REPUBLIC OF TURKEY
BINGOL UNIVERSITY
INSTITUTE OF SCIENCE

PHYSIOLOGICAL EFFECT OF MICROALGAE *Chlorella* sp. ON THE COMMON CARP (*Cyprinus Carpio* L.)

MASTER THESIS

Sanaa Hoshyar ABID

BIOLOGY

THESIS SUPERVISOR

Assoc. Prof. Dr. Mustafa KOYUN
Co. Supervisor: Assist. Proff. Dr. Nasreen Mohialddin ABDULRAHMAN

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This thesis was unanimously approved by the following jury on 17.01.2018

Assoc. Prof. Dr.	Assist. Prof. Dr.	Assist. Prof. Dr.
President of the Jury	**Member**	**Member**

I confirm the result above

Prof. Dr. İbrahim Y. ERDOĞAN
Director of the institute

PREFACE

First of all, I praise God, the almighty for providing me this opportunity and granting me the ability to proceed successfully. This thesis appears in its current form due to the assistance and guidance of several people.

I would like to take this chance for expressing my deep and honest gratitude and thankfulness to my supervisor, Assoc. Prof. Dr. Mustafa KOYUN, for his help and encouragement during the whole period of my research.

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CONTENT

LIST OF ABBREVIATIONS

HCT	:	Hematocrit
SGR	:	Specific growth rate
FCR	:	Feed conversion ratio
HDL-C	:	High density lipoproteins cholesterol
LDL-C	:	Low density lipoproteins cholesterol
ASP	:	Aquatic Species Program
HUFA	:	Highly unsaturated fatty acids
PUFAs	:	Poly-Unsaturated Fatty Acids
DHA	:	Docosahexaenoic acid
EPA	:	Eicosapentaenoic acid
MCHC	:	Mean corpuscular hemoglobin concentration
MCV	:	Mean corpuscular volume
PER	:	Protein efficiency ratio
PCV	:	Packed cell volume
DWG	:	Daily weight gain
RWG	:	Relative weight gain
CBC	:	complete blood count
MON	:	Monocyte
LYM	:	Lymphocyte
GRAN	:	Granulocyte
PLT	:	Platelet
FER	:	Food Efficiency Ratio
RDW	:	Red blood cell distribution width

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MİKROALG *Chlorella* sp.'NİN PULLU SAZAN (*Cyprinus carpio*) ÜZERİNE FİZYOLOJİK ETKİSİ

ÖZET

Bu çalışma, 105 gün boyunca balık yemine farklı seviyelerde mikroalg *Chlorella* spp. katkısının etkisini değerlendirmek için yürütülmüştür. Balık ağırlıkları 35,7 gr'dan başlayarak 35-45 gr arasında değişiklik göstermiştir. *Chlorella* spp'nin dört farklı seviyesinin etkisini test etmek için 21 günlük yemleme denemelerinden önce balıkların laboratuvar şartlarına uyumu sağlandı ve kontrol peletleri (%29 protein) ile beslendiler. Kontrol grubu (T1) "0" *Chlorella*, (T2) 2,5gm *Chlorella*/kg, (T3) 5gm *chlorella*/kg ve (T4) 7,5gm *chlorella*/kg ile beslendi. İçerisinde günde iki kez deneysel yemle beslenen beş yavru sazanın bulunduğu akvaryumların her birinde üç kez tekrarlayan uygulamalar yapıldı. Bu çalışma alg katkılı balık yemleri ile balıkları beslenmenin incelenen büyüme performansında, kilo alımında ve günlük büyüme hızında belirgin biçimde değişiklik gösterdiğini, diğer uygulamalara kıyasla T3 ve T4'ün ilgili büyüme oranlarının önemli ölçüde daha yüksek olduğunu açıkça göstermiştir. *Chlorella*'nın balık yem katkı maddesi olarak kullanılması sonucu yem kullanımını etkilediği, Gıda Dönüşüm Oranında T3 ve T4'ün diğer uygulamalara göre anlamlı olarak düşük olduğu, Gıda Verim Oranında uygulamalar arasında anlamlı bir farklılık gözlenmediği, Protein Verimliliği Oranında T3 ve T4'ün diğer uygulamalara göre anlamlı derecede yüksek olduğu görülmüştür. Denemede kullanılan sazan balıklarını yosunla beslemek, incelenen biyolojik parametreleri belirgin biçimde değiştirmiştir. Hepatosomatik (Karaciğer/Vücut) indeksinde tüm uygulamalar kontrol grubundan anlamlı bir şekilde farklı, Spleenosomatik (Dalak/Vücut) indeksinde kontrol ve T4 diğerlerinden daha yüksek, Gillsomatic (Solungaç/Vücut) indeksinde T2 ve T4'ün anlamlı farklılık gösterdiği, T4'ün Kidneysomatik (Böbrek/Vücut) indeksi'ndeki diğer uygulamalara göre anlamlı derecede yüksek olduğu görülmüştür. Bağırsak Ağırlık Indeksi T2 dışındaki tüm uygulamalarda anlamlı olarak farklıydı, T4'te Bağırsak Uzunluğu Indeksi diğer gruplara göre anlamlı derecede yüksekti ve T3'teki uygulama grupları arasında kondisyon faktörleri önemli derecede farklı bulundu. Karkas (Viskeri) balık ağırlıkları T2 ve T3'ün her birinde belirgin olarak daha yüksek bulunmuştur. Denemede kullanılan balıklarının etinin kimyasal analizinde (yakın analiz), T4'ün protein ve kül oranı, T4 ve T3'ün lipid ve nem oranı anlamlı derecede yüksekti. Balık yemlerine *Chlorella* eklenmesi tüm seviyelerde etin rengini ve sululuk oranını kontrol grubundan farklı olarak kabul edilebilir derecede etkilemiştir. Bu çalışma, WBC ve lenfositlerin T2'de diğer uygulamalara göre anlamlı derecede yüksek olduğunu, kontrol ve T4'teki monosit ve granülositlerin diğer uygulamalara göre anlamlı derecede yüksek olduğu, kontrol ve T2'nin diğerlerinden daha düşük olduğunu göstermiştir. RBC ve HGB, T3 ve T4'ün her birinde anlamlı derecede yüksek; HCT, diğer tedavilere kıyasla T4'teki tedaviler arasında anlamlı olarak farklılık göstermiştir. MCV ve MCHC, T2 ve T3'de anlamlı derecede yüksek bulunmuştur. Glikoz, T4'te diğerlerinden daha yüksek; Kolesterol, T1 ve T2'de anlamlı derecede yüksek, Trigliserid T1'de diğerlerinden daha yüksek, HDL-C ve LDL-C diğer uygulamalara kıyasla T2'de anlamlı olarak düşük bulunmuştur. Spesifik büyüme oranı, Tazelik ve MCH'nin her birinde önemli bir farklılık gözlemlenmemiştir.

Anahtar Kelimeler: *Cyprinus carpio*, *chlorella* spp. büyüme, karkas, kan parametreleri.

PHYSIOLOGICAL EFFECT OF MICROALGAE *Chlorella* sp. ON THE COMMON CARP (*Cyprinus carpio*)

ABSTRACT

This study was carried out to evaluate the effect of adding the microalgae *Chlorella* spp. with different levels at fish meal for 105 days. The size of fish was varying and the weight ranged between 35-45 g with initial weight was 35.7g. The fish were acclimated to laboratory conditions and fed with control pellets (29% protein) prior to the feeding trials for 21 days, to test the effect of four different levels of the algae *Chlorella* spp. The control treatment T1 without *Chlorella* spp., (T2) with 2.5 gm Chlorella/kg diet, (T3) 5 gm *Chlorella* /kg diet, and (T4) 7.5 gm *Chlorella*/kg diet. Each treatments in three replicates in which five fingerlings common carp were stocked in each aquarium which fed the experimental diets twice daily. The present study clearly showed that feeding algae as a feed additive to fish remarkably change the studied growth performance, in weight gain and daily growth rate was significantly higher as compared to other treatments, relative growth rate T3 and T4 were higher significantly than others. The results of using the *Chlorella* algae as a feed additive to fish affect feed utilization, in Food Conversion Ratio T3 and T4 were significantly lower than other treatments, in Food Efficiency Ratio no significant differences observed among treatments, Protein Efficiency ratio the T3 and T4 were significantly higher than other treatments. Feeding algae to common carp remarkably change the studied biological parameters, in Hepatosomatic index all treatments were significantly differ than the control, Spleenosomatic index the control and T4 were higher significantly than others, in Gillsomatic index the T2 and T4 were differ significantly, while T4 was significantly higher than other treatments in Kidneysomatic Index. The Intestine weight index was differ significantly in all treatments except of T2, Intestine length index in T4 was significantly higher than other treatments and condition factors differ significantly among treatments in T3. Fish weight without viscera was significantly higher in each of T2 and T3. The chemical analyses (proximate analyses) of common carp meat, T4 was higher significantly in protein and ash ratio, in lipid and moisture T4 and T3 were higher significantly. The adding of Chlorella to fish diets in all levels significantly affect meat color, Juiciness and complete acceptable than the control. The present study showed that, WBC and lymphocytes were higher significantly in T2 than other treatments, monocytes and granulocytes in control and T4 were significantly higher than other treatments, the control and T2 were lower significantly than others. RBC and HGB were higher significantly in each of T3 and T4, HCT differ significantly among treatments in T4 as compared to other treatments. MCV and MCHC were higher significantly in T2 and T3. Glucose was higher significantly than others in T4; Cholesterol was higher significantly in T1 and T2, while in Triglyceride T1 higher significantly than others, in HDL-C and LDL-C T2 was significantly lower as compared to other treatments. No significant differences observed in each of specific growth rate, Freshness, and MCH.

Keywords: *Cyprinus carpio*, *Chlorella* spp. growth, carcass, blood parameter.

1. INTRODUCTION

For those who are raising fish, it is critical from a number of perspectives to consider what to feed them. For maximal growth, fish nutrition needs to be tailored to the species and the stage of development. Given that fish feed is one of the highest operating costs of an aquaculture system, it is necessary to maximize the feed conversion ratio and use costly feed ingredients judiciously. Ultimately, the goal should be to optimize the nutritional composition of fish for consumption, since fish represent the main source of long-chain omega-3 fatty acids (LC ω-3 FA) in the human diet (FAO 2006).

World aquaculture production of fish accounted for 44.1% of total production (including for non-food uses) from capture fisheries and aquaculture in 2014, up from 42.1 % in 2012 and 31.1 % in 2004. All continents have shown a general trend of an increasing share of aquaculture production in total fish production, although in Oceania this share has declined in the last three years (Qiu 2014).

Photosynthetic organisms, including plants, algae, and some photosynthetic bacteria, efficiently utilize the energy from the sun to convert water and CO_2 from the air into biomass. The Aquatic Species Program (ASP) at SERI1 was initiated as a long term, basic research effort to produce renewable fuels and chemicals from biomass. It emphasized the use of photosynthetic organisms from aquatic environments, especially species that grow in environments unsuitable for crop production. Early in the program, macroalgae, microalgae, and emergent were investigated for their ability to make lipids (as a feedstock for liquid fuel or chemical production) or carbohydrates (for fermentation into ethanol or anaerobic digestion for methane production) (Sheehan 1998).

Many microalgae (microscopic, photosynthetic organisms that live in saline or freshwater environments), produce lipids as the primary storage molecule. By the early 1980s, the decision was made to focus ASP research efforts on the use of microalgal lipids for the production of fuels and other energy products, there was significant interest in the development of microalgal lipids for biodiesel production (Solomon et al. 2007).

Cyanobacteria are members of a group identified as eubacteria or true bacteria. For a long time they were not known as bacteria, further often being referred to as blue-green algae. Bacteria have no structured nucleus. Cyanobacteria are classified as bacteria, not algae, because their genetic material is not organized in a membrane-bound nucleus. Cyanobacteria disparate other bacteria, they have chlorophyll and utilize the sun as an energy source. They are frequently referred to as blue-greens; while the first Cyanobacteria identified were bluefish-green in color. Though, not all members are this color. Some are dark green or olive, and others are even purplish in color (Future 2016).

These micro algae are the cell organisms that be able to easily grow in both fresh water and sea water. In calculation to high levels of provitamin A, dried micro algae can provide various other nutrients including proteins, minerals, antioxidants, and vitamins. World production of delicate algae and algae products to be used as nutritional supplements, food additives, functional foods, and medicines has reached thousands of tons per year (Lee 1997; Gershwin and Belay 2000). Since of their potential use as biofuel production will definitely increase however, many of the dietary intervention studies for nutritional rehabilitation of malnutrition are of poor-methodological quality and have to be interpreted with caution (Halidou et al. 2008).

One of the biggest problems in front of the use of fish nutrition, in many aquaculture operations today, feed financial statement more than half of the variable operating cost (NRC 1993). As a result, the potential use of unconventional food stuffs such as algae, for substitution the high cost food stuffs such as fish meal is very important. Algae have attention as a likely option protein source for cultured fish, particular in tropical and subtropical developing countries where algae production rates are high and their higher protein, vitamins and essential fatty acids contents (El-Hindawy et al. 2006).

Different types of algae, specifically microalgae, that could become more prevalent in food supplements and nutraceuticals are *Nostoc, Botryococcus, Anabaena,* *Chlamydomonas, Scenedesmus, Synechococcus, Parietochloris, and Porphyridium* etc. due to the capability of producing necessary vitamins including: A (Retinol), B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B6 (Pyridoxine), B9 (Folic acid), B12 (Cobalamin), C (L-Ascorbic acid), D, E (Tocopherol), and H (Biotin). Also, these organisms concentrate essential elements including: Potassium, Zinc, Iodine, Selenium, Iron, Manganese, Copper, Phosphorus, Sodium, Nitrogen, Magnesium, Cobalt, Molybdenum, Sulfur and Calcium. Algae are also high producers of essential amino acids and Omega 6 (Arachidonic acid) and Omega 3 (docosahexaenoic acid, eicosapentaenoic acid) fatty acids (Simoons 1991). Due to their abundant production of beneficial compounds and nutritive contents, the market for increased algae production for nutraceuticals is lucrative and imminent. As proper nourishment is a growing concern with increasing world populations, easy to produce and cost-effective sources that can rapidly produce large amounts of nutritional value are needed. Algae can provide a significant source of a diverse number of critical nutrients to support human health. Algae are ubiquitous throughout the world and have persisted and thrived in numerous types of environments. The adaptations they have developed and propagated are accompanied by benefits to organisms up the food chain. Many of these unique characteristics (carotenoids, micronutrient accumulation, amino acids etc.) have led to an extensive base of compounds that are critical in human health. Discovering of these algae and contained compounds is in its infancy, though numerous beneficial products are currently present. The goal of this article is to review the current status of nutraceuticals products and food supplements when it regards common cultured microalgae production and use as well as to outline the positive health benefits documented from these algae (Bishop and Zubeck 2012).

Common carp *C. carpio* L., 1758 has been a popular aquaculture fish for more than 2000 years, is one of the most commercially important and widely cultivated freshwater fish in the world, contributing to 11% of the total world freshwater aquaculture production (FAO 2010). More than 90% of this production comes from Asia, where common carp is cultured in various pond aquaculture systems. Similarly, common carp might alter its food preference and behavior in response to changing food resources (Adamek et al. 2003; Rahman et al. 2006; 2008).

The purpose of the present study was to evaluate the utilization of different algae *Chlorella* levels in the diet on the productive performance of the common carp *Cyprinus carpio* under Iraqi conditions especially in Sulaimaniyah.

2. LITREATURE REVIEW

2.1. Importance of Fish and Aquaculture

Fisheries have always played a very significant socio-economic role in many countries and communities, as a subsistence produce, fish is a vital resource towards poverty reduction and food security for most poor households (FAO 2010). Income generated from the fisheries sector or through fish trade is the most important indirect contribution to food security, in sub-Sahara Africa, fishing and fish related employment provides both part-time and full-time jobs to 6 and 9 million people, respectively. There are about 43.5 million fishers in the world, and there are at least four other people associated with each fisher in fish-related jobs, including processors, traders and small-scale operators, thus, the fishing industry supports over 170 million people with income (FAO 2002).

In 2006, fisheries and aquaculture produced a total of 143.6 million tons of fish (FAO 2009), 81.9 million tons from marine capture fisheries, 10.1 million tons from inland capture fisheries, 31.6 million tons from inland aquaculture and 20.1 million tons from marine aquaculture. China is by far the largest producer of fish, producing 51.5 million tons of fish in 2006, 17.1 million tons from capture fisheries and 34.4 million tons from aquaculture (FAO 2009). The Asia–Pacific region dominates both fisheries and aquaculture, particularly in terms of the number of people working in these sectors: 86% of fishers and fish farmers worldwide live in Asia, with the greatest numbers in China (8.1 million fishers and 4.5 million fish farmers) (FAO 2009). Asia is also a major producer of fish, accounting for 52% of the world's wild caught fish, while aquaculture in the Asia–Pacific region accounts for 89% of world production by quantity and 77% by value (FAO 2009).

One of the most complicated challenges facing profitable fish producers is the constant paired act necessary to continue a stable connection among the fish, water an microscopic flora and fauna in their pond systems. In environment, where densities of fish and other living organisms are low, complex ecological systems maintain this fragile stability to avoid explosive shifts in populations and the negative property that they can have on the entirety systems. In commercial fish construction ponds, although, natural hauling capacities are significantly exceeded, and a lot overloaded synthetic ecology is conventional between a variety of organisms and the environment in which they live **(**Brunson et al. 1994**).**

The nutritional benefits of fish and fish oil consumption on human health, including the prevention of cancer, diabetes and heart diseases, have been well established, as public awareness about the health benefits of fish consumption continues to increase, the global demand for aquatic foods is also expected to continue to rise (Gina 2009; Heidarsdottir et al. 2010).

2.2. Using of Algae as a Supplement to Enhance the Nutritional Value of Formulated Feeds

Using feeds in aquaculture (occasionally referred to as aquafeeds) commonly increases yield. In contrast to maximize cost-effectiveness, it is predominantly constructive in small-scale aquaculture to use locally available materials, either as ingredients (raw materials) in compound aquafeeds or as sole feedstuffs. There is also a vital need to seek valuable ingredients that can either partially or entirely substitute marine ingredients as protein sources in animal feedstuffs commonly, in particular in aquafeeds (Hasan and Chakrabarti 2009). Algae have been part of the human diet for thousands of years, based on archaeological evidence from 14000 yBP in Chile (Dillehay et al. 2008) and early written accounts (e.g., in China, 300 A.D.; in Ireland, 600 A.D.; Newton 1951; Tseng 1981; Aaronson 1986; Turner 2003; Gantar and Svircev 2008; Craigie 2010). In North America, the Tsimshian first Nations' people named the month of May for the time of year when they harvested the important food crop of Pyropia. More contemporaneously, the global harvest of sea-weeds in 2013 was estimated at US $6.7 billion, and over 95%

was produced in mariculture, with China and Indonesia being the top producers (FAO 2015).

In addition to macroalgae, some microalgae are cultivated for foods and food additives (Fournier et al. 2005; Gantar and Svircev 2008; Chacón-Lee and Gonzalez Marino 2010; FAO 2016). The FAO (2014) estimated that 38% of the 23.8 millionth of seaweeds in the 2012 global harvest was eaten by humans in forms recognizable to them as seaweeds (e.g., kelps, nori/laver), not counting additional consumption of hydrocolloids (e.g., agars, alginates, and carrageenan) used as thickening agents in foods and beverages. Human consumption of algal foods varies by nation, with Japanese diets representing a re-cent (2010–2014) annual per capita consumption ranging from 9.6 (2014) to 11.0 (2010) g macro algae day^{-1} (MHLW 2014). Generally, the development towards rising nutritional demand for algal products a universal basis stems from a better focus on health and wider applies of food additives. In addition to their dietary value, algae increasingly are being marketed as functional foods or B nutraceuticals; these provisos have no legal category in many nations however describe foods that contain bioactive compounds, or photochemical, that may promote health beyond the role of basic nutrition e.g., anti-inflammatories, disease prevention; (Bagchi 2006; Hafting et al. 2012). The path from algal research to the launching of new food products or dietary supplements is strongly affected by industrial, regulatory, and nutritional considerations (Borowitzka 2013 and Finley et al. 2014). The widespread interest in algal foods and/or their functional food potential is evident in numerous recent reviews (Warrand 2006; MacArtain et al. 2007; Kulshreshtha et al. 2008; Bocanegra et al. 2009; Cottin et al. 2011; Pangestuti and Kim 2011; Stengel et al. 2011 ; Cornish et al. 2015; Hafting et al. 2015).

Various researches description the prospective nutritional or bioactive substance of different algae but some fewer studies quantify the bioavailability of nutrients and photochemical from algal foods. Our intention is to evaluate and assess what is known about different food components (i.e., proteins, polysaccharides, lipids, vitamins, minerals, and anti-oxidants, potential toxicants) in the context of improving knowledge about the efficacy of algal foods. There are rich opportunities for phycologists to collaborate with other scientists and clinicians in this emerging field from algal B prospecting to defining nutritional value, bioaccessibility, and subsequent bioactivity, to the design and construction of mid-large cultivation systems for production of commercial-scale product (Wells et al. 2016).

2.2.1. Vitamins and Minerals

In the view of consumers, the concept of sustainable, “chemical free” and organic farming has become very appealing, including using the natural forms of vitamins and minerals instead of the synthetically produced ones. Both micro- and macroalgae have potential as mineral additives to replace the inorganic mineral salts that are most commonly used in the animal feed industry, it has been suggested that the natural forms are more bio-available to the animal than the synthetic forms and can be even altered or manipulated via the process of bio-absorption (Doucha et al. 2009). Minerals rich seaweed has been incorporated in commercial salmon feeds at 15 % in lieu of manufactured vitamin and mineral pre-mixes (Kraan and Mair 2010). Final tests suggested that salmon fed the “seaweed” feeds appeared to be healthier, more active; flavor and texture were improved which may have been due to the bromophenolic compounds found in seaweeds. Elsewhere, *Enteromorpha prolifera* and *Cladophora* sp., when added to the feeds of laying hens, positively influenced egg weight and egg shell thickness (Richmond 2004). The vitamin substance of algal biomass can be different radically among species. Ascorbic acid shows the greatest variability according to Brown and Miller (1992), although this may have been due to differences in processing, drying and storage of algae, as ascorbic acid is very sensitive to heat. This highlights the drawback of supplying essential micronutrients via natural sources, i.e. there is too much variability arising from the combined effects of different algal species, growing season, culture conditions, and processing methods to reliably supply the required micronutrients in a pre-determined fashion. Accordingly, algal biomass mainly offers a supplementary source rather than a complete replacement for manufactured minerals or vitamins in animal feeds (Richmond 2004).

