Biological and Applied Environmental Research

Vol. 7(1): 109-129, 2023

https://doi.org/10.51304/baer.2023.7.1.109

ISSN: 2002-6153
Biol. Appl. Environ. Res.
www.baerj.com
editor@baerj.com

Effect of Salinity on Survival, Energy Metabolism and Some Hematological Indices in Common Carp (*Cyprinus carpio* L.) Juveniles Fed Potassium Chloride and Growth Hormone Diet

Mustafa A. A. Albadran*, Fatima A. M. Sultan & Salah M. Najim

Department of Fisheries and Marine Resources, University of Basrah, Basrah, Iraq

*Corresponding author: hederfatm@gmail.com

Abstract: The juveniles of common carp *Cyprinus carpio* with a weight of 44.82±11.82 g exposed to salinity of 7 and 15 g/l to study the effect of additive potassium chloride and growth hormone to the diet on salinity tolerance, as well as their effects on some hematological and biochemical measurements. Five experimental diets were set as controls: free added diet T1, diet contained two levels of potassium chloride 3% (T2) and 7% (T3), as well as two levels of growth hormone: 1% (T4) and 3% (T5). The experimental diets had a crude protein content of 27.5% and a crude lipid content of 8.02%. The results showed that fishes which were fed potassium chloride shows a high survival rate at salinities of 7 g/l and 15 g/l and fewer changes in blood glucose levels than those that were fed the growth hormone. Protein levels were lower and energy consumption was lower in fishes fed potassium chloride. Liver enzymes: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphates (ALP) have significantly raised, correlated with the food additive and levels of salinity. According to the current study, a diet supplemented with potassium chloride and growth hormone could reduce salinity stress in common carp and improve fish survival.

Keywords: Potassium chloride, Growth hormone, Common carp, AST, ALT, ALP, Oxygen consumption

Introduction

Fishes can make many physiological changes in response to stressors to maintain homeostasis, osmolality and hematology (Carragher & Rees, 1994; McDonald & Milligan, 1997). In aquaculture, salinity is an important abiotic factor and the optimal level can affect growth and survival success (Ruscoe et al., 2004). Different species have varying levels of salinity tolerance (Larsen et al., 2012). The physiological responses of various freshwater and marine fish species to high and low salinity levels in the aquatic environment have been studied (Hasenbein et al., 2013; Sinha et al., 2015).

Common carp, *Cyprinus carpio* is a member of the family Cyprinidae. It is the most widely cultivated freshwater species in the world (El-Saidy & Gaber, 2005),

Received Oct. 9, 2021, accepted Jan. 7, 2023

although it could adapt to different levels of water salinities. Hwang et al. (2018) demonstrated that ionic regulation organs such as the gills, kidneys and intestines help bony fishes maintain plasma ion balance. As a result, the most important salts in osmotic regulation are Na^+ and K^+. Ion regulation is an energy-consuming process that is taken from the fish body. Several studies have shown that during salinity adaptation, fishes consume 10% to 50% of the energy required for ion regulation (Mozanzadeh et al., 2021; Shukry et al., 2021). Guo et al. (2020) found that carbohydrate and glucose metabolisms improved the efficiency of energy supply transfer in fishes during salt acclimatization. Previous studies examining the relationship between water salinity and energy metabolism in fishes were conducted by Makaras et al. (2020) and Zhu et al. (2021).

Salinity stress can increase glucose metabolism and enhance glycolysis in the liver due to the increased energy demand to regulate the concentration of ions between the fish body fluids and the aquatic environment. Changes in salinity have been shown to alter teleosts' normal hematological characteristics, increasing plasma corticosteroids and glucose levels (Barton & Iwama. 1991; Yada et al., 2002) and lowering some blood parameter levels while increasing others. These changes may affect oxygen consumption. In various aquatic species, both hematological and biochemical parameters have been widely used as indicators of general health (Ninh et al., 2014; Tran-Ngoc et al., 2016). Many studies have been carried out to estimate the effects of salinity changes on fish physiology (Boeuf & Payan, 2001; Gholampoor et al., 2011).

The current study aimed at investigating the effects of various levels of potassium chloride and growth hormone as food additives in the diet of common carp on salinity tolerance, as well as their effects on some hematological and biochemical measurements.

Materials and Methods

Fishes and Experimental design

Two hundred juvenile common carp Cyprinus *carpio* (mean body weight 44.82±11.82 g. and mean body length 12.5±2.33 cm) were obtained from the earthen ponds of the Aquaculture Unit, College of Agriculture, University of Basrah. Fishes that appeared to be in good health were transported to the lab and acclimatized in dechlorinated water for one week before being fed a basal fish diet. Following acclimation, the fishes were randomly divided into two equal groups with different salinity levels (7 g/l, and 15 g/l) for another 70 days. Each group consists of 100 fishes distributed among 10 aquaria (30×30×60 cm) with a water capacity of 50L, the experimental salinities were obtained by adding a specific amount of marine salt to achieve salinities of 7g/l and 15g/l. To avoid any changes, the salinity of the water was measured daily. The experiment was carried out in natural photoperiod, and the fishes were fed twice daily by 3% of the total stock biomass.

Preparation of Experimental Diet

Five experimental diets were set as controls (free added diet T1), and another examined diet contained two levels of potassium chloride: 3 % (T2) and 7 % (T3), as well as two levels of growth hormone: 1% (T4) and 3% (T5). The experimental diets had a crude protein content of 27.5% and a crude lipid content of 8.02% (Table 1). The proximate analysis of chemical composition for the tested diets was estimated according to AOAC (2000).

Table 1: The formulation and composition of the experimental diet (%).

Diet	Diet (% addition)				
	Diet T1	Diet T2	Diet T3	DT4	DT5
Fishmeal	25	25	25	25	25
Soybean	18	18	18	18	18
Barley flour	15	15	15	15	15
Vegetable oil	2	2	2	2	2
Wheat flour	38	38	38	38	38
Mixture of vitamins and minerals	5	5	5	5	5
KCl	0	3	7	0	0
Growth hormone	0	0	0	1	3
Proximate composition (%)					
Moisture	7.2	7.2	7.2	7.2	7.2
Fat	8.02	8.02	8.02	8.02	8.02
Protein	27.5	27.5	27.5	27.5	27.5
Ash	12.6	12.6	12.6	12.6	12.6
Carbohydrate	51.88	51.88	51.88	51.88	51.88

Surviving Rate

The survival rate of common carp fed different level of potassium chloride and growth hormone introduced to different salinities (7 and 15 g/l) were determined after 70 days of salinity exposure as follow:

Survival Rate (%) = total fish survived/ total fish stocked × 100

Blood Sampling

Before sampling, the fishes were fasted for 24 hours. To reduce handling stress, blood was collected from the caudal vein with a 3 ml syringe in less than 3 minutes. The blood was transferred to an anticoagulant-free tube for biochemical analysis.

Biochemical Measurement

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman & Frankel (1957). Total serum protein was determined according to Doumas et al. (1981). Plasma glucose concentrations were measured by the glucose oxidase method (Barham & Trinder, 1972). Alkaline Phosphates Activity levels were measured according to Pan et al. (1997).

Oxygen Consumption

The oxygen consumption was based on the decrease in the levels of dissolved oxygen in the water in closed containers based on Nordlie & Leffer (1976). One fish of known weight was placed in a conical, opaque glass container of one-liter capacity, filled with oxygen-saturated water. The fishes from different treatments (T1, T2, T3, T4 and T5) were transported at the following water salinities: 7 and 15 g/l for 24 hours to acclimatization. The level of oxygen was measured at frequent intervals (every half an hour) until the dissolved oxygen concentration dropped to 60% of the saturation level. The level of dissolved oxygen was measured by the YSI 559MPS device. The level of oxygen consumed was estimated in mg O_2/kg/hr. According to Brett (1972), the consumed oxygen was converted to mg O_2/kg/hr into energy according to the following equation:

1 mg O_2/kg/hr is equivalent to 0.00337 kcal/kg/hr.

Statistical Analysis

SPPS (ver. 22, USA) was used for statistical analysis of the data. Data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences between the means.

Results

After 14 days of exposure to the salinity of 7g/l and 15g/l, the survival rate of common carp fed different levels of potassium chloride and growth hormone showed significant differences ($P<0.05$). A higher survival rate was recorded in T3 (7% potassium chloride) at salinities of 7 g/l and 15 g/l. (Figure 1).

