T. R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF FISHERIES ENGINEERING

UTILIZATION OF *CHLORELLA* AND *DAPHNIA* AS NATURAL FOOD SOURCES AND THEIR COMBINATION AS A FEED SUPPLEMENT COMPARED TO COMMERCIAL FEED FOR COMMON CARP (*Cyprinus carpio*)

M.Sc. THESIS

PREPARED BY: Amanj Ibrahim BAIZ
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SECOND SUPERVISOR: Asst. Prof. Dr. Nasreen M. ABDULRAHMAN

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled **"Utilization Of *Chlorella* And *Daphnia* As Natural Food Sources And Their Combination As A Feed Supplement Compared To Commercial Feed For Common Carp (*Cyprinus Carpio*)"** presented by Amanj Ibrahim BAIZ under supervision of Asst. Prof. Dr. Ş. Şenol PARUĞ in the Faculty of Fisheries has been accepted as a M. Sc. thesis according to Legislations of Graduate Higher Education on 14.05.2018.. with unanimity / ~~majority~~ of votes members of jury.

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on 18/05/2018 with decision number 2018/25-I

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THESIS STATEMENT

All information presented in the thesis were obtained according to the ethical behaviours and academic rules frame, And also, all kinds of statement and source of information that does not belong to me in this work prepared in accordance with the rules of thesis were cited to the source of information at all.

Signature

Amanj Ibrahim BAIZ

ABSTRACT

UTILIZATION OF *CHLORELLA* AND *DAPHNIA* AS NATURAL FOOD SOURCES AND THEIR COMBINATION AS A FEED SUPPLEMENT COMPARED TO COMMERCIAL FEED FOR COMMON CARP (*Cyprinus carpio*)

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This study was carried out to evaluate the effect of adding the two natural food sources, phytoplankton *Chlorella* spp., zooplankton *Daphnia* spp. and their combination as a feed supplement and compared them to commercial feed for common carp (*Cyprinus carpio* L.) fingerlings with different levels. The study was performed in fish laboratory of Animal Sciences Department/ College of Agricultural Sciences/ University of Sulaimani in Iraq, for 12 weeks. The size of fish were varying and the weight ranged between (25.07-37.21 g) with mean initial weight (28.58 g). The fish were acclimated to laboratory conditions and fed with control pellets (28.06 % protein) prior to the feeding trials for 21 days, to test the effect of two different levels of the *Chlorella* spp., zooplankton *Daphnia* spp. alone and in combination on growth performance, feed utilization, blood parameters, biological parameters, chemical composition and organoleptic (sensory) evaluation of fingerlings. The control treatment (T1) fish were fed a commercial diet without adding any amount of *Chlorella* and *Daphnia* , while in other six treatments fish fed control diet with adding *Chlorella* spp. powder and freeze-dried *Daphnia* spp. as following: (T2) with 25 g *Chlorella*/ kg diet, (T3) with 50 g *Chlorella*/ kg diet, while, in (T4) with 25 g *Daphnia*/ kg diet, (T5) with 50 g *Daphnia*/ kg diet, (T6) with 25 g *Chlorella* + 25 g *Daphnia*/ kg diet and (T7) with 50 g *Chlorella* + 50 g *Daphnia*/ kg diet. Each treatment had three replicates in which six fingerlings common carp were stocked in each 100 L tank which feed the experimental diets twice daily. The results showed that both *Chlorella* spp., *Daphnia* spp. and their combination were very suitable feed supplements for common carp.

Keywords: Biological, Blood parameters, Common carp (*C. carpio*), *Chlorella* spp., Chemical composition, *Daphnia* spp., Feed utilization, Fish growth, Organoleptic evaluation.

ÖZET

DOĞAL BİR BESİN KAYNAĞI OLARAK *CHLORELLA* VE *DAPHNİA* VE BUNLARIN KOMBİNASYONLARININ KULLANILMASI VE SAZAN BALIĞI (*Cyprinus carpio)* YETİŞTİRİCİLİĞİNDE TİCARİ YEMLER İLE KARŞILAŞTIRILMASI

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Mayıs 2018, 90 sayfa

Bu çalışma doğal iki besin kaynağı olan fitoplanton *Chlorella* spp. ile zooplankton *Daphnia* spp.'nin ve kombinasyonlarının gıda katkısı olarak kullanılmasının etkilerinin değerlendirilmesi ve bunların pullu sazan (*Cyprinus carpio* L.) yavruları (fingerling) için kullanılan ticari yemlerle karşılaştırılması amacıyla gerçekleştirilmiştir. Çalışma Irak'taki Süleymaniye Üniversitesi Ziraat Bilimleri Fakültesi Hayvan Bilimleri Bölümü balık laboratuvarlarında, 12 hafta süre ile gerçekleştirilmiştir. Çalışmada kullanılan balıkların boyutları değişken olup, ağırlıkları 25,07 ile 37,21 g arasında değişmektedir ve ortlama başlangıç ağırlığı 28,58 g'dır. Balıklar önce 21 gün boyunca laboratuvar şartlarına alıştırılmış olup, bu süre boyunca ve beslenme deneyleri başlatılmadan önce kontrol peletleri ile (% 28.06 protein) beslenmişlerdir. Ardından iki farklı dozda *Chlorella* spp. ve *Daphnia* spp. ile katkılandırılmış yemler ayrı ayrı ve birlikte verilerek, balıkların büyüme performansları, yemden faydalanmaları, kan değerleri, biyolojik değerleri ve kimyasal kompozisyonları incelenmiş ve fingerlinglerin organoleptik (duyusal) değerlendirmeleri yapılmıştır. Kontrol grubu (T1) balıklara hiç *Chlorella* veya *Daphnia* kullanılmadan yalnızca ticari yemler verilirken, diğer altı gruptaki balıklar kontrol diyetine ilaveten, belirtilen değerlerde *Chlorella* spp. tozu ve dondurulup kurutulmuş *Daphnia* spp. eklenerek beslenmişlerdir: (T2) 25 g *Chlorella*/ kg diyeti ile, (T3) 50 g *Chlorella*/kg diyeti ile, (T4) 25 g *Daphnia*/ kg diyeti ile (T5) 50 g *Daphnia*/ kg diyeti ile, (T6) 25 g *Chlorella* + 25 g *Daphnia*/kg diyeti ile, ve (T7) 50 g *Chlorella* + 50 g *Daphnia*/kg diyeti ile. Her besleme grubu için üçer tekerrür yapılmış ve 100 L'lik tanklarda altışar pullu sazan fingerlingi stoklanıp, deneysel yemlerle günde iki defa beslenmişlerdir. Sonuçlar, *Chlorella* spp., *Daphnia* spp. ve onların kombinasyonunun pullu sazan için çok uygun yem takviyeleri olduklarını göstermiştir. Her tanka hava kompresörleri yardımı ile uygun şekilde havalandırma da temin edilmiştir.

Anahtar kelimeler: Balıklarda büyüme, Biyolojik değerler, *Chlorella* spp., *Daphnia* spp., Kan, Kimyasal bileşim ve organoleptik değerlendirme, Pullu sazan (*C. carpio*), Yemden yararlanma.

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Abbreviations	Description
a.m.	Ante meridiem
p.m.	Post meridiem
TDS	Total Dissolved Solids
pH	Potential of Hydrogen
FAO	Food and Agricultural Organization
HLPE	High Level Panel of Experts
ppm	Parts per million
PUFA	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
mmt	Million metric tons
SCP	Single-cell protein
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
NPU	Net protein utilization
CGF	*Chlorella* growth factor
NRC	National research council
AOAC	Association of official analytical chemists

Symbols	Description
L	Liter
dL	Deciliter
$\mathbf{m^3}$	Cubic meter
%	Percentage
Kg	Kilogram

Symbols	**Description**
Kcal	Kilocalorie
˚C	Centigrade
mg/L	Milligram/ liter
pg	Pico gram
g/dL	Gram/ deciliter
fL	Femtoliter
t	Ton
mm	Millimeter
µm	Micrometer
µg	Microgram
β	Beta
mg	Milligram

1. INTRODUCTION

The fastest rising food-production technology in the world is aquaculture according to Anderson et al. (2017). Subasinghe et al. (2009) stated that for eliminating malnutrition and starvation via providing fish and other aquatic products which are high protein content, essential fatty acids and vitamins and minerals, aquaculture have significant role in international efforts.

Internationally, in comparison to other main meat sources, seafood (both farmed and wildly caught) is a bigger source of animal protein, as reported by Anderson et al. (2017). In many regions in the world, predominantly in developing countries, sea food is the major source of animal protein (Anderson et al., 2017). Both Smith et al. (2010) and Anderson et al. (2017) demonstrated that aquaculture contributes to the improvement of food security either indirectly via economic development from export trade or directly throughout local consumption. In addition, it also changes the global food consumption pattern. Furthermore, aquaculture has significant utilization for public health (Mozaffarian and Rimm, 2006; Anderson et al., 2017).

Nowadays, in accordance to Becker (2013b) approximately forty percent of the whole aquaculture productions are definitely reliant upon commercial feedstuff. That is particularly accurate in the circumstance of meat-eating types, whose feed comprises enormous amounts of aquatic inputs in the form of fishmeal, such as salmon, shrimp and trout (Becker, 2013b). Becker (2013b) indicated that utilizing the amount of commercial feeds differs by different fish farms. For example, while salmon and trout farms used 100 % commercial feed, marine shrimp and carp farms utilized 83 % and 38 % commercial feed respectively. Thus, unsurprisingly phytoplankton, especially microalgae, have an imperative capability in the raising of marine creatures such as mollusks, fish and shrimp (Becker, 2013b). Badwy et al. (2008) exposed that unconventional feed ingredients like algae has been increasingly utilized as feed inputs and replaced of expensive feedstuffs, for instance fishmeal.

According to Casal (2006) currently from 120 countries in world, common carp is established in around 91 countries. Furthermore, common carp has great adaptability to a wide variety of environmental circumstances (Khan et al., 2016).

Dadebo et al. (2015) declared that *C. carpio*'s native dissemination covers an enormous region from Eurasia eastward across Russia and China in lakes, slowly moving or motionless waters, reservoirs and permanent wetlands, commonly with silt bottoms.

The significant constituents of freshwater and marine water-column ecosystems are planktons. Great contributions are made by planktons to global biogeochemical cycling. In addition, through 'pumping' carbon to the deep sea, they are also ameliorating the accumulation of carbon dioxide in the atmosphere. The combinations of these functions are endangered from climate-associated physiological effect upon single organisms and broadly on dispersal of plankton communities (Brierley, 2017).

As natural feeds encompass entire essential nutrients for the development and growth of fish, they are imperative in the diet of pond fish (Tuchapska and Krazhan, 2014). They revealed that integration of artificial feeds; fish growth and immunity are vastly affected by the portion of natural feeds in fish diet. Tuchapska and Krazhan (2014) declared that artificial farming of aquatic organisms is the chief method for assuring procurement of natural feeds for fish feeding at various periods of their growth.

The nutritional value of feed composites is optimized by the role of technology.

Nevertheless, particular additives might be essential for safeguarding the nutritional value of feeds and the product quality (Chebbaki et al., 2010). Aba et al (2012) presented that the major component of production cost in aquaculture is feed, as the cost can reach up to 60 % of the production cost of farmed fish. Therefore, the improvement formulation of a food economy affects the development of the fish farming sector, however it has to comply with the requirements of the fish in terms of quantity and quality (Aba et al., 2012). In accordance to Sirakov et al. (2015) algae is one of the easily reached and comparatively low-cost food constituent that can answer effectively the encounter question raised by fish farming sector. The necessity for sources of nutrition safer compared to traditional animal products has refreshed interest largely in plants and predominantly in microalgae (Muller-Feuga, 2000; Hemaiswarya et al., 2011).

In fish farming (aquaculture), algae are applied for different utilization. However, Becker (2013b) reported that the major applications are associated to nutrition, for example providing basic nutrients, as a source of pigments to color the flesh (of salmonids) or the skin for other biological objectives. Algae might be utilized *in toto*, which is an unprocessed sole constituent for building up tiny food chains or in the formula of dried substances in pellets or otherwise treated preparations (Becker, 2013b).

Concerning to aquatic wildlife in the open sea, microalgae certainly have a vital nutritional role. Therefore, they have crucial impact on fish farming (Guedes and Malcata, 2012). As live feed for the entire development phases of bivalve mollusks for larval/early juvenile stages of abalone, crustaceans and some fish species and for zooplankton microalgae are largely utilized in fish farming food webs (Guedes and Malcata, 2012).

Brown (2002) and Sirakov et al. (2015) reported that the microalgae have to have good nutrient composition and free of poisons that could pose threat through transfering up the food chain. In fish farming, the chief microalgae utilization is associated with their use for feed purposes (Sirakov et al., 2015). According to Becker (2007) and Sirakov et al. (2015) presently 30 % of the world production of algae is used for animal feed. However, FAO (2009) and Sirakov et al. (2015) demonstrated that in fish farming algae is primarily utilized for larval fish, crustaceans and mollusks.

In aquaculture, the chief microalgae utilizations are related to as food additive or nutrition to color the flesh of salmonids and to induce other biological activities. *Chlorella, Pavlova, Tetraselmis, Phaeodactylum, Isochrysis, Nannochloropsis, Chaetoceros, Thalassiosira* and *Skeletonema* are the most commonly utilized genera of microalgae (Radhakrishnan et al., 2015).

The algae biomass appropriateness as a valued feed replacement or supplement for conventional sources of protein such as fish meal, rice bran and soybean meal is demonstrated by abundance toxicological and nutritional evaluations (Becker, 2007). Becker (2007) declared that because the integration of algae into poultry feed have the most encouraging prospect for their commercial utilization in animal feeding, the objective domestic animal is poultry. Nonetheless, the micro-algae application in aquaculture is another rising market. Approximately 30 percent of the present global algal production is approximated to be sold for animal feed utilization (Becker, 2007).

Predominantly because *Chlorella* has long been consumed by human as a nutraceutical and food additives, it is acknowledged as a safe food ingredient globally (Guccione et al., 2014; Becker, 2007).

Liu and Hu (2013) Unveiled that once *Chlorella* is cultivated under favorable environmental conditions, the biomass of *Chlorella* might comprise of 12–15 % lipids and 10–15 % carbohydrates. The C16 and C18 fatty acid groups such as C16:0, C16:2, C18:1, C18:2 and C18:3 found in main lipids in *Chlorella* (Liu and Hu, 2013). In addition, multivitamins, specifically provitamin A, vitamins B1, B2, B6, B12, C, and E, niacin, folic acid, and pantothenic acid are also found in *Chlorella*. Furthermore, *Chlorella* contains some minerals such as potassium, sodium, calcium, iron, magnesium, zinc, manganese and copper (Guedes et al., 2010). Moreover, chlorophyll *a* and *b*, in conjunction with a variety of carotenoids such as *β*-carotene, neoxanthin, violaxanthin, lutein, zeaxanthin, and antheraxanthin are also included in *Chlorella* (Liu and Hu, 2013). The most ample carotenoid in *Chlorella* cells is Lutein. It might amass around 0.45 percent of the dry weight of cell (Liu and Hu, 2013).

Globally, fish farming, which frequently resorts to microalgae as feed, is a rising commercial activity (Guedes et al., 2010). According to Borowitzka (1997) and Guedes et al (2010) the main employment of microalgae in the industry of aquaculture are either feed directly to fish, crustacean and bivalves or as feed for zooplankton, which are consecutively utilized as share of the crustaceans and fish diet. Because microalgae are ultimately applied as feed and the nutritional quality have an essential role in animals, the growth rate, researches concerning the biochemical composition of microalgae are favorable (Guedes et al., 2010). In the raising of the entire phases of marine bivalve mollusks, larval phases of several marine gastropods, and the larvae of several marine fish species, penaeid zooplankton and shrimps, microalgae are important (Becker, 2013b).

Hemaiswarya et al. (2011) stated that in aquaculture microalgae have various utilizations. For aquaculture, the major employment of microalgae are related to nutrition (as the only constituent or like food supplement to basic nutrients) to color the flesh of salmonids and to induce other biological activities (Hemaiswarya et al., 2011). According to Muller-Feuga (2000) examples of aquatic animals that feed on Microalgae are echinoderms, the larvae of mollusks and crustaceans in addition to the live prey of several fish larvae. Because of the positive impact of algae has on weight gain, improved the deposition of protein and triglyceride in muscle, enhanced immunity system, reduced output of nitrogen into the ecosystem, augmented digestibility of fish, hunger tolerance, physiological activity and quality of the carcass, the interest of algae utilization as an additive in fish farming has increased dramatically (Sirakov et al., 2015).

Kibria et al. (1997) revealed that in addition to the importance of zooplankton as a food source for various fish species, feeding zooplanktons also enhance the texture and flavor of fish. Kibria et al. (1997) stated that algae can be a low-cost ingredient to substitute costly fishmeal and an alternative to more costly brine shrimp. Daphnids, similar to other invertebrate creatures, have become keystones of research of biology systems for methodologies and alternative testing. This encourages the utilizations of Daphnids for prescreening tests prior vertebrate testing, for practical and ethical purposes (Siciliano et al., 2015).

Pereira et al. (2007) stated that a significant food resource to planktivorous fish is the cladocerans especially those belong genus *Daphnia*. Thus, it has a crucial role in the energy transmission from primary producers (phytoplankton) to upper stages of the food web.

This research aims to compare the utilization of chlorella and daphnia as natural food sources and their combination as a food supplement to commercial feed for common carp (*Cyprinus carpio*).

2. LITERATURE REVIEW

The total mortality is reduced by 17 % and the risk of coronary death is decreasedby 36 % by consuming fish, particularly species with high the omega-3 fatty acids content such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), once or twice per week (Mozaffarian and Rimm, 2006). According to Schwab et al (2014) cardiovascular disease and diabetes are decreased with the decline in the consumption of saturated fats. Mainly because of the content of long chain polyunsaturated omega-3 fatty acids, fish are regarded as nutritionally valuable part of the human diet. Therefore, consumption of fish is recommended twice per week (Tilami and Sampels, 2018). It is confirmed that these fatty acids are vital in human diet and is involved in several metabolic functions (Tilami and Sampels, 2018).

2.1. Aquaculture

Through enhancing revenues, offering work opportunities and escalating the returns on resource utilization, aquaculture could make substantial take part in development process (Subasinghe et al., 2009). Aquaculture and fisheries, particularly minor-scale fisheries, are internationally major source of livelihoods and employment. According to HLPE (2014) approximately 800 million persons, including employees and their families, partly or completely rely upon aquaculture, fisheries and associated industries as a source of revenue. In the current years, the importance of aquaculture has escalated through the diet improvement of the people, creating work opportunity in country side and substituting the import through foreign exchange conservation (Adewumi, 2015). Anderson et al. (2017) stated that this growth has overwhelmingly altered the way seafood is generated, operated and consumed. In addition, in world food manufacturing, it has redefined the role of seafood as well.

As a significant agricultural industry, the Blue Revolution (Aquaculture) has truly just started (Anderson et al., 2017). It is also stated that additional efficiency development via selective breeding, controlling diseases, improved nutrition, and farm management has significant potential (Anderson et al., 2017). Aquaculture merely accounted for around 4 percent of total agricultural production in the 1970 while the majority of seafood was yielded from wild stocks. However, when the harvesting wild-fish reached plateau in the late 1980s, and further considerable development was improbable, seafood farming (aquaculture) started growing (Anderson et al., 2017). FAO (2016) and Anderson et al. (2017) stated that seafood farming exceeded wild-stock landings as a food source.

According to Anderson (2002) and Anderson et al. (2017) when seafood farmers improved their ability in controlling the process of production and manage the market, the significance of aquaculture has escalated. Between 1970 and 2013, seafood farmers have obviously taken benefit of their opportunities in development of global production of aquaculture. During that period, the production of aquaculture has escalated from approximately 4 mmt to 69.3 mmt (FAO, 2016; Anderson et al., 2017).

Asche et al. (2013) and Anderson et al. (2017) reported that the production of aquaculture is enormously diverse, for instance there are multinational companies and small-scale family farms. In addition, in terms production systems, there are extremely multifaceted closed production systems and basic extensive systems. Furthermore, there are breeding variations as well from simple algae to bivalves to crustaceans to finfish (Anderson et al., 2017).

In terrestrial animal production, improvements were greatly grounded upon the applied technologies in disease controlling, feed preparation and breeding (Anderson et al., 2017). Then, globally for other freshwater and marine species, effective techniques were adjusted and advanced (Asche, 2008; Anderson et al., 2017). Anderson et al. (2017) stated that the competitiveness of aquaculture products and the sector's capability for developing novel markets for aquatic products were improved greatly by technology.

The production of aquaculture might increase to 94 million metric tons (mmt) by 2030 as forecasted the Fish to 2030 Project. This indicates around 60 % growth compared to 2010 (Kobayashi et al., 2015). In comparison to the previous decades, it is a lower growth rate, nonetheless is probable to retain position of aquaculture as the quickest developing technology of food-production (Anderson et al., 2017). Asche et al (2016), Knapp and Rubino (2016) expressed that the composition of regional production, the species to be farmed include natural endowments, management systems, trade obstacles, and seafood demands are the main factors that will impact the aquaculture's growth rate. Probably, demand is the most significant factor that is affected by growth of population, consumer wealth and price (Anderson et al., 2017).

FAO (2016) projected that in 2014 around 57 million individuals were involved in the primary sector of aquaculture and capture fisheries. Approximately a quarter of those people were partly involved and 36 % were fully engaged. However, the rest were either infrequent fishers or of unspecified status. The percentage of employees involved in aquaculture augmented from 17 % to 33 % from 1990 to 2014, however, since 2010 numbers have stayed comparatively constant (FAO, 2016). More than 80 % of the world inhabitants who involved in the aquaculture and fisheries industry were in Asia (FAO, 2016). The most increase in aquaculture production in 2025 will be Freshwater species like catfish, carp and tilapia. They will comprise around 60 % of the entire aquaculture production (FAO, 2016). In 2008 carps production (*Cyprinidae*, 20.4 mt, or by 71.1 %) dominated the freshwater fishes production (FAO, 2010).

2.2. Common Carp (*Cyprinus carpio*)

Around 4000 to 5000 years ago, fish production in controlled environment took place in China. The earthen ponds were utilized for farming common carp (*Cyprinus carpio*). These carps, which are strong omnivorous, lived upon naturally grown plankton, algae, snails and detritus in the ponds (Gjedrem and Baranski, 2009).

Khan et al. (2016) pronounced that the most common cyprinid species that produces a substantial share of inland production freshwater fish is Common carp. This type is presented to inland waters for instance lakes, dam lakes and streams in diverse areas. In several European and numerous Asian countries common carp *C. carpio* is regarded to be a vital aquaculture species (Rahman, 2015). Rahman (2015) stated that Common carp (*C. carpio*) belongs to the order Cypriniformes and the family Cyprinidae, which is regarded the bigest family of freshwater fish.

