

## EFFECT OF PARTIAL SUBSTITUTION SPIRULINA INSTEAD OF SOYBEAN MEAL IN COMMON CARP *CYPRINUS CARPIO* L. DIET ON SOME BLOOD PICTURE AND SOME BIOCHEMICAL CRITERIA

M. A. Mohammad  
Assist. Prof.

W. A. Qasab Bashi  
Researcher

Dept. Animal Production, College of Agriculture, University of Mosul,

Email:drmahmoud@uomosul.ed.iq

### ABSTRACT

This study conducted to find the effect of partial replacement of soybean meal by *Spirulina Arthospira platensis* on some blood picture and biochemical criteria in common carp *Cyprinus carpio*. Fish were fed on five experimental diets contained spirulina with ratio 1.45%, 2.90%, 3.54% and 6.10% of the total diet as well as control ( without spirulina) for 56 days . Results of the statistical analysis showed a significant decrease ( $p \leq 0.05$ ) in the concentration of hemoglobin in treated fish fed on spirulina diets, meanwhile a significantly differences ( $p \leq 0.05$ ) for stress index and the albumin criteria for fish fed at fifth diet comparing with control diet. The aspartate amino transferase for fish fed on the control diet increased significantly ( $p \leq 0.05$ ) compared for other experimental diets. The results of the current study show that replacing the soybean meal with spirulina by 11.8 % (3.54 of the total diet) improved the studied criteria compared to the control diet.

Key words: algae, common carp, ALT, AST, creatinine.

Part of M.Sc. thesis of the 2<sup>nd</sup> author.

محمد وباشي

مجلة العلوم الزراعية العراقية -2020: 51: (6) 1740-1746

تأثير الاستبدال الجزئي لكسبة فول الصويا بالسبايرولينا *Spirulina* في عليقة اسماك الكارب الشائع *Cyprinus carpio*

L. على الدم وبعض المعايير الكيموحيوية

ورقاء عادل أحمد قصاب باشي\*

محمود أحمد محمد

باحث

أستاذ مساعد

قسم الانتاج الحيواني / كلية الزراعة والغابات/ جامعة الموصل

المستخلص

نفذت الدراسة لمعرفة مدى تأثير الاحلال الجزئي لكسبة فول الصويا بالسبايرولينا *Arthospira platensis* على بعض معايير الصورة الدموية والكيمياء الحياتية في اسماك الكارب الشائع *Cyprinus carpio*. غذيت الاسماك على خمسة علائق تجريبية احتوت على السبايرولينا بنسبة 1.45 و 2.9 و 3.54 و 6.1% من العليقة الكلية ولمدة 56 يوما فضلا عن عليقة السيطرة الخالية من السبايرولينا. اشارت نتائج التحليل الاحصائي وجود انخفاض معنوي ( $p \leq 0.05$ ) في تركيز خضاب الدم في مجموعة الاسماك المغذاة على العليقة الثالثة والرابعة والخامسة وتفق معنوي ( $p \leq 0.05$ ) لدليل الإجهاد stress index وألالبومين للأسماك المغذاة على العليقة الخامسة مقارنة بعليقة السيطرة. ارتفعت قيمة أنزيم Aspartate Amino Transferase للأسماك المغذاة على عليقة السيطرة معنوياً ( $p \leq 0.05$ ) مقارنة بالأسماك المغذاة على العلائق التجريبية الأخرى. أظهرت نتائج الدراسة الحالية أن استبدال كسبة فول الصويا بالسبايرولينا بنسبة 11.8 % (3.54) من العليقة الكلية) قد أدى تحسن في المعايير المدروسة مقارنة بعليقة السيطرة.

كلمات مفتاحية: طحالب ، الكارب الشائع ، *Arthospira platensis* ، ALT ، AST ، كرياتينين .