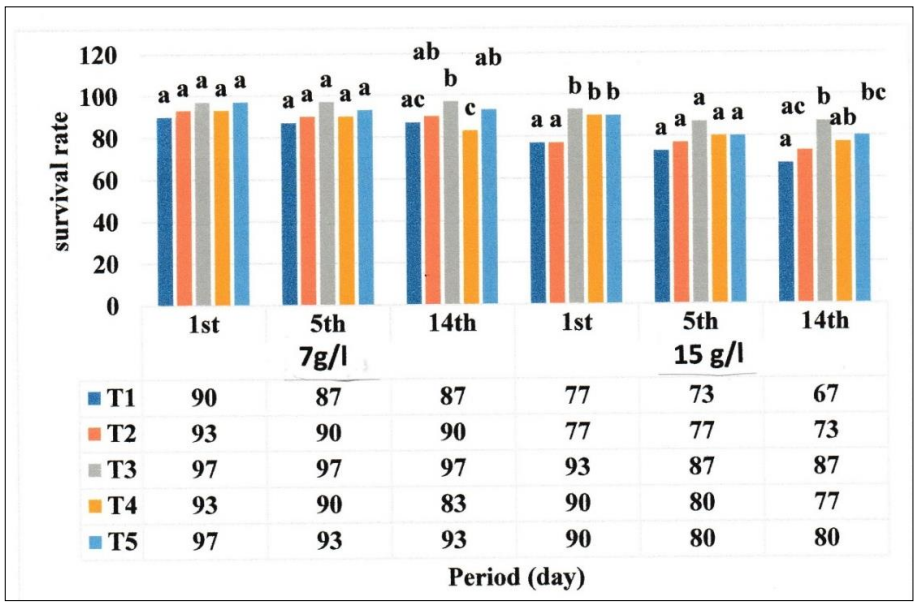


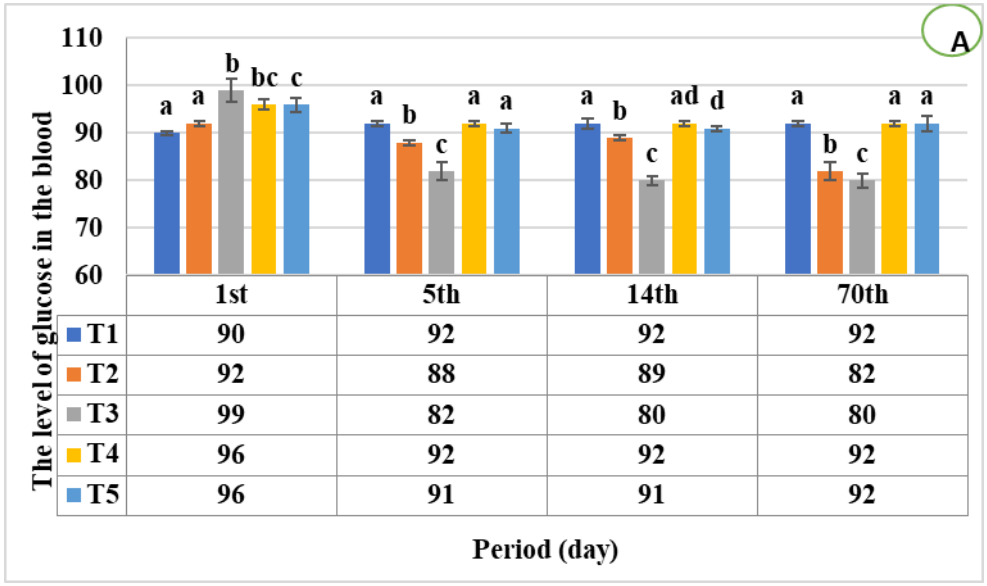
Figure 1: Survival rates of common carp juveniles in different treatments at a salinity of 7 and 15 g/l.

Plasma Glucose Level

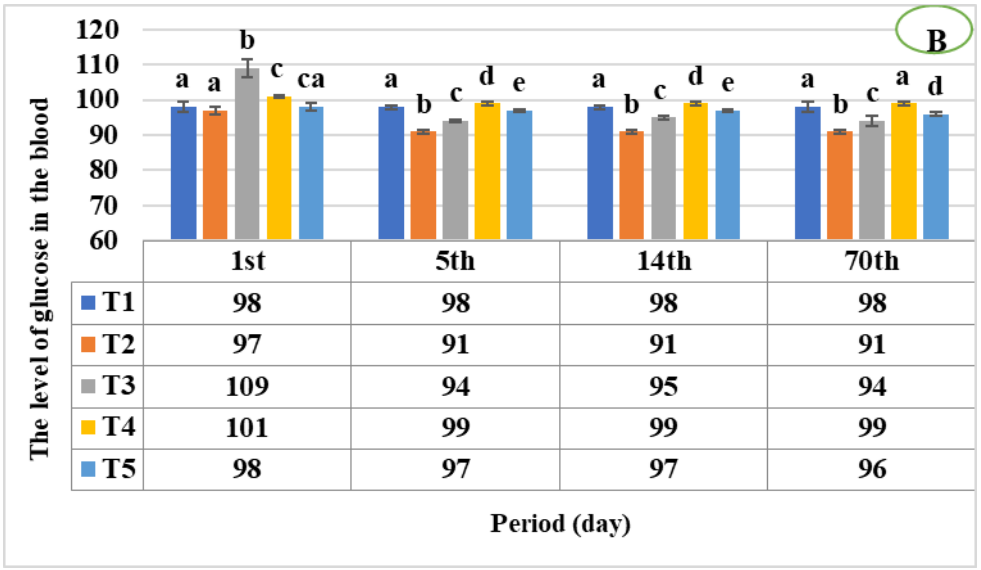
Figures 2A and B showed the level of glucose in the blood of common carp fed different levels of potassium chloride and growth hormone during the 1st, 5th, 14th, and 70th days after being exposed to the salinity of 7 and 15 g/l. A fluctuating level of glucose in the blood was observed throughout the experiment. On the 70th day of the experiment, a significant decrease ($P<0.05$) was found in the glucose level in treatment T2 and T3 (82 and 80 mg/100 ml, respectively) compared to the control treatment (T1) at a salinity of 7 g/l, while no difference ($P>0.05$) was observed in the treatment T4 and T5 (99 and 96 mg/l, respectively) compared to the control. At a salinity of 15 g/l, there was a fluctuation in blood glucose values during the 1st day of exposure in all treatments (T1, T2, T3, T4 and T5). There was a significant decrease ($P<0.05$) on the 5th and 14th days in the treatments (T2, T3, T4 and T5) compared to the control sample (T1). A significant decrease ($P<0.05$) was recorded in treatments T2, T3 and T5 (91, 94, and 96 mg/100 ml, respectively) and a significant increase ($P<0.05$) in T4 (99 mg/100 ml) compared to the control T1 (98 mg/100 ml) after 70 days from the beginning of the experiment at a salinity of 15 g/l (Figure 2B).

Total Proteins

Figures 3A and B showed the total protein in the blood plasma of common carp on the 1st, 5th, 14th and 70th days of exposure to a salinity of 7 and 15 g/l. There were no significant differences ($P>0.05$) in the levels of total protein in the blood plasma on the 5th and 14th days after salinity exposure between T3 and control (T1), while significant differences ($P<0.05$) were found in T2, T4 and T5 compared with the control. Seventy days after the start of the experiment, a significant decrease ($P<0.05$) was found in T2 and T4 and insignificant decrease ($P>0.05$) in treatments T3 and T5 compared to the control sample (T1). Total protein values of 5.43, 5.77, 5.56 and 5.71 mg/100 ml were recorded in T2, T3, T4 and T5, respectively compared to the control T1 (5.8 mg/100 ml) at the salinity of 7 g/l (Figure 3A). The values of total protein in fishes exposed to a salinity of 15 g/l fluctuated during the 1st, 5th and 14th days in all treatments (T1, T2, T3, T4 and T5). A significant decrease ($P<0.05$) was found in the treatments (T2, T3, T4 and T5) compared to the control sample (T1) after 70th days of exposure to salinity of 15 g/l (Figure 3B).