2.2.2. Pigments

The carotenoids are a class of yellow, orange or red naturally occurring pigments, which are distributed everywhere in the living world. Only the microorganisms, fungi, algae and higher plants are able to synthesis carotenoids de novo, therefore animals rely on the pigment or closely related precursor being supplied in their diets, which in nature would have passed on through the food chain (Garner et al. 2010). Farmed salmonid fish therefore require supplementation of dietary astaxanthin to achieve the pink color of the fillet. Artificial carotenoids are mostly used for this purpose in profitable aquaculture, although algae-derived carotenoids can also communicate pigmentation effectively (Garner et al. 2010). *Chlorella* sp. and *Spirulina* sp. are commonly incorporated into feeds for ornamental fish, where coloration and healthy appearance is the main market criterion (Sergejevová and Masojídek 2011). Seaweeds are the favorite feed of sea urchins in natural world and in an aquaculture setting, carotenoid-rich sources such as *Ulva* sp. And *Gracilaria* sp. are necessary to enhance the orange color of the gonads that consumers prefer (Al-Badri 2010).

2.2.3. Fatty Acids

Farmed fish and shellfish offer rich sources of long chain, highly unsaturated fatty acids (HUFA), due to the inclusion of fishmeal and fish oil in formulated aquafeeds. HUFA are crucial to human health and play an important role in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, and other inflammatory and autoimmune disorders. Due to the global shortage of fish oil and fishmeal, researchers are looking increasingly into alternative sources of lipid, including from algal biomass (Atalah et al. 2007). Despite the typically low lipid content of seaweeds, Dantagnan et al. (2009) reported that Macrocystis pyrifera meal enhanced the level of PUFAs in trout flesh, when included in the diet at a level of 6 %.

2.2.4. As a Potential Feed Ingredient – Source of Protein and Energy

Typical compositions of feed and feed/gain ratio are summarized in Table (2.1) for several farmed terrestrial and aquatic animal species. This table just provides an overview, as different feed formulations are used depending on the production stage of the target species. Since protein is generally one of the most expensive feed ingredients, targeted rations are used and the amounts of protein in the diet are reduced as the animals grow. As can be seen, feeds for aquatic animals are more energy and nutrient dense than those for terrestrial animals. Due to this, fish need to be fed less to support each unit of growth, as is indicated by the lower feed conversion ratio (FCR) (FAO 2010).

Table 2.1. Typical composition of commercially available feed ingredients and algae species (per dry matter) (Ayoola 2010)

	% Crude Protein	% Crude Lipid	% Crude Carbohydrate*	% Ash	Gross Energy MJ/kg
Fishmeal	63.0	11.0	-	15.8	20.1
Poultry meal	58.0	11.3	-	18.9	19.1
Corn-gluten	62.0	5.0	18.5	4.8	21.3
Soybean	44.0	2.2	39.0	6.1	18.2
Wheat meal	12.2	2.9	69.0	1.6	16.8
Spirulina	58.0	11.6	10.8	13.4	20.1
Chlorella	52.0	7.5	24.3	8.2	19.3
Tetraselmis	27.2	14.0	45.4	11.5	18.0
Gracilaria sp[1]	34.0	1.5	37.1	26.9	13.4
Gracilaria sp[2]	10.0	0.9	50.1	34.0	11.2
Ulva lactuca[1]	37.4	2.8	42.2	17.4	15.7
Ulva lactuca[2]	12.5	1.0	57.0	24.5	11.2
Schizochytrium[3]	12.5	40.2	38.9	8.4	25.6

Carbohydrates calculated as the difference % DM – (% protein + % lipid + % ash)
[1] Cultured in effluent of fish tanks
[2] Collected from natural habitat
[3] Commercial product, Martek Biosciences

To help in assessing algae as a potential source of protein and energy in the form of carbohydrates and lipids, Table 2.1 compares the typical nutritional profiles of commercially available animal feed ingredients with some selected micro- and macroalgae. In totaling to quantifying the gross composition of feed ingredients, awareness of their digestibility is required in order to evaluate the nutritional value (Ayoola 2010).

2.3. Definition and Composition of Algae

To be a viable alternative, a candidate ingredient must possess certain characteristics including wide availability and a competitive price. It must also be easy to handle ship and store. Several materials have been tested as alternative protein sources, such as animal by-products, single cell proteins including micro algae, bacterial single cell protein (El-Sayed 1994; Mazurkiewicz 2009). Algae can grow in places that are unsuited for agriculture, such as desert, wastelands or unfertile coastal areas. Microalgae have evolved in the hostile environment of the primordial earth where there was not so much oxygen in the atmosphere (Kovavc et al. 2013).Rapid growth rates can be achieved with the optimal light exposure (intensity and wavelength), pH, temperature, mixing speed, change of substrate composition, and the ratio of the concentration of dissolved oxygen and CO_2 in the medium (Cheah et al. 2015). These conditions will favor algal growth (production of biomass), and protein production. During unfavorable growth conditions, algae will start to store energy in the form of lipids and carbohydrates. To achieve this, we need to stress algae by limiting nitrogen and\or phosphorus sources, their primary nutrient sources. Other stress factors include temperature increase, excessive exposure to light, and high iron content (Singh and Singh 2015). To achieve desired biomass composition (lipid, proteins, carbohydrates and pigment content), development of various growth techniques is necessary.

Algae are photosynthetic organisms and they are the ultimate source of both cellular carbon and chemical energy for other organisms. Therefore, they often called primary producers. Generally they categorized as macroalgae (seaweed) and microalgae (unicellular). For the growth of microalgae need light, carbon dioxide and nutrients. The microalgae are cultivated and use for food, for production of useful compounds, as biofilters to remove nutrients and other pollutants from wastewaters, in cosmetic and pharmaceutical industry and in aquaculture purpose. Also microalgae are potentially good sources for biofuel production because of their high oil content and rapid biomass production (Velichkova et al. 2012; Sharma et al. 2013; Hattab and Ghaly 2014).

2.3.1. Microalgae

Higher plants cannot meet all animal requirements; some of them contain anti-nutritive components, (for example soy) that make them unsuitable for the feed industry. The micro-algae could be therefore a solution. Even though the principle of cultivation of *Chlorella* is relative simple, high production costs did not allow for the industrial production for large scale and wide use in animal or human nourishment in northern countries, wherever closed cultivation system have to be built. Since photosynthesis, and thus plant growth, requires sunlight micro-algae can only be grown economically using natural sunshine. Only in countries like Japan or Taiwan do the climatic conditions allow for economically viable cultivation in open tanks, this is one of the reasons that micro-algae have been used in these countries for centuries. However the simplicity of the cultivation systems has acceptable the producers to commercialize the micro-algae, and today the international annually micro-algae production value reaches 500 Million US $. *Chlorella vulgaris* belongs to one of about 30 cultivated algae species and is being sold as a nutritional supplement in powder or tablets form, or as a component in cookies, noodles and other foodstuff, nowadays. Another commercial use of the green micro-algae is in the cosmetics industry (FAO 2009). Diverse morphological and physiological characteristics of microalgae are enabling their use in the production of protein, vitamins, antioxidants, drugs, immunostimulants, biofuels and food supplements. These physiological characteristic was the main reason for intensive research on microalgae cells in recent years. Being singe celled gives them the opportunity to spread on the much wider surface maximizing use of sunlight contrary to plants that has limited surface and position (Lum et al. 2013). Their simple cell structure will ensure the rapid and successful growth of under various conditions. This characteristic enables them to be present in the most diverse ecosystems (sea, rivers, lakes, lagoons) and the habitats with unfavorable environmental conditions for other species (Chu 2012).

Microalgae are the source of energy run through the aquatic food chain and are greatly regarded for their dietary value. Microalgae are widely used in aquaculture and are a few of the most essential feed sources for special groups of commercially vital aquatic organisms in both marine aquaculture and freshwater. Microalgae are frequently used as a food source for marine herbivores and in the first feeding process of some carnivorous larvae. Microalgae are consumed mostly whole as a basic diet component or as a food additive to supply basic nutrients (FAO 2009). Microalgae as a source of PUFA (polyunsaturated fatty acid), high quality proteins and other nutrients could partially be a substitute for fish proteins, fish oil in feeds and feed supplements. The production of microalgae is commonly intended associated to the achievement of aquaculture and enough production is generally regarded as a constraint and challenge to finfish and shellfish production. As the aquaculture industry develops, concepts such as engineering waste management in aquaculture systems, nutrients recycling and feed conversion have become of particular interest. The obtainable saleable production systems for microalgae are well recognized and are based on simple technology, even though innovative culture systems maintain to be developed gradually as understanding of microalgae biology and the necessities of a large-scale algae culture system continue to improve (FAO 2009).The utilize of live microalgae to eliminate excess dissolved nutrients from aquaculture effluents is an efficient and cost effective waste water treatment method (Velichkova et al. 2014). Microalgae contain numerous bioactive compounds that can be harnessed for commercial use. The pigment responsible for the pink color of salmon and trout is the carotenoid astaxanthin and one of the natural astaxanthin sources is the freshwater green alga *Haematococcus pluvialis* (Lorenz and Cysewski 2000). On the other hand a small number of some microalgae release toxins which can cause problems in the freshwater aquaculture of both vertebrates (fish) and invertebrates (shellfish). Severe blooms of even non-toxic algae can spell disaster for cultured hydrobionts, because the blooms reduce the oxygen in the shallow waters of a lot of aquaculture systems. This review was done in order to establish the positive and negative importance of microalgae for the aquaculture due to the growing significance of this sector. Microalgae consists of a wide range of nourishing compounds as well as protein, vitamins, essential amino acids, pigments and minerals, which has involved world interest for considering it as a dietary additive (Becker 2007). Microalgae could be used successfully as a good monitors or indicators to determine the quantity and quality of pollutants in the water. Surface water rich with highly nutrients contain many types of microorganisms, the use of algae as fish feed source was first mentioned by (Broun 1980), they found positive growth performance in all fish groups feed diets containing algae cells (Robinson et al. 1998).

Table 2.2. Classes, genera, and species of major currently named microalgae grown for food in fish aquaculture, and their main utilization. Synonymous names are in brackets (Richmond 2004)

Class	Genus	Species	Main utilization
Cyanophyceae (blue-green algae)	*Arthrospira (Spirulina)*	*platensis, maxima*	OGF
Chlorophyceae (green algae)	*Chlorella* *Dunaliella* *Nannochloris* *Haematococcus*	*minutissima,* *virginica, grossii* *tertiolecta, salina* *atomus pluvialis*	FLPF, GW
Prasinophyceae (scaled green algae)	*Tetraselmis (Platymonas)* *Pyramimonas*	*suecica, striata, chuii* *virginica*	FLPF, GW
Eustigmatophyceae	*Nannochloropsis*	*Oculata*	FLPF, GW
Prymnesiophyceae (Haptophyceae)	*Isochrysis* *Pavlova(Monochrysis)*	*galbana, aff. galbana* *'Tahiti ' (T-iso)* *lutheri, salina*	FLPF, GW
Dinophyceae (dinoflagellates)	*Crypthecodinium*	*Cohnii*	SDLPF
Thraustochytriidae	*Schizochytrium*	sp.	SDLPF

OGF: On growing formulation; FLPF: Fresh live prey feed; GW: Green water; SDLPF: Spray dried live prey feed.

Microalgae are photosynthetic microscopic organisms that are found in both freshwater and marine water environments. Microalgae find uses as food and as live feed in aquaculture for production of bivalve mollusks, for juvenile stages of abalone, crustaceans and some fish species and for zooplankton used in aquaculture food chains. Advantageous supplements from micro-algae involve a vital market in which compounds such as astaxanthin, β-carotene, polyunsaturated fatty acid (PUFA) such as DHA and EPA and polysaccharides such as β-glucan dominate. The dominating species of microalgae in commercial production includes *Isochrysis*, *Chaetoceros*, *Chlorella*, *Arthrospira Spirulina* and *Dunaliella.* In the recent article it has been paying attention on the using of microalgae (marine, freshwater and other such habitats) in viable and industrial sector to harness the growing demands of such unexplored natural resources (Priyadarshani and Rath 2012).

2.3.2. Chlorella

Chlorella is a single-celled fresh water micro alga with grass-like odor. Its characteristic emerald-green color and pleasant grass odor is due to its high content of chlorophyll, in fact the highest compared to any known plant. The name "*Chlorella*" was consequent from the Latin words 'chlor' for green and 'ella' for small. It has a size of 2 – 8 microns which makes it possible to be observed only under a microscope. It is roughly equal in size as human red blood cell but differs in shape where *Chlorella* is spherical whereas the human red blood cell is disc-shaped. *Chlorella* reproduces at a very fast rate, renewing into four new cells in every 17 – 24 hours. This remarkable ability of reproduction shows that it is very high in 'qi' or vital energy (DOE 2016).

Microalgae use by indigenous populations has occurred for centuries. However, the cultivation of microalgae is only a few decades old and among the 30000 species that are believed to exist, only a few thousands strains are kept in collections, a few hundred are investigated for chemical content and just a handful are cultivated in industrial quantities (Borowitzka 1999; Chaumont 1993; Radmer and Parker 1994; Olaizola 2003) Some of the most biotechnologically relevant microalgae are the green algae (Chlorophycea) *Chlorella* vulgaris, *Haematococcus pluvialis*, *Dunaliella salina* and the Cyanobacteria *Spirulina maxima* which are already widely commercialized and used, essentially as dietary supplements for humans and as animal feed additives. *Chlorella vulgaris* has been used as an alternative medicine in the Far East since ancient times and it is known as a traditional food in the Orient. It is widely produced and marketed as a food supplement in many countries, including China, Japan, Europe and the US, despite not possessing GRAS status.

Chlorella spp. is being considered as a potential source of a wide spectrum of nutrients (e.g. carotenoids, vitamins, minerals) being widely used in the healthy food market as well as for animal feed and aquaculture. *Chlorella* spp. is important as a health-promoting factor on many kinds of disorders such as gastric ulcers, wounds, constipation, anemia, hypertension, diabetes infant malnutrition and neurosis (Yamaguchi 1996). It is also attributed a preventive action against atherosclerosis and hypercholesterolemia by glycolipids and phospholipids, and antitumor actions by glycoproteins, peptides and nucleotides (Yamaguchi 1996). However, the most important substance in *Chlorella* spp. seems to be a beta-1,3-glucan, which is an active immunostimulator, a free-radical scavenger and a reducer of blood lipids (Spolaore et al. 2006). As photosynthetic organisms, these groups play a key role in the productivity of oceans and constitute the basis of the marine food chain. Among numerous alga genera, *Spirulina* and *Chlorella* deserve extraordinary consideration due to their significance as human food and there *in vitro* and *in vivo* antioxidant potential, These algae can be broadly developed to obtain a protein rich material of alimentary use (foodstuff for diet complementation) or industrial use (blue pigments, emulsifiers, thickening and gelling agent) (Habib et al. 2008; Hasan and Chakrabarti 2009). Yamaguchi (1996), improved that among the microalgae, *Chlorella*, belonging to the Chlorophyta, Chlorophyceae and *Chlorella*, are broadly distributed in the environment, especially in fresh water. Yamaguchi, referred *Chlorella* can live by photoautotrophy and heterotrophism by outside carbon source. Therefore *Chlorella* is easily cultivated in laboratory and possesses highly applied value (Yamaguchi 1996). Tanaka et al. (1998) proved that *Chlorella* contains important comfortable of protein, vitamins, polysaccharide, lipid, minerals and other dietary substances, and those ingredients possess great bioactivity concerning in numerous physiological. Found that the extraction of *Chlorella* could increase the CD4 cell number to inhibit the neoplasm metastasis and progression. Temporarily, the *Chlorella* could guard the mice against *Listeria* monocytogenes disease (Dantas et al. 1999). *Chlorella* is sphere-shaped single celled freshwater micro-algae. *Chlorella* has potentials as component of portion or natural ASUH (safe, healthy, whole and halal) feed enhancement for it contains nutrition and active element, decreases cholesterol level and resulting darker yolk. *Chlorella vulgaris* is kind of green algae which, its economic potential needs to be revealed. Variety of components of increasing media is one of factors determining quality of microalgae. In terms of mass production, it is significant to find correct, cheap and easy to feed nourishment for breeders.

Chlorella vulgaris grew well at technical medium 10% of Phytos, crude protein 57.63%, fat 5.84%, b Carotene 6.44 mg/gram, Vitamin C 4.12 mg/gram and vitamin E 1.32 mg/gram. *Chlorella vulgaris* potential to be natural and ASUH feed supplement and Phytos can be used as nutrition for mass production (Marlida and Purwati 2014). *Chlorella vulgaris* is a spherical, unicellular microalga, with a diameter of 2-10 μM that grows in fresh water conditions. It shows rapid growth during favorable conditions, and it is resistant to invaders and harsh environment. The minimal conditions necessary for algae growth, in the water medium are light and CO_2. By changing the medium and modifying conditions, their growth is accelerated and targets the production of the particular set of compounds (Safi et al. 2014).

2.4. Chlorella as a Feed Supplement for Humans

Microalgae, with their rapid growth rates and utilization of renewable resources (Borowitzka 1997) are efficient producers of high-protein biomass. Microalgae are a vast group of photosynthetic heterotrophic organisms which contain essential amino acids, protein, minerals, vitamins, chlorophylls and some kinds of antioxidants and bioactive substances (Kwak et al. 2012). Due to these properties, microalgae had been applied in areas of food and medicine. Recently, the immunostimulating properties of microalgae have attracted the interest of researchers.

The *Chlorella* could protect the mice against *Listeria monocytogenes* infection by increasing T-helper-1 cell (Hasegawa et al. 1994; Dantas et al. 1999). Tanaka et al. (1998) found that the *Chlorella* extraction could increase the CD4+ cell number to inhibit the neoplasm metastasis and progression. In addition, *Chlorella* could induce the activation and maturation of human monocyte-derived dendritic cells through NF-αB and PI3K/MAPK pathways (Chou et al. 2012). Studies in mammals have provided great references for the application of microalgae in fish farming. The utilize of algal biomass, which is a sustainable resource of a lot of valuable active substances with a broad range of applications in agriculture, includes sustainable agriculture and manufacturing and meets both ecological and economic objectives, measured as a protection beside pollution and risks from agricultural activities. The size of the productivity of microalgal biomass is determined now as 5 thousand tons per year (dry weight), which gives the market value of $500 MM (Spolaore et al. 2006; Muller-Feuga 2000) Because of these nutrients and the values of feeds, microalgae can be incorporated into the diets of various animals, fish, and domestic animals and in animal breeding (Brown et al.1997; Navarro et al. 2001; Martinez- Fernandez et al. 2006). The use of algae as feed materials for animals is more common than their use in the human diet. A large number of nutritional and toxicological evaluations showed the algal biomass could be used as a valuable feed supplement, which can successfully replace conventional sources of protein (soy, fish meal, rice bran, etc.) (Becker 2007). Seaweeds are also a source of dietary minerals such as sodium, potassium, iodine as well as fibre. Another potential area, where the use of seaweeds becomes important, is their supplementation in order to improve the texture of foods (Chojnacka et al. 2012).There is therefore a role for both national governments as well as intergovernmental organizations to re-evaluate the potential of *Spirulina* to fulfill both their own food security needs as well as a tool for their overseas development and emergency response efforts (Habib et al. 2008). Algae are an important source of vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants, etc. (Pulz and Gross 2004; Svircev 2005; Blazencic 2007; Gouveia et al. 2014). The strong potential of microalgae stems from the facts that they are not as well studied as agricultural crops, they can be cultivated in are as unsuitable for plants (with less or no seasonality required), and in comparison with plants, some species have several fold higher production. Since they utilize sunlight energy more efficiently, their potential for the production of valuable compounds or biomass is widely recognized and they can be used to enhance the nutritional value of food and feed.

2.5. Feeding Algae to Fish

A study was undertaken to evaluate the use of *Spirulina* (*Arthrospir platensis)* as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus*. The growth-promoting influence of *Spirulina* was observed with fish. No significant changes in fish survival among the different treatments, although *Spirulina* supplementation increased protein deposition in fish body especially when fed on 1.25 – 5.0 g/kg diet. No important differences in lipid and residue contents were observed among the different treatments. The physiological parameters were improved when fish fed *Spirulina* supplement. However, the highest red blood cells (RBC), white blood cells (WBC), and nitro blue tetrazolium (NBT) values were obtained at 5.0 - 10.0 g *Spirulina*/kg diet; meanwhile the lowest values were obtained at control. Total fish mortality 10-days after IP injection with *A. hydrophila* and its count after incubation with fish serum decreased with the increase of *Spirulina* level in fish diets. The lowest fish mortality and bacterial counts were obtained when fish fed 5.0 - 10.0 g *Spirulina*/kg. These results indicate that

Spirulina supplementation is promising for disease prevention in tilapia culture, and the optimum level of *Spirulina* in fish diet is 5.0 - 10.0 g per kg diet**.** (Abdel-Tawwab et al. 2008). Replacement fishmeal with 10% Spirulina in the study of Al-Koye (2013) had excellent affect in all growth parameters like weight gain, daily growth rate, specific growth rate, relative growth rate and had good effect on productivity especially food efficiency ratio, survival. In fish carcass had effect on protein also, in fish diet had effect on lipid. In blood parameters had excellent effect on (WBC) White Blood Cell, (HB) hemoglobin, (MCH) Mean Corpuscular Hemoglobin, (MCHC) Mean Corpuscular Hemoglobin Concentration, (MCV) Mean Corpuscular Volume also in bacterial total count in rear water of aquarium and fish intestine (T3) had great affect. Different *Ulva* level in the diet of (El-tawil 2010) were used, the highest significant ($P< 0.05$) values of protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) were receive through the fish maintained at 10-15 and 20% nutritional *Ulva*. Therefore, green seaweeds (*Ulva* sp.) could be supplemented to red tilapia (*Oreochromis* sp.) diet at optimum level of 15% to improve growth performance without any adverse effect on feed efficiency or survival rate.

This research of (Abdel-wraith et al. 2016) aimed to evaluate the property of diet containing the green macroalgae, *Ulva lactuca*, on the growth performance, feed consumption and body composition of African catfish *Clarias gariepinus*. Significant differences were evident in weight gain, specific growth rate and feed utilization. Fish fed with a diet containing 20% or 30% *U. lactuca* meal had inferior development performance and feed consumption. Protein productive value, protein efficiency ratio, daily dry feed intake and total feed intake were also significantly lower in fish fed with D3 and D4 than in the control D1 and D2. Overall, the results of the experiment revealed that African catfish fed a diet with *U. lactuca* included at 20% and 30% levels showed poorer growth and feed utilization than the control group and fish fed diets containing 10% of *U. lactuca***.**

In recent times, a few feedstuffs and feed additives used to improve lipid metabolism to reduce body lipid and improve carcass quality (Nakagawa and Montgomery 2007). A few dietary macroalgae meals are enhanced the growth, physiological activity, lipid metabolism, stress response, disease resistance and carcass quality of various fish species

(Ergün et al. 2009; Güroy et al. 2011, 2013). Güroy et al. (2011), showed that addition of dietary low level *Ulva* meal has been found to develop growth performance and lipid deposition for several fish species counting rainbow trout *Oncorhynchus mykiss* and tilapia *Oreochromis niloticus* (Güroy et al. 2007; Azaza et al. 2008; Ergün et al. 2009). *Ulva* is a best supply of protein, minerals, vitamins and pigments, particularly rich in vitamin C (Ortiz et al. 2006; Garcia-Casal et al. 2007). Addition of *Ulva* meal showed significant improvements in the growth performance, feed utilization, total crude protein content and highly unsaturated fatty acids percentages which have positive impact on human health. The fish group received 7.5% *U. fasciata* showed better responses than the other fish groups except for the group received 10 % *U. fasciata,* a plateau was observed in almost all tested parameters by the increment of *Ulva* inclusion level from 7.5 to 10%. The study showed the beneficial effect of *U. fasciata* as a feed additive on the growth performances and stress tolerance of red tilapia (Norhan et al. 2009).

