

## IMPACTS OF *CHLORELLA VULGARIS* SUPPLEMENTATION TO CHICKEN DRINKING WATER ON AMINO ACIDS, FATTY ACIDS, MINERALS CONTENT OF BROILER CHICKEN MEATS

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### SUMMARY

The influence of green alga *Chlorella vulgaris* supplementation to drinking water was investigated on hematology, oxidative status and meat quality represented by amino acids, fatty acids and minerals content of muscles. One hundred thirty-five, one week broiler chicks (Cobb breed) with mean body weight of 200±0.30g were randomly divided into three groups (freezing and thawing algal culture (G1), fresh algal culture (G2), that added five gm/liters in drinking water, control (G3)) and were allocated to three pen replicates (15 birds each) for each of the three treatments in a completely randomized design. Diets were subedited to be iso-nitrogenous and iso-caloric to cover all recommended. All groups were fed this diet. Two groups of each supplemented chicken drinking water by freezing and thawing (G1) and fresh alga *Chlorella vulgaris* (G2). The feeding trial was performed for 42 days. Blood samples collected during slaughtered of the experiment. The results indicated that *Chlorella* alga supplementation enhanced hemoglobin, meat quality was valuable in G1 and G2 than control and all treatments had differed in content and amount of fatty acids and amino acids.

Conclusion: *Chlorella vulgaris* increased total WBC count and hemoglobin, decreasing serum malondialdehyde concentration. The biotechnological implementation of *Chlorella* alga in broiler chicks drinking water as antioxidants activity to keep against free radicals cellular harm from stress, and as immuno-modulator is claiming to explore. The content and amount of both amino acids and fatty acids are varied in broiler chick carcasses with the supplementation methods of algae to drinking water.

**Keywords:** broiler chicks; algae; *Chlorella*; meat quality; amino acids; fatty acids.

### INTRODUCTION

Poultry meat is an essential supply of food protein (Rymer and Givens, 2005). The poultry sector faces expensive ratio price and poultry product containing high cholesterol and chemical residue resulting from additive component fed broiler to close its growth, chemical residue caused many diseases of people (Salvia et al., 2014). So, it is should seek alternative food. Supplementation of microalgae to chicken food is safe and healthy (Chen et al., 2011). *Chlorella vulgaris* is green alga, unicellular, and includes more contents of proteins, chlorophyll and micronutrients and simplicity for cultivation with high productivity (Buono et al., 2014). The contents of *Chlorella* are proteins, essential amino acids, vitamins, minerals, and antioxidant (Schubert, 1988). The biomass of *Chlorella* is a very high source of pigments such as carotenoids (Batista et al., 2013). Owing to high protein contents of *Chlorella*, it and soybean meal were used as a supplemental feed instead of a fish meal for growing chicks (Lipstein and Hurwitz 1983). Immune modulatory activity is stimulated when broiler chickens feed by *Chlorella vulgaris* (Kotrbacke et al., 1994). The food additive with *Chlorella* had valuable effects, on growth, antioxidant activity, immuno-modulation and tissue rebuilding (Guzmgn et al., 2001). Supplementation

the chicken food by *Chlorella* sp stimulates nonspecific host defense mechanisms and inhibit bacterial growth (Yan et al., 2012). Also, it had effects on intestinal microbial varieties (Janczyk et al., 2009). The phagocytic efficacy of leucocytes was increased and lymphatic tissue advancement when boiler chicken fed with 0.5% *Chlorella* (Kotrbaček et al., 1994). Fresh liquid *Chlorella vulgaris* involved 1% dietary level has positively affected on immune feature (e.g. the number of white blood cells and lymphocytes) (Kang et al., 2013). The purpose of study was to improve meat quality (amino acids and fats) of broiler chicks by *Chlorella vulgaris* supplementation to drinking water

## **MATERIALS AND METHODS**

### ***Algal source:***

Green alga *Chlorella vulgaris* was possessed from microbiology Lab., GEBRI, University of Sadat City. For 15 days under light and dark natural days at 25±1 °C. An alga was cultured in Kuhl medium (Kuhl, 1962).

### ***Experimental design:***

A sum of 135 one week broiler chicks “Cobb breed Massachusetts, USA” were randomly divided into three groups (freezing and thawing algal culture (G1)), fresh alga culture (G2), that added five gm/liters in drinking water, Control (G3) and were allocated to three pen replicates (15 birds each) for each of the three groups in a completely randomized design. Treated drinking water of alga was freely obtainable. Diets were formulated to be iso-nitrogenous and iso-caloric to cover all recommended.

