Int. J. Aquat. Biol. (2023) 11(5): 449-456
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Some physiological and nutritional responses of common carp *Cyprinus carpio* juveniles to osmotic stress

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Abstract: The effect of salinity (<1, 7, and 15 ppt) on some physiological and nutritional parameters of common carp (*Cyprinus carpio*) juveniles was examined in two trials. In the first trial, the activity of the common liver enzymes (alkaline phosphatase ALP, Aspartate transaminase AST, and Alanine transaminase ALT) in the blood serum, and total plasma protein level on the 1st, 7th, and 14th day of exposure, using 90 fish (14.05±2.01 g) were studied. In the second trial, the growth and feed efficiency performance (weight gain WG, relative growth rate RGR, specific growth rate SGR, and feed conversion ratio FCR), and apparent digestibility coefficients ADCs of dry matter, nutrients, and energy were investigated during ten ten-week rearing period using another 90 fish (15.71±1.59 g). The results showed that the activity of the ALP was increased significantly ($P \leq 0.05$) with increasing salinity on the 1st day, and continued to the following 7th and 14th day periods. AST in 7 and 15 ppt showed significantly ($P \leq 0.05$) higher activity levels compared with 1 ppt on the 1st day, similar differences were found on the 7th and 14th day for 15 ppt, but not for 7 ppt during the same periods. ALT exhibited significantly ($P \leq 0.05$) higher activity in 7 and 15 ppt relative to <1 ppt during all periods. Total plasma protein fluctuated slightly ($P > 0.05$) on the 1st and 7th day and decreased significantly ($P \leq 0.05$) in 15 ppt only on the 14th day. Significantly ($P \leq 0.05$) better specific growth rate SGR and feed conversion ratio FCR were observed in the lowest salinity (<1 ppt) while the worst in the highest (15 ppt). The ADCs of dry matter, nutrients, and digestible energy were decreased significantly ($P \leq 0.05$) with increasing water salinity.

Article history:
Received 3 April 2023
Accepted 30 May 2023
Available online 25 October 2023

Keywords:
Salinity
Liver enzymes
Plasma protein
Growth
FCR

Introduction

Climate change causes a continuous decrease in freshwater resources and a change in its quality, especially the high levels of salinity, which exposes fish to osmotic stress (UNESCO, 2021). The changes in the salinity of the water bodies cause osmotic stress due to their effects on physiological homeostasis and routine biological processes (Kültz, 2015) .The increase in water salinity is a source of environmental stress, which stimulates several responses in fish to deal with the physiological changes, such as changes in the concentration of hormones, the concentration of basic substances in the plasma, the change in the size and number of blood cells, the functional changes that are observed in the members of the osmoregulatory system, and other functions involving the digestive system (Teles et al., 2021).

Salt stress occurs with rapid and sudden changes in the salt concentrations of the aquatic environment, for example, due to tidal flows, rainstorms, droughts, or evaporation from small bodies of water. However, gradual changes in salt concentration can also cause osmotic stress in aquatic environments if levels exceed limits that reduce the resistance of resident organisms (Evans and Kültz, 2020). Moving or transporting some fish species from freshwater to seawater causes some hormonal and physiological changes as well as a loss of energy, which may eventually affect the growth of the fish (Whitfield, 2015). Salinity is one of the most important environmental factors influencing the survival, growth, and distribution of fish. Many studies show that high levels of salinity increase energy requirements and decrease feeding rates in fish to maintain internal stability and maintenance (Takei et al., 2014; Takei and Hwang, 2016).

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DOI: https://doi.org/10.22034/ijab.v11i5.2070

Table 1. Ingredients and proximate composition of the trial diet.

Ingredients	%	chemical composition	
Fish meal	30	Moisture %	8.70±0.35
Soybean meal	35	Protein %	34.84±1.77
Barley	10	Ether extract %	8.59±0.31
Yellow corn	10	Carbohydrate %	40.78±1.92
Wheat bran	10	Ash %	7.09±0.11
Binder CMC	2	Gross energy (Kcal/100 g)	437.01±0.97
Vitamin and mineral premix	3	P:E ratio (mg protein/Kcal)	79.73±4.22