One of the main commercially significant and broadly farmed freshwater fish in the globe is the Common carp (*C. carpio*) (Rahman et al., 2010). Khan et al. (2016) expressed that due to outstanding growth rate, omnivorous habit, breeding in restrained waters, robust nature and easily adapted to artificial feeds, common carp (*C. carpio*) is greatly preferred for farming in ponds in Asia, Near and Far East, in mixture with other fishes or alone. Worldwide, common carp (*C. carpio*) represented the third largest production (around 3 mt) among entire species of freshwater in 2005 (Rahman and Meyer, 2009). Asia, where *C. carpio* are farmed in earthen ponds comprising a diversity of naturally growing foods which are added via nutrient fertilization and artificial feeds, produced more than 90 percent of this amount (Rahman and Meyer, 2009).

2.3. Plankton and Its Role in Aquaculture

Taxonomically, plankton comprised of different animals, bacteria, plants and viruses (Brierley, 2017). Even though the amplifying period of zooplankton (normally ranging between weeks and months) is substantially longer compared to phytoplankton (ranging between hours and days), worldwide, the zooplankton and phytoplankton are equivalent in the biomasses (Brierley, 2017). The plankton are mainly sub-millimeter to millimeter in dimension, however in a group their sizes extend numerous orders of magnitude. In addition, zooplankton comprise of jellyfish with tentacles trailing several metres (Brierley, 2017).

The commencement of spring brings a fast rise in activity of phytoplankton in lakes, oceans and seas at temperate latitudes. Furthermore, in illuminated surface waters which are rich in necessary nutrients such as silicate, nitrate and phosphate, photosynthesis is promoted by rising temperature and increasing sunlight subsequent vertical combination by waves and wind throughout storms periods of the earlier winter. Moreover, it is stated that visibility in water is reduces by the Chlorophyll proliferation in the phytoplankton the rapid surface waters greening in spring is named the ‘spring bloom’ (Brierley, 2017).

Brierley (2017) while in freshwater and marine pelagic ecosystems the major primary producers are plankton, the major primary customers are zooplankton. Some essential characteristics like proper nutritional value, a edible cell wall that makes the components obtainable, appropriate size and shape for ingestion, elevated growth rates, mass farming suitability, resists the fluctuation of growth conditions and finally nontoxicity should exist in microalgae to be appropriate for use in aquaculture (Becker, 2013b). There are two approaches for distinguishing the algae for aquaculture utilization. The first approach is by phytoplankton's natural populations which either as they are from mass cultures supplemented by nutrients or found in nature. The second approach as the algal monocultures, cultivated inside or outside. It is grown inside for producing algal cultures that is free of bacteria or decreasing the danger of presenting pathogens into the aquacultures or for producing a high-quality feed with identified nutritional possessions (Becker, 2013b). Hundreds of microalgal species have been examined as feed, however merely a few found a broad utilization in aquaculture. Around 40 microalgal species have been separated and investigated and are cultivated as pure strains intensively in farming systems (Becker, 2013b).

In general, the main components of plankton are tiny animals, zooplankton, which are drifting on the flows as well as unicellular plants, phytoplankton (Brierley, 2017). The aquatic creatures which populate the water column (specifically, whose living far from the seabed, in the *pelagic* realm) is called Plankton. It is originated from the Greek *planao* which means 'to wander'. They have weak ability of swimming and almost immobile, however they horizontally moved at the sympathy of streams (the current velocities of the ocean are extremely varied between 0.1 and 1 m/s. Nonetheless, the swimming capability of crustacean zooplankton is merely around tens of meters/h (Brierley, 2017). According to Das et al. (2012) zooplankton consists of animal origin plankters while Phytoplankton comprises of non-photosynthetic plants or saproplankton (for example fungi and bacteria) and chlorophyll bearing organisms (for example, *Microcystis, Volvox, Eudorina, Oscillatoria*). It primarily consists of the crustaceans planktonic forms (*Artemia* spp.,), cladoceran (*Moina* spp., *Daphnia* spp., *Ceriodaphnia* sp. etc.), ostracoda (*Cypris*, *Stenocypris*, *Eucypris* etc.), copepods (*Mesocyclpos leuckarti*, *M. hyalinus*, *Microcyclops varicans*, *Heliodiaptomus viduus*), protozoans (for instance *Arcella* sp., *Difflugia* sp., *Actinophrys* sp., *Vorticella* sp. etc.) and rotifers (such as *Brachionus* spp., *Keratella* sp., *Asplanchna brightwelli*, *Polyarthra vulgaris*, *Filinia opoliensis* etc.) and their larvae (Das et al., 2012). Treece and Davis (2000) stated that in general Cladocerans are not existed in brackish water as they are mainly freshwater zooplankters. Most of Cladocerans do not endure higher salinities than three ppt. Because live feeds are capable of swimming in the water column, they are continuously accessible to the larvae (David, 2003). Nevertheless, regularly in clear pond water natural fish food organisms are not plentiful; however they are plentiful in ponds having greenish water. The existence of phytoplankton and other natural food organisms are indicated by the green color (Das et al., 2012).

Even though natural fish food contains low amount of carbohydrates (containing between 3 to 4.79 % of carbohydrates on dry matter), it is the major source of lipids and protein in the fish diet, (Anton-Pardo and Adámek, 2015). Therefore, cereals are regularly added to carp ponds as a supplementary feeds (Kibria et al., 1997; Anton-Pardo and Adámek, 2015). In spite of that, for both growth and survival of the fish natural food has been displayed to have substantial assistances (Anton-Pardo and Adámek, 2015). According to Anton-Pardo and Adámek (2015) zooplankton decompose easier compared to dry food and thus might be more edible for fish larvae because it contains comparatively low quantity of dry matter (around 10 %).

2.4. Using of Algae in Aquaculture

For other organisms, algae, which are photosynthetic organisms, are the significant source of both chemical energy and cellular carbon. Hence, algae are regularly termed primary producers (Sirakov et al., 2015). In general, algae are classified in to two main categories: microalgae (unicellular) and macroalgae (seaweed). Microalgae required carbon dioxide, sunlight and nutrients for growth, and they are cultivated and utilized for several applications such as food, production of valuable composites, removing contaminants and other nutrients from wastewaters as biofilters, in cosmetic and pharmaceutical industry and in fish farming purpose (Sirakov et al., 2015). In addition, due to high oil content in microalgae and fast biomass generation, they are possibly a decented sources for the production of biofuel (Sirakov et al., 2015).

As microalgae have greater photosynthetic efficacies, greater harvests and growth rates, and required for few space of land for cultivation and have the capability to grow in salty waters and in arid and barren land areas, they might demonstrate as an alternative to terrestrial crops (Lakaniemi et al., 2011). Safi et al. (2014) expressed that microalgae, similar to other phytoplankton, have nutritional benefits.

The protein that removed from combined or pure cultures of yeast, algae, bacteria or fungi and utilized as a replacement for the conventional protein sources exploited for the consumption of animal and human is called Single-cell protein (SCP) (Barka and Blecker, 2016). Throughout the second half of the twentieth century, microalgae were a source of concern to the most of those engaged in food domains agricultural sector and have been recognized as major consistent protein sources (Barka and Blecker, 2016). The protein content of several microalgal sources are bigger compared to conventional plant or animal sources, for instance *Spirulina paltensis* have 65 % of protein content which is bigger than that of peanuts (26 %), soy flour (37 %), dried skimmed milk (36 %), beef (22 %), fish (24 %), and chicken (24 %), (Moorhead et al., 2011; Barka and Blecker, 2016). According to Moorhead et al (2011) the first plants that appeared on the planet were algae.

During the last forty year, hundreds of species of microalgae have been examined as food, however the number of species that obtained prevalent utilization in aquaculture were less than twenty (Table 2.1) (Brown, 2002).

Brown (2002) declared that for microalgae to be beneficial aquaculture species have to have numerous important characteristics. Such attributes are a suitable size for consumption which varied between 1 and 15 µm for filter feeders; 10 and 100 µm for grazers and easily break down (Brown, 2002). In addition, they have to have fast rates of growth, be submissive to mass culture, and resistance to the fluctuations of the culture's temperature, light and nutrients that might happen in hatchery systems (Brown, 2002). Lastly, microalgae have to possess a decent composition of nutrients, and be free of poisons which may transfer up the food chain (Brown, 2002). *Tetraselmis*, *Chlorella*, *Scenedesmus*, *Pavlova*, *Nannochloropsis*, *Phaeodactylum*, *Chaetoceros*, *Thalassiosira* and *Skeletonema* are the most commonly utilized genus of microalgae in aquaculture (Sirakov et al., 2015). According to Sirakov et al. (2015) these genus of microalgae have required attributes to be beneficial for aquaculture such resistance to fluctuation in nutrients, temperature and light and fast growth rates.

There are various bioactive composites in the microalgae that can be controlled and used for commercial application (Sirakov et al., 2015). The freshwater green alga *Haematococcus pluvialis* is one of the sources of natural astaxanthin which controls the pink color of trout and salmon (Sirakov et al., 2015).

Table 2.1. Microalgae commonly used in aquaculture, either as individual diets or components of mixed diets (++ denotes more popular than +)

Microalgae	Bivalve mollusks	Crustacean larvae	Juvenile abalone	Zooplankton (used for crustacean, fish larvae)
Isochrysis sp. (T.ISO)	++	+		++
Pavlova lutheri	++	+		++
Chaetoceros calcitrans	++	++		+
C. muelleri or C. gracilis	+	++		+
Thalassiosira pseudonana	+	+		
Skeletonema spp.	+	++		
Tetraselmis suecica	+	+		++
Rhodomonas spp.	+			
Pyramimonas spp.	+			
Navicula spp.	+	+	++	
Nitzschia spp.		+	++	
Cocconeis spp.			+	
Amphora spp.			+	
Nannochloropsis spp.				++

In the recent years, searching for novel alternative of protein has increased because of the escalation in the demand on protein and the high price of fish meal (Sirakov et al., 2015). There is an escalating demand for new vision on the prospective of alternative sources of protein in fish feeds assuming the world needs for fish oil and fish meal for aquaculture (Chebbaki et al., 2010).

Currently, either as natural food pigments or nutritional enhancements, microalgae added to snack foods, pasta or drinks as well (Priyadarshani and Rath, 2012). They are commercially cultured for the utilization like treasured chemical sources such as betacarotene and healthy food (Borowitzka, 1997). In the commercial raising of numerous marine animals, particularly the spat and larvae of bivalve mollusks, penaeid prawn larvae and natural fish food organisms like rotifers which, consecutively, are utilized in rearing the larvae of marine finfish and crustaceans, microalgae are a significant source of food and feed supplements (Borowitzka, 1997). As algae are the source of natural food of these animals, its significance in aquaculture is not astonishing (Borowitzka, 1997). Live algae are still the optimum and the favored source of food even though microencapsulated feeds and yeasts are considered alternatives to algae (Borowitzka, 1997).

Unialgal cultures and natural populations of phytoplankton are the two major sources of algal species that utilized in aquaculture. When a high quality feed source with recognized nutritional characteristics is needed, unialgal cultures are vital. Furthermore, natural populations of phytoplankton are either found in nature or from cultures enhanced via supplying nutrients (Borowitzka, 1997). The expense is the major restriction on production microbial for aquaculture (Borowitzka, 1997). According to Borowitzka (1997) the costs of producing alga for example *Spirulina*, *Dunaliella* and *Chlorella* cost around \$US 15–20 kg^{-1} dry algae. The high expenses of algal culture and the related issues with culture stability is solved by dedicating units for algae production, using effective culture techniques, providing qualified staff, a total quality management program, that could accomplish economies of scale, therefore supplying hatcheries with a inexpensive, dependable and high quality source of algae (Borowitzka, 1997).

Despite the being the important food sources, microalgae might have a significant role in improving the quality of cultured animal species. For achieving the flesh colour in salmonids, it requires the carotenoids additives, particularly astaxanthin, in their diet (Borowitzka, 1997). The main factors determining microalgae's nutritional value are vitamin and protein content (Radhakrishnan et al., 2015).

The content of protein, fat, total carbohydrate, ash and crude fiber were 46.7 %, 14.8 %, 11.6 %, 17.5 % , 9.30 %, respectively and the content nucleic acid content (RNA 2.63 % and DNA 1.72 %). In addition, the content vitamins group antioxidant B6 was 0.05(µg \g dry weight), B12 was 0.08 (µg \g dry weight), E was 2.25 (µg \g dry weight), C was 16.0 (µg \g dry weight), and B-carotene 2384.0 (µg \g dry weight) (Badwy et al., 2008). In accordance to Radhakrishnan et al. (2015) for *Marobrachium rosenbergii* post larval culture, the partial substitution of fish meal with *Chlorella vulgaris* is preferable.

In commercial aqua feeds, the main source of protein is fishmeal. There is an interest in evolving alternatives to this limited constituent due to the drop in fishery resources that goes in the production of fishmeal and the rapid escalation in price of fishmeal (Radhakrishnan et al., 2015). For the aquatic feed industry, discovering and examining alternative sources of lipid and protein are imperative (Kiron et al., 2012; Radhakrishnan et al., 2015). Furthermore, Radhakrishnan et al. (2015) stated the other major important factors are the content of polyunsaturated fatty acids (PUFA) (eicosapentaenoic acid (EPA), arachidonic acid and docosahexaenoic acid (DHA). Around fifty years ago, so called "protein gap" was predicted to be closed by the mass production of some microalgae that are rich in protein (Becker, 2007).

Nutritional research and widespread analyses have indicated that these high quality algal proteins are equivalent to conventional vegetable proteins (Becker, 2007). Nonetheless, because of high production expenses and technical complications incorporating components of the algae into edible food process, to the present day the proliferation of protein of algae is still in its initial stages, most of the preparations of microalgae are commercialized as animal feed, health food and cosmetics (Becker, 2007). Currently, these microscopic organisms, such as *Spirulina platensis* and *Chlorella vulgaris*, are still used as food additive (Fradique et al., 2010; AL-Koye, 2013; Safi et al., 2014). In addition, Safi et al. (2014) reported that the products of these micro-organisms are utilized for diverse purposes such as animal feed, colors, medicines, cosmetics and aquaculture. Several illnesses like wounds, gastric ulcers, constipation, hypertension, anemia, diabetes, infant neurosis and malnutrition are assisted by the health benefits of *Chlorella* (Yamaguchi, 1997; Fradique et al., 2010). Noguchi et al. (2014) expressed that *Chlorella* have the advantageous impact on the quality of life of breast cancer patients. It is also stated that *Chlorella* extract drink impacts the vitality status in breast cancer patients.

Currently, healthy people, cancer patients and chronic disease patients broadly utilize *Chlorella* is as nutritional complement (Noguchi et al., 2014). As well as the health benefits, it is also significant source of natural colorants, such as carotenoids (Fradique et al., 2010). In the last twenty years, the consequent of upsurge in oil expenses, the decrease of fossil fuel reserves and the concern of global warming motivated the utilization of microalgae (Safi et al., 2014). In the process of photosynthesis, after capturing sunlight microalgae produce around 50 % of atmospheric oxygen on the planet and absorb enormous quantities of carbon dioxide as a main feed. Thus, because of remarkable capacity of microalgae in absorbing carbon dioxide and converting them to food, potential biofuel, feed and extremely added value components, it highly important to grow them beside combustion power plants (Safi et al., 2014). Becker (2007) declared that the micro-algae utilization of as animal feed is new.

Becker (2007) and Xu et al. (2014) demonstrated that microalgae have fascinated the global interest for regarding them as a nutritional additive due to their broad spectrum of nutritious composites comprising vitamins, protein, essential amino acids, pigments and minerals. Enzyme inhibitors are the novel objective of bioactive composites in microalgae (Yamaguchi, 1997). All over the globe, even though the search in microalgae for enzyme inhibitors is still very restricted, the exploration in other microorganisms has previously been widespread and numerous beneficial antibiotics have been attained (Yamaguchi, 1997).

One of the few microalgae that utilized for human consumption is *Chlorella* (Guccione et al., 2014). In the early 1960s, Nihon *Chlorella* Inc. in Japan commercially introduced *Chlorella*, thus it is considered one of the most ancient microalgae species utilized in the human diet (Barka and Blecker, 2016). According to Barka and Blecker (2016) because *Chlorella* comprises β-1, 3-glucan, an immunoactive constituent, it is initially was farmed for its utilization as a health food and after that in marine aquaculture. In the 1990s, the entire production quantity was around 2,000 tones annually (Barka and Blecker, 2016). There are two modes (heterotrophic or mixotrophic) for *Chlorella* mass production. In comparison to heterotrophic, production yield is higher in mixotrophic (Barka and Blecker, 2016). Beside the carbon dioxide, the acetic acid can be supplied to the medium as an organic source of carbon in the mixotrophic production (Barka and Blecker, 2016).

However, carbon is added via the sole organic source of carbon in the heterotrophic mode, (Barka and Blecker, 2016). Primarily in the past, the in vivo examinations on its potential as protein source and food have been conducted (Guccione et al., 2014). Nevertheless, the biomass of *Chlorella* that gained via mass production of heterotrophic displays a better quality for ingesting as a health food. Furthermore, it does not exhibit pollutants and it is rich in useful phytochemicals (Barka and Blecker, 2016).

The mixotrophic and autotrophic modes mass production of *Chlorella* species exposed that a the maximum levels of biomass productivity was exhibited by mixotrophic fed-batch farming with glucose and a supply of air in dark cycles (561 mg.l^{-1}.d^{-1}) and fatty-acid methyl-ester (168 mg.l^{-1}.d^{-1}) (Praveenkumar et al., 2014; Barka and Blecker, 2016).

Barka and Blecker (2016) expressed that the *Chlorella* species is a green microalga of the phylum chlorophyta and has a spherical form with a diameter of around 2 to 10 μm. Among the strains of *Chlorella* species, content of protein varied widely (for example *Chlorella vulgaris* contains around 58 % protein while *Chlorella spaerckii* has 6.87 % protein and *Chlorella spaerckii* and *Chlorella ovalis* contain around 10.87 % of protein) (Becker, 2007; Barka and Blecker, 2016).

Kotrbáček et al. (2015) presented that comparative easy cultivation, high efficiency and high proteins and other valuable components content are some attributes of unicellular freshwater microalgae of the genus *Chlorella*. The content of protein, polysaccharide, lipid, minerals, vitamins and other nutritional constituents are high in *Chlorella*, and these components have abundant bioactivity including in several physiological activities (Xu et al., 2014). It is shown that *Chlorella* might be utilized as source of protein for animal feed and human food because it contains around 51-58 % the protein content of dry matter in addition to containing numerous essential amino acids (Becker, 2007). Xu et al. (2014) stated that the addition of various percentage of *Chlorella* in the of gibel carp's (*Carassius auratus gibelio*) basal feed can obviously exhibits the impact of *Chlorella* on the digestive enzyme, blood parameters and growth performance and demonstrate that it could be a decent option for utilizing as an supplements for fish feed. As an autotrophical organism, algae produce their food via obtaining carbon from CO, energy from artificial light or sunlight, and nutrients from the available carbohydrates in the medium (Nangul and Bhatia, 2013). *Chlorella*, belonging to the *Chlorophyceae, Chlorella* and *Chlorophyta* are the most broadly dispersed species among the microalgae in the nature particularly in fresh waters. According to Xu et al. (2014), It can live either heterotrophically by external source of carbon or photoautotrophically. Thus, it is effortlessly farmed in lab and owns extremely useful value (Yamaguchi, 1997; Xu et al., 2014).

At present, human food is the main focus of *Chlorella* utilization. The studies *Chlorella* utilization in lower vertebrate was less (Xu et al., 2014). Nangul and Bhatia (2013) showed that the main source for high value biological molecules like polyunsaturated fatty acids, pigments and proteins are algae. *Chlorella*, *Spirulina*

(Arthrospira) and *Dunaliella* are the three major commercially utilized algae species for protein production, with a protein content of 55 %, 65 % and 57 % correspondingly (Nangul and Bhatia, 2013). In biotechnology, strains of *Chlorella* are being employed for different utilizations (Nangul and Bhatia, 2013).

Nangul and Bhatia (2013) stated that with lowest reliance upon water, climate and soil conditions, single-cell protein (SCP) or microbial proteins can be quickly grown on substrates. Numerous animals and humans toxicological research investigated the utilization of microalgae as food and medication supplements, and lastly nutritional quality standards and legislative provisions for ensuring safety in the utilization of the biomass of algae (Becker, 2013a). A species of unicellular green microalgae, *Chlorella*, has drawn the observation of commercial and scientific interest (Liu and Hu, 2013). A constant *Chlorella* industry mainly for animal feed and human nutrition has been caused by the accomplishment of mass culture of *Chlorella* mixotrophically, heterotrophically and photoautotrophically (Liu and Hu, 2013). *Chlorella* has a nominee for biofuels and bioremediation because of its capability of quickly uptake and convert nutrients (for example phosphorous and nitrogen) from waste streams and carbon dioxide and produce huge quantity of lipids (Liu and Hu, 2013). Nevertheless, the present production systems and processes of *Chlorella* are unrealistic potential utilizations because they are not energy-efficient neither cost effective. Therefore, there are the requirements for advancement and innovations in evolving next generation technologies for the production of *Chlorella* (Liu and Hu, 2013).

In 1890, Beijerinck was the first to describe *Chlorella* with *Chlorella vulgaris* being the kind species (Liu and Hu, 2013). Sub sequently, a huge quantity of *Chlorella* species were separated and categorized. The cells of *Chlorella* have ellipsoidal or spherical form, and it has a diameter varying between 2 and 10 μm (Liu and Hu, 2013). *Chlorella* cells are dispersed in various habitats like seawater, freshwater, and soil. Furthermore, they are free-living or interdependently with protozoa and lichens. Throughout asexual autospore production, *Chlorella* reproduces itself (Liu and Hu, 2013). Liu and Hu (2013) reported that growth (escalating the size of cell), ripening (escalating the number of nuclear substances for mitosis), and division (isolating the mother cell into daughter cells) are the three stages involved in the process of reproduction. Instantaneously the mother cell wall ruptured and released 2–16 autospores (Liu and Hu, 2013).

In the literature, more than 100 strains of *Chlorella* are stated (Liu and Hu, 2013). Liu and Hu (2013) reviewed the genus of *Chlorella* and regarded it as a polyphyletic assemblage distributed through two categories of Chlorophyta (for example Trebouxiophyceae and Chlorophyceae). Under preferred growing circumstances, *Chlorella* can yield a huge quantitie of protein (more than 60 percent of dry weight of the cell). *Chlorella* contains the entire amino acids recognized to be necessary for animal and human nutrition such as Phenylalanine[a], Tyrosine, Arginine [a], Lysine[a], Histidine[a], Isoleucine[a], Methionine[a] Leucine[a],, Valine [a], Alanine, Proline, Gultamic acid, Glycine, Serine, Threonine[a] ,Aspartic acid, Cystine and Tryptophane[a].