### ***Sampling and analysis:***

At 42 days of age, four birds from each treatment were chosen and blood samples were collected from the slaughtered birds “slaughtered according, by cutting the jugular veins and carotid arteries of both sides of the neck just caudal to the larynx” in heparinized tubes. Blood serum was separated by centrifugation (Shimadzu UV-1601) with 3000 rpm for 15 minutes and stocked for immediate hematology analysis. The portion of each blood sample was used to measure hemoglobin (Hb) (Linne and Ringsrud, 1992) and white blood cell (WBC) counts. To separate plasma and blood, samples were centrifuged at 2,000 xg at 4°C for 20 min and stored at -20°C until measurement of plasma composition. Spectrophotometric analysis by Ultra violet-Visible spectroscopy of plasma was used with a spectrophotometer Shimadzu UV-1601 PC spectrophotometer, (England) in the range 200-800.

### ***Metal estimation:***

The mineral levels of serum were analyzed by using atomic absorption spectroscopy (Perkin Elmer A Analyst 100 AAS).

### ***Analysis of meat samples:***

Meat samples were taken from the carcasses of birds slaughtered and subjected to chemical analysis. Meat samples were dried at 60°C for 48 hrs to determine lipids, total fats, and amino acids.

### ***Amino acid analysis:***

Amino acids were analyzed by HPLC method (Pasquale et al., 2001). HPLC grade acetonitrile ethanol and methanol were used, ammonium acetate were all analytical grade from Carlo Erba (Milan, Italy), phenyl isothiocyanate (PITC) & triethylamine purchased from Sigma-Aldrich (St. Louis, MO, USA)

### ***Amino acid standard:***

Glutamic acid, Aspartic acid, Glycine, Serine, Histidine, Taurine, Alanine, Valine, Arginine, Methionine, Proline, Tyrosine, Threonine, Leucine, Isoleucine, Lysine, and cysteine were purchased from Sigma (St. Louis, MO, USA).

***Sample preparation:***

Phenyl-isothiocyanate (PITC) was accomplished by resuspended samples in buffer content in acetonitrile: ethanol: triethylamine: water (10:5:2:3), the sample was refined by centrifugation, vaporized under decreased pressure and resuspended in 100  $\mu$ L of buffer. After 5 minutes of incubation with 5  $\mu$ L of PITC, the sample was evaporated, and then resuspended in 250  $\mu$ L of 50 mM ammonium acetate buffer (pH 6.5) containing 10  $\mu$ L methanol. A twenty  $\mu$ L sample were used for HPLC analysis.

***Chromatographic Equipment and condition:***

High performance liquid chromatographic (HPLC) Agilent HPLC (USA) 1260 infinity that consisted of a quaternary pump and UV detector equipped with sampler TCC, under computer control unit was used. Mobile phase gradient between two solvents in which solvent B varied from 5%-70% (w/v) over 20 min run time, solvent A 50 mM ammonium acetate buffer, PH 6.5. Solvent B 100 mM ammonium acetate: acetonitrile50:50, pH 6.5. Flow rate 2ml / min. UV detected at 254nm, Column was maintained at 50 °C through the chromatography. Separations were carried out on a RP-18 column (250- $\times$  4-mm, 5- $\mu$ m i.d.) protected with a guard column of the same material (Merck).

***Identification of the lipids content of breast:***

***Lipids samples preparation:***

The lipids matter was prepared from the different groups by extraction with petroleum ether 60-80°C (4 x50 ml). The petroleum ether extract was concentrated under reduced pressure at 30°C till dryness. It has yellowish brown colors and aromatic odor. It stored in an amber colored container and kept under nitrogen in refrigerator till analysis. The lipid fraction was studied physically with regard to its odor, taste, it's solubility in petroleum ether, ether, benzene, chloroform, acetone solubility in warm methyl and ethyl alcohol was also tested and preparation of the lipids samples according to a method described by USP Pharmacopeia, (2017). The samples were injected on GC/MS.

***GC/ MS analysis of lipid matter:***

The chemical contents of samples were done by using Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA), capillary column TG-5MS (30 m x 0.25 mm x 0.25  $\mu$ m film thickness). The column temperature was started at 50°C for 5 min and then rose by 5°C /min to 280°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min.

