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Effect of Adding Cortisol and Thyroxin Hormones to the Grass carp "*Ctenopharyngodon idella* (Val., 1844)" Diets on Some Physiological and Nutritional Parameters During Salt Acclimatization

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ABSTRACT

The impact of adding cortisol and thyroxin hormones was examined in the present study in the grass carp "*Ctenopharyngodon idella* (Val., 1844)" diets on some physiological and nutritional parameters during salt acclimatization. Seven diets with the same nitrogen and caloric content were prepared: one control diet vs. six experimental diets. Grass carp (24.80± 2.01g) were obtained from the Aquaculture Unit in the College of Agriculture- University of Basrah. Fish were maintained under laboratory conditions (salinity= 1.3g/ l) for one week in plastic aquaria. Fishes were fed twice daily on feed containing 35% protein with a feeding rate of 3% of their body weight. Grass carp were acclimatized to the target salinity of 10g/ L by the addition of sea salt. The growth parameters showed that weight gain was recorded at the highest value in T7 (5.22g), which was significantly different ($P \leq 0.05$) from the other treatments (3.93, 3.53, 4.02, 4.08, and 4.38 for T2, T3, T4, T5 and T6, respectively), while the lowest value was noted in the control treatment. The same trends were found for relative growth rate (RGR), specific growth rate (SGR), daily growth rate (DGR), and feed conversion ratio (FCR). The effects of thyroxin and cortisol hormones on sodium ion in blood plasma indicated a significant ($P \leq 0.05$) decline in all treatments compared to the control treatment in the first day of transportation and continued to the 10^{th} day in treatments T3, T4, T6 and T7. Similarly, a significant ($P \leq 0.05$) effect was found for thyroxin hormone in potassium ion in blood plasma in treatments T2, T3, and T4 with control treatment from the first day of transportation in 10g/ l salinity, while for the cortisol hormone, a significant ($P \leq 0.05$) decline was detected in treatments T6 and T7 start from the first day and continued to the 10^{th} day in treatment T7 compared to the control treatment. Sodium and potassium ions concentration in the muscles showed no significant ($P > 0.05$) differences among all treatments with the control treatment on the first day, but a significant ($P \leq 0.05$) decline in the 10^{th} day in some treatments compared to the control treatment. Water content of muscles during the transportation in 10g/ l salinity showed a significant ($P \leq 0.05$) increase in treatment T3 compared to the control treatment in the thyroxin hormone treatment in the 10^{th} day. However, in the cortisol hormone treatment, a significant ($P \leq 0.05$) decrease was recorded on the 10^{th} day in treatment T7 compared to other treatments. No effect was noticed of the thyroxin and cortisol hormones on the packed cell volume (PCV).

INTRODUCTION

Some functional activities in fish are so complex that could be triggered by many internal and external activators **(Reindl & Sheridan, 2012; Douros *et al.*, 2017)**. The state of internal

metabolism in fish is governed preferentially by environmental signals and inherent indicators to confirm the suitability of surrounding parameters for different biological activities, including feeding, growth, reproduction or energy regulation **(Kageyama *et al.*, 2010; Reindl & Sheridan, 2012)**. Food is considered one of the most important factors among the external indicators which may have an effect on both feeding behavior and growth in fish **(Crespo *et al.*, 2017)**. These processes are strictly controlled by both the availability of food and its components, which reflects primarily on hormones engaging in endocrine regulation **(Bertucci *et al.*, 2019)**. Therefore, endocrine indices may not only provide useful growth biomarkers but also provide an endocrine basis for better understanding growth variation, which will assist producers in developing efficient cultured fish farming systems **(Picha *et al.*, 2008)**. Amongst endocrine is thyroid gland, which is considered a key metabolic regulator in the body of in all vertebrates animals. Thyroid hormones influence many physiological processes, such as growth, development, metabolism, and morphogenesis **(McAninch & Bianco, 2014; Rabah *et al.*, 2019)**. Nevertheless, in most fish, the thyroid role is not completely understood, even though there is homology in genetic mechanisms between mammals and fish **(Zoeller *et al.*, 2007; van de Pol *et al.*, 2017)**. Osmoregulation and metabolite regulation are indispensable physiological processes that control life activities at the cellular and systemic levels. The intricate control of these dynamic processes is intricate due to the interplay of hormones such as thyroid hormones and cortisol. The precise adjustment of metabolic and osmotic regulations, along with their interconnected coordination, primarily involves facilitating both immediate and extended effects. This mechanism aids fish in overcoming challenges and promoting their well-being **(Subhash Peter, 2011; Albadran *et al.*, 2023)**. Regulation of these dynamic processes is complex due to the interaction of hormones such as thyroid hormone and cortisol. Cortisol, a major glucocorticoid is synthesized in the internal tissue of fish; when fish are under stress, cortisol plays an important role in maintaining the balance of both physiological and biochemical processes **(Wendelaar Bong, 1997)**. There are few information about cortisol and its roles in regulating food intake in animal models despite the clear correlation between responses of stress, elevated levels of plasma cortisol and lower levels of food intake. An interaction could be suggested between both plasma levels of thyroxin and cortisol during periods of osmotic regulation **(Arjona *et al.*, 2008)**. There is a noticable joint control between cortisol and thyroid hormones in fish to regulate energy transfer for supporting processes of osmotic regulation and enhancing the activity of sodium pump during periods of chronic regulation **(Flik *et al.*, 2006)**. There is a significant effect of elevated water salinity on the activity of thyroid hormones, which reflects in decreased tri-iodothyronine and increased thyroxin serum levels in grass carp in experimental settings **(Peyghan *et al.*, 2013)**.