The study of (Khalafalla 2015) was carried out to investigate the effect of green algae *Ulva lactuca* and red algae *Pterocladia capillacea* at 0.0, 2.5 and 5% on growth performance, feed utilization, carcass composition and blood indices of Nile tilapia, fingerlings. All the growth performance parameters and feed utilization values of experimental fish were increased significantly ($P \leq 0.05$) by both of algae supplementation. Diet supplemented with 5% of *Ulva lactuca* had acceptable growth parameters compared with other diets. Fish fed supplemented diets had slight increases and decreases for carcass protein and lipids without significant differences ($P \geq 0.05$). Also, no significant differences ($P > 0.05$) were obtained for serum total protein, albumin and globulin and liver activity. It could be summarized that, algae supplementation particularly at five percent of *Ulva lactuca* point can improves growth parameters and remains composition lacking undesirable effects on liver action and blood metabolites.

In a twelve week feeding experiment, the result of two algae meals (*Cystoseira barbata* or *Ulva rigida*) on feed ingestion, development, and nutrient use of juvenile Nile tilapia, was investigated. The maximum values for weight gain were for fish fed the 5% *Cystoseira* diet, control diet, and 5% Ulva diet (156%, 151%, and 150%, respectively), however the values were not considerably different ($P > 0.05$) compared to other treatments, excluding for the fish fed on the 15% *Ulva* diet ($P < 0.05$), which exhibited the lowest weight gain. Fish fed the diet consist of 15% *Ulva* meal showed the low feed change ratio (FCR). Protein and energy utilization contribute to decrease in the groups fed the algae meals at the maximum supplementation stage of 15%. Carcass lipid levels decreased with increasing levels of Ulva meal, while an increase in carcass lipid level with increasing levels of *Cystoseira* meal was observed ($P<0.05$). The consequences recommended that *Cystoseira barbata* or *Ulva rigida* meals could be used in little percentages in tilapia diets (Güroy et al. 2007).

Chemical composition of three species of Chlorophyta, *Ulva lactuca*, *Ulva fasciata* and *Ulvaria oxysperma*, was determined. *Ulvaria oxysperma* showed humidity (16-20%), ash (17-31% dry-base), proteins (6-10%db), lipids (0.5-3.2%db), fibers (3-12%db) and carbohydrates (46-72%db) which corresponded to 192-270 kcal.100g-1 (wetbase). *U. lactuca* (15-18%db) and *U. fasciata* (13-16%db) revealed grades slightly higher for proteins, but with similar energetic contents. Natural blades of *U. lactuca* and of *U. fasciata* were more rigid than blades of *U. oxysperma* (Padua et al. 2004).

Apayd et al. (2010), study is to examine the contents of fundamental and poisonous trace component by the energy-dispersive X-ray fluorescence spectrometry (EDXRF) in seaweed (*Ulva lactuca*) from collected eight different regions of Istanbul (Turkey) in the years of 2006 and 2007. It has been analyzed by the samples using two annular radioactive sources and an Ultra-LEGe detector. A radioisotope energized X-ray fluorescence analysis via the method of several standard additions was applied for the elemental analysis of seaweed samples. The results demonstrated that these seaweeds contain some critical element, but no deadly element.

Rybak et al. (2012) analyzed the capacity of freshwater taxa of the genus *Ulva* (Ulvaceae, Chlorophyta) to serve as bioindicators of metal in rivers and lakes. Changes in heavy metal (Ni, Cd and Pb) and alkaline earth metal (Ca and Mg) concentrations in freshwater *Ulva thalli* were investigated during the period from June to August 2010. The study was conducted in two ecosystems in Western Poland, the Nielba river (six sites) and the Malta Lake (10 sites). Three components were collected for each sample, including water, sediment and *Ulva thalli*. The average concentrations of metals in the water sample and in the macroalgae decreased in the following order: Ca>Mg> Ni>Pb> Cd. The sediment revealed a slightly altered order: Ca>Mg> Pb> Ni>Cd. Ca and Mg were found at the highest concentrations in *U. thalli* due to the presence of carbonate on its surface. Among the examined heavy metals in *U. thalli*, Ni was in the highest concentration, and Cd found in the lowest concentration. There were statistically significant correlations between the levels of metals in macroalgae, water and sediment. Freshwater populations of Ulva exhibited a greater efficiency to bioaccumulate nickel as compared to species derived from marine ecosystems.

2.6. Nutritional Considerations of Algal Usage

To penetrate the animal feed market and be economically viable, algae must compete with equivalent feed ingredients, mainly fishmeal and oilseed meals (soya, etc.). Algae that have thus far been used as animal feeds include species of green, blue-green and pigmented flagellates, the advantage being that artificially cultivated algae are highly effective producers of protein as far as use of land and water resources are concerned (Berend et al. 1980). Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced radically in current years with the expansion of new, impartial commercial food that support optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products (Craig et al. 2009).

The protein content of *Chlorella* is 51-58% and contains several important amino acids, viewing that *Chlorella* can also be used as protein resource for human food and animal diets (Becker 2007). However, current applications of *Chlorella* mainly focus in human food. The research on its application in lower vertebrate was less. Gibel carp (*Carassius auratus gibelio*) is an essential inexpensive food fish in China. Many studies have been carried on its nutrition, immunization and development (Xue and Cui 2001, Hu et al. 2008, Van Campenhuout et al. 2010), however the utilization of *Chlorella* in this species is not reported until now. Their work designed six diets contain different levels of *Chlorella* to treat the Gibel carp and then the growth performance, blood parameters and digestive enzyme were detected. Tartiel and Badawy (2005) found that crude protein content of *Chlorella* spp. was 46.7% , the crude fat content was 14.8%, total carbohydrate content was 11.6% , ash was 17.5% , crude fiber 9.30 %, nucleic acid content (RNA 2.63% and DNA 1.72%), and vitamins group antioxidant B_6, B_{12} , E, C, B-carotene (µg/g dry weight). Dawah et al. (2002) reported that, five amino acids (aspartic acid, serine, alanine, leucine and glycine) were collectively responsible for 50% or more of the total dry matter content of *Chlorella* and *Scenedesmus* algae, the same authors add that algae protein like other single cell protein are deficient in sulfur-amino acids. Zeinhom (2004) found that fish fed diet containing 15% algae increased significant by the digestibility coefficient of dry matter (92.5%), crude protein (87.63%), ether extract (88.45%) and energy (81.41%). Natural food still remains the major feed for Tilapia rearing so a timely supply of microalgae in sufficient quantity ensures the success of a tilapia hatchery. Amar et al. (2004) found that microalgae extraction could enhance the innate immunity of rainbow trout (O*ncorhynchus mykiss*). Cerezuela et al. (2012) found that three orally administered microalgae (*Nannochloropsis gaditana*, *Tetraselmis chuii* and *Phaeodactylum tricornutum*) could enhance gilthead seabream (*Sparus aurata*) defence activity. *Chlorella* could be used as a good additive and could promote the growth performance and physiological parameters of Gibel carp (*Carassius auratus gibelio*) (Xu et al. 2014). However, the effects of *Chlorella* on the immunity of aquaculture species remain limited. The green microalga *Chlorella vulgaris* was exposed to monochromatic light at six different wavelengths in order to study the effect on biomass productivity and fatty acid content. A significantly higher amount of biomass by produced in the treatments with yellow, red and white light compared with blue, green and purple light. There were also significant differences in total lipid content and fatty acid profile between the treatments. The green light regime gave the lowest concentration of lipids, but increased the concentration of polyunsaturated fatty acids. Their results indicate that light quality plays a role in biofuel formation and that blue light receptors are most probably involved (Hultberg 2014). In the study of the effects of dietary *Chlorella* on the immune status of Gibel carp, *Carassius auratus gibelio*, were evaluated. Results showed that *Chlorella* could increase the levels of immunoglobulin M and D, interleukin-22 and chemokine (C-C motif) ligand 5 in some tissues, which indicated that dietary *Chlorella* can be involved in regulating adaptive and innate immunity (Zhang et al. 2016). This study of Abdulrahman (2014) was conducted to evaluate the effect of replacing fishmeal with *Spirulina* spp. in four different levels 0%, 5%, 10%, 15% and 20%, as T1, T2, T3, T4and T5 respectively on Carcass means weight (CMW) with head and without peripheral organs and CMW without head and peripheral organs, where the third and fifth treatment give the higher value in CMW with head and without peripheral organs, and the fifth treatment obtain significant differences in CMW without head and peripheral organs as compared to other treatment. When regarding the chemical composition in which the higher significant difference found in the T3 and T5 in crude protein, the T5 significantly differ in crude fat as compared with other treatments.

The study of (Sungchul 2016) was conducted to investigate the effects of *Chlorella* powder (CHP) as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Six experimental diets were supplemented with *Chlorella* powder (CHP) at 0, 0.5, 1.0, 1.5, 2.0 and 4.0% (CHP0, CHP0.5, CHP1.0, CHP1.5, CHP2.0 and CHP4.0, respectively) of diet as a dry matter basis. These results suggest that the optimum dietary CHP supplementation level could be approximately 0.5% of diet for positive effects on growth and feed utilization without any negative effects on blood parameters and body composition in juvenile Korean rockfish.

The effect of adding different levels of the alga *Spirulina* spp. in fish laboratory of Animal Production Department, Sulaimani University, Iraq was studied by Abdulrahman (2014). The control treatment T1 with 0% *Spirulina* spp., (T2) adding 1 gm *Spirulina*/kg diet, (T3) with 3 gm *Spirulina*/kg diet, and (T4) with 5 gm *Spirulina* /kg diet. Results determined that forth treatment advanced significant than other treatments in weight gain 6.89, Daily growth rate 0.17, Specific growth rate 0.147, Relative growth rate 15.31 and Food change ratio 2.14 for the control; while, Food effectiveness ratio showed significance in T3 and T4 (62.48 and 62.47) correspondingly.

3. MATERIAL METHOD

3.1. Experimental Animal

The experiment will conducted for 105 days and for these purpose 200 fingerlings common carp *C. carpio* Figure 3.1 were brought from a local aquarium fish supplier located in Daqoq, in middle of Iraq. The size of fish was varying and the weights ranged between 35-45 g. The fish were sorted depending on size then weighed and put in experimental plastic tanks. Mean initial weight was 35.7g. The fish were acclimated to laboratory conditions and fed with control pellets (29% protein) prior to the feeding trials for 21 days.

Figure 3.1. The reared fish common carp *Cyprinus carpio*

3.2. Experimental System and Design

Twelve plastic tanks (100 L) were used in this trial. Each tank was provided with a proper continuous aeration. Each aquarium was stocked with five fish and fed two times a day. The numbers of treatments in the trial were four with three replicates for each. The tanks (replicates) were randomly allocated to minimize differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the aquaria. Also, a daily cleaning by siphon method was applied to remove remained particles from the system as shown in Figure 3.2.

Figure 3.2. Experimental tank

In T1 fish were fed a diet with 0% *Chlorella*, while in T2, fish were fed a diet with 2.5gm *Chlorella*/kg diet, T3 represents the third treatment, in which fish were fed on a diet with 5gm *Chlorella*/kg diet, and in T4 fish were fed a diet with 7.5 gm *Chlorella*/kg diet, as showed in Figure 3.3.

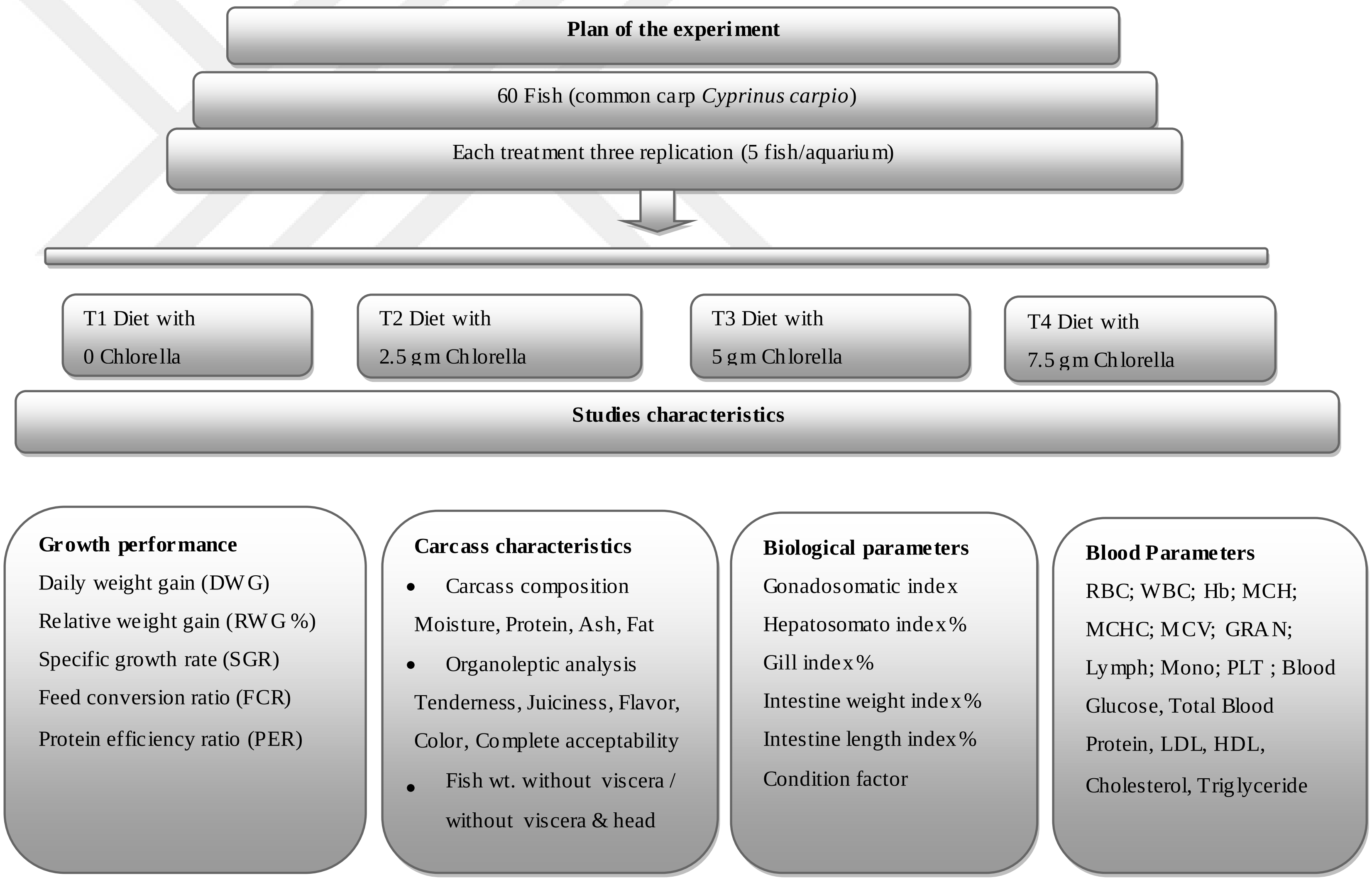


Figure 3.3. Shows the experimental design of the study

3.3. Diet Formulation

Experimental diets were prepared with animal protein concentrate, wheat bran, soybean, barley, yellow corn and *Chlorella*, and the chemical composition of the different diet shown in Table 3.1. The ingredients were mixed with water to obtain dough. Then, the dough was passed through an electrical mincer for pelleting by using Kenwood Multi-processors. The pellets were dried at room temperature for a few days and crushed to yield fine particles. The fish were fed 2 times a day, once was at 9:00 am and another time at 2:00 pm. Feeding rate 3% of biomass depending on satiation level. Fish were individually weighed bimonthly. The feeding trial continued for 12 weeks.

Table 3.1. Chemical composition of the different diet by NRC (1993, 1994)

Ingredients	**Crude protein %**	**Crude fat %**	**Dry matter %**	**Crude Fiber %**	**Energy KG/Kg**
Animal protein concentrate	40	5	92.9	2.2	2107
Yellow corn	8.9	3.6	89	2.2	3400
Soybean meal	48	1.1	89	7	2230
Barley	11	1.9	89	5.5	2640
Wheat bran	15.7	4	89	11	1300

Table 3.2. Composition of experimental diet

Ingredients	**Percentage** (%)
Yellow corn	15%
Wheat bran	18%
Soya bean meal 48%	40%
Concentration protein	10%
Barley	15%
Vitamins + Minerals Mix	2%
Total	100
Calculated chemical composition	
Crud protein	29.011
Metabolisable energy (kcal/kg feed)	2304.7
%Arginine	0.2394
%Lysine	0.25375
%Methionine + cysteine	0.12872
%Threonine	0.017
%Tryptophan	0.029

3.4. Used Chlorella

Organic *Chlorella* powder from (*Chlorella pyrenoidosa*) a product packed by Nukraft, 433 Caledonian Road, London N7 9BG UK. The nutritional information as labelled has shown in table (3.3) and figure (3.4) as below:

Table 3.3. The nutritional information of used *Chlorella*

Component	Per 100 gm
Energy	418 Kcal
Protein	55
Fat	15
Carbohydrate	19.5
Fiber	12.5
Salt	0.1

Figure 3.4. Showed the *Chlorella* used in the present study

3.5. Growth Parameters

The individual body weight (g) and total body length (cm) for all fish per treatment (60 fishes) will measured bimonthly. The feed consumption of each treatment was recorded and readjusted according to the obtained biomass at every treatment weekly. The average body weight gain (ABGW) as (g/fish) was estimated according to the following equation:

Body weight gain (g/fish) = Mean of weight (g) at the end of the experimental period – weight (g) at the beginning of the experimental period (Schmalhusen 1926).

Daily weight gain (DWG) = Gain / experimental period (Schmalhusen 1926).

Relative weight gain (RWG %) = Gain / initial weight x 100 (Brown 1957).

Specific growth rate (SGR) = (In W_1 – In W_0) / T) x 100 (Uten 1978).

W_1: final weight W0: initial weight T: time between $W_{1\ \text{and}}$ $W_{0.}$

Feed conversion ratio (FCR) =Total feed fed (g/fish) / total wet weight gain (g/fish) (Uten 1978).

Protein efficiency ratio (PER) = Total wet weight gain (g/fish) / amount of protein fed (g/fish) (Uten 1978).

3.6. Blood Parameters

At the end of the experimental period, all fish are randomly taken from each experimental group. All fish samples are weighed individually. The blood samples from each fish of the different groups were collected by suction of the caudal peduncle. Whole blood samples were collected in small plastic vials containing heparin for determination of hemoglobin (Hb). The hemoglobin (%) concentrations were determined by using the hematology analyzer BC-2800 is a compact, fully automatic hematology analyzer with 19 parameters for complete blood count (CBC) test.

RBC (Red Blood Cell; 10^{12} cells/l); WBC (White Blood Cell; 10^9cells/l); Hb (Hemoglobin; g/l); MCH (Mean Corpuscular Hemoglobin; pg); MCHC (Mean Corpuscular Hemoglobin Concentration; g/l); MCV (Mean Corpuscular Volume; fl); GRAN (Granulocyte; %); Lymph (Lymphocyte; %); Mon (Monocyte; %); PLT (Platelet; 10^9 cells/l).

Blood serum is being separated from fish whole blood by centrifugation for 3-5 minutes at 4500g. Serum samples were used for the purpose of conducting experimental tests of Blood Cholesterol (Cholesterol mg/dl), Blood Glucose (Glucose mg/dl), Total Blood Protein (Total Protein g/dL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglyceride and Cholesterol by using a hematological analyzing device type ACCENT 200 Poland's origin.

3.7. Biological Parameters

After blood samples collection, all the fish samples are scarified and soon the abdominal cavity is opened to remove, gonads and liver to be weighed at once. The gonad and liver indices were calculated as follow:

Gonadosomatic index (GSI) % = Gonads weight (g)/ Body weight (g) x 100

Hepatosomato index % = liver weight (g)/body weight (g) x 100 (Lagler, 1956).

Gill index % = Gill weight (g)/body weight (g) x 100

Intestine weight index % = Intestine weight (g)/body weight (g) x 100

Intestine length index % = Intestine length (g)/body length (g) x 100

Condition factor = Fish weight / Body length^3

3.8. Chemical Composition

All fish samples are used for the chemical analysis of the muscle (Moisture %, crude protein %, ether extract % and ash %) according to AOAC (2000) analytical methods. Sensory analyses will be performed by a panel of seven experienced assessors. The fish meat fillets specimens are placed in open aluminum boxes and cooked for 15 min in an oven pre-heated at 200°C; each member of the panel will filled a sensory evaluation table, as shown in Table 3.4.

Table 3.4. Organoleptic (Sensory) evaluation form

Treatments	Tenderness	Juiciness	Color	Flavor	Overall acceptability
T1					
T2					
T3					
T4					
5 =extremely like; 4 = like; 3 = neither like nor dislike; 2 = dislike; 1 = extremely dislike					

3.9. Statistical Analysis

Analysis of variance is conducted using the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 One way (ANOVA). Fisher's L.S.D test's was used to compare between means of the control and experiment treatments. The model of analysis was as follows:

$Y_{ij} = \mu + T_i + E_{ij}$

μ = the overall mean.

T_i= the effect of treatment.

E_{ij}= the random error.

4. RESULT AND DISCUSSION

4.1. Growth Performance

The present study clearly showed that feeding algae as a feed additive to fish remarkably change the studied growth performance as shown in Table 4.1, in weight gain and daily growth rate T4 (7.5 gm *Chlorella*/kg diet) was significantly higher with 35.070 and 0.418 respectively as compared to other treatments as shown in Figures 4.1 and 4.2, relative growth rate T3 (5 gm *Chlorella*/kg diet) and T4 (7.5 gm *Chlorella*/kg diet) were higher significantly than others as shown in Figure 4.3, no significant differences observed in specific growth rate among treatments as observed in Figure 4.4.

Table 4.1. Effect of adding *Chlorella* in different levels on common carp *C. carpio* growth performance

Treatments	Weight gain	Daily Growth Rate	Relative Growth Rate	Specific Growth Rate
T1 (control)	24.547 bc ± 0.059	0.292 bc ± 0.059	61.090 b ± 0.120	0.339 a ± 0.097
T2 (2.5 g *Chlorella*/kg diet)	18.595 c ± 0.030	0.221 c ± 0.030	47.041 b ± 0.153	0.314 a ± 0.232
T3 (5 g *Chlorella*/kg diet)	28.380 b ± 0.036	0.338 b ± 0.036	71.921 ab ± 0.138	0.385 a ± 0.105
T4 (7.5 g *Chlorella*/kg diet)	35.070 a ± 0.186	0.418 a ± 0.186	88.878 a ± 0.250	0.423 a ± 0.180

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).