***Statistical analyses:***

The data were analyzed by the completely randomized design by the general linear models (GLM) procedure of statistical analysis system and the differences among means were determined using Duncan's Multiple Range test. The statistical model was as following:

$Y_{ij} = \mu + T_i + e_{ij}$  Where:  $Y_{ij}$  = the individual observation.  $\mu$  = the overall mean.  $T_i$  = treatment effect  
 $e_{ij}$  = the experimental error.

## **RESULTS AND DISCUSSION**

***Plasma spectrum:***

Various plasma and cellular contents reflect physiological and pathological changes that take place in the tissues (Gunasekaran&Uthra, 2008). The UV-visible spectroscopic method has been employed to study the healthy and diseased blood samples, spectroscopic techniques can be efficiently employed as a diagnostic tool in clinical chemistry and it can be an alternative technique in a clinical analysis (Gunasekaran et al., 2008). The spectrophotometric analysis of the blood plasma was applied to examine the difference between control and treatments. Figure (1) represents that there is very small variation between control and chicken treatments. The peaks were obtained at 405,400.5 and 405.5 with control, fresh algae and freezing algae, respectively. This denotes that there is no a significant change between control and treatments. The UV spectrum gives information about the absorption and scattering characteristic of suspensions. This data can be used to deduce the spectrum in terms of the allocation of particle sizes, shape and the chemical contents in the sample (Motrescu et al., 2006).

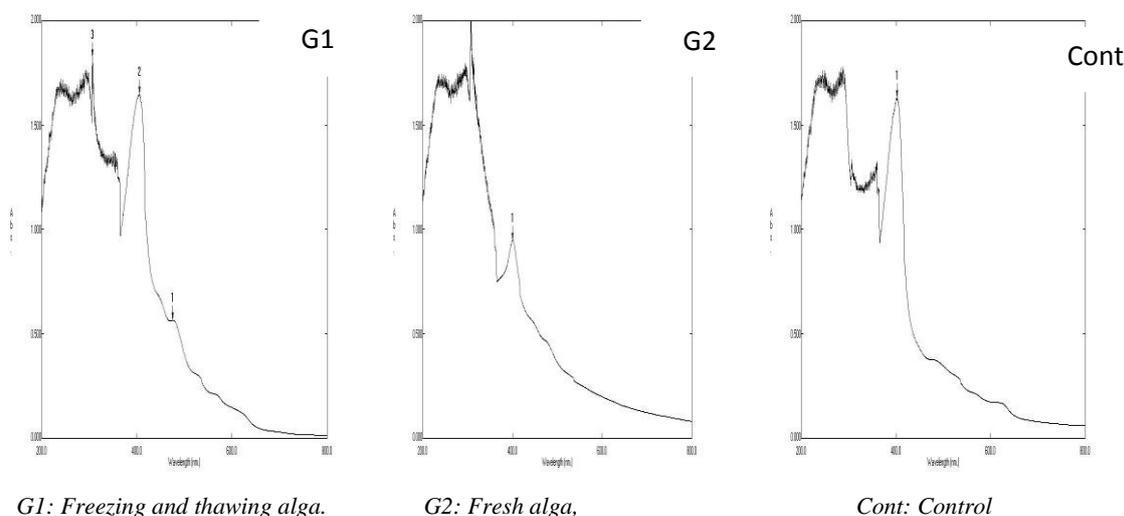


Fig. (1): UV Visible spectra of broiler chicken plasma affected by *Chlorella vulgaris*

**Hematological and Anti-oxidative activities:**

Table (1) shows WBC count Hb band MDA concentrations of broiler chicks through the experiment. Total WBC and Hb concentrations were higher ( $P<0.05$ ) in broiler chicks treated by *Chlorella* alga treatments compared to the control. *Chlorella vulgaris* could act as a natural immune stimulant (Saberi et al., 2017). There was a significant reduction ( $P<0.05$ ) in MDA levels in both groups treated with *Chlorella* alga supplemented to watering compared with control. This result is in a confirmed to Aizzat et al. (2010) that they declared *C. vulgaris* decreased MDA scale in STZ-induced diabetic rats in parallel to the control group. *Chlorella vulgaris* is able to reduce free radical spoilage by directly performing as a free radical scavenger and by obliquely enhancement antioxidant enzyme activities in animal's exposure to lead (Yun et al., 2011).