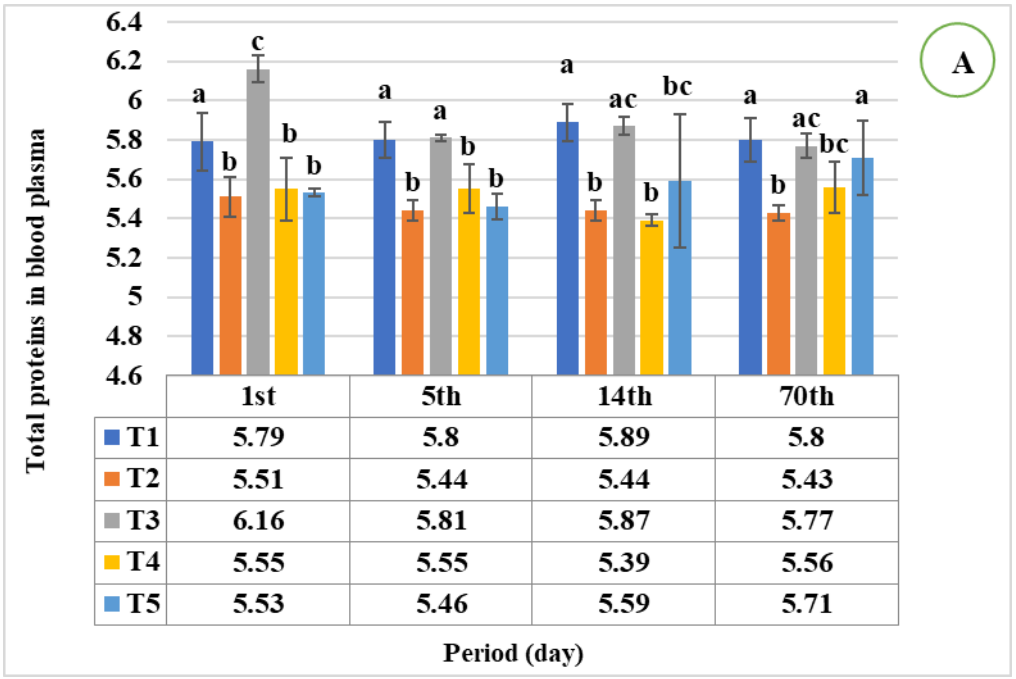


	1st	5th	14th	70th
T1	90	92	92	92
T2	92	88	89	82
T3	99	82	80	80
T4	96	92	92	92
T5	96	91	91	92

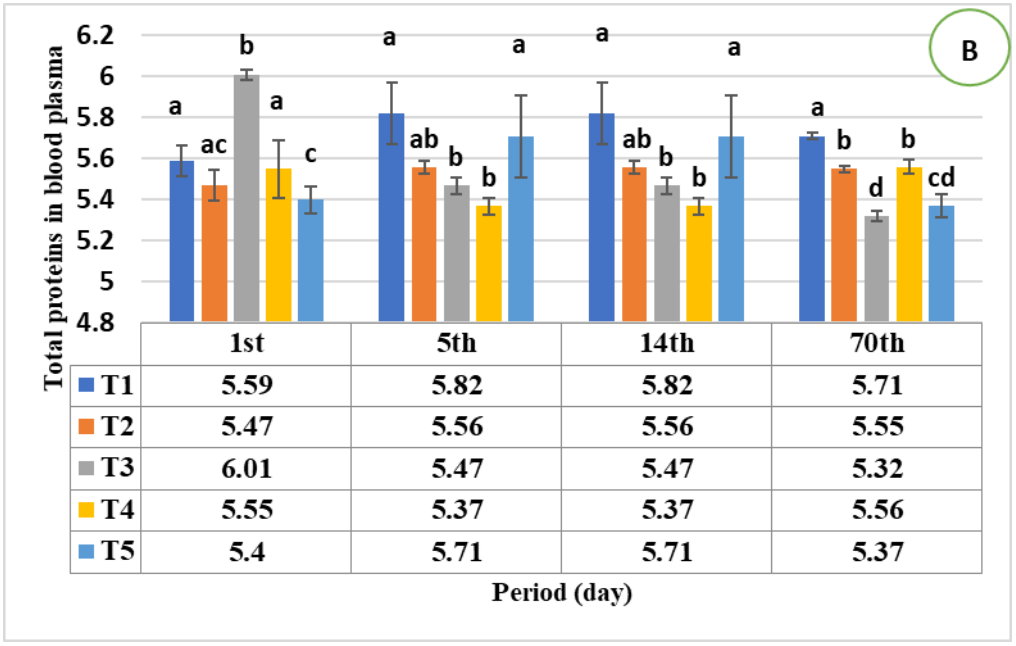


	1st	5th	14th	70th
T1	98	98	98	98
T2	97	91	91	91
T3	109	94	95	94
T4	101	99	99	99
T5	98	97	97	96

Figure 2: The level of glucose in the blood (mg/100 ml) for juvenile common carp in different treatments exposed to salinity: A, 7 g/l and B, 15 g/l.



	1st	5th	14th	70th
T1	5.79	5.8	5.89	5.8
T2	5.51	5.44	5.44	5.43
T3	6.16	5.81	5.87	5.77
T4	5.55	5.55	5.39	5.56
T5	5.53	5.46	5.59	5.71



	1st	5th	14th	70th
T1	5.59	5.82	5.82	5.71
T2	5.47	5.56	5.56	5.55
T3	6.01	5.47	5.47	5.32
T4	5.55	5.37	5.37	5.56
T5	5.4	5.71	5.71	5.37

Figure 3: Total protein levels in blood plasma (mg/100 ml) of juvenile common carp in different salt treatments: A, 7 g/l and B, 15 g/l.

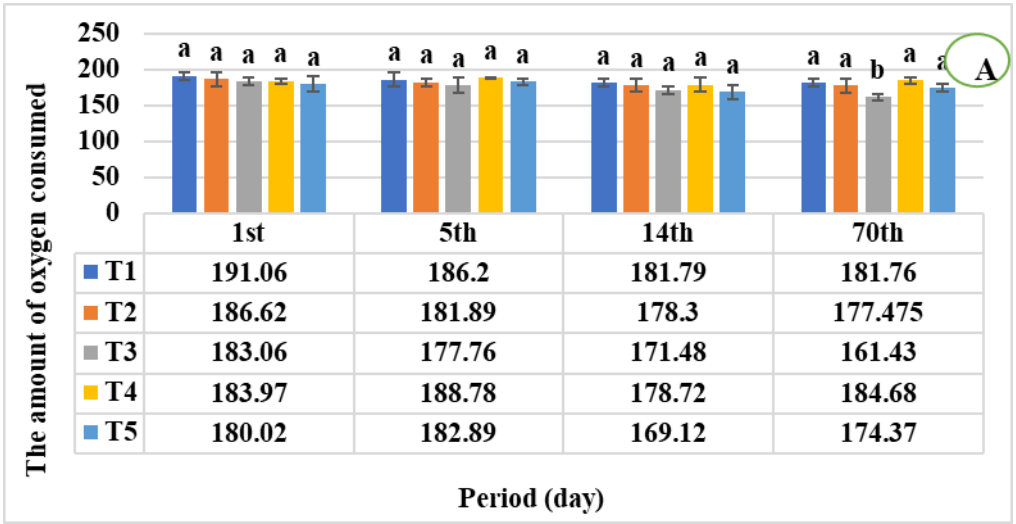
Oxygen Consumption

Figures 4A and B showed the level of oxygen consumed by common carp on the 1st, 5th, 14th and 70th days after transfer to a salinity of 7 and 15 g/l. There were no significant differences (P>0.05) in the level of oxygen consumed on the 1st, 5th and 14t days after exposure to a different level of salinity in all treatments (T1, T2, T3, T4 and T5) compared with the control treatment. Seventy days after the start of the experiment, a significant decrease (P<0.05) was found in treatment T3 compared to the control sample (T1), where a value of 161.43 mg O_2/kg/hr was recorded compared with the control treatment (181.86 mg O_2/kg/hr), while no significant differences were observed. (P>0.05) between treatments T2, T4 and T5 compared with the control at a salinity of 7 g/l (Figure 4A).