Locally, many studies deal with grass carp, but the hormonal aspect was absent from these studies. This experiment was designed to demonstrate the effect of using thyroxin and cortisol as a nutritional stimulant to increase the ability of fish to confrontation with changes in salinity and overcome the osmotic shock stage in grass carp. Additionally, it aimed to evaluate their effect on

some nutritional and physiological parameters to ascertain if thyroxin and cortisol hormone were involved in the osmoregulatory response of fish.

MATERIALS AND METHODS

Experimental diets

Seven diets which have the same nitrogen and caloric content i.e. one control diet vs. six experimental diets are displayed in Table (1).

Table 1. The ingredients of the experimental diets with the additives

Ingredient	Control (T1)	T2	T٣	T4	T5	T6	T7
Fish meal (%)	24	24	24	24	24	24	24
Soybean meal (%)	٢٣	٢٣	٢٣	٢٣	٢٣	٢٣	٢٣
Wheat flour (%)	29	29	29	29	29	29	29
Wheat bran (%)	20	20	20	20	20	20	20
Starch (%)	2	2	2	2	2	2	2
Vitamins & minerals premix (%)	2	2	2	2	2	2	2
Thyroxin (mg/kg feed)	0	5	10	15	0	0	0
Cortisol(mg/kg feed)	0	0	0	0	5	10	15

Experimental fish, feeding, and sampling

Grass carp (24.80± 2.01g) were obtained from the Aquaculture Unit in the College of Agriculture- University of Basrah. Fish were maintained under laboratory conditions (salinity = 1.3g/ l) for one week in plastic aquaria, and fed twice daily on feed containing 35% protein, with a feeding ratio 3% of their body weight. Then, 105 healthy fish were randomly distributed into seven treatments, each with three replicates of 5 fish per tank and stocked in 21 glass tanks (60L). Grass carp were acclimated for seven days to the studied salinity of 10g/ L using sea salt. The water in plastic aquaria was thoroughly aerated and also double-filtered before use, and salinity was checked every day by means of a salinity meter (Mond 720, Germany). Throughout the periods of both acclimation and experiment, temperature was maintained at 23± 3°C; the dissolved oxygen was maintained above 8.6mg/ L, while pH was 7.3± 0.2. Approximately, one-half of the aquaria water was daily exchanged with new aerated water using studied salinity (10g/ l). Additionally, any uneaten feed and feces were daily removed using a siphon tube.

At the end of the experiment which lasted ten weeks, before sampling all fish were deprived of feed for 24h and counted. Growth indicators were measured by the following equations:

Weight gain (WG g) = (W2 − W1)

Daily growth rate (DGR g day^{-1}) = W2 – W1 / day

Relative growth rate (RGR %) = (W2 – W1 / W1) ×١٠٠

Specific growth rate (SGR % day^{-1}) = [ln (W2) − ln (W1)] × 100/days

Feed conversion ratio (FCR) = Food weight / (W2 − W1)
Survival (%) = (N1 − N2) / N1 × 100

Where, N1, N2 is the initial and final fish number, W1, W2 is the initial and the final average weight.

