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EFFECT OF PROTEIN CONTENT ON CONDITION FACTOR AND SOME BLOOD PARAMETERS OF GRASS CARP *Ctenopharyngodon idella* CULTIVATED IN EARTHEN PONDS

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Abstract

The current experiment was conducted in Basra Governorate in the ponds of the Aquaculture Unit at the Agricultural Research Station-College of Agriculture-University of Basra, located in Al-Haritha District, for the period from 4/3/2020 to 6/12/2020. Grass carp fingerlings (3760 individuals) with an average weight of 16 ± 2.75 g and an average total length of 12.1 ± 0.1 cm, were used in the study. These fingerlings were divided into four treatments, and each treatment with 2 replicates in 2 ponds of 600 m^2. Four diets were manufactured with different protein ratios (T_1 20, T_2 25, T_3 30 and T_4 35 %). The aim of current experiment was to find out the optimal proportion of protein in grass carp diet, which achieve the best health and condition factor. The results of current experiment revealed the grow that the beginning of the experiment was asymmetric-negative (b = 2.8543), and the statistical analysis proved this value recorded a significant difference ($P \leq 0.05$) from the t-test for the ideal value 3. Also, the growth was negative asymmetric for all treatments at the end of the experiment, where the value of (b) ranged from 2.6459 in T_2 to 2.8871 in T_4, with significant differences ($P \leq 0.05$) compared to the ideal value 3. Statistical analysis of the results for the modified condition factor (Kb) showed that there were significant differences ($P \leq 0.05$) between fish cultivate at the beginning and end of the experiment and also significant differences ($P \leq 0.05$) among the four treatments. Statistical analysis of relative condition factor (Kn) indicates that there were no significant differences ($P > 0.05$) between fish at the beginning and end of the experiment, as well as between the four treatments at the end of the experiment. It was found through the statistical analysis of the results that there were significant differences ($P \leq 0.05$) for all blood measurements among the fish before the experiment: 3.96 g/100 ml, HCT 13.90 %, RBC 1.76 and after the experiment. Statistical analysis of the results proved also that there were no significant differences ($P > 0.05$) in blood measurements for all treatments.

Key words: *Ctenopharyngodon idella*, Grass carp, Protein content and Blood parameters.

1. Introduction

Fish has been economically important in all countries of the world since ancient times, as the importance of fish reaches in some countries and regions to the extent that it constitutes a high percentage of the daily food for human, as well as being exploited in many industries such as feed, fertilizers, dyes, oils, gums and some medical preparations (Al-Shkakrchy and Ahemed, 2013). Grass carp, *Ctenopharyngodon idella* is a native fish of East Asian large rivers and was transferred to other regions in 1945 mainly to control aquatic vegetation (Taher, 2020a).

Grass carp, *Ctenopharyngodon idella* in the past was belonging to the family Cyprinidae and it was belonging to Xenocypridinae family

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according to the recent phylogenetic studies (Tan and Armbruster, 2018). The global demand for aquatic-source foods is constantly rising, and this is not only due to the growing population, but also because the preference has become for healthy foods, in addition to the fact that fish is a good food through its contribution to high-quality protein and nutrients important for human health (Abimorad and Carneiro, 2007). Since the aquaculture is the fastest growing sector in global food production, as it reached around 122.6 million tons in 2020, which is more than half of the total global production of fish, which reached 214 million tons, this rapid growth of fish farming made it possible to be a major source of future supplies for food fish, instead of commercial fisheries (FAO, 2022). Because animal protein, especially fish protein, is the most expensive component of feed in the diet manufactured for feeding fish, so our goal in our current study is to determine the optimal percentage of protein that is included in the composition of the diet of grass carp fish, which achieves the rapid and required growth and the use of the condition factor and some blood parameters to determine the appropriateness of this ratio proposed protein and its effect on the health status of grass carp.