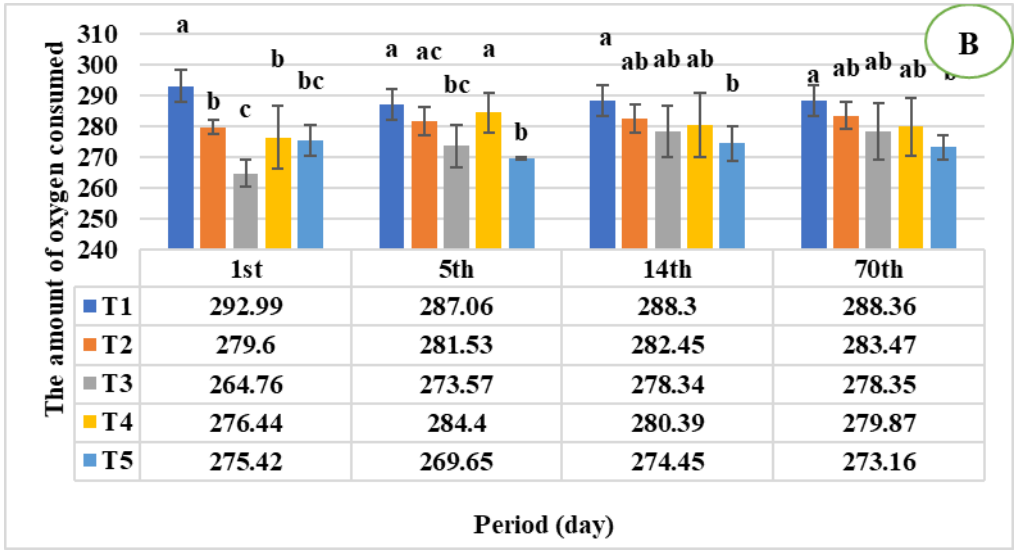
There was an increase in the level of oxygen consumption in the control treatment during the 1st, 5th and 14th days of the experiment compared to treatments T2, T3, T4 and T5. Seventy days after the start of the experiment, a significant decrease (P<0.05) was found in the treatment T5 compared to T1, which recorded a value of 273.16 mg O_2/kg/hr compared to the control sample (288.36 mg O_2/kg/hr) as in Figure 4B.

Energy Consumption

Figures 5A and B showed the amount of energy consumed in common carp on the 1st, 5th, 14th and 70th days after transfer to a salinity of 7 and 15 g/l. There were no significant differences (P>0.05) in the amount of energy consumed on the 1st, 5th and 14th days of exposure to different salinity in all treatments (T2, T3, T4 and T5) compared to the control treatment (T1). Seventy days after the start of the experiment, a significant decrease (P<0.05) was found in treatment T3 compared to the control (T1), with a value of 0.544 kcal/kg/hr was recorded compared with the control treatment of 0.613 kcal/kg/hr, while no significant differences (P>0.05) were observed between treatments T2, T4 and T5 compared with the control T1 at a salinity of 7 g/l (Figure 5A). There was an increase in the amount of energy consumed in the control sample during the 1st, 5th and 14th days of salinity exposure compared to the treatments (T2, T3, T4, and T5). Seventy days after the start of the experiment, a significant decrease (P<0.05) was found in treatment T5 compared to the control T1, with a value of 0.921 kcal/kg/hr was recorded compared to the control T1, the value of 0.972 kcal/kg/hr (Figure 5B).



	1st	5th	14th	70th
T1	191.06	186.2	181.79	181.76
T2	186.62	181.89	178.3	177.475
T3	183.06	177.76	171.48	161.43
T4	183.97	188.78	178.72	184.68
T5	180.02	182.89	169.12	174.37



	1st	5th	14th	70th
T1	292.99	287.06	288.3	288.36
T2	279.6	281.53	282.45	283.47
T3	264.76	273.57	278.34	278.35
T4	276.44	284.4	280.39	279.87
T5	275.42	269.65	274.45	273.16

Figure 4: The level of oxygen consumed ($mgO_2/kg/hr.$) for juvenile common carp in different treatments exposed to a salinity: A, 7 g/l) and B, 15 g/l.

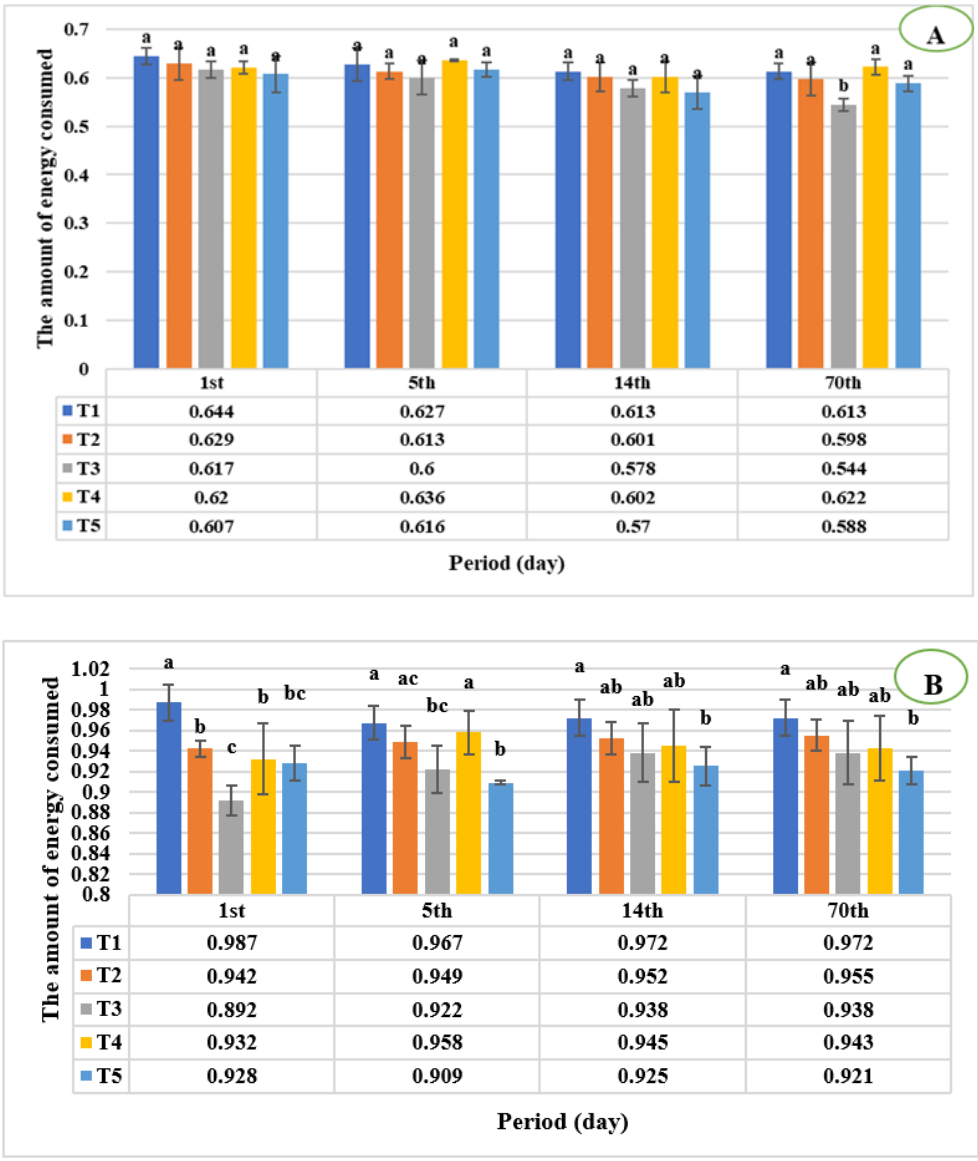


Figure 5: The amount of energy consumed (kcal/kg/hr) for juvenile common carp in different treatments exposed to the salinity of A, 7 g/l and B, 15 g/l.

Alkaline Phosphatase (ALP)

Figures 6A and B showed the level of alkaline phosphatase ALP enzyme in the blood plasma on the 1st, 5th, 14th and 70th days after transfer to a salinity of 7 and 15 g/l.

The level of alkaline phosphatase enzyme ALP at a salinity of 7 g/l decreased significantly ($P<0.05$) throughout the experiment period in all treatments (T2, T3, T4 and T5) when compared to the control (T1). Seventy days after starting experiments values of 20.49, 20.18, 20.61 and 20.12 IU/l were recorded,

respectively, compared with the control treatment of 26.67 IU/l at the a salinity of 7 g/l (Figure 6A). There was a significant decrease ($P \leq 0.05$) in the concentration of alkaline phosphatase at a concentration of 15 g/l throughout the experiment period in all treatments (T2, T3, T4 and T5) compared with the control treatment (T1). Seventy days after the start of the experiment, values of 22.77, 20.88, 22.32 and 21.18 IU/l were recorded, respectively, compared with the control treatment of 23.44 IU/l at the salinity of 15 g/l (Figure 6B).

Aspartate Transaminase (AST)

Figure 7A and B showed the level of AST enzyme in the blood plasma on the 1st, 5th, 14th and 70th days after transfer to a salinity of 7 and 15 g/l. A fluctuation in AST level was observed in the treatments T1, T2, T3, T4 and T5 after fourteen days from the start of the experiment. There was a significant decrease ($P<0.05$) in the level of AST enzyme at a salinity of 7 g/l in all treatments (T2, T3, T4 and T5) compared with the control treatment (T1), after seventy days from the start of the experiment, values of 43.33, 43.74, 41.76 and 43.24 IU/l, respectively, were recorded compared with the control treatment of 45.31 IU/l at a salinity of 7 g/l (Figure 7A). There was a significant increase ($P<0.05$) in the level of AST enzyme in the blood plasma at a salinity of 15 g/l throughout the experiment in T4 compared to the control treatment (T1). Seventy days after the start of the experiment, a value of 60.7 IU/l was recorded, compared with the control treatment (54.97 IU/l) at the salinity of 15 g/l (Figure 7B).