The fish were transferred to 21 plastic tanks, each tank having a capacity of 30 liters of water and a pre-prepared salt concentration of 10g/ L, with three replicates for each treatment and 5 fish in each tank. The duration of the salt tolerance experiment was ten days, taking into account maintaining water quality by providing artificial aeration to the tanks, as well as changing 25% of the tank water from time to time. Samples were taken from the fish on the first day (24 hours after the start of the experiment) and the fifth and tenth days after the start of the experiment. The following physiological measurements were taken: the volume of compressed blood cells, the concentration of sodium ions (Na^+) and potassium (K^+) in the blood serum and muscles, as well as the water content in the muscles.

Physiological parameter

Blood sampling

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

Assay Na^+ and K^+ in plasma, and muscles

Both Na^+ and K^+ ions were estimated using a flame–photometer spectrometer model CL378 after calibrating it with standard solutions of NaCl, and KCl. In the same context, according to **Bath and Eddy (1979)**, the concentration of ions in the muscles can be measured, which is summarized as follows: 0.1g of dry, ground muscle tissue was taken, then the sample was placed in a test tube and 5ml of dilute nitric acid (0.2N) was added to it. It was left for 24 hours in the room with continuous shaking. To complete the extraction process, the tubes were placed in the centrifuge (JANET2KIT5) at a speed of 3500rpm (1507RCF) for 15 minutes. However, 1ml of the filtrate was taken and the volume was supplemented to 10ml with deionized distilled water. Samples were kept under freezing (–12°C) in plastic bottles until they were measured in the same way as measuring blood plasma ions above.

Water content of muscles

Following the removal of scales and skin from the region beneath the dorsal fin, a sample of muscle tissue was extracted and rinsed with distilled water to eliminate any external salts. Subsequently, the muscle tissue was dried using filter paper, and its wet weight was measured using a precise balance. The meat was then subjected to oven drying at 105°C for 24 hours until a constant dry weight was achieved. The percentage of water content in the muscle was determined using the following equation:

$$\text{Water content of muscles (\%)} = \frac{Wet\ weight\ of\ meat\ (g) - Dry\ weight\ meat\ (g)}{Wet\ weight\ meat\ (g)} \times 100$$

Statistical analysis

Experimental data were expressed as mean ± standard deviation. Significant differences between treatments were tested by one-way ANOVA. Significantly differed treatments were compared using LSD test at P= 0.05 applying SPSS software V.26.

RESULTS

The growth parameters presented in Table (2) showed that the initial average weight was 24.80± 2.01g without significant differences (P> 0.05) between all treatments; the final weight was 29.86± 5.00g in T7 that differed significantly ($P\leq$ 0.05) between all treatments except T6 and T5, while it was 28.17± 1.53g in the control treatment. The weight gain recorded the highest value in T7 (5.22g), which was significantly different ($P\leq$ 0.05) compared with the other treatments; while, the control treatment recorded the lowest value. Additionally, the same trends were found for RGR, SGR and DGR.

The feed conversion ratio (FCR) ranged from 7.18 in T7 to 10.22 in T3, while T7 differed significantly ($P\leq$ 0.05) from all studied treatments, except T6 and T5.

Table 2. Growth parameters of grass carp fed diets with thyroxin and cortisol hormone

	Treatment						
	Control (T1)	**10 g/l**					
		T2	**T3**	**T4**	**T5**	**T6**	**T7**
W1	25.05 ±2.10 a	24.59 ±1.36 a	24.81 ±1.45 a	24.82 ±0.40 a	24.81 ±1.43 a	24.87 ±2.03 a	24.64 ±5.22 a
W2	28.17 ±1.53 c	28.52 ±2.65 bc	28.34 ±2.85 bc	28.84 ±0.85 bc	28.89 ±2.09 abc	29.25 ±3.21 ab	29.86 ±5.00 a
WG	3.12 ±0.64 c	3.93 ±3.91 bd	3.53 ±2.02 bd	4.02 ±0.70 cd	4.08 ±1.08 bc	4.38 ±1.28 b	5.22 ±1.50 a
RGR	14.25 ±0.75 c	15.99 ±3.36 bc	14.21 ±1.58 c	16.21 ±0.55 bc	16.40 ±0.82 bc	17.60 ±0.78 b	21.22 ±1.74 a
SGR	0.26 ±0.01 c	0.29 ±0.05 bc	0.26 ±0.02 c	0.30 ±0.01 bc	0.30 ±0.01 bc	0.32 ±0.٣1 b	0.38 ±0.١2 a
DGR	0.07 ±0.٢1 c	0.08 ±0.١2 bc	0.07 ±0.١1 c	0.08 ±0.01 bc	0.08 ±0.01bc	0.09 ±0.01 b	0.10 ±0.01 a
FCR	9.95 ±0.35c	9.10 ±1.73 bc	10.22 ±1.25 c	8.79 ±0.40 bc	8.71 ±0.40 abc	8.41 ±0.30 ab	7.18 ±0.57 a

Different letters in one row indicate significant differences ($P\leq$ 0.05).