In the late 1940s, *Chlorella*'s mass cultivation commenced virtually simultaneously in Japan, Germany and United States (Burlew, 1964; Liu and Hu, 2013). It has broadly been utilized as additives in animal feed and nutritional food (Liu and Hu, 2013). Liu and Hu (2013) displayed that the major producers of *Chlorella* with a yearly production of around 3500 tons are Taiwan and Japan. Currently, the globe's leader in using *Chlorella* is Japan (Safi et al., 2014). It is mainly utilized for medical purposes as it exhibited to possess immune-modulating and anti-cancer possessions (Safi et al., 2014). Safi et al. (2014) unveiled that rabbits, rats and mice have displayed protection properties against haematopoiesis subsequent feeding *Chlorella* in the form of powder. *Chlorella* also decreases age-related illnesses such as hypertension, cardiovascular illnesses and cataract. In addition, it minimizes the danger of atherosclerosis and stimulates production of collagen for skin (Safi et al., 2014).

Generally, for human ingestion *Chlorella* is produced in the form of tablets, dried powder or capsules (Liu and Hu, 2013). The algal biomass digestibility is a crucial necessity for its application. However, in the mono gastric creatures the firm cellulosic cell wall confines the admission to the contents of the cell in the gastrointestinal tract (Kotrbáček et al., 2015). Becker (2007) and Kotrbáček et al. (2015) described that net protein utilization (NPU) of sun-dried *Chlorella* increased less than 30 % to around 68 % subsequent partial rupturing of the wall of the cell. When 90 percent of cells were disrupted, a highest digestibility of about 80 % was accomplished (Kotrbáček et al., 2015).

A substantial escalation in the phagocytic activity of broiler chicken leukocytes in response to feed supplemented with 0.5 % disintegrated *Chlorella* was discovered by Kotrbáček et al. (2015). Superior nutritional utilization and declined mortality rate was observed in duck fed enriched ratio with 0.2 and 0.3 % *Chlorella* (Kotrbáček et al., 2015). Traditionally in breeding of ornamental fish and aquaculture, the *Chlorella* has been utilized (Kotrbáček et al., 2015). Gouveia et al. (1996, 2002) and Kotrbáček et al. (2015) indicated that *Chlorella* not merely impact the coloration of the skin but also affects the color and muscle tissue composition that is utilized for human consumption. Throughout the larval phases of several fish species, Microscopic algae are highly significant which frequently denoted to as the green water impact (Müller-Fuega, 2000; Kotrbáček et al., 2015). In addition, it is also important for shrimps, mollusks and rotifers that are vital constituents of the food chain of fish (Borowitzka, 1997; Kotrbáček et al., 2015).

A positive impact might be achieved from supplementing animal feeds with small and economically acceptable concentration of *Chlorella* biomass. This might related to the enhancement of palatability of the feed, feed intake and feed conversion (Kotrbáček et al., 2015). In addition, it also affects on improved minerals digestibility instead of a direct impact on somatic developments (Kotrbáček et al., 2015).

When using non-disintegrated algal biomass, the quantity of algae has to be folded for attaining a similar effect of algal supplementation (Kotrbáček et al., 2015). According to Kotrbáček et al. (2015) supplementing feeds with 1 % *Chlorella* caused an important however noticeable tendency to minor juvenile mortality. The algae are highly cost for using broadly as a protein additive in animal ratio. However, supplementation of *Chlorella* biomass in animal ratio can effectively impact the performance and growth even when utilized in tiny, economically acceptable concentration (Kotrbáček et al., 2015). This is because of the attendance of antioxidants, pigments, vitamins, provitamins, and a growth constituent recognized as the *Chlorella* Growth Factor (CGF). Thus, it motivates or improves the immunity system, escalates feed intake and utilizations and encourages reproduction. Furthermore, *Chlorella* biomass utilization might upsurge the value of animal products for human consumption (Kotrbáček et al., 2015). According to Guccione et al. (2014) one of the major examined microalgae in the world is *Chlorella* (Chlorophyta, Trebouxiophyceae) is cultivated commercially by more than 70 corporations. The *Chlorella* commercialization as a food product is not yet developed widely (Guccione et al., 2014).

2.5. Microalgae Role in Aquaculture

As a method of supplementing zooplankton for feeding fish and larvae, microalgae play a great role in aquaculture (Hemaiswarya et al., 2011). Rotifers *Brachionus plicatilis* and *Artemia salina* are most frequently used zooplankton (Hemaiswarya et al., 2011). However, cladocerans (*Moina macrocopa*, *Daphnia* spp.) and copepods (*Euterpina acutifrons*, *Tigriopus japonicus*) are also utilized but less frequently (Hemaiswarya et al., 2011).

According to Kiron et al. (2012) in commercial marine feeds, fishmeal is the major protein source. As discovering replacement for fishmeal in aqua feeds is difficult, identifying the suitability of the available algae co-product is also challenging (Kiron et al., 2012). The protein in the microalgae might be a reasonable alternative to fishmeal protein because it has a decent quality and good profiles of amino acid similar to that of other reference food proteins (Becker, 2007; Kiron et al., 2012; AL-Koye, 2013). Furthermore, microalgae might be a perfect substitution for fishmeal in marine feeds, as they are the source of whole photosynthetically stable carbon in the food web of marine creatures (Kiron et al., 2012).

Many studies exhibit that animal growth fed with feed composed of a combination of numerous species of algae is frequently better to that gained via feeding merely a single species of algae. Perhaps, this might be because of the fact that a certain alga might have deficiency in some nutrients, whereas the other one might comprise those nutrients (Yamaguchi, 1997; Becker, 2013b). Producing microalgae for the utilization as feed is separated into intensive monoculture and extensive culture. The first approach is utilized for larval phases of bivalves, shrimp and certain fish species, however, the second method is for growth of bivalves, carp, and shrimp (Becker, 2013a).

In aquaculture, microalgae are mainly applied for providing feed. Nevertheless, they assist in stabilizing and improving the medium’s quality (Becker, 2013b). Thus, phytoplankton supplementation into raising ponds, the greenwater technique, regularly brings about superior survival and growth rates compared to the conventional clear-water technique. Perhaps, this is because of the production of oxygen and the stabilization of pH (Becker, 2013b). Moreover, algal compounds excretion demonstrated a positive impact, because they are controlling and regulating bacterial pollution, in addition to immune motivation possessions and contributing probiotic impact (Becker, 2013a).

Hemaiswarya et al. (2011) the microalgal production for aquaculture extended to 1,000 tons (62 %, 21 % and 16 % for mollusks, shrimps and fish respectively). Because of chemical composition microalgae, they can be utilized for enhancing the nutritional value of animal feed and human food (Fradique et al., 2010).

The main kinds of microalgae that have been utilized effectively for producing high levels of useful composites like protein, lipids and colorants are *Chlorella* sp., *Dunaliella* sp. and *Spirulina* sp. (Sirakov et al., 2015). *Spirulina* sp. and *Chlorella* sp. are frequently comprised into feeds for ornamental fish, where pigmentation and healthy appearance is the major market standard (Sergejevová and Masojídek, 2012; Sirakov et al., 2015). *Chaetoceros*, *Tetraselmis*, *Thalassiosira*, *Nannochloropsis* and *Isochrysis*, are genera of microalgae for larval feeds (Sirakov et al., 2015).

The (Table 2.2) displays a list of main species of algae presently utilized as feed for various groups of commercially significant marine organisms, comprising species of flagellated, diatoms and chlorococcalean green algae and filamentous blue-green algae, different in volume from around 5 µm (*Chlorella*) to more than 100 µm (*Spirulina*) (Becker, 2013b).

Table 2.2. Microalgae used as feed in aquaculture

Algal species	Field of application
Bacillariophyceae	
Skeletonema costatum	B, B, D
Thalassiosira pseudonana	B, A, D
Phaeodactylum tricornutum	B, A, D, C, F
Chaetoceros affinis, *Chaetoceros calcitrans*, *Chaetoceros muelleri*	B, A, D, F
Cylindrotheca closterium	B
Bellerochea polymorpha	D
Actinocyclus normanii	D
Nitzschia closterium, Nitzschia paleacea	F
Cyclotella nana	F
Amphora ovalis	B
Cocconeis duplex	E
Navicula sp.	A, D, E
Haptophyceae	
Isochrysis affinis galbana, *Isochrysis tahiti*	B, A, D, C, F
Pseudoisochrysis paradoxa	A, D, C
Dicrateria sp.	D
Cricosphaera elongata	D
Coccolithus huxleyi	D
Olisthodiscus luteus	I
Pavlova lutheri, *Pavlova pinguis*	A, D, F, G
Chrysophyceae	
Pyramimonas virginica	A, D
Micromonas pussila	D
Chryptophyceae	
Cryptomonas	D
Rhodomonas salina	A, D
Chroomonas salina	D
Eustigmatophyceae	
Nannochloropsis sp.	I
Xanthophyceae	
Olisthodiscus luteus	D
Cyanophyceae	
Spirulina (Arthrospira) platensis	B, D, F, G
Chlorophyceae	
Tetraselmis suecica	B, A, D, E, F, G
Chlorella sp.	A, C, F, G, I
Scenedesmus obliquus, Scenedesmus quadricauda	I, G, F
Dunaliella tertiolecta	D, F, G
Chlamydomonas khaki	A, D, I, G, I
Chlorococcum sp.	D

2.6. Microalgae in Feeding Trials

Several microalgae types in feeding examination with fish escalate growth (protein accretion), feed application, physiological activity, stress response, hunger resistance, disease tolerance, and quality of carcass (Mustafa and Nakagawa, 1995; Khani et al., 2017a). Partial substitution of fishmeal with *spirulina* powder (% 5, % 10, % 15, and % 20) was examined by AL-Koye (2013). The results indicated that % 15 and % 20 replacements had superior impact on the fingerlings of Carp *C. Carpio* regarding feed utilization, growth, blood parameters. *Chlorella* is one of the most extensively utilized microalgae in aquaculture. It contains elevated concentration of important macronutrients for example vitamins, protein, colorants and unidentified *Chlorella* growth elements (Khani et al., 2017a). Koi (*C. carpio*), is non-invasive and an omnivore fish. Their main feeds are insects, plants, worm, snails, and algae (Khani et al., 2017a). Becker (2013a) compared the general nutritional characteristics chemical composition of diverse algae that regularly utilized in aquaculture.

Protein, selected carotenoids, lipids and significant polyunsaturated fatty acids are drawn the most attention. In relation to limitations and opportunities involved in the algal feed preparation, these composites in different algal classes are compared. Furthermore, it described approaches for preserving microalgae-containing feed preparations. In aquaculture, particular attention is given to the role of *Spirulina* sp., which it the most general cyanobacteria. In the marine food chain, microalgae are at the base and provide the production of renewable fishing resources (Becker, 2013b).

Mixture of various algal species offers superior stable nutrition and enhanced fish growth better compared to a feed constitute of merely single algal species (Sirakov et al., 2015). For microalgae strain to be utilizes in aquaculture must possess some characteristics such as easily cultured, free of toxic, contains high nutritional value with precise cell volume and form and a edible cell wall for making nutrients accessible (Sirakov et al., 2015).

2.7. Phytoplankton

Because of the seasonal periodicity, environmental circumstances and biotic interrelations, accumulations of zooplankton and phytoplankton in natural waters are much easily distinguished (Krzebietke, 2017).

According to Brierley (2017) they are accounted for around half of worldwide yearly primary production and are grazed by zooplankton. This sequentially will become appropriate sized foodstuffs for predators comprising great whales and commercially important fish.

Brierley (2017) stated that the phytoplankton comprises photosynthetic flagellate protists (dinofl agellates) and cyanobacteria along with single-celled plants, containing coccolithophores and diatoms. The single-celled marine and freshwater microalgae and other plant-like organisms are main components of Phytoplankton (Creswell, 2010). Mainly in pharmaceuticals production, diet supplements, colorants, biofuels and aquaculture feeds, Phytoplanktons are utilized. In the primary larval phases of crustaceans, the feed bivalve mollusks (all life phases) and the zooplankton (for example copepods, rotifers) which are utilized as live food in hatcheries of fish, phytoplanktons are cultured (Creswell, 2010).

As the principal producer, phytoplanktons have a critical role in an aquatic ecosystem, for example the basic component in a conventional food web (Krzebietke, 2017). Moreover, for the food web of microbial, flagella-ted algae are crucial as well (Krzebietke, 2017). The assemblages of phytoplankton are distinguished via their high capability for providing several useful biologically active ingredients and phytonutrients for example amino acids, enzymes,fatty acids, sterols, organic carotenoids, chlorophyll, trace elements, minerals, antioxidants and vitamins (Guedes and Malcata 2012; Krzebietke, 2017). Krzebietke (2017) expressed that in a marine ecosystem, a basic conventional food web encompasses phytoplankton as main food for zooplankton. In addition, the zooplankton described as food for planktivorous fish. Consecutively, the planktivorous fish are depicted as food for predatory fish. Similarly, the phytoplankton's nutritional value is conveyed to zooplankton, subsequently to fish and lastly to humans (with fish consumed) (Krzebietke, 2017). The fish larvae of some fish families such as Terapontidae Sparidae, Gobiidae and Clupeidae are mostly herbivorous. The main food in their diet comprises of phytoplankton, other algae and plant-like matter in a percentage of (56.3-82.5 %), (0.9-11.7 %) and (7.3-15.1 %) respectively (Krzebietke, 2017).

Likewise, while a large roach larvae (the family Cyprinidae) (12 mm) have a diet with only 16.3 % phytoplankton of this food sources, smaller larvae with the size of 7-8 mm completely feed on phytoplankton (Krzebietke, 2017). Nonetheless, roach larvae with a body length of about 8 mm can feed on both zooplankton and phytoplankton (Krzebietke, 2017). Rotifers, cladocerans are the types of zooplankton that are imperative for larval fish (water fleas) (Morris and Mischke, 1999).

Via the process of photosynthesis Utilizing carbon dioxide, light as an energy source and nutrients from the water, phytoplankton produces cellular constituents (Creswell, 2010). According to Pal and Choudhury (2014), in water bodies, the foundation of the natural food chain is phytoplankton community. Thus, they support the natural fauna comprising the fish populations. Simultaneously, around 70 percent of the atmospheric oxygen in the world is produced phytoplankton community (Pal and Choudhury, 2014).

In the aquatic environment approximately 45 % of world's primary production takes place (roughly 50 x 10^{15} g carbon yearly). Furthermore, around 25 % of the inorganic carbon is fixed in the ocean every year by planktonic diatoms. Phytoplankton draws CO_2 from the atmosphere by the photosynthesis process (Brierley, 2017). Around 40 % of the entire carbon dioxide, which produced by human activities, has entered the oceans. This caused an increase in the level of the atmospheric carbon dioxide around 200 ppm fewer compared the case anthropogenic effect. Phytoplankton draws CO_2 from the atmosphere by the photosynthesis process (Brierley, 2017).

Phytoplanktons have negative effects even though they are the vital base of the majority of marine ecosystems (Brierley, 2017). Damaging algal blooms, which occasionally named (red tides), is caused by the propagation of several dinoflagellates (for example, *Karenia* spp.) and diatoms (such as *Pseudonitzschia* spp.). Thus, in that case, they negatively impact the quality of water and could be directly poisonous to human swimmers, or cause paralytic shellfish poisoning. In addition, they might also cause ill impacts, occasionally fatal, on farmed fish and wildlife (Brierley, 2017).

Because of the high growth rates, high photosynthetic productivities and no requirement for outside organic carbon source, microalgae (for example, *Dunaliella tertiolecta* - a marine microalga and *Chlorella vulgaris* - a fresh water microalga) are an encouraging feedstock for production of bioenergy and biofuel (Lakaniemi et al., 2011). *Chlorella* is unicellular green algal genus that reproduces at a fast proportion. For more than fifteen years, this genus has been utilized as a source of food and nutritional supplement (Noguchi et al., 2014).

Scientifically, *Chlorella* is classified as the following:

Domain: Eukaryota,

Kingdom: Protista,

Division: Chlorophyta,

Class: Trebouxiophyceae,

Order: Chlorellales,

Family: Chlorellaceae,

Genus: *Chlorella* (Safi et al., 2014).

According to Kwak et al. (2012) *Chlorella* contains numerous beneficial substances such as essential amino acids, protein, dietary fiber, minerals, vitamins, and variety bioactive substances, antioxidants and chlorophylls. Internationally, *Chlorella* has been a prevalent food source, particularly in Taiwan, Korea and Japan (Kwak et al., 2012).

Strains of microalgae such as *galbana* and genera *Tetraselmis, Chlorella, Dunaliella, Haematococcus, Chaetoceros, Skeletonema, Thalossiosira, Navicula, Amphora,* which rich in nutrients, are presently widely farmed for crustacean, bivalve, rotifer or finfish larvae aquaculture (Krzebietke, 2017).

For reducing the present of nutrient content in wastewater treatment, *Chlorella* sp. was categorized as a biological apparatus (Ahmad et al., 2013). Simultaneously, *Chlorella* sp. biomass that is the side products of the handling procedure could be sold as valuable yields (Ahmad et al., 2013). Furthermore, up taking different greenhouse gasses and heavy metals that present in the wastewater are treated by phytoremediation (Ahmad et al., 2013). Thus, it takes part in overcoming environmentally associated issues of local wastewater stated around the globe (Ahmad et al., 2013).

Different pharmacological impacts of *Chlorella* have been articulated in human experiments as well as in animal models (Kwak et al., 2012). In hypercholesterolemic patients who intake *Chlorella*, The level of blood cholesterol was decreased (Kwak et al., 2012). In addition, consuming *Chlorella* resulted in a beneficial impact on hyperlipemia (Kwak et al., 2012). Moreover, blood pressure in mildly hypertensive individuals was decreased by *Chlorella* consumption (Kwak et al., 2012).

Apart from the beneficial effect, *Chlorella* also reduces the level of blood glucose in diabetic animal models, has substantial antioxidant impacts (Kwak et al., 2012). Indeed, the effects of *Chlorella* or *Chlorella* extract in the modulation of immune responses against cancers and bacterial and viral infection are indicated by in vitro and animal studies (Kwak et al., 2012). Nevertheless, in humans, principally in uninfected normal individuals, there were no clear indications on the impact of supplementation of *Chlorella* on immune response (Kwak et al., 2012).

Some species of microalgae *Chlorella* and *Arthrospira* are well-known in the market of the skin care (Priyadarshani and Rath, 2012). Priyadarshani and Rath (2012) stated that extracts of microalgae can be largely found in the products of skin and face care, for example anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers. Besides, sun protection and hair care products also contain microalgae (Priyadarshani and Rath, 2012). Priyadarshani and Rath (2012) indicated that *Mastocarpus stellatus*, *Nannochloropsis oculata*, *Chondrus crispus*, *Alaria esculenta*, *Ascophyllum nodosum*, *Spirulina platensis*, *Dunaliella salina* and *Chlorella vulgaris* are the main types of microalgae.

Ahmad et al. (2013) revealed that the biological treatment that utilizes any types of plants either marine or terrestrial plant is called phytoremediation. Because of the role of phytoremediation in remediating pollution such as heavy metals, high nutrient level and also positive contribution to the environment, this kind of biological treatment has obtained its popularity (Ahmad et al., 2013). Owing to a multiplicity of reactions, microalgae are acknowledged as the major encouraging candidate for bioprocess (Ahmad et al., 2013). Because microalgae are capable of serving a multiple role like bioremediation and producing biomass for production of biofuel with concomitant carbon sequestration, microalgae utilization is desirable (Ahmad et al., 2013).

Additionally, as wastewater remediation by microalgae does not discharge any secondary contamination as long as the produced biomass is uninterruptedly reused and efficient nutrient recycling is maintained, it is considered an eco-friendly procedure (Ahmad et al., 2013). Discharging unprocessed municipal, industrial and agricultural wastewater causes severe ecological encounters to the receiving water bodies (Ahmad et al., 2013).

Ahmad et al. (2013) stated that eutrophication is caused by the impact of discharging wastewater which high amount of organic composites and inorganic chemicals like nitrates and phosphates. Via employing microalgae where the wastewater is used as feed for maintaining the growth of microalgae, the issue of eutrophication could be solved globaly (Ahmad et al., 2013). During the treatment phase, biodiesel feedstock production will be enabled by the gathering of biomass for downstream processing (Ahmad et al., 2013).

Such results have confirmed that microalgae are capability in removing nutrients from wastewater for meeting the stringent necessities consistent with international standards (Ahmad et al., 2013).

Spirulina are the most important blue-green algae in around 30,000 species (Moorhead et al., 2011). In contrast to the cell of other plants that comprise firm cellulose, the cells of *Spirulina* have no nucleus and soft walls are and effortlessly processed (Moorhead et al., 2011). Barka and Blecker (2016) revealed that when microalgae like *Dunaliella*, *Spirulina*, *Scenedesmu* and *Chlorella* processed appropriately, have an attractive taste. Therefore, *Spirulina* is assimilated into several kinds of food. The *Chlorella* algae and *Spirulina* biomass are utilized as nutritional additive are marketed as liquids, tablets and capsules and which (Priyadarshani and Rath, 2012).

2.8. Zooplankton

It contains more than 50 % protein of the dry matter for several groups. In addition, zooplankton is also comprises around 10 % lipids and momentous quantity of unsaturated fatty acids (Anton-Pardo and Adámek, 2015). Thus, in comparison to other diets, natural food might supply a high nutritional content (Anton-Pardo and Adámek, 2015). Even though rotifers inclined to display lower protein content (around 25 % to 50 % of dry matter, some genera such as *Daphnia*, *Moina* and Cyclops contain higher than 50 % protein of the dry weight (Aragao et al., 2004; Conceicao et al., 2010; Jeeja et al., 2011; Yin et al., 2013; Anton-Pardo and Adámek, 2015).

In accordance to Kibria et al. (1997) and Anton-Pardo and Adámek (2015) generally, the percentage of essential amino acids in most investigated rotifer B. plicatilis are marginally less than 50 % of the whole amino acids while this percentage is slightly higher than 50 % in copepods and cladocerans. The amount of lipid is around 10 % of dry matter in zooplankton. It is stated that zooplankton lower amount of saturated fatty acids than unsaturated fatty acids (Kibria et al., 1997; Bogut et al., 2010; Anton-Pardo and Adámek, 2015). Body weight gain and survival rate are improved by the upsurge in the application of live zooplankton as food for fish (Sharma and Chakrabarti, 1999; Anton-Pardo and Adámek, 2015).

Reduced expenses in comparison fishmeal; the rapid growth rate of zooplankton, causing high densities over short periods; and high feeding efficiency are some other advantages of natural food (Kibria et al., 1997; Anton-Pardo and Adámek, 2015).

Some investigators combined live foods and dry diet for rearing cyprinid larvae (Dabrowski et al., 1983).