Alanine Transaminase (ALT)

Figures 8 A and B showed the level of ALT enzyme in the blood plasma on the 1st, 5th, 14th and 70th days after transfer to a salinity of 7 and 15 g/l.

A fluctuation in the concentration of ALT enzyme values was observed in all treatments T1, T2, T3, T4 and T5 after fourteen days from the start of the experiment. There was a significant decrease ($P<0.05$) in the level of ALT enzyme at a salinity of 7 g/l in T4 compared to the control (T1) after seventy days from the start of the experiment, a value of 6.67 IU/l were recorded, compared to the control treatment 6.65 IU/l in the saline concentration of 7 g/l, while there were no significant differences ($P>0.05$) between treatments T1, T2, T3 and T5 (Figure 8A). There was a significant increase ($P \leq 0.05$) in the concentration of ALT enzyme in the blood plasma at a concentration of 15 g/l throughout the trial period in treatments T2, T3, T4 and T5 compared with the control treatment (T1). Seventy days after the start of the experiment, values of 8.48, 8.53, 8.46 and 8.06 IU/l were recorded, respectively, compared with the control treatment of 7.86 IU/l at a salinity of 15 g/l (Figure 8B).

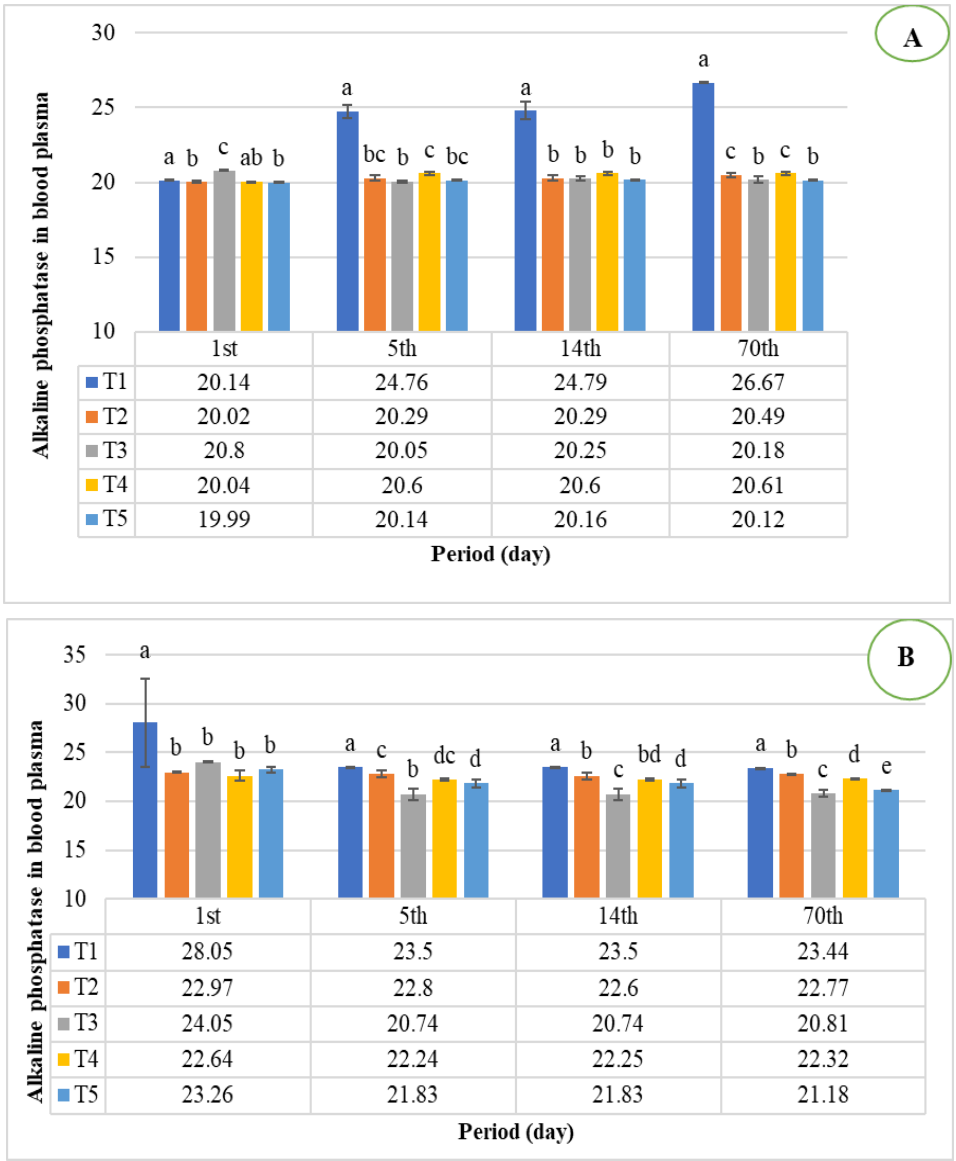
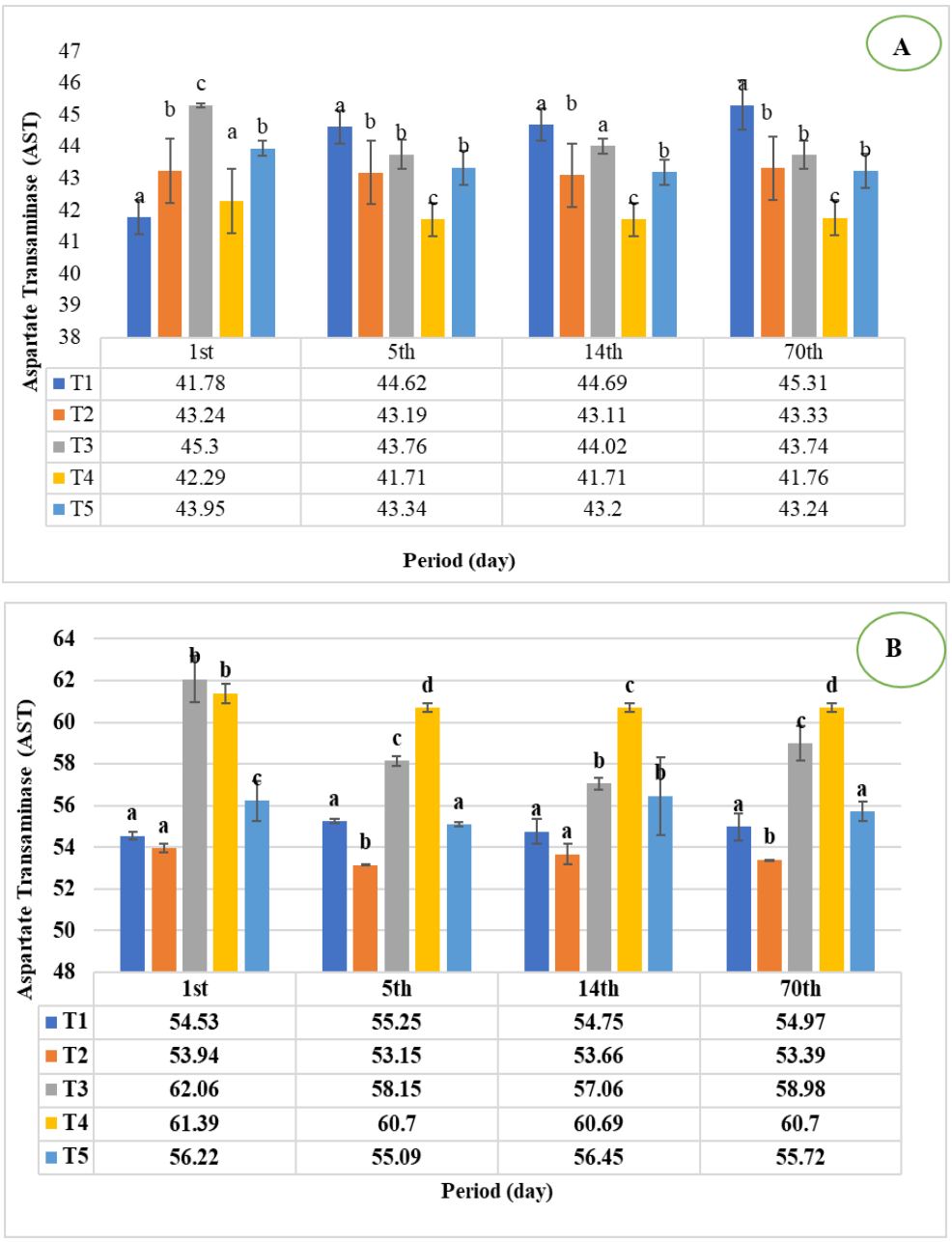


Figure 6: The level of alkaline phosphatase (IU/l) of juvenile common carp in different treatments exposed to salinity: A, 7 g/l and B, 15 g/l.

