The effects of *Chlorella vulgaris* supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (*Cyprinus carpio*)

Khani M.[1]; Soltani M.[2*]; Shamsaie Mehrjan M.[1]; Foroudi F.[3]; Ghaeni M.[4]

Received: October 2016 Accepted: February 2017

1- Department of Fisheries, College of Agricultural and Natural Resources, Science and Research Branch, Islamic Azad University, P.O.Box 14515/775,Tehran,Iran.
2- Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3- Department of Animal sciences, Islamic Azad University, Tehran, Iran.
4- Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

[*]Corresponding author's Email: msoltani@ut.ac.ir

Keywords: Koi carp, *Chlorella vulgaris*, Growth performance.

Introduction

Microalgae are a wide group of photosynthetic heterotrophic organisms consisting of vital amino acids, protein, minerals, vitamins, chlorophylls and some forms of antioxidants and bioactive substances (Yamaguchi, 1996; Kwak *et al*., 2012). According to Takeuchi *et al*. (2002), microalgae are valuable in aquaculture and have been utilized as live feeds for larval or juvenile crustaceans and finfish, for all bivalve mollusks including oysters, scallops, clams and mussels and as a feed for zooplankton in aquaculture. Microalgae are well provided sources of vitamins, fundamental amino acids, minerals, vital fatty acids, and carotenoid pigments for aquatic animals (Takeuchi *et al*., 2002). Many kinds of microalgae in feeding trials with fish increase growth (protein accretion), feed utilization, physiological activity, stress response, starvation tolerance, disease resistance, carcass quality (Mustafa and Nakagawa, 1995), carotenoid , and protein sources for shrimp (Patnaik *et al*., 2006; Regunathan and Wesley, 2006).

Yamaguchi (1996) maintains that among the microalgae, Chlorella, associated with the Chlorophyta, *Chlorophyceae* and *Chlorella*, broadly exist in the nature, particularly in fresh water. Moreover, one of the most widely employed microalgae in aquaculture is Chlorella containing high levels of vital macronutrients such as

protein, vitamins, pigments and unknown Chlorella growth factors (Ajiboye, Yakubu and Adams, 2012). Kay (1991) points out that *Chlorella vulgaris* is a unicellular green algae observed in both fresh and marine water and also it is employed as food supplements. Considerable attention has recently been drawn to the use of microalgae for improving functional food, as microalgae produces a great variety of nutrients that are vital for human health. The nutritional value of *C. vulgaris* was fundamentally identified in the 1950s-1960s (Lubitz, 1963), and was also examined as a food source in Japan, United States and Germany after World War II (Miyachi, 1995; Ashraf *et al*., 2011).

The protein content of Chlorella is 51-58% and consists of many essential amino acids, demonstrating that Chlorella could be employed as a protein source for human food and animal diets (Becker, 2007). However, the current use of Chlorella mainly is in human food. Up to now, far too little attention has been paid to its use in lower vertebrates. In fact, koi is a domestic type of common carp with the scientific name of *Cyprinus carpio* that is usually kept in pools and water gardens for ornamental uses and sometimes they are called Japanese carp. In 1999, George suggested that koi is a kind of valuable ornamental fish and its high price is the cause that makes it different it from other ornamental species.

Koi carp tolerates high scopes of temperature but the best temperature scope for them is between18-24 centigrade degree. This fish grows until 90 cm (36 inch) and sometimes more. It is noteworthy to note that this fish in aquarium doesn't reach this size. koi (*Cyprinus carpio*), is an omnivore and non-invasive fish. Plants, insects, snails, worm, and algae are part of their main diets. So, in the present study, we prepared five diets containing different levels of chlorella to treat the koi carp in order to examine growth performance, blood parameters and digestive enzymes. The results provided the basis for the use of chlorella as an additive in koi carp.

Materials and methods

Fish culture and feeding trial

Koi fish (average weight 29.8±0.5 g), taken from a local fish farm (Varamin, Tehran Province, Iran) were randomly stocked into 15 tanks (250 L) at a density of 15 fish per tank (3 tanks per treatment). During the experiments, one-third of the water volume was exchanged once a week. The water temperature was 20˚C, dissolved oxygen content >5 mg/L, NH_3<0.05 mg/L, H_2S <0.1 mg/L and pH 6.8- 7.2 during the course of the experiment.

