

Effect of starvation on oxygen consumption and some blood parameters of the common carp (*Cyprinus carpio* L.)

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Abstract. The experiment was conducted using 48 fish, common carp *Cyprinus carpio* L. (average weight 41.51 ± 3.51 g). Fish were distributed on 6 aquaria each one represents a starvation period and contained 8 individuals. After two weeks of acclimatization on commercial diet to reduce individual differences in nutritional status, fish were exposed to starvation periods (2, 4, 6, 8, and 10 weeks). Fish weighed every two weeks with the sample of 5 fish for measurements of some blood parameters (Haematocrit PCV, haemoglobin Hb, total number of erythrocytes and leukocytes count, total protein, plasma glucose level) and the rate of oxygen and energy consumption. Body weight rates decreased gradually as a direct result of the starvation process. All the studied parameters except for plasma glucose level decreased significantly ($P \leq 0.01$) after six weeks of starvation, and the decrease continued to the end of experiment which lasted for ten weeks.

Key word: starvation, oxygen consumption, blood parameters, common carp.

Introduction

Food availability may fluctuate widely in many aquatic environments, as a consequence, many aquatic animals, including fishes, may frequently experience periods of starvation. Natural starvation events can be caused by tidal, diel or seasonal cycles, long-term ecological change, and severe anthropogenic disturbance (41). Moreover, feed restriction or deprivation for short periods may also be adopted by fish farmers as a management strategy to reduce mortality due to disease outbreaks.(55) or to solve water quality problems and reduce handling stress (15). Studies on fish starvation are important for better understanding of the growth biology of

fish in wild state (1). Many species of fish are subjected to a natural starvation period during part of the year and have developed an ability to survive without food. (38 , 60). A variety of strategies for surviving to different periods of food deprivation have been adopted by fish, including metabolic, hormonal and behavioral responses. As a rule, fish appear to use catabolic energy conservation strategies which meet their caloric needs but minimize their tissue energy loss (45).

Tissue glycogen levels and plasma glucose concentrations are usually defended during starvation. (42, 35, 6, 20, 45). However, the nature of metabolic changes in starvation depends on the species and duration of the

fasting period. Certain fish such as goldfish, rainbow trout (4) carp, (3) and porgy (51) preserve glycogen stores while metabolizing lipids and/or proteins. Alternatively, other species, such as cod (22), tilapia (25) and coho salmon (32) preserve protein and lipid while partially depleting glycogen stores.

Effects of starvation on the biochemical composition of blood in the Antarctic fish *Notothenia coriiceps* showed a significant increase in glucose levels (60). Short-term food deprivation (7days) and re-feeding (2 days) in tench, demonstrated a significant reduction of plasma glucose levels, supporting the key role of liver glycogen as energy depot consumed during fasting (16).

During starving (long lasting deprivation of food) a very highly significantly decrease in erythrocytes count, leukocytes count, haematocrit and hemoglobin content noted as a consequence of anemia in the starved fish (59, 40, 21, 11). In contrast, (33) reported no changes in European eel after prolonged starvation. Moreover, (28) and (29) found a significant increase after starvation due to the release of RBCs from erythropietic storage. In contrast, prolonged fasting induced a decrease in leucocytic count as a result of impaired immunological defense in burbot fish (56), European eel (29) and *Tilapia nilotica* (52).

Because fish can be expose to periods of starvation during it is life as a result of breeding, changes in temperature and lack of food with the fact that starvation is very influential in the life of fish for of its direct impact on metabolic rate aquarium and the above procedures applied.

and oxygen consumption, growth and survival rate, the present study aimed to investigate the effect of starvation of common carp on some physiological and biochemical parameters.

Materials and methods

Starvation experiment was conducted in the laboratory and lasted for ten weeks represented in five periods of starvation, each with two weeks. Six glass aquaria (60 x 40 x 30 cm) were used which were covered with plastic nets to prevent fish from jumping out. The volume of water in each aquarium was approximately 60 liters and equipped with aerators where quarter of the water volume replaced daily. First aquarium considered as control, while the remaining five aquaria allocated to the five periods of starvation. (48 fish) Fish (common carp *Cyprinus carpio*) used in the experiment brought from the Fish Farming Station at the Marine Science Center. The average weight of fish used in the experiment was 41.51 ± 3.51 g. Eight fish transferred into each aquarium after an acclimation for two weeks on laboratory conditions where fish fed on a commercial diet. The experiment started from 5/11/2012 until 20/1/2013. This period, which lasted 10 weeks, was divided to five periods of starvation, each of two weeks. Fish in control aquarium weighed and samples were taken for estimating parameters (Haematocrit PCV, blood haemoglobin Hb, total number of erythrocytes and leukocytes count, total protein of blood plasma, the level of glucose in the blood and the amount of oxygen and energy consumption) at the end of acclimation. After each period of starvation all fish removed from the