.



	1st	5th	14th	70th
T1	41.78	44.62	44.69	45.31
T2	43.24	43.19	43.11	43.33
T3	45.3	43.76	44.02	43.74
T4	42.29	41.71	41.71	41.76
T5	43.95	43.34	43.2	43.24

	1st	5th	14th	70th
T1	54.53	55.25	54.75	54.97
T2	53.94	53.15	53.66	53.39
T3	62.06	58.15	57.06	58.98
T4	61.39	60.7	60.69	60.7
T5	56.22	55.09	56.45	55.72

Figure 7: The level of AST enzyme (IU/l) in the blood plasma of juvenile common carp in different treatments exposed to salinity: A, 7 g/l and B, 15 g/l.

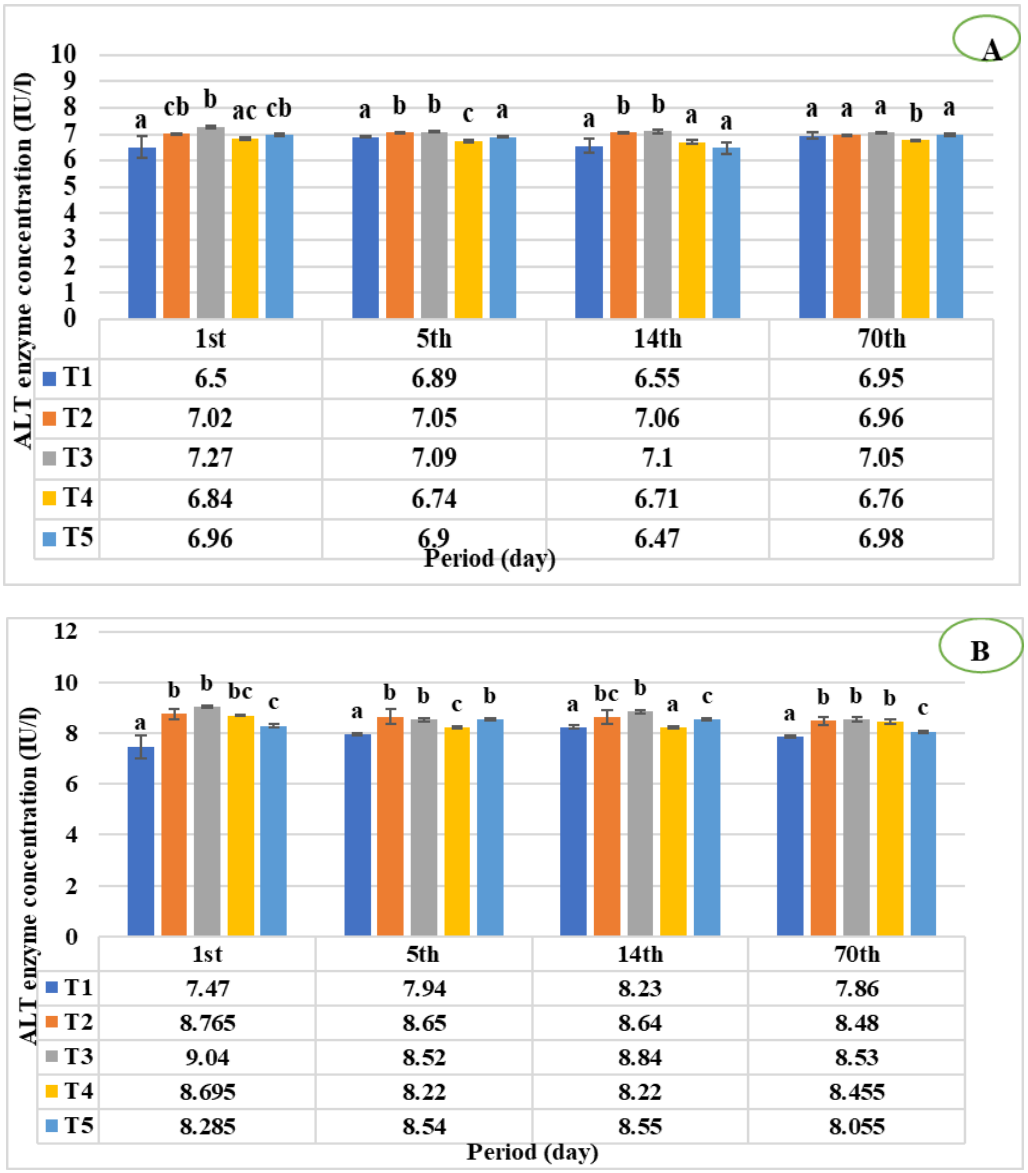


Figure 8: The level of ALT enzyme (IU/l) of juvenile common carp in different treatments exposed to salinity: A, 7 g/l) and B, 15 g/l).

Discussion

Survival Rates

It is well known in aquatic organisms that environmental factors and diet play a crucial role in growth and survival rate (Mommsen, 1998). Salinity stress is a major risk in the freshwater aquaculture production sector, causing a physiological disturbance and harming performance and fish survival (Küçük et al., 2013). The current study was carried out to increase the utilization of inland saline water resources for the cultivation of freshwater fishes. According to the current study, a

diet supplemented with potassium chloride and growth hormone could reduce salinity stress in common carp and improve fish survival.

This is consistent with many previous studies that indicated the ability of carps to withstand salinity levels, particularly when improving their abilities to regulate osmosis and control the internal stability of fluids and body ions in contrast to the high ionic and saline concentrations in the surrounding environment (Ahmed & Jaffar, 2013). The effects of adding potassium chloride and other salts to fish feed before salinity exposure include some mechanisms proposed by Al-Saadi & Al-Kashali (2015). Many studies have shown that a group of growth hormones can positively affect fish's ability to deal with osmotic changes in the environment (Reindl & Sheridan, 2012).

Blood Glucose Level

In terms of a fish physiological state, glucose is an important stress indicator because its levels can fluctuate depending on the fish's physiological state, particularly when it is subjected to stress such as osmotic stress or a lack of oxygen in the water. In a metabolic or hormonal response of the fish's body to the stressor, glucose concentrations can be greatly reduced (Makaras et al., 2020; Zhu et al., 2021).

Fishes fed potassium chloride as a feed additive experienced more changes in blood glucose levels than that fed growth hormone as a feed additive. This indicates that potassium chloride had a greater and clearer effect on reducing osmotic stress on fishes and assisting them in dealing with the high salinity of the surrounding environment. This could be due to the rate with which salt dissolves and absorbs, as opposed to hormone growth, which may take longer to show its effects, which differed significantly and were less than the effects of salt even after fourteen days of exposing fishes to salinity (Mozanzadeh et al., 2021; Shukry et al., 2021).

A rise in glycogenolysis could explain the decrease in glucose levels observed in fishes fed potassium chloride (Kavya et al., 2016). Because the liver is the primary site of glycogen/glucose turnover in fishes, liver metabolism may be increased during osmotic adaptation to ensure that glucose is available to fuel other metabolic and osmoregulatory tissues such as the gills and kidneys (Vijayan et al., 1996). Fishes raised at extreme salinities may use more glucose to meet the higher energy requirements of various tissues involved directly or indirectly in osmoregulatory work (Laiz-Carrión et al., 2003; Sangiao-Alvarellos et al., 2005).

Total Proteins Content

Total proteins are one of the most important components of blood plasma. Its main source is proteins found in fish food. The liver is the main factory for specialized proteins, particularly albumin and globulin, though some immune globulins can be produced by large lymphoid white blood cells in response to a pathological condition (Peyghan et al., 2014).