Fig. (1A, B) illustrates the effect of thyroxin and cortisol hormones on sodium ion in blood plasma. A significant decline ($P\leq$ 0.05) was observed in all treatments compared to the control treatment in the first day of transportation and continued to the 10th day in treatments T3,

T4, T6, and T7 compared to the control treatment. Furthermore, a significant ($P \leq 0.05$) effect was observed for thyroxin hormone in potassium ion in blood plasma (Fig. 2A, B). This effect was evident from the first day of transportation in 10g/ l salinity in treatments T2, T3 and T4 compared to the control treatment. Meanwhile, for cortisol hormone, the significant ($P \leq 0.05$) decline was observed in treatments T٦ and T٧ on the first day, and this trend continued until the 10^{th} day in treatment T٧ compared to the control treatment.

Fig. (3A, B) shows the effect of thyroxin and cortisol hormones on the sodium ion concentration in the muscles; no significant differences ($P > 0.05$) were noticed between all treatments and the control treatment in the first day, but a significant ($P \leq 0.05$) decline was observed in the 10^{th} day in all treatments compared to the control treatment , except T4.

Fig. (4A, B) exhibits the effect of thyroxin and cortisol hormones on the potassium ion concentration in the muscles; it was noticed that no significant differences ($P > 0.05$) were observed between all treatments and the control treatment in the first day, but a significant decline ($P \leq 0.05$) was recorded in the 10^{th} day in treatments T2, T3 and T4, however no significant differences ($P > 0.05$) were found between treatments T5, T6 and T7 compared to the control treatment.

Fig. (5A, B) displays the effect of thyroxin and cortisol hormones on the water content of muscles during the transportation in 10g/ l salinity. A significant increase ($P \leq 0.05$) was observed in treatment T3 compared to the control treatment in thyroxin hormone treatment in the 10^{th} day. In cortisol hormone treatment, a significant difference ($P \leq 0.05$) was recirded in the 10^{th} day in treatment T7 compared to other treatments.

No effect of thyroxin and cortisol hormones on the packed cell volume (PCV) was observed during transportation in 10g/ l salinity, as shown in Fig. (6A, B). For both hormones, no significant differences ($P > 0.05$) were noted between all treatments compared to the control treatment.