Enzymes control the metabolism of living organisms, and therefore any change, even a slight value, in the enzymatic activities would lead to a disruption of the metabolism process (Balasubramanian and Kumar, 2013). The most important of these enzymes are aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are produced mainly by the liver, although they are present in other tissues such as the heart, muscles, kidneys, gills, and other organs. They belong to non-functional plasma enzymes that are usually present inside cells and are released in the case of damage (Dawood et al., 2021; Fernández-Muela et al., 2023). Hepatic enzymes AST and ALT act as indicators of changes in physiological state or stress. These enzymes have been used frequently to demonstrate damage in fish liver tissue. The normal level of the two enzymes in the blood plasma is affected by some environmental factors, such as temperature and salinity. Several recent studies revealed significant changes in their effectiveness in fish during salt adaptation (Al-Khshali and Al-Hilali, 2019; Nabi et al., 2022). Alkaline phosphatase (ALP) is an enzyme that catalyzes the hydrolysis of monophosphate esters (Abedi et al., 2013). Temperature, photoperiod, salinity, and physiological state have an impact on its activity in fishes. The activity of ALP increases in eels adapted to seawater compared to those adapted to freshwater, therefore it is considered a biological indicator of the acclimatization success to saltwater (Mozanzadeh et al., 2021; Zhou et al., 2022).

Salinity negatively affects fish growth and feed digestibility, especially during direct exposure to salinity fluctuations. It was suggested that the main factor governing this effect is osmoregulatory stress, which is reflected in the whole metabolic balance (Tran-Ngoc et al., 2017; Mozanzadeh et al., 2021). Therefore, this study aimed to assess the effect of water salinity on survival, growth, nutritional criteria, and activity of the common liver enzymes, alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) in the blood serum, and total plasma protein level in common carp, *Cyprinus carpio* juvenile.

Materials and Methods

Fish and experimental design: Common carp juveniles were obtained from the fish ponds of the Marine Science Center, University of Basrah. Two trials were conducted, during the first trial, serum ALP, AST, ALT, and total plasma protein levels were measured on the 1st, 7th, and 14th day (n = 90 fish; 4.05±2.01g). In the second trial, growth, apparent digestibility coefficients ADCs, and survival rate were studied for ten weeks (n = 90, 15.71±1.59 g). Before each trial, fish were not fed for one day before stocking. Then, the fish were acclimated to laboratory conditions for one week. Fish were introduced into nine (10 fish each) plastic tanks, each containing 45 liters of water. There were three salinity treatments, tap water (<1), 7, and 15 ppt using marine salt, each treatment was replicated three times. The fish were fed twice daily at a feeding intensity of 3% of body weight with a locally prepared 35% protein pelleted feed (Table 1) during the acclimatization and experimental periods.

Trial 1. Measurement of enzyme activity and plasma protein level

Blood sampling: With a 3 ml syringe, blood samples were taken via the heart, and the blood samples were

transferred into 10 ml Falcone tubes. Two types of Falcone tubes were used, the first contains no additives, allowing the blood coagulation process to separate the serum for the determination of enzyme concentrations. After separation, serum samples were transferred to other Falcone tubes and kept at -20°C until enzyme activity measurements. The second Falcone tube contains EDTA as an anticoagulant to separate the plasma for the determination of the total plasma protein level. Both tubes were placed in a centrifuge at a speed of 1000 rpm to separate the serum and plasma.

ALP: The enzyme level was measured using an alkaline phosphatase ELISA Kit from Human mbH company, Germany. Samples were read using a spectrophotometer at a wavelength of 400 nm according to the manufacturer's instructions. The calculation was from the sum of three readings, divided by their number, and multiplied by the constant factor, according to the manufacturer's protocol, using the equation of ALP IU/L = ΔA/min. × 3433.

AST: The enzyme level was measured using an aspartate aminotransferase ELISA Kit from Human mbH company, Germany. Samples were read using a spectrophotometer at a wavelength of 340 nm according to the manufacturer's instructions. The calculation was from the sum of three readings, divided by their number, and multiplied by the constant factor, according to the manufacturer's protocol, using the equation of AST IU/L = ΔA/min. × 1667.

ALT: The enzyme level was measured using an alanine aminotransferase assay kit from Randox Laboratory, Northern Ireland. Samples were read using a spectrophotometer at a wavelength of 550 nm according to the manufacturer's instructions. The calculation was done by comparing the results with a standard table provided in the manufacturer's instructions.

