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Effect of Dietary Chlorella on the Growth Performance and Physiological Parameters of Gibel carp, *Carassius auratus gibelio*

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Abstract

In the present study, a chlorella-free control diet (C) and five experimental diets supplemented with 0.4~2.0% *Chlorella* were formulated. The gibel carp (*Carassius auratus gibelio*), one of the commercial fish in China, were fed with the designed diets for 60 days. At the end of trials, parameters of growth performance, blood and the digestive enzyme were analyzed. The results showed that the optimal dietary level of *Chlorella* as an additive in gibel carp diet was 0.8~1.2%. The growth performance including RGR, SGR, PER and PDR in 0.8 and 1.2% chlorella-adding group were significantly higher than that of control group ($P<0.05$). Meanwhile, some blood parameters including total protein, lysozyme in these two groups were also higher than in the control group. Furthermore, the chlorella could increase the digestive enzyme, e.g. amylase, lipase and protease in hepatopancreas and intestine. Our study confirmed that the *Chlorella* could be used as additive in gibel carp farming and the optimal adding level of *Chlorella* was 0.8%-1.2%, which could promote the growth performance, immune response and the activity of digestive enzyme.

Keywords: *Carassius auratus gibelio*, Chlorella, additive, growth performance.

Introduction

Microalgae consists of a broad spectrum of nutritious compounds including protein, vitamins, essential amino acids, minerals and pigments, which has attracted world interest for considering it as a dietary additive (Becker, 2007). Among the microalgae, *Chlorella*, belonging to the *Chlorophyta*, *Chlorophyceae* and *Chlorella*, are widely distributed in the nature, especially in fresh water. *Chlorella* can survive by photoautotrophy and heterotrophism by external carbon source. So, *Chlorella* is easily cultured in laboratory and possesses highly applied value (Yamaguchi, 1996).

It has been proved that *Chlorella* contains high content of protein, lipid, polysaccharide, vitamins, minerals and other nutritional substances, and those ingredients possess great bioactivity involving in many physiological activity. Tanaka *et al* (1998) found that the extraction of *Chlorella* could increase the CD4 cell number to inhibit the neoplasm metastasis and progression. Meanwhile, the *Chlorella* could protect the mice against *Listeria monocytogenes* infection (Dantas *et al*., 1999). 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, 2007). However, current applications of *Chlorella* mainly focus in human food. The research on its application in lower vertebrate was less.

Gibel carp (*Carassius auratus gibelio*) is an important economical food fish in China. Many studies have been carried on its nutrition, immunization and development (Xue and Cui, 2001; Hu *et al*., 2008; Van Campenhuout *et al*., 2010), but the application of chlorella in this species is not reported until now. So, in the present work we designed six diets that contain different levels of chlorella to treat the gibel carp and then the growth performance, blood parameters and digestive enzyme were detected. Those results provided the basis for the application of chlorella as additive in gibel carp.

Materials and Methods

Experimental Animals and Feeding

Gibel carp were purchased from a local fish farm and transferred to our laboratory using car equipped with automatic aerator. Fish were reared in 18 tanks (diameter 70 cm, water volume 250 L) with a temperature-controlled and water-recirculated system.

Fish were acclimated with the commercial diets to the new rearing conditions for 14 days.

After acclimation, the fish (Avg. weight 29.91±0.11 g) were weighed after being starved for 2 days to allow the gut to empty and then 540 fish were randomly divided into nine tanks (30 fish per tank). The tanks were then randomly divided into six groups (three tanks per group), with one control group and five experimental groups. The control group was fed with the basal diet without *Chlorella*, while the five experimental groups were fed with the diets supplemented with 0.4, 0.8, 1.2, 1.6 and 2.0% *Chlorella* dry powder, respectively. The ingredients and proximate composition of the experimental diets were shown in Table 1. The feeding trial lasted for 60 days. Fish were fed three times a day at a daily feeding rate of 8% of body weight at 6:30, 13:30 and 18:30 h. During the experiments, one-third of the water volume was exchanged once a week. The water temperature was 26˚C, dissolved oxygen content >5 mg/L, NH_3<0.05 mg/L, H_2S <0.1 mg/L and pH 6.8-7.8 during the course of the experiment.