Feeding preparation

A basal diet was formulated for koi fish (Table 1); this basal diet served as the control diet and the experimental diets were produced by supplementation of the basal formulation with varying levels of chlorella (2, 5, 7, and 10 g kg-1). The ingredients were blended thoroughly in a mixer and pelleted using a meat grinder. The pelleted diets were air-dried, ground and sieved to produce a suitable crumble (2 mm). Then the feed was stored at 4°C until feeding trials began. The experimental fish were weighted every 15 days in order to adjust the daily feed rate which was 3 g kg^{-1} of the total biomass. The fish were fed three times daily to apparent satiation for 60 days (Akrami *et al*., 2010). The chemical composition of formulated diets was determined according to standard AOAC (AOAC, 1990) methodology.

Growth and feeding performance

In order to measure the growth parameters, weight and length of all fish were measured at every 15 day intervals. After an 8-week feeding period, Weight Gain (g kg^{-1}), Specific Growth Rate (SGR g kg^{-1} /day), Feed Conversion Ratio (FCR), Condition Factor (CF g/cm3) and Survival Rate (g kg-1) were calculated according to the following equations (Bekcan *et al.*, 2006): WG (g kg^{-1})=(Wt−W0)× 100/W0, SGR= (Ln Wt−Ln W0)×100/t , FCR=dry feed fed in g/Wet weight gain in g, CF=100× Wt/Lt3, Survival rate = (Nt/N0) × 100. Here Wt and W0 are final and initial body weights (g) respectively, t is duration of experimental days, N0 is the initial number of fish and Nt is the final number of fish.

Blood parameters measurements

Albumin in plasma was determined calorimetrically according to Wotton and Freeman (1982).Serum total protein content was measured using a micro protein determination method (C-690; Sigma). Glocuse, cholsterol, and triglyceride were measured using enzymic methods, and hepatopancrease enzyme (ALT, AST, LDH) was measured using kinetic colorimetric method and ALP via kinetic enzymic method according to Johnson *et al* (1999).

The activity of digestive enzymes in the liver and gut

To assess the effects of Chlorella on digestive enzyme activity, 9 fish from each group (3 fish per tank) were anesthetized with MS_{222} (50 mg/L) and tissues including intestine and hepatopancreas were immediately separated. The intestines were emptied and washed with ice-cold phosphate buffer (pH 7.0, 200 mM) for three times. Then the hepatopancreas and intestine were excised and homogenized in ice-cold 200 mM PBS buffer and centrifuged at 5000 g for 5 min at 4˚C to collect the supernatants. The activity of amylase, lipase and protease were detected with the kits purchased from Jiancheng

biotechnology company (Nanjing, China).

Chemical analysis of diets and C. vulgaris incorporated feed (g kg^{-1})

The chemical composition of formulated diets (Table 1) and *C. vulgaris* incorporated feed (Table 2) were determined according to standard AOAC methodology (AOAC, 1990). Crude protein content was determined by Kjeldahl method using Auto Kjeldahl System, crude lipid content by soxhlet extraction method, ash content in a furnace muffler (550°C for 4 h), moisture content in a dry oven (105°C for 24 h) and crude fiber content using an automatic analyzer (Fibertec, Sweden) (AOAC, 1990).

Table: 1 Ingredients and proximate composition of the experimental diets (g kg^{-1}).

Ingredients	Control	CL2%	CL5%	CL7%	CL10%
Fish meal	150.0	130.0	100.0	80.0	50.0
Chlorella vulgaris	0	20.0	50.0	70.0	100.0
Soy bean meal	170.0	170.0	170.0	170.0	170.0
Soy bean full-fat	80.0	80.0	80.0	80.0	80.0
Solvent-extracted cotton seed meal	110.0	110.0	110.0	110.0	110.0
Wheat shorts	250.0	250.0	250.0	250.0	250.0
Wheat flour	150.0	150.0	150.0	150.0	150.0
Attapulgite meal	40.0	40.0	40.0	40.0	40.0
Vitamin/minerals premix[a]	10.0	10.0	10.0	10.0	10.0
Soy bean oil	20.0	20.0	20.0	20.0	20.0
Analyzed proximate composition(in % dry matter)					
Crude protein	37.59	37.40	37.21	37.34	37.12
Crude lipid	6.90	6.56	6.44	6.12	6.10
Ash	11.49	10.88	10.20	10.01	9.44
Moisture	8.82	8.45	8.46	8.62	8.70