1. Haematocrit (PCV).

The haematocrit estimated by Micro-heamatocrit method as blood withdrawn from fish peduncle with the head upside to help the flow of blood from the caudal artery and/or vein in to capillaries with size 1.1-1.75mm containing a anti-clotting agent. 75% of tube volume filled with blood and one end of the tube closed by a special wax placed in the micro-centrifuge type (MSE) for a period of (2-3) minutes and speed (10000 rpm) to separate the plasma from the cells and measuring the proportion of compressed blood volume using a special ruler Micro-Capillary Reader type DAMON \ IEC.

2. The percentage of blood haemoglobin

The method of Sally Sahli used in estimating the proportion of blood haemoglobin according to (24) which depend on the conversion of haemoglobin to acidic hematite using hydrochloric acid (0.1N). A tube device was filled to the mark 20% with the acid and the blood withdrawn to the mark with mixing and then diluted by distilled water until matching the color to comparison and the result expressed as percentage.

3.Total number of erythrocytes and leukocytes count:

To calculate the total number of red and white blood cells using the method of (36), Haemocytometer device was used which consists of a pipette and a glass slide type " Improved Neubaure " where blood for red blood cells withdrawn by its pipette to the mark 0.5 and the blood diluted by Hymes solution to mark 101. While the white blood cells diluted by Natt-Herrigs solution to the mark 11. Count chamber in the glass slide filled with the solution after placing pipette at

angle of 45° with the slide and cover then tested by compound microscope with power 40X.

4. Total proteins in blood plasma.

total proteins in blood plasma was estimated according to Biuret method (23) using Biuret Kit (from French RANDOX company). In this method copper ions in the middle baseband react with Protein peptide composing colored compound .Then absorbance read on optical Palmttiyav spectro photometer at a wavelength of 546 nm and an estimated blood plasma proteins in unit g/100 ml or unit g/l, according to the following equation

Total protein concentration=

Absorbance of the sample

————— × concentration of standard
Absorbency standard

5. Blood glucose level.

To calculate the percentage of glucose in the blood of fish ACCU-CHEK ACTIVE Irish-made device (GNO2278729) was used. Fish peduncle cut to make the blood flow continuously and a drop of blood placed on the exact location of the device tape installed in the special place after reset device by the slide of the device and the result read directly.

6. Oxygen consumption rate.

The method used for measuring fish oxygen consumption depend on the decline in the amount of dissolved oxygen in water using closed system according to (46) in which a single fish with a known weight (41.51+3.51g) put in a conical glass container (1 liter) full with oxygen saturated water. The acclimated fish

transported to the container and left for 24 hours for confinement acclimation. At experiment starting aeration stopped and the container closed tightly to prevent leakage of oxygen into the container. The amount of oxygen was measured at frequent intervals (30 minutes) until low concentration of dissolved oxygen achieved 60% of saturation. The consumption expressed as $\text{mgO}_2/\text{kgfishwt./h}$. According to (9), oxygen consumption can be converted into energy consumption using $0.00337 \text{ kcal/kg/h}$ equivalent to 1 mg/kg/h .

Data were analyzed statistically using analysis of variance ANOVA (Completely randomized design) with five replicates per treatment to compare the blood parameters during different periods of starvation with a level of significance 0.01 (2), using statistical software SPSS (Statistical package for social sciences, version 16.0.)