2. Materials and Methods

Study area

The current study was conducted in Basra Governorate in the earthen ponds of the Aquaculture Unit at the Agricultural Research Station - College of Agriculture - University of Basra, located in Al-Hartha District, for the period from 4/3/2020 to 6/12/2020.

Study fish

Totally, 3760 grass carp fingerlings were used, with an average weight of 16 ± 2.75 g and an average length of 12.1 ± 0.1 cm.

Earthen culture ponds

Eight earthen ponds, with an area of 600 m^2 per pond, with an average depth of 2 meters were used. The ponds were dried before culturing processes. One pond was filled with water two days before the arrival of fish to reduce the presence of natural enemies to a minimum. All fish were placed in this pond and left for three days in order to acclimatization before dividing them into the eight ponds, which were filled with water later. Nearly, 15 – 25 % of the pond water is replaced daily and about 75 % of water replaced during periodic sampling in order to facilitate the process of catching fish samples.

Fish feed

Four commercial pellets diets (diameter of 4 - 6 mm) were manufactured by the feed plant of Agricultural Consultant Office belonging to Agriculture College with different ratio of crude protein (20, 25, 30 and 35) % for treatments T1, T2, T3 and T4 respectively by using different feed ingredients (Table - 1). Fish were fed on these diets throughout cultivation period. The chemical analysis of the different diets was carried out in the Al-Ghadeer Laboratory that located in the center of Babylon Governorate, and three replicates were taken from each of the four treatments.

Environmental factors

Water temperature of the ponds was measured with the periodic fish sampling during the period of the experiment using a Chinese-made mercury thermometer, to the nearest degree Celsius. The salinity and pH of the ponds were also measured by a German device produced by Yasa company, and the dissolved oxygen was measured using a digital device of the type Lovibond Senso Direct 150.

Fish weight measurements

About 10 % of the total fish number were caught randomly by beach trawls approximately every 20 days (sometimes increased according to the available conditions) from each pond and weighed in the farm using an electronic scale to the nearest gram. Feeding ratio ranged between 3 – 5 % of total fish weight, and the amount of daily feed provided to the fish is adjusted according to the new weights. The fish were handily fed three times a day (morning, noon and afternoon) at the same feeding places for each pond.

Condition Factor (K)

The condition Factor (K) of grass carp was estimated using the following equations:

a) Fulton condition Factor, K value calculated according to Froese (2006): $K3 = 100\ w/L^3$.
b) The Modified condition Factor parameter was estimated according to Ricker, 1975): Kb = 100W/L^b.
c) The relative condition factor (Kn) for fish was calculated monthly according to the equation of (LeCren, 1951): Kn = W/wˆ where W is the actual weight of the fish and wˆ is the weight of the fish calculated from the length and weight equation.

Blood measurements

Blood samples were taken from grass carp with three replicates before the experiment and three replicates for each treatment after the end of the experiment from the caudal vein after cutting the fish tail with a sharp medical blade and placing the fish at an angle of 45° with a blood collection tube with a capacity of 2 ml containing a substance (Heparin) to prevent blood clotting. The tube was moved gently after closing it in a rotational movement for 2 - 3 minutes and the treatment and duplicate number was fixed on it. The replicates of each treatment were placed in an independent plastic bag to avoid mixing between the replicates of the different treatments. The samples were placed in the refrigerator until they were transferred a few hours later to Al-Laith Laboratory in the Five-mile area of Basra Governorate. The necessary tests were carried out before and after the experiment in the same laboratory in an advanced American-made device (GENEX) that contains standard information for various blood tests, and usually 0.2 ml is drawn from each sample, and it deals with it within one minute to get the paper results out through a printer linked to the device. Clove oil was used two drops per liter of water for ten minutes to anesthetize the fish before killing them (Al-Faiz *et al*., 2014; Alashaab *et al*., 2017 and Al-Niaeem *et al*., 2017). The following checks were performed:

a) Measurement of hemoglobin (Hb)
b) Hematocrit Test (HCT)
c) Red blood cell measurement (RBC)
d) White blood cell measurement (WBC)

Statistical Analysis

The results of current experiment was conducted with a completely randomized design, and the differences between the means were tested by analysis of variance (ANOVA). The significant differences were tested by LSD test at 0.5 % probability level by SPSS program Ver. 26.