\*جزء مستل من رسالة الماجستير للباحث الثاني

## INTRODUCTION

Aquaculture contributes to meet the increasing needs of the population of the aquatic organism, including fish (4), because the commercial fishing from open water suffers from a decrease in its contribution to filling this deficit to 50% of the consumer needs of seafood in all parts of the world (16). Common carp is very much favored for cultivation in ponds in Iraq, Asia, Near East, mono or polyculture with other fishes, because of its excellent growth and feed efficiency rate, omnivorous habit, hardy culture and easy adaptation to artificial feeds. Consequently, this fish was introduced into many countries throughout the world, including Europe, Australia and North America(20) with very different ecological conditions and variable growth rates, so that probably genetic varieties of the GH gene might be of adaptive importance(2). Per capita consumption of fish in Iraq was 4 kg per year, while the average Arab per capita consumption of aquatic organisms had been estimated at 10.97 kg / year compared to 19.9 kg /capita worldwide (6). It known that the soybean meal is low in its sulfur amino acid contents as well as, the presence of a number of inhibitors nutrition, including gossypol; trypsin inhibitor; hemagglutinating agents; glucosinolate; phytic acid and erucic acid that reduced the utilization of soybean meal by fish (17). These anti-factors of nutrition have a negative impact on the viability of digestion of nutrients, including protein (10). Recently, the use of spirulina as food additives to improve the nutritional value of traditional diets popularized, through its contribution to support the growth and food efficiency, protein and digestion of vegetarian food (35). As well as being plant origin, known higher its content of vitamin B complex and vitamin A (33). Spirulina mainly consists of nutrients from crude protein, carbohydrates, essential fatty acids, minerals and pigments such as carotenoids ( $\beta$ -carotene) and chlorophyll (3 and 19), in addition to containing all the essential amino acids and it is rich in minerals. The advantages of this bio-preparation qualify it to be a competitor to traditional foods, as it commercially produced in many countries of the world in Bangladesh, India and the United

States of America (15). It has a unique quality to detoxify chelate heavy metal, as well as another dry algae (5). The present study aimed to find the effect of partial substitution of soybean meal by blue-green algae on blood picture and efficiency of ALT, AST enzyme and creatine.

## MATERIALS AND METHODS

**Experimental fish:** The study was conducted at the Fish Laboratory of Animal Production, College of Agriculture and Forestry, University of Mosul. A total of 120 fingerlings of common carp *Cyprinus carpio* with average weight of 15 g. with no visible signs of disease or morbidity, and acclimated to laboratory condition for 15 days before beginning of the experiment. Fish were briefly bathed in NaCl for 5min for remove all external parasite if present.

### Diet preparation

Eight week feeding experiment were started using a commercial diet .

Diet(1): Used the same commercial basal diet for the control group.

Diet (2): Basal diet supplemented with 1.45% spirulina and 27.5% of soybean meal from the total diet.

Diet (3): Basal diet supplemented with 2.9% spirulina and 25% of soybean meal from the total diet.

Diet (4): Basal diet supplemented with 3.54% spirulina and 22.5% of soybean meal from the total diet.

Diet (5) : Basal diet supplemented with 6.1% spirulina and 20% of soybean meal from the total diet. Each of the five treatments was fed the formulated diets at a twice daily rate of (3%) body weight throughout the experiment. Fish fed on equal diets with crude protein, which amounted to 27% and metabolic energy of 13.48 MJ / kg. Table (1) and Table(2) showed the components and chemical composition of the experimental diets respectively.

**The aquarium used:** The class aquarium used in the experiment, measuring 60×40×40 cm., using de-chlorinated tap water filled the aquarium to reach 70L., water was replaced 20-30% every 24 hr. after feeding in order to maintain a healthy environment with enough oxygen. The following physical and chemical

characteristic such as temperature (24- 26°C); pH (7.7-7.8); Oxygen (5.5-6.0 mg/l).