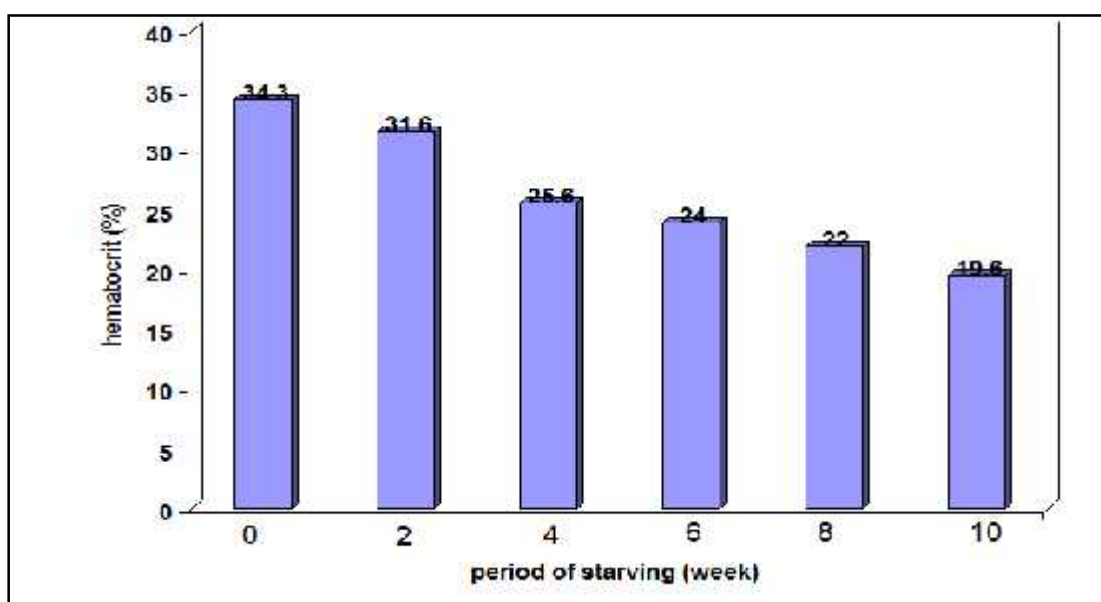
Results

No mortality recorded as a result of starvation during the experiments period, but body weight rates decreased gradually as a direct result of the starvation process (Table 1). In this study, it was demonstrated that during starvation of the com-

mon carp the values of haematocrit, haemoglobin content, erythrocytes and leukocytes count and total protein of blood plasma declined. After 2 and 4 weeks of starvation little fluctuations were noted but the major fluctuation was recorded after 6 weeks of starvation in all the above parameters (Figs. 1, 2, 3, 4 and 5). It was noticed that there are a significant reduction ($P \leq 0.01$) in the values of haematocrit, haemoglobin content, erythrocytes and leukocytes count and total protein of blood plasma during the starvation period, the values of these parameters was, 19% , $5.1 \text{ g}/100 \text{ ml}$, $1.21 \times 10^6 / \text{mm}^3$, $12.87 \times 10^3 / \text{mm}^3$, $47.96 \text{ g}/100 \text{ ml}$ respectively at the end of starvation period in comparison with control (at the starting of starvation period). It was noticed that there is rise the level of glucose in the blood at the starting of starvation period Until the sixth week of the experiment and then continued to decline to the end of the experiment, having valued $77 \text{ mg}/100 \text{ ml}$ (Figs. 6). The results also showed a marked effect on the amount of oxygen and energy consumption. All the studied parameters decreased significantly ($P \leq 0.01$) (Figs. 7 and 8) and the decrease continued to the end of experiment which lasted for ten weeks. The decrease was significant ($P < 0.01$) at week ten compared to week one.

Table 1: Changes in body weight (g) of common carp starved for different periods.

Treatment	Period of starving (week)					
	0	2	4	6	8	10
1	364.42	-	-	-	-	-
2	309.02	281.05	-	-	-	-
3	324.02	303.20	279.43	-	-	-
4	326.06	309.07	292.52	268.75	-	-
5	368.08	347.30	327.66	310.65	308.29	-
6	301.11	284.10	265.72	246.08	240.72	229.33
Average	332.12	304.94	291.33	275.16	274.51	229.33