Table – 1: The proportions of the main components included in the composition of the experiment diets

Ingredients	Ratio %			
	T1	**T2**	**T3**	**T4**
Fish meal	5	15	25	38
Soybean meal	22	22	22	22
Starch	2	2	2	2
Wheat flour	29	29	29	29
Wheat bran	40	30	20	7
Mixture of vitamins and minerals	2	2	2	2

3. Results

Table - 2 shows the measurements taken for some environmental factors during the experiment. The water temperature was the most variable and the highest temperature was recorded 38 °C in August and the lowest 17 °C in March, while the changes in the pH were limited and ranged between 7.4 in March and 8.7 in August. The salinity of the water in ponds ranged from 3.3 g/L in March to 6.0 g/L in August, while the highest dissolved oxygen concentration was 7.7 mg/L at March and the lowest concentration was 4.3 mg/L at August. Figure (1) represents the relationship between length and weight at the beginning of the experiment, as the value of (b) was equal to 2.8543, and this value recorded a significant difference ($P<0.05$) for the t-test from the ideal value of 3, while the coefficient of determination (R2) was 0.8203 and the growth is asymmetric negative.

Figures (2) represent the length-weight relationship for the different treatments at the end of the experiment. The value of (b) for these relationships ranged from 2.6459 in the second treatment to 2.8871 in the fourth treatment, and this confirms the existence of significant differences ($P\leq0.05$) for the t-test compared to the ideal value 3, while the determination value ranged from 0.8358 in the first treatment to 0.9801 in the fourth treatment. The growth in the first treatment was negative asymmetric, as the difference was significant ($P\leq0.05$), where the b value was 2.7789 and the coefficient of determination was 0.8358, As well as the other treatments, all of their results indicated negative asymmetric growth with significant differences ($P\leq0.05$) for the t-test compared to the ideal value of 3. In the second treatment, the value of b was 2.6459 and the coefficient of determination was 0.9394, and in the third treatment the value of b was 2.7737 and the coefficient of determination was 0.9328, while in the treatment T4, the value of b was 2.8871 and the coefficient of determination was 0.9801.

Table (3) shows three types of Condition factor for grass carp at the beginning and end of the experiment. Statistical analysis proved that there were significant differences ($P\leq0.05$) in Fulton's condition factor (K3) between fish at the beginning and end of the experiment for the four treatments whose relationships differed in the percentage of crude protein. The third treatment was significantly ($P\leq0.05$) superior to fish in the rest of the treatments, and there were no significant differences ($P > 0.05$) between these three treatments. Statistical analysis of the results of the Modified condition factor (Kb) showed that there were significant differences ($P\leq0.05$) between fish reared at the beginning and end of the experiment. Significant differences ($P\leq0.05$) were found in the Condition factor among the four treatments. Statistical analysis indicates the results of the relative condition factor (Kn) that there are no significant differences ($P>0.05$) between fish that were cultured at the beginning and end of the experiment, as well as between the four treatments at the end of the experiment.