Sampling and Chemical Analysis

At the beginning of the trial, the fish in each tank were mass weighed for the determination of the initial body weight (IBW). At the end of the 60-day period, the fish were starved for 24 h and batch weighed for the final body weight (FBW). The blood from five fish per tank was collected and send to Tinghu hospital (Yancheng, Jiangsu province, China) for determining the blood parameters. The diet and fish muscle samples were freeze dried and the proximate composition were analyzed according to the standard methods (Association of the Official Analytical Chemists 1990). The growth performances were calculated using the following formula:

WG (weight gain) =FBW (final body weight, g)-IBW (initial body weight, g)

RGR (relative gain rate, %)=[FBW (final body weight, g)-IBW (initial body weight, g)]/IBW×100

SGR (specific growth rate, %)=[(lnFBW-lnIBW)/number of feeding days]×100

FCR (feed conversion ratio)=[total feed supplied g DM/WG (g)]

PER (protein efficiency ratio)=[WG (g)/total protein fed (g DM)]

PDR (protein deposition ratio)=100×[FBW×FTP (final total protein of whole fish)-IBW×ITP (initial total protein of whole fish)]/ total protein in diets

The Activity of Digestive Enzymes in 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 MS222 (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).

Statistical Analysis

The final data were expressed as mean values ± standard error (SE) and analyzed by one-way analysis of variance (ANOVA). Percentage data were arcsine transformed before analysis of variance. Duncan's multiple range tests were analyzed among different group means. The significant level was set as $P<0.05$. All statistical analyses were performed using the SPSS11.0.

Results

Effect of *Chlorella* on the Growth Performance of Gibel Carp

The growth performance of fish fed with the experimental diets was summarized in Table 1. It was clear that the supplement of *Chlorella* could

Table 1. Performance for gibel carp fed with different experimental diets n=15; $\overline{X} \pm$ S.E

Diets	IBW	FBW	WG	RGR	SGR	PER	PDR	FCR
Control	30.01 ±0.099^{a}	58.164 ±0.39^{b}	28.15 ±0.32^{b}	93.80 ±0.87^{b}	1.10 ±0.01^{b}	1.31 ±0.01^{c}	92.43 ±0.33^{d}	2.27 ±0.01^{a}
0.4% Chlorella	29.98 ±0.12^{a}	60.27 ±0.90^{b}	30.29 ±1.01^{b}	101.07 ±3.77^{b}	1.16 ±0.03^{b}	1.38 ±0.02^{bc}	97.55 ±1.73^{c}	2.20 ±0.01^{a}
0.8% Chlorella	29.90 ±0.08^{a}	63.75 ±1.96^{a}	33.85 ±1.96^{a}	113.22 ±6.59^{a}	1.26 ±0.05^{a}	1.41 ±0.04^{ab}	99.68 ±2.44^{bc}	2.04 ±0.06^{b}
1.2% Chlorella	29.99 ±0.11^{a}	63.93 ±0.04^{a}	33.94 ±0.08^{a}	113.19 ±0.69^{a}	1.26 ±0.01^{a}	1.48 ±0.02^{a}	106.04 ±0.86^{a}	1.96 ±0.01^{b}
1.6% Chlorella	29.77 ±0.08^{a}	65.76 ±0.87^{a}	36.00 ±0.93^{a}	120.96 ±3.37^{a}	1.32 ±0.02^{a}	1.48 ±0.02^{a}	105.85 ±1.68^{a}	1.96 ±0.02^{b}
2.0% Chlorella	29.83 ±0.14^{a}	65.64 ±0.60^{a}	35.81 ±0.50^{a}	120.02 ±1.38^{a}	1.31 ±0.01^{a}	1.45 ±0.03^{a}	103.25 ±1.94^{ab}	2.01 ±0.05^{b}

Note: Values (mean ± SE) in the same column not sharing a common superscript letter are significantly different ($P<0.05$).

significantly increase the growth performance of gibel carp. During the 60-day trial, fish fed with 0.8% *Chlorella* grew from 29.90±0.08 g to 63.75 ± 1.96 g, with a RGR of 113.22±6.59 and a SGR of 1.26±0.05. The PER and PDR in 0.8% *Chlorella* group were 1.41±0.04 and 99.68±2.44, respectively. The RGR, SGR, PER and PDR in 0.8% *Chlorella* group were all higher than that of control group ($P<0.05$), except the FCR were lower than that of control group ($P<0.05$). As the *Chlorella* content increased to 1.2%, the RGR, SGR, PER and PDR also increased correspondingly, while the FCR still remained lower than that of control group ($P<0.05$). Compared with 1.2% chlorella group, the RGR, SGR, PER, PDR and FCR in 1.6% and 2.0% *Chlorella* groups remained unchanged ($P>0.05$), but these results still shared statistic significance with the corresponding results in the control group ($P<0.05$).