**Figure (1): changes in haematocrit (%) in the blood of common carp fish during periods of starvation.**

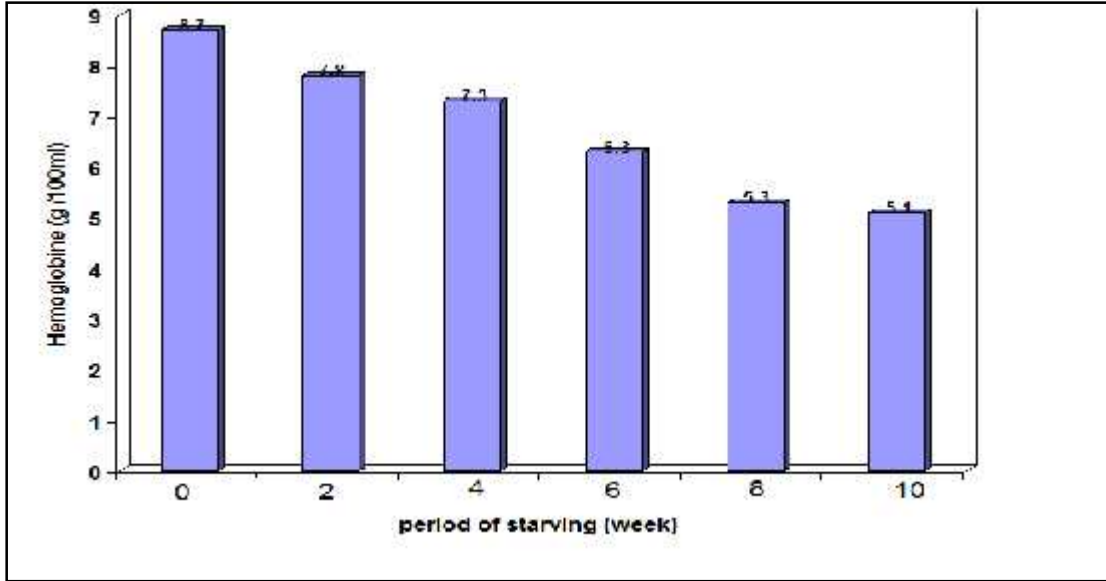


Figure (2): changes in hemoglobin in the blood of common carp fish during periods of starvation.



Figure (3): changes in erythrocytes count in the blood of common carp fish during periods of starvation.

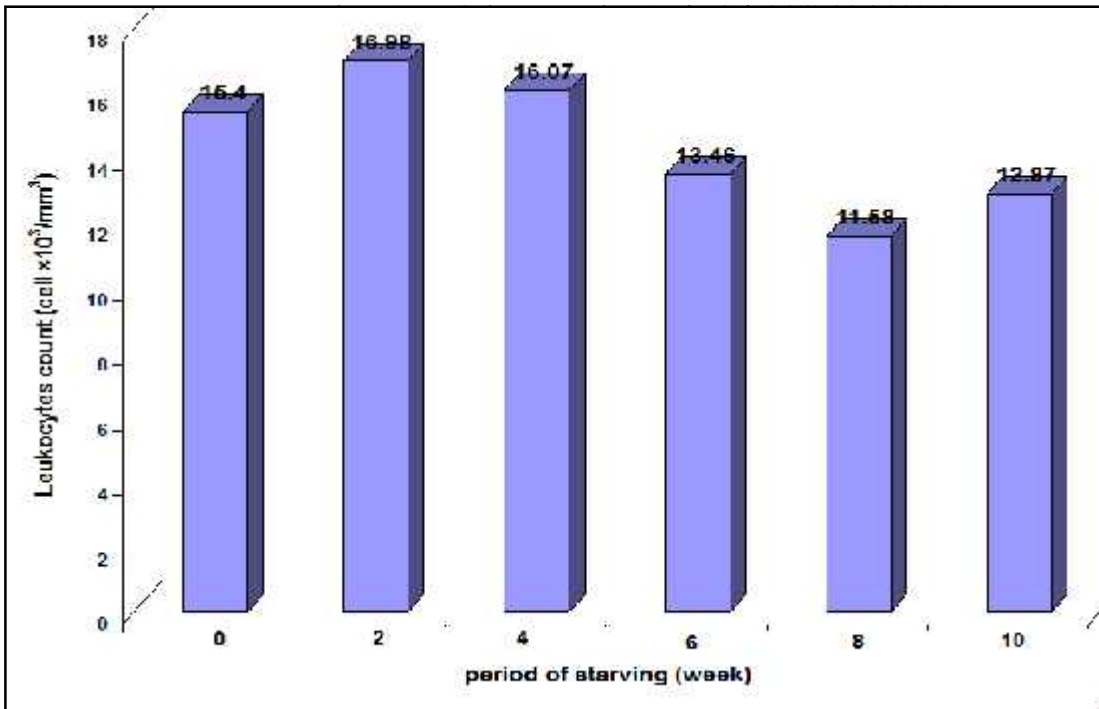


Figure (4): changes in leukocytes count in the blood of common carp fish during periods of starvation.