Total plasma protein: The total plasma protein concentration was measured using a total protein ELISA kit from Human mbH company, Germany. Samples were read using a spectrophotometer at a wavelength of 580 nm according to the manufacturer's instructions. The calculation was done by comparing the results with the provided standard in the manufacturer's instructions using the following equation:

Protein concentration (mg/100cm^3) = (sample absorption /standard solution absorption) × 8

Growth and survival rates: All fish were weighed in each tank on days 1, 35, and 70 to regulate the feed and to track growth. Feeding was stopped 24 hours before fish weight measurement. According to the following formula, weight gain WG, relative growth rate RGR, specific growth rate SGR, feed conversion ratio FCR, and survival rate were measured.

WG g = FW g – IW g

RGR % = (WG g / IW g) × 100

SGR %/day = ((ln FW g - ln IW g) × 100) / t

FCR = FC g / WG g

Survival rate% = (Number of fish at the end of the trial / Number of fish at the beginning of the trial) × 100, where FW is the final weight, IW is the initial weight, t is the trial period in days, and FC is feed consumed.

Digestibility: During the second experiment, the indirect method of determining apparent digestibility coefficients (ADCs) was used, and chromic oxide at a concentration of 1% dry weight as an inert indicator was included in the diet. Fishes were acclimated to the diet for a week prior to collection of feces. Voided feed partials and feces were removed one-hour post-feeding through siphoning. Thereafter, feces collection started at 20-minute intervals. Feces collected from each tank were pooled and then kept frozen at –20°C. At the end of the collection period, frozen fecal was dried at 60°C for 72 h in the oven and then stored at 4°C for further analysis. The quantity of chromic oxide in the feed and fecal samples was estimated by wet acid digestion procedure with concentrated nitric and perchloric acids (Furukawa and Tsukahara, 1966), and the absorption was measured by atomic absorption spectrometry at 357.9 nm. The apparent digestibility coefficients (ADCs) were calculated according to the following equations:

Total apparent digestibility coefficient% = 100 – (100 × (indicator in feed% / indicator in feces %)

Nutrient apparent digestibility coefficient% = 100 – (100 × (indicator in feed % / indicator in feces%) × (nutrient in feces% / nutrient in feed%)

Statistical analysis: Mean and standard deviation (Mean±SD) for all data were calculated. Data were

Table 2. The growth and feed efficiency performance of common carp juveniles in different salinities (tap water, 7ppt, and 15ppt).

Parameters	Tap water	7ppt	15ppt
IW (g)	15.13±1.89[a]	16.67±1.42[a]	15.32±1.55[a]
FW (g)	35.15±3.07[a]	23.46±1.85[b]	16.29±1.56[c]
WG (g)	20.02±1.25[a]	6.80±0.46[b]	0.96±0.02[c]
RGR (%)	133.08±9.26[a]	40.84±1.57[b]	6.32±0.54[c]
SGR (%/day)	1.21±0.06[a]	0.49±0.02[b]	0.09±0.01[c]
FCR	2.26±0.10[a]	5.31±0.17[b]	29.51±2.49[c]
Survival (%)	100	100	80

Values in each row with the different superscript letters indicate a significant ($P \leq 0.05$) difference.

subjected to one-way analysis of variance (ANOVA) at a probability level of 0.05, applied Duncan's multiple range test as a means of comparison, using IBM SPSS statistics 26 software.

Results

The results of salinity treatments (7 and 15 ppt) showed changes from the 1st day of exposure up to the 14th day compared to the control group (<1 ppt). ALP activity in the blood serum of the juveniles, which have been transferred from freshwater to 7 and 15 ppt salinity showed that the level of the enzyme was raised with increasing salinity from 19.33 IU/L in tap water to 21.80 and 24.13 IU/L in 7 and 15 ppt, respectively during 1st day of the exposure. The same pattern was observed during the following 7th and 14th day periods. Also, the enzyme level continued to rise in 7 and 15 ppt treatments to reach 36.10 and 42.97 IU/L on the 14th day, respectively. The results indicated significant ($P \leq 0.05$) differences among all treatments in the three periods (Fig. 1).