Table (4) shows the results of hematological measurements of grass carp before and after the experiment. These measurements included all of the Hemoglobin (Hb), the volume of red blood cells (Hematocrit Test, HCT), Red Blood Cell (RBC) and White Blood Cell (WBC). The values of the previous tests before the experiment were: Hb 3.96±0.50 g/100 ml, HCT 13.90 % ± 1.05, RBC 1.76 ± 0.33 and after the experiment for the first treatment: Hb 8.43 ± 1.25 g/100 ml, HCT 27.63 ± 3.66%, RBC 2.76 ± 0.27 (10^6 cell/mm³), WBC 43.60 ± 5.29 (10^6 cell/mm³) and for the second treatment: Hb 7.73 ± 0.63 g/100 ml, HCT 25.26 ±1.70%, RBC 2.68 ± 0.27 (10^6 cell/mm³), WBC 39.20±5.75 (10^6 cell/mm³), third treatment: Hb 7.70 ±0.70 g/100mL, HCT 26.13 ±1.64%, RBC 2.64 ± 0.25 (10^6 cell/mm³), WBC 42.93 ± 7.05 (10^6 cell/mm³), for the fourth treatment, the values were as follows: Hb 8.10 ±0.26 g/100 ml, HCT 26.23 ± 0.73 %, RBC 2.79 ± 0.14 (10^6 cell/mm³), and WBC ± 42.96 0.86 (10^6 cell/mm³). It is clear from the statistical analysis of the results that there were significant differences ($P\leq0.05$) for all blood measurements between fish before the experiment and fish after the experiment, while no significant difference ($P>0.05$) was observed for the same characteristics among the four treatments after the experiment.

Table (2) Some environmental factors of water during the experiment

Sampling date	Environmental factors			
	Water temperature (°C)	pH	Salinity (PSU)	Dissolved oxygen (mg/L)
8/3/2020	17	7.4	3.3	7.7
3/4	21	7.8	4.1	7.4
23/4	23	7.9	4.3	7.1
12/5	26	8.1	4.7	6.7
2/6	28	8.2	5.0	6.5
22/6	28	8.4	5.5	6.3
12/7	33	8.5	5.7	6.0
1/8	38	8.7	6.0	4.3
8/9	28	8.3	5.7	6.0
28/9	27	8.1	5.4	6.5
18/10	24	7.6	5.1	7.5
11/11	22	7.7	4.0	7.6
6/12	18	7.5	3.6	7.7

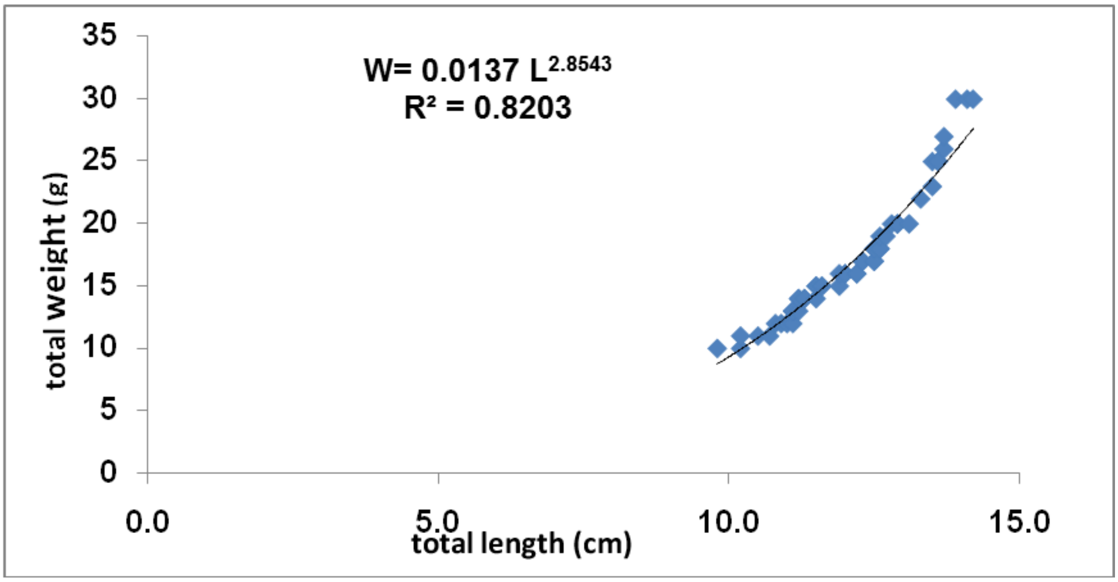

Figure - 1: The relationship between length and weight of grass carp at the beginning of the experiment