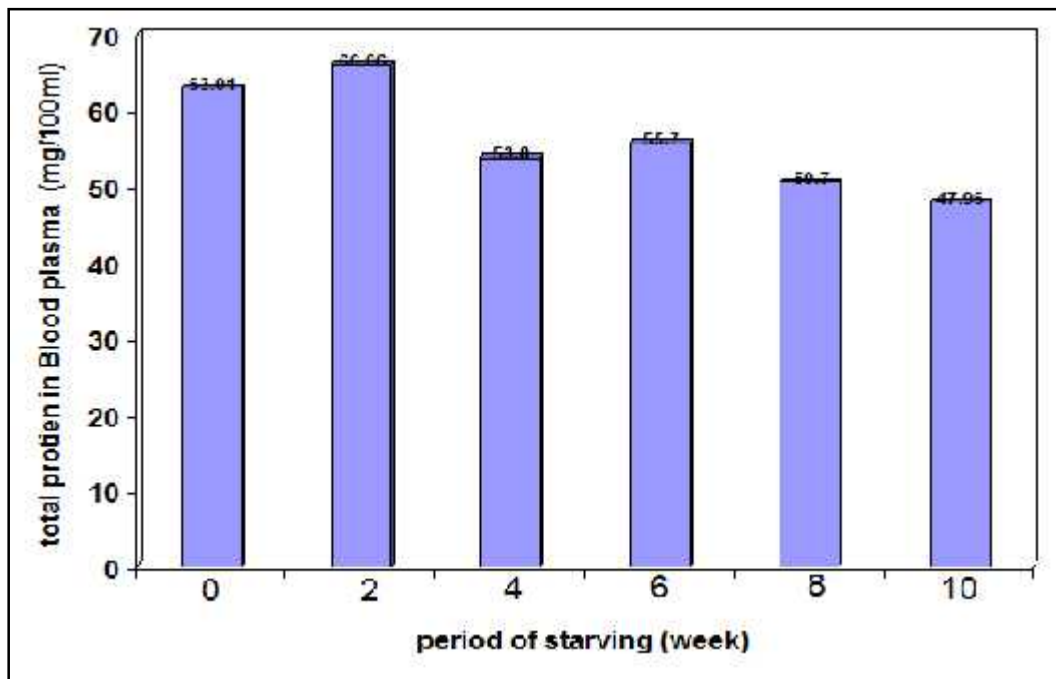


Figure (5): changes in total protein in blood plasma of common carp fish during periods of starvation.

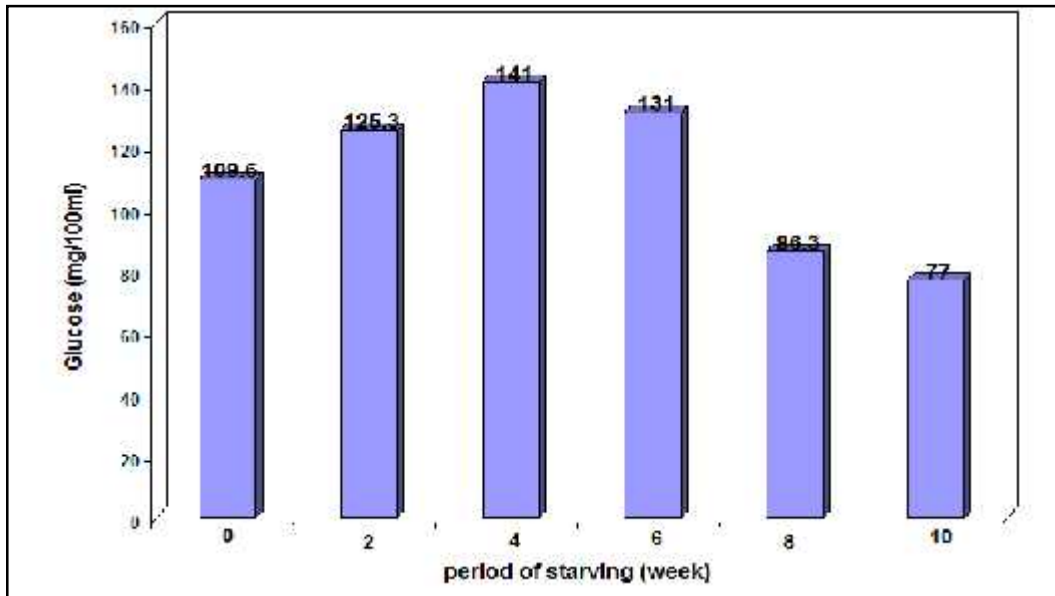


Figure (6): changes in glucose in the blood of common carp fish during periods of starvation.