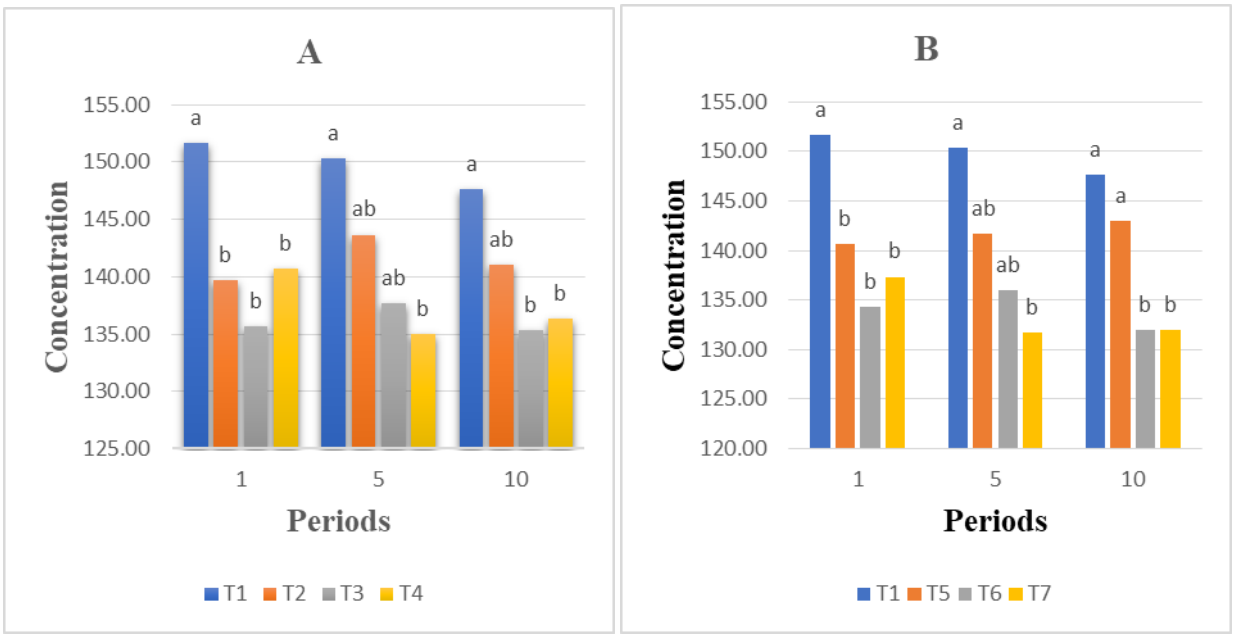

Fig. 1. Sodium ions concentration in blood plasma (mmol/ L) showing: (A) Thyroxin and (B) Cortisol

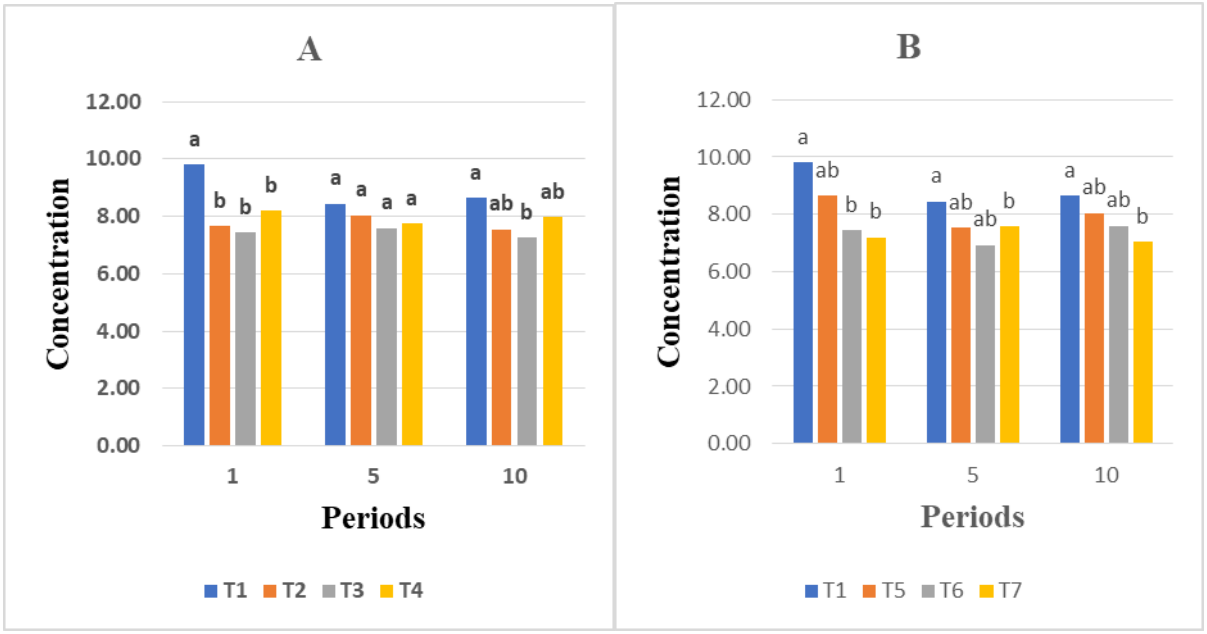

Fig. 2. Potassium ions concentration in blood plasma (mmol/ L) showing: (A) Thyroxin and (B) Cortisol