a Mineral premix (mg or g kg^{-1} diet): KCl, 200 mg; KI (1%), 60 mg; $CoCl^2.6H_2O$(1%), 7 mg; $CuSO_4.5H_2O$, 14 mg; $FeSO4.H_2O$, 400 mg; $ZnSO_4.H_2O$, 200 mg; $MnSO_4.H_2O$,80 mg; $Na_2SeO_3.5H_2O$(1%), 65 mg; $MgSO_4.7H_2O$, 3000 mg; $Ca(H_2PO_4)2.H_2O$, 20 g; NaCl, 136 mg; Zoelite, 5.84 g.b vitamin premix (mg or g kg^{-1} diet): thiamin,12 mg; riboflavin, 5 mg; pyridoxine HCL, 6 mg; vitamin B12, 0.05 mg; vitamin K3, 5 mg; inositol,100 mg; pantothenic acid, 30 mg; niacin acid 35 mg; folic acid, 2 mg, biotin, 0.06 mg; retinol acetate, 25 mg; cholecalciferol, 5 mg; alpha-tocopherol, 40 mg; ascorbic acid, 500 mg; ethoxyquin 150 mg, wheat middling 19.09 g.c Crude protein, lipid, ash, gross energy are expressed on a dry matter basis andgiven as means (n ¼ 2).

Table 2: Proximate composition of *Chlorella vulgaris* incorporated feed (g kg^{-1}).

Measurement factors	Control	CL_2%	CL-5%	CL-7%	CL-10%
Crude protein	420.20	414.70	409.30	403.80	398.40
Carbohydrate	204.80	206.80	208.80	210.50	212.70
Lipid (%)	147.00	136.30	134.10	132.40	131.30
Ash (%)	111.86	123.30	130.00	137.30	142.00
Moisture (%)	99.30	96.00	93.30	90.46	87.00
Gross energy (kcal kg^{-1})	3187.71	2930.13	2713.09	2651.96	2379.96

Chemical composition of *C. vulgaris*: crude protein – 55.70%; carbohydrate – 15.28%: Lipid – 10.65%; Ash – 9.00%; Moisture 6.30%. * Becosules capsules (Each capsule contains); Thiamine mononitrat (IP): 10 mg; Riboflavin (IP): 10 mg; Pyridoxine hydrochloride (IP); 3 mg Vitamin B12 (as tablets 1:100) (IP): 15 mcg; Niacinamide (IP): 100mg; Calcium pantothenate (IP): 50 mg Folic acid (IP): 1.5mg; Biotin USP (IP): 100 mcg; Ascorbic acid (IP): 150mg.

Statistical analysis

All data were shown as the mean±SD. All data were confirmed in terms of normality after transformation (ASIN). Data were analyzed by one-way analysis of variance using the statistical software SPSS version 18.0. Subsequently, significant differences between the groups were determined using Duncan's new multiple range test. Data are presented as treatment means ± standard deviation (SD). Differences were considered significant when $p<0.05$.

Results and discussion

The effects of *Chlorella* on the growth performance of koi carp fed with the experimental diets are summarized in Table 3. It was clear that the supplement of *Chlorella* could significantly increase growth performance of koi carp. During the 8 week trial, fish fed 5% Chlorella grew from 29.8 ± 0.5 g to 98.06 ± 0.73g, with a RGR of 141±1 and a SGR of 1.47±0.05. The PER and LER in 5% Chlorella group were 1.05±1.01, respectively. The PGR, SGR, PER, and LER in 5% Chlorella group were all higher than that of the control group ($p<0.05$), and only the FCR was lower than that of the control group ($p<0.05$).