**Table 1. Dietary ingredients composition of the experimental diets containing different percentages of spirulina *Arthospira platensis***

Diets	(1) Control	(2) Spirulina 1.45%	(3) spirulina 2.9%	(4) spirulina 3.54%	(5) spirulina 6.1%
<b>Ingredients</b>					
Spirulina	-	1.45	2.9	3.54	6.1
Animal protein	10	10	10	10	10
Soybean meal	30	27.5	25	22.5	20
Local barley	20	19.45	21	22.46	21
Yellow corn	18.5	19	19.5	19	20.2
Wheat bran	19	20.1	19.1	20	20
Food salt	1	1	1	1	1
Vita. & Miner. Mix.	0.5	0.5	0.5	0.5	0.5
Lime stone	0.5	0.5	0.5	0.5	0.5
binder (Bentonite)	0.5	0.5	0.5	0.5	0.5

**Table 2. The chemical composition (%DM) of experimental diets.**

Diets	(1) Control	(2) spirulina 1.45%(2)	(3) spirulina 2.9%	(4) spirulina 3.54%	(5) spirulina 6.1%	Spirulina (determine)
<b>Nutrients</b>						
Dry matter	89.25	89.26	89.26	89.24	89.24	95.9
Crude protein	29.0	28.3	28.75	28.79	28.99	63.0
Ether extract	2.90	2.95	2.90	2.99	2.90	3.34
Crude fiber	5.95	5.95	5.68	5.63	5.88	10.0
Ash	6.97	6.85	6.97	6.89	6.89	11.67
Nitrogen free extract	55.18	55.95	55.70	55.70	55.34	11.99
ME (MJ/Kg)	13.43	13.46	13.51	13.51	13.53	14.31

\*Calculated according to equation: ME (MJ/Kg) = Protein X 18.8 + Fat X 33.5 + NFE X13.8 (31).

### Hematological examinations

Blood was collected in the end of the experiment from the caudal vein, using a sterile disposable plastic syringe 3 ml, transferred immediately to a test tube containing (EDTA) (Ethylene di amine tetra-acetic acid) for studying hematological parameters, including the Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and Haemoglobin concentration (Hb), these parameters were determined as described by (8). Blood smears were prepared; methyl alcohol was stabilized at 100% concentration for 10 minutes, then stained with kimza dye and then lymphocytes and heterophil cells calculated in 100 white cells and according to the following equation.

### Stress index

Stress index calculated according to following equation (9):

$$\text{Stress index} = \text{Heterophil} / \text{Lymphocyte}$$

### Biochemical parameters Total

#### Proteins

Biuret method used to the examine through a test kit that described by (34). Total protein =  $\frac{\text{O.D.Sample}}{\text{O.D.Standard}} \times n \text{ g/dl}$ ;  $n=5$ ,  $\text{g/l}$ :  $n=50$  The method described by (18) adopted to measure

total protein concentration using a special reagent produced by the French Company Biolabo. (11) used. A special reagent produced by the French company Biolabo used to measure the albumin concentration. The following equation adopted to measure the concentration of globulin: Globulin concentration (g/100 mL blood) = total protein concentration - albumin concentration. The enzyme transporter of the alanine amino transferase (ALT) was measured by the method described by (27) using a special reagent from Rondex. The enzyme transporter of the aspartic acid (AST) transferase Aspartate Amino measured based on the (27) and a special reagent from the Rondex Company, were used. Biolabo creatinine measurement kit, French production, used to determine the colorimetric reaction of creatinine with alkaline bionate to the unknown sample in the spectrophotometer and at a wavelength of 490 nm (22).

### Statistical analysis

Complete Randomized Design (CRD) used in data analysis by the Statistical Package for Social Science (32) in analyzing the effect of experimental coefficients on the studied criteria, and the significant differences

between the mean characteristics of the characters examined by the multiple-ranged Duncan's test (13).

