DOI: 10.22059/ijvm.2018.244747.1004856 *Iranian Journal of Veterinary Medicine*

Effect of Adding Microalgae *Chlorella* sp. on Some Biological Parameters and Proximate Analysis of Common Carp *Cyprinus Carpio* L.

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Abstract:

BACKGROUND: Chlorella is commonly used as a dietary supplement. Various studies and researches have been done regarding the intervention of microalgae as foods for a long time. And, it is found that Chlorella have potential health benefits, in terms of their protein and antioxidant content. Thus, with the objective of formulating nutritional supplements, this algae is being harvested in artificial ponds on a large scale and could be used in different ways such as feed supplement or a replacement with a source of protein.

OBJECTIVES: evaluate the effect of feed containing various ratios of Chlorella on some biological parameters in common carp.

METHODS: The experiment was conducted for 105 days and for this purpose 200 common carp fingerlings (*C. carpio* L.) were brought from a local fish aquarium in Iraq. Mean initial weight was 35.7g. The fish were acclimated to laboratory conditions and fed with control pellets (29% protein) prior to the feeding trials for 21 days in fish laboratory/Dept. of Animal Sciences/College of Agricultural Sciences/University of Sulaimani. In T1 fish were fed a diet with 0 Chlorella gm/kg ,while in T2, fish were fed a diet with 2.5 Chlorella gm/kg, T3 represents the third treatment, in which fish were fed a diet with 5 Chlorella gm/kg, and in T4 fish were fed a diet with 7.5 Chlorella gm/kg.

RESULTS: The present study clearly showed that feeding algae as a feed additive to fish remarkably changed the studied biological parameters, in Hepatosomatic index all treatments were significantly different from the control, Spleenosomatic index of the control and T4 were significantly higher than others, in Gonadosomatic index the T2 and T4 significantly differed, while T4 was significantly higher than other treatments in Kidneysomatic index. Fish weight without viscera and weight without viscera & head differ significantly among treatments with the addition of Chlorella the diet.

CONCLUSIONS: Chlorella can be used as protein rich sources to replace fishmeal or as feeding additives in the diets of common carp fingerlings with different levels. Chlorella products are safe and of good quality.

Keywords:

Common carp, Gonadosomatic index, Hepatosomatic index, Kidneysomatic index, Microalgae chlorella

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Received: 7 March 2017

Accepted: 23 June 2017

Introduction

Feed accounts for more than half of the variable operating costs in many aquaculture operations today (NRC, 1993). Therefore, the potential use of unconventional feed ingredients such as algae, as feed inputs as a replacement of high cost feed stuffs such as fishmeal has been increasing. Algae have gained attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production is high and have good protein, vitamins and essential fatty acids contents (El-Hindawyet al. 2006). The use of microalgae as fish feed inputs has been studied with encouraging results. Broun, (1980), reported positive growth performance in fish feed diets containing algae cells. Zeinhom. (2004) found that fish fed diet containing 15% algae increased significantly by the digestibility coefficient of dry matter (92.5%), crude protein (87.63%), ether extract (88.45%) and energy (81.41%).

Chlorella is a green alga, belonging to the division Chlorophyta. It is eukaryotic and unicellular, which synthesizes its food by the process of photosynthesis. In brief, chlorella is a more advanced form, as compared to Chlorella. Whereas Chlorella is a spiral shaped blue green algae (BGA), belonging to the division Cyanophyta. It is prokaryotic in cell organization, but possesses chlorophyll for photosynthesis (Al-Koye, 2013).

This study was designed to evaluate the use of Chlorella as feed additive in practical diets for common carp in terms of biological parameters. So the objective of this research is to evaluate the effect of feed containing various ratios of Chlorella on the growth of the common carp and to study some biological parameters of the common carp that have been fed various ratios of Chlorella, and evaluate their effect on some biological parameters in common carp.

Materials and Methods

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

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

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

Table 1. Chemical composition of the different diet.

