2 and T4 on the fourteenth day of transfer.

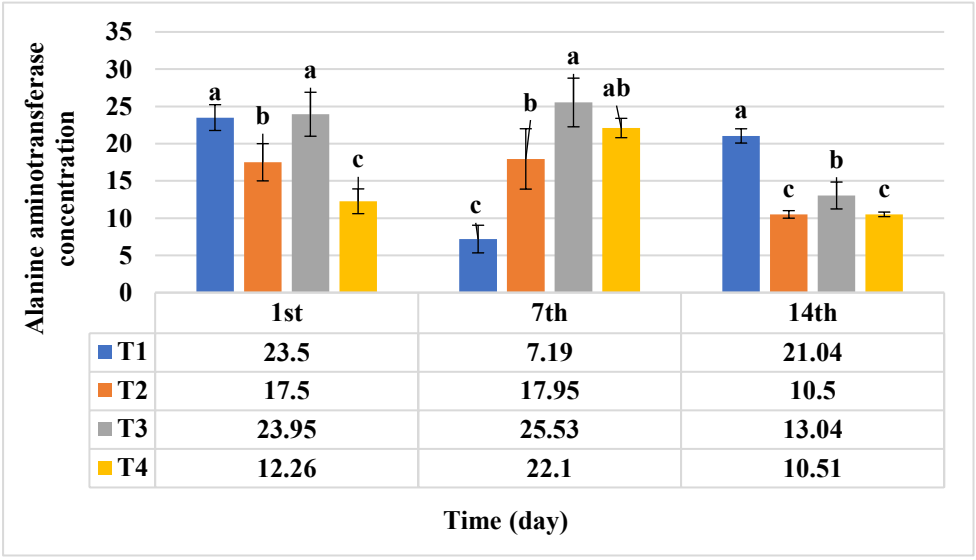

Figure (4) ALT enzyme concentration (IU/L) of grass carp fingerlings in different treatments in 10PSU salt concentration.

V. Discussion

Packed Cell Volume (PCV):

It was observed in the current study that there was a significant increase in PCV in T1 on the fourteenth day of transfer to saline compared to T2, T3 and T4. There was a significant increase in PCV, it can be explained on the basis of the difference in osmotic status that results from the high level of salinity, leads to the loss of water from extracellular fluids to the external environment, causing a state of dehydration (Plaut, 1998). The increased of PCV percentage was likely due to higher salinity levels, leads to an increase in the number of red blood cells, for the purpose of meeting the increasing demand for oxygen consumption, which

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was sufficient to expend additional energy against osmotic and ionic stress (Brown *et al*., 2001; Prodocimo *et al*., 2008).

The results of the current study in terms of the increase in PCV percentage in T1 were consistent with what was reported by Jaafar (2010) in a study conducted on grass carp fish to demonstrate their salt tolerance, it was evident that there was an increase in PCV percentage to (34 and 39)% when exposed to the two salt concentrations (5 and 10) PSU compared to the control sample (liquefied water) 30.33%. Al-Khshali (2017) in his study on grass carp indicated an increase in PCV with a gradual increase in water salinity, it increased to (30, 34 and 37)% in salt concentrations (4, 8 and 12) PSU compared to the control treatment (liquefaction water) 27%.

Previous studies indicated opposite results, as they showed a decrease in PCV percentage with the increase in salinity, or it was not affected. Morgan & Iwama (1991) reported a decrease in PCV with an increase in salinity in Chinook salmon, this was explained on the basis of a change in the number or size of red blood cells or perhaps due to a change in PCV of blood plasma depending on the type of fish. The golden perch *Liza carinata*, PCV did not show a correlation with environmental salinity (Ahmed, 2002). Magill & Sayer (2004) showed that the PCV of *Gadus morhua* juveniles, it did not change over a wide range of variation in salinity level.