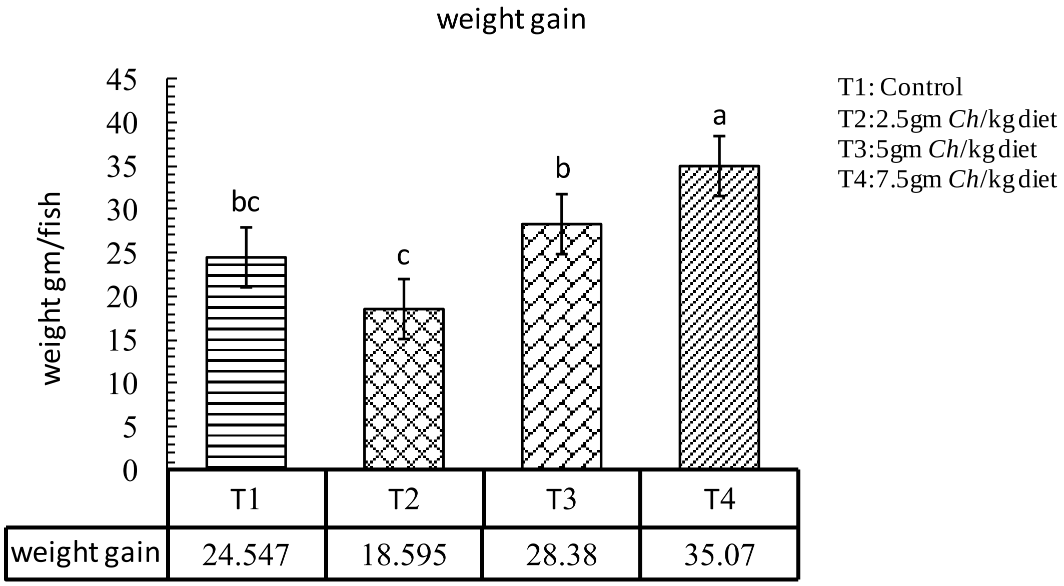


Figure 4.1. The effect of adding *Chlorella* in weight gain of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

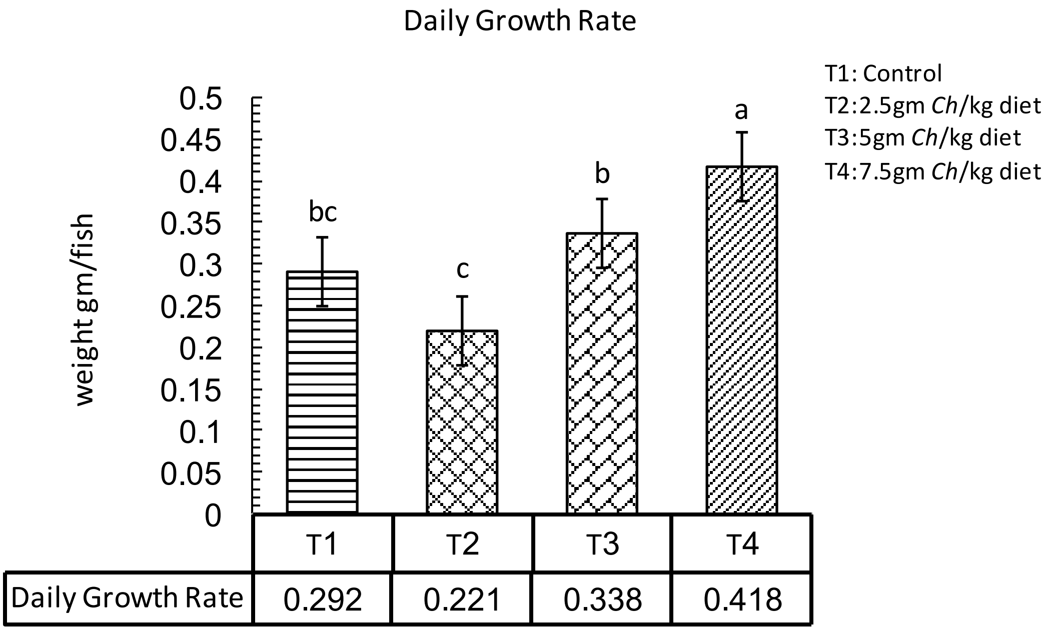
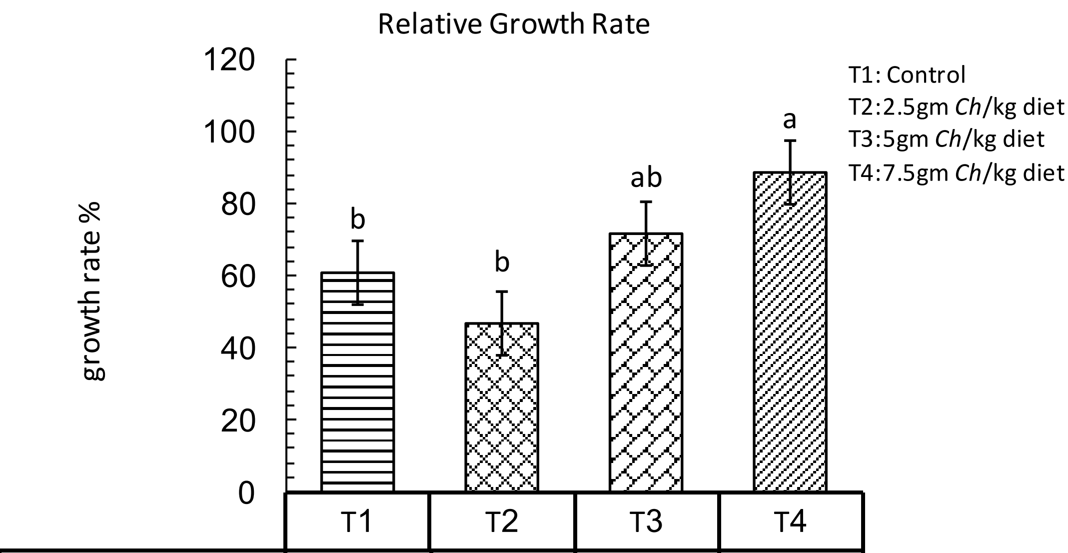
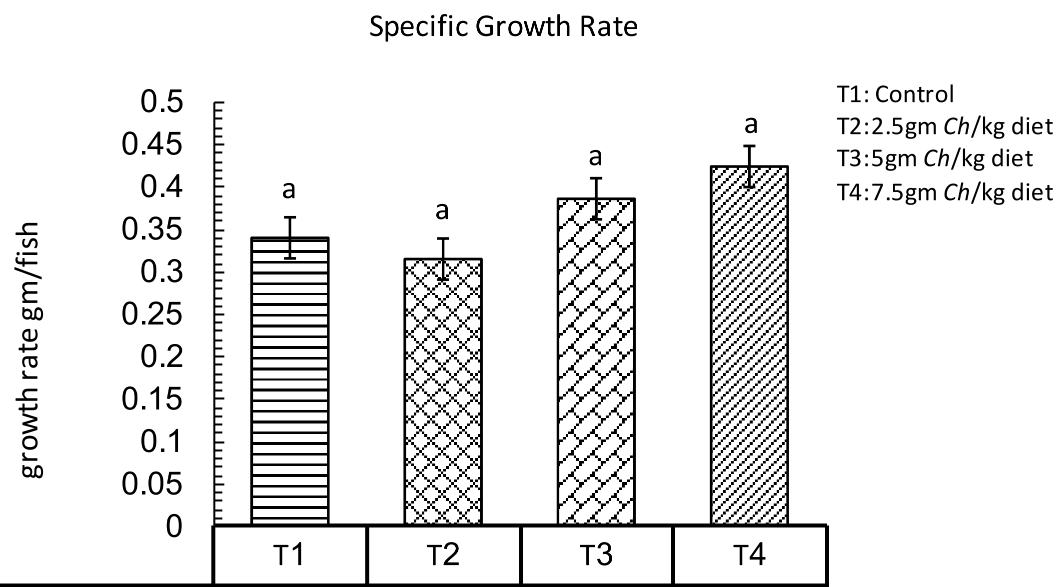


Figure 4.2. The effect of adding *Chlorella* in daily growth rate of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)



	T1	T2	T3	T4
Relative Growth Rate	61.090	47.041	71.921	88.878

Figure 4.3. The effect of adding *Chlorella* in relative growth rate of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)



	T1	T2	T3	T4
Specific Growth Rate	0.339	0.314	0.385	0.423

Figure 4.4. The effect of adding *Chlorella* in specific growth rate of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)

Dietary supplementation of 5% *Chlorella* powder significantly enhanced growth and feed intake of koi fish. The results (5% *Chlorella* supplementation) confirm the findings on *Girella pametato* (Nakazoe et al. 1986), the nibber (Nakazoe et al. 1986), (5% *Spirulina* supplementation) for *Girella pametato* (Nakazoe et al. 1986), (5% *Spirulina* supplementation) for *Pagrus major* (Mustafa et al. 1994; 1997; Nakagawa et al. 2000), and (5% *Spirulina* supplementation) for Striped jack (*Pseudocaranx denta*) (Liao et al. 1990; Watanabe et al. 1990) and this agree with the present study in which highest amount added to the diet was best significantly in growth performance.

These results differ from some findings on red sea bream (Yone et al. 1986; Mustafa et al. 1995), rainbow trout (Sommer et al. 1992), and Japanese flounder (Xu et al. 1993) that were fed on 2% *Chlorella* supplemented diets. Gibel carps *Carassius auratus gibelio* (Bloch) displayed a significant increase in growth parameters when fed *Chlorella* incorporated diets at inclusion levels of up to 1.2% (Xu et al. 2014). Positive effects on growth and skin coloration were also observed in freshwater sterlet, *Acipenser ruthenus* L. at 2.5% *Chlorella* inclusion (Sergejevova and Masojıdek 2013). Similarly, *Seriola quinquero fista* had a significant increase in growth parameters when fed *Chlorella* diets at 0.5 levels (Nakagawa et al. 1985).

The comparable growth performance in koi fish fed *Chlorella* diets may be attributed to growth promoters, such as sufficient amounts of macronutrients and naturally occurring bioactive ingredients (*Chlorella* growth factor (CGF)) that are present in *C. vulgaris* (Yamaguchi 1996; Badwy et al. 2008; Khani et al. 2017). Also the evident on growth enhancement may be due to high digestibility of the microalgae (Anderson et al. 1979).

4.2. Feed Utilization

The results of using the *Chlorella* algae as a feed additive to fish remarkably change the studied feed utilization as shown in Table 4.2, in Food Conversion Ratio T3 (5 gm *Chlorella*/kg diet) and T4 (7.5 gm *Chlorella*/kg diet) were significantly lower than other treatments as shown in Figure 4.5, in Food Efficiency Ratio no significant differences observed among treatments as observed in Figure 4.6, Protein Efficiency ratio the T3 (5 gm *Chlorella*/kg diet) and T4 (7.5 gm *Chlorella*/kg diet) were significantly higher than other treatments as shown in Figure 4.7.

Table 4.2. Effect of adding *Chlorella* in different levels on common carp *C. carpio* feed utilization

Treatments	Food Conversion Ratio	Food Efficiency Ratio	Protein Efficiency ratio
T1 (control)	0.557 ab ± 0.081	1.803 ab ± 0.078	621.554 bc ± 0.078
T2 (2.5 g *Chlorella*/kg diet)	0.633 a ± 0.226	1.414 a ± 0.101	487.545 c ± 0.101
T3 (5 g *Chlorella*/kg diet)	0.492 ab ± 0.064	2.100 ab ± 0.051	702.412 ab ± 0.064
T4 (7.5 g *Chlorella*/kg diet)	0.456 b ± 0.184	2.406 a	829.301 a ± 0.220

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).

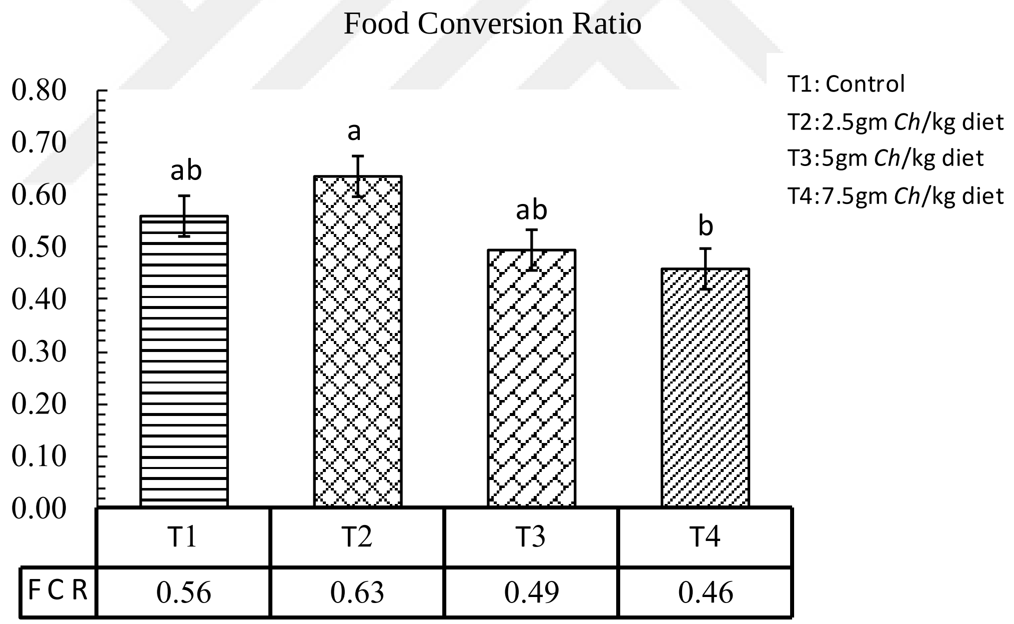


Figure 4.5. The effect of adding *Chlorella* in food conversion ratio of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)

The higher amount of *Chlorella* lower diet eaten that reflects in protein efficiency ratio, different results were obtained when different species of microalgae were incorporable in fish diet. Stanley and Jones (2003) recorded poor growth and FCR for grass carp when fed on *spirogyra* sp. *Tilapia aurea* and big mouth buffalo fed on *Spirulina* and *spirogyra* and showed high growth rate of 29g dry weight/kg for 28 days, 14g/kg body weight respectively; FCR was 2.0, 10, respectively.

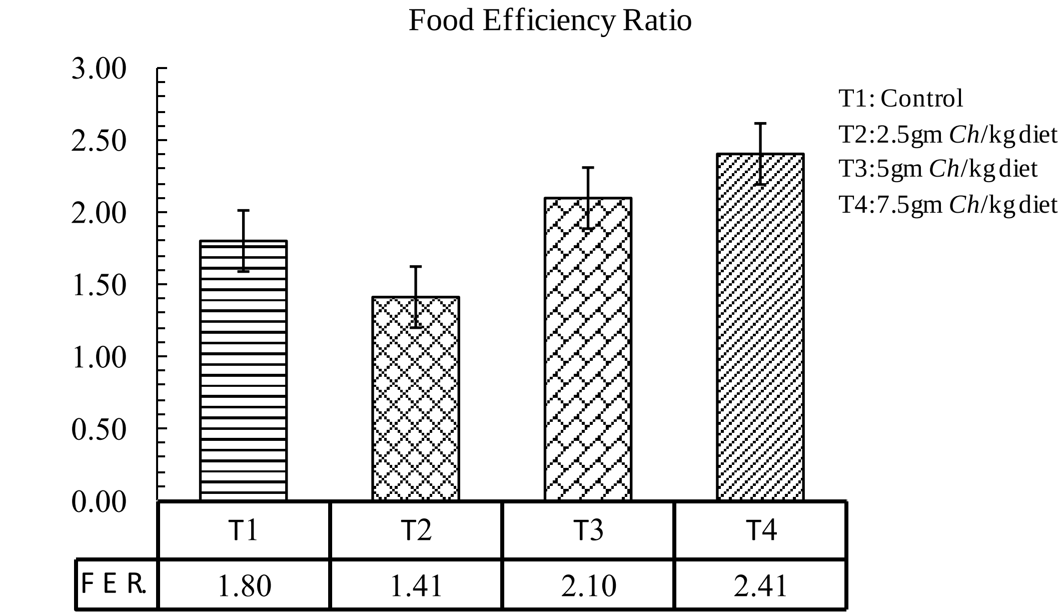


Figure 4.6. The effect of adding *Chlorella* in food efficiency ratio of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

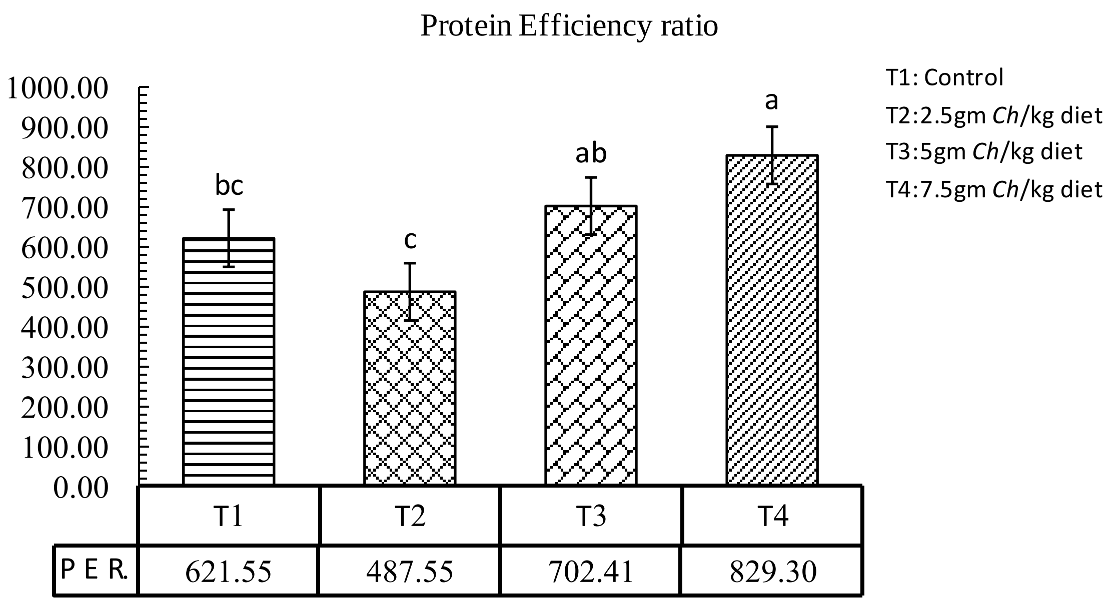


Figure 4.7. The effect of adding *Chlorella* in protein efficiency ratio of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The study of Badwy et al. (2005) was designed to investigate the effect of partial replacement of fish meal with dried microalgae (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) diets on fish growth performance, feed efficiency and body composition. Results indicated that, growth performance, feed conversion ratio and protein productive value were significantly ($P<0.05$) higher in fish feed diets containing 50% of both *Chlorella* spp. and *Scenedesmus* spp., whereas fish feed diets containing 75% algae had significance lower performance ($P< 0.05$). This finding was in agreement with the results of the present work.

Zeinhom (2004) found that, Inclusion of algae in fish diets significantly ($P<0.05$) increased the live body weight (39.69 g) and improved the FCR (2.33) and Nandeesha et al. (1998) reported that body weight gain of Nile tilapia (*O. niloticus*) increased linearly with increasing the level of algae in fish diet at levels less than 20%. It was suggested that the use of various protein sources in combination was more effective than a single source in replacing fish meal in carp diet (Hasan 1986; Hossain and Jauncey 1990). Earlier reviews of Mustafa and Nakagawa (1995) confirmed that the supplementation of macro and micro algae meal enhanced growth, feed utilization, lipid metabolism, body composition, disease resistance and carcass quality of a variety of fishes.

4.3. Biological Parameters (Health Aspects)

The present study clearly showed that feeding algae to common carp remarkably change the studied biological parameters as shown in Table 4.3, in Hepatosomatic index all treatments were significantly differ than the control as shown in Figure 4.8, Spleenosomatic index the control and T4 (7.5 gm *Chlorella*/kg diet) were higher significantly than others as shown in Figure 4.9, in Gillsomatic index the T2 (2.5 gm *Chlorella*/kg diet) and T4 (7.5 gm *Chlorella*/kg diet) were differ significantly as shown in Figure 4.10, while T4 (7.5 gm *Chlorella*/kg diet) was significantly higher than other treatments in Kidneysomatic index as shown in Figure 4.11.

Table 4.3. Effect of adding *Chlorella* in different levels on some biological indices of common carp *C. carpio*

Treatments	Hepatosomatic index	Spleenosomatic index	Gillsomatic index	Kidneysomatic Index
T1 (control)	2.697 b ± 0.075	0.504 ab ± 0.115	3.386 b ± 0.071	0.433 c ± 0.311
T2 (2.5 g *Chlorella*/kg diet)	3.444 a ± 0.111	0.457 b ± 0.138	4.295 a ± 0.063	0.737 b ± 0.140
T3 (5 g *Chlorella*/kg diet)	3.336 a ± 0.122	0.351 c ± 0.149	3.514 b ± 0.122	0.862 b ± 0.015
T4 (7.5 g *Chlorella*/kg diet)	2.982 ab ± 0.049	0.555 a ± 0.099	4.654 a ± 0.110	1.221 a ± 0.111

Mean values with different superscripts within a column differ significantly (P≤0.05).

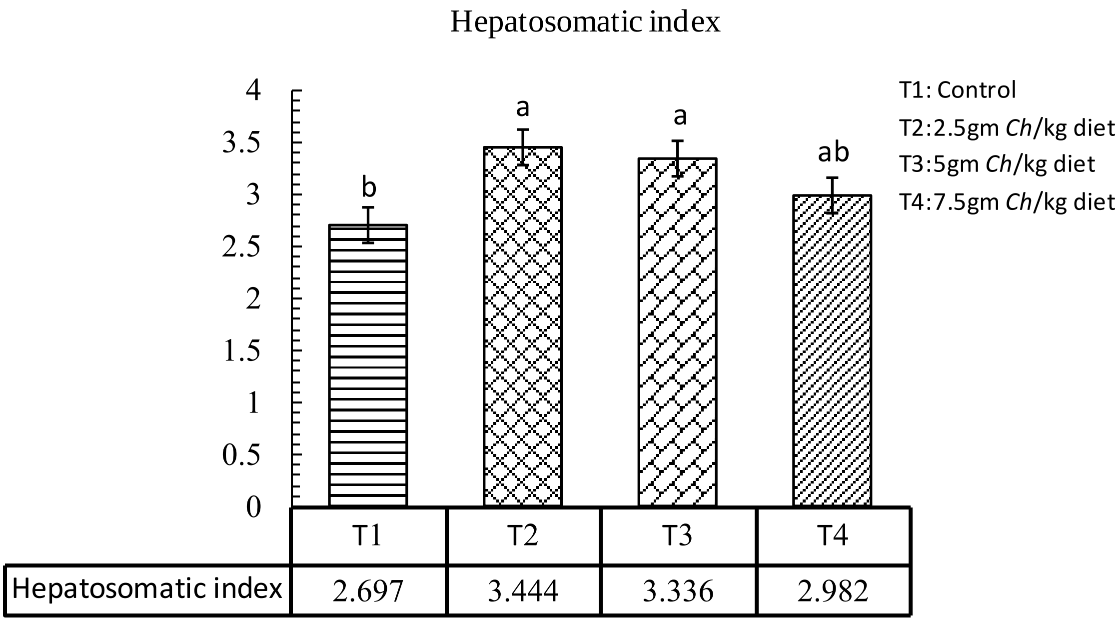


Figure 4.8. The effect of adding *Chlorella* in Hepatosomatic index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Mustafa et al. (1995) mention that the addition of algae is known to delay the absorption of dietary nutrients and improve carbohydrate and protein utilization; in addition, there may be other factors which stimulate the metabolism and growth. Liver glycogen, which is a readily available energy source, was also higher in groups fed algae. A high protein or high carbohydrate diet generally leads to high glycogen deposition in liver, Therefore, the high glycogen accumulation and high growth performance in the fish fed algae might be due to the effective absorption of nutrients and this may be the reason for increasing Hepatosomatic index in all treatments fish than the control.

