Effect of starvation and crowding on some physiological parameters of young common carp (*Cyprinus carpio L.*)

Shaima Saleh Mahmood

Animal Science Department, College of Agricultural engineering sciences, University of Sulaimani, Sulaimani, Iraq
***Corresponding author e-mail: shaima.mahmood@univsul.edu.iq**
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Abstract

Fish frequently undergo stress during aquaculture or experimental operations, which can change any physiological parameter and have an impact on the success of rearing the fish or the outcomes of the research.The purpose of this study was to investigate how different stressors affected some physiological parameters of common carp (*Cyprinus carpio L.*). A total of 162 common carp with body weights ranging from 60.4 to 62.8 g which were evenly distributed in plastic aquaria were subjected to three stressor factors: crowding, starvation, and a combination of starvation and crowding for durations of 24, 72, and 144 hours. Cortisol levels were 167.55, 183.8, 212.8 nmol/L, and 222.65 nmol/L, respectively, for control, crowding, starvation and (crowding+starvation). Unlike haematocrit, which was consistently lower in starved fish, hemoglobin content did not appear to be influenced by starvation. Stressors such as starvation, crowding, and their combination had a substantial influence on typical automotive physiological measures such as cortisol level, blood glucose and hematocrit.

Keywords: Crowding, Hematology, Cortisol, Starvation, Carp.

Introduction

Stress as a condition of diminished fitness or any external agent that challenges an organism's homeostasis or threatens survival [1]. Aquaculture-related stresses can also increase fish illness susceptibility [2]. Aquaculture practices can cause stress and reduce welfare for farmed fish. Factors such as genetics, environmental factors, stocking density, malnutrition, starvation, cataracts, deformities, transport, handling, selection, and overcrowding can all contribute to welfare reduction [3].

The degree and kind of behavioural and physiological response to stress can differ among fish species, strains and individuals [4]. Stress can cause physiological changes, including neuroendocrine responses like catecholamine release and activation of the corticotrophin internal axis, as well as secondary responses like haematological, metabolic, and osmoregulatory changes [5,6].

All vertebrates, including fish, experience stress as a natural part of their lives**.** Stress levels indicate a fish's ability to adapt to adverse stimuli and maintain homeostasis**.** This data is then incorporated into evolutionary and ecological theory to better

understand how animals adapt to, or can adapt to, future stresses. Measuring stress helps us understand carryover effects [7], parenting impacts [8], and life history variance [9,10].

Stocking density, as a fundamental production parameter, may optimize our use of the aquatic environment while also influencing the economic advantages of production. However, stocking density creates intense competition for dissolved oxygen, food intake, and the biological niche in aquatic habitats. Previous studies have shown that high stocking density inhibits growth and reduces immunity and survival rate of aquatic species. Final weight, survival rate, weight gain rate, specific growth rate, and feed utilization rate all decrease significantly as stocking density increases [11, 12, 13, 14].

Food limitation is expected to occur in all animals due to a variety of variables, including population competition, uneven geographical distribution of food, seasonal fluctuations, and ecological instability [15, 16]. Unlike other vertebrates, numerous fish species have demonstrated the ability to survive short or lengthy periods of famine [17]. During fasting, fish experience changes in the depletion of endogenous energy reserves, immunological state, and the expression of genes implicated in several metabolic pathways [18, 19, 20].

The current study sought to clarify the effects of various stressors such as overcrowding and starvation on some physiological parameters of common carp (*C. Carpio L.*).

Materials and Methods

Ethical Approval

The procedure and the way of treatment with live fish in the experiment were approved by ethic committee at the college of agricultural engineering science /University of Sulaimani.

Time and Place

The experiment was done at the animal science research facility at college of agricultural engineering science/ University of Sulaimani/ Sulaimani/Iraq. The experiment was conducted on 1/6/2023.

Research Materials

(Cobas e 411) was supplied from Roche and manufactured which made in Switzerland. Chinese's air compressors, Hailea aco-318 made in China was used for aeration during experiment.