Ingredients	Crude protein %	Crude fat %	Dry matter %	Crude Fiber %	Energy Kg/Kg	Percentage %
Animal protein concentrate	40	5	92.9	2.2	2107	10%
Yellow corn	8.9	3.6	89	2.2	3400	15%
Soybean meal	48	1.1	89	7	2230	40%
Barely	11	1.9	89	5.5	2640	15%
Wheat bran	15.7	4	89	11	1300	18%
Vitamins + Minerals	---	---	---	---	---	2%
Calculated chemical composition						
Crud protein				29.011		
Metabolizable energy (kcal/kg feed)				2304.7		
%Arginine				0.2394		
%Lysine				0.25375		
%Methionine + cysteine				0.12872		
%Threonine				0.017		
%Tryptophan				0.029		

Table 2. The nutritional information of used Chlorella.

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

Diet formulation: Experimental diets were prepared with Animal concentrate, wheat bran, soybean, barley and Chlorella, and the chemical composition of the different diets shown in Table 1. The ingredients were mixed with water to obtain dough. Then, the dough was passed through an electric mincer for pelleting by using Kenwood Multi-processors. The pellets were dried at room temperature for a few days and crushed to yield fine particles. The fish were fed 2 times a day, once at 9:00 am and another time at 2:00 pm. Feeding rate started with 3% of biomass depending on satiation level. Fish were individually weighed bimonthly. The feeding amount was then recalculated according to weekly weights. The feeding trial continued for 12 weeks.

Used Chlorella: Organic Chlorella powder from (Chlorella pyrenoidosa) a product packed by Nukraft, 433 Caledonian Road, London N7 9BG UK. The nutritional information as labelled isshown in Table 2 as below:

Biological (Health Aspects) parameters: All the fish samples were scarified and the abdominal cavity quickly opened in order to remove all organs to be weighed at once. They were calculated as follow:

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

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

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

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

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

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

Condition factor= Fish weight / Body length3

Proximate analyses: All fish samples are used for the chemical analysis of the muscle (Moisture %, crude protein%, ether extract% and ash%) according to AOAC

Table 3. Effect of adding Chlorella in different levels on some biological indices of common carp *Cyprinus carpio* L. Mean values with different superscripts within a column differ significantly (P≤0.05).

Treatments	Hepatosomatic index	Spleenosomatic index	Gillsomatic index	Kidneysomatic index
T1	2.697 ^{b}	0.504 ^{ab}	3.386 ^{b}	0.433 ^{c}
T2	3.444 ^{a}	0.457 ^{b}	4.295 ^{a}	0.737 ^{b}
T3	3.336 ^{a}	0.351 ^{c}	3.514 ^{b}	0.862 ^{b}
T4	2.982 ^{ab}	0.555 ^{a}	4.654 ^{a}	1.221 ^{a}

Table 4. Effect of adding Chlorella in different levels on Intestine weight and length index, and Condition factor of common carp *Cyprinus carpio* L. Mean values with different superscripts within a column differ significantly (P≤0.05).

Treatments	Intestine weight index	Intestine length index	Condition factor
T1	3.566 ^{a}	36.999 ^{c}	1.729 ^{b}
T2	2.587 ^{b}	43.414 ^{b}	1.641 ^{c}
T3	3.263 ^{a}	37.637 ^{c}	1.847 ^{a}
T4	3.445 ^{a}	49.593 ^{a}	1.723 ^{b}

Table 5. Effect of adding Chlorella in different levels on fish meat indices of common carp *Cyprinus carpio* L. Mean values with different superscripts within a column differ significantly (P≤0.05).

Treatments	Fish wt. without viscera	Fish wt. without viscera & head
T1	81.930 ^{b}	61.310 ^{a}
T2	83.395 ^{a}	59.471 ^{ab}
T3	83.505 ^{a}	60.717 ^{ab}
T4	80.145 ^{c}	58.063 ^{b}

(2000) analytical methods.

Fish wt. without viscera% = Fish weight without viscera (g)/body weight (g) x 100

Fish wt. without viscera & head % = Fish weight without viscera & head (g)/body weight (g) x 100

Statistical analysis: Analysis of variance is conducted using the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 one way (ANOVA). Fisher's LSD test was used to compare between means of the control and experiment treatments.