### RESULTS AND DISCUSSION

Results of the statistical analysis of PCV cells showed that there were no significant differences among fish fed on different experimental diets, which ranged between 20 to 24% (Table 3). These results are consistent with (24) in the absence of changes in the blood parameter of Catfish, *Clarias macrocephalus* x *Clarias gariepinus*, fed on spirulina diet. While (14) reported an increase

in PCV of red tilapia hybrids *Oreochromis niloticus* x *Oreochromis mossambicus* fed a diet supplemented with spirulina. While a significant of of hemoglobin concentration ( $P \leq 0.05$ ) was observed for fish fed to the second and control diets compared with other experimental diets (Table 3) which reached 75.2 and 78.1, respectively. These results are consistent with (1). They indicated a significant increase in the hemoglobin concentration of common carp fed on a diet supplemented with spirulina.

**Table 3. Effect of substitution of different levels of Spirulina on PCV, concentration of Hemoglobin and stress index in common carp (Means  $\pm$  SE).**

Treatments	T1 Control	T2 spirulina 1.45%	T3 spirulina 2.9%	T4 spirulina 3.54%	T5 spirulina 6.1%
PCV %	0.24 $\pm$ 0.01 a	0.24 $\pm$ 0.01a	0.21 $\pm$ 0.11 a	0.20 $\pm$ 0.01 a	0.20 $\pm$ 0.01 a
Hb g /100 ml	78 $\pm$ 1.00 a	75 $\pm$ 2.00 a	63 $\pm$ 1.00 b	59 $\pm$ 2.00 b	63 $\pm$ 1.00 b
Stress index %	28.04 $\pm$ 0.06 b	25.72 $\pm$ 0.3c	24.49 $\pm$ 0.14d	23.62 $\pm$ 0.01 e	35.72 $\pm$ 0.1 a

Means with different small letters in the same row are significantly different  $p \leq 0.05$

Results in Table 3 showed the effect of spirulina on the stress index of fish fed on different experimental diets. A significant increase ( $P \leq 0.05$ ) observed in the stress index criterion for the treatment of fifth diet containing spirulina 6.1% (35.62) compared with control diet (28.04), which was higher than for other experimental diets (Table 3). Results obtained previously (25), that the best weight gain and nutritional conversion ratio were obtained when feeding fish on the fourth diet (3.54% spirulina), and this was shown by the results of the stress index, which reached 23.62%, were the percentage of stress index indicates an increase or decrease in white blood cells, especially Heterophil and lymphocytes, which reflected positively on the growth of fish that fed on fourth diet.

### Concentration of total protein, albumin, and globulin

Results of Table 4 showed that no significant differences ( $P \leq 0.05$ ) in total protein

concentration among the different treatments. While it's found there were a significant differences ( $P \leq 0.05$ ) in the concentration of albumin (g / 100 ml) for fish fed on the fifth diet (spirulina with 6.1% of the total diet) as it reached 3.65, which differed significantly ( $P \leq 0.05$ ) from the experimental diets (except for the second diet). The results of the statistical analysis of the standard globulin concentration (g / 100 ml) showed that there were no significant differences ( $P \leq 0.05$ ) among the different experimental treatments. Their rates ranged between 1.31-2.16 of fourth diet and control diet respectively. Abdulrahman (1) reported a significant increase in the level of total protein for fish fed on control diet compared with fish fed on diet containing 5 g of spirulina / kg. Sherif et al. (30) indicated a high level of total protein and globulin in groups containing spirulina and a decrease in albumin concentration in diets containing spirulina

**Table 4. Effect of substitution of different levels of Spirulina on total protein, albumin and globulin concentration of common carp (Means ± SE).**

Treatments	T1 Control	T2 spirulina 1.45%	T3 spirulina 2.9%	T4 spirulina 3.54%	T5 spirulina 6.1%
<b>Parametrs</b>					
Total protein gm/100ml	4.76 ±0.14 a	4.69 ±0.21 a	3.78±0.14 a	4.31±0.01 a	5.25±0.21 a
Albumin gm/100ml	2.60±0.00 b	2.75±0.35 b	63±0.35 b	3.00±0.10 ab	3.65±0.25 a
Globulin gm/100ml	2.16±0.14 a	1.94±0.14 a	1.58±0.04 a	1.31±0.09 a	1.60±0.40 a

Means with different small letters in the same row are significantly different ( $p \leq 0.05$ )

It also found that fish fed with diets contained 10-5 g of spirulina / kg, which gave a high percentage of protein, albumin and globulin concentration compared with fish fed at the control diet. (28 and 26).