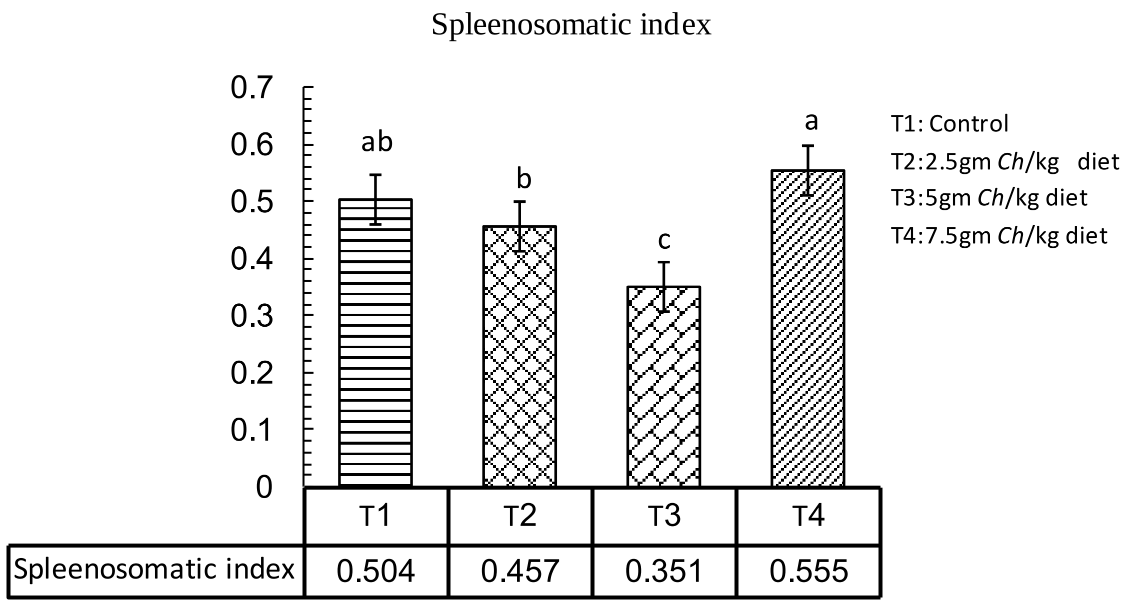


Figure 4.9. The effect of adding *Chlorella* in Spleenosomatic index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

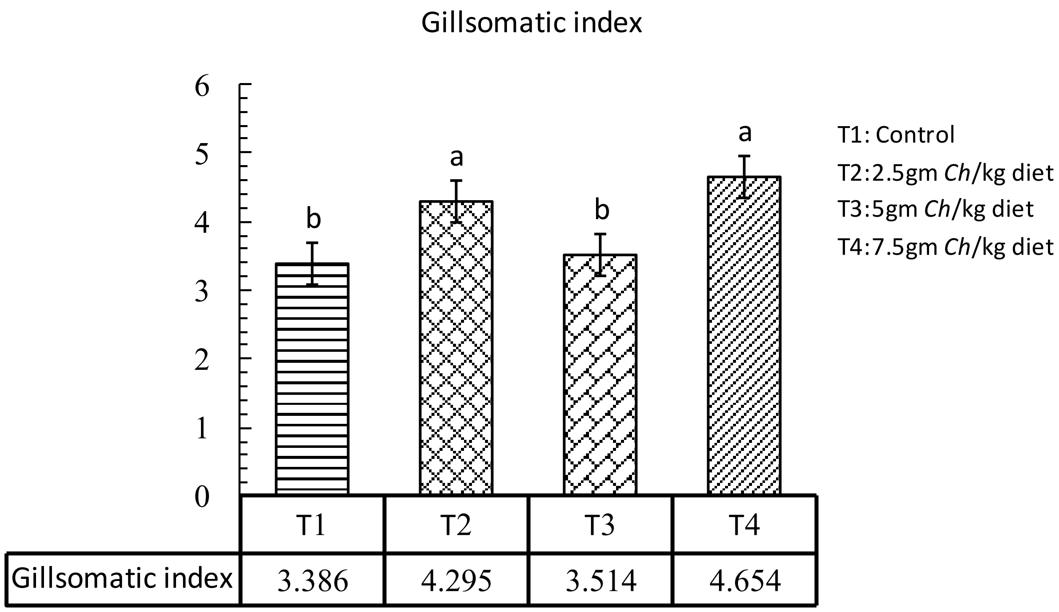


Figure 4.10. The effect of adding *Chlorella* in Gillsomatic index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

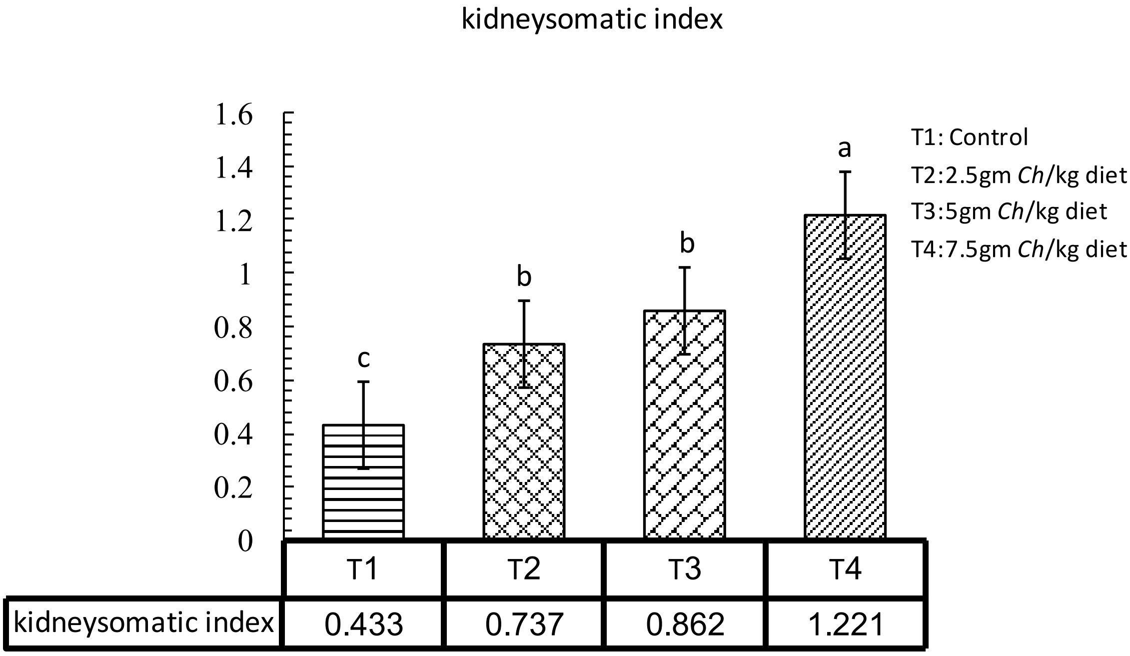


Figure 4.11. The effect of adding *Chlorella* in Kidneysomatic index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Analysis of digestive enzyme activity is a reliable methodology that can be used as an indicator of digestive processes and nutritional condition of fish (Abolfathi et al. 2012). In the study of Xu et al. (2014) found that the dietary *Chlorella* could significantly increase the digestive enzyme in the hepatopancreas and intestine of Gibel carp *Carassius auratus gibelio*, suggesting the *Chlorella* could enhance the diet utilization rate by increasing the activity of digestive enzyme.

Many fishes occasionally ingest many species of algae, even carnivorous fishes. Nevertheless, ecologists have not regarded algae as an important food source for fishes (Radhakrishnan et al. 2015). The results of the present experiment suggest that algae promote physiological activities such as the increasing of the physio-biological parameters as observed in Table 9, and indicate the efficacy of using dietary algae as a feed additive for cultured fishes.

The intestine weight index was differ significantly in all treatments except of T2 as shown in Figure 4.12, Intestine length index in T4 (7.5 gm *Chlorella*/kg diet) was significantly higher than other treatments as shown in Figure 4.13 and 4.14 condition factors higher significantly among treatments in T3 (5 gm *Chlorella*/kg diet) as cleared in Table 4.4.

Table 4.4. Effect of adding *Chlorella* in different levels on Intestine weight and length index, and Condition factor of common carp *C. carpio*

Treatments	**Intestine weight index**	**Intestine length index**	**Condition factor**
T1(control)	3.566 a ± 0.092	36.999 c ± 0.040	1.729 b ± 0.019
T2(2.5 g *Chlorella*/kg diet)	2.587 b ± 0.244	43.414 b ± 0.048	1.641 c ± 0.014
T3(5 g *Chlorella*/kg diet)	3.263 a ± 0.044	37.637 c ± 0.100	1.847 a ± 0.013
T4(7.5 g *Chlorella*/kg diet)	3.445 a ± 0.116	49.593 a ± 0.049	1.723 b ± 0.009

Mean values with different superscripts within a column differ significantly (P≤0.05).

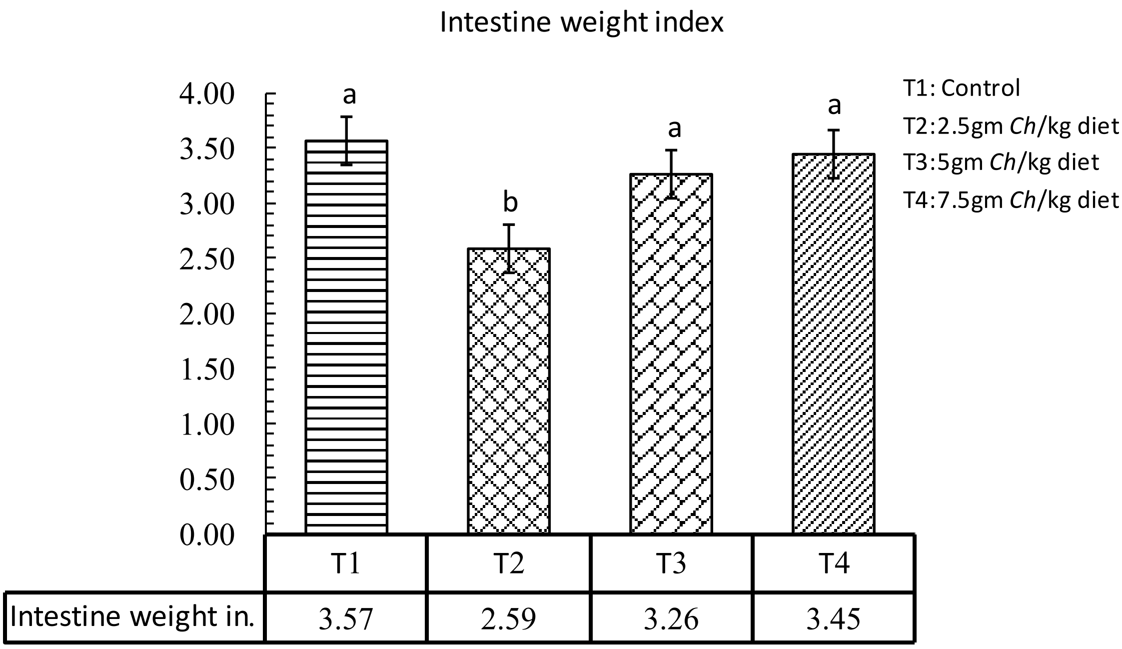
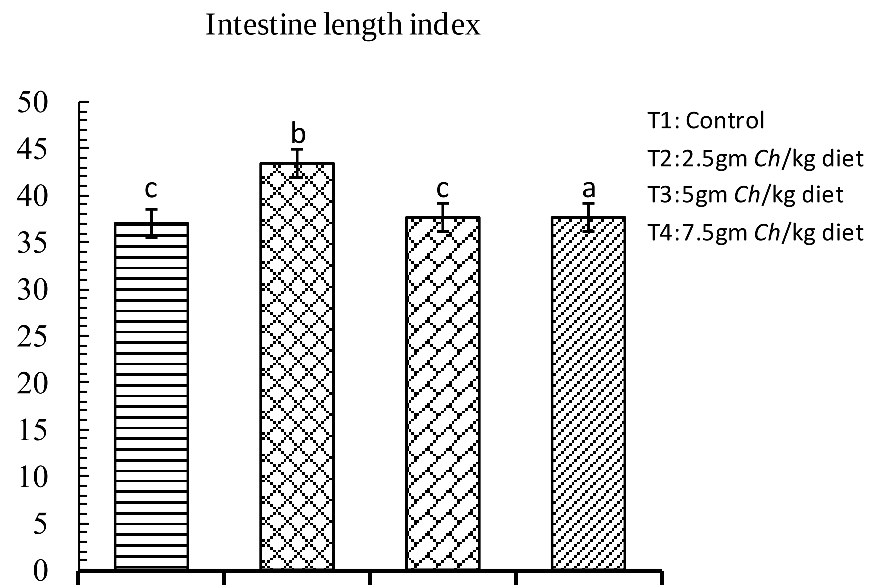


Figure 4.12. The effect of adding *Chlorella* in intestine weight index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The results of Xu et al. (2014) indicated that *Chlorella* can be a good choice for using as an additive for fish diets. Due to high contents of protein, some microalgae have been used as fish meal substitution in fish diet. It has been found that the survival rate and protein content of juvenile tilapia (*Oreochromis niloticus*) increased greatly after only feed with *Spirulina* for 63 days. The results were in accordance with the observation of Palmegiano et al. (2005). It might be the bioactive ingredients e.g. *Chlorella* growth factor (CGF) that promote the growth of fish (Yamaguchi 1996). And this may be the reasons of increasing fish length intestine with increasing the concentration of *Chlorella* in the diets of present study.



	T1	T2	T3	T4
Intestine length in.	36.999	43.414	37.637	37.637

Figure 4.13. The effect of adding *Chlorella* in intestine length index of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

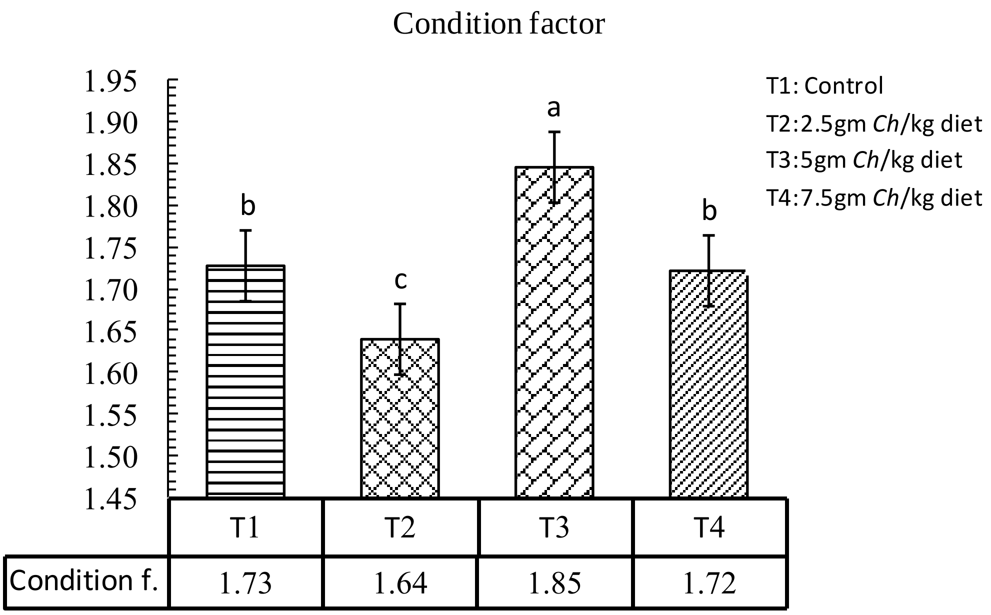


Figure 4.14. The effect of adding *Chlorella* in Condition factor of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The protein content of *Chlorella* is 51-58% and contains many essential amino acids, showing that *Chlorella* could also be used as protein source for human food and animal diets. However, current applications of *Chlorella* mainly focus in human food. The research on its application in lower vertebrate was less. The potential for microalgae to enhance nutritional content of conventional food preparations and to act as probiotic agents that positively affect the health of humans and animals has a broad spectrum (Becker 2007).

Today, microalgae, marketed as health food or food supplement, are commonly sold in the form of tablets, capsules, and liquids. Algae are also incorporated in pastas, snack foods, candy bars or gums, in drink mixes, and beverages, etc., either as nutritious supplement, or as source of natural food colorant (FAO 2016). The results of Zhang et al. (2014) showed that the *Chlorella* could significantly increase the growth of Gibel carp, *e.g.* for fish fed with 0.8% *Chlorella* the body weight increased from 29.90±0.08 to 63.75±1.96 g with a WG of 33.85±1.96 g, which was higher than that of control group (P<0.05). The immune system represents a nodal point in the balance between animal health and disease (Barreda et al. 2014). Previous studies had found that *Chlorella* could be involved in the regulation of animal adaptive and innate immunity. Zhang et al. (2014)

found that the *Chlorella* could significantly increase the serum IgM and IgD levels of Gibel carp. Increasing of IgD, one of the immunoglobins involved in mucosal defense (Salians et al. 2011), suggested that *Chlorella* might play some role in the mucosal immunity.

4.4. Meat Indices and Proximate Analyses

Fish weight without viscera was significantly higher in each of T2 (2.5 gm *Chlorella*/kg diet) and T3 (5 gm *Chlorella*/kg diet) with 83.395 and 83.505 respectively as shown in Figure 4.15, weight without viscera and head differ significantly among treatments with the addition of *Chlorella* the diet as observed in Figure 4.16.

Table 4.5. Effect of adding *Chlorella* in different levels on fish meat indices of common carp *C. carpio*

Treatments	**Fish wt. without viscera**	**Fish wt. without viscera and head**
T1 (control)	81.930 b ± 0.003	61.310 a ± 0.029
T2 (2.5 g *Chlorella*/kg diet)	83.395 a ± 0.008	59.471 ab ± 0.018
T3 (5 g *Chlorella*/kg diet)	83.505 a ± 0.003	60.717 ab ± 0.010
T4 (7.5 g *Chlorella*/kg diet)	80.145 c ± 0.015	58.063 b ± 0.047

Mean values with different superscripts within a column differ significantly (P≤0.05).

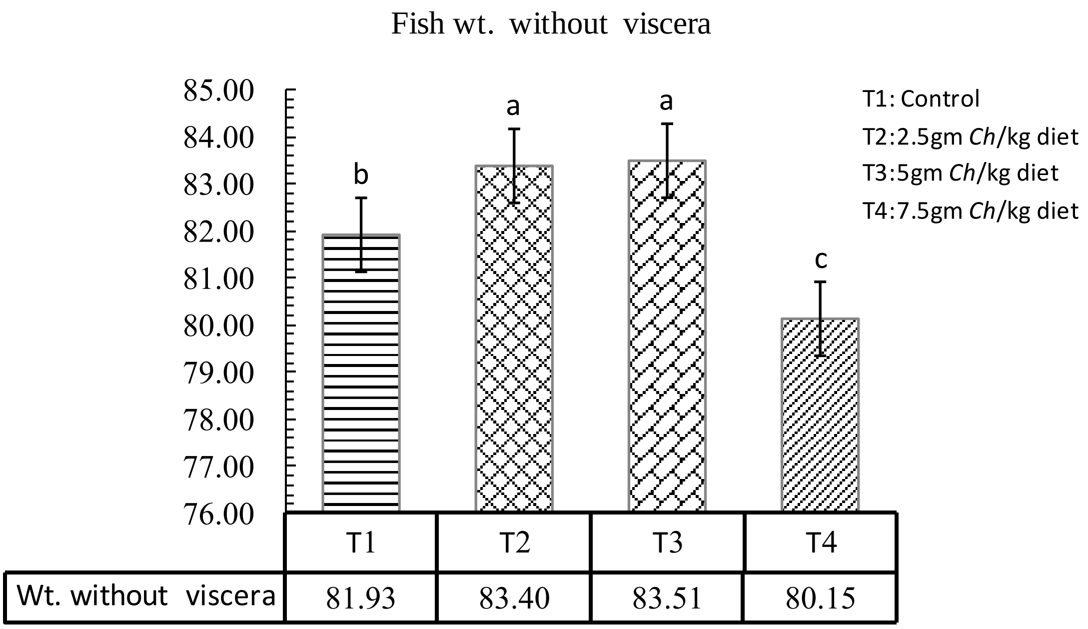


Figure 4.15. The effect of adding *Chlorella* in fish weight without viscera of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

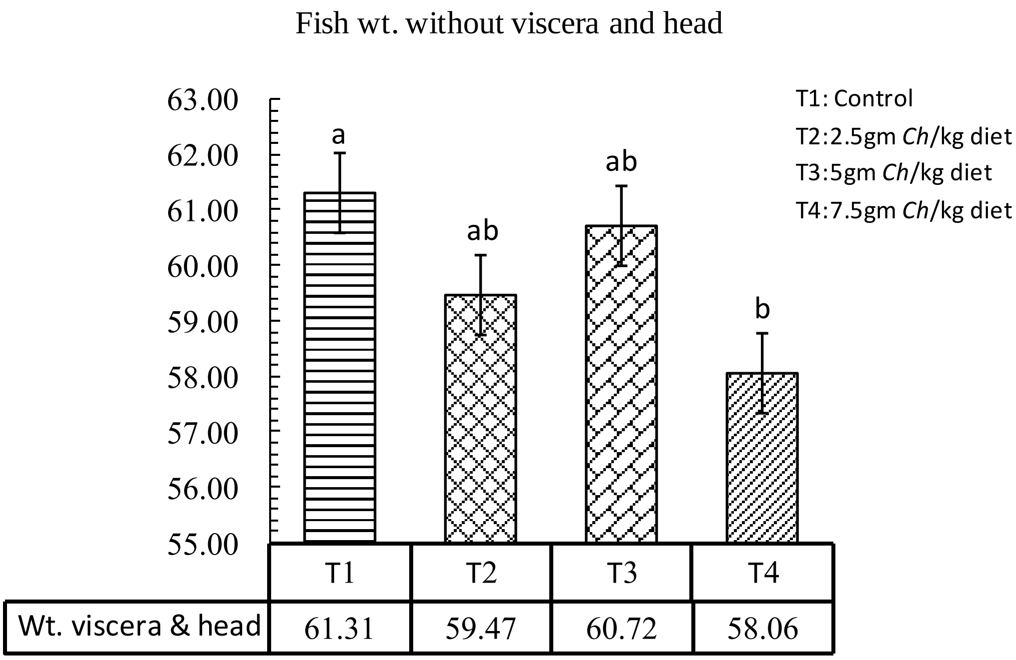


Figure 4.16. The effect of adding *Chlorella* in Fish weight without viscera and head of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Badwy et al. (2008) study the effect of partial replacement of fish meal with dried microalgae (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) diets on fish growth and body composition. In the study of Kim et al. (2002) found that dietary supplementation of 2% *Chlorella* powder significantly improved growth and feed utilization of juvenile flounder. Several factors contribute to the nutritional value of a microalga – including its size and shape, and digestibility as related to cell wall structure and composition, as well as biochemical composition (e.g. accumulation compounds, enzymes and toxins) and specific requirements of the target animal. For this reason, several studies have attempted to correlate the nutritional value of microalgae to their chemical profile.