It is vital to identify the level of nutrients in their natural food organisms for improving artificial diet formulae for fish larvae (Dabrowski and Rusiecki, 1983). Slight information is obtainable on the content of free amino acid in several species of zooplankton even though composition of the amino acid of these organisms has been inspected with respect to their nutritional value to fish (Dabrowski and Rusiecki, 1983). suitability of the natural fish food organisms for all ontogenic stages (larvae, juvenile, adult) of fishes are determined by chemical evaluation of natural fish food organisms (Dabrowski and Rusiecki, 1983).

Copepods and Cladocerans have various peak reproductive times even though they have comparable lifespans of around 50 day (Morris and Mischke, 1999). Furthermore, while cladocerans necessitate 14-15 days to reach the peak reproductive capacity while copepods need 24 days (Allan, 1976; Morris and Mischke, 1999). The two important cladocerans as live food are *Daphnia* and *Moina* (Das et al., 2012). The first one is found in freshwater ponds, tanks and lakes, around the globe. *Daphnia* swims via fast jerks of the two large antennules (Das et al., 2012).

Several of digestive enzymes, for example proteases, cellulase, amylase, peptidases and lipase are obtainable in *Daphnia* (Das et al., 2012). *Daphnia* functions as live food for advanced phases of fishes because it is bigger in volume compared to *Moina* (Das et al., 2012). Furthermore, the predominant inhabitants of temporary ditches or ponds are *Moina* (Das et al., 2012). In comparison to *Daphnia*, *Moina* is smaller in volume (0.5 to 2 mm). Because *Moina* contains 70 % more protein, it is considered as decent substitution for Artemia in aqua hatcheries (Das et al., 2012). In several hatcheries and in the maintenance, *Moina* has also been widely employed as live food. Furthermore, it is commercially important in aquarium fish cultures (Martins et al., 2007; Das et al., 2012).

Allan (1976) stated that the water flea *Daphnia* characterizes the life history of cladocerans which comprises of parthenogenetic and sexual generations (Allan, 1976). In freshwater, sexually and parthenogenetic reproducing the inhabitants of zooplankton are both represented decently (Allan, 1976).

When evaluating the nutritional value based on the net protein utilization (NPU) and protein efficiency ratio (PER) for feeding l-2 g common carp juveniles, the nauplii of *Artemia salina* and rotifers, *Bruchionus plicatilis* is inferior to casein (Dabrowski and Rusiecki, 1983). In addition, casein is incapable to assist growth and survival of carp larvae, when utilized as a main dietary ingredient. Nonetheless, for stomachless carp larvaec both *Artemia* nauplii and rotifers are outstanding foods (Dabrowski and Rusiecki, 1983).

The nutritional necessities of fish larvae are provided by utilizing live, frozen or freeze-dried plankton as initial food, either alone or in mixture with artificial feeds (Jungwirth et al., 1989; Verga and Böhm, 1992). Modest results were obtained when frozen and freeze-dried zooplanktons have been utilized as experimental food for fish larvae (Verga and Böhm, 1992). The frozen and freeze-dried zooplanktons have regularly confirmed to be inappropriate to rear fish larvae because the enzyme activity and free amino acids (FAA) of frozen and freeze-dried zooplankton leached rapidly when introduced into water (Verga and Böhm, 1992). Grabner et al. (1981) revealed the rate of leaching reduced considerably by pelleting freeze-dried plankton.

The ineffectiveness of some plankton organisms (such as *Daphnia sp*. with 7.5 % protein of wet weight) are demonstrated when they are utilized as exclusive feed in the intensive propagation of fingerlings (Jungwirth et al., 1989). Therefore, the future alternative, for rearing Danube salmon and other species that are hard or impossible to raise initial phases of their development, is the mass propagation approach utilizing dry diet with supplementary freeze-dried zooplankton (Jungwirth et al., 1989).

the alterations happening in the plankton subsequent to freezing, drying and storing and introducing the treated material into water must be examined for understanding the unsuitability of frozen (or freeze-dried) plankton as a food for fish larvae (Grabner et al., 1981).

Kar et al. (2017) indicated that the fingerlings of various fish species are reared with the live or processed zooplankton for achieving wanted growth in commercial aquaculture. According to Kar et al. (2017) zooplankton, as a live food, is vital for the growth and survival of the fish larvae because it contains high quality protein and lipid. The zooplankton culture is being stimulated regardless of the fish species, as the fish larvae obtains the complete nutritional requirements or at least in major part from the zooplankton food resources (Kar et al., 2017). In the raising of the larval fish, although the utilization of cladocerans and calanoid copepods are observed in some instances, the *Artemia* and rotifers are the most commonly utilized (Kar et al., 2017). Though, viability of the culture has to be taken into consideration before promoting the zooplankton species as live food for the fish culture (Kar et al., 2017).

For the purpose of increasing the nutritional quality with improved protein and lipid content that might simplify the growth of the fish larvae the culture of the zooplankton is conducted (Kar et al., 2017). The preferred concentration of nutrient can be manipulated in the concerned species promoting the growth of the fish species because of the advantage of incorporating the precursor molecules via the zooplankton food (Kar et al., 2017). The zooplankton culture is achieved in wastewater with slight or no contaminant accumulation from the ambient environment (Kar et al., 2017).

In aquatic poisonousness testing, water fleas have long being utilized because of the following reasons: they are easily handled, they spend their complete life in water, they are prolific, they have a moderately short lifespan, and they are imperative members of marine food chains (Tatarazako and Oda, 2007; Siciliano et al., 2015). In addition, water fleas are known to be quite sensitive to many chemicals, including heavy metals (Tatarazako and Oda, 2007).

The body weight of initial carp larvae at a particular growing stage depends on diet (Sharma and Chakrabarti, 1999). Sharma and Chakrabarti (1999) reported that larvae of Carp *C. carpio* fed formulated feeds are less advanced morphologically compared to those fed zooplankton at the same age. In addition, Sharma and Chakrabarti (1999) discovered that the mean body weight of common carp and the plankton intake are directly related.

Greater profusion of plankton food and enhanced quality of water resulted in the highest growth and production of the larvae of common carp (Sharma and Chakrabarti, 1999). According to Bogut et al. (2010), the ideal diet for carp juveniles is natural food. Planktonic rotifers, copepods and cladocerans are among the freshwater zooplankton that widely utilized in juvenile carp feeding (Bogut et al., 2010).

A single big compound eye and two pairs of extremely branched antennae are the most noticeable *Daphnia's* outer features (Tatarazako and Oda, 2007). The antennae are utilized for movement (Tatarazako and Oda, 2007). An apparent shell protects thorax and abdomen. A number of pairs of legs with setae (hairs) are evident via the shell (Tatarazako and Oda, 2007). For directing bacteria, protozoa, organic detritus and algae to the mouth, water current is created by the moving legs (Tatarazako and Oda, 2007).

Tatarazako and Oda (2007) and Siciliano et al. (2015) expressed that *Daphnia* spp. are tiny planktonic crustaceans (1-5 mm long) which usually named water fleas. In addition, they are suborder of Cladocera that are omnipresent in aquatic environments of freshwater. *Daphnia* is either sexual or clonally reproduced (Siciliano et al., 2015). Siciliano et al (2015) demonstrated that, water flea reproduces via parthenogenesis under satisfactory environments. In this case, females of *Daphnia* produce clonal offspring asexually. However, when the environmental conditions are not favorable a number of females produce haploid eggs that require fertilization by males (Siciliano et al., 2015). The fertilized eggs surrounded by a number of protecting films (the ephippium) and stay in a dormant state. Thus, they are able to survive severe conditions for tens of years before hatching (Siciliano et al., 2015).

Since the beginning of 1900's, one of the most broadly selected marine invertebrates for ecotoxicology is Cladoceran daphnids (Siciliano et al., 2015). Because the fact that *Daphnia*, primarily *D.magna* and *D.pulex* are regarded as high sensitivity analytical apparatuses to screen poisonousness of common environmental chemicals and observing of effluents and polluted waters, the widespread studies utilized *Daphnia* , primarily *D.magna* and *D.pulex* as bio-indicators in severe poisonousness (Siciliano et al., 2015). Furthermore, in the safety assessment of chemical in Japan, in accordance to the modified Chemical Substances Control Law, the *D. magna* are utilized in acute and chronic toxicity tests (National Institute of Technology and Evaluation in Japan 2004) (Tatarazako and Oda, 2007).

The chemical compositions of zooplankton, the development of zooplankton-based dry diets and the impact of the fish meal substitution with zooplankton meal for commercial aquaculture species required further investigations (Kibria et al., 1997).

3. MATERIAL AND METHODS

3.1. Materials

3.1.1. Experimental animal

The experiment was conducted for 12 weeks (84 days) on 126 young common carp *C. carpio* L. (Figure 3. 1) which were brought from a local aquarium fish supplier located in Daqoq Middle of Iraq. The weight of fish varied between (25.07-37.21 g). The fish were distributed among experimental aquaria with mean initial weight 28.58 g. They were pre-acclimated to laboratory conditions and fed with commercial pellets (their chemical composition and Percentage of ingredients are shown in Table 3. 1, Table 3. 2) for 21 days prior to the feeding trials.

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

Ingredients	Crude Protein %	Crude Fat %	Dry Matter %	Crude Fiber %	Energy Kcal/ 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
Barely	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	28.06
Metabolizable energy (kcal/kg feed)	2242.7
%Arginine	0.2394
%Lysine	0.25375
%Methionine + cysteine	0.12872
%Threonine	0.017
%Tryptophan	0.029

3.1.2. Experimental system

Twenty-one plastic tanks (100 L) were used in this trial representing seven treatments with 3 replicates each, water quality shown in (Table 3.3). Each tank was provided with a proper continuous aeration by Chinese's air compressors, Hailea ACO-318 (power: 45 watt, air flow: 70L /min), Hailea ACO-328 (power: 55 watt, air flow: 82L /min), Resun ACO-010 (power: 200 watt, air flow: 0.135 m^3 /min) and eleven small aquarium air pumps, Luckiness 828 (power: 5 watt, air flow: 3.5L /min). Each tank was stocked with six fish. The tanks (replicates) were randomly allocated to minimize differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the tank. In addition, a daily cleaning by pumping method was applied to remove remained particles from the system as shown in (Figure 3. 1).

The experimental trial would include seven treatments with three replicates; each contains six fish per tank as follows:

T1: Commercial diet without any supplement,

T2: adding 25 g *Chlorella* powder /kg diet,

T3: adding 50 g *Chlorella* powder /kg diet,

T4: adding 25 g Freeze dried *Daphnia* /kg diet,

T5: adding 50 g Freeze dried *Daphnia* /kg diet, T6: adding 25 g *Chlorella* powder + 25 g Freeze dried *Daphnia* /kg diet,

T7: adding 50 g *Chlorella* powder + 50 g Freeze dried *Daphnia* /kg diet.

Table 3.3. Water quality parameters in the experimental tanks

Parameters	Values
Temperature (°C)	28±1
Dissolved Oxygen (mg/L)	4±1
Hardness (ppm)	330±10
pH	7±0.5
Total Dissolved Solids (TDS) (ppm)	230±10

Figure 3.1. Experimental, system, tank and fish.

In T1 fish were fed a diet (commercial diet) without adding any amount of *Chlorella* and *Daphnia* , while in T2, fish were fed a diet with 25 g *Chlorella* powder /kg diet, T3 represents the third treatment, in which fish were fed on a diet 50 g *Chlorella* powder/ kg diet, while, in T4 fish were fed a diet 25 g freeze-dried *Daphnia*/ kg diet, and T5 50 g freeze-dried *Daphnia*/ kg diet, T6 was 25 g *Chlorella* powder + 25 g freeze-dried *Daphnia*/ kg diet, the T7 50 g *Chlorella* powder + 50 g freeze-dried *Daphnia*/ kg diet.

3.1.3. Diet formulation

Experimental diets composed of a standard commercial diet type found in Sulaimani city markets in north region of Iraq, enriched with *Chlorella* powder, freeze-dried *Daphnia* and their combination, the chemical composition of the different diets are shown in (Table 3.4). 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, as shown in (Figure 3.2). The pellets were dried at room temperature for a few days and crushed to yield fine particles. Fish were fed twice a day at 9:00 a.m. and 2:00 p.m. with a ratio of 3 % of body weight. Fish in every tank were weighed together every two weeks. The feeding amount was then recalculated according to new weights. The feeding trial continued for 12 weeks.

Table 3.4. Chemical composition of all seven experimental diets

Treatment	Moisture %	Protein %	Fat %	Ash %
T1 (control commercial diet without any supplement)	4.39	28.06	2.48	4.11
T2 (25g *Chlorella*/ kg diet)	4.17	28.23	2.89	4.34
T3 (50g *Chlorella*/ kg diet)	4.17	28.66	3.28	4.56
T4 (25g *daphnia*/ kg diet)	4.15	28.17	2.78	4.73
T5 (50g *daphnia*/ kg diet)	4.72	28.38	2.97	4.98
T6 (25g *Chlorella* + 25g *daphnia*/ kg diet)	4.55	28.89	3.18	4.78
T7 (50g *Chlorella* + 50g *daphnia*/ kg diet)	4.37	29.15	3.59	5.31

Treatments: 1 2 3 4 5 6 7

Figure 3.2. Feed maker machine and all seven feed types.

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 3.5).

Table 3.5. Nutritional information of used *Chlorella*

component	Per 100 g
Energy	418 Kcal
Protein	55 g
Fat	15 g
Carbohydrate	19.5 g
Fiber	12.5 g
Salt	0.1 g

Freeze dried *Daphnia* were natural dried *Daphnia* packed by DLS fish food brand in UK, DLS Aquatics and Pets, 369 Osmaston park road, Derby, DE24 8DB, The nutritional information as labelled has shown in (Table 3.6).

Table 3.6. Nutritional information of used *Daphnia*

Component	Percentage (%)
Crude protein	48 %
Crude fat	9 %
Crude fiber	7 %
Ash	18 %
Humidity	5 %

The photos of *Chlorella* powder and freeze dried *Daphnia* were added to diets shown in (Figure 3.3)

Figure 3.3. Used *Chlorella* and *Daphnia*.

3.2. Methods

3.2.1. Experimental design

In this study six fields of characteristics were studied: growth performance, feed utilization, blood parameters, biological parameters, Carcass composition and Organoleptic (Sensory) characteristics. The Experimental Design showed in (Figure 3.4).

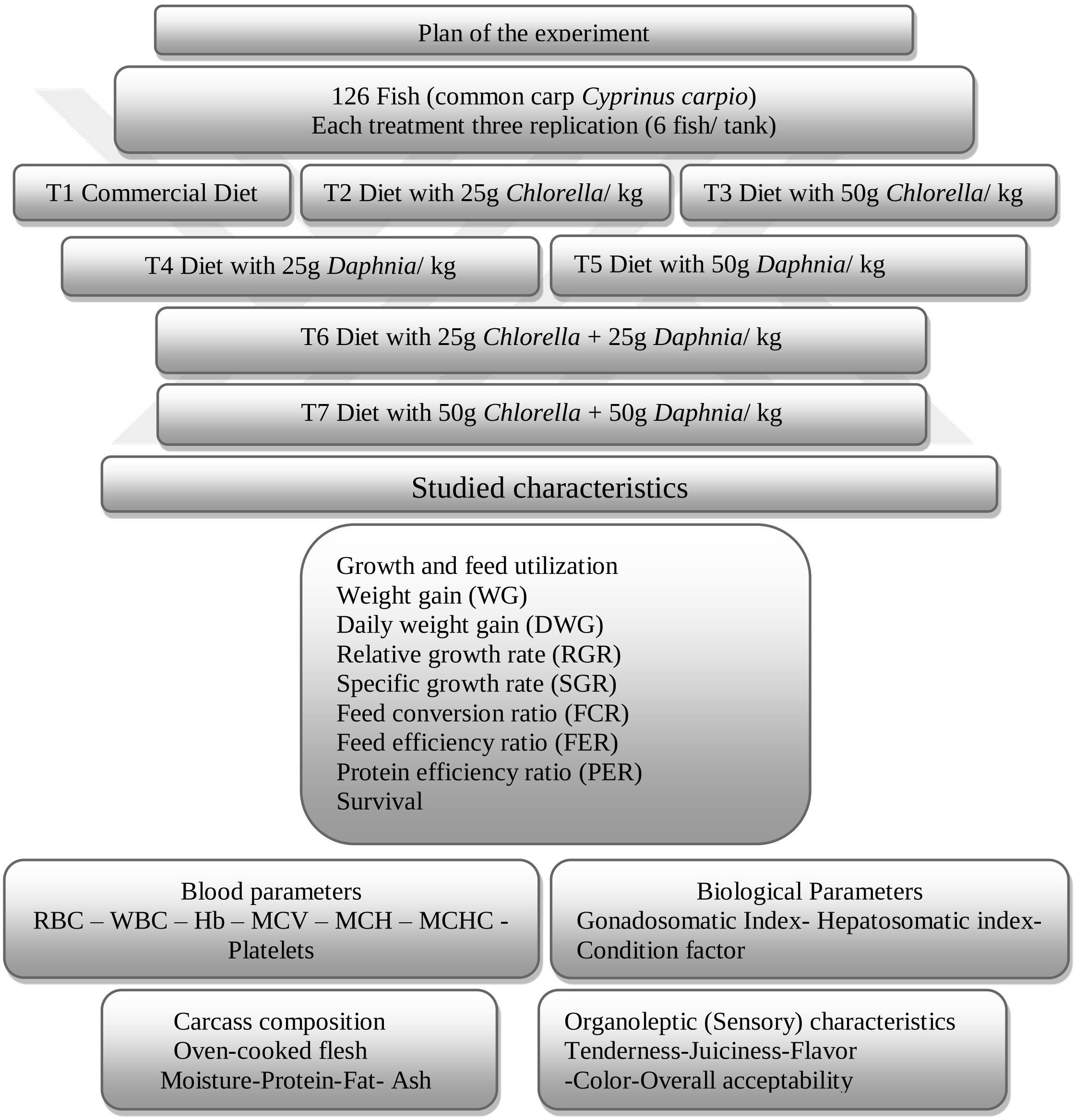


Figure 3.4. Experimental design of the study.

3.2.2. Growth and feed utilization parameters

For calculating these parameters, fish were together weighed (g) for all treatments once two weeks. Feed consumption of each treatment was recorded and readjusted according to the obtained biomass at every two weeks.

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).

Weight gain (g/fish) = W2 – W1 (3.1)

Where:

W_2: Fish weight (g) at the end of experimental period

W_1: Fish weight (g) at the beginning of the experimental period

Daily weight gain (DWG) (g/day) = Weight Gain / experimental period

= W2 – W_1 / T (Schmalhusen, 1926). (3.2)

T: time between W2 and W1 (84 days)

Relative growth rate (RGR %) = Weight Gain / Initial weight x 100

= W2 – W_1 / W_1 x 100 (Brown, 1957). (3.3)

Specific growth rate (SGR) = ([ln final body weight–ln initial body weight] /experimental period) X100

= ((In W2 – In W1) / T) x 100 (Lagler, 1956). (3.4)

Survival rate (%) =No. of fish at the end / No. of fish at the beginning x 100. (3.5)

Feed conversion ratio (FCR) =Total feed fed (g) / total wet weight gain (g) (Uten, 1978). (3.6)

Feed efficiency ratio (FER) = Total weight gain (g) / Total feed fed (g) (Uten, 1978). (3.7)

Protein efficiency ratio (PER) = Total wet weight gain (g/fish) / amount of protein fed (g/fish) (Uten, 1978). (3.8)

3.2.3. Blood parameters

At the end of the experimental period, three fish were randomly taken from each experimental group. All fish samples were weighed and measured their length individually. The blood samples from each fish of the different groups were collected by catting of the caudal vein. Whole blood samples were collected in small plastic vials containing heparin and stored under cooling condition (AL-Koye, 2013), all CBC test (complete blood count) were determined by using the Spincell 3 Automatic Hematology Analyzer, Spain origin and prior to analysis for determination of:

Red Blood Cells (RBCs: 10^{12}cells/L), Mean Corpuscular Hemoglobin (MCH; pg), Mean Corpuscular Hemoglobin Concentration (MCHC; g/dL), Mean Corpuscular Volume (MCV; fL), Hemoglobin (Hb; g/dL) and Platelet (PLT; 10^{9}cells/L).

Note: Results of the White Blood Cells (WBCs) weren't shown because they weren't appeared in the analysis.

3.2.4. Biological parameters

After blood samples collection, all the fish samples were scarified and soon the abdominal cavity was opened to remove gonads and liver to be weighed at once. The gonad, liver indices and Condition factor was calculated using the cube law, and were calculated as follows (Lagler, 1956).

Condition factor = Fish weight/ total length^3 (Lagler, 1956). (3.9)

Gonadosomatic index (GSI) % = Gonads weight (g)/ Body weight (g) x 100 (Lagler, 1956). (3.10)

Hepatosomatic index (HSI) % = liver weight (g) / body weight (g) x 100 (Lagler, 1956). (3.11)

3.2.5. Carcass characteristics

Whole fish samples were taken after the feeding experiment and dried (oven-cooked flesh) for further chemical analysis. Analysis methods followed the standard methods outlined by AOAC (2000) for proximate composition of moisture, protein, fat and ash.

3.2.6. Organoleptic (sensory) evaluation

Sensory analyses were performed by a panel of seven experienced assessors. The fish meat fillets specimens were placed in open aluminum boxes and cooked for 40 min in an oven pre-heated at 100°C, after cooking seven teaching staff of the Department of Animal Science- College of Agricultural sciences /University of Sulaimani were randomly determined as a sensory evaluation panel (AOAC, 2000). Each member of the panel has filled a sensory evaluation, as shown in (Table 3.7).

Table 3.7. Sensory evaluation form used for organoleptic evaluation

Treatments	Color	Flavor	Tenderness	Juiciness	Overall acceptability
T1					
T2					
T3					
T4					
T5					
T6					
T7					

5 =extremely like; 4 = like; 3 = neither like nor dislike; 2 = dislike; 1 = extremely dislike

3.2.7. Statistical analysis

The experimental was conducted using One Way ANOVA by completely randomized design (CRD) and general linear models (GLM) procedure of XLSTAT 2016 Version.02.28451. Duncan's test was used to compare between treatments mean of the experiment (Duncan, 1955). The model of analysis was as follows:

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

i = (1, 2, 3, 4, 5, 6, 7…..treatment)

j = (1, 2, 3….replication)

μ = the overall mean.

T_i = the effect of treatment (i).

E_{ij}= the random error.

4. RESULTS AND DISCUSSION

4.1. Growth Performance

The present study demonstrated that the inclusion of *Chlorella* and *Daphnia* to fish diet change the growth performance in which it increase the final weight gain, daily weight gain, relative growth rate and specific growth rate as shown in (Table 4.1). No there was no significant variations monitored in initial weights, the final weight gain in T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg and T3 diet with 50 g *Chlorella* /kg, were 238.633 g and 232.570 g respectively, which where differ significantly (P≤ 0.05) than other treatments.