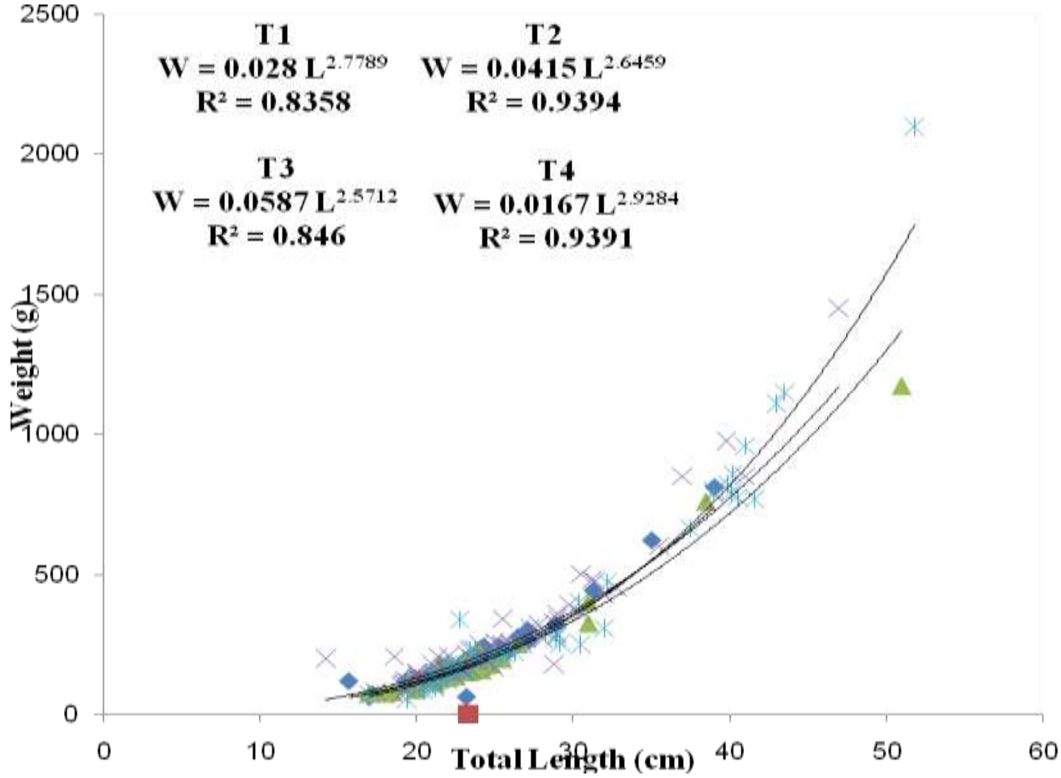

Figure (2) Relationship of length and weight for grass carp at the end of the experiment.

Table (3) Condition factors for grass carp at the beginning and after the experiment.

Treatments	Condition factors		
	Relative (Kn)	Modified (Kb)	Fulton (K3)
Beginning of experiment	1.00 a ±0.10	1.37 e ±0.14	0.95 c ±0.10
T1	1.00 ±0.08 a	2.82 ±0.22 c	1.40 ±0.10 b
T2	1.02 ±0.10a	4.23 ±0.45 a	1.39 ±0.16 b
T3	1.02 ±0.12a	3.19 ±0.39 b	1.52 ±0.20 a
T4	1.03 ±0.21a	1.99 ±0.42 d	1.37 ±0.30 b

Different letters in the same column show significant differences (P≤0.05).

Table (4) Hematological measurements for grass carp before and after the experiment.

Treatments				**before the experiment**	**hematological measurements**
T4	T3	**T2**	T1		
8.10 b ±0.26	7.70 b ±0.70	7.73 b ±0.63	8.43 b ±1.25	3.96 a ±0.50	Hb (g/100ml)
26.23 b ±0.73	26.13 b ±1.64	25.26 b ±1.70	27.63 b ±3.66	13.90 a ±1.05	HCT%
2.79b 10^{6} × ±0.14	2.64b 10^{6} × ±0.25	2.68b10^{6} × ±0.27	2.76 10^{6} × b ±0.27	1.76±0.33 10^{6} × a	RBC (cell/mm³)
42.96±10^{6} ×0.86b	42.93±10^{6} ×7.05b	39.20±10^{6} ×5.75 b	43.60$10^{6}$ × ±5.29 b	10.2 10^{6} × ±1.46a	WBC (cell/mm³)

Different letters in the same column show significant differences (P≤0.05).