The results of blood parameters of fish fed with experimental diets are summarized in Table 4. As it can be seen, the total protein and albumin of fish in the 5% Chlorella group increased when compared with the control group ($p<0.05$). The serum cholesterol and triglyceride level of fish fed 5% chlorella were lower than that of fish in the control group ($p<0.05$). The ALT and AST of fish in different groups had the normal level pattern ($p>0.05$).The fish fed 2% chlorella experienced the most significant difference in cholesterol and triglyceride levels. The activity of three digestive enzymes including amylase, lipase and protease in hepatopancreas and intestine were examined, and the results were summarized in Table 4.

Fish fed with 5% had the most significant difference with C 2%, 7%, 10% groups as well as control one ($p<0.05$). It should be noted that there was no significant correlation between glocuse and the amount of *C. vulgaris* in diets ($p>0.05$).

In the present study, the different contents of Chlorella were added in the basal diet of Koi fish and growth performance, blood parameters and digestive enzyme were examined. The results indicated that Chlorella can be a good choice as an additive for fish diets. Due to supreme level of crude protein, it possesses significant concentration of polysaccharides, lipid, minerals and other bioactive components involved in many physiological activities (Xu *et al.*, 2014).

Table 3: Growth factors and survival of Koi carp fed on different levels of *Chlorella*.

Factors	Treatments				
	Control	**CL-2%**	**CL-5%**	**CL-7%**	**CL-10%**
Initial weight (g)	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5
Final weight (g)	57.8± 0.48 ^{d}	62.1 ± 1.15 ^{d}	98/06 ± 0.73^{a}	83.29 ± 0.08 ^{b}	85.39 ± 0.83^{c}
SGR	1.15 ± 0.01 ^{d}	1.17 ± 0.02 ^{d}	1.47 ^{a}	1.39 ± 0.02 ^{b}	1.23 ± 0.01^{c}
CF	1.27 ± 0.02 ^{b}	1.2 ± 0.03^{b}	1.42 ± 0.04 ^{a}	1.35 ± 0.05 ^{ab}	1.33 ± 0.06 ^{ab}
RGR	99.42 ± 1.45 ^{d}	103± 1.58 ^{d}	141± 1 ^{a}	133 ± 1.43 ^{b}	106± 2.56 ^{c}
WG (%)	29.7 ± 0.43 ^{d}	29.15 ± 0.18 ^{d}	41.9 ± 0.2^{a}	39.01 ± 1 ^{b}	32.01 ± 0.14^{c}
BWG	199± 1.65 ^{d}	201 ± 2.68 ^{d}	240± 1 ^{a}	231 ± 3.45 ^{b}	206 ± 1.86 ^{c}
VL	583 ± 8.33 ^{b}	607 ± 25.8 ^{ab}	642 ± 19.4 ^{a}	649± 19.33 ^{a}	595 ± 33.4 ^{b}
SR (%)	96.7	96.7	96.7	93.4	96.7
FCR	2.97 ± 0.01^{a}	2.0 ± 9.05 ^{a}	2.0 ± 61.02^{b}	2.0 ± 61.01^{c}	2.0 ± 1.01^{d}
PER	0.76± 0.03^{d}	0.0 ± 79.01^{d}	0.0 ± 85.02 ^{c}	0 ± 1.04^{b}	1.0 ± 05.02^{a}
LER	3.09 ± 0.05^{d}	3.0 ± 16.06 ^{d}	3.0 ± 39.05 ^{c}	4.0 ± 21.01 ^{b}	4.0 ± 51.01 ^{a}
EER	0.0 2	0.0 2	0.0 2	0.0 3	0.0 3

Values are means of triplicate groups ±SEM. Means along a row with different letters are significantly different (p<0.05). SGR=Special growth rate; CF=condition factor; RGR=relative growth rate; WG=weight gain; BWG=body weight gain; VL=velocity length; SR= survival rate, FCR=Food conversion rate PER=Protein efficiency rate, LER=Lipid efficiency rate, (EER). EER=Energy efficiency rate.

Table 4: Measurement factors and survival in Koi fed on different levels of Chlorella.