Table 4.1. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on growth performance for common carp (*Cyprinus carpio*)

Treatments	Initial weight (g)	Final weight gain (g)	Daily weight gain (g/day)	Relative growth rate (g/ day%)	Specific growth rate/day	Survival %
T1	169.863 a ± 5.7	155.910 d ± 6.79	1.857 d ± 0.08	91.967 e ± 4.93	0.333 d ± 0.01	100.000 a ± 0.00
T2	173.777 a ± 4.51	175.487 cd ± 11.29	2.090 cd ± 0.13	100.907 de ± 5.34	0.360 cd ± 0.02	100.000 a ± 0.00
T3	172.977 a ± 3.98	232.570 a ± 12.33	2.770 a ± 0.15	134.280 ab ± 4.26	0.437 ab ± 0.01	100.000 a ± 0.00
T4	170.597 a ± 3.16	192.437 bc ± 1.61	2.290 bc ± 0.02	112.907 cd ± 2.85	0.393 bc ± 0.01	100.000 a ± 0.00
T5	172.777 a ± 4.45	199.410 bc ± 14.82	2.373 bc ± 0.17	115.980 bcd ± 11.21	0.397 bc ± 0.03	100.000 a ± 0.00
T6	170.023 a ± 3.1	219.087 ab ± 6.07	2.607 ab ± 0.07	129.053 abc ± 5.71	0.430 ab ± 0.01	100.000 a ± 0.00
T7	170.527 a ± 2.23	238.633 a ± 10.36	2.840 a ± 0.12	140.033 a ± 6.89	0.453 a ± 0.01	100.000 a ± 0.00

Mean values with different superscripts within a column differ significantly (P≤0.05).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg.

In daily weight gain T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg and T3 diet with 50 g *Chlorella* /kg were higher significantly (P≤ 0.05) other treatments, the relative growth rate in T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg was growth better significantly than other treatments while the specific growth rate in T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg. Survivals for all treatments in the study were 100 %.

The results in present study agree with those showed by Abdulrahman (2014a) with adding 5 g/ kg diet *Spirulina* spp. powder to common carp (*C. carpio*) fingerlings feed for 42 days, weight gain, specific growth rate, daily growth rate, relative growth rate significantly higher (P<0.05) than other treatments where fish fed with (0, 1, 3 g *Spirulina* spp. /kg diet). Badwy et al. (2008) the presence of *chlorella* spp. powder in diets of Nile tilapia (*Oreochromis niloticus*) fingerlings significantly (P<0.05) increased fingerlings' growth performance in terms of; final body weight, body weight gain, daily weight gain, relative weight gain and rate of specific growth, where fish meal replaced with alga meal (*chlorella* powder) in four different percentages (10 %, 25 %, 50 % and 75 %). However, the highest values observed with 50 % replacement. Similarly inclusion of *Scenedesmus* spp. microalga in Nile tilapia (*O. niloticus*) diets improved performance of growth, while fishfeed diets comprising seventy five percent algae had smaller significant performances (P < 0.05). Those results are similar with the results of present study in fish gain weight.

Zeinhom (2004) showed that the addition of algae in diets of fish augmented the live body weight (39.69 g), body weight gain (26.46 g), daily weight gain (0.29 g) and specific growth ratio (1.22) significantly, Nandeesha et al. (1998) reported that increasing the gain of body weight of Nile tilapia (*O. niloticus*) linearly depend on rising the concentration of algae in diet of fish at concentration less than 20 percent. Abid **(**2018) with adding 7.5 g /kg diet *Chlorella* powder in common carp (*C. carpio*) fingerlings feed for 105 days, weight gain (35.070 g), daily growth rate (0.418 g), relative growth rate (88.878), specific growth rate (0.423) significantly higher (P<0.05) than other treatments where fish fed with (0, 2.5, 5 g *Chlorella* spp. /kg diet). According to Abu Zead (2001) supplementing fish diets with water primrose, water hyacinth and algae enhanced performance of growth of both Common carp and Nile tilapia. Our findings agreed partially with those Ibrahim (2001) and Dawah et al. (2002 b) as they revealed that the growth performance of Nile tilapia (*O. niloticus*) is improved by the addition of algae in fish diets.

These results in disagreement with some findings, when fish fed on 2 % *Chlorella* supplemented diets such as, red sea bream (Mustafa et al., 1994), rainbow trout (Sommer et al., 1992), and Japanese flounder (Xu et al., 1993). Xu et al. (2014) stated that the growth parameters was amplified significantly when Gibel carps *Carassius auratus gibelio* fed *Chlorella* combined diets at the presence of up to 1.2 percent. Sergejevová and Masojídek (2012) found that, 2.5 % *Chlorella* inclusion in freshwater starlet *Acipenser ruthenus* L. diets showed positive impact upon skin coloration and growth.

The findings of present research partially agreed with the findings of Navarro and Sarasquete (1998) who reported that there were significant differences (P<0.05) when the algae 20 %, aquatic plant 25 % and yeast 10 % in fish diets.

Also, Olvera-Novoa et al. (1998) expressed that the supplimentation of microalgae *Spirulina* in fish diets for Tilapia *mossambicus* less than 30 and 50 % correspondingly, did not cuase significant differences (P<0.05) in daily body weight gain.

Zhang et al. (2014) exhibited that the growth of Gibel carp can be increased significantly by the addition of *Chlorella*. For example, the body weight gain augmented from 29.90 ± 0.08 to 63.75 ± 1.96 g when fish fed with 0.8 % *Chlorella* which was greater compared to that of control group (P<0.05).

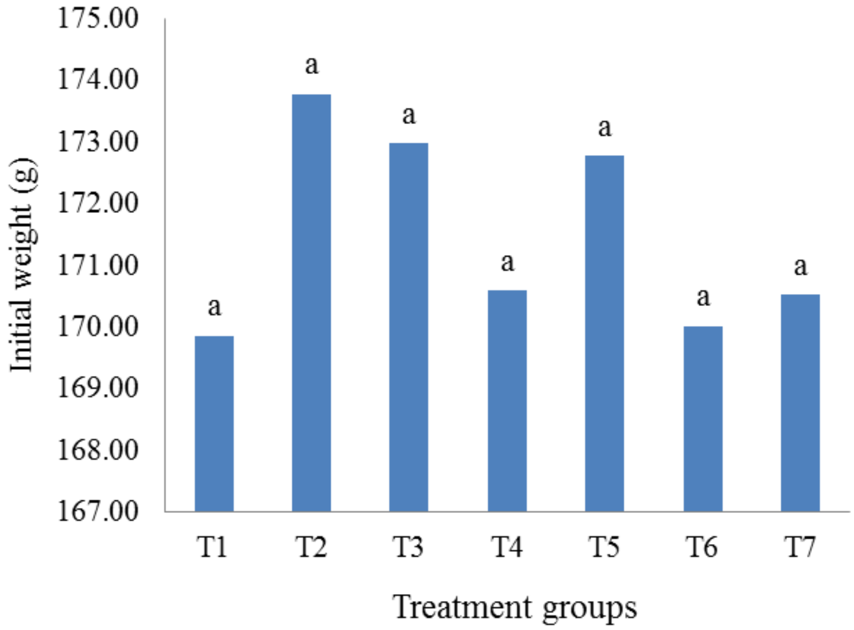
A comparable growth performance in koi fish fed *Chlorella* diets resulted from the presence of the growth promoters, like adequate quantities of macronutrients and naturally occurring bioactive ingredients (*Chlorella* growth factor (CGF)) that are present in *C. vulgaris* (Yamaguchi, 1997; Badwy et al., 2008; Khani et al., 2017a, b). Besides, high digestibility of the microalgae is apparent on growth improvement (Anderson et al., 1979).

In both dry and fresh *Daphnia magna* mass, the content of protein related to 1.18 and 39.24 %, correspondingly, with regard to the content of protein, *Daphnia magna* is an ideal feed for carp and other omnivorous fishes, also Concerning the crude fat content in both fresh and dry *Daphnia magna* mass 0.15 and 4.98 %, respectively, therefore *Daphnia magna* complies the necessities of adults of carp nevertheless carp juveniles and fry necessitate greater amount (around 8 %), the level of omega–3 fatty acids, which is necessary for the carp, the proportion between omega–3 and omega–6 fatty acids (5.68:1), in addition to the proportion between saturated and unsaturated fatty acids (3.54:1) in the lipids of *Daphnia magna* body totally comply the feeding necessity of all growth categories of carp (Bogut et al., 2010). Except partial deficit of phenylalanine, all essential amino acids are existence in suitable amounts for the nutrition of all growth classes of carp and other omnivorous fishes (Bogut et al., 2010).

Analysis of Grabner et al. (1981) showed that freezing of freeze-drying and by storage at -18 °C even for very long times, the activities of proteases and of enzymes of intermediary metabolism, of zooplankton are not declined. Therefore, for hatchlings of white-fish, carp, lake char fish which usually necessitate live plankton for growth, frozen or freeze-dried plankton could be an outstanding food source.

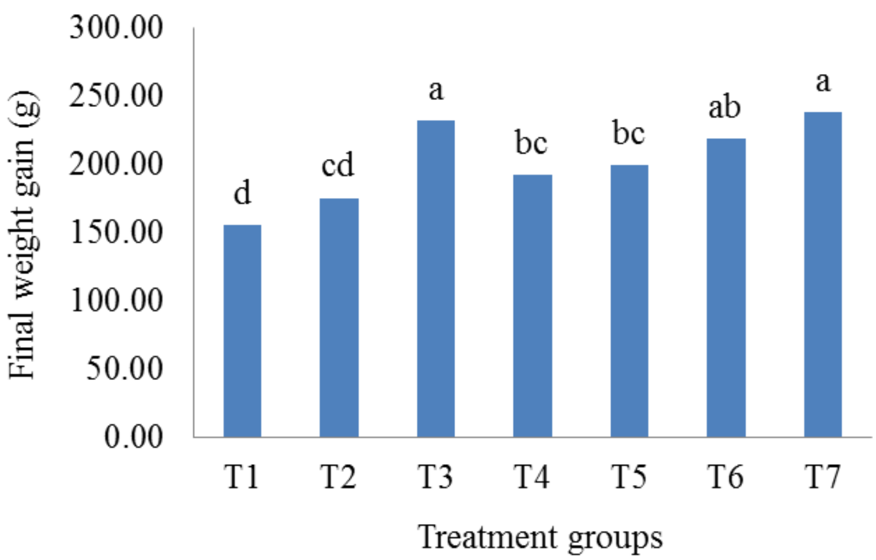
Freeze-dried zooplankton supplementation by (8 %) to salmon starter food and arctic char starter food for danube salmon *(Haxho hucho* L.) fry immensely enhanced the circumstance of the fish in comparison to those fed onely on salmon starter food and arctic char starter food, survival and growth rate was increased significantly with addition of (8 %) of freeze-dried *Artemia cysts* to salmon starter food for danube salmon *(Haxho hucho* L.) fry feed and the coefficient of weight variance was reduced, mortality was not increased significantly throughout the week 2 among fry fed diets salmon starter food with (8 %) of freeze-dried zooplankton (copepods and cladocerans, ratio 1:6) and arctic char starter food with (8 %) of freeze-dried zooplankton (copepods and cladocerans, ratio 1:6), and during the 4^{th}-5^{th} week among fry fed diet salmon starter food with (8 %) of freeze-dried zooplankton (copepods and cladocerans, ratio 1:6) (Verga and Böhm, 1992).

With regard to combination of *Chlorella* powder and freeze dried *Daphnia* in both T6 diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg and T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg, and because of the lack of references or previous researches on these combination in fish feeds and their effects on growth performance no comparison of our results with previous reviews done.



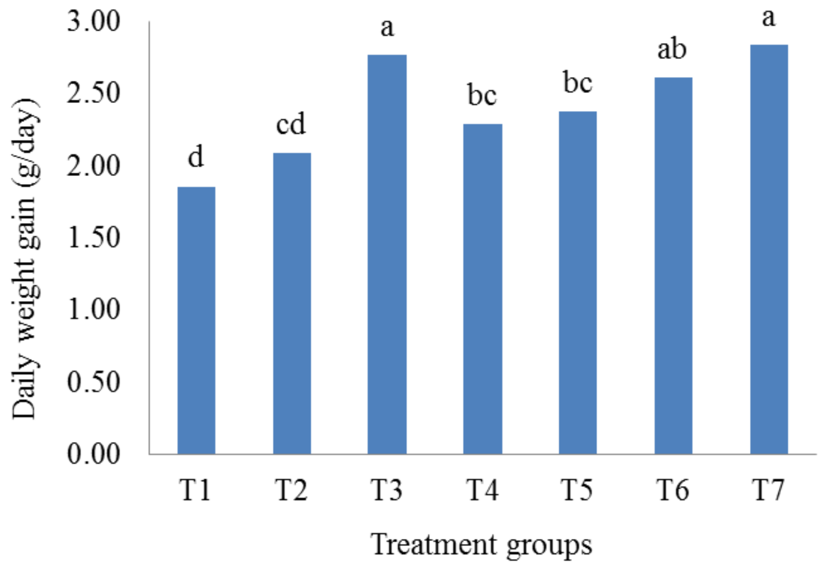
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.1. Initial weights of the experimental fish.



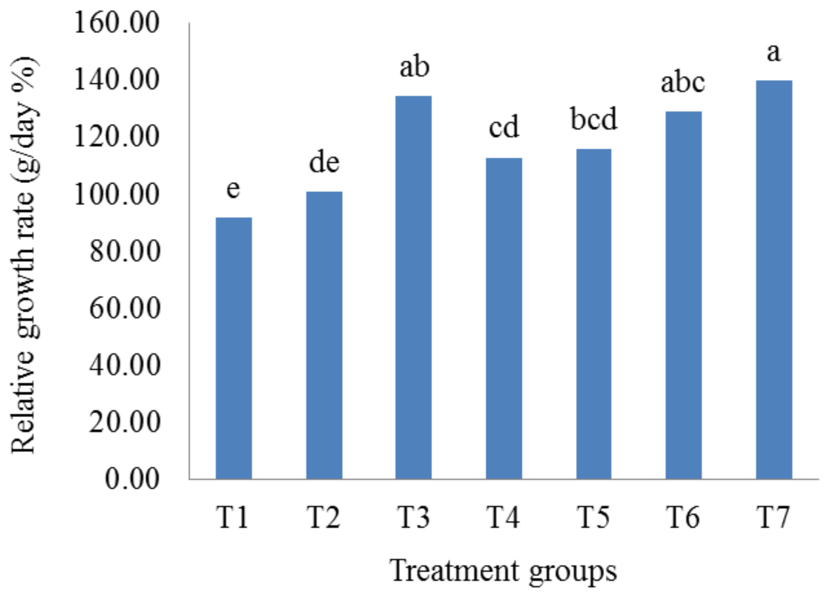
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.2. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on final weight gain for Common Carp (*Cyprinus carpio*).



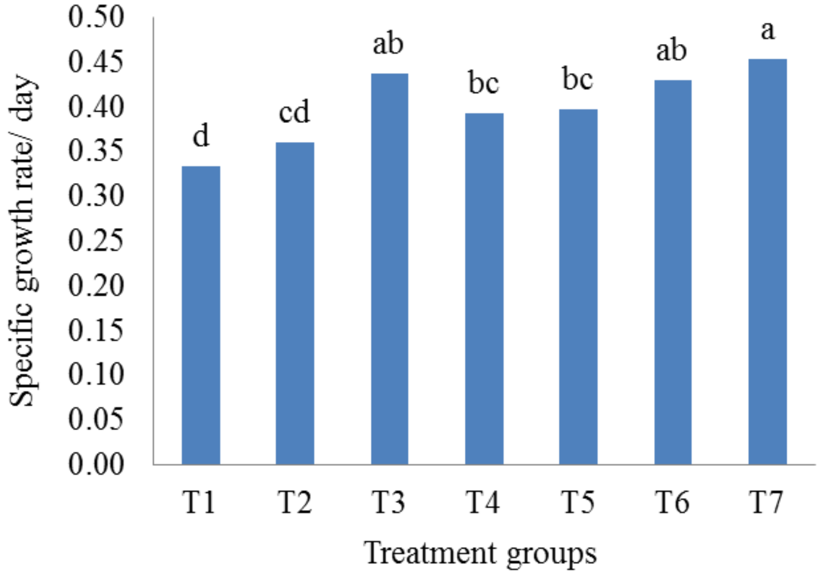
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.3. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on daily growth rate for Common Carp (*Cyprinus carpio*).



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

Figure 4.4. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on relative growth rate for Common Carp (*Cyprinus carpio*).



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

Figure 4.5. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on specific growth rate for Common Carp (*Cyprinus carpio*).

4.2. Feed Utilization

The findings in (Table 4.2) displays that the no adding any amount of *Chlorella* and *Daphnia* in T1 diet affect ratio of feed conversion in which T1 had differed significantly (P≤0.05) compared to other treatments. Combination of 50 g *Chlorella* and 50 g *Daphnia*/ kg affect the ratio of protein and feed efficiency, T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg in the ratio of protein and feed efficiency were higher significantly when paralleled to other treatments.

Badwy et al. (2008) where they showed that lower significant value of feed intake that obtained by Nile tilapia (*O. niloticus*) fingerlings fed diet with replacing fishmeal with 50 % *Chlorella* spp. meal. However, the average values of feed conversion ratio and Protein efficiency ratio significantly increased (P<0.05) with rising of (*Chlorella* spp.) meal substitution from 0 to 50 %. Similar results recorded when replaced fishmeal in diets with 50 % *Scenedesmus* spp., while diets of fish comprising 75 percent algae had significantly lower performance (P < 0.05).

Dawah et al. (2002a) discovered that, the ratio of Protein and feed efficiency superior as the fish were fed on artificial diets with 10 % and 20 % dried algae.

Table 4.2. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on utilization of feed for common carp (*Cyprinus carpio*)

Treatments	Feed conversion ratio	Feed efficiency ratio	Protein efficiency ratio
T1	3.581 a ± 0.15	0.280 d ± 0.01	5.557 e ± 0.24
T2	3.305 ab ± 0.2	0.305 cd ± 0.02	6.217 de ± 0.4
T3	2.618 c ± 0.05	0.382 ab ± 0.01	8.115 ab ± 0.43
T4	3.013 bc ± 0.04	0.332 bcd ± 0	6.831 cd ± 0.06
T5	2.952 bc ± 0.27	0.344 bc ± 0.03	7.027 bcd ± 0.52
T6	2.740 c ± 0.11	0.366 ab ± 0.01	7.584 abc ± 0.21
T7	2.523 c ± 0.1	0.398 a ± 0.02	8.186 a ± 0.36

Mean values with different superscripts within a column differ significantly (P≤0.05).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg.

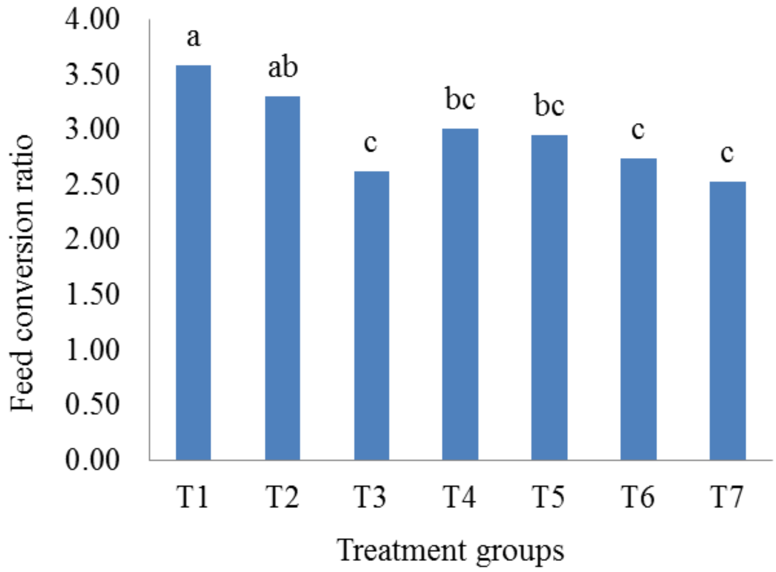
Abid (2018) food conversion ratio (0.633) higher significantly (P≤ 0.05) with adding 2.5 g /kg diet *Chlorella* spp. powder to common carp (*C. carpio*) fingerlings feed for 105 days compared to other treatments where fish fed diet (0, 5, 7.5 g/ kg diet) of *Chlorella* spp., food Efficiency ratio (2.406) and Protein Efficiency ratio (829.301) in group fed (7.5 g /kg diet) of Chlorella spp. higher significantly than other treatments. when grass carp fed on *spirogyra* sp. *Tilapia aurea* and big mouth buffalo *Ictiobus cyprinellus* fed on *Spirulina* and *spirogyra*, Stanley and Jones (1976) noted poor growth and food conversion ratio for displayed high rate of growth of 29 g dry weight/ kg for four weeks, 14 g/ kg body weight correspondingly; food conversion ratio was 2.0, 10, correspondingly.

Our results were approved by Ibrahim (2001) and Abu Zead (2001) who discovered that when Nile tilapia and common carp fed on diets comprising aquatic plant and algae, the ratio of protein efficiency varied between 1.1 and 1.7. However, Zeinhom (2004) discovered that, addition of algae in fish diets insignificantly (P<0.05) enhanced the feed conversion ratio (2.33) and protein efficiency ratio (1.34) while feed consumption was increased significantly. The results in present study agree with those showed by Abdulrahman (2014a) food efficiency ratio higher significantly (P≤ 0.05) with adding 5 g and 3 g *Spirulina* spp. powder to common carp (*C. carpio*) fingerlings feed for 42 days compared to control group where fish fed diet without any amount of *Spirulina* spp., and Food conversion ratio in control group higher significantly than other treatments. Mustafa and Nakagawa (1995) declared that the addition of micro and macro algae meal improve growth, the utilization of feed, metabolism of lipid, body composition, disease resistance and quality of carcass of a diversity of fishes.

Trout showed better growth and feed efficiency when fed on Antarctic krill meal than fish meal; also the protein efficiency ratio was better with the krill meal (Kibria et al., 1997). Hossain and Jauncey (1990) suggested the mixture of different sources of protein compared to the use a single protein source in substituting fish meal in carp diet because it was more effective.