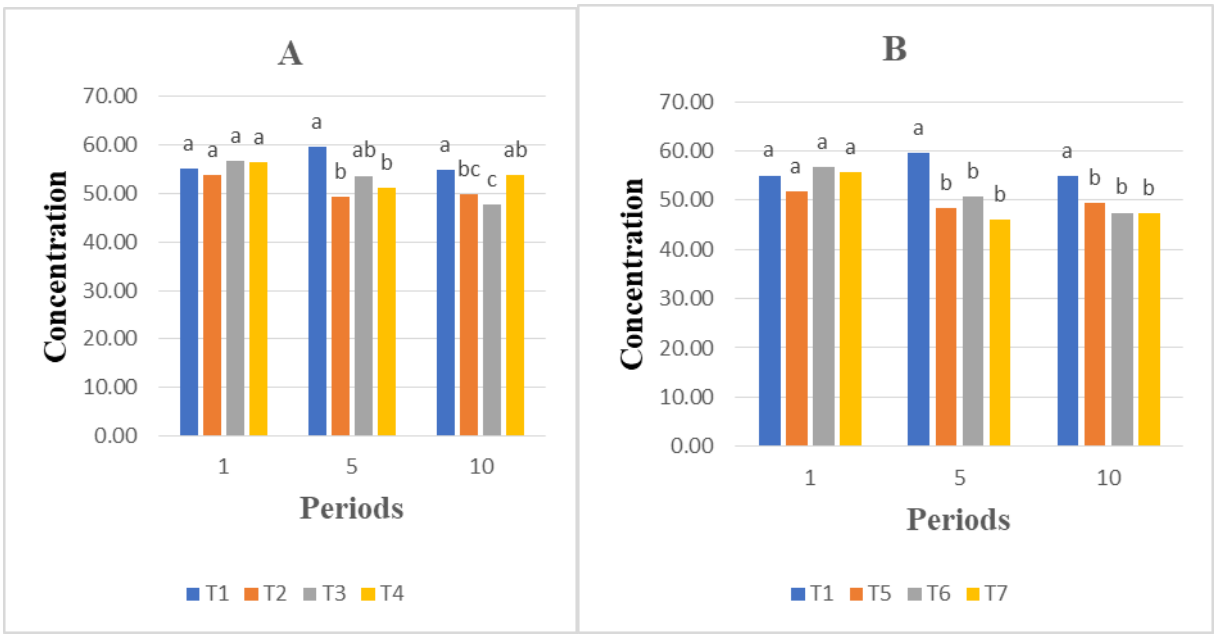

Fig. 3. Sodium ions concentration in muscles (mmol/ 1 kg tissue water) showing: (A) Thyroxin and (B) Cortisol

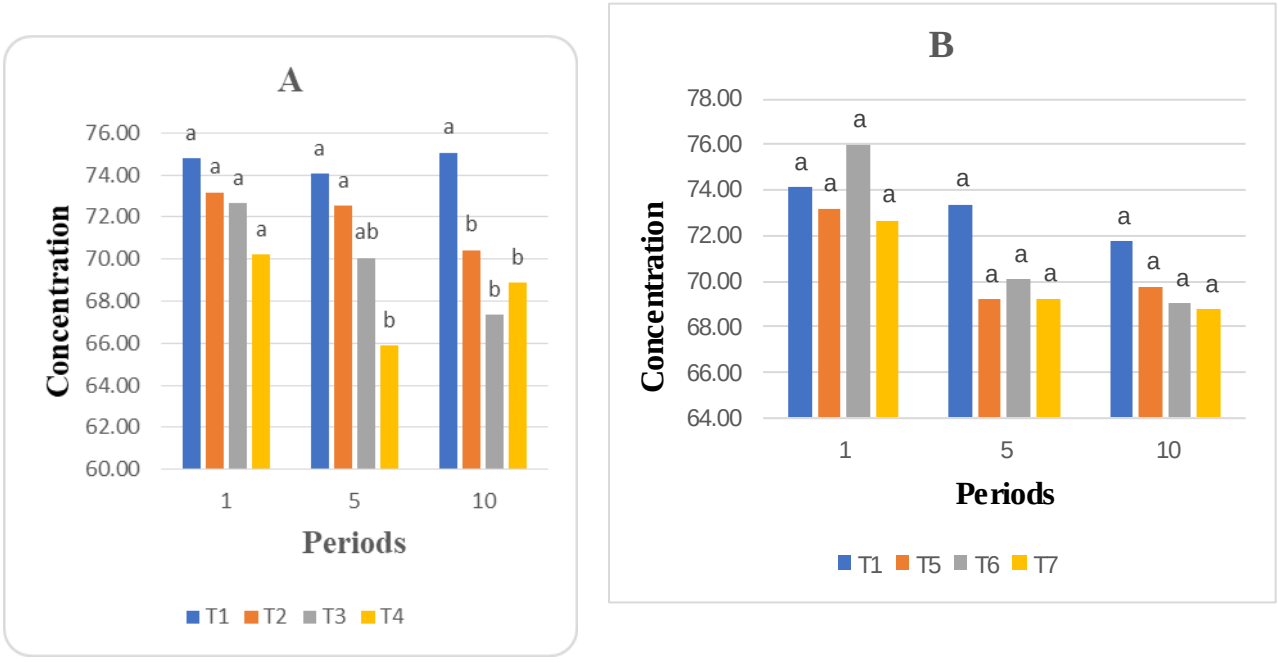

Fig. 4. Potassium ions concentration in muscles (mmol/1 kg tissue water) showing: (A) Thyroxin and (B) Cortisol