However, results from feeding experiments are often difficult to interpret because of the confounding effects of other formulation additives. An examination of literature data – including those pertaining to microalga-based, compounded diet emulsions, have meanwhile allowed a few general conclusions to be reached. According to the results obtained from the chemical analyses (proximate analyses) of common carp meat, T4 (7.5 gm *Chlorella*/kg diet) was higher significantly in protein and ash ratio as shown in Figures 4.17 and 4.18, in lipid and moisture T4 (7.5 gm *Chlorella*/kg diet) and T3 (5 gm *Chlorella*/kg diet) were higher significantly as showed in Figures 4.19 and 4.20.

Table 4.6. Effect of adding *Chlorella* in different levels on proximate analyses of common carp *C. carpio* meat

Treatment	Protein %	Lipids %	Ash %	Moisture %
T1 (control)	20.91 cd ± 0.67	1.23 c ± 0.99	0.87 c ± 0.009	71.54 c ± 0.002
T2 (2.5 g *Chlorella*/kg diet)	21.93 c ± 0.98	0.87 d ± 0.87	0.83 c± 0.006	71.64 c ±0.003
T3 (5 g *Chlorella*/kg diet)	23.04 b ± 0.98	1.57 a ± 0.97	1.56 b ± 0.008	74. 97 ab ± 0.002
T4 (7.5 g *Chlorella*/kg diet)	25.85 a ± 0.21	1.47 a ±0.99	1.97 a ± 0.006	75.53 a ± 0.002

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).

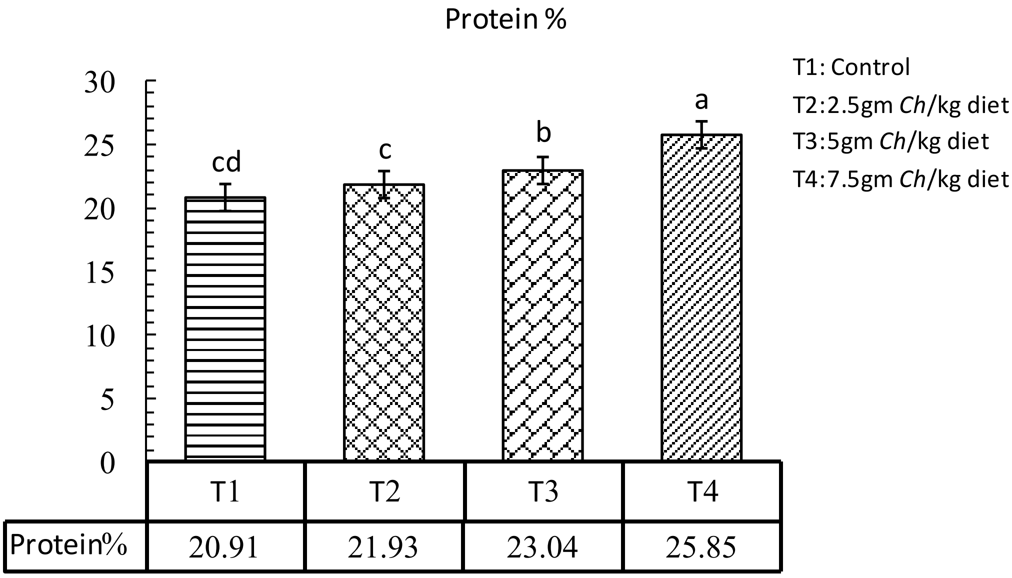


Figure 4.17. The effect of adding *Chlorella* in % Protein of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

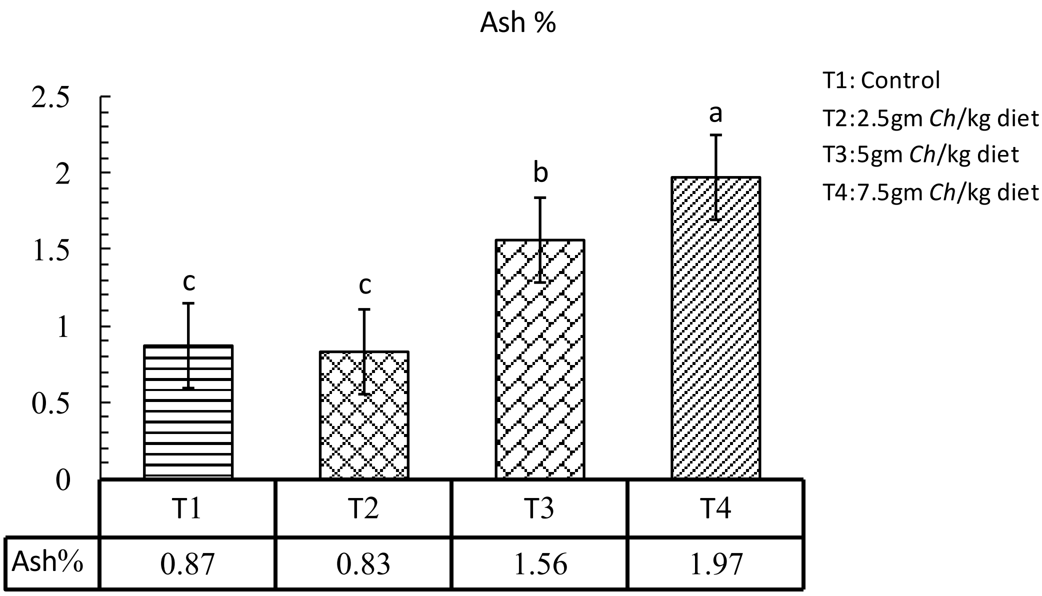


Figure 4.18. The effect of adding *Chlorella* in % Ash of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Algae with high nutritional value have remarkable potential as fish feed. Previous finding by Khatoon et al. (2010) suggested that algal feed could be a better supplement for animal protein like Tubificidae. Initially it was revealed from the proximate composition analysis of the formulated feeds as well as the carcass study that the value added feeds had higher ash content than the control feed indicating more minerals in algal feeds, which resulted into high deposition of nutrients in the VAF fed fishes, confirming that the experimental feeds enhanced more utilization of the dietary nutrients (Mukherjee et al. 2011). The high carcass protein content of the fishes from experimental feeding groups might have been contributed by the higher protein content of the experimental diets and this confirmed by the results of Mukherjee et al. (2011). The exchange of dietary proteins with growth and its utilization was also reported by Rajbanshi and Mumtazuddin (1989) for Indian major carp (*L. rohita*) when fed with different dietary protein levels, and The high dietary carbohydrate content of the experimental feeds resulted into high muscle glycogen content.

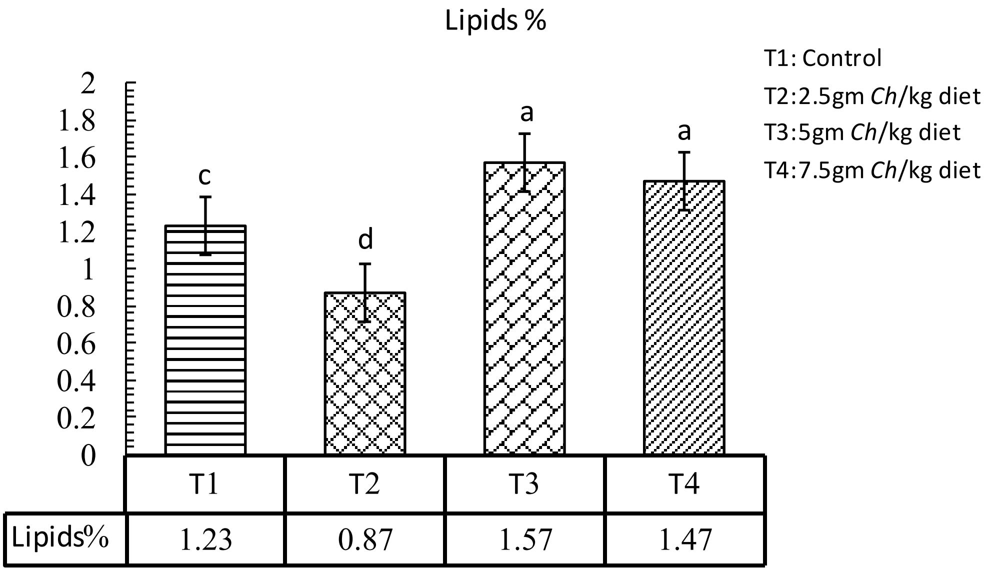
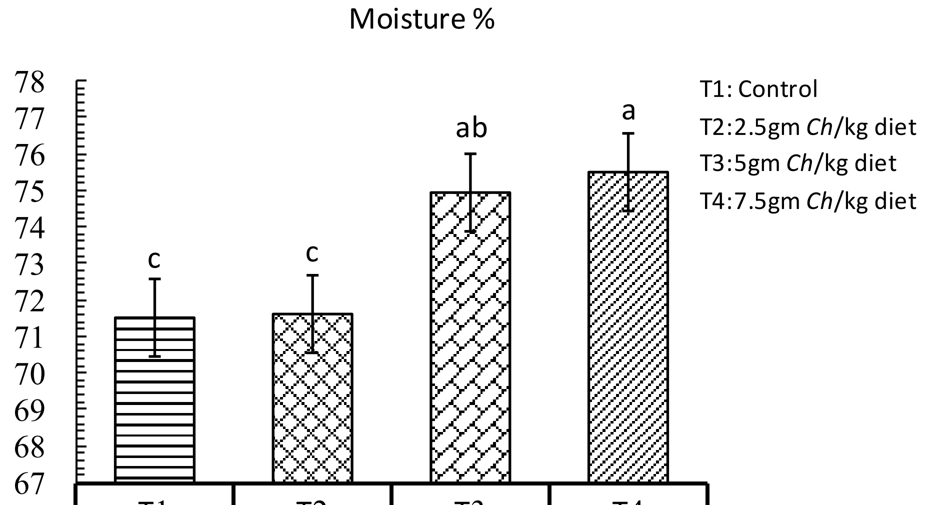


Figure 4.19. The effect of adding *Chlorella* in % Lipid of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)



	T1	T2	T3	T4
Moisture%	71.54	71.64	74.97	75.53

Figure 4.20. The effect of adding *Chlorella* in % Moisture of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The muscle lipid content of the fish group fed with control feed increased significantly (P< 0.05) over the 12 weeks of experimental tenure in comparison to the other experimental feeds, this might probably due to lipid deposition in the body of fishes fed with control feed. Deposition of high body lipid content in the fish fed on high-energy containing diet was also reported by Mahata et al. (1994) and Nandeesha et al. (1990). The high lipid deposition in control diet group had a negative correlation with body weight gain which was supportive to the findings that the high energy content in the diets might have a negative correlation with weight gain and specific growth rate (Daniels and Robinson 1986). On the other hand optimum lipid levels resulted in improved growth rates, feed conversion ratios, nutrient utilization, and reduced nitrogen excretion (Yigit et al. 2002; Martins et al. 2007). Both the control and T2 had a lipid content of much lower than the optimum level and they resulted into a low body lipid deposition and an improved growth rate in all aspect. Therefore, the study confirmed the fact that the high lipid containing diet such as control feed used in this experiment might have a deleterious effect on fish body growth as excessive lipid could lead to decreased feed intake and might reduce the utilization of other nutrients, resulting in poor growth performance (Hemre and Sandnes 1999). Control diet resulted into high body lipid deposition which led to poor growth of the fishes. The present experiment thus suggested that the efficacy of the algal protein incorporated diets was higher than the control one in terms of growth performances. Therefore, the locally available algal genera can be used in combination with the control feed to achieve a comparable or even better result in carp farming which may have a high commercial value in long term.

As shown in Table 4.7 adding *Chlorella* to fish diets in all levels significantly affect meat color, Juiciness, flavor and complete acceptable than the control as shown in Figures 4.21, 4.22, 4.23 and 4.24, no significant differences in Freshness as shown in Figure 4.25.

Table 4.7. Effect of adding *Chlorella* in different levels on Organoleptic evaluation of common carp *C. carpio*

Treatment	Color	Freshness	Juiciness	Flavor	Complete acceptable
T1 (control)	1.47 b ± 0.001	2.03 a ± 0.006	1.87 c ± 0.006	3.65 ab ± 0.09	2.16 b ± 0.06
T2 (2.5 g *Chlorella*/kg diet)	2.01 ab ± 0.002	2.54 a ± 0.005	2.63 ab ± 0.007	2.99 c ± 0.08	2.65 ab ± 0.05
T3 (5 g *Chlorella*/kg diet)	2.24 ab ± 0.001	2.23 a ± 0.005	2.56 ab ± 0.006	3.80 ab ± 0.09	2.80 a ± 0.05
T4 (7.5 g *Chlorella*/kg diet)	2.53 a ± 0.002	2.45 a ± 0.004	2.79 a ± 0.006	3.99 a ± 0.09	2.74 a ± 0.06

Mean values with different superscripts within a column differ significantly (P≤0.05).

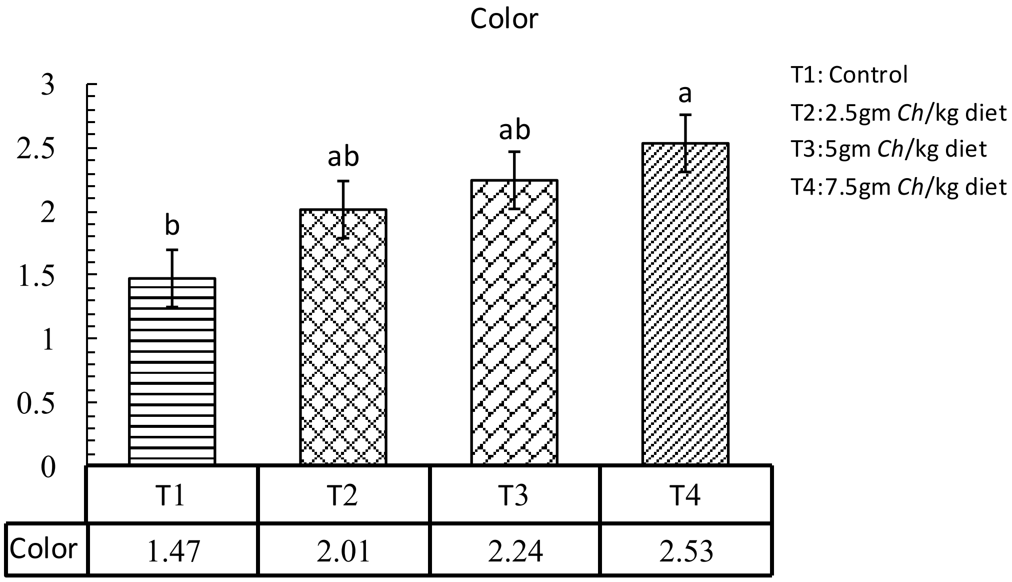


Figure 4.21. The effect of adding *Chlorella* in color of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

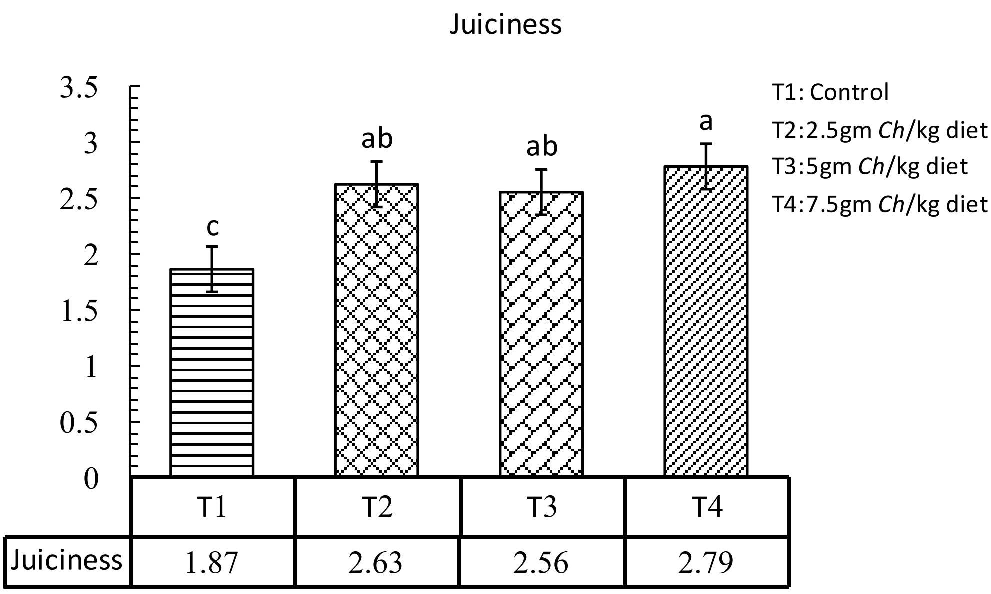


Figure 4.22. The effect of adding *Chlorella* in Juiciness of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

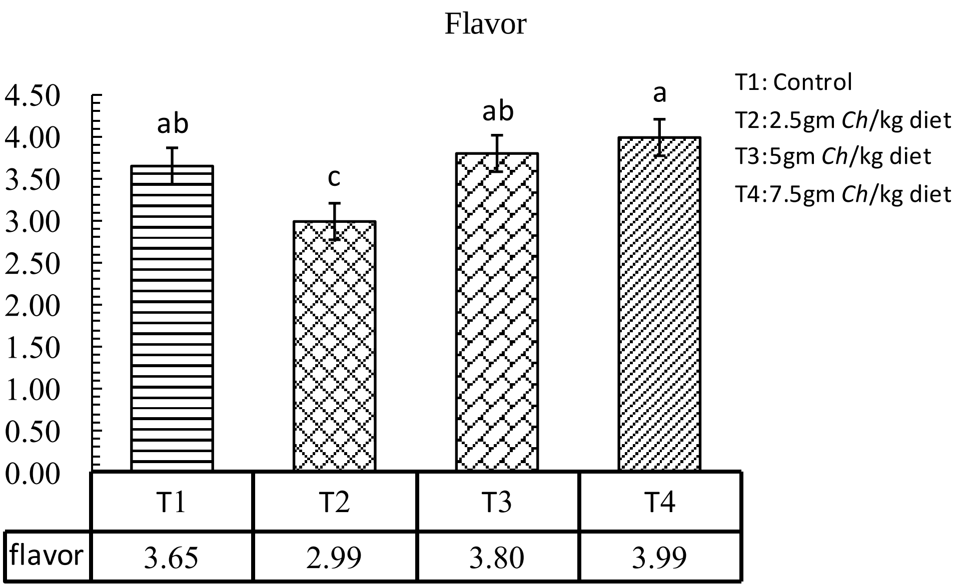
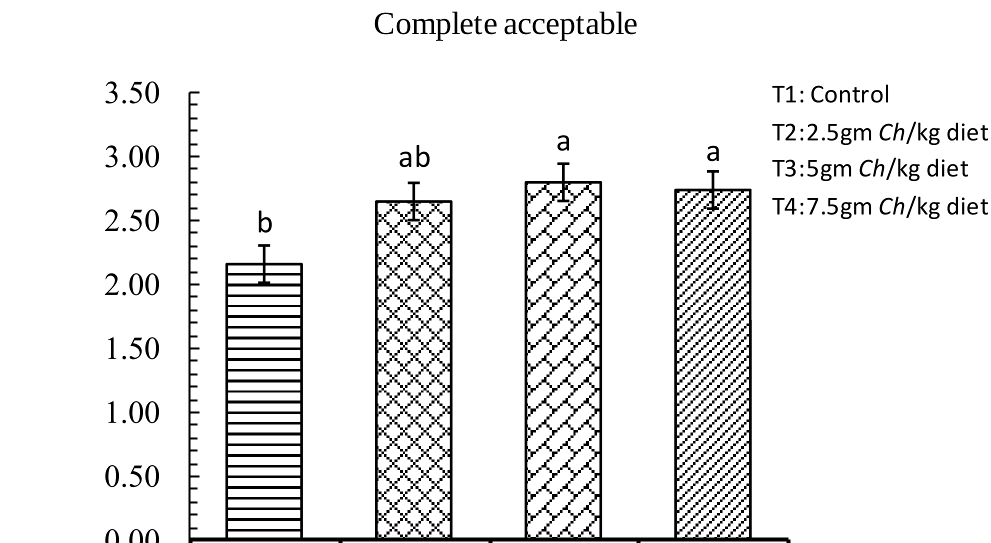
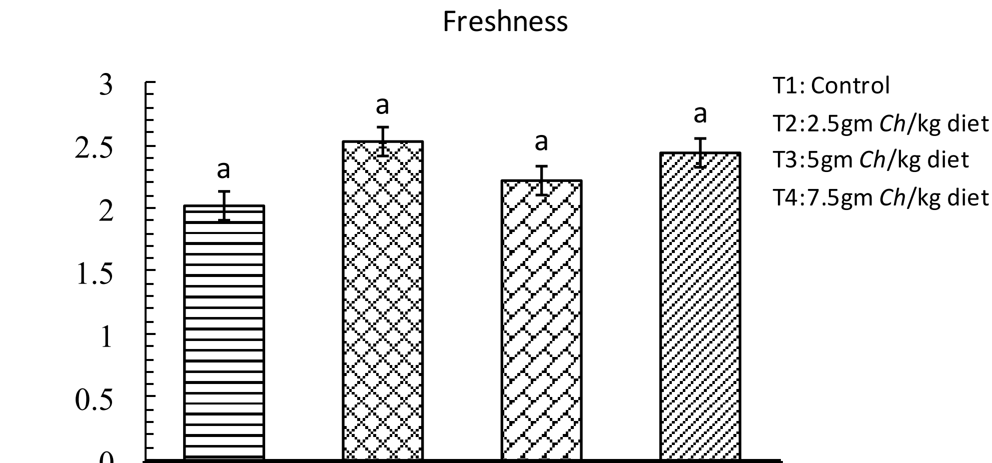


Figure 4.23. The effect of adding *Chlorella* in Flavor of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)



	T1	T2	T3	T4
C. acceptable	2.16	2.65	2.80	2.74

Figure 4.24. The effect of adding *Chlorella* in Complete acceptable of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)



	T1	T2	T3	T4
Freshness	2.03	2.54	2.23	2.45

Figure 4.25. The effect of adding *Chlorella* in Freshness of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

4.5. Blood Picture

The present study showed that, WBC and lymphocytes were higher significantly in T2 (2.5 gm *Chlorella*/kg diet) than other treatments as shown in Figures 4.26 and 4.27, also shown in Figure 4.28 and 4.29 monocytes and granulocytes in control and T4 (7.5 gm *Chlorella*/kg diet) were significantly higher than other treatments, the control and T2 were lower significantly than others, as shown in Table 4.8.