Results

The present study clearly showed that feeding algae as a feed additive to fish remarkably changed the studied biological parameters as shown in Table 3, in HSI all treatments were significantly different from the control, SSI of the control and T4 were significantly higher than the others, in GSI the T2 and T4 differed significantly while T4 was significantly higher than other treatments in KSI.

The Intestine weight index, Intestine length index and condition factors differ significantly among treatments as shown in Table 4.

Fish weight without viscera and weight without viscera & head differ significantly among treatments with the addition of Chlorella the diet as observed in Table 5.

Discussion

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

index in all fish treatments other than the control. Many fishes occasionally ingest many species of algae, even carnivorous fishes. Nevertheless, ecologists have not regarded algae as an important food source for fishes (Radhakrishnan et at., 2015). The results of the present experiment suggest that algae promote physiological activities such as the increasing of the physio-biological parameters as observed in Table 3, and indicate the efficacy of using dietary algae as a feed additive for cultured fishes.

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

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

The protein content of Chlorella is 51-58% and contains many essential amino acids, showing that Chlorella could also be used as protein source for human food and animal diets (Becker, 2004). However, current applications of Chlorella mainly focus on human food. The research on its application in lower vertebrate was less. The potential for microalgae to enhance nutritional content of conventional food preparations and to act as probiotic agents that positively affect the health of humans and animals has a broad spectrum.

Today, microalgae, marketed as health food or food supplement, are commonly sold in the form of tablets, capsules, and liquids. Algae are also incorporated in pastas, snack foods, candy bars or gums, in drink mixes, and beverages, etc., either as nutritional supplement, or as source of natural food colorant (FAO, 2016).

The results of Zhang et al., (2014) showed that the Chlorella could significantly increase the growth of gibel carp, e.g. for fish fed with 0.8% Chlorella the body weight increased from 29.90±0.08 to 63.75±1.96 g with a WG of 33.85±1.96 g, which was higher than that of control group ($P<0.05$). The immune system represents a nodal point in the balance between animal health and disease (Barreda et at., 2014). Previous studies had found that Chlorella could be involved in the regulation of animal adaptive and innate immunity. Zhang, et al., (2014) found that the Chlorella could significantly increase the serum IgM and IgD levels of gibel carp. Increasing of IgD, one of the immunoglobins involved in mucosal defense (Salians et at., 2011), suggested that Chlorella might play some role in the mucosal immunity.

Zeinhom, (2004) found that, inclusion of algae in fish diets significantly ($P<0.05$) increased the live body weight (39.69 g) and

improved the FCR (2.33), and Nandeesha, et at. (1998) reported that body weight gain of Nile tilapia (*O. niloticus*) increased linearly with increasing the level of algae in fish diet at levels less than 20%.

Badwy et al., (2008) studied the effect of partial replacement of fish meal with dried microalgae (*Chlorella* spp and Scenedesmus spp) in Nile tilapia (Oreochromis niloticus) diets on fish growth performance, feed efficiency and body composition. In the study of Kim et al., (2002) found that dietary supplementation of 2% Chlorella powder significantly improved growth and feed utilization of juvenile flounder.

Several factors contribute to the nutritional value of a microalga – including its size and shape, and digestibility as related to cell wall structure and composition, as well as biochemical composition (e.g. accumulation compounds, enzymes and toxins) and specific requirements of the target animal. For this reason, several studies have attempted to correlate the nutritional value of microalgae to their chemical profile.

However, results from feeding experiments are often difficult to interpret because of the confounding effects of other formulation additives. An examination of literature data – including those pertaining to microalga-based, compounded diet emulsions, have meanwhile allowed a few general conclusions to be reached.

Conclusiosn: As aquaculture continues to develop, there will be an increasing need to use alternative plant proteins in aquaculture diets so that aqua eco-systems will be sustainable. Chlorella can be used as protein rich sources to replace fishmeal or as feeding additives in the diets of common carp fingerlings with different levels. Products that are safe and of good quality.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۳، ۲۰۵-۱۹۹

اثر افزودن میکروالگای کلرلا اسپ. بر روی برخی از پارامترهای بیولوژیکی و تجزیه و تحلیل تقریبی کپور معمولی *Cyprinus carpio* L.