The present study shows a decrease in protein levels of T2 and T3 at a salinity of 7 g/l and 15 g/l concerning the T1. In contrast, other studies have found either no

change or an increase in protein levels as salinity increases. The potential importance of increased serum protein as a fuel for tissues during osmotic acclimation has yet to be investigated, but it could be related to a metabolic reallocation of energy resources once carbohydrate stores have been depleted. Amino acids appear to be important in allowing fishes to adjust to varying salinities, either as energy sources or as important osmolytes for cell volume regulation (Aragão et al., 2010). Thus, changes in the concentrations of total proteins in the blood plasma have a direct reflection on the response to osmotic shock and the various osmotic regulation processes in fishes (Coeurdacier et al., 2011).

The concentration of total proteins in blood plasma is also influenced by the amount of water in the blood. A decrease in blood water content increases the concentration of dissolved solids, including various proteins. Thus, total proteins in blood plasma can be regarded as an important indicator of rapid and short-term fluctuations in the osmotic regulation process in response to changes in the high salinity of the environment (Al-Khshali & Al-Hilalli, 2017).

Liver Enzymes

The liver is the body's main chemical laboratory, where it performs numerous functions such as protein synthesis, glycogen storage and fat synthesis. Functional enzymes, each of which plays a unique role in the decomposition or synthesis of various substances within the liver (Al-Khshali & Al-Hilali, 2019; Al-Khashali & Al-Shawi, 2021). AST and ALT, found primarily in fish hepatocytes and cardiomyocytes, play important roles in protein metabolism. When the liver and myocardium are damaged or their permeability increases, AST and ALT are released into the blood, resulting in increased blood transaminase activity. The activities of serum AST and ALT can therefore be used to monitor the health status of fishes (Wang et al., 2005). In the present study, significantly elevated values of AST and ALT, correlated with the food additive and levels of salinity, indicate increased permeability of the hepatocytes and cellular leakage. The increase in ALP enzyme levels could be caused by an increase in salinity concentrations because the ALP enzyme is one of the important enzymes in the osmoregulation process; it plays a role in ion transfer. The entry of large amounts of water into the fish body may harm the osmotic pressure and cause a high permeability between the cell membranes inside the fish body, causing the ALP to spread throughout the fish body in an attempt to regulate osmosis. The fluctuation of salinity frequently causes a variety of physiological stress responses, disrupting the balance of serum hormones, energy metabolism, and electrolytes in aquatic animals (Al-Khshali & Al Hilali, 2019). The ALP enzyme, which is found in the membrane of the intestinal epithelium, plays an important role in the adaptation of fishes to saline water, as an increase in the level of this enzyme has been observed 2-3 times when fishes are exposed to saline water (Oide, 1970). ALP can also be used as a biological indicator of the salinity adaptation process's success (Ahmed et al., 2004).

Oxygen and Energy Consumed

Oxygen plays the most important role in energy production by breaking down the bonds in food and converting them into energy, and because the body requires a certain amount of energy to achieve internal stability, fishes require more oxygen to provide more energy. Metabolism includes chemical reactions that occur in living organisms, and the most common method of indirectly detecting metabolic rate is the measurement of oxygen consumed. Changing the oxygen consumption rate can identify the energy required by the body to adapt to changes in salinity (Tseng & Hwang, 2008). Changes in fish respiratory rate are one of the most common physiological responses to salinity stress. Under conditions of environmental imbalance, the change in oxygen consumption rate is commonly used to estimate the change in metabolic rate (Dube & Hosetti, 2010). The reason for the change in oxygen consumption rate as salinity levels change is due to increased activity in active ion transport and activity of the Na/k/ ATPase in gills, which increases the energy needed for the completion of the osmoregulation process (Sangiao-Alvarellos et al., 2005). The current findings of this study revealed that as salinity increased, so did the rate of oxygen consumption, which was accompanied by an increase in the amount of energy expended. Ahmed & Jaffar (2013) mentioned that the reason for the change in the rate of oxygen consumption that follows the change in the levels of salinity is a result of the change in the effective transport of ions, which increases the energy requirements for the completion of the osmosis regulation process. The results of previous studies of different species of fishes showed an increase in the rate of oxygen consumption, with an increase in the rate of oxygen consumption in the salinity of the environment (Ern et al., 2014; Takei et al., 2014; Al-Saadi & Al-Kashali, 2015; Reed, 2015). The increase in oxygen consumption is caused by an increase in metabolic rate to meet the high energy requirements associated with the osmosis situation caused by exposing the fishes to high levels of salinity. This is a natural condition caused by saline stress and increased energy demand by fishes for osmoregulation.

References

Ahmed, S.M.; Al-Dubiakel, A.Y. & Mohamed, F.A. (2004). Changes in alkaline phosphatase activity in the intestine of *Liza abu* juveniles during salinity acclimation. Iraqi J. Aquac., 11: 17-27.

Ahmed, S.M. & Jaffar, R.S. (2013). Effect of salt stress on energy usage and growth in grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and common carp *Cyprinus carpio* L. juveniles. Iraqi J. Aquac., 10(1): 1-24. (In Arabic).

Al-Khashali, M.Sh. & Al-Shawi, S.A.S. (2013). Effect of salt stress on ALT and AST enzymes activity and cortisol level in adults of *Carassius auratus*. Pak. J. Nutr., 12(1): 97-100. DOI:10.3923/pjn.2013.97.100.

Al-Khshali, M.S. & Al-Hilalli, H.A. (2017). Effect of gradual high salinity on some stress parameters (glucose, total protein and lactate) in blood plasma of common carp. Iraqi J. Agric. Sci., 48(2): 573-581. DOI:10.36103/ ijas.v48i2.424. (In Arabic).

Al-Khshali, M.S. & Al Hilali, H.A. (2019). Some physiological changes (ALP, AST and ALT) of common carp (*Cyprinus carpio*) caused by high salinity. Biochem. Cell. Arch., 19(2): 4605-4610. DOI:10.35124/bca.2019.19.2.4605.

Al-Saadi, D.A.A. & Al-Kashali, K.S. (2015). Effect of salty feeding in sodium and potassium ions concentration in blood plasma and packed cell blood volume (PCV) in common carp (*Cyprinus carpio*). Iraqi J. Vet. Med., 39(2): 25-29. (In Arabic).

AOAC (2000). Official methods of analysis. J. AOCA Int., 83(2):305-310.

Aragão, C.; Costas, B.; Vargas-Chacoff, L.; Ruiz-Jarabo, I.; Dinis, M.T.; Mancera, J.M. & Conceição, L.E.C. (2010). Changes in plasma amino acid levels in a euryhaline fish exposed to different environmental salinities. Amino Acids, 38: 311-317.

Barham, D. & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst., 97(151): 142-5. DOI:10.1039/an9729700142. PMID: 5037807.

Barton, B.A. & Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis., 1: 3-26.

Boeuf, G. & Payan, P. (2001). How should salinity influence fish growth? Comp. Biochem. Physiol., C.130: 411-423.

Brett, J.R. (1972). The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respir. Physiol., 14(1-2): 151-170. DOI:10.1016/0034-5687(72)90025-4.

Carragher, J.F. & Rees, C.M. (1994) Primary and secondary stress responses in golden perch, *Macquaria ambigua*. Comp. Biochem. Physiol., Part A: Physiol., 107(1): 49-56.

Coeurdacier, J.-L.; Dutto, G.; Gasset, E. & Blancheton, J.-P. (2011). Is total serum protein a good indicator for welfare in reared sea bass (*Dicentrarchus labrax*)? Aquat. Living Resour., 24(2): 121-127. DOI:10.1051/alr/2011130.

Doumas, B.T.; Bayse, D.D.; Borner, K.; Carter, R.J.; Elevitch, F.; Garber, C.C.; Graby, R.A.; Hause, L.L.; Mather, A.; Peters Jr., T. & Rand, R.N. (1981). A candidate reference method for determination of total protein in serum. II. Test for transferability. Clin. Chem., 27(10): 1651-1654.

Dube, P.N., Hosetti, B.B. (2010). Behavior, surveillance and oxygen consumption in the fresh water fish *Labeo rohita* (Hamilton) exposed to sodium cyanide. Biotech. Anim. Husb., 26(2-1): 91-103.

El-Saidy, D.M. & Gaber, M. (2005). Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. Aquac. Res., 36: 163-171.

Ern, R.; Huong, D.T.T.; Cong, N.V.; Bayley, M. & Wang, T. (2014). Effect of salinity on oxygen consumption in fishes: A review. J. Fish Biol., 84(4): 1210-1220. DOI:10.1111/jfb.12330.

Gholampoor, T.E.; Imanpoor, M.R.; Shabanpoor, B. & Hosseini, S.A. (2011). The study of growth performance, body composition and some blood parameters of *Rutilus frisii* kutum (Kamenskii, 1901) fingerlings at different salinities. J. Agric. Sci. Technol., 13(6): 869-876.