Treatment	Criteria				
	Control	**CL-2%**	**CL-5%**	**CL-7%**	**CL-10%**
Initial weight (g)	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5	29.8 ± 0.5
Final weight (g)	57.8± 0.48 ^{d}	62.1 ± 1.15 ^{d}	98/06 ± 0.73^{a}	83.29 ± 0.08 ^{b}	85.39 ± 0.83^{c}
SGR	1.15 ± 0.01 ^{d}	1.17 ± 0.02 ^{d}	1.47 ^{a}	1.39 ± 0.02 ^{b}	1.23 ± 0.01^{c}
CF	1.27 ± 0.02 ^{b}	1.2 ± 0.03^{b}	1.42 ± 0.04 ^{a}	1.35 ± 0.05 ^{ab}	1.33 ± 0.06 ^{ab}
RGR	99.42 ± 1.45 ^{d}	103± 1.58 ^{d}	141± 1 ^{a}	133 ± 1.43 ^{b}	106± 2.56 ^{c}
WG %	29.7 ± 0.43 ^{d}	29.15 ± 0.18 ^{d}	41.9 ± 0.2^{a}	39.01 ± 1 ^{b}	32.01 ± 0.14^{c}
BWG	199± 1.65 ^{d}	201 ± 2.68 ^{d}	240± 1 ^{a}	231 ± 3.45 ^{b}	206 ± 1.86 ^{c}
VL	583 ± 8.33 ^{b}	607 ± 25.8 ^{ab}	642 ± 19.4 ^{a}	649± 19.33 ^{a}	595 ± 33.4 ^{b}
SR %	96.7	96/7	96/7	93/4	96/7

Values are means of triplicate groups ±SEM. Means along a row with different letters are significantly different (p<0.05). SGR, special growth rate ; CF, condition factor; RGR, relative growth rate; WG %, weight gain; BWG , body weight gain; VL ,velocity length; SR %, survival rate.

In this study, dietary supplementation of 5% *Chlorella* powder significantly enhanced growth and feed intake of koi fish. The results (5% Chlorella supplementation) confirm the findings on *Girella pametato* (Nakazoe *et al*. ,1986), the nibber (Nakazoe *et al*., 1986), (5% Spirulina supplementation) for *Girella pametato* (Nakazoe *et al*., 1986), (5% Spirulina supplementation) for *Pagrus major* (Mustafa *et al*., 1994, 1995, 1997; Nakagawa *et al*., 2000), and (5% Spirulina supplementation) for Striped jack (*Pseudocaranx denta*) (Liao *et al*., 1990; Watanabe *et al*., 1990).

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

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

In addition, the group fed on 5% chlorella in this study had the most significant level of protein and albumin. The albumins are synthesized by the liver and served as protein transporters (Anderson *et al*., 1979). Also, high level of serum protein is the result of liver function improvement. Moreover, the Chlorella could increase the total serum protein (TP), albumin in the immune response of koi carp. Similarly, Yildiz *et al*. (2002) and Yu *et al*. (2006) hold a view that the increase of serum protein level is an appropriate factor for displaying the immune condition of the fish.

Our findings revealed that the dietary addition of Chlorella could increase the immune response. It is noteworthy to say that studies concerning dietary inclusion of *C. vulgaris* as a functional ingredient or as an immunostimulant in koi fish are limited.

C. vulgaris, as many other microalgae, is known to have a number of immunostimulants in the form polysaccharides, which are substances inducing immune activity in different animals (Huang, Zhou and Zhang, 2006). Its ability to improve immune function has been already proven in

gibel carps (Zhang, Giu, Xu, Gao, Shao and Qi, 2014).