Research Design

The fish were maintained in natural daylight and fed commercial pelleted rations at a rate of 3% of body weight twice daily except starved groups. The fish were separated into four groups (162 fish) with three replicates for each group, which were evenly distributed in plastic aquaria as follows:

The control group, Group I, was kept in appropriate and normal environmental conditions (30 fish).

Group II: crowding was put in plastic aquaria (51 fish).
Group III: Starved fish received regular consistent environmental conditions except that they were starved throughout the trial (30 fish).
Group IIII: crowding and starvation (51 fish).

Work Procedure

Sampling

Blood samples from caudal vein were obtained 24 hours, 72 hours, and 144 hours after the trial began, using heparin as an anticoagulant.

The plasma was divided by centrifugation at 3000 rpm and kept at -20 C for hormonal as well as biochemical analysis. After the plasma was separated, the packed cells were washed three times with 2 ml of 0.65% saline solutions and centrifuged at 3000 rpm for ten minutes [21].

Hormonal assay of cortisol

Cortisol hormone test was performed using the Radioimmuno test (RIA) technology (Cobas e 411) supplied from Roche and produced by Hitachi High-Technologies Corporation [22].

Experimental fish

162 fish were transported from a fish pond in Qaladze/Sulaimani/Iraq. The weights of the fish ranged from 60.4 to 62.8 grams, with a mean beginning weight of 61.7 grams, fish were dispersed among experimental plastic tanks. Prior to the real feeding experiments, 21 days of laboratory pre-acclimation and feeding with commercial pellets (their proportion of components and chemical makeup are shown in tables 1 and 2) were completed. Twelve plastic tanks (70 L water) were used in this trial for four treatments each with three replicates. Proper continuous aeration added to each tank by using Chinese's air compressors, Hailea aco-318. The replicates were randomly placed to reduced differences among treatments. A daily cleaning by siphoning method was applied to remove remained feeds and feces from the system.

Table (1): Chemical composition of the different diet by NRC (1993).

Ingredients	Crude protein %	Crude fat %	Dry matter %	Crude fiber %	Energy kcal/ kg
Animal protein Concentrate	40	5	92.9	2.2	2107
Yellow corn	8.9	3.6	89	2.2	3400
Soybean meal	48	1.1	89	7	2230
Barely	11	1.9	89	5.5	2640
Wheat bran	15.7	4	89	11	1300

Table (2): Composition of experimental diet.

Ingredients (%)	Control 1st treatment	Overcrowded 2nd treatment
Yellow corn	15 %	15 %
Wheat bran	15 %	15 %
Animal concentrate protein	20 %	20 %
Barley	15 %	15 %
Soya bean meal 48%	35 %	35 %
Total	100%	100%
Crud protein	28.06	
Gross energy (kcal/kg feed)	2242.7	

Data Analysis

The trial was carried out using Xlstat 2019 version 54614's one-way (Anova) with completely randomized design (CRD) and general linear models GLM) approach. Duncan's test was used to compare the means of different treatments.

Results and Discussion

Figure 1 shows the values of cortisol that obtained after 24, 72 and 144 hours in fasted and overcrowded fish. After 24 hours of stressor exposure, cortisol concentrations were started to increase compare to control group, there were a significant difference ($p< 0.05$) among treatments, also, noted that after 72 of exposure levels of cortisol slightly decreased compare to 24 hours of exposure to stressors factors. At 144 hours of exposure cortisol level decreased significantly there was a significant difference among treatment (2,3,4) with control group. [23] Defined stress in fish as a summary of physiological reactions occurring when an animal tries to maintain its homeostasis. In the first phase of organism's response to the effect of stressors, endocrine changes appear as primary reactions, considering, above all, the catecholamins and glucocorticoids. They are utilized in controlling the organism and they cause metabolic and osmotic changes, health changes and other changes considered as secondary ones [5,24,25,26]. In agreement with the present study [27] suggested that plasmatic levels of cortisol increase quickly after exposure to an acute stress and the standard conditions are restored in few hours.