Guo, T.; Yang, Y.; Meng, F.; Wang, S.; Xia, S.; Qian, Y.; Li, M. & Wang, R. (2020). Effects of low salinity on gill and liver glycogen metabolism of great blue-spotted mudskippers (*Boleophthalmus pectinirostris*). Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol., 230: 108709. DOI:10.1016/ j.cbpc.2020.108709.

Hasenbein, M.; Komoroske, L.M.; Connon, R.E.; Geist, J. & Fangue, N.A. (2013). Turbidity and salinity affect feeding performance and physiological stress in the endangered delta smelt. Integr. Comp. Biol., 53: 620-634.

Hwang, J.; Kim, S.; Seo, Y.; Lee, K.; Park, C.; Choi, Y.; Kim, D.; Gilad, A.A. & Choi, J. (2018). Mechanisms of salinity control in sea bass. Biotechnol. Bioprocess Eng., 23(3): 271-277. DOI:10.1007/s12257-018-0049-3.

Kavya, K.; Jadesh, M. & Kulkarni, R. (2016). Hematology and serum biochemical changes in response to change in saline concentration in fresh water fish *Notopterus notopterus*. World Sci. News, 32: 49.

Küçük, S.; Karul, A.; Yildirim, Ş. & Gamsiz, K. (2013). Effects of salinity on growth and metabolism in blue tilapia (*Oreochromis aureus*). Afr. J. Biotechnol., 12(19): 2715-2721.

Laiz-Carrión, R. & Mancera, J.M. (2019). Energy metabolism and osmotic acclimation in teleost fish. In: Baldisserotto, B.; Mancera, J.M. & Kapoor, B.G. (Eds.). Fish osmoregulation, Sci. Publ., Enfield: 277-307.

Larsen, P.F.; Nielsen, E.E.; Meier, K.; Olsvik, P.A.; Hansen, M.M. & Loeschcke, V. (2012). Differences in salinity tolerance and gene expression between two populations of Atlantic cod (*Gadus morhua*) in response to salinity stress. Biochem. Genet., 50: 454-466.

Makaras, T.; Razumienė, J.; Gurevičienė, V.; Šakinytė, I.; Stankevičiūtė, M. & Kazlauskienė, N. (2020). A new approach of stress evaluation in fish using β-d-Glucose measurement in fish holding-water. Ecol. Indic., 109: 105829. DOI: 10.1016/j.ecolind.2019.105829.

McDonald, G. & Milligan, L. (1997). Ionic, osmotic and acid-base regulation in stress. Fish Stress Health Aquac., 62: 119-145.

Mommsen, T.P. (1998). Growth and metabolism. In: Evans, D.H. (ed.). The physiology of fishes, 2nd edition, CRC Press, Boca Raton: 65-97.

Mozanzadeh, M.T.; Safari, O.; Oosooli, R.; Mehrjooyan, S.; Najafabadi, M.Z.; Hoseini, S.J.; Saghavi, H. & Monem, J. (2021). The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*). Aquaculture, 534: 736329. DOI:10.1016/j.aquaculture.2020.736329.

Ninh, N.H.; Thoa, N.P.; Knibb, W. & Nguyen, N.H. (2014). Selection for enhanced growth performance of Nile tilapia (*Oreochromis niloticus*) in brackish water (15–20ppt) in Vietnam. Aquaculture, 428: 1-6.

Nordlie, F.G. & Leffler, C.W. (1976). Ionic regulation and the energetics of osmoregulation in *Mugil cephalus* Linn. Comp. Biochem. Physiol. Part A: Physiol., 51(1): 125-131. DOI:10.1016/0300-9629(76)90065-7.

Oide, M. (1970). Purification and some properties of alkaline phosphatase from intestinal mucosa of the eel adapted to fresh water or sea water. Comp. Biochem. Physiol., 36(2): 241-252.

Pan, X.R.; Li, G.W.; Hu, Y.H.; Wang, J.X.; Yang, W.Y.; An, Z.X.; Hu, Z.X.; Lin, J.; Xiao, J.Z.; Cao, H.B.; Liu, P.A.; Jiang. X.G.; Jiang, Y.Y.; Wang, J.P.; Zheng, H.; Zhang, H.; Bennett, P.H. & Howard, B.V. (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: The Da Qing IGT and diabetes study. Diabetes Care, 20(4): 537-544. DOI: 10.2337/diacare.20.4.537.

Peyghan, R.; Khadjeh, G.H. & Enayati, A. (2014). Effect of water salinity on total protein and electrophoretic pattern of serum proteins of grass carp, *Ctenopharyngodon idella*. Vet. Res. Forum, 5(3): 225-229.

Reed, P.J. (2015). Hormonal effects on expression of osmoregulatory genes in the gill of Japanese medaka (*Oryzias latipes*). Biological Sciences Undergraduate Honors Theses. Retrieved from https://scholarworks.uark.edu/biscuht/5.

Reitman, A. & Frankel, S. (1957). Determination of aspartate glutamic aminotransferase and alanine aminotransferase. Am. J. Clin. Pathol., 28: 56.

Reindl, K.M. & Sheridan, M.A. (2012). Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 163(3-4): 231-245. DOI:10.1016/j.cbpa.2012.08.003.

Ruscoe, I.M.; Shelley, C.C, & Williams, G.R. (2004). The combined effects of temperature and salinity on growth and survival of juvenile mud crabs (*Scylla serrata* Forskal). Aquaculture, 238: 239-247.

Sangiao-Alvarellos, S.; Arjona, F.J.; Martin del Rio; M.P.; Miguez, J.M.; Mancera, J.M. & Soengas, J.L. (2005). Time course of osmoregulation and metabolic changes during osmotic acclimation in *Sparus auratus*. J. Exp. Biol., 208: 4291-4304.

Shukry, M.; Abd El-Kader, M.F.; Hendam, B.M.; Dawood, M.A.O.; Farrag, F.A.; Aboelenin, S.M.; Soliman, M.M. & Abdel-Latif, H.M.R. (2021). Dietary *Aspergillus oryzae* modulates serum biochemical indices, immune responses, oxidative stress, and transcription of HSP70 and cytokine genes in Nile tilapia exposed to salinity stress. Animals, 11(6): 1621. DOI:10.3390/ani11061621.

Sinha, A.K.; AbdElgawad, H.; Zinta, G.; Dasan, A.F.; Rasoloniriana, R.; Asard. H.; Blust, R. & De Boeck, G. (2015). Nutritional status as the key modulator of antioxidant responses induced by high environmental ammonia and salinity stress in European sea bass (*Dicentrarchus labrax*). PloS One, 10(8): e0135091. DOI:10.1371/journal.pone.0135091.

Takei, Y.; Hiroi, J.; Takahashi, H. & Sakamoto, T. (2014). Diverse mechanisms for body fluid regulation in teleost fishes. Am. J. Physiol. Regul. Integr. Comp. Physiol., 307(7): R778-R792 .DOI:10.1152/ajpregu.00104.2014.

Tran-Ngoc, K.T.; Schrama, J.W.; Le, M.T.; Nguyen, T.H.; Roem, A.J. & Verreth, J. A. (2017). Salinity and diet composition affect digestibility and intestinal morphology in Nile tilapia (*Oreochromis oticus*). Aquaculture, 469: 36-43.

Tseng, Y.C. & Hwang, P.P. (2008). Some insights into energy metabolism for osmoregulation in fish. Comp. Biochem. Physiol. C- Toxicol. Pharmacol., 148(4): 419-429.

Vijayan, M.; Mommsen, T.; Glemet, H. & Moon, T. (1996). Metabolic effects of cortisol treatment in a marine teleost, the sea raven. J. Exp. Biol., 199: 1509-1514.

Wang, K.; Li, F.D. & Sun, Y.Z. (2005) Single or combined effects of fructo and mannan oligosaccharide supplements. Aquac. Nutr., 17: 902-911.

Yada, T.; Uchida, K.; Kajimura, S.; Azuma, T.; Hirano, T. & Grau, E. (2002). Immunomodulatory effects of prolactin and growth hormone in the tilapia, *Oreochromis mossambicus*. J. Endocrinol., 173: 483-492.

Zhu, J.; Wang, X.; Bu, X.; Wang, C.; Pan, J.; Li, E.; Shi, Q.; Zhang, M.; Qin, J.G.. & Chen, L. (2021). Relationship between myo-inositol synthesis and carbohydrate metabolism changes in Mozambique tilapia (*Oreochromis mossambicus*) under acute hypersaline stress. Aquaculture, 532: 736005. DOI: 10.1016/j.aquaculture.2020.736005.