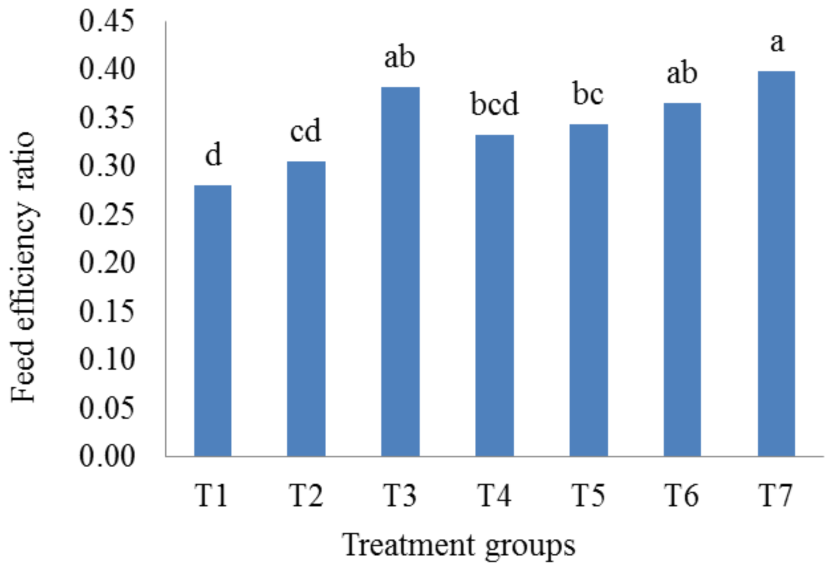
Some aspects have also been stated to take part to the monitored differences in the fishes' growth responses. Hasan and Macintosh (1992) unveiled that the performance of growth of common carp (*Cyprinus carpio*, Cyprinidae) differed with the difference in the diets acceptability. It could be because of the attractive color, racy flavor and decent composition of the nutrient of the experimental feeds (Mukherjee et al., 2011).

With regard to use of freeze dried *Daphnia* and combination of *Chlorella* powder with freeze dried *daphnia* in fish diets, because of the lack of references or previous researches on these combinations in fish diets and their effects on feed utilization no comparison done with other results.



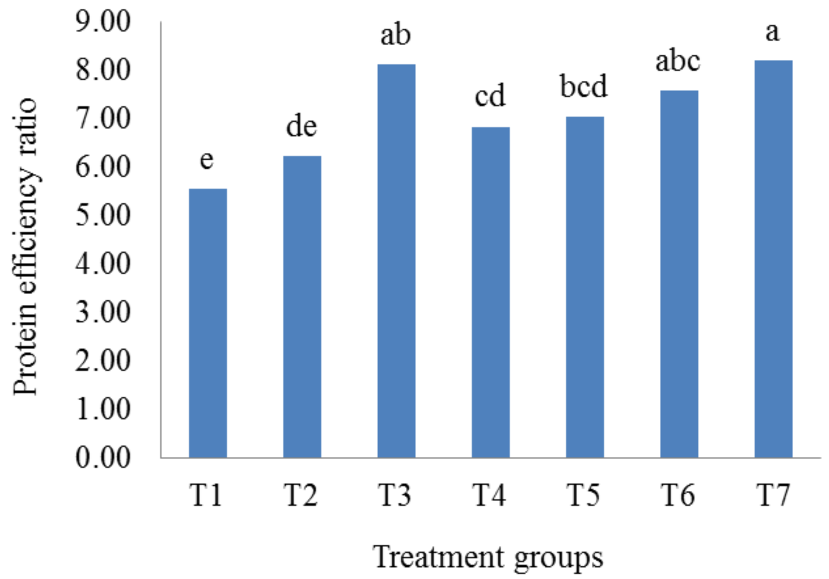
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.6. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on feed conversion ratio for Common Carp (*Cyprinus carpio*).



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

Figure 4.7. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on feed efficiency ratio for Common Carp (*Cyprinus carpio*).



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

Figure 4.8. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on protein efficiency ratio for Common Carp (*Cyprinus carpio*).

4.3. Blood Parameters

Table 4.3 shows that hematological parameters were not different significantly (P≤0.05) of all treatments in red blood cells (RBCs). The data regarded of hemoglobin (HGB), T5 diet with 50 g *Daphnia* /kg, T3 diet with 50 g *Chlorella* /kg and T4 diet with 25 g *Daphnia* /kg were higher respectively than other treatments significantly (*P<0.05*). among all the treatments, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were not different significantly (P≤0.05).The Platelets (PLT) were 52.333 and 52.000 (10^9 cells/L), for the T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg and T5 diet with 50 g *Daphnia* /kg respectively were different significantly (P≤0.05) from other dietary treatments.

Table 4.3. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on blood parameters for common carp (*Cyprinus carpio*)

Treatments	RBCs (10^{12}cells/L)	HGB (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^9 cells/L)
T1	2.377 a ± 0.15	12.133 b ± 0.29	169.100 a ± 0.81	60.200 a ± 2.8	34.067 a ± 1.3	16.500 c ± 1.44
T2	2.617 a ± 0.12	13.667 ab ± 0.74	168.533 a ± 4.89	57.033 a ± 2.42	35.067 a ± 0.45	18.000 c ± 2
T3	2.327 a ± 0.1	14.700 a ± 0.91	164.200 a ± 3.48	58.700 a ± 0.35	34.700 a ± 0.81	37.000 b ± 1.15
T4	2.573 a ± 0.02	14.367 a ± 0.26	170.833 a ± 4.72	55.500 a ± 0.87	33.100 a ± 0.6	37.333 b ± 6.74
T5	2.593 a ± 0.04	14.767 a ± 0.3	165.100 a ± 2.86	58.633 a ± 4.01	35.167 a ± 3.03	52.000 a ± 4.04
T6	2.417 a ± 0.09	14.400 a ± 0.55	161.033 a ± 1.32	59.567 a ± 1.53	35.533 a ± 0.69	39.000 b ± 2.89
T7	2.373 a ± 0.14	13.400 ab ± 0.38	167.933 a ± 2.28	55.500 a ± 3.39	35.200 a ± 1.63	52.333 a ± 6.12

Mean values with different superscripts within a column differ significantly (P≤0.05).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg.

Al-Koye (2013) showed that, replacing fishmeal with 10, 15, 20 % *Spirulina* in 200 fingerlings common carp (*C. carpio*) diet for 105 days, increased blood parameters (HGB, MCH, MCHC, MCV and PLT) than other treatments where fish fed with replacing (0, 5 % *Spirulina*), and there were significant differences (P≤0.05) for all treatment in (RBCs).

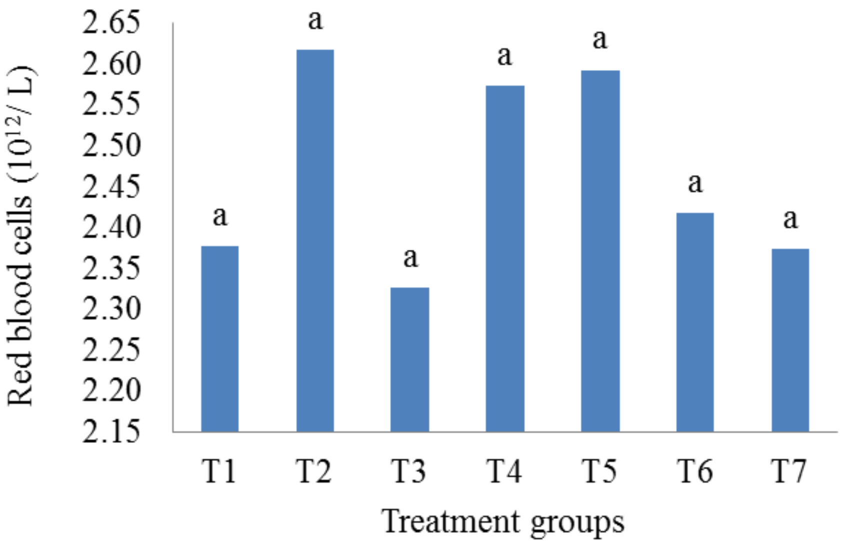
It was estimated that about 30 % of microalgae production was sold for animal feed purposes (Safi et al., 2014), due to the increasing demand for food with natural composition instead of synthesized ingredients (Becker, 2007). With regards to *Chlorella vulgaris* which contains important amount of carotenoids 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., 1996, 2002).

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). RBC in teleost is related to the oxygen requirement (Zanjani et al., 1967). For evaluating possible undesired collateral impacts (anemia) motivated by immunostimulants managed in supplemented feed, RBC count is a normally utilized parameter among the immune cell parameters (Morera et al., 2011). The activity of innate immune parameters can be impacted by several internal and external factors. While several food additives and immunostimulants might improve various innate factors, temperature alterations, stress handling and crowding might have suppressive impacts on innate parameters. WBCs are the immune-competent cells of immune system which play critical roles to both infectious and non-infectious diseases (Magnado´ ttir, 2006). Physiologically, Hb and HCT are vital for fish survival, being straightly associated with the blood capacity in oxygen binding (Bielek and Strauss 1993). Obviously under such conditions the animal metabolism is improved resulting in a better growth performance (Khani et al., 2017a).

Zhang et al. (2014) discovered that serum IgM and IgD levels of Gibel carp increased significantly by *Chlorella*. Salians et al. (2011) expressed that increase of IgD, one of the immunoglobins involved in mucosal defense, proposed that *Chlorella* could have some role in the mucosal immunity. These outcomes demonstrate that *Chlorella* might encompass in the fish innate immunity regulation via improving several gene expressions, demonstrates that *Chlorella* might be elaborated in fish innate and adaptive immunity regulation as wekk as might be utilized as a suppliment in fish diets.

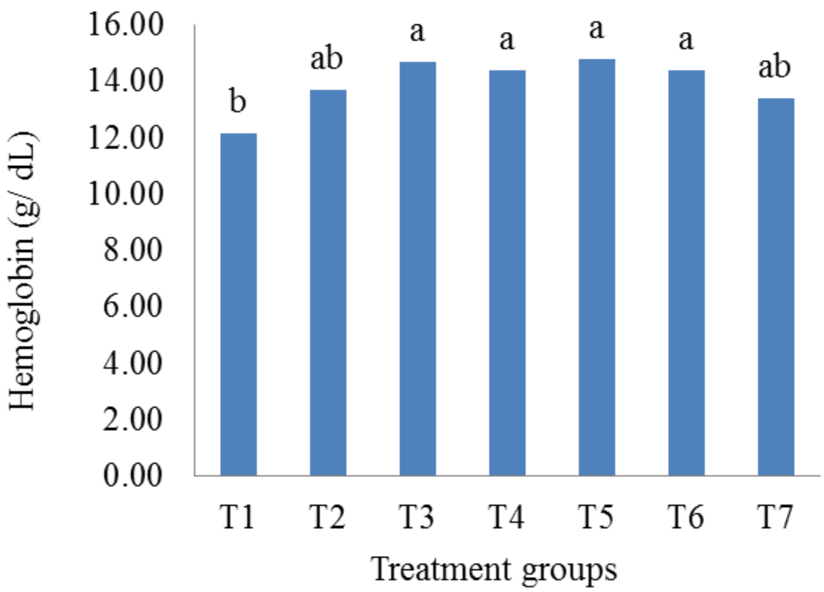
Differently, Farahi et al. (2010, 2012) and Najafpour et al. (2012) reported the dissimilar results with present study in their works. No significant effect of dietary inclusion of basil on Lymphocyte, Monocyte, Heterophil and Eosinophil percentages was obtained, but results indicated the outstanding 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 significant differences with control group (diet without garlic).

The results demonstrated that for fish diets, *Chlorella* can be a decent choice as an additive. it possesses significant level of polysaccharides, minerals, lipid and other bioactive constituents integrated in several physiological activities Because of supreme concentration of crude protein, (Xu et al., 2014; Khani et al., 2017b).



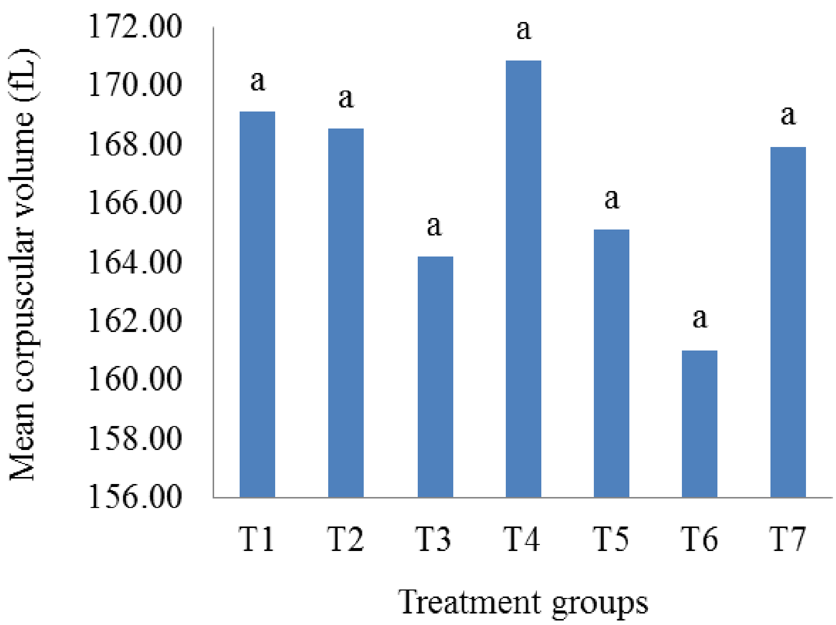
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.9. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on red blood cells for Common Carp (*Cyprinus carpio*).



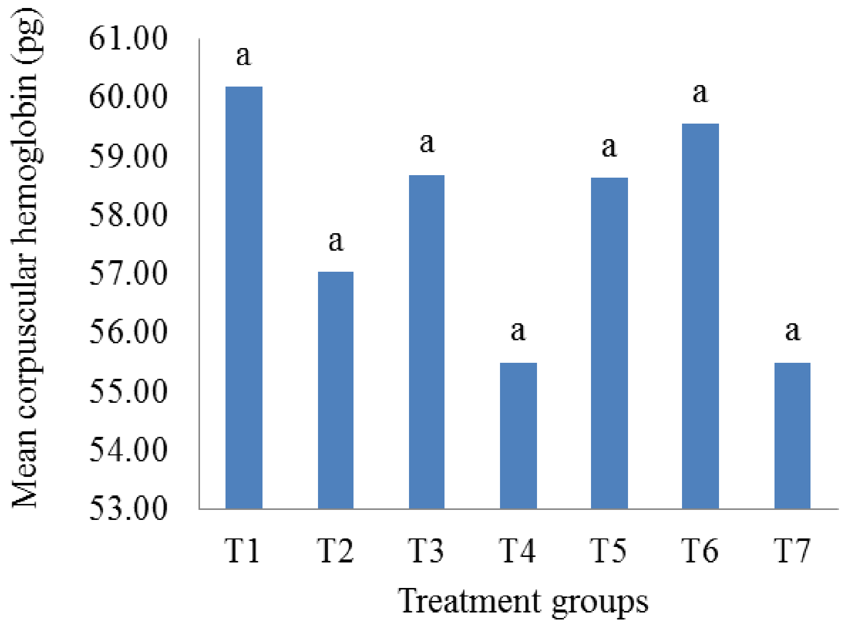
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.10. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on hemoglobin for Common Carp (*Cyprinus carpio*).



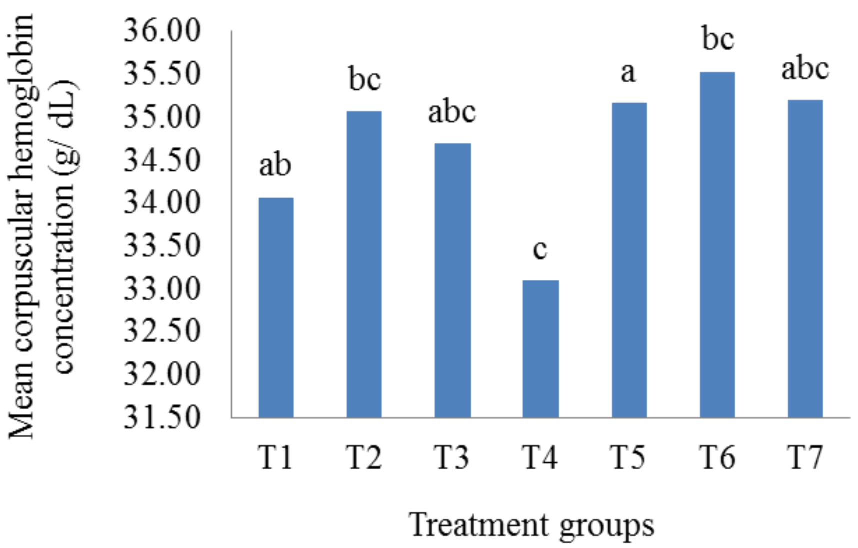
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.11. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on mean corpuscular volume for Common Carp (*Cyprinus carpio*).



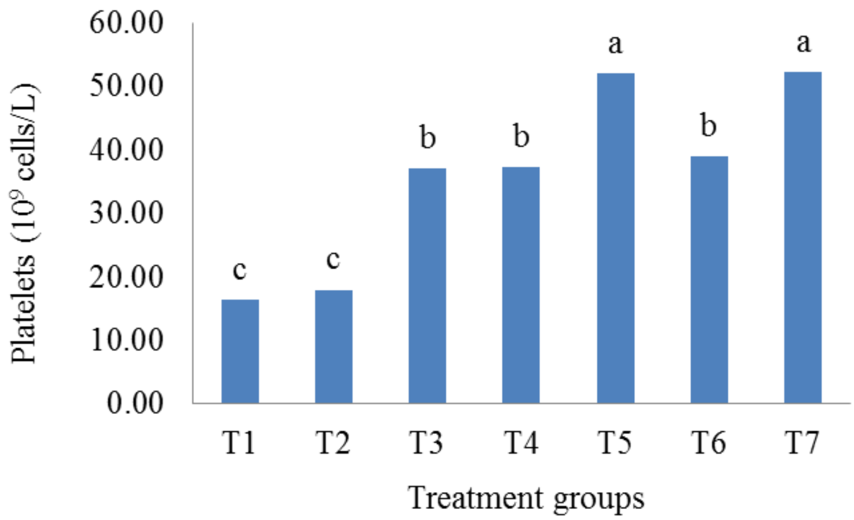
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.12. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on mean corpuscular hemoglobin for Common Carp (*Cyprinus carpio*).



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

Figure 4.13. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on mean corpuscular hemoglobin concentration for Common Carp (*Cyprinus carpio*).



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

Figure 4.14. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on platelets for Common Carp (*Cyprinus carpio*).

4.4. Biological Parameters

Results in the (Table 4.4) shows there were no differences significantly (P≤0.05) of all treatment in hepatosomato index and condition factor, gonadosomato index in T4 diet with 25 g *Daphnia* /kg, T2 diet with 25 g *Chlorella* /kg and T5 diet with 50 g *Daphnia* /kg differed significantly (P≤0.05) compared to other treatments.

Table 4.4. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on biological parameters for common carp (*Cyprinus carpio*)

Treatments	Hepatosomatic index	Gonadosomatic index	Condition factor
T1	3.203 a ± 0.23	0.633 b ± 0.11	0.017 a ± 0.0006
T2	3.090 a ± 0.5	2.287 a ± 0.29	0.017 a ± 0.0003
T3	2.967 a ± 0.05	0.917 b ± 0.1	0.017 a ± 0.0007
T4	2.903 a ± 0.31	2.823 a ± 0.73	0.017 a ± 0.0006
T5	2.607 a ± 0.41	2.267 a ± 0.28	0.017 a ± 0.0003
T6	2.687 a ± 0.48	0.833 b ± 0.21	0.017 a ± 0.0015
T7	2.737 a ± 0.23	1.007 b ± 0.14	0.015 a ± 0.0003

Mean values with different superscripts within a column differ significantly (P≤0.05).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg.

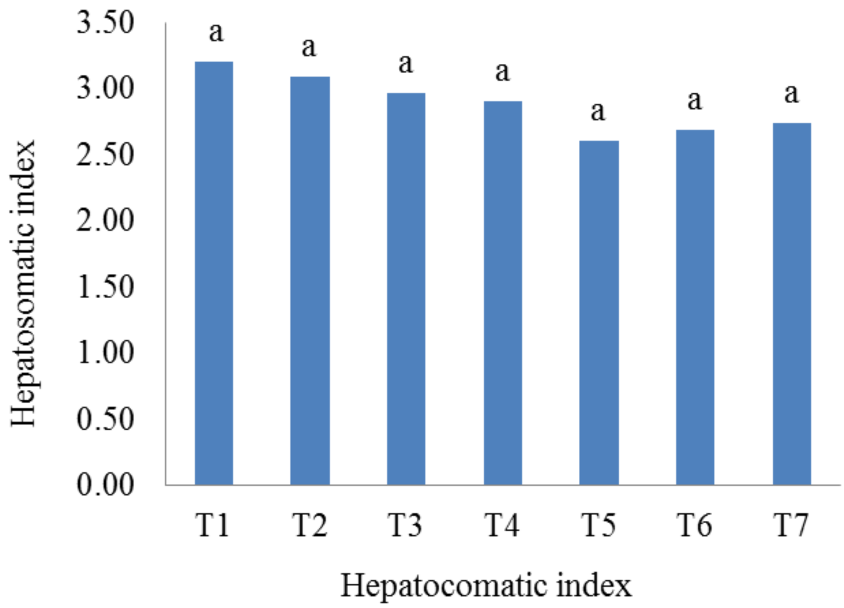
Abid (2018) with adding 2.5 g and 5 g/ kg diet *Chlorella* spp. powder to common carp (*C. carpio*) fingerlings feed for 105 days, Hepatosomatic index (3.444) and (3.336) were significantly higher ($P<0.05$) in comparason to other treatments where fish fed diet (0, 7.5 g /kg diet) of *Chlorella* spp.. According to Abolfathi et al. (2012) the main process in animal metabolism are digestion together with absorption because it determines the obtainability of nutrients required for entirre biological functions. Bolasina et al. (2007) stated that is a simple and dependable methodology that can be utilized as an indicator of digestive processes and nutritional condition of larvae is the analysis of digestive enzyme activity. Xu et al. (2014) found that the digestive enzyme in the hepatopancreas and intestine of Gibel carp *Carassius auratus gibelio* can increase significantly by the dietary *Chlorella*. This suggests that the *Chlorella* could improve the feed intake via increasing the activity of digestive enzyme.

Xu et al. (2014) showed that because of high protein content, *Chlorella* can be a good option for utilizing as an additive for fish diets. In fish diet, several microalgae have been utilized like fish meal replacement. Takeuchi et al. (2002) indicated that juvenile tilapia (*O. niloticus*) grew normally via feeding onley on raw *Spirulina* at the feed transition stage. Raw *Spirulina* seemed to be effective as a universal feed for early juvenile tilapia although the feed efficiency and specific growth rate of tilapia fed on *Spirulina* were lower compared those of the control. The protein content survival rate and of juvenile tilapia (*Oreochromis niloticus*) increased significantly after two months of feeding with *Spirulina*. Palmegiano et al. (2005) had monitored similar results for growing sturgeon (*Acipenser baeri*). Yamaguchi (1997) stated that the bioactive ingredients for example *Chlorella* growth factor (CGF) might encourage fish growth. And this may be the reasons of increasing fish length intestine with increasing the concentration of *Chlorella* in the diets of present study. Al-Koye (2013) showed that liver index (2.629) and gonadosomatic (6.489) were higher significantly ($P<0.05$) with replacing fishmeal with 10 % *Spirulina* in 200 fingerlings common carp (*C. carpio*) diet for 105 days than other treatments where fish fed with replacing (0, 5,15, 20 % *Spirulina*).