Furthermore, the Chlorella could decrease the level of blood cholesterol, not the glucose of koi carp, demonstrating that the Chlorella might be involved in the metabolism of lipid. The same results were also found by Güroy *et al.* (2011). According to the study by Kim *et al*. (2007), adding *C. vulgaris* powder in *Paralichthys olivaceus* ‘ diet resulted in improving growth performance, and serum cholesterol level. Examination of digestive enzyme activity is a reliable method that can be employed as a symbol of digestive processes and nutritional condition of fish (Abolfathi *et al*., 2012). In the current study, we concluded that the dietary Chlorella could significantly increase the digestive enzyme in the hepatopancreas and intestine of koi carp, suggesting that Chlorella could enhance the diet utilization rate by increasing the activity of digestive enzyme. Overall, Xu *et al*. (2014) claimed that *C. vulgaris* could increase digestive enzymes and results in improving growth performance and immune response. Similarly, Radhakrishnan *et al*. (2015) reported that, the growth and level of digestive enzymatic activities of *M. rosenbergii* fed *C. vulgaris* meal up to 50% level increased significantly. Nandeesha *et al*. (1998) reported that Spirulina inclusion diets fed to *C. carpio* had significantly increased hepatopancreas protease, amylase and lipase activity when compared to the control. Also, Umesh *et al*. (1994) reported that 50% of *S. platens* dietary inclusion significantly improved the protein digestibility in common carp *C. carpio*. Nandeesha *et al*. (1994) reported that 25, 50, 75 and 100% level of fishmeal replacement with *S.platens*i significantly increased the digestibility in catla, rohu and common carp mixed culture. These positive effects of Chlorella powder on growth and feed utilization could demonstrate that several substances included in microalgae perform as dietary protein, minerals, vitamins, fiber, feeding attractants, antioxidants and unknown growth factors (Mustafa and Nakagawa, 1995; Nakagawa, 1997).

In sum, this study suggests that Chlorella could be used as a feed additive in koi carp farming. The optimal adding level of dietary *Chlorella* was 5%. Hence, dietary *Chlorella* could promote the growth performance, blood factors and the activity of digestive enzyme.

References

Abolfathis, M., Hajimoradloo, A., Ghorbani, R. and Zamani, A., 2012. Effect of starvation and refeeding on digestive enzyme activities in juvenile roach, *Rutilus rutilus caspicus. Comparative Biochemistry and Physiology A: Molecular Integrative Physiology*, 161(**2**), 166-173. Doi: 10.1016/j.cbpa.2011.10.020.

Ajiboye, O., Yakubu A. and Adams T., 2012. A perspective on the ingestion and nutritional effects of feed additives in farmed fish species.

World Journal of Fisheries and Marine Sciences, 4, 87-101.

Akrami, R., 2010. The effects of mannan oligosaccharide on growth,survival, body composition and resistance of Caspian Sea white fish (*Rutilus rutilus*). *Journal of Marine Science and Technology*, 21, 8.

Anderson, D.P., Robertson, B.S. and Dixon O.W., 1979. Plaque-forming cells and humoral antibody in rainbow trout (*Salmo gairdneri*) induced by immersion in a *Yersinia rockery* O-antigen preparation. *Journal of the Fisheries Research Board of Canada*, 36(**6**), 636-639. Doi: 10.1139/f79-092.

AOAC (Association of Official Analytical Chemists).1990. Official methods of analysis of the Association of Official Analytical Chemists, 15th edition. AOAC, Inc., Arlington, Virginia, USA.

Ashraf, M., Javaid, M., Rashid, T., Ayub, M., Zafar, A., Ali, S., 2011. Replacement of expensive pure nutritive media with low cost commercial fertilizers for mass culture of freshwater algae, Chlorella vulgaris. *International Journal of Biological Agriculture*, 13, 44-49.

Badwy, E.M. and Ibrahim, M.M., Zeinhom, M.M., 2008. Partial replacement of fishmeal with dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) diets. pp. 801-811. 8th International Symposium on Tilapia in Aquaculture. Central Laboratory for Aquaculture Research, Agricultural Research Center, Ministry of Agriculture, Egypt.

Becker, E.W., 2007. Micro-algae as a source of protein. *Biotchnology Advances*, 25, 207-210.

Güroy, D., Güroy, B., Merrifield, D.L., Ergün, S., Tekinay, A.A. and Yiğit, M., 2011. Effect of dietary *Ulva* and *Spirulina* on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period. *Journal of Animal Physiology and Animal Nutrition*, 95, 320-327. Doi: 10.1111/j.1439-0396.2010.01057.x

Huang, X., Zhou H. and Zhang, H., 2006. The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, *Fennero penaeus* chinensis. *Fish and Shellfish Immunology*, 20, 750–757.

Johnson, S.L. and Zon, L.I., 1999. Genetic backgrounds and some standard stocks and strains used in zebrafish developmental biology and genetics. *Methods in Cell Biology*, 60, 357-359.