**Table (1): Haemoglobin concentration (g/dl), total WBC counts (per mm) and MDA affected by *Chlorella vulgaris*.**

Item	treatments		
	Control	G1	G2
WBC	11225 <sup>b</sup>	16625 <sup>a</sup>	13312 <sup>a</sup>
HG (gm)	10.62 <sup>b</sup>	13.37 <sup>a</sup>	12.83 <sup>a</sup>
MDA	15.7 <sup>a</sup>	14.2 <sup>b</sup>	9.4 <sup>c</sup>

Means with the same letter are not significantly different

Hb = Haemoglobin. WBC = White blood cells. MDA = Reduced malondialdehyde

**Minerals in serum:**

Table 2 shows that the effect of fresh (G2) and freezing, thawing (G1) green alga *Chlorella vulgaris* supplementation to drinking water on metals content ( Fe, Zn, and K) of serum broiler chicken. The results demonstrated that the maximum amount of Fe and Zn were present in broiler chicken drinking freezing and thawing *Chlorella vulgaris* and no significant change of Zinc between three groups. The most important role of potassium is maintaining body acid-base balance as well as osmotic pressure in body fluids, activation of an intracellular enzyme, participation in protein synthesis and carbohydrate metabolisms, preserving normal heart function by favors heart muscle relaxation (Baloš et al., 2016). Irons are an important nutrient which contents of haemoglobin in red blood cells (RBC) which circulates

oxygen around the body and keep oxygen in muscles and tissues, and also as of enzymes that are complimentary for energy metabolism, the metabolism of proteins and nucleotides, and the manufacture of proteins, tissues, hormones and neurotransmitters (Prentice et al.,2010). *Chlorella* has the high content of potassium, iron which is important for heart-healthy, blood circulation and formulation otherwise *Chlorella* has sufficient amount of zinc which keep healthy immune function (Świątkiewicz et al., 2015).

**Table (2): Minerals content of broiler chicken as affected by *Chlorella vulgaris*.**

Minerals ppm	treatments		
	Control	G1	G2
Fe	2.18 <sup>b</sup>	3.56 <sup>a</sup>	2.91 <sup>b</sup>
Zn	1.48	1.50	1.46
K	33.6 <sup>a</sup>	34.5 <sup>a</sup>	24.1 <sup>b</sup>

Means with the same letter are not significantly different

**Meat quality:**

**Amino acids of meat:**

Current information depicts the biological worth of a food protein is attached to its amino acid components (Mitchell, 1942). Amino acids are divided into two types as essential amino acids which risks to health if they are deficient and nonessential amino acids (Kamiya, 2002). The essential amino acids which are nutritionally essential for optimal animal growth are arginine, histidine, lysine, tyrosine, tryptophan, phenylalanine, serine, threonine, cysteine, isoleucine and methionine (Kamiya, 2002). Consequently, not only to recognize the quantitative and qualitative necessities of the human for vital amino acids but also the quantitative components of food proteins which are generally applied to accomplish these body needs (Rose et al., 1942).

**Table (3): Effect of *Chlorellavulgaris* on quantitative and qualitative amino acids present in different groups of broiler chicken**

Amino acids	Rt	G1	G2	Control	(LOD)	(C,C)
Cysteine	1.70	2	1.9	2.4	0.3	0.999
Aspartic acid	2.89	-	2.03	3.47	0.17	0.999
Glutamic acid	3.39	-	-	-	0.16	0.999
Serine	5.12	-	4.15	3.62	0.14	0.999
Glycine	5.58	-	3.05	1.2	0.15	0.999
Histadine	6.65	-	9.3	4.56	0.16	0.999
Taurine	7.63	-	3.5	-	0.10	0.999
Alanine	8.02	-	9.3	-	0.13	0.999
Arginine	9.19	-	10.6	-	0.11	0.999
Proline	9.47	1.8	-	-	0.09	0.999
Tyrosine	10.38	-	3.7	3.7	0.10	0.999
Valine	12.33	9.9	5.5	1.6	0.10	0.999
Methionine	13.01	9.2	2.7	1.9	0.11	0.999
Threonine	13.31	-	4	4.5	0.12	0.999
Leucine	15.14	6.1	19.9	18.7	0.10	0.999
Isoleucine	15.31	11.6	-	-	0.09	0.999
Lysine	16.13	3	5.8	5.8	0.08	0.999

The data of derivatizations are summarized: the reported parameters are retention time (Rt),

Concentration range, limit of detection (LOD) and correlation coefficient (C, C).

G1 group watering with freezing and thawing alga G2 group watering fresh alga.