The occurrence of some changes in PCV percentage during transfer to saline does not provide a specific pattern or clear response to salt changes or to the effect of taurine, because all values fall within natural limits (Marshall, 1995; Al-Khshali, 2017). However, adding taurine at a rate of 1 and 2% may contribute to maintaining the PCV, did not increasing them with increased salinity. Banerjee *et al*. (2019) showed that the erythrocytes of *Clarias magur* fish possess a highly effective taurine-dependent regulation mechanism, to resist changes in cellular volume under isotropic conditions, as a unique adaptive strategy to defend against osmotic changes in the cellular volume of erythrocytes.

Alkaline phosphatase enzyme

The results of the current study showed a fluctuation in the level of the alkaline phosphatase enzyme, it increased on the first and seventh days of transfer to salinity and in all treatments compared to the fourteenth day of transfer. Its value in T1 was significantly higher compared to other treatments supported with taurine on the fourteenth day of transportation. Its high value at the beginning of the transfer to salinity can be explained by the salt stress resulting from exposure of freshwater fish to high salinities, change the permeability of cellular membranes, leads to increased leakage of some enzymes into the blood (Bahjat and Shaaban, 1985). One of these enzymes was the alkaline phosphatase (ALP), the activity of which is affected by multiple factors, it affects the chemical composition of blood plasma and tissues, such as temperature, nutrition, salinity, etc. (Folmer *et al*., 1992; Al-Khshali, 2011).

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The results of the current study indicate that some organs of grass carp were damaged due to the salinity stress they were exposed to, because an increase in the enzyme alkaline phosphatase in the blood indicates damage and disturbance in the functions of the liver, kidneys, and gills. A decrease in this enzyme is linked to a disturbance in membrane transport in the cell (Nemcsok & Benedeczky, 1995; Bernet *et al*., 2001; Huang *et al*., 2021; Liu *et al*., 2020).

The results of the current study agreed with Huang *et al*. (2021) reported that alkaline phosphatase activity, it increased significantly in T1 not fortified with taurine and the treatment containing 0.5% of taurine, while it decreased on the seventh and fourteenth days of acclimation to salinity and returned to its values in fresh water, the low values were much lower in the other treatments supplemented with (1 and 2)% of taurine.

Aminotransferase enzymes (AST and ALT):

Measuring enzyme activity in fish can be used to determine changes, affecting water quality and pollution, an increase in enzymes in plasma, it may indicate the presence of liver necrosis and tissue damage (Shahsavani *et al*., 2010). As shown by Vijayan *et al*. (1996) transferred tilapia fish from fresh water to sea water for two weeks, caused a significant increase in the activity of AST and ALT enzymes in the liver, an increase in the process of protein catabolism in the liver was observed more in fish found in salt water than in fish found in fresh water. This increases the need for energy and the catabolism process is accompanied by an increase in the activity of the enzymes AST and ALT in the liver (French *et al*., 1983; Vijayan *et al*., 1997). The results of the current study showed that there was a fluctuation between an increase and a decrease in the levels of the AST and ALT enzymes on the first and seventh days of transfer to salinity, while there was a decrease in their level on the fourteenth day of transportation in the treatments supported with taurine compared to T1, which represented the highest value.

The results of the current study agreed with Huang *et al*. (2021) regarding the increase in the levels of both AST and ALT enzymes on the first day of transferring rainbow trout to seawater in both the control treatment and the treatments supplemented with (0.5 and 1)% taurine, while their value in the transaction supported by 2% of taurine was higher than the rest of the treatments, explained that the level of both enzymes decreased significantly on the fourteenth day of transportation, and the treatment supplemented with 2% taurine was also higher than the rest of the treatments. The results of the current study were not consistent with what was reported by Li *et al*. (2021) in his study on *P. stellatus*, it has been shown that taurine supplements cause an increase in the activity of amino acid transfer enzymes (AST and ALT).

It was concluded from the study that adding taurine at level 1, 2, and 3%, it has very little effect on the physiological traits, did not help grass carp fingerlings to tolerate salt, to resist the stress stage.

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