Nowadays, generally the microalgae that marketed as food additives or health food are traded in the formula of liquids, capsules and tablets. FAO (2016) reported that either as source of natural food colorant or as nutritious additives, algae are integrated in drink mixes and beverages, snack foods, pastas, candy bars or gums. *Chlorella* can be utilized as protein source for human food and animal diets because it contains around 60 % of protein and contains several essential amino acids. Nonetheless, present utilizations of *Chlorella* mostly concentrate on human food. In lower vertebrate, the research on utilizations of *Chlorella* is limited. For enhancing nutritional content of conventional food preparations and for acting as probiotic agents that positively impact human and animal health, microalgae has a potential broad spectrum (Becker, 2007).

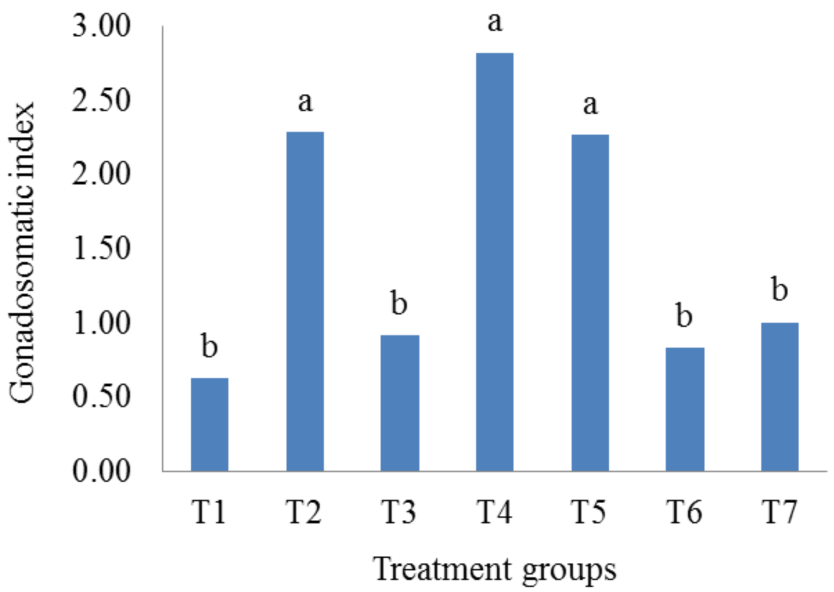
Barreda et al. (2014) expressed that in the balance between disease and animal health, the immune system characterizes a nodal point. Earlier research had discovered that *Chlorella* might be involved in the control of animal adaptive and innate immunity (Zhang et al., 2014) discovered that the *Chlorella* can significantly escalate the serum of two classes of immunoglobulin (Ig) M and D concentrations of Gibel carp. Salinas et al. (2011) expressed that *Chlorella* might have a role in the mucosal immunity because it increases the IgD, which is the immunoglobins involved in mucosal defense.

In the study of Kim et al. (2002) found that feed utilization and growth of juvenile flounder were improved significantly by dietary supplementation of 2 % *Chlorella* powder. However, the dietary *Chlorella* inclusion did not significantly affect the hepatosomatic index (HSI) and condition factor (CF) of flounder. Shape and size, and digestibility as associated with the structure and composition of cell wall and biochemical composition (for example. accumulation compounds, enzymes and toxins) and specific requirements of the target animal are some factors tat contribute to the nutritional value of a microalga. Thus, numerous researches have tried to associate the chemical profile of microalgae to their nutritional value.



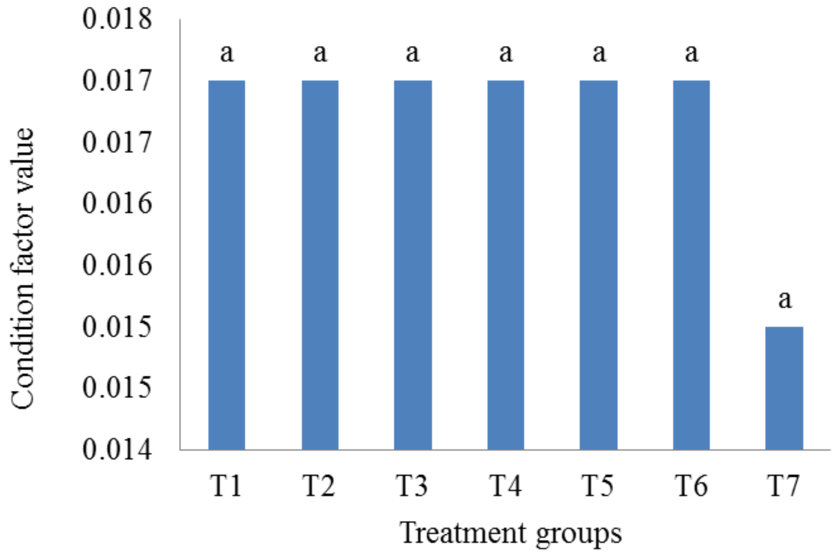
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.15. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on hepatosomatic index for Common Carp (*Cyprinus carpio*).



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

Figure 4.16. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on gonadosomatic index for Common Carp (*Cyprinus carpio*).



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

Figure 4.17. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on condition factor for Common Carp (*Cyprinus carpio*).

4.5. Chemical Composition

However, results from feeding experiments were 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.

The data in (Table 4.5) revealed that fish carcass composition of carp fed on various levels of *Chlorella*, *Daphnia* and their combination. Percentage of protein reached higher significant (P≤0.05) value in T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg as compared with the other treatments. Fat composition of fish in T5 diet with 50 g *Daphnia* /kg, T4 diet with 25 g *Daphnia* /kg, T7 diet with 50 g *Chlorella* + 50 g *Daphnia*/ kg and T6 diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg were significantly (P≤0.05) different as compared with the other treatments. As for moisture and ash data, moisture reached its maximum level in T1 commercial diet without any supplement (control diet) which was higher significantly than other treatments and ash reached its maximum level in T6 diet with 25 g *Chlorella* + 25 g *Daphnia*/ kg.

Table 4.5. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on carcass characteristics for common carp (*Cyprinus carpio*)

Treatments	Protein %	Fat %	Moisture %	Ash %
T1	19.593 d ± 0.25	2.489 b ± 0.15	76.375 a ± 0.11	1.061 ab ± 0.07
T2	21.742 c ± 0.38	3.212 b ± 0.26	73.615 b ± 0.29	1.011 b ± 0.05
T3	23.282 b ± 0.26	3.222 b ± 0.39	71.838 c ± 0.64	1.093 ab ± 0.03
T4	21.295 c ± 0.27	4.800 a ± 0.09	72.417 bc ± 0.41	1.104 ab ± 0.05
T5	23.084 b ± 0.39	4.864 a ± 0.05	70.435 d ± 0.39	1.084 ab ± 0.02
T6	23.753 b ± 0.16	4.135 a ± 0.25	70.458 d ± 0.62	1.238 a ± 0.08
T7	24.976 a ± 0.2	4.738 a ± 0.31	68.790 e ± 0.19	1.093 ab ± 0.05

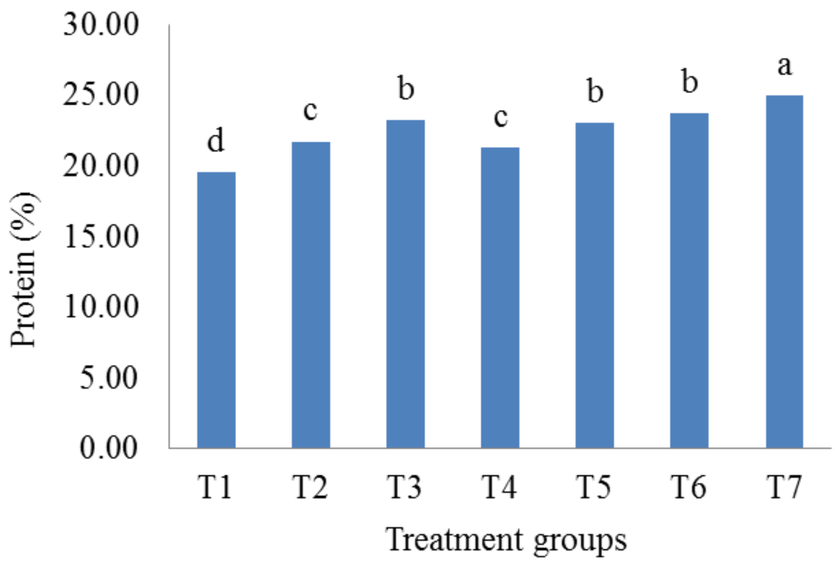
Mean values with different superscripts within a column differ significantly ($P \leq 0.05$).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia* /kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia* /kg.

Badwy et al. (2008) found that, Nile tilapia (*O. niloticus*) fingerlings fed diet comprising fifty percent algae substitution with fishmeal in both algae species (*Chlorella* spp. and *Scenedesmus* spp.) exhibited the biggest significant (P<0.05) dry matter, protein body content and lowest significant (P<0.05) fat and gross energy body content. Including algae from 10, 25, 50 to 75 % in the fish feeding, protein content and dry matter were enhanced apart from fish fed diet comprising seventy five percent algae substitution that are lower protein content and body dry matter compared to control group (diet without algae). That's in agreement with our results. Zeinhom (2004) showed similar results, who discovered that 15 % algae suplimentation caused the bigger value of crude protein, chemical composition of the dry matter and ash by the amount of (64.12), (28.11) and (19.76) respectively. However, utilizing 25 % algae caused greater value of gross energy (540.7) and ether extract (21.4). Abdulrahman (2014b) showed that the carcass mean weight (with head and without peripheral organs CMW (54.343); without head and peripheral organs (37.608), crude protein (25.145), crude fat (39.045) and ash (1.177) were higher significantly (P<0.05) with replacing fishmeal with 20 % *Spirulina* in 200 fingerlings common carp (*C. carpio*) diet for 105 days than other treatments where fish fed with replacing (0, 5, 10, 15 % *Spirulina*).

Our results were in partially confermed by Appler and Jauncy (1983). They reported that while content of fat declined with increasing algae in fish diet, the content of moisture declined with increasing algae concentration in fish diet. Our results were in disagreement with those were observed by Abu Zead (2001) who stated that increasing replacement by aquatic plant less than 20 % for tilapia and less than 30 % for common carp resulted in the increase the dry matter of tilapia and common carp. However, the contents of fat were conflicting trend by the contents of protein.

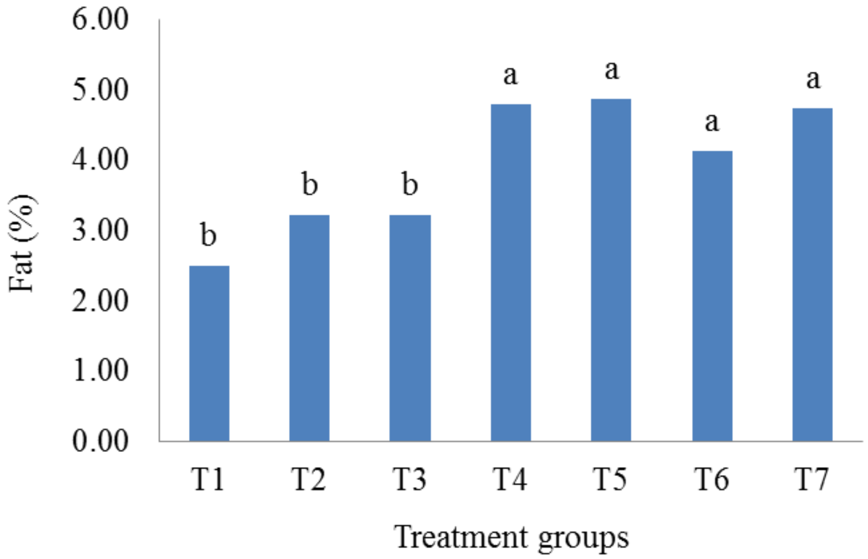
Khatoon et al. (2010) stated that Algal species with varying nutritional value have extraordinary potential as feed for fish. Abid (2018) with adding 7.5 g/kg diet *Chlorella* spp. powder to common carp (*C. carpio*) fingerlings feed for 105 days, Protein, (25.85 %), Lipids (1.47 %), Ash (1.97 %) and Moisture (75.53 %) were significantly higher ($P<0.05$) in comparison to other treatments where fish diet (0, 2.5, 5 g /kg diet) of *Chlorella* spp.. Badwy et al. (2008) investigated fish growth and body composition via fractional substitutions of fish meal with dried microalgae (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*O. niloticus*) diets. Primarily, from the proximate composition analysis of the formulated feeds and the carcass research, it was found that the suplimented feeds had ggreater content of ash compared to the control feed demonstrating higher minerals in algal feeds, which caused elevated nutrients deposition in the suplimented feed fed fishes. This confirms that the experimental feeds improved higher utilization of the dietary nutrients (Mukherjee et al., 2011).The high content of protein of the experimental diets might caused high protein content carcass of the fishes from experimental feeding groups, high content of muscle glycogen could be resulted from high dietary carbohydrate content of the experimental feeds and these confirmed by the results of Mukherjee et al. (2011). The earlier discovery by Khatoon et al. (2010) displaying fewer application of the minerals in highest concentration by more nutrient comprising experimental algal feed (*N. ellipsosporum*: *Navicula minima*: *Daphnia*, 2:1:1) compared to the control (*Daphnia*) as established via the content of ash. This conflicting out comes might be because of the fishes ability in utilizing dietary nutrients might varied among species.

In the group that fed on control diet (commercial diet without any supplement) the muscle fat content significantly increased ($P< 0.05$) through the 84 days of experimental period compared to the other experimental diets, this could be because of the deposition of lipid in the fishes body fed with control diet. Also, according Nandeesha et al. (1990, 1998) the deposition of elevated content of lipid in the fish fed on high energy containing diet. The high deposition of lipid in control diet group and T2 (control diet with 25 g *chlorella* powder /kg diet) negativly correlated with body weight gain. This supported the results that the high content of energy in the diets could negativly correlate with specific growth rate and weight gain (Daniels and Robinson, 1986). In contrast, Yigit et al. (2002) and Martins et al. (2007) reported that ideal concentration of lipid caused an enhanced nutrient utilization, growth rates, feed conversion ratios, and decreased excretion of nitrogen. The content of lipid of the control and T2 were much lesser compared the optimum concentration. They cuased low deposition of the body lipid and an enhance rate of growth in the entire aspects. So, the research acknowledged that the diet with high lipid content such as control feed utilized in this investigation could have a harmful impact on growth of fish since large amount of lipid might reduce feed consumption and could decrease the application of other nutrients, causing poor performance of growth (Hemre and Sandnes, 1999). Control feed caused elevated deposition of lipid which resulted in deprived growth of the fishes. Therefore, the current study recommended that the efficiency of the algal protein combined diets was greater compared the control group regarding the performances of growth. Consequently, in mixture with the control feed, the locally obtainable algal types can be utilized for obtaining a comparable or even better outcom in carp farming which might have a high value commercially in long period.



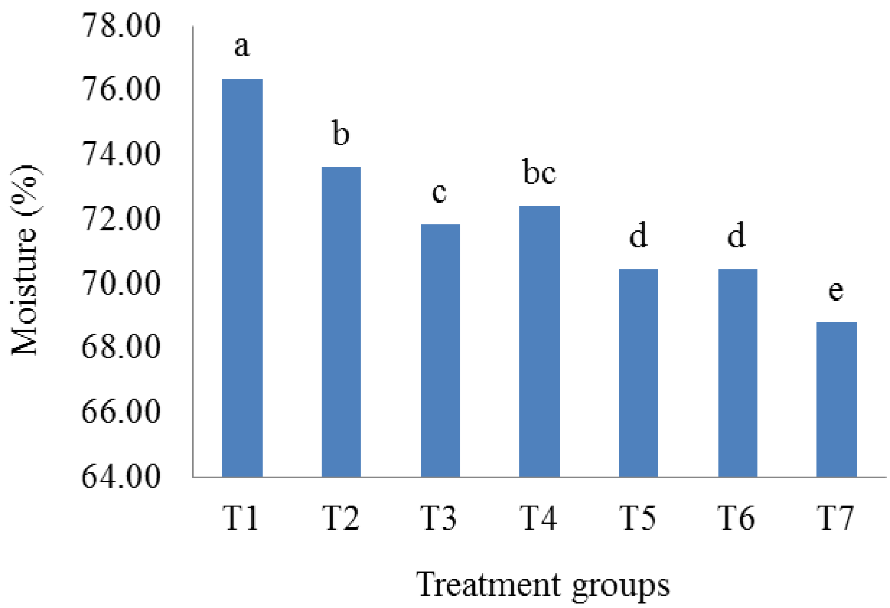
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.18. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on protein for Common Carp (*Cyprinus carpio*).



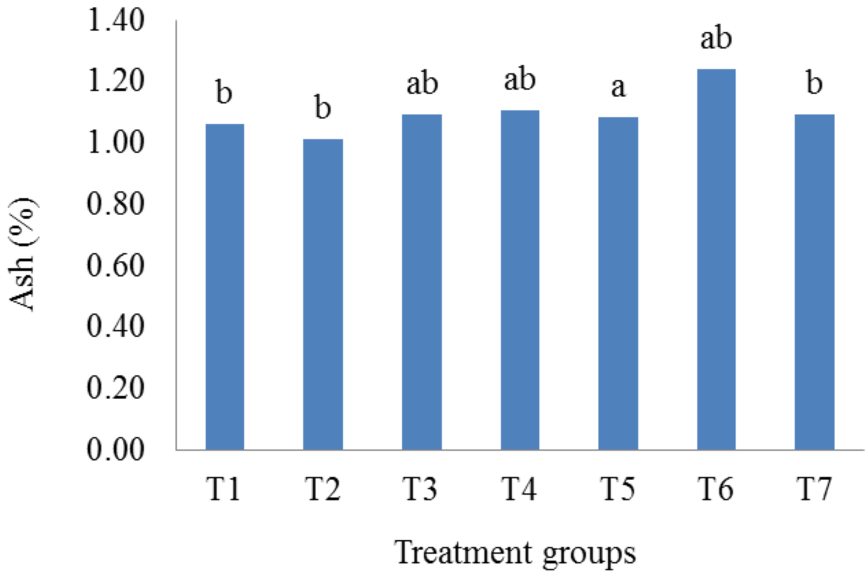
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.19. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on fat for Common Carp (*Cyprinus carpio*).



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

Figure 4.20. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on moisture for Common Carp (*Cyprinus carpio*).



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

Figure 4.21. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on ash for Common Carp (*Cyprinus carpio*).

4.6. Organoleptic (Sensory) Evaluation

The (Table 4.4) displays that in Juiciness the T5 diet with 50 g *Daphnia* /kg was significantly higher (P<0.05) compared to other treatments. There were no significant differences (P>0.05) regarding the Color, Flavor, Tenderness and Overall acceptability among treatments.

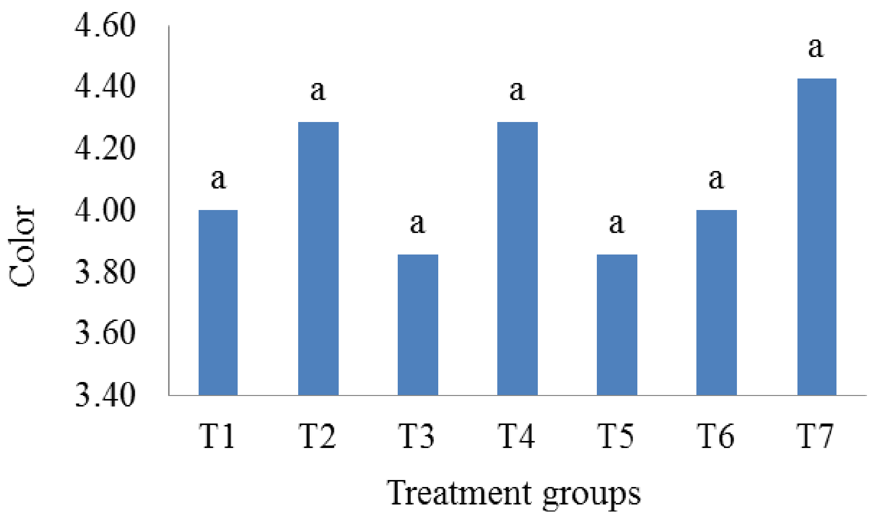
Al-Koye (2013) showed that, with replacing fishmeal with 10, 15, 20 % *Spirulina* in 200 fingerlings common carp (*C. carpio*) diet for 105 days, 10 % replacing significantly (P≤0.05) impacted color of the flesh of *C. carpio* and no significant effect observed with different levels of replacement in flavor, Juiciness, Complete acceptable and freshness, those results partial agreement with present study.

Abid (2018) adding 7.5 g *Chlorella* spp. /kg diet to common carp (*C. carpio*) fingerlings feed for 105 days, had a significant (P≤0.05) effect on color, freshness, juiciness, flavor, complete acceptable observed results disagree observed results disagree with present study. With regards to feeding fish on freeze dried *Daphian* and its effect on Organoleptic (sensory) evaluation we can't compare present results with others because of lack of references.