The activity of AST in the blood serum is shown in Figure 2. AST activity in the blood serum of the fish exposed to 7 (45.31 IU/L) and 15 (64.3 5IU/L) ppt salinity showed significantly ($P \leq 0.05$) higher compared to the control one (35.97 IU/L) during 1st day. 15 ppt treatment indicates similar differences compared to the control on the 7th and 14th day. No significant ($P > 0.05$) differences were noticed between the 7 ppt and tap water treatments during the same periods.

The enzyme activity of ALT in the blood serum is shown in Figure 3. Blood serum ALT enzyme exhibited significantly ($P \leq 0.05$) higher levels in common carp exposed to 7 ppt (7.09 IU/L) and 15 ppt (9.10 IU/L) salinity concentrations relative to the tap water (3.51

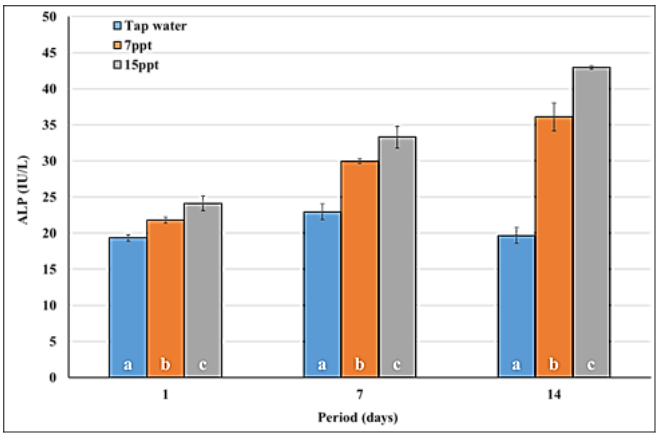

Figure 1. Alkaline phosphatase enzyme (ALP) activity in the blood serum of common carp juveniles within different periods and salt concentrations. Different letters at the inside base of the columns indicate significant ($P \leq 0.05$) differences between the three treatments during each period.

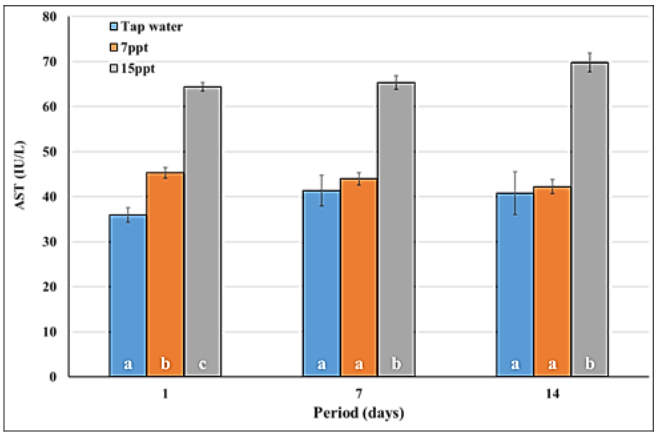

Figure 2. Aspartate amino transferase enzyme (AST) activity in the blood serum of common carp juveniles within different periods and salt concentrations. Different letters at the inside base of the columns indicate significant ($P \leq 0.05$) differences between the three treatments during each period.

IU/L) treatment during 1st day of the exposure. The following two (7th and 14th day) periods revealed the same trend of the differences. Additionally, on the 14th day, the enzyme activity rose in the 7 and 15 ppt

Table 3. The apparent digestibility coefficients (ADCs) and digestible energy of common carp juveniles in different salinities (tap water, 7ppt, and 15ppt).

	Tap water	7 ppt	15 ppt
ADC $_{\text{Dry matter}}$ (%)	88.33±1.63^{a}	62.89±1.92^{b}	44.02±2.06^{c}
ADC $_{\text{Crude protein}}$ (%)	86.39±1.31^{a}	65.17±2.43^{b}	38.76±2.64^{c}
ADC $_{\text{Crude lipid}}$ (%)	93.89±2.86^{a}	66.16±2.33^{b}	55.09±1.86^{c}
ADC $_{\text{Carbohydrate}}$ (%)	79.97±1.54^{a}	60.93±1.41^{b}	50.58±3.30^{c}
DE/GE (%)	85.27±1.67^{a}	63.72±2.02^{b}	46.20±2.75^{c}
DE (Kcal/g diet)	3.73±0.07^{a}	2.78±0.09^{b}	2.02±0.12^{c}

Values in each row with the different superscript letters indicate a significant (P≤0.05) difference.