Table 4.8. Effect of adding *Chlorella* in different levels on some blood parameters of common carp *C. carpio*

Treatments	WBC	LYM	MON	GRA
T1 (control)	112.717 ab ± 0.110	67.200 c ± 0.024	28.000 a ± 0.056	16.900 ab ± 0.126
T2 (2.5 g *Chlorella*/kg diet)	124.000 a ± 0.032	88.967 a ± 0.030	20.933 c ± 0.072	10.233 c ± 0.250
T3 (5 g *Chlorella*/kg diet)	107.100 b ± 0.046	75.333 b ± 0.053	23.733 b ± 0.027	14.000 b ± 0.093
T4 (7.5 g *Chlorella*/kg diet)	118.350 ab ± 0.028	68.767 c ± 0.023	27.700 a ± 0.026	17.800 a ± 0.073

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).

It is estimated that about 30% of microalgal production is used for animal feed due to the increasing demand for food with natural composition instead of synthesized ingredients (Safi et al. 2014). In this regards, *C. vulgaris* contains important amount of carotenoids and other nutrients (considered as nutrient-dense super food) and after feeding it to fish, it showed interesting pigmentation potential for fish flesh, together with enhancing health and increasing its life expectancy (Gouveia et al. 1999, 2002).

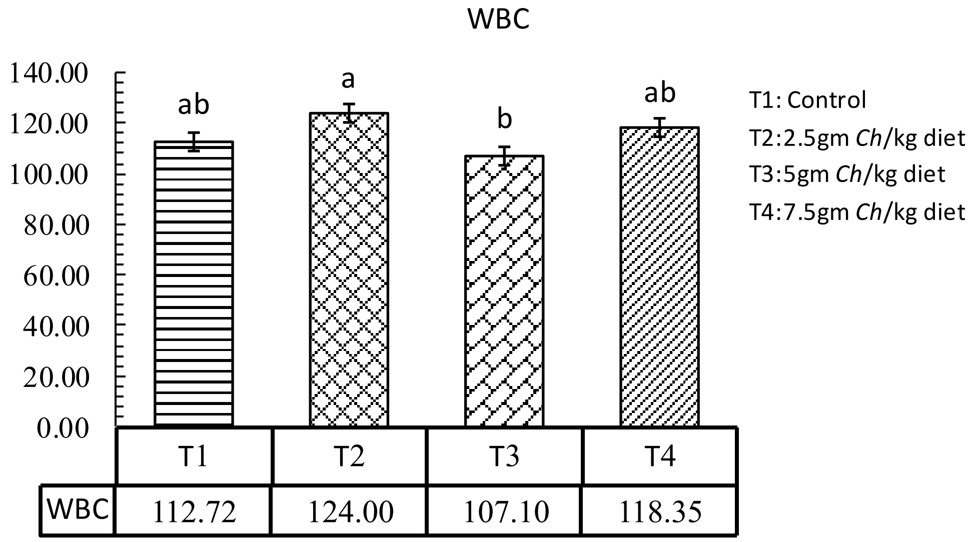


Figure 4.26. The effect of adding *Chlorella* in WBC of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

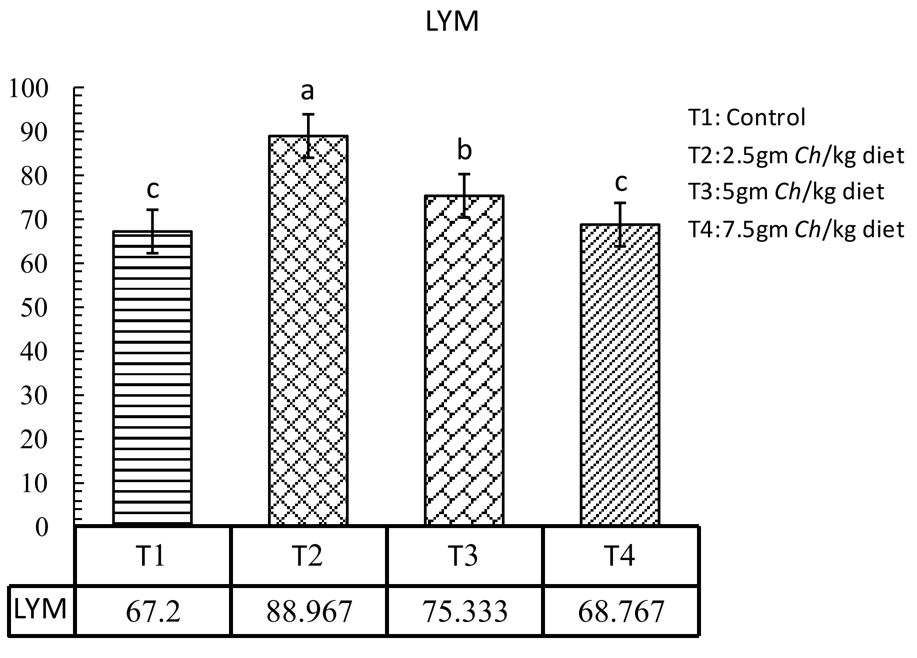


Figure 4.27. The effect of adding *Chlorella* in LYM of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Hematological parameters are influenced by species, age, sexual maturity, health condition, nutritional quality of the target fish and the environmental conditions (Bielek and Strauss 1993). Based on the results, the application of CV in feed of Koi carp showed proliferation of all measured hematological parameters. Supplementation of CV in koi carp feed was led a rising in RBC level. RBC in teleost is related to the oxygen requirement (Zanjani et al. 1967) and in the present study, its increase can be considered as positive effect made by CV. Among the immune cell parameters, RBC count is a frequently used parameter to evaluate possible undesired collateral effects (anemia) provoked by immunostimulants administered in supplemented feed (Morera et al. 2011). WBCs are the immune-competent cells of immune system which play critical roles to both infectious and non-infectious diseases (Magandottir 2006).

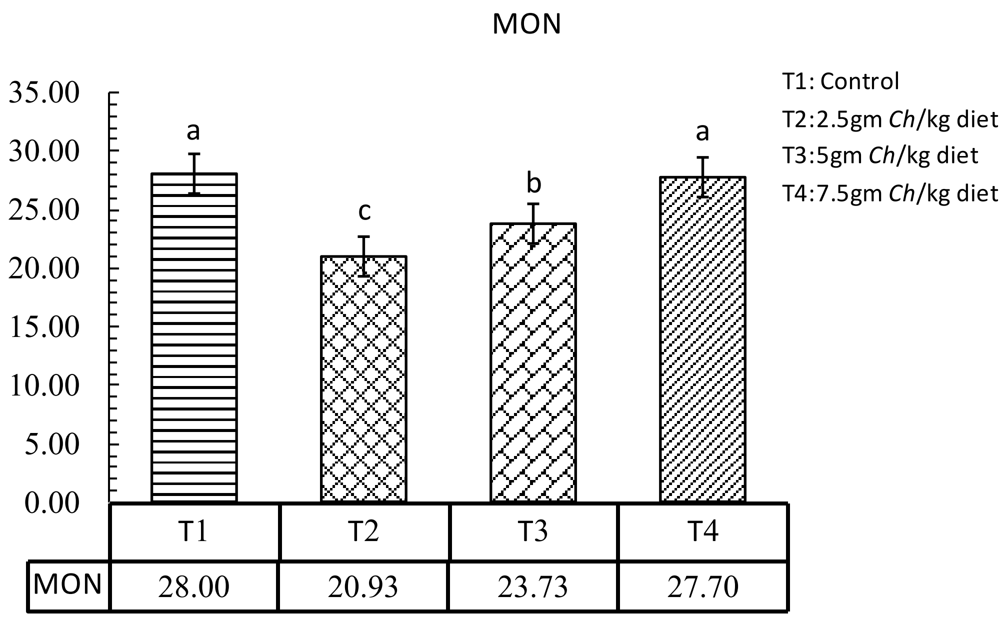


Figure 4.28. The effect of adding *Chlorella* in MON of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

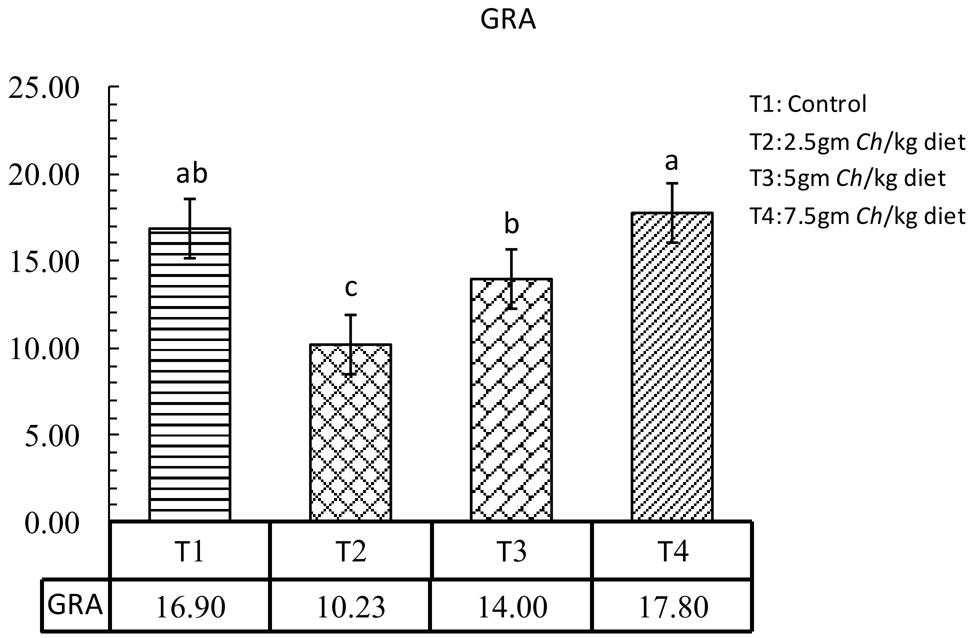


Figure 4.29. The effect of adding *Chlorella* in GRA of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

As shown in Figure 4.30 and 4.31 RBC and HGB were higher significantly in each of T3 (5 gm *Chlorella*/kg diet) and T4 (7.5 gm *Chlorella*/kg diet), also shown in Figure 4.32 HCT differ significantly among treatments in T4 (7.5 gm *Chlorella*/kg diet) as compared to other treatments as cleared in Table 4.9.

Table 4.9. Effect of adding *Chlorella* in different levels on some blood parameters of common carp *C. carpio*

Treatments	**RBC**	**HGB**	**HCT**
T1 (control)	1.327 b ± 0.133	9.125 b ± 0.081	38.000 b ± 0.030
T2 (2.5 g *Chlorella*/kg diet)	1.323 b ± 0.064	7.925 c ± 0.089	26.767 c ± 0.058
T3 (5 g *Chlorella*/kg diet)	1.707 a ± 0.035	10.633 a ± 0.014	37.867 b ± 0.020
T4 (7.5 g *Chlorella*/kg diet)	1.560 ab ± 0.082	11.367 a ± 0.072	42.475 a ± 0.069

Mean values with different superscripts within a column differ significantly (P≤0.05).

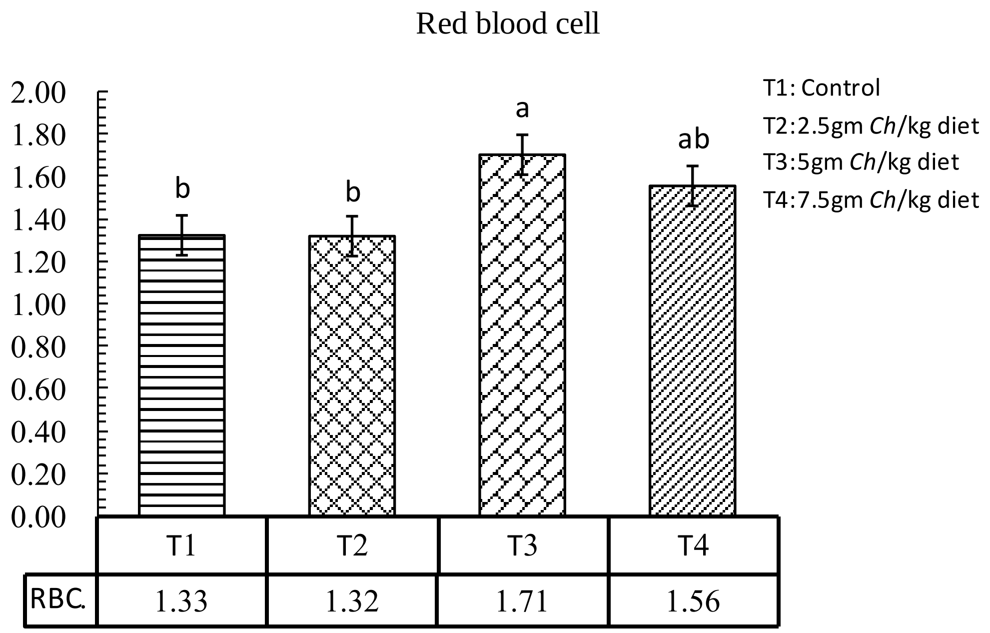


Figure 4.30. The effect of adding *Chlorella* in RBC of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

An enhancement in the WBC population size was seen in fish fed CV at different concentrations. Such enhancement is in part be due to the positive effects of some ingredients of CV e.g. vitamins and glucans available in the cell wall of CV. Therefore, inclusion of CV in the fish feed can provide a stimulatory role for the fish immune status i.e. increasing in phagocytosis capacity and cell-mediated immune responses resulting in an increasing in the fish resistance to the diseases and disorders (Feldman et al. 2000; Montero-Rocha et al. 2005; Sakai 1999; Parra et al. 2015). This is supported by a significant increase in the levels of IgM, lysozyme and C4 in the fish fed CV. It has been shown that *Chlorella* could be involved in the regulation of animal adaptive and innate immunity. For instance, Cerezuela et al. (2012) found that microalgae's could increase the expression of major histocompatibility complex class I (MHC I) of gilthead seabream resulting in the stimulation of cytotoxic cells.

Therefore, an improving in these immune parameters clearly suggests a positive role of CV administration (up to 10% of the diet) on the mucosal immunity of fish (Magnadottir 2006; Dunkelberger and Song 2010). Physiologically, Hb and HCT are crucial to the survival of fish, being directly related to the oxygen binding capacity of blood (Bielek and Strauss 1993). Obviously under such conditions the animal metabolism is improved resulting in a better growth performance (Khani et al. 2017).

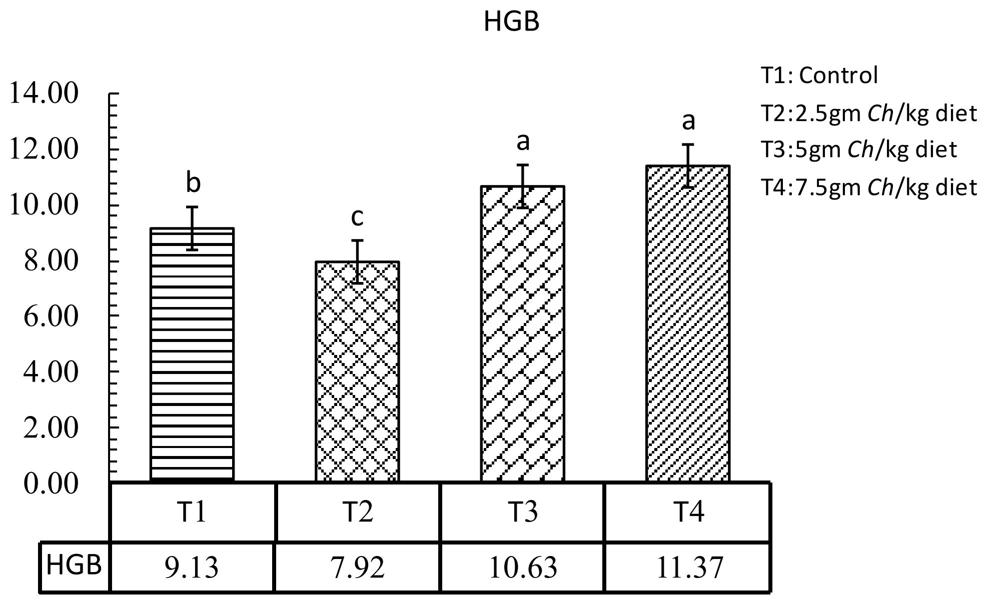


Figure 4.31. The effect of adding *Chlorella* in HGB of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

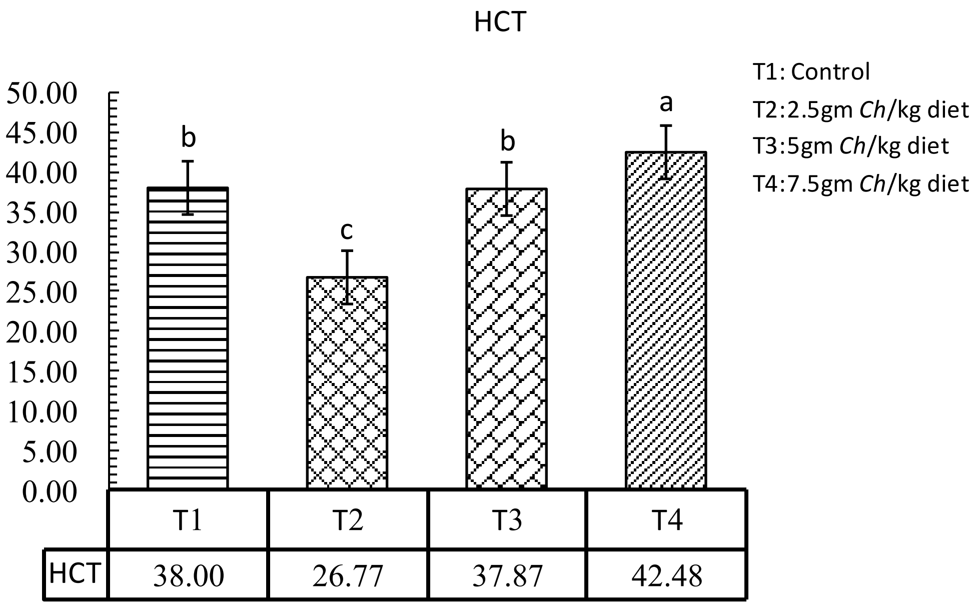


Figure 4.32. The effect of adding *Chlorella* in HCT of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Zhang et al. (2014) found that the *Chlorella* could significantly increase the serum IgM and IgD levels of Gibel carp. Increasing of IgD, one of the immunoglobins involved in mucosal defense (Salians et al. 2011), suggested that *Chlorella* might play some role in the mucosal immunity. These results indicate that *Chlorella* might involve in the regulation of fish innate immunity by enhancing some gene expressions, indicate that *Chlorella* could be involved in the regulation of fish innate and adaptive immunity and be used as an additive in fish diets.

MCV was higher significantly in T2 (2.5 gm *Chlorella*/kg diet) as shown in Figure 4.33, MCHC was higher significantly in each of T2 (2.5 gm *Chlorella*/kg diet) and T3 (5 gm *Chlorella*/kg diet) as shown in Figure 4.34, RDW differ significantly in T4 (7.5 gm *Chlorella*/kg diet) than other treatments as shown in Figure 4.35, also observed in Figure 4.36 no significant differences observed in MCH as cleared in Table 4.10.

Table 4.10. Effect of adding *Chlorella* in different levels on some blood Parameters of common carp *C. carpio*

Treatments	MCV	MCH	MCHC	RDW
T1 (control)	227.700 b ± 0.009	60.267 a ± 0.027	26.333 b ± 0.015	8.175 b ± 0.112
T2 (2.5 g *Chlorella*/kg diet)	235.233 a ± 0.026	62.967 a ± 0.073	28.033 a ± 0.018	6.033 c ± 0.108
T3 (5 g *Chlorella*/kg diet)	208.567 c ± 0.007	60.733 a ± 0.020	27.167 ab ± 0.028	8.925 b ± 0.060
T4 (7.5 g *Chlorella*/kg diet)	226.800 b ± 0.008	62.033 a ± 0.038	26.825 b ± 0.011	11.000 a ± 0.000

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).

Similarly, Farahi et al. (2010, 2012) and Najafpour et al. (2012) reported the same results in their works. No significant effect of dietary inclusion of basil on Lymphocyte, Monocyte, Heterophil and Eosinophil percentages was obtained, but results indicated the outstanding effect of dietary inclusion of basil on MCV, MCH and MCHC. Farahi et al. (2010) revealed that MCH was increased significantly by diet supplemented with garlic; however, the amount of MCV and MCHC had no remarkable differences with control group (without garlic).