4. Discussion

The environmental factors measured in this study were suitable for the culture of grass carp, except for a few days when the temperature rose to 38 °C, and forcing to stopped the feeding trials in order to avoid stress arising from high temperature and reduce the heat emitted by the fish as a result of feed metabolism. Taher (2020b) found that the best rate of food intake for grass carp at a temperature of 25 °C. Muhammed (2022a) showed that the range required to achieve the best growth is at a temperature of 19.6 - 27.2 °C and a salinity level less than 10 g/l. The highest concentration of salinity was recorded in the current study at the beginning of the eighth month, and it reached 6.0 g /l, and the lowest concentration (3.3 g/l) during the third month. Jaafar and Ahmed (2011) showed that the grass carp fish did not tolerate salt concentrations higher than 10 g/l, especially in the case of direct exposure to these concentrations, while Ahmed and Jaafar (2013) found that young grass carp did not tolerate sudden changes in salinity at 5 g/l.

The nutritional requirements of young grass carp differ from those of adult fish, as juveniles require higher protein content than adults (Shireman and Maceina, 1981). Determining the specific nutritional needs of grass carp, especially protein, has been a subject of contention among researchers. Köprücü and Sertel (2012) concluded that the percentage of protein required for the growth of grass carp larvae was 33 %, fingerlings 30 %, and adults 25 %. Khan *et al*. (2004) adopted a protein percentage close to some of the parameters of the current study for fingerlings and adults, which are 42 and 35 % respectively, while Liang *et al*. (2022) showed that 34.13 - 38.31 % protein is the optimal rate for the growth of juvenile grass carp. Ghazala *et al*. (2011) showed that less than 40 % is the best protein level for grass carp growth, while Taher (2017) determined 25 % as the best protein for grass carp growth and also showed that the percentages 27 % and 30 % did not lead to an increase in the weight of grass carp and these percentages were close to the percentages of some of the treatments of the current study. Nekoubin and Sudagar (2012) found results differ from current results, where they don't noticed any significant differences (P>0.05) in growth of fishes fed on diets of 25 and 35 % protein ratio.

The relationship of length and weight is of great importance in the field of fish life and reproduction (Moutopoulos and Stergiou, 2002). If the value of (b = 3), the growth is isometric, while b > 3 or b < 3, the growth is asymmetric (Naeem and Salam, 2005), therefore the growth is expressed as non-standard (Khan and Khan, 2014). From results obtained in current study, it is clear that the growth is asymmetric negative through the relationship of length and weight at the beginning of the experiment as well as at the end of the experiment because the value of b is less than 3. The negative growth in the current study could be due to the fish's low of acceptability or palatability of the manufactured diets because grass carp are vegetarian and usually prefer to eat green feed such as Alfalfa *Medica gosativa*, Azolla *Azolla* spp., Duckweeds *Lemnna minor* and other aquatic and wild plants that are accepted to be eaten by grass carp. Previous result was confirmed by Zolfinejad *et al*. (2017) that grass carp fish preferred water Duckweeds and Alfalfa plant, as well as Muhammed *et al*. (2022b) indicated that grass carp fish preferred eating Alfalfa over manufactured diets. The results of current study are in agreement with the results of Taher (2020a) on grass carp fish cultivated in earthen ponds, where the growth was negative asymmetric for all treatments. The results of the current study are similar to those of Taher *et al*. (2021) on common carp *Cyprinus carpio* and grass carp, where the growth of grass carp was asymmetric and negative, as were most of the treatments of common carp, which were cultured under similar conditions. The results of the current study are in agreement with those of Riberio *et al*. (2015), who recorded significant differences ($P \geq 0.05$) in the length-weight relationship for different treatments of grass carp and the growth in all treatments is asymmetric and negative. The results of the current study differed with the results of the study of Bhosale and Bhilave (2014) and the study of Chitrakar and Parajuli (2017) on grass carp in that the growth in them was asymmetrically positive when feeding on manufactured diets.