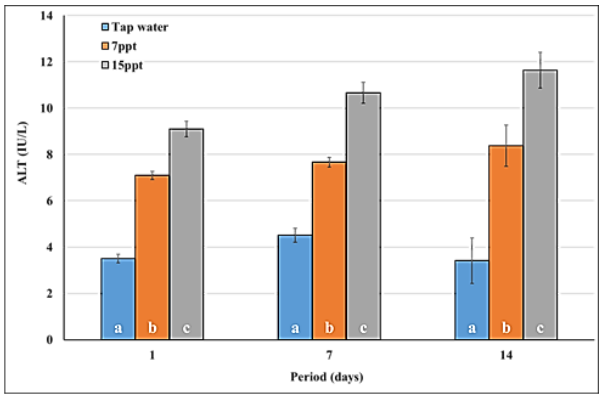

Figure 3. Alanine amino transferase enzyme (ALT) activity in the blood serum of common carp juveniles within different periods and salt concentrations. Different letters at the inside base of the columns indicate significant (P≤0.05) differences between the three treatments during each period.

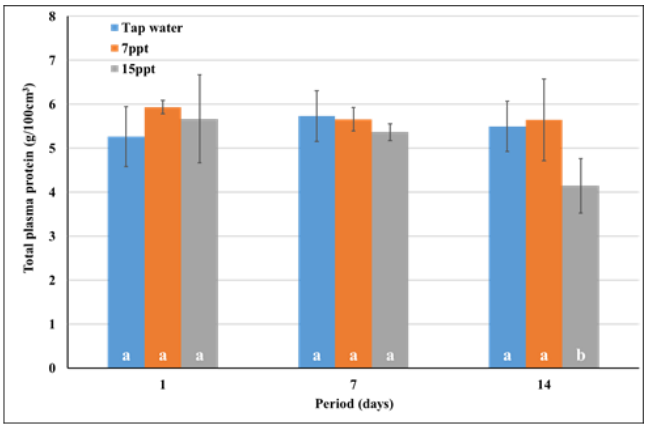

Figure 4. Total plasma protein in the blood plasma of common carp juveniles within different periods and salt concentrations. Different letters at the inside base of the columns indicate significant (P≤0.05) differences between the three treatments during each period.

treatments, achieving 8.38 and 11.63 IU/L, respectively.

The results (Fig. 4) exhibited that, the average of total plasma protein in the control, 7, and 15 ppt treatments were 5.26, 5.93, and 5.67 g/100cm^3 during the first day of the exposure, respectively. Furthermore, the results from the second period (7th day) coincided with those from the 1st day. The results showed that no significant (P≤0.05) differences were found in the levels of total serum 1st and 7th day periods. The level of plasma total protein of common carp juveniles in the 15 ppt treatments (4.15 g/100cm^3) was decreased significantly (P≤0.05) in comparison with the tap water (5.37 g/100cm^3) and 7 ppt (5.67 g/100cm^3) during the last period (14th day).

A significantly (P≤0.05) better growth and feed efficiency performance in terms of weight gain WG, relative growth rate RGR, specific growth rate SGR, and feed conversion ratio FCR were observed in the lowest salinity treatment (tap water) while the worst were observed in the 15 ppt treatments (Table 2).

Table 3 displays the apparent digestibility coefficient (ADC) of dry matter, protein, lipid, and carbohydrate of common carp juveniles in different salinities (tap water, 7 ppt, and 15 ppt). The ADC of dry matter, nutrients, and digestible energy of fish in tap water treatment were significantly (P≤0.05) higher than that of fish in 7 and 15 ppt treatments during the 90-day trial.

Discussions

The liver is one of the important glands in the body that has great importance in the process of metabolism (Mitra and Metcalf, 2009). The cells convert carbohydrates into glucose which is used to obtain energy in the cycle of oxidative phosphorylation, the excess sugar stored in the liver in the form of glycogen or triglycerides to regulate the level of sugar in the blood (Dorcas and Solomon, 2014). Elevation of the alkaline phosphatase enzyme in the blood indicates damage and disturbance in the functions of the liver, kidneys, and gills, while the decrease of this enzyme is associated

with a disorder of the membrane transport in the cell (Ahmmed et al., 2017; Kim et al., 2021).