عبدالرحمن نسرین محی الدین* عبید سنا هشیار خیدیر اریان عزیز عمر بینایی بختیار هما راشد دشتی بهمن بها الدین لاوک هیوا

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(دریافت مقاله: ۱۶ اسفند ماه ۱۳۹۶، پذیرش نهایی: ۲ خرداد ماه ۱۳۹۷)

چکیده

زمینه مطالعه: کلرلا به عنوان مکمل‌های غذایی مورد اســتفاده قرار می‌گیرد. مطالعات و تحقیقات مختلف در مورد مداخلات میکروالگ‌ها به عنوان مواد غذایی از مدت‌ها قبل انجام شــده اســت. و مشــخص شده است که کلرلا از نظر میزان پروتئین و آنتی اکسیدان، دارای مزایایی بالقوه برای سلامتی است. بنابراین، با هدف فرمول کردن مکمل‌های غذایی، این جلبک‌ها در حوضچه‌های مصنوعی در مقیاس وسیع برداشت می‌شوند و می‌توانند به شیوه‌ای متفاوت از جمله مکمل غذایی یا جایگزینی با منبع پروتئین استفاده شوند.

هدف: ارزیابی اثر تغذیه حاوی نسبت‌های مختلف کلرلا بر برخی از پارامترهای بیولوژیکی در کپور معمولی.

روش کار: این آزمایش به مدت ۱۰۵ روز انجام شد و برای این منظور ۲۰۰ عدد کپور معمولی انگشت قد آکواریوم ماهی محلی در عراق آورده شد. میانگین وزن اولیه ۳۵/۷g بود. ماهی‌ها با شرایط آزمایشگاهی سازگار شدند و به مدت ۲۱ روز در آزمایشگاه ماهی / گروه علوم حیوانی / دانشکده علوم کشاورزی / دانشگاه سلیمانیه با پلت‌های کنترل (۲۹٪ پروتئین) تغذیه شدند. در گروه T۱ ماهی با رژیم غذایی ۰ کلرلا گرم/کیلوگرم تغذیه شد، در حالی که در T۲ ماهی با رژیم غذایی حاوی کلرلا ۲/۵ kg/kg تغذیه شد، T۳ نشان دهنده درمان سوم بود که در آن ماهی‌ها با رژیم غذایی ۵ کلرلا گرم / کیلوگرم رژیم غذایی و در گروه T۴ ماهی با رژیم غذایی حاوی ۷/۵ کلرلا گرم در کیلو گرم تغذیه شد.

نتایج: مطالعه حاضر به وضوح نشان داد که تغذیه جلبک‌ها به عنوان افزودنی خوراکی به طور قابل توجهی پارامترهای بیولوژیکی مورد مطالعه را تغییر داده است. در شاخص Hepatosomatic همه تیمارها به طور معنی‌داری نسبت به شاهد متفاوت بودند، شاخص Spleenosomatic شــاهد و T۴ نســبت به سایر موارد بیشتر بود، در شــاخص Gonadosomatic T۲ و T۴ به طور معنی‌داری متفاوت بودند، در حالی که T۴ به طور معنی‌داری بیشتر از سایر تیمارها در شاخص Kidneysomatic بود. وزن ماهی بدون علف احشاء و وزن بدون علف احشاء و سر در بین تیمارها با افزودن کلرلا در رژیم غذایی تفاوت معنی‌داری دارد.

نتیجه‌گیری نهایی: کلرلا می‌تواند به عنوان منابع غنی از پروتئین برای جایگزینی غذاهای ماهی و یا افزودنی مواد غذایی در رژیم‌های کپور معمولی انگشت قد با سطوح مختلف استفاده شود. کلرلا از محصولات ایمن و با کیفیت خوب می‌باشد.

واژه‌های کلیدی: کپور معمولی، شاخص Gonadosomatic، شاخص Hepatosomatic، شاخص Kidneysomatic، کلرلا

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