#### The effect of spirulina on ALT, AST and creatinine activities

Results of the statistical analysis confirmed the presence of significant differences ( $P \leq 0.05$ ) in the effectiveness of the ALT enzyme for fish fed on the third diet (23.95), which increased significantly ( $P \leq 0.05$ ) compared to control diet (21.65) and other experimental diets (Table 5). Whereas, the results of the statistical analysis of the effectiveness of AST enzyme indicated a significant superiority of the fish fed on the control diet (23.10), which significantly ( $P \leq 0.05$ ) increase compare to other experimental treatments. Table 5 noticed that the values of these two criteria decrease with the increase in the percentage of spirulina

in the experimental diets. Significant decrease ( $P \leq 0.05$ ) was recorded in serum ALT activity values for all treatments containing spirulina (exception of the group fed on spirulina with 2.9%), a significant increase ( $P \leq 0.05$ ) was observed in the effectiveness of this enzyme compared to the control treatment and the other treatments. While a significant increase ( $P \leq 0.05$ ) observed in the effectiveness of the AST enzyme in the serum of fish of the control group compared to other experimental treatments. The results of the statistical analysis of the effectiveness of creatinine enzyme, showed significant increase ( $P \leq 0.05$ ) for fish fed on spirulina in its various proportions. The highest percentage was in the blood of fish fed on the second diet (90.20 mg/dl) compared with the other treatments 69.85, 87.55, 72.40, 86.10 mg/dl for third, fourth, and fifth, respectively.

**Table 5. Effect of substitution of different levels of Spirulina on ALT, AST and creatinine transporting enzymes for common carp (Means ± SE).**

Treatments	T1 Control	T2 spirulina 1.45%	T3 Spirulina 2.9%	T4 spirulina 3.54%	T5 spirulina 6.1%
<b>Parametrs</b>					
AL IU/L	21.65 ±0.15 b	18.80 ±0.15 c	23.95 0.01 a	13.65±0.0 1 d	11.30±0.1 a
AST IU/L	23.10 ±0.10 a	17.45±0.01 c	18.40 ±0.10 b	12.65±0.10 d	10.30±0.1 d
Creatinine g/dl	69.85±0.02 e	90.20±0.10 a	87.55±0.02 b	72.40±0.10 d	86.10±0.1 c

Means with different small letters in the same row are significantly different ( $p \leq 0.05$ )

Results of this study were agree with the results of (21) that indicated a decrease in the level of ALT and AST enzymes. While (29) and (30) not found negative effect of spirulina in the level of liver enzymes of tilapia fish *Oreochromis niloticus*. The effectiveness of these enzymes is an indication of the vital functions and effectiveness of the liver and therefore the effectiveness of these enzymes in

fish serum within safe limits may be due to the role of spirulina in protecting the liver from cellular harm (7), as biochemical indicators of liver enzymes (ALT and AST) are affected with many internal and external factors (23). Generally, liver enzymes decreased for fish fed at spirulina compared with control diet. This is due to spirulina works as a good antioxidant against free ions that are released

as a result of stress factors, including the metabolism that takes place inside cells. When the level of free ions released in greater quantities than the level of antioxidants increases, it leads to the destruction of body cells and the increase in the secretion of liver enzymes.

### Conclusion

The results of the current study showed that replacing the soybean meal with spirulina by 11.8% (3.54 of the total diet) improves the studied characteristics compared to the control diet

### Acknowledgment

The author are very grateful to university of Mosul College of Agriculture and Forestry / for their provided facilities, which helped to improve quality for this work.