In the current study, a significant increase was observed in the activity of ALP enzyme in the blood serum of common carp juveniles transferred to 7 and 15 ppt salinity compared to the control sample. This increase in enzyme activity could be a primary response to the increased secretion of adrenaline and cortisol, which leads to physiological changes in fish, especially in liver and blood serum enzymes (Georgieva et al., 2014). ALP enzyme has a major role in salt acclimatization in fish. Ahmed (2004) found that an increase in the level of thyroxine in blood plasma during salt acclimation leads to an increase in the activity of ALP. ALP leaks into the blood plasma from the liver as a result of its damage, and this situation is an indicator of a disease state, or a state of stress because it has no function in the blood plasma (Shahsavani et al., 2010).

The current study showed the highest level of AST and ALT enzyme activities by increasing salinity, as a significant increase in both enzyme activities was observed in fish transferred to 15 ppt salinity. Fish stress leads to an increase in endogenous oxidative stress, which changes the permeability of cell membranes, and therefore, causes an increase in the leakage of AST and ALT enzymes into the blood (Dawood et al., 2021). The transfer of European sea bass, *Dicentrarchus labrax*, from salinity 10 to 15 ppt for 60 days has caused a significant decrease in the activity of plasma AST and ALT enzymes (Goda et al., 2019).

The increase in plasma proteins was observed in the current study, and this explains the increase in fish's need for energy. The catabolism process is accompanied by an increase in the activity of AST and ALT enzymes in the liver (Lala et al., 2023). Alam et al. (2019) indicated that the high concentration of these enzymes is an indicator of gluconeogenesis which occurs during starvation and intense moving activity. The concentration of total proteins in the blood plasma is also an indicator of a healthy liver, as it is responsible for the formation of most of the proteins present in the blood serum. The blood proteins in fish consist mainly of fibrinogen, blood plasma proteins albumin, and globulin (Yuasa and Hatai, 1995). Proteins exist in fixed proportions under normal conditions, but these ratios are subject to change if the fish are exposed to a disease or environmental physiological changes; total plasma proteins take different patterns during the acclimatization of salt-tolerant fish with high salinity (Martinez-Porchas et al., 2009). Sangiao-Alvarellos et al. (2003) found an increase in the levels of plasma proteins of gilthead sea bream *Sparus aurata* with the increase in water salinity. In contrast, Kelly and Woo (1999) found a decrease in the levels of plasma proteins of silver sea bream *S. sarba* with the increase in water salinity. Whereas, Malik et al. (2020) noticed no changes in the levels of plasma proteins of common carp *C. carpio* with the changes in water salinity. In the current study, the transferring of common carp juveniles to higher water salinities (15 ppt 14th day from the start of the experiment) caused a significant decrease in the concentration of total protein in blood plasma compared to the salinity of tap water and 7 ppt. The reasons for this may be attributed to the effect of stress and the increasing requirements for energy for osmotic regulation which is associated with a decrease in the fish appetite (Al-Akel and Shamsi, 2000). The results of this study agreed with many other studies, as a general decrease in the level of total plasma protein was observed significantly with an increase in salinity from 15 to 20 g/l in *Acipenser naccarii* (Martínez-Alvarez et al., 2002).

The osmotic stress due to the high salinity of the water pushes the fish to allocate part of the energy obtained from food to meet the requirements of the osmotic regulation processes, and this, in turn, leads to a reduction in the amounts of energy available for growth and is reflected in the decrease of the growth. Based on our findings, the growth rates decreased by more than two times, while the feed conversion coefficient decreased by about 3 times at the beginning, and with increasing the salinity concentration, growth almost stopped, and feeding was no longer feasible. Mangat and Hundal (2014) and Mubarik et al. (2019) observed similar negative responses in common carp fish that were exposed to rising water salinities, and

attributed these effects to the weak appetite of fish and the negative effect on food digestion parameters, as also happened in the current study, which leads to significant imbalances in the energy balance in the body that often ends with the death of fish after varying periods due to failure to provide the energy needs necessary to sustain the various vital activities.

It can be concluded that common carp *C. carpio* juveniles have medium to weak resistance to changes in water salinity. This appears through negative physiological and nutritional responses, which necessitates the need to provide an appropriate environment, especially in terms of water salinity levels, in the culture environment of this species to ensure the success of the productivity and economic feasibility of the culture process.

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