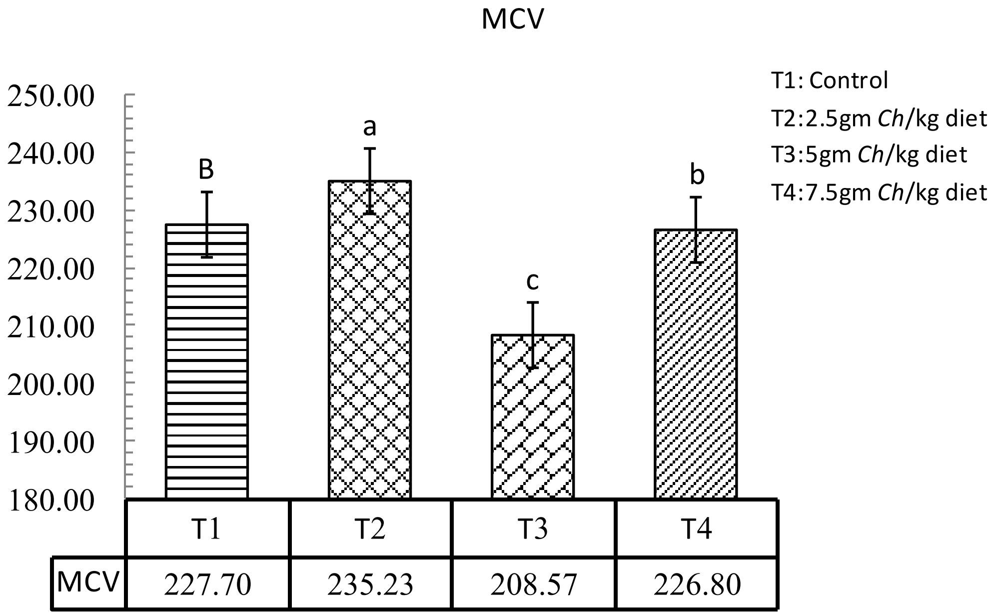


Figure 4.33. The effect of adding *Chlorella* in MCV of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

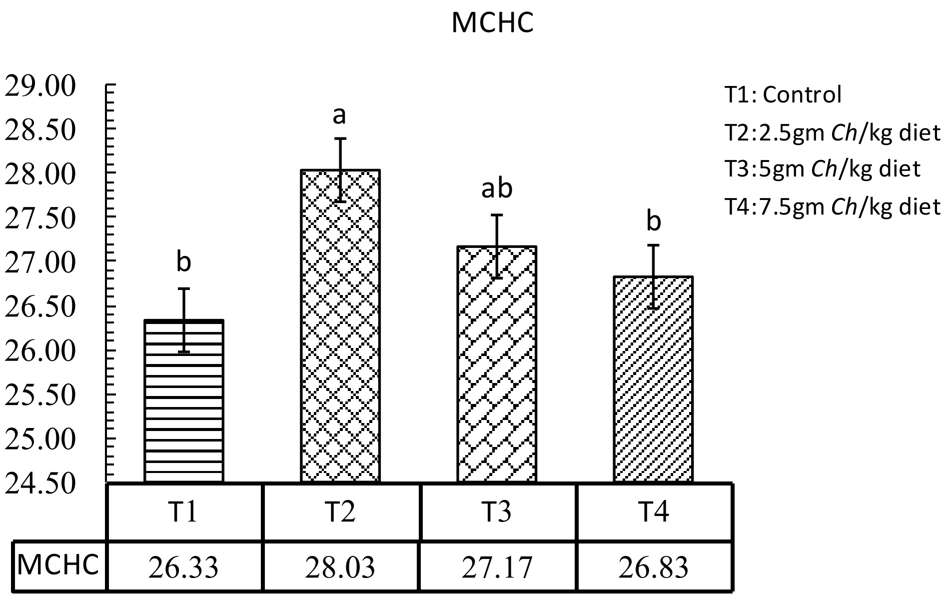


Figure 4.34. The effect of adding *Chlorella* in MCHC of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

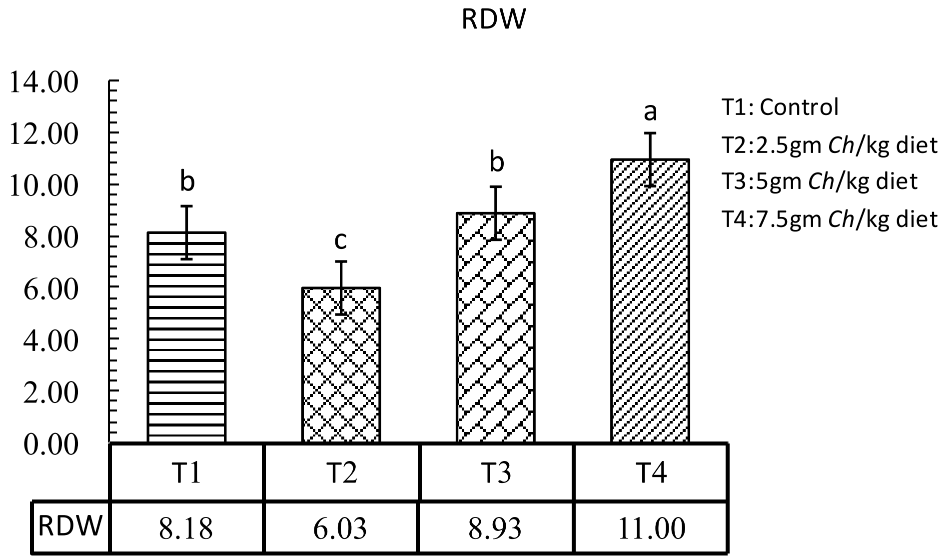


Figure 4.35. The effect of adding *Chlorella* in RDW of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

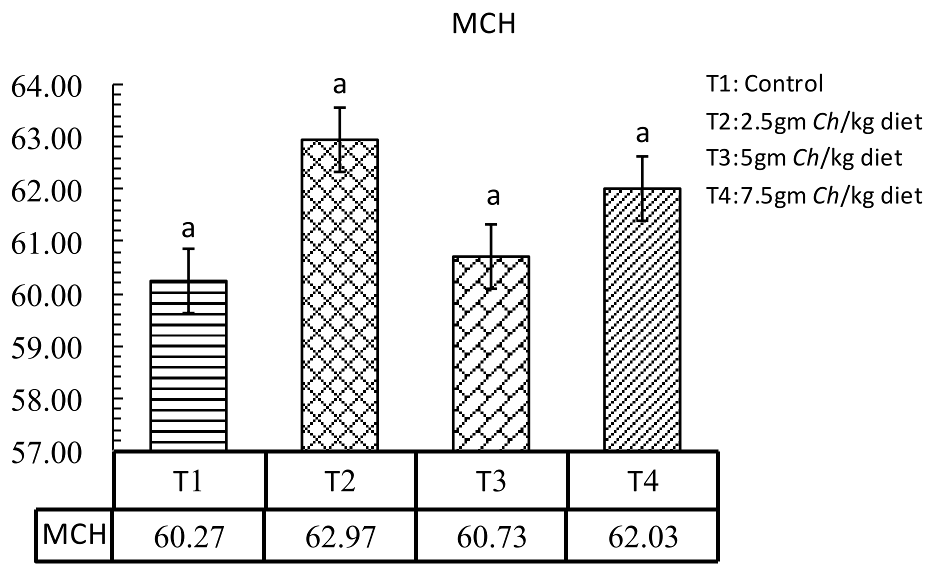


Figure 4.36. The effect of adding *Chlorella* in MCH of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The results indicated that *Chlorella* can be a good choice as an additive for fish diets. Due to supreme level of crude protein, it possesses significant concentration of polysaccharides, lipid, minerals and other bioactive components involved in many physiological activities (Xu et al. 2014; Khani et al. 2017).

As shown in Figure 4.37 Glucose was higher significantly than others in T4; also shown in Figure 4.38 Total protein differ significantly among treatments with the addition of *Chlorella* the diet as shown in Table 4.11.

Table 4.11. Effect of adding *Chlorella* in different levels on blood biochemical of common carp *C. carpio*

Treatments	**Blood Glucose**	**Blood Total protein**
T1 (control)	101.000 a ± 0.368	2.825 a ± 0.025
T2 (2.5 g *Chlorella*/kg diet)	105.333 a ± 0.304	2.800 a ± 0.000
T3 (5 g *Chlorella*/kg diet)	104.000 a ± 0.244	3.100 a ± 0.100
T4 (7.5 g *Chlorella*/kg diet)	113.333 a ± 0.231	3.167 a ± 0.167

Mean values with different superscripts within a column differ significantly (P≤0.05).

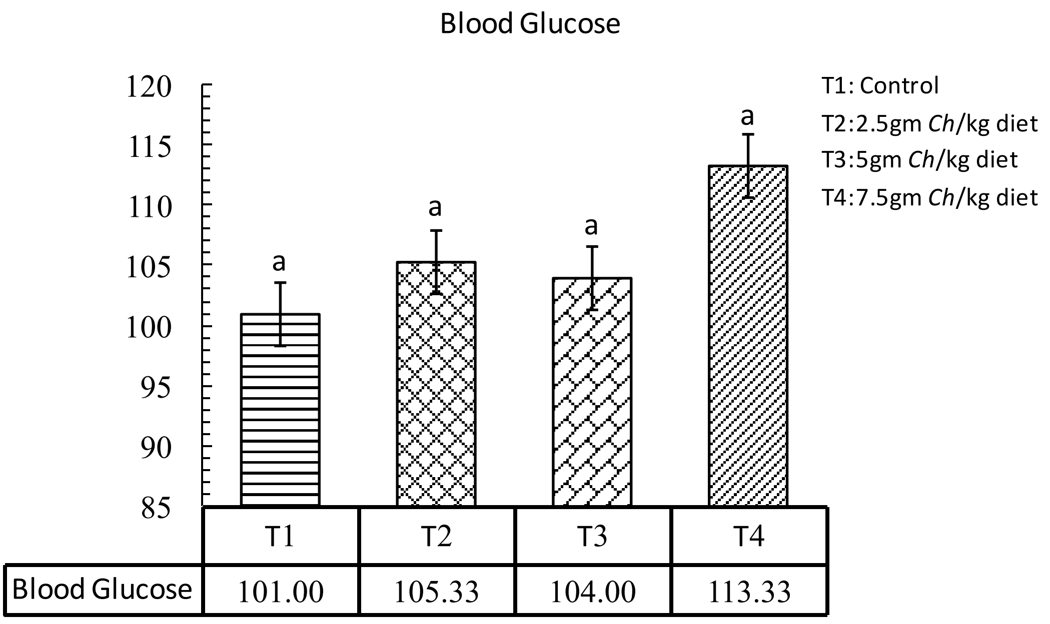
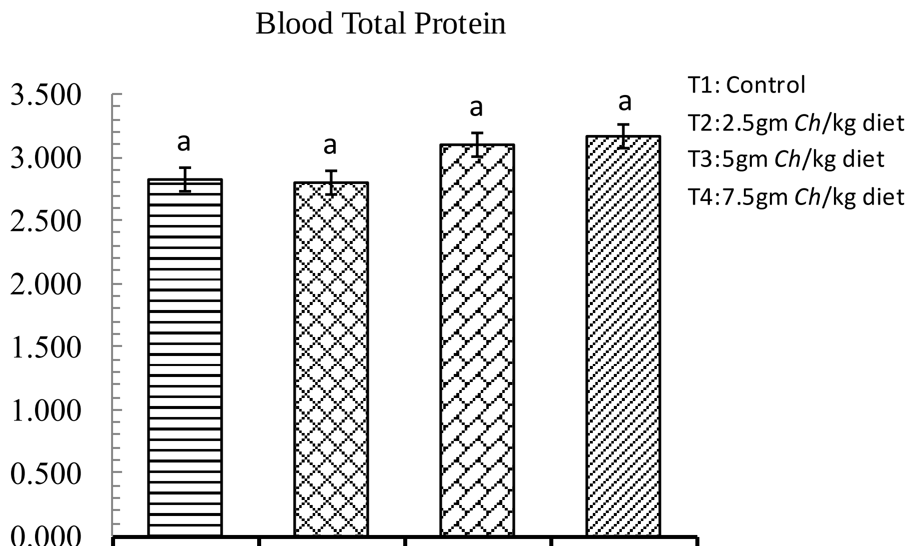


Figure 4.37. The effect of adding *Chlorella* in Blood Glucose of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

The results of blood parameters of fish fed with experimental diets are summarized in Table 4. As it can be seen, the total protein and albumin of fish in the 5% *Chlorella* group increased when compared with the control group ($p<0.05$). High level of serum protein is the result of liver function improvement. Moreover, the *Chlorella* could increase the total serum protein (TP), albumin in the immune response of koi carp. Similarly, Yildiz et al. (2002) and Yu et al. (2006) hold a view that the increase of serum protein level is an appropriate factor for displaying the immune condition of the fish.



	T1	T2	T3	T4
Blood Total protein	2.825	2.800	3.100	3.167

Figure 4.38. The effect of adding *Chlorella* in Blood Total Protein of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)

Cholesterol was higher significantly in T1 and T2 as shown in Figure 4.39, while in Triglyceride T1 higher significantly than others as shown in Figure 4.40, in HDL-C and LDL-C second treatment was significantly lower as compared to other treatments as shown in Figures 4.41 and 4.42.

Table 4.12. Effect of adding *Chlorella* in different levels on blood lipid profiles of common carp *C. carpio*

Treatments	Cholesterol	Triglyceride	HDL-C	LDL-C
T1 (control)	114.000 ab ± 0.125	342.000 a ± 0.394	42.667 b ± 0.289	1.010 b ± 2.538
T2 (2.5 g *Chlorella*/kg diet)	120.000 a ± 0.499	150.000 b ± 0.634	35.667 c ± 0.187	0.367 b ± 1.640
T3 (5 g *Chlorella*/kg diet)	95.333 bc ± 0.322	143.500 b ± 0.341	52.667 a ± 0.172	3.585 a ± 1.484
T4 (7.5 g *Chlorella*/kg diet)	85.000 c ± 0.309	144.000 b ± 0.397	50.000 a ± 0.439	3.385 a ± 1.702

Mean values with different superscripts within a column differ significantly (P≤0.05).

The serum cholesterol and triglyceride level of fish fed 5% *Chlorella* were lower than that of fish in the control group (p<0.05). The ALT and AST of fish in different groups had the normal level pattern (p>0.05).The fish fed 2% *Chlorella* experienced the most significant difference in cholesterol and triglyceride levels. The activity of three digestive enzymes including amylase, lipase and protease in hepatopancreas and intestine were examined, and the results were summarized in Table 4.

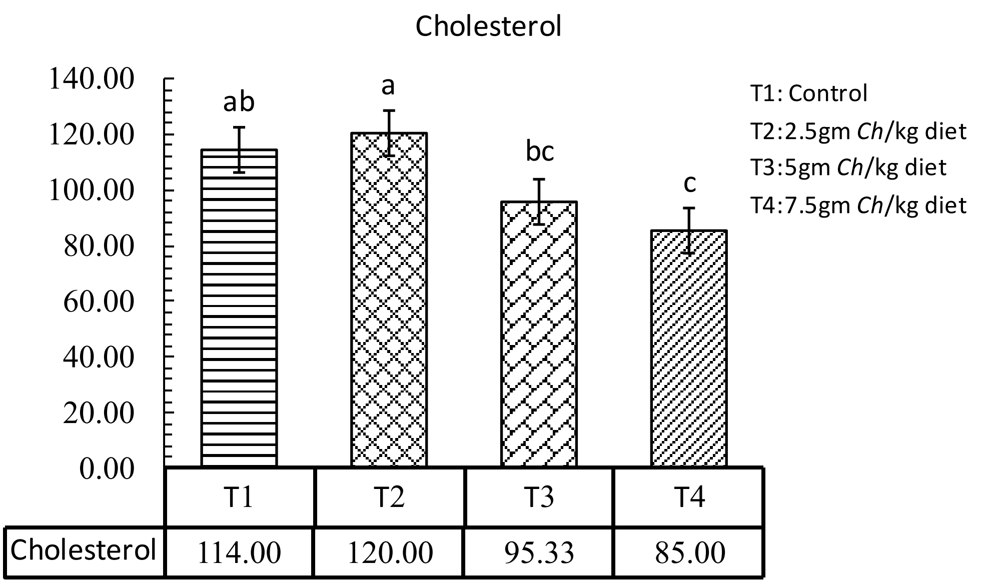


Figure 4.39. The effect of adding *Chlorella* in Cholesterol of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

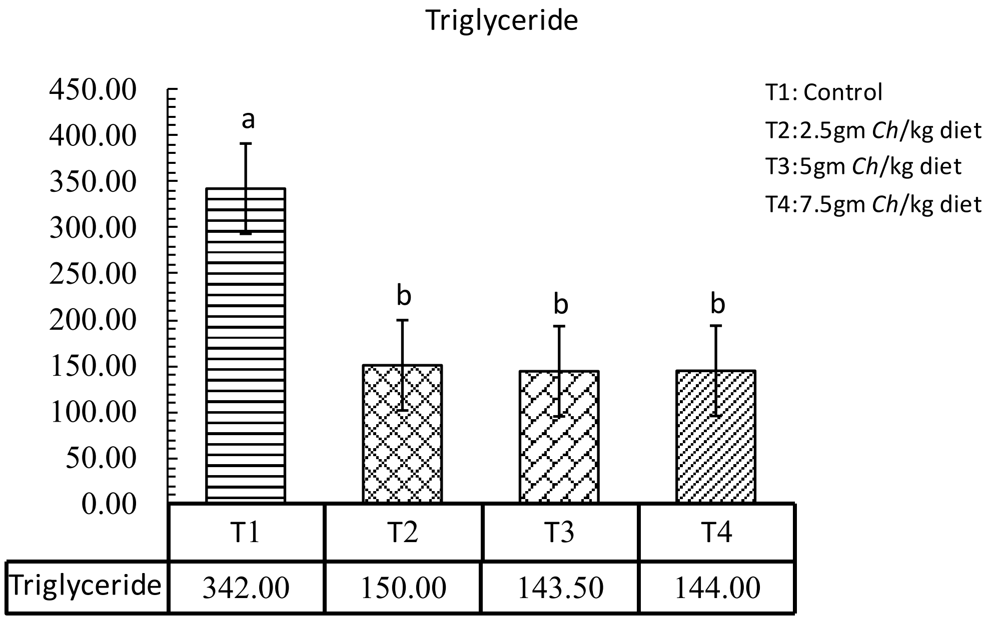


Figure 4.40. The effect of adding *Chlorella* in Triglyceride of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

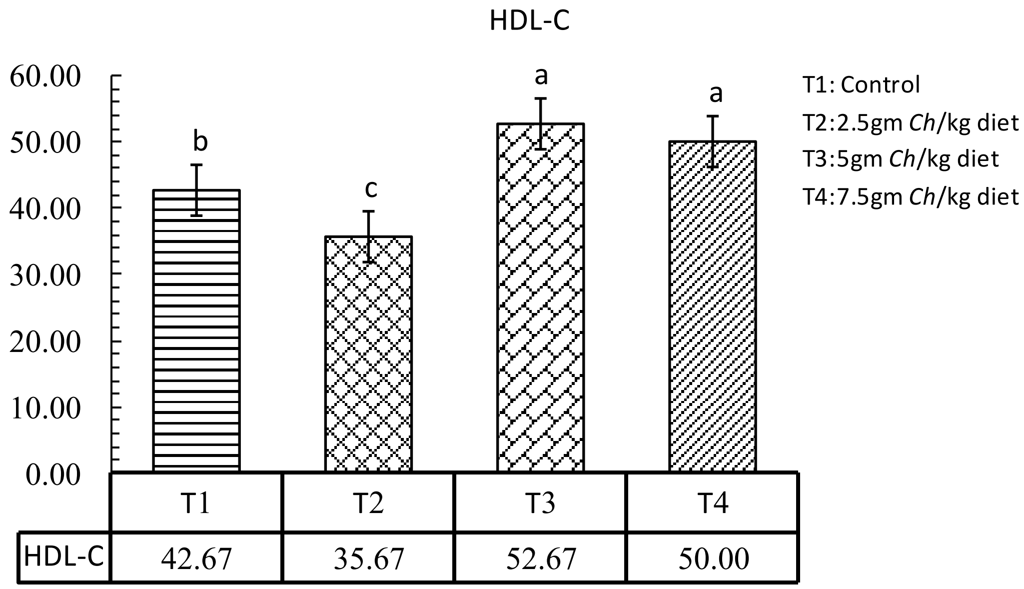


Figure 4.41. The effect of adding *Chlorella* in HDL-C of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

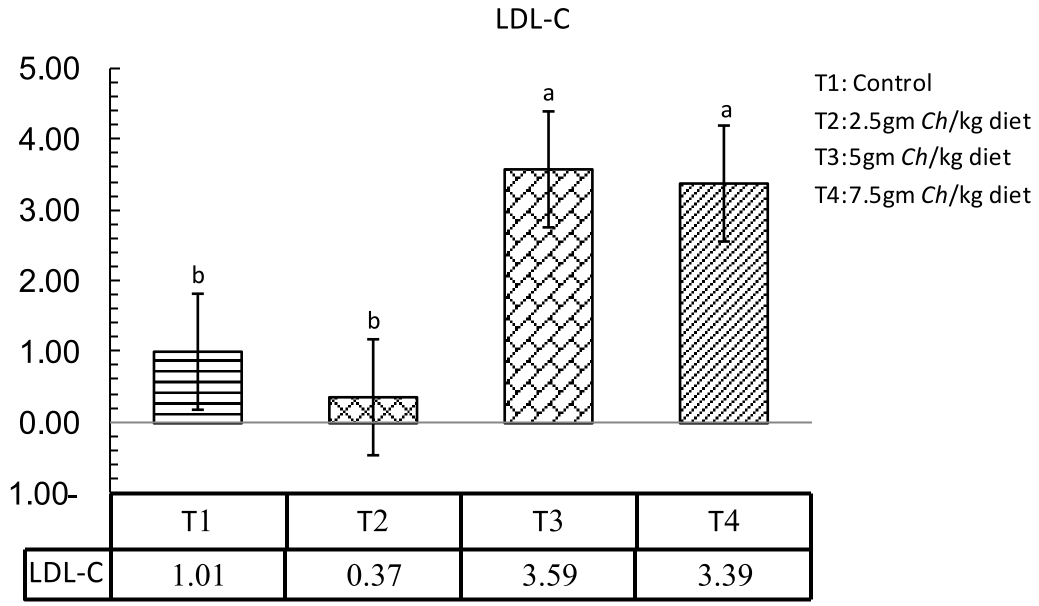


Figure 4.42. The effect of adding *Chlorella* in LDL-C of common carp *C. carpio*, Mean values with different superscripts within a column differ significantly (P≤0.05)

Fish fed with 5% had the most significant difference with C 2%, 7%, 10% groups as well as control one (p<0.05). It should be noted that there was no significant correlation between glucose and the amount of *C. vulgaris* in diets (p>0.05). In the present study, the different contents of *Chlorella* were added in the basal diet of Koi fish and growth performance, blood parameters and digestive enzyme were examined. The results indicated that *Chlorella* can be a good choice as an additive for fish diets. Due to supreme level of crude protein, it possesses significant concentration of polysaccharides, lipid, minerals and other bioactive components involved in many physiological activities (Xu et al. 2014). Furthermore, the *Chlorella* could decrease the level of blood cholesterol, not the glucose of koi carp, demonstrating that the *Chlorella* might be involved in the metabolism of lipid. The same results were also found by Güroy et al. (2011). According to the study by Kim et al. (2007), adding *C. vulgaris* powder in *Paralichthys olivaceus* diet resulted in improving growth performance, and serum cholesterol level.

5. CONCLUSION

Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health.

The protein content of *Chlorella* is 51-58% and contains many essential amino acids, showing that *Chlorella* could also be used as protein source for human food and animal diets. Also can additive to the diets of common carp in different levels that affect it.

(T4) algae as feed additive with 7.5 gm *Chlorella*/ kg diet had excellent affect in growth performance like weight gain, daily growth rate and relative growth rate. Also had affect in feed utilization like Protein Efficiency ratio. In biological parameters had effect on Spleenosomatic index and Kidneysomatic index in the intestine length index had affected. According to the results obtained from the chemical analyses (proximate analyses) of common carp meat had good affect in each of protein, lipids, ash and moisture. Also had affect in the blood parameter like monocytes, granulocytes, RBC, HGB and glucose.

(T3) algae as feed additive with 5 gm *Chlorella*/ kg diet had affect in relative growth rate. Also had good affect in feed utilization like protein efficiency ratio. In condition factor had excellent affect, also had effect on Fish weight without viscera in fish meat indices. In lipid and moisture had good effect on proximate analysis. Also had affect in blood parameter on RBC, HGB and MCHC.

(T2) algae as feed additive with 2.5 gm *Chlorella*/ kg diet had affect in Fish weight without viscera. In blood parameter had great effect on WBC, Lymphocytes, MCV, MCHC, Cholesterol, HDL-C and LDL-C.

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CURRICULUM VITAE

Full name: Sanaa Hoshyar ABID

Address: Qaladza/ Sulaimany/ IRAQ

Date of birth: 23/1/1993

Gender : Female

Marital stage: Married

Email address: hoshyar.sana@yahoo.com

Telephone: +9647501480073 Korek Telecom / IRAQ

I was born in Halabja – Sulaimani IRAQ in 1993 . I completed primary , secondary and preparatory school education in Halabja . I graduated from the Department of general science Faculty of Basic Eucation in the University of Salahaddin 2011 -2014 . I enrolled at the Postgraduate Programme in Bingol University in the Department of Biology in september 2015, and completed my research in January 2018.