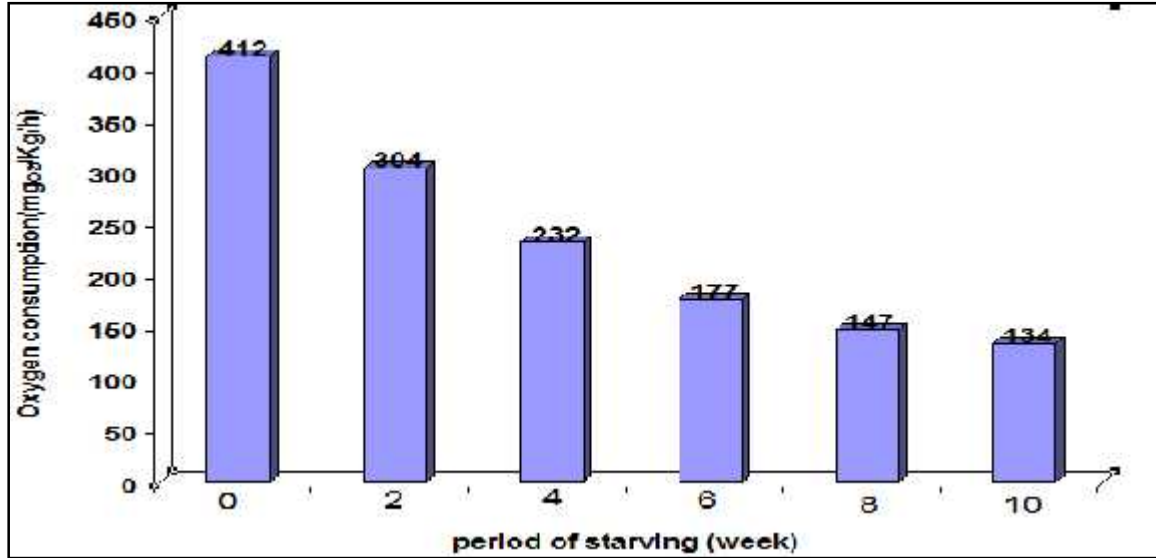


Figure (7): changes in oxygen consumption of common carp fish during periods of starvation

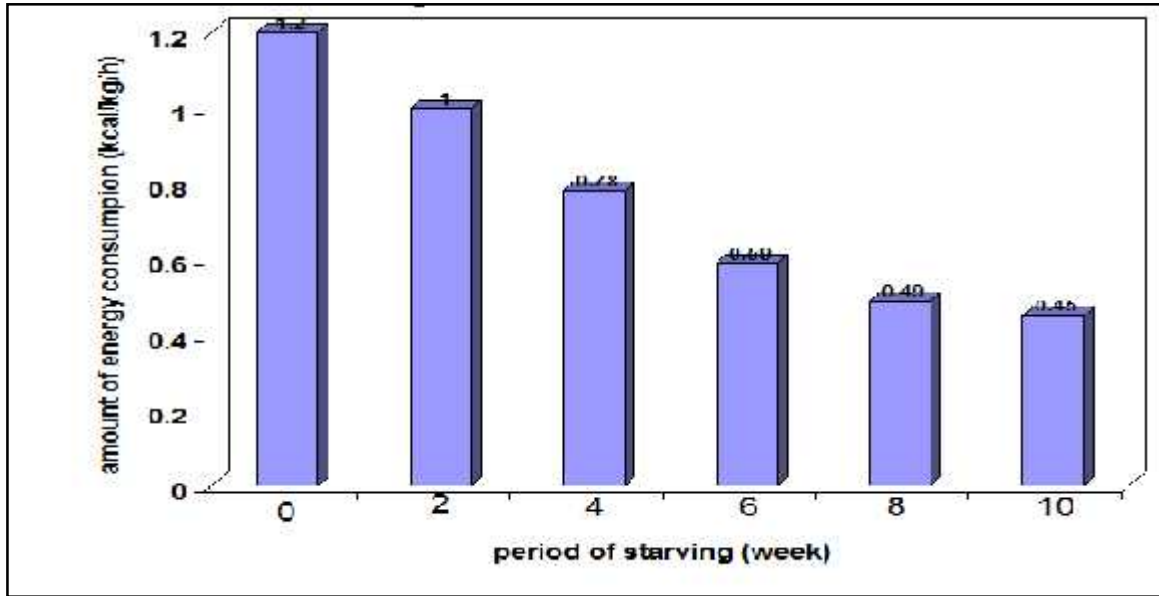


Figure (8): changes in amount of energy consumption of common carp fish during periods of starvation.