The results in Table (3) indicated that the muscles of broiler chicken that drinking by freezing, thawing(G1), fresh *Chlorella vulgaris* (G2) and control contain essential amino acids percentage of total proteins as Serine (-, 4.15 and 3.62), Histidine (-, 9.3 and 4.56), Arginine (-,10.6, -), Threonine (-,4 and

4,5), Leucine (6.1,19.9 and 18.7), Isoleucine(11.6,-,-),Lysine(3,5.8 and 5.8) respectively. The fresh alga *Chlorella vulgaris* supplementation to drinking water is the best treatments to store essential amino acids in broiler chicken muscles among freezing, thawing alga and control. Amino acids are stimulation muscle protein anabolism (Volpi et al., 2003). Regulate insulin and growth hormone secretion (Lorraine and Katrin, 2006), help in protein formation in tissue and melanin synthesis (Jun et al., 2010). The function of amino acids is modulating cholesterol metabolism, caring liver from toxic factors, reducing blood pressure and blood ammonia (Volpi et al., 2003). *Chlorella* contains 42-58% of protein per dry weight (Seyfabadi et al., 2011).

**Investigation of lipid:**

The results show that the highest content of abdominal fats in controls group among the other groups, the low content of abdominal fats was present in the group was treated with freezing and thawing *C. vulgaris*. The lipids of different treatments were yellowish brown in color, semi-solid having faint odor. Results of lipids data of broiler chicks as influenced by *C. vulgaris* using different experimental treatment control, freezing and thawing algae and fresh algae are presented in Table 4. Data were referred that there is no significant change among treatments and control.

The results obtained in the Table (5) investigated that there is a difference in quantitative and qualitative fatty acid profile in lipids matter among treated broiler chicks and control. Oleic acid methyl ester, Palmitic acid methyl ester, and 9-Hexadecenoic acid, methyl ester (Methyl palmitoleate) are present in all treated broiler chicks and control.

**Table (4): The quantity of fatty acids of tested samples.**

NO. of sample	The petroleum ether extract	% Fatty acids
G1	0.375 mg / 5gm	7.5
G2	0.429 mg / 5gm	8.58
Control	0.388 mg/ 5gm	7.76

G1 group watering with freezing and thawing alga,  
G2 group watering fresh alga.

**Table (5): GC/ MS for analysis of lipid matter of broiler chicks muscles.**

No.	RI	Sample Name			Compound name
		G1	G2	Control	
1	1727	2.94	-	-	Tetradecanoic acid, methyl ester.
2	1928.1	23.32	22.48	24.17	Palmitic acid, methyl ester.
3	1932	4.78	3.77	4.98	9-Hexadecenoic acid, methyl ester (Methyl palmitoleate).
4	2021	16.75	-	-	Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate).
5	2113	4.38	-	-	9, 12, 15-Octadecatrienoic acid, 2, 3-dihydroxypropyl ester.
6	2140	38.24	34.15	42.13	Oleic acid, methyl ester.
7	2145	-	27.39	23.13	9, 12-Octadecadienoic acid, methyl ester.
8	2285	3.81	3.5	-	1-Heptatriacotanol.
9	2652	-	2.71	-	Cholestan-3-ol, 2-methylene-, (3á,5à)-
10	3094	-	1.64	1.77	Ethyl iso-allocholate.
11	3145	2.46	-	-	7, 8-Epoxy lanostan-11-ol, 3-acetoxy.
12	3350	3.29	-	-	Tricyclo [20.8.0.0(7, 16)] triacontane, 1 (22), 7 (16)-diepoxy.
13	3508	-	1.52	1.81	17-Pentatriacontene.
14	4149	-	2.59	1.75	Oleic acid, 3-(octadecyloxy) propyl ester.
Total	-	99.97	99.75	99.74	

G1 group watering with freezing and thawing alga. G2 group watering fresh alga,  
RI: Retention index (kovats index)

Oleic acid (omega-9) is the maximum widespread monounsaturated fatty acid in groups 1, 2 and control by the levels 38.24, 34.15 and 42.13, respectively. Milićević (2014) reported that oleic acid is the most widespread in human cells and vegetables and have a maximum scale (42.85%) was noticed in the meat of chicken. Oleic acids (omega-9) have anticancer and integrated, into cell membrane phospholipids, where it is vital for suitable membrane fluidity.