### REFERENCES

1. Abdulrahman, N.M., H.J. Hama, Sh.R. Hama, B.R. Hassan, and P.J. Nader. 2019. Effect of microalgae *Spirulina* spp. as food additive on some biological and blood parameters of common carp *Cyprinus carpio* L. Iraqi J. of Vet. Med.Sci. 1 (33):27-31
2. Al-Khshali, M. A.N. and M. A.N.Al-Azzawy.2018. Relationship of growth hormone gene with some productive traits of common carp *Cyprinus carpio* L. Iraqi J. of Agric. Sci.49(6):1011-1017
3. Ali, SH. K. and A. M. Saleh.2012. Spirulina- An over view. Int. J. of Pharm. and Pharmaceutical Sci. 3(4): 9-15
4. Allan, G. 2004. Fish for Feed vs. Fish for Food. In: A. G. Brown (ed.) Fish, Record Aquaculture and Food Security: Sustaining Fish as a Food Supply. A Conference Conducted by the ATSE Crawford Fund, Parliament House Canberra. pp: 20-26
5. Al-Qaisi, M.R.Z., R. A. Abdul-Jabbar, A. A. Al-Hussieny. 2019. Reduction of some heavy elements of some polluted water using the biological adsorption technique by dry algae. Iraqi J. of Agric. Sci. 50(4):1162- 1173.
6. Arab Organization for Agriculture Development (AOAD). 2017. Arab Strategy for Agricultural Development 2017-2037., League of Arab States, Republic of Sudan, Khartoum. pp: 44.
7. Bhattacharyya, S. and Mehta, P. 2012. The hepatoprotective potential of spirulina and vitamin C supplementation in cisplatin toxicity. Food Funct. 3:164-169
8. Blaxhall, P.C. and K.W. Daisley.1973. Routine Haematological Methods for Use with Fish Blood. J. of Fish Biol. 5: 771-781
9. Coles, R.J. 1986. Absorption, transport and hepatic metabolism of copper and zinc. Special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65:238 – 309
10. Dersjant-Li, Y. 2002. The Use of Soy Protein in Aqua Feeds. Nutrition Acuicola VI. Memorias del VI Simposium Interacional de Nutricion, September. =
11. Doumas, B.T., W.A. Watson and H.G. Briggs .1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chime. Acta. 31: 87-96.
12. Drabkin, D.L. and J.H. Austin (1935). J. Biol. Chem., PP:112-151.
- 13- Duncan, C.B. (1955). Multiple Rang and Multiple “F” Test. Biometric, 11: 1-12
14. El-Sheekh, M., I. El-Shourbagy; S. Shalaby and S. Hosny .2014. Effect of feeding *Arthrospira platensis* (*Spirulina*) on growth and carcass composition of hybrid red tilapia *Oreochromis niloticus x Oreochromis mossambicus*. Turkish J. of Fish. and Aqua. Sci. 14: 471-478
15. Food and Agriculture Organization(FAO). 2008. Production and Use of Spirulina as Food for Humans and Feeds for Domestic Animals and Fish. Department of Aquaculture, Bangladesh, Agricultural University Mymensingh, Bangladesh. pp: 1-41
16. Food and Agriculture Organization(FAO). 2009. Report of the Global Conference on Small-scale Fisheries. Bangkok, Thailand, 13-17 October 2008. Rome. pp: 190
17. Francis, G., H.P.S. Makkar and K.Becker. 2001. Effects of cyclic and regular feeding of a *Quillaja saponin* supplemented diet on growth and metabolism of common carp *Cyprinus carpio* L. Fish Physiol. Biochem. 24: 343-350
18. Gornall , A.C., C.J. Bardawill and M.M. David.1949. J. Biol. Chem. 177(2):751-766.
19. Kapoor, R. and U. Mehta. 1993. Effect of Supplementation of blue green algae (*Spirulina*) on outcome of pregnancy in rats. Human Nutr. 43(1): 29-35
20. Khan, M.N., K. Shahzad, A. Chatta, M. Sohail, M. Piria, and T. Treer. 2016. A review

of introduction of common carp *Cyprinus carpio* in Pakistan: Origin, purpose, impact and management. Croat. J. Fish. 74: 71-80