Cortisol is widely used both as long term and as short-term stress condition index, even if it may be influenced by species, feeding, reproductive cycles, seasonal cycles, photoperiod, husbandry condition and sampling [28], and multiple stress condition seems to amplify the cortisol response [29]. In addition, teleostean fishes lack aldosterone and mineral regulatory processes seem under dominant control by cortisol [30]. The results of the present work revealed that plasma glucose levels was increased significantly in both starvation and overcrowding group which might be re-

sult from the increased level of catecholamines and cortisol as they are considered the principle hormones in controlling carbohydrate metabolism [31].

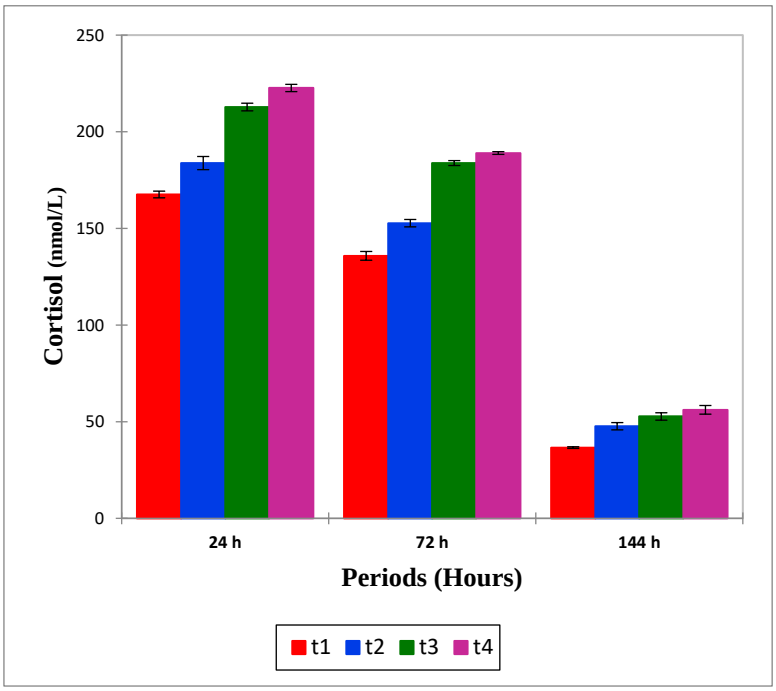

Figure (1): Changes in cortisol (nmol/ L) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Following a similar pattern to cortisol concentrations, blood glucose levels rose in starved and crowded fish compared to the control group. There were significant differences ($p< 0.05$) among control group with treated groups, level of blood glucose started to increase with time of exposure after slightly started to decrease. Control group got lower level of glucose compare with other groups (figure 2). These results are in agreement with the finding of [24] who proved that stress might increase secretion of catecholamines which initially suppressed insulin secretion and subsequently increasing plasma levels of glucose [32].