The condition factor is an easy way for fish farmers to estimate the growth rate of fish inside fish farms and through it the level of nutrition or type of nutrition in the farm can be changed, which varies according to the density of fish per cubic meter, and each type of fish has a different condition factor within a certain range, the condition factor also varies from another fish strain within the same species (Das *et al*., 2019). Fulton's condition coefficient (K3) at the beginning of the experiment (0.95) and for the four treatments at the end of the experiment ranged between 1.37 - 1.52, and the Modified condition coefficient (Kb) at the beginning of the experiment (1.37), and for the treatments it ranged between 1.99 - 4.23, and the relative condition coefficient (Kn) was at the beginning of the experiment 1.00 and ranged between 1.00 - 1.03 at the end of the experiment. The results of the current experiment are similar to what was found by Taher (2020a) in his study of the relative condition co-efficient (Kn) on grass carp, as it was 1.02 at the beginning of the study and 1.0, 1.01 and 1. 01 for the three treatments at the end of experiment, while the results of the same researcher for the same study differed when testing the Modified condition coefficient (Kb), which was 1.15 at the beginning of the experiment and at the end of the experiment (1.28, 2.16 and 2.72) for the three treatments respectively. Also, the values of the relative condition coefficient (Kn) in current study differed with the results of Das *et al*. (2019).

Blood analyses provide essential information on the physiological aspects of the animal, including the activity of the neuroendocrine glands and immunity, the immediate and long-term effects due to poor culture conditions, possible diseases and the genetic predisposition of the fish (Seibel *et al*., 2021), and that these responses can be determined in the form of changes in the concentration of hormones or the concentration of basic substances in the plasma or the change in the size and numbers of blood cells (Ahmed and Jaffar, 2013). Ahmad and Ahmad (2020) stated that low protein in fish diets leads to a decrease in hemoglobin and RBC and the volume of HCT in fish blood. It is clear from the statistical analysis of the current results that there were significant differences ($P \leq 0.05$) for all blood measurements between fish before the experiment and after the experiment, while there was no significant difference ($P > 0.05$) for the same characteristics among the four treatments after the experiment. This is considered normal with regard to the difference between the beginning and end of the experiment as a result of the difference in age and size that the fish reached at the end of the experiment, and therefore the proportion of different blood components is expected to increase. The results of Pieterse *et al*. (1981) an approach to some of the parameters of our current study in their study of the blood parameters of grass carp to identify the physiological state of these fishes before their settlement in South Africa within the Savannah ecosystem project at that time in order to control the growth of aquatic plants and at the same time obtain a promising food product where they were the recorded blood parameters are (HCT 20.50 %, Hb 8.6 g/100 ml and RBC 1.6 (10^6 cell/mm³).The results of current study were similar to the findings of Ejraei *et al*. (2015) on male and female of grass carp and its age ranged between 6 - 24 months, with no significant differences(P>0.05) between adult fish in blood components, but the WBC values differed significantly (P≥0.05) between the two studies, and this may be due to the different environments and the exposure of fish to salt or heat stress. Restiannasab *et al*. (2016) also obtained similar results to some of our current study results, in their study of the blood parameters of healthy and infected grass carp with a type of parasite, the values of some of these parameters were in healthy grass carp, red blood cells RBC (10^6 cell/mm³) 2.25, hemoglobin Hb 10 g/100 ml and HCT 27.5%, so the researchers noticed an increase in the number of WBC in infected fish as a result of the immune response, and lower of other measures such as RBC and Hb.

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