The second common fatty acids that found in chicken are saturated fatty acid Palmitic acid methyl ester. Amid palmitic acid and stearic acid, helps in reducing cardiovascular diseases (Krishnaveni et al, 1984). Palmitic acid acts as antioxidant, hypocholesterolemic, nematocide, pesticide, lubricant and anti-androgenic (Dhayabaran and Thangarathinam, 2016). Tetra decanoic acid, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate), 9, 12, 15-Octadecatrienoic acid, 2, 3-dihydroxypropyl ester, 7, 8-Epoxy lanostan-11-ol, 3-acetoxy and Tricyclo [20.8.0.0(7, 16)] triacontane, 1(22), 7(16)-diepoxy are found only in G1. Tetradecanoic acid, methyl ester acts as antibacterial and antifungal (Chandrasekaran, 2011). Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate) used against skin cancer protein (Elaiyaraja and Chandramohan, 2016). Cholestan-3-ol, 2-methylene-, (3 $\alpha$ ,5 $\alpha$ ) are found in G2. Cholestan-3-ol, 2-methylene is steroid compounds have Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma and Antiarthritic activity (Thanga et al., 2012). *Chlorella vulgaris* lipid components are nonanoic acid, decanoic acid, palmitic acid, stearic acid, oleic acid, linoleic acid with this chain length C9: 0, 10:0, 16:0, 18:0, 18:1, and 18:2, respectively (Aguoru and Okibe, 2015).

## CONCLUSIONS

*Chlorella vulgaris* increased total WBC count and hemoglobin, decreasing serum malondialdehyde concentration. The biotechnological implementation of *Chlorella* alga in broiler chicks drinking water as antioxidants activity to keep against free radicals cellular harm from stress, and as immuno-modulator is claiming to explore. The content and amount of both amino acids and fatty acids are varied in broiler chick carcasses with the supplementation methods of algae to drinking water. Furthermore, experiment with included of *Chlorella* algae level in water and are eligibility while to estimate the nutritional value of *Chlorella* alga perfectly and precisely.

### *Compliance with ethical standards:*

Conflict of interest we declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no conflict of interest.

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## تأثير اضافة طحلب الكلورلا فى مياه الشرب على محتوى الاحماض الامينية والدهنية والمعادن فى لحم دجاج التسمين

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تضمنت هذه الدراسة تجربة لبحث تأثير اضافة 5 جم من طحلب الكلورلا/لتر ماء الشرب على بعض فسيولوجيا الدم, جودة اللحم من حيث المحتوى من الاحماض الامينية وكذلك الاحماض الدهنية وبعض العناصر المعدنية فى العضلات فى سلالة الكب.

تم تقسيم 135 ككتوت من سلالة كب عشوائياً إلى 3 مجموعات كل مجموعة من 45 طائراً ؛ كل مجموعة مقسمة الي ثلاث مكررات بكل مكرر 15 طائراً.

المجموعة الأولى: ككتايت السمين غديت على العليقة الاساسية بالأضافة الى 5جم من طحلب الكلورلا/لتر فى مياه الشرب (تجميد وتذويب الطحالب).

المجموعة الثانية : ككتايت السمين غديت على العليقة الاساسية بالأضافة الى 5جم من طحلب الكلورلا/لتر فى مياه الشرب (طحالب طازجة).

المجموعة الثالثة: ككتايت السمين غديت على العليقة الاساسية بدون إضافات لماء الشرب (عليقة المقارنة)

أجريت التجربة لمدة 42 يوم.

ويمكن تلخيص أهم النتائج التي تم التوصل إليها فيما يلي:

- 1- تحسن معنوي في الهيموجلوبين وكرات الدم البيضاء للمجموعات التجريبية عن مجموعة المقارنة , انخفاض معنوي فى تركيز MDA للمجموعات التجريبية عن مجموعة المقارنة خلال فترة التجربة.
  - 2- تحسن غير معنوي في نسبة الحديد والزنك فى المجموعة التجريبية الاولى G<sub>1</sub> عن G<sub>2</sub> ومجموعة المقارنة.
  - 3- المجموعة التجريبية الثانية G<sub>2</sub> أفضل المجموعات فى تخزين الاحماض الامينية الاساسية
  - 4- يوجد اختلاف فى محتوى الاحماض الدهنية للمعاملات التجريبية
- بشكل عام ، اعتمادا على النتائج التي تم الحصول عليها فى هذه الدراسة ، يمكن التوصية بأن طحلب الكلورلا ذو قيمة غذائية لدجاج التسمين .