Kay, R.A., 1991. Microalgae as food and supplement. Critical reviews. *Food Science Nutrition*, 30, 555-573.

Kim, S.C., Anderson, A.T. and Arthington, J.D., 2007. Optimizing nitrogen utilization in growing steers fed forage diets supplemented with dried citrus pulp. *Journal of Animal Sciences*, 85(**10**), 2548-2555.

Kwak, J.H., Baek, S.H., Woo, Y., Han, J.K., Kim,B.G., Kim, O.Y.

and Lee, J.H., 2012. Beneficialimmunostimulatory effect of short-term Chlorella supplementation: enhancement of natural killer cell activity and earlyinflammatory response (randomized, double-blinded placebo-controlled trial. *Journal of Nutirition*, 11, 53.

Liao, W.-L., Takeuchi T., Watanabe T. Yamaguchi K., 1990. Eifect of dietary Spirulina supplementation on extractive nitrogenous constituent and sensory test of cultured striped jack flesh. *Journal of the Tokyo University of Fisheries*, 77(**2**), 241-246.

Lubitz, J.A., 1963. The protein quality, digestibility, and composition of algae, chlorella 71105. *Journal of Food Science*, 28, 229 -232.

Miyachi S., 1995. Diversity of microalgae and their possible application, environmental impacts of aquatic biotechnology, OECD, Paris, France, pp. 28-31.

Mustafa, M.G., Umino, T. and Nakagawa, H., 1994. The effect of Spirulina feeding on muscle protein deposition in red sea bream,Pagrus major. *Journal of Applied Ichthyology*, 10, 141-145.

Mustafa, M.G. and Nakagawa, H., 1995. A review: dietary benefits of algae as an additive in fish feed. *Israeli Journal of Aquaculture-bamidgeh*, 47,155-162.

Mustafa, M.G., Umino, T., and Nakagawa, H., 1997. Limited synergistic effect of dietary Spirulina on vitamin C nutrition of red sea bream Pagrus major. *Journal of Marine Biotechnology*, 5, 129-132.

Nakagawa, H., Kumai, H., Nakamura, M. and Kasahara, S., 1982. Effect of feeding of Chlorella-extract supplement in diet on cultured yellow tail. II. Effect on resisting power monitored by blood parameters against air dipping. *Suisanzoshoku*, 30, 76-83 (In Japanese with English abstract).

Nakagawa, S., Yoshida, S., Hirao, Y., Kasuga, S. and Fuwa, T. 1985. Biological effects of biosynthetic human EGF on the growth of mammalian cells in vitro. Differentiation. 29, 284-288.

Nakagawa, H., Kumai, H. Nakamura, M. and Kasahara, S., 1985. Effect of algae supplemented diet on serum and body constituents of cultured yellow tail. *Bulletin of the Japanese Scientific Fisheries*, 51, 279-286 (In Japanese with English abstract).

Nakagawa, H., 1997. Effect of dietary algae on improvement of lipid metabolism in fish. *Biomedicine and Pharmacotherapy*, 51(**8**), 345-348

Nakagawa, H., Mustafa, M.G, Takii, K, Umino, T. and Kumai, H., 2000. Effect of dietary cateechin and *Spirulina* on vitamin C metabolism in red sea bream. *Fisheries Science*, 66, 321- 326.

Nakazoe, J., Kimura, S., Yokoyoma, M. and Iida, H., 1986. Effect of the supplementation of algae or lipids to the diets on the growth and body composition of nibber, *Girella punctate* Gray. *Bulletin of the Tokai*

Regional Fisheries Research Laboratory, 120, 43-51.

Nandeesha, M.C., De Silva, S.S., Krishnamoorthy, D., Dathathri, K., 1994. Use of mixed feeding schedules in fish culture. I. field trails on catla, rohu and common carp. *Aquacultural Fish Management*, 25, 659-670.

Nandeesha, M.C., Gangadhar, B., Varghese, T.J. and Keshavanath, P., 1998. Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. *Aquacultural Research*

Regunathan, C. and Wesley, S.G., 2006. Pigment deficiency correction in shrimp broodstock using Spirulina as a carotenoid source. *Aquaculture Nutrition*, 12, 425–432.