Table 4.6. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on organoleptic evaluation for common carp (*Cyprinus carpio*)

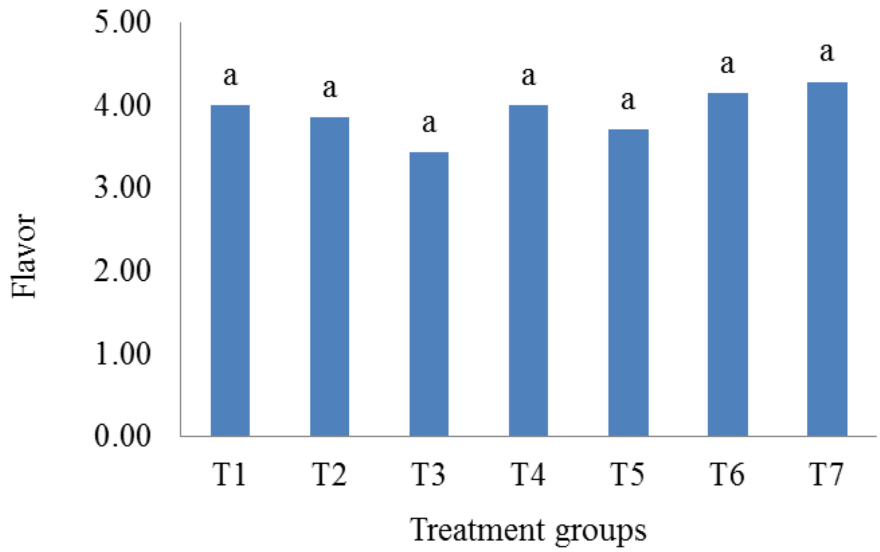
Treatments	Color	Flavor	Tenderness	Juiciness	Overall acceptability
T1	4.000 a ± 0.38	4.000 a ± 0.31	3.429 a ± 0.2	3.429 b ± 0.2	3.857 a ± 0.4
T2	4.286 a ± 0.29	3.857 a ± 0.26	4.000 a ± 0.31	3.571 ab ± 0.3	4.000 a ± 0.31
T3	3.857 a ± 0.34	3.429 a ± 0.3	3.857 a ± 0.26	4.143 ab ± 0.34	3.571 a ± 0.37
T4	4.286 a ± 0.29	4.000 a ± 0.31	4.000 a ± 0.38	4.143 ab ± 0.34	4.000 a ± 0.44
T5	3.857 a ± 0.14	3.714 a ± 0.18	4.143 a ± 0.26	4.286 a ± 0.18	4.000 a ± 0.31
T6	4.000 a ± 0.44	4.143 a ± 0.34	3.857 a ± 0.14	3.857 ab ± 0.14	3.857 a ± 0.34
T7	4.429 a ± 0.3	4.286 a ± 0.29	3.857 a ± 0.26	3.571 ab ± 0.2	3.714 a ± 0.29

Mean values with different superscripts within a column differ significantly (P≤0.05).
T1: Commercial diet without any supplement (Control); T2: Diet with 25 g *Chlorella* /kg; T3: Diet with 50 g *Chlorella* /kg: T4: Diet with 25 g *Daphnia* /kg; T5: Diet with 50 g *Daphnia* /kg; T6: Diet with 25 g *Chlorella* + 25 g *Daphnia* /kg; T7: Diet with 50 g *Chlorella* + 50 g *Daphnia* /kg.



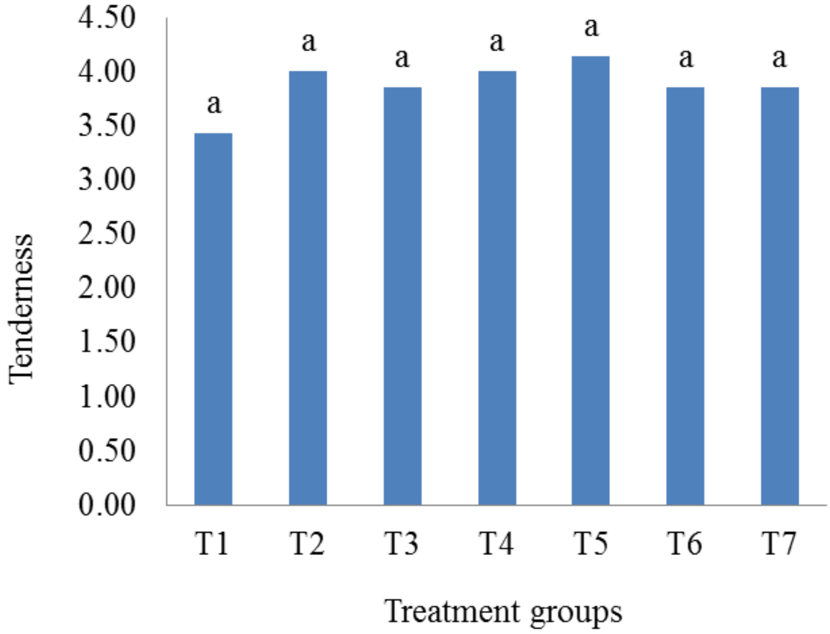
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.22. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on color for Common Carp (*Cyprinus carpio*).



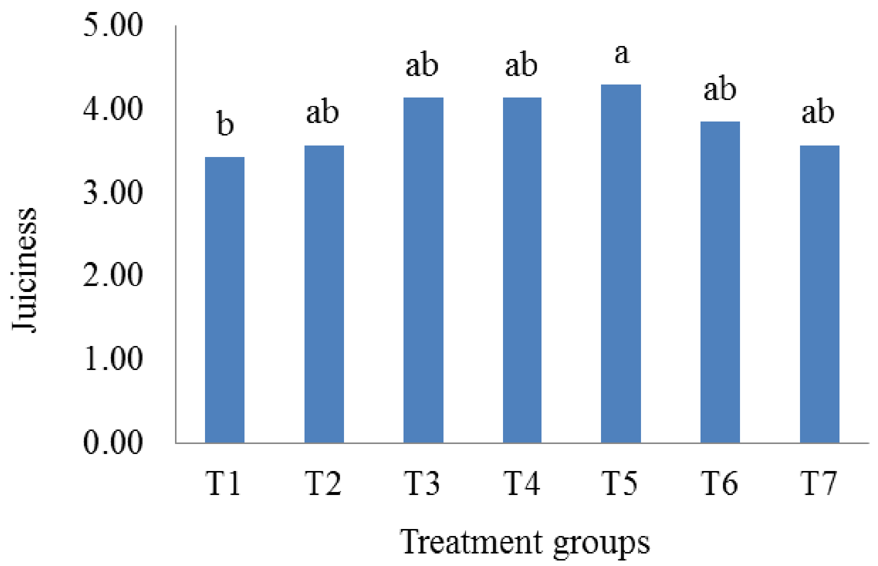
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.23. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on flavor for Common Carp (*Cyprinus carpio*).



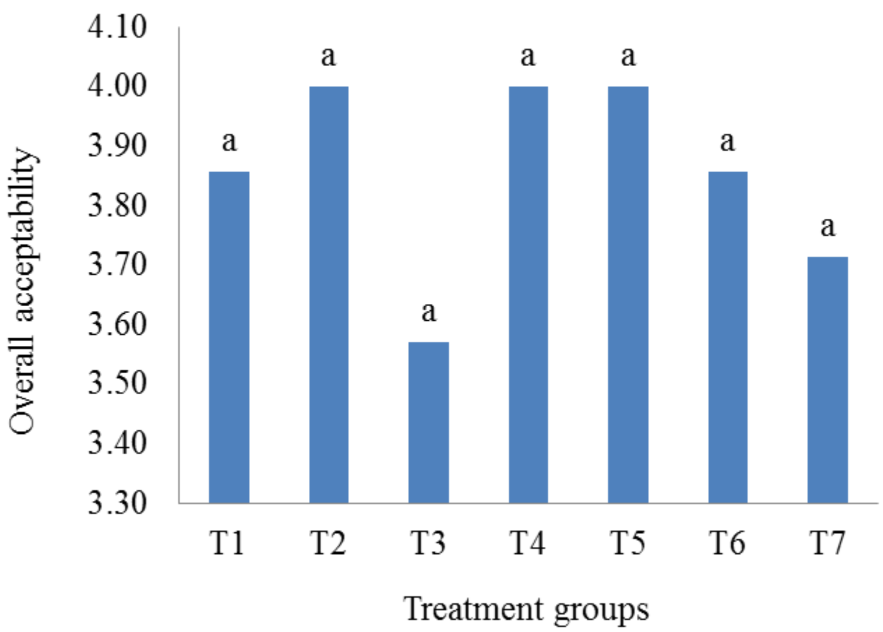
Mean values with different superscripts within a column differ significantly (P≤0.05).

Figure 4.24. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on tenderness for Common Carp (*Cyprinus carpio*).



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

Figure 4.25. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on juiciness for Common Carp (*Cyprinus carpio*).



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

Figure 4.26. Effect of *Chlorella*, *Daphnia* and their combination as a feed supplement on overall acceptability for Common Carp (*Cyprinus carpio*).

5. CONCLUSION

In the current research, the obtained results meet with the projected objectives. Nonetheless, new research subjects are opened from the summarized conclusions.

The inclusion of *Chlorella* and *Daphnia* to fish diet changed the growth performance in which it increase the daily and final weight gain, and specific and relative growth rate.

The *Chlorella* and *Daphnia* utilization caused a reduction in costs of feed conversion ratio which has a substantial role in determining the cost of aquaculture, dietary *Chlorella* and *Daphnia* escalates the Feed efficiency ratio and declines feed conversion ratio.

In accordance to the findings of chemical composition, various statements acquired wherein the high proportion of *Chlorella* and *Daphnia* significantly affect the protein and fat ratio.

In the studied blood parameters, there were diverse impacts of the treatment.

The outcomes demonstrated that *Chlorella* could be a decent option as an additive for fish feed.

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EXTENDENT TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

DOĞAL BİR BESİN KAYNAĞI OLARAK *CHLORELLA* VE *DAPHNİA* VE BUNLARIN KOMBİNASYONLARININ KULLANILMASI VE SAZAN BALIĞI (*Cyprinus carpio)* YETİŞTİRİCİLİĞİNDE TİCARİ YEMLER İLE KARŞILAŞTIRILMASI

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GİRİŞ

Bu çalışma doğal iki besin kaynağı olan fitoplanton *Chlorella* spp. ile zooplankton *Daphnia* spp.'nin ve kombinasyonlarının gıda katkısı olarak kullanılmasının etkilerinin değerlendirilmesi ve bunların pullu sazan (*Cyprinus carpio* L.) yavruları (fingerling) için kullanılan ticari yemlerle karşılaştırılması amacıyla gerçekleştirilmiştir. Çalışma Irak'taki Süleymaniye Üniversitesi Ziraat Bilimleri Fakültesi Hayvan Bilimleri Bölümü Balık Laboratuvarı'nda 12 hafta süre ile gerçekleştirilmiştir. Çalışmada kullanılan balıkların boyutları değişken olup, ağırlıkları 25,07 ile 37,21 g arasında değişmektedir ve ortlama başlangıç ağırlığı 28,58 g'dır. Balıklar önce 21 gün boyunca laboratuvar şartlarına alıştırılmış olup, bu süre boyunca ve beslenme deneyleri başlatılmadan önce kontrol peletleri ile (% 28.06 protein) beslenmişlerdir. Ardından iki farklı dozda *Chlorella* spp. ve *Daphnia* spp. ile katkılandırılmış yemler ayrı ayrı ve birlikte verilerek, balıkların büyüme performansları, yemden faydalanmaları, kan değerleri, biyolojik değerleri ve kimyasal kompozisyonları incelenmiş ve fingerlinglerin organoleptik (duyusal) değerlendirmeleri yapılmıştır. Kontrol grubu (T1) balıklara hiç *Chlorella* veya *Daphnia* kullanılmadan yalnızca ticari yemler verilirken, diğer altı gruptaki balıklar kontrol diyetine ilaveten, belirtilen değerlerde *Chlorella* spp. tozu ve dondurulup kurutulmuş *Daphnia* spp. eklenerek beslenmişlerdir: (T2) 25 g *Chlorella*/ kg diyeti ile, (T3) 50 g *Chlorella*/kg diyeti ile, (T4) 25 g *Daphnia*/ kg diyeti ile (T5) 50 g *Daphnia*/ kg diyeti ile, (T6) 25 g *Chlorella* +

25 g *Daphnia*/kg diyeti ile, ve (T7) 50 g *Chlorella* + 50 g *Daphnia*/kg diyeti ile. Her besleme grubu için üçer tekerrür yapılmış ve 100 L'lik tanklarda altışar pullu sazan fingerlingi stoklanıp, deneysel yemlerle günde iki defa beslenmişlerdir.. Her tanka hava kompresörleri yardımı ile uygun şekilde havalandırma da temin edilmiştir.

MATERYAL VE YÖNTEM

Deneysel diyet Irak'ın kuzey kesimlerinde yer alan Süleymaniye şehri pazarlarında bulunabilen sıradan ticari yemlerden sağlanmış olup, bunların kimyasal bileşenleri ve yüzdeleri aşağıda verilmiştir (Çizelge 1). *Chlorella, Daphnia,* ve bunların birlikte kullanılmaları ile elde edilen toplamda 7 çeşit yemin bileşenleri de Çizelge 2'de verilmiştir. Bileşenler birbirlerine eklenip üzerine su ilave edilerek hamur haline getirilmiştir. Ardından hamur elektrikli bir doğrayıcıdan geçirilmiş ve Kenwood Mutli-işleyicileri kullanılarak pelet haline getirilmiştir.

Çizelge 1. Deneysel Diyetin bileşenleri

İçerik	Yüzdesi (%)
Sarı mısır	15%
Buğday kepeği	18%
Soya küspesi 48%	40%
Konsantre protein	10%
Arpa	15%
Vitamin + Mineral Karışımı	2%
Toplam	100
Hesaplanmış Kimyasal Bileşim	
Ham protein	28.06
Metabolize edilebilir enerji (kcal/kg feed)	2242.7
%Arjinin	0.2394
%Lisin	0.25375
%Metiyonin + sistein	0.12872
%Treonin	0.017
%Triptofan	0.029

Çizelge 2. Yedi Diyetin Tümünün Kimyasal Bileşimleri

Besin grubu	Nem %	Protein %	Yağ %	Kül %
T1 (ilavesiz ticari kontrol grubu diyeti)	4.39	28.06	2.48	4.11
T2 (25g *Chlorella*/ kg diyeti)	4.17	28.23	2.89	4.34
T3 (50g *Chlorella*/ kg diyeti)	4.17	28.66	3.28	4.56
T4 (25g *daphnia*/ kg diyeti)	4.15	28.17	2.78	4.73
T5 (50g *daphnia*/ kg diyeti)	4.72	28.38	2.97	4.98
T6 (25g *Chlorella* + 25g *daphnia*/ kg diyeti)	4.55	28.89	3.18	4.78
T7 (50g *Chlorella* + 50g *daphnia*/ kg diyeti)	4.37	29.15	3.59	5.31

Nukraft, UK tarafından üretilen ambalajlı bir üründen elde edilen organik *Chlorella* tozu, Çizelge 3'te verilen şekilde yemlere eklenmiştir.

Çizelge 3. Kullanılan *Chlorella'*nın besinsel değerleri

Bileşen	Her 100 g başına
Enerji	418 Kcal
Protein	55 g
Yağ	15 g
Karbonhidrat	19.5 g
Lif	12.5 g
Tuz	0.1 g

Dondurularak kurutulmuş *Daphnia* ise doğal yollarla kurutulmuş DLS balık markalı UK menşeili bir ürün olup detayları Çizelge 4'te verildiği şekilde yemlere ilave edilmiştir.

Çizelge 4. Kullanılan *Daphnia'*nın besinsel değerleri

Bileşen	Yüzdesi (%)
protein	48 %
Yağ	9 %
Lif	7 %
Kül	18 %
Nem	5 %

Deneysel sürecin sonunda, her deney grubundan üçer adet balık rastgele seçilmiştir. Tüm balık numuneleri ayrı ayrı tartılmıştır. Farklı gruplara ait her bir balıktan, kuyruk damarı yoluyla kan numuneleri toplanmıştır. Kan numunelerinin tümü heparin içeren küçük plastik şişelere alınmış olup, şişeler soğuk şartlarda depolanmış ve Spincell3 Automatik Hematology Analyzer kullanılarak kan değerleri tespit edilmiştir.

Kan numuneleri alındıktan sonra, tüm numune balıkların hayatları sonlandırılarak karın boşlukları açılıp eşey organları ve karaciğerleri tartılmak, indekslerinin çıkarılması ve Kondisyon Faktörü'nün hesaplanması amacıyla alınmıştır.

Beslenme deneyinden sonra ayrıca tüm balık numuneleri kurutularak (fırında) daha derin kimyasal analize de tabi tutulmuştur. AOAC (2000) ile ana hatları belirlenen standart analiz yöntemleri kullanılarak yaklaşık protein, yağ, kül ve nem değerleri tespit edilmiştir.

Organoleptik (duyusal) muayeneler yedi tecrübeli değerlendiriciden oluşan bir heyet tarafından gerçekleştirilmiştir. Fileto haline getirilmiş balık numunesi etleri açık alüminyum kutulara konarak, önceden ısıtılmış fırında 100 °C'de 40 dakika boyunca pişirilmiştir. Pişirmenin ardından Süleymaniye Üniversitesi Ziraat Bilimleri Hayvan Bilimleri Bölümünden rastgele seçilen yedi öğretim görevlisi ile bir duyusal değerlendirme heyeti kurulmuştur.

SONUÇ

Deneysel diyete *Chlorella, Daphnia,* ve bunların kombinasyonunun eklenmesinin özellikle büyüme performansı, yemden faydalanma ve karkas özellikleri başta olmak üzere çalışılan tüm özelliklerde etkisi olduğu gözlenmiştir. Büyüme performansı, yemden faydalanma ve kimyasal bileşimler aşağıdaki üç tabloda (Çizelge 5, Çizelge 6 ve Çizelge 7) gösterilmektedir.

Çizelge 5. *Chlorella*, *Daphnia*'nın teker teker ve birlikte gıda katkısı olarak kullanılmasının pullu sazanların (*Cyprinus carpio*) büyüme performansları üzerindeki etkileri

Besin Grupları	İlk ağırlık (g)	Nihai ağırlık kazanımı (g)	Günlük ağırlık kazanımı (g/gün)	Nispi büyüme oranı (g/ gün%)	Özgün büyüme oranı/gün	Hayatta kalma %
T1	169.863 a ± 5.7	155.910 d ± 6.79	1.857 d ± 0.08	91.967 e ± 4.93	0.333 d ± 0.01	100.000 a ± 0.00
T2	173.777 a ± 4.51	175.487 cd ± 11.29	2.090 cd ± 0.13	100.907 de ± 5.34	0.360 cd ± 0.02	100.000 a ± 0.00
T3	172.977 a ± 3.98	232.570 a ± 12.33	2.770 a ± 0.15	134.280 ab ± 4.26	0.437 ab ± 0.01	100.000 a ± 0.00
T4	170.597 a ± 3.16	192.437 bc ± 1.61	2.290 bc ± 0.02	112.907 cd ± 2.85	0.393 bc ± 0.01	100.000 a ± 0.00
T5	172.777 a ± 4.45	199.410 bc ± 14.82	2.373 bc ± 0.17	115.980 bcd ± 11.21	0.397 bc ± 0.03	100.000 a ± 0.00
T6	170.023 a ± 3.1	219.087 ab ± 6.07	2.607 ab ± 0.07	129.053 abc ± 5.71	0.430 ab ± 0.01	100.000 a ± 0.00
T7	170.527 a ± 2.23	238.633 a ± 10.36	2.840 a ± 0.12	140.033 a ± 6.89	0.453 a ± 0.01	100.000 a ± 0.00

Üst indis bulunan ortalama değerlerin farkları istatistiki açıdan anlamlıdır ($P \leq 0.05$).
T1: katkısız ticari diyet (Kontrol); (T2) 25 g *Chlorella*/ kg diyeti, (T3) 50 g *Chlorella*/kg diyeti, (T4) 25 g *Daphnia*/ kg diyeti, (T5) 50 g *Daphnia*/ kg diyeti, (T6) 25 g *Chlorella* + 25 g *Daphnia*/kg diyeti, ve (T7) 50 g *Chlorella* + 50 g *Daphnia*/kg diyeti.

Çizelge 6. *Chlorella*, *Daphnia*'nın teker teker ve birlikte gıda katkısı olarak kullanılmasının pullu sazanların (*Cyprinus carpio*) yemden faydalanmaları üzerindeki etkileri

Besin Grupları	Yem dönüşüm oranı	Yem verimlilik oranı	Protein verimlilik oranı
T1	3.581 a ± 0.15	0.280 d ± 0.01	5.557 e ± 0.24
T2	3.305 ab ± 0.2	0.305 cd ± 0.02	6.217 de ± 0.4
T3	2.618 c ± 0.05	0.382 ab ± 0.01	8.115 ab ± 0.43
T4	3.013 bc ± 0.04	0.332 bcd ± 0	6.831 cd ± 0.06
T5	2.952 bc ± 0.27	0.344 bc ± 0.03	7.027 bcd ± 0.52
T6	2.740 c ± 0.11	0.366 ab ± 0.01	7.584 abc ± 0.21
T7	2.523 c ± 0.1	0.398 a ± 0.02	8.186 a ± 0.36

Üst indis bulunan ortalama değerlerin farkları istatistiki açıdan anlamlıdır ($P \leq 0.05$).
T1: katkısız ticari diyet (Kontrol); (T2) 25 g *Chlorella*/ kg diyeti, (T3) 50 g *Chlorella*/kg diyeti, (T4) 25 g *Daphnia*/ kg diyeti, (T5) 50 g *Daphnia*/ kg diyeti, (T6) 25 g *Chlorella* + 25 g *Daphnia*/kg diyeti, ve (T7) 50 g *Chlorella* + 50 g *Daphnia*/kg diyeti.

Çizelge 7. *Chlorella*, *Daphnia*'nın teker teker ve birlikte gıda katkısı olarak kullanılmasının pullu sazanların (*Cyprinus carpio*) karkas özellikleri üzerindeki etkileri

Besin Grupları	Protein %	Yağ %	Nem %	Kül %
T1	19.593 d ± 0.25	2.489 b ± 0.15	76.375 a ± 0.11	1.061 ab ± 0.07
T2	21.742 c ± 0.38	3.212 b ± 0.26	73.615 b ± 0.29	1.011 b ± 0.05
T3	23.282 b ± 0.26	3.222 b ± 0.39	71.838 c ± 0.64	1.093 ab ± 0.03
T4	21.295 c ± 0.27	4.800 a ± 0.09	72.417 bc ± 0.41	1.104 ab ± 0.05
T5	23.084 b ± 0.39	4.864 a ± 0.05	70.435 d ± 0.39	1.084 ab ± 0.02
T6	23.753 b ± 0.16	4.135 a ± 0.25	70.458 d ± 0.62	1.238 a ± 0.08
T7	24.976 a ± 0.2	4.738 a ± 0.31	68.790 e ± 0.19	1.093 ab ± 0.05

Üst indis bulunan ortalama değerlerin farkları istatistiki açıdan anlamlıdır ($P \leq 0.05$).
T1: katkısız ticari diyet (Kontrol); (T2) 25 g *Chlorella*/ kg diyeti, (T3) 50 g *Chlorella*/kg diyeti, (T4) 25 g *Daphnia*/ kg diyeti, (T5) 50 g *Daphnia*/ kg diyeti, (T6) 25 g *Chlorella* + 25 g *Daphnia*/kg diyeti, ve (T7) 50 g *Chlorella* + 50 g *Daphnia*/kg diyeti.

CURRICULUM VITAE

Amanj Ibrahim BAIZ was born in 1980 in Sarchnar of Sulaymaniyah-Iraq. He completed his primary, secondary and high school in Hajiawa town of Sulaymaniyah in 2000-2001. He started his college study in 2001-2002 in Animal Science Department/ College of Agricultural Science/ University of Sulaimani and graduated in the year 2004-2005. He began to study his master degree in February 2015 in Department of Fisheries Engineering in Van Yuzuncu Yil University in Turkey.

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