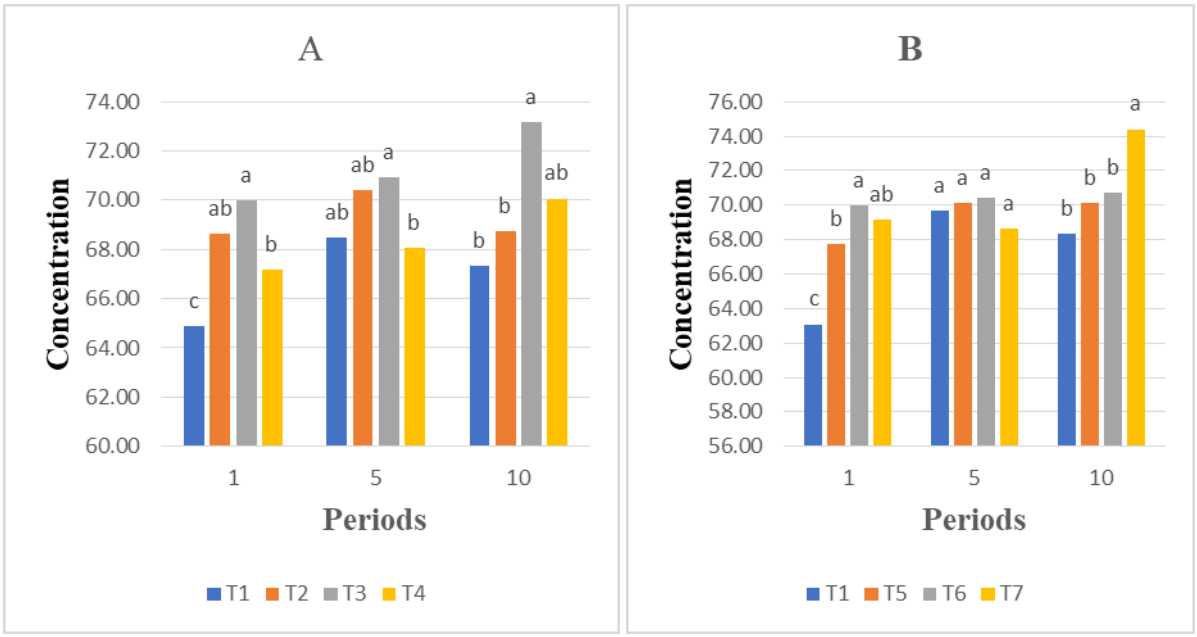

Fig. 5. Water content in muscles (%) showing: (A) Thyroxin and (B) Cortisol

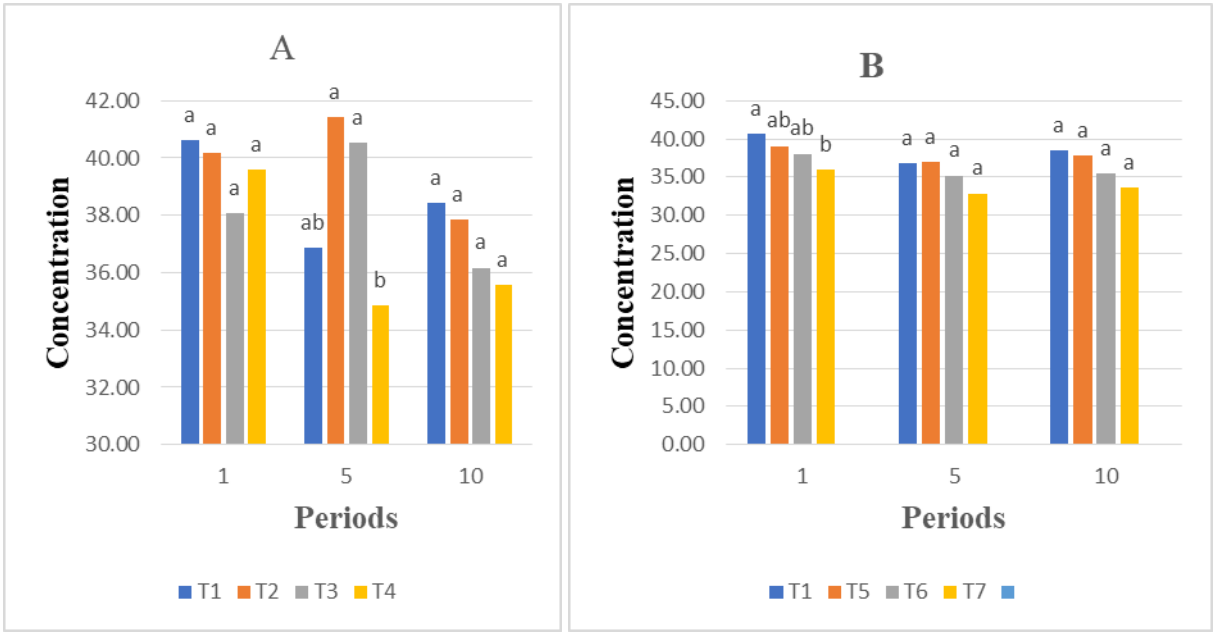

Fig. 6. Packed cell volume PCV (%) showing: (A) Thyroxin and (B) Cortisol

DISCUSSION

Fish body growth is a crucial characteristic that significantly impacts an individual's ability to survive and reproduce. This aspect holds significant implications for population dynamics, ecological interactions, and evolutionary processes. The regulation of the fish's body growth is controlled by the GH/ IGF endocrine axis, which is impacted by a range of factors including

nutrition, feeding habits, hormones involved in reproduction, and abiotic factors such as temperature, oxygen levels, and salinity **(Canosa & Bertucci, 2023)**.

Negative effects were noted on fish growth, reproduction, survival rates, metabolism, and immunity when saline-alkali fluctuations exceeded its osmoregulation capacity **(Sultan & Ahmed, 2014; Shi *et al.*, 2023)**. Furthermore, these variations can have an effect on the performance of fish, the quality of their flesh, and the overall production in the field of aquaculture. Freshwater fish species, like grass carp, tend to experience higher growth rates and better physiological performance when they are cultivated in low salinity environments compared to high salinity environments. This is due to the trade-off in resource allocation between energy expended on osmoregulation and growth of energy in the optimal salinity conditions **(Liu *et al.*, 2023)**. However, when fish deviate from both ionic and osmotic homeostasis due to critical salinity conditions, it has the potential to disrupt their energy supply and hormone production **(Tsuzuki *et al.*, 2007; Shi *et al.*, 2023)**.

It was observed from the present results that thyroid hormone supplementation promotes growth in grass carp since thyroid hormones are necessary to stimulate growth hormone (GH) secretion in the pituitary gland in fish **(Cao *et al.*, 2022)**. Interactions between nutrition and thyroid hormone status was established in many fish species. High thyroid activity, associated with increased food intake, has been recorded in the green sunfish (*Lepomis cyanellus*) **(Deal & Volkoff, 2020)**. This is consistent with the current results, where the feed conversion ratio (FCR) was the best in treatment T3.