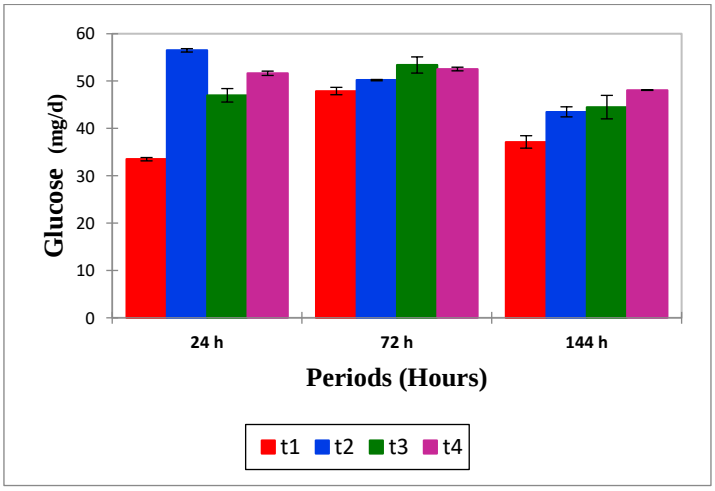

Figure (2). Changes in glucose (mg/ d) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure 3 shows the impact of starvation and overcrowding on white blood cells after 24, 72, 144 hours of exposure to stressor factors. At 24 hours of exposure there were significant differences among treatment, while there were no significant differences among treatments at 72 of exposure of stressor factors. At 144 hours of exposure there was insignificant difference among control group with treatment 2 while there was a significant difference among treatment 3, 4 with treatment 1 and 2. Generally, the fish under starvation has a weaker immune system than fish with appropriate feeding. Thus, the starved fish is prone to pathogen attacks and usually its WBC level is higher than fish with adequate feeding [33].

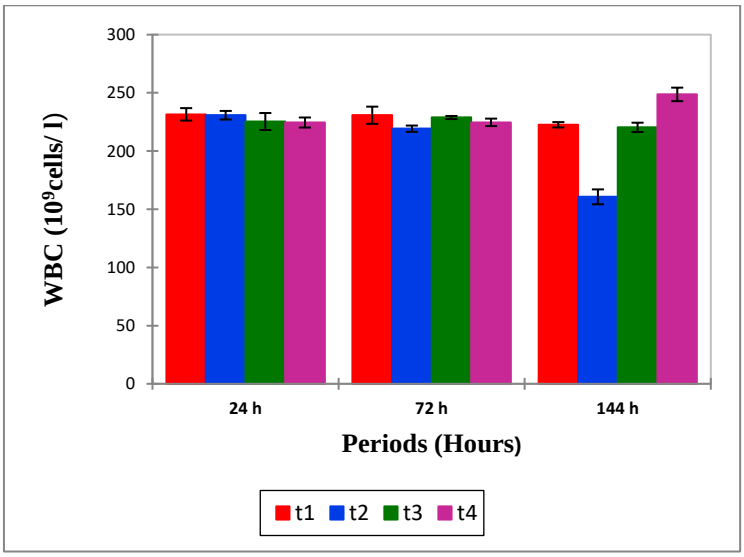

Figure (3): Changes in WBC (10^9cells/ l) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure 4 shows the impact of fasting and crowding on RBC (10^{12}cells/ l) after 24. 72 and 144 hours of exposure. During the trial, the values of RBC were recorded at low number and significant differences ($p < 0.05$) among treatments were seen it. With continuing to exposure the value of RBC were increased significantly compared with previous period.

The number of RBCs is an indicator of oxygen transfer efficiency from respiratory organs to tissues [34, 35]. Therefore, changes in the number RBC could be associated with changes in metabolic levels. Also the RBC count shows the status of the fish immune system. Some studies demonstrated that the fish immune system could be affected by its nutritional situation [36, 37].