21. Kim, S, S. Rahimnejad, K. Kim; B. Lee, and K. Lee.2013. Effects of dietary supplementation of Spirulina and quercetin on growth, innate immune responses, disease resistance against *Edwardsiella tarda*, and dietary antioxidant capacity in the juvenile olive flounder *Paralichthys olivaceus*. Fish Aqua. Sci. 16(1):1-8

22. Luna, L.G. 1968. Manual of Histological Staining Methods of Armed Forces Institute of Pathology, Third Ed. Mc Graw- Hill Book G. New York. 4:158-169

23. Marzouk, M.S., M. Mostafa, N.A.Ibrahim, Pick, F.R. S.Mahmoud and M.S. Sharaf. 2013. Effect of freshwater toxic and nontoxic cyanobacteria, microcystins aeruginosa strains on some biochemical parameters of *Oreochromis niloticus*. Egypt. J. Aquat. Biol. and Fish. 17: 55-68

24. Phromkunthong, W. and Pipattanawattanakul, A.2005. Effects of *Spirulina* sp. on growth performance and antibody levels in hybrid catfish, *Clarias macrocephalus* x *Clarias gariepinus* (Burchell). Songklanakar. J. Sci., Technol. 27(1):115-132

25. Qasab Bashi, W and A. M. A. Mohammad .2019. Effect of Partial Substitution Spirulina Instead of Soybean Meal in Common Carp *Cyprinus carpio* L. Diet on Production and Blood Criteria 1- Growth and Food Utilization. Mesopotamia Journal of Agric. Vol.(47), Supplement 1, 2019 Proceeding of 3<sup>rd</sup> International Agri., Conference, College of Agric. and Forestry, Univ. of Mosul and College of Agri. Engineering Sciences, Univ. of Duhok 2-3 October.

26. Qureshi, M. A. and R. A.Ali, 1996. *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. Immunopharm. mmunotoxicol. 18(3): 457-463

27. Reitman, A. and S. Frankel. 1957. Determination of aspartate amino transferase and alanine aminotransferase. Am. J. Clin. Path. 28: 56-63

28. Savidov, N. 2004. Evaluation and development of aquaponics production and product market capabilities in Alberta. IDS Initiatives Fund Final Report, Project, 679056201: 190

29. Schaperclaus, W., H. Kulow and K. Schreckenbach.1992. Fish Disease. A.A. Balkema, Rotterdam, the Netherlands

30. Sherif, A.H., M.M. EL-Sheekh and S. Abdel-Halim .2012. Effect of *Spirulina* algae on the health status and growth performance of Nile tilapia *Oreochromis niloticus* cultured at Kafr El-Sheikh Governorate. Egypt. J. Basic Appl. Physiol. 11(1):57-68.

31. Smith, R. G. 1971. A method for measuring digestibility and metabolizable of energy of feeds. Progressive Fish- Culturist. 33: 132 - 134.

32. Statistical Package for Social Science SPSS (2001), Version 10, SPSS Inc, U.S.A.

33.Tadros, M.G. and D. Macelroy. 1998.Characterization of Spirulina Biomass for CELSS Diet Potential. Alabama Aem. Univ. pp:33

34. Weichselbaum, T.E. 1946. An accurate and rapid method for the determination of proteins in small amounts of blood, serum and plasma. Am. J. of Clinica. Pathol. 16: 40-49

35. Wu, Q;L. Liu; A.Miron; B.Klímová; D.Wan; K. Kuča .2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. Arch Toxicol. 90(8):1817-1840.