Most freshwater fish species such as grass carp exhibited higher growth rate and improved physiological performance when cultivated in a low-salinity environment, compared to a high-salinity environment **(Liu *et al.*, 2023)**. Hence, it has been noted that fish growth is relatively homogeneous. This is since there is a trade-off in resource allocation between energy spent on osmoregulation for growth and development in the preferred salinity environment. However, thyroid hormone addition may enhance growth in grass carp since this hormone is necessary to stimulate growth hormone secretion in the pituitary gland in many fish **(Cao *et al.*, 2022)**, and interactions between nutrition and thyroid status have been confirmed in many species. In the green sunfish (*Lepomis cyanellus*), hyperthyroidism is associated with the increased food intake **(Deal & Volkoff, 2020)**, and it was also observed in the present results that FCR was preferable to the T3 treatment.

Cortisol is a major effector of the HPA axis, which plays an important role in the development, energy balance, and behavior performance **(Denver, 2009)**. The most notorious effects of cortisol are to reduce hepatic expression of the growth hormone receptors Ghr1 and Ghr2 and to reduce serum levels of IGF-1 and hepatic expression of IGF-1 **(Reindl & Sheridan, 2012)**. This is consistent with the present results, as indicated by the absence of significant differences in growth in the treatments that included cortisol. The same trend was observed for the feed conversion ratio (FCR) which increased. This may be attributed to the hormone itself and its elevated levels **(Conde-Sieira *et al.*, 2018)**.

In conclusion, this study highlights the importance of thyroid and cortisol hormones as an important additive to overcome the osmotic shock and stress in grass carp in high salinity waters. Implementing these hormones could substantially enhance fish culture economics by addressing this challenge.

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