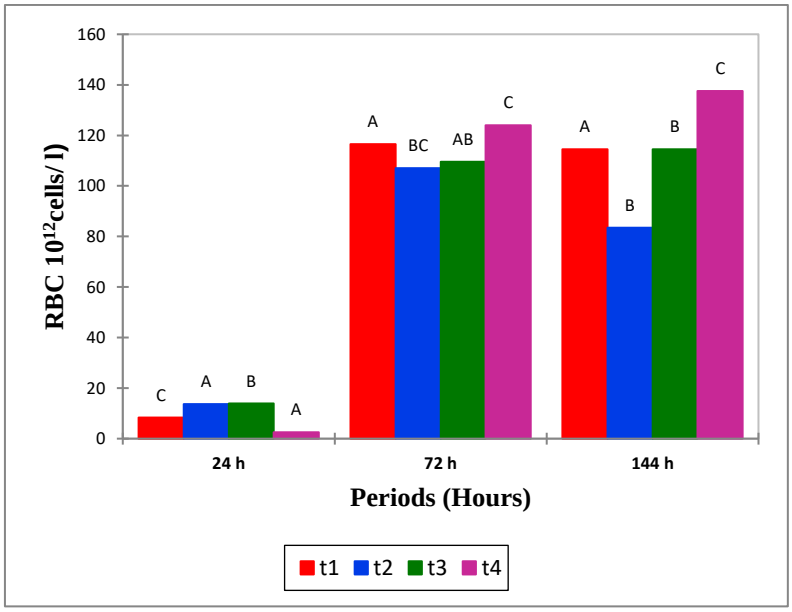

Figure (4): Changes in RBC (10^{12}cells/ l) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure 5 shows the impact of starvation and crowding on HB after 24, 72 and 144 hours of exposure. There was no significant difference in HB values at all times of exposure for stress situations. In this study, the hemoglobin content did not seem to be affected by starvation, unlike hematocrit, which was always lower in fish that had undergone starvation. Conflicting results exist in the scientific literature concerning the effects of starvation on blood hemoglobin content and hematocrit value.

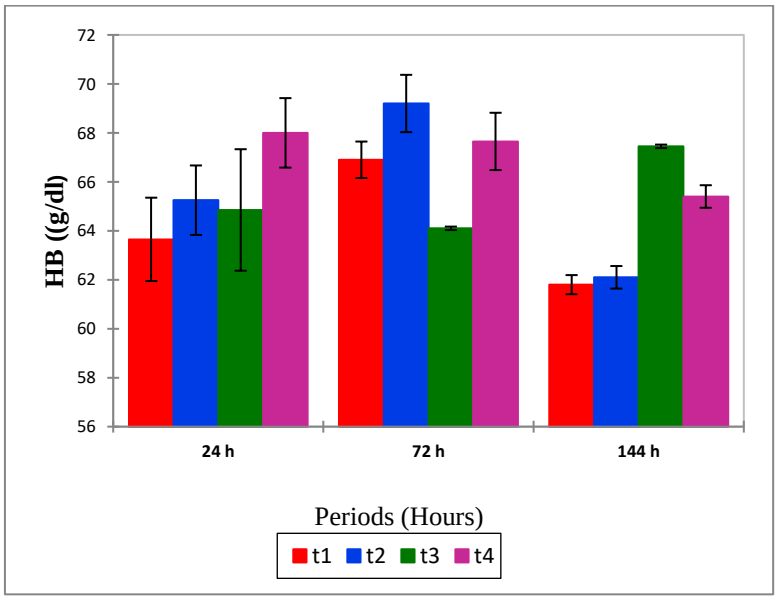

Figure (5): Changes in HB ((g/dl) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure (6,7) show changes in the values of MCH and MCHC during trials. At 24 hours of exposure there was a significant difference among control group with other treatments for MCH value while at the 72 hours of exposure there was no significant difference was recorded among treatments. For MCHC values control group was significantly differing with other treatment at all times of exposure.

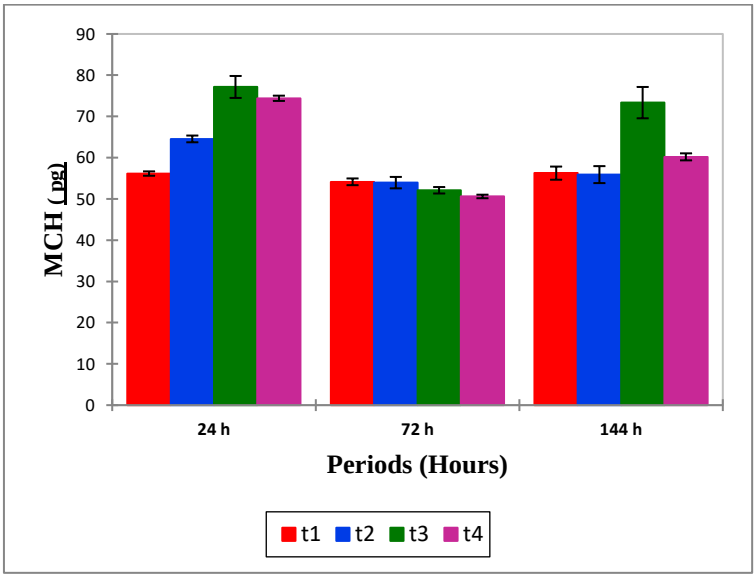

Figure (6): Changes in MCH (pg) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