Discussion

Literature concerning the effects of starvation on haematological and biochemical parameters in fish is often in conflict. In fact, the physiological effects of starvation may vary considerably in relation to fish species and age, as well as to the length of the period of food deprivation. Experimentally induced starvation could be compared to a condition of (chronic exposure) to stressors, which can lead to decreases in growth and other physiological characteristics (61). Starving resulted in body weight reduction. (21)and(54) showed a significant reduction in total body weight of common carp fish at starvation for 10 weeks as that observed in the present study. A similar result, with a 15% loss of the initial weight, was observed in gilthead sea bream after 46 days of starvation (47). Specimens of traíra, *Hoplias malabaricus*, were

starved for up to 240 days. Two groups were refed after 90 and 240 days of food deprivation. Body mass (Wt) decreased during the starvation periods (49). In contrast, body mass were not considerably affected by fasting for 40 days in the Atlantic salmon (31) or by fasting for 1-2 months in the yellow perch (18), confirming that fish response to starvation may vary according to the species .

In this study, it was demonstrated that during starvation of the common carp the values of erythrocytes count, leukocytes count, haemoglobin content and haematocrit declined. After 2 and 4 weeks of starvation little fluctuations were noted but the major fluctuation was recorded after 6 weeks of starvation in all the above parameters. Conflicting results exist in the scientific literature concerning the effects of starvation on blood haemoglobin con-

tent, haematocrit value and total number of erythrocytes and leukocytes count. In fact, (30) reported a decrease in the haematocrit and haemoglobin contents in starved carp and rainbow trout, respectively. In blackspot sea bream, haematocrit values decreased over time from 41.2 ± 3.2 to 29.5 ± 4.2 % in the starved fish. (10). On the other hand, (29) reported an increase in the haematocrit value in response to starvation periods of 90 and 145 days in the Japanese eel, the burbot and the European eel, respectively. In sea bass, the haematocrit values measured 0 and 31 days after starvation varied between 32.3 ± 2.1 and 40.3 ± 1.8 %, respectively (10). Whereas (11) observed no effect on blood haemoglobin which measured in *Anguilla anguilla* after 31, 42 and 58 days of starvation, However, marked decreases observed in the erythrocyte count, haematocrit, and haemoglobin content in the carp subjected to long-term starving (10). A 5- month starving , led to progressing anemia: the erythrocyte count, haematocrit, and haemoglobin content dropped down to $0.83 \times 10^3 /\text{mm}^3$ blood, 20.3%, and 3.78g/100ml respectively.(59). During starvation the values of erythrocytes, leukocytes counts, haemoglobin content and haematocrit declined. After 15, 21 and 27 days of starvation little fluctuations were noted but the major fluctuation was recorded after 33 days of starvation in all the above parameters,(48). As noted, Changes in erythrocytes numbers, haemoglobin content, haematocrit value, based on weekly samples from a group of starved fish were investigated. After 8 weeks of starvation, erythrocytes were found to be most sensitive to starvation. from week 5 onwards a sharp

decline in these cell populations was noted. The leukocytes showed a change parallel to erythrocytes(39). Adult specimens of traíra (*Hoplias malabaricus* Bloch) were subjected to long-term starvation (30 to 240 days). Counting of immature erythrocytes in peripheral blood showed that erythropoiesis decreased significantly during the first 30 days of food deprivation. The results suggested that a process of senescence takes place in the preexistent red blood cells and that the cells are not replaced during starvation. After 240 days of starvation, *H. malabaricus* had a significantly reduced number of red blood cells and haematocrit (50). These parameters did not recover after refeeding (after 90 and 240 days of starvation). This hypometabolic state in response to food deprivation contributed to energy conservation during these periods. Traíras fish can survive food deprivation for periods of up to 180 days without reductions in metabolism and when they do become hypometabolic, normal metabolic rates are rapidly restored upon refeeding (49). Effects of starvation on the biochemical composition of blood reflected in a decrease in blood crude protein content (21, 54). Erythrocyte numbers in blood of vertebrates ranged from 1 to $5 \times 10^6/\text{mm}^3$ and followed a descending evolutionary scale where the fish have the lowest cell counts (12, 53). This relationship can be further expanded including metabolic activity (8). Thus within the vertebrate group the erythrocytes appear to have significant differences in terms of circulating numbers and longevity which may reflect the metabolic needs of the species (5, 14, 37).