Radhakrishnan, S., Bhavan, P.S., Seenivasan, C., Shanthim R., Poongodi, R., 2015. Influence of medicinal herbs (*Alteranthera sessilis, Ecliptaalba* and *Cissus qudrangularis*) on growth and biochemical parameters of the freshwater prawn *Macrobrachium rosenbergii. Aquaculture International.* (Doi: 10.1007/s10499-013-9666-1).

Patnaik, S., Samocha, T.M., Davis, D.A., Bullis, R.A. and Browdy, C.L.,2006. The use of HUFA rich algal meals in diets for Litopenaeus vannamei. *Aquaculture Nutrition*, 12, 395–401.

Sergejevova, M. and Masojıdek, J., 2013. Chlorella biomass as feed supplement for freshwater fish: sterlet, *Acipenser ruthenus*. *Aquaculture Research*, 44, 157–159.

Sommer, U., 1992. Phosphorous-limited Daphnia: Intraspecific facilitation instead of ompetition. *Limnology and Oceanography*, 37, 966–973.

Takeuchi, T., Lu, J., Yoshizaki, G. and Satoh, S., 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fisheries Science*, 68, 34-40. Doi: 10.1046/j.1444-2906.2002.00386.

Twigg, D., 2001. How to Keep Koi: An Essential Guide. Wiley, ISBN-13: 9780764562426.

Umesh, N.R., Dathatri, K., Nandeesha, M.C., Gangadhara, B., Varghese, T.J., 1994. Digestibility of dry matter and protein from *Spirulina platensis* by common carp, *Cyprinus carpio*, with a note on time of faeces collection in digestibility estimations. In: De Silva SS (Ed.), Fish nutrition Research Asia, Proceedings of the Fifth Asian Fish Nutrition Workshop, Asian Fisheries Society, Manilla, pp. 81-84.

Watanabe, Y., Liao, W.L., Takeuchi, T. and Yamamoto, H., 1990. Effect of dietary *Spirulina* supplementation on growth performance and flesh lipids of cultured striped jack. *Journal of the Tokyo University of Fisheries*, 77, 231-239. (In Japanese with English abstract).

Wotton, I.D. and Freeman, H., 1982. Microanalysis in medical biochemistry. Churchill, New York, USA.

Xu, B.T., Yamasaki, S. and Hirata, H., 1993. Supplementary Ulvu sp. var. meal level in diet of Japanese flounder, Puralichrhys olivaceus. Suisanzo-shoku 41, 461-468.

Xu, W., Gao, Z., Qi, Z., Qiu, M., Peng, J. and Shao, R., 2014. Effect of dietary Chlorella on the growth performance and physiological parameters of gibel carp, *Carassius auratus gibelio*. *Turkish Journal of Fisheries and Aquatic Sciences*, 14, 53–57.

Yamaguchi, K., 1996. Recent advance in micro-algal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *Journal of Applied Phycology*, 8(**6**), 487-490. Doi: 10.1007/BF02186327

Yildiz, F.H., Liu, X.S., Heydorn, A., and Schoolnik, G.K., 2002. Molecular analysis of rugosity in a vibrio chlorea O1 El Tor phase variant. *Molecular Microbiology*, 53, 497-515. Doi: 10.1111/j.1365-2958. 2004.04154.

Yone, Y., Furuichi, M. and Urano, K., 1986. Effects of dietary wakame *Undaria pinnatifida* and *Ascophyllum nodosum* supplements on growth, feed efficiency and proximate compositions of liver and muscle of red sea bream. Nippon Suisan Gakkaishi, 52, 1465- 1468.

Yu, J., Starr, D.A., Wu, X., Parkhurst, S.M., Zhuang, Y., Xu, R. and Han, M., 2006. The KASH domain protein MSP-300 plays an essential role in nuclear anchoring during Drosophila oogenesis. *Developmental Biology*, 289(**2**), 336-345. Doi: 10.1016/j.ydbio.2005.10.027

Zhang, Q., Giu, M., Xu, W., Gao, Z., Shao, R. and Qi, Z., 2014. Effects of dietary administration of Chlorella on the immune status of Gibel carp, *Carassius auratus* gibelio. *Italian Journal of Animal Science*, 13, 653–656.