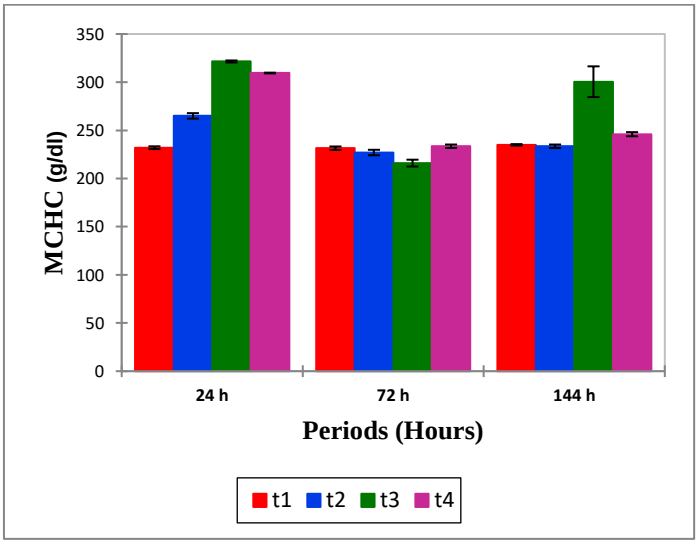

Figure (7): Changes in MCHC (g/dl) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure (8) show the values of PLT during trial, according to the result significant differences were recorded among treatments at 24 and 144 hour of exposure while at 72 hours of exposure only significant difference was seen among treatment 3 with other treatments.

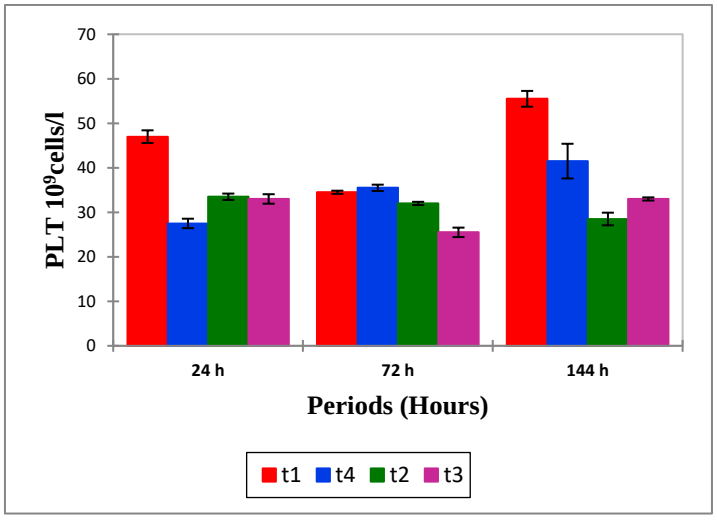

Figure (8): Changes in PLT (10^9cells/l) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

At 24 hours of exposure to stressor factors only significant difference were noted among treatment 3 with other treatments for MCV. At 72 hours of trial significant decrease in the value of MCV were noted while at the 144 hours of exposure there were no significant differences among treatments for MCV (figure 9). According to our results, the lower values of MCH and MCV were observed in starved, although its value was not occasionally significant compared to some other experimental groups. One assumption could be dehydration of the blood due to starvation as reported previously by [38]. In such situations, the volume of each RBC decreases and its hemoglobin content is concentrated.