Starvation is known to induce different responses on blood glucose level, depending on how long the starvation lasts, as well as on the species-specific differences in the metabolism and its regulation. Starvation resulted in significant changes in levels of

the carp blood serum components analyzed. An increase of the blood glucose content was observed during the initial 6 weeks of starvation, followed by stabilization of glucose content at week 8 at a lower level and remained henceforth unchanged until the termination of the experiment. This effect may be indicative of an adaptive response of the fish body to new, nutritionally disadvantageous situation and can also evidence for mobilization of glycogen reserves (19). In starved cod, plasma glucose levels are maintained by reducing the rate of glucose utilization and/or increasing glycogen depots, as it occurs rainbow trout (17). In the European eel, *Anguilla anguilla*, glucose levels in the blood plasma were measured after 31, 42 and 58 days of starvation, and compared to those of fed fish. Starvation led in glycemia have been shown in other species under different periods of food deprivation (58, 17, 7). A significant decrease in plasma glucose levels was observed in the sturgeon after fasting for a period of 10 weeks (26), and in carp after fasting for 6 weeks (19). In the European eel, a decrease in blood glucose was observed after fasting for 95 days (33) and for 47 days (13). During early stages of fasting (1 week) carp mobilized liver glycogen depots. This strategy for supplying energy during short starvation periods adopted by carp is in line with other fish species that also use liver glycogen for the maintenance of metabolic functions during early starvation (22, 58, 25, 32). the effect of starvation on the histological structure of the common carp (*Cyprinus carpio*) liver, a progressive reduction of glycogen granules and lipid vacuoles was also observed after 10 weeks (3). Moreover, a fall in plasma glucose levels was clearly produced b

to an increase in glucose levels in starved eels by day 42 (11). Suggesting that fasted fish were able to maintain their value of glycaemia by enhancing gluconeogenesis. The good gluconeogenic capability in the starved eels suggested their adaptive response to food shortage by means of mobilization of non carbohydrate sources in order to preserve their glucose homeostasis. In starved eels, (57) reported the preservation of normal glycaemia by the activation of glycogenolysis in the liver and proteolysis of muscle proteins, both acting as stabilizing factors. During the starvation period, enhanced gluconeogenesis rates have been observed in many species of teleosts (64, 44, 43, 18). Significant decrease in plasma glucose levels was observed in the common carp fasting for a period of 10 weeks. Similar decreases

fasting in carp. Concomitant decrease in plasma glucose in carp after starvation indicates that the active glycogenolysis produced to counteract fasting was not enough to maintain the glycemia. Length of starvation period was associated with a significant decrease in the oxygen consumption (49). The present study revealed that the effect of starvation on the metabolic rate of *C. carpio* can be observed for longer than a week into the starvation period; however, keeping in mind that long-term starvation causes stress in fish. Under such conditions, the energy expenditure on active metabolism increases, and this may affect the results. This can be explained by changes in the biochemical composition of fish tissues and in the relative size of various organs. A reduction in ribosome numbers, protein synthesis rate, and metabolic activity were observed in animals that are starved for a long period (34 , 27). The metabolic rate reduction observed in small pike-

perch starved for 26 days might have resulted from such changes. (62). Average oxygen consumption in *Stizostedion lucioperca*, decreased from 355.70 mg O₂ kg⁻¹h⁻¹ in fish fed to 163.22 mg O₂ kg⁻¹h⁻¹ in starved fish (63). Further starvation resulted in a reduction of oxygen consumption which can be explained by changes in the biochemical composition of fish tissues and the relative size of various organs (63).

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تأثير التجويع على معدل استهلاك الاوكسجين وبعض القياسات الدموية في اسماك الكارب الشائع *Cyprinus carpio* L.

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المستخلص. استخدمت في التجربة 48 سمكة كارب شائع *Cyprinus carpio* (معدل الوزن 41.51 ± 3.51 غم)، وزعت الأسماك على ستة أحواض (كل حوض يمثل فترة تجويع) بمعدل ثمانية أسماك لكل حوض، بعد فترة أقلمة لمدة أسبوعين غذيت خلالها الأسماك لحد الإشباع على عليقة تجارية لتقليل الفروقات الفردية في الحالة التغذوية بين الأسماك قبل بدء التجربة، عرضت بعدها الأسماك للتجويع على فترات 2 و 4 و 6 و 8 و 10 اسابيع، وزنت الأسماك كل أسبوعين مع أخذ عينة (5 اسماك) لاجراء القياسات المطلوبة لبعض مقاييس الدم (مكداس الدم الهيموغلوبين و العدد الكلي لكريات الدم الحمراء والبيضاءو البروتين الكلي ومستوى سكر الدم) ومعدل استهلاك الاوكسجين والطاقة. معدل وزن الجسم انخفض تدريجيا كنتيجة مباشرة لعملية التجويع. كما لوحظ انخفاض معنوي ($p \leq 0.01$) في اغلب القياسات المذكورة اعلاه بعد أربعة أسابيع من المجاعة، و الانخفاض استمر حتى نهاية التجربة التي استمرت لمدة عشرة أسابيع.