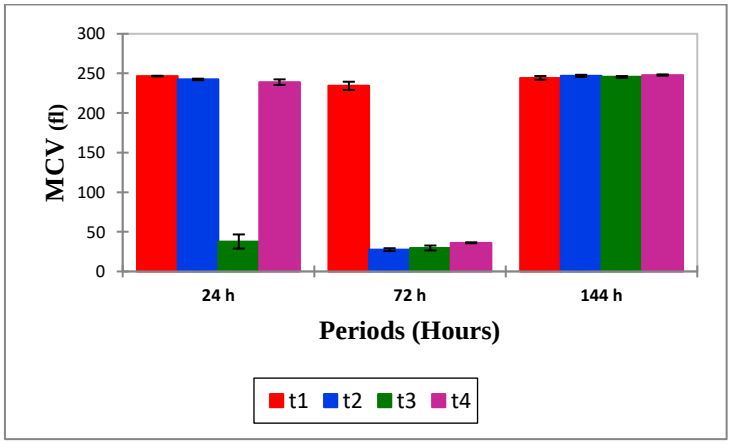

Figure (9): Changes in MCV (fl) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

The hematocrit level (% red blood cells) was significantly lower in treatment 4 (starvation and crowding) fish compared to other groups at 24 hour of exposure. At 72 and 144 hours of exposure HCT (%) was started to increase slightly among treatments (figure 10). [39] reported an increase in the hematocrit value in response to

starvation periods of 90, 145 and 47 days in the Japanese eel, the burbot and the European eel, respectively. On the other hand, [40,41] reported a decrease in the hematocrit and hemoglobin contents in starved carp and rainbow trout, respectively, whereas Larsson and Lewander (1973) [42] showed that starvation did not affect the hematocrit and hemoglobin values of the European eel which had fasted for 150 days.

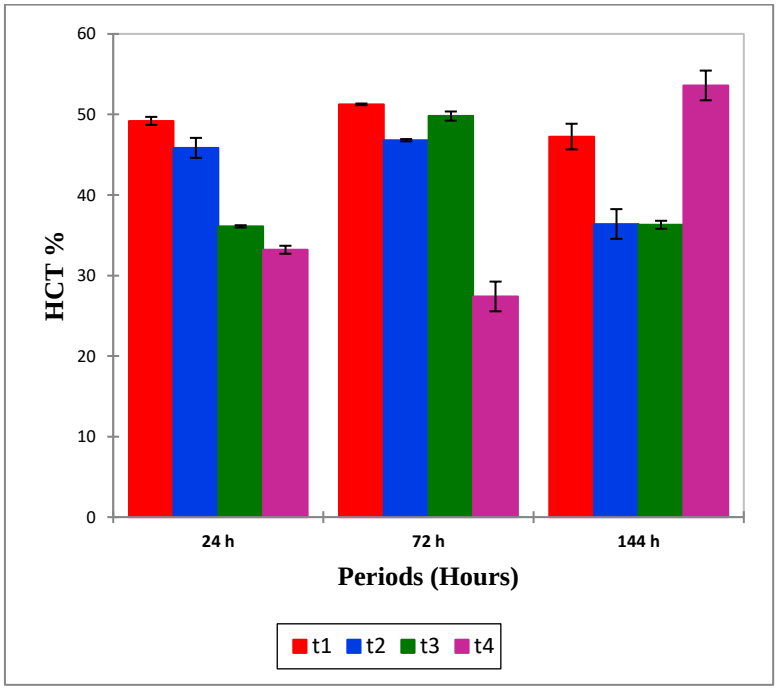

Figure (10): Changes in HCT (%) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

Figure (11, 12) show changes in the values of lymph number and lymph percentage during trials. At 24 hours of exposure there was a significant difference among control group with other treatments for lymph number while at the 72 hours of exposure there was no significant difference was recorded among treatments; also, there was a significant difference at 144 hours of exposure among treatment 1 and treatment 3. For lymph percentage control group was significantly differing with other treatment at 24, 144 hours of exposure. Furthermore, had no significant effect on the WBC count and therefore the percentage of lymphocytes, neutrophils, eosinophils and monocytes in the Persian sturgeon juveniles. Leukocytes were found to be most sensitive to starvation [43]. While lymphocytes are recognized as immunocompetent cells and can affect immune responses in fish (Ellis, 1977) [44]. The increase in total leukocyte values was observed in an air breathing fish Channa punctatus Bloch) [43] and traíra (Hoplias malabaricus Bloch) [38] during starvation. In contrast, a successive decrease in the white blood cells (WBC) count during the starvation period was observed in the European eel, Anguilla *anguilla* L. [39].

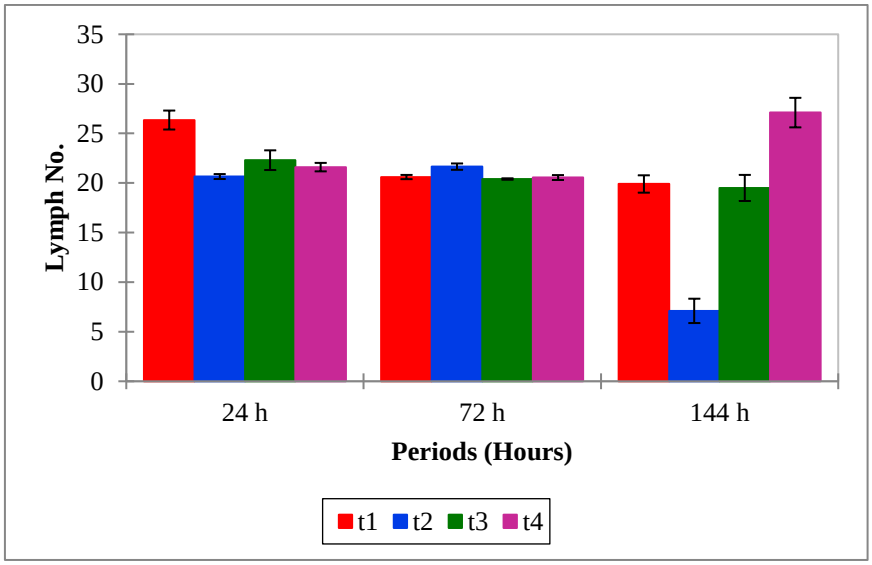

Figure (11): Changes in lymph number (10^9cells/l) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

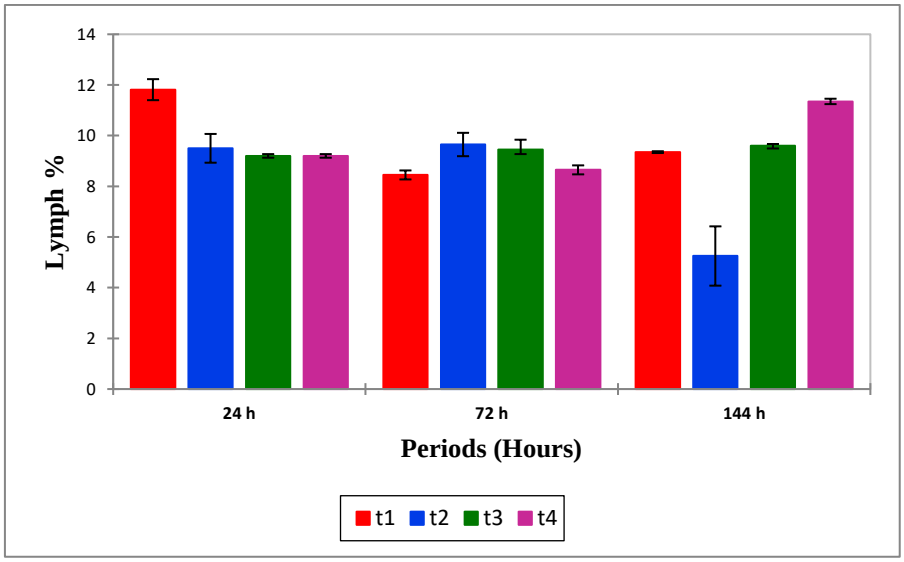

Figure (12): Changes in lymph (%) of young common carp (*C.Carpio*) after the exposure for crowding and starvation for the periods of (24, 72, 144 hours).

According to experiment findings, many stressors have a considerable impact on the physiological parameters of common carp. Stressors can have an impact on fish physiology in both the short and long term, and it is the primary responsibility of aquacultureists to overcome stressors in fish farms.

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