Effect of *Chlorella* on the Blood Parameters of Gibel Carp

The blood parameters of fish fed with the experimental diets were shown in Table 2. Analysis of the data in Table 2 suggested that the dietary chlorella mainly affected the parameters involved in protein/lipid metabolism and immunity of gibel carp. The total protein of fish in 0.4-2.0% *Chlorella* groups increased when compared with the control group ($P<0.05$). The cholesterol of fish in 1.6% and 2.0% *Chlorella* group were lower than that of fish in control group and 0.4%-, 0.8%- and 1.2%-*Chlorella* group ($P<0.05$). The ALT and AST of fish in different groups exerted an increasing and then back to normal level pattern ($P>0.05$). The immunity parameters including POD, SOD and lysozyme exerted an increasing pattern with the increase of dietary *Chlorella* ($P<0.05$).

Effect of *Chlorella* on the Digestive Enzyme

The activity of three digestive enzyme including amylase, lipase and protease in hepatopancreas and intestine were examined, and the results were summarized in Table 3. Compared with the control group, these three enzymes exerted an increasing pattern with the increase of dietary *Chlorella* ($P<0.05$). The amylase, lipase and protease in hepatopancreas of fish in control group were 286.23 ± 2.85, 29.32 ± 0.62 and 17.34 ± 0.24 u per mg protein, while the activity of these three enzyme in fish fed with 2.0% *Chlorella* group were 404.11±26.98, 41.74±3.46 and 26.62±0.45 u per mg protein, which increased nearly 1.4-, 1.4-, and 1.53 folds, respectively. Compared with the control group, the activity of these three enzymes in gut of fish in 2.0% *Chlorella* group increased 1.5-, 1.6- and 1.1-fold.

Discussion

In the present study, the different contents of *Chlorella* were added in the basal diet of gibel carp and the effects of *Chlorella* on the growth performance, blood parameters and digestive enzyme were detected. Our results indicated that chlorella can be a good choice for using as an additive for fish diets.

Due to high contents of protein, some microalgae have been used as fish meal substitution in fish diet. It has been found that the survival rate and protein content of juvenile tilapia (*Oreochromis niloticus*) increased greatly after only feed with *Spirulina* for 63 days (Takeuchi *et al*., 2002). Palmegiano *et al.* (2008) also found that *Spirulina* combined with plant oil could be a good alternative to sturgeon diet. Here, we found that the dietary adding microalgae could significantly improve the growth performance of fish, e.g. the RGR nearly increase 113.19% in the diet adding 1.2% chlorella. Our results were in accordance with the observation of Palmegiano *et al* (2005). It might be the bioactive ingredients e.g. chlorella growth factor (CGF) that promote the growth of fish (Yamaguchi, 1996). We also found that it was not better to add more chlorella in diet. Compared with fish fed with basal diet the performance of fish fed with 0.4% chlorella diet remained unchanged ($P>0.05$). Meanwhile, the performance in 1.6-2.0% *Chlorella* group shared no significantly changes with that of 1.2% *Chlorella* group. So we recommended the optimal adding of *Chlorella* was 0.8-1.2%.

0.8-1.2% *Chlorella* in diets could significantly

Table 2. Blood parameters of fish fed with experimental diets

	Control	0.4% Chlorella	0.8% Chlorella	1.2% Chlorella	1.6% Chlorella	2.0% Chlorella
Total protein (TP)	$56.75±1.20^{c}$	$57.45±0.73^{bc}$	$61.60±2.40^{ab}$	$63.58±1.13^{a}$	$62.87±1.46^{a}$	$61.40±1.13^{ab}$
Albumin	$33.15±1.60^{b}$	$32.98±0.88^{b}$	$35.35±0.97^{ab}$	$36.63±0.41^{a}$	$36.83±0.66^{a}$	$35.20±0.78^{ab}$
Globins	$23.02±0.71^{b}$	$24.30±0.62^{ab}$	$25.67±1.05^{ab}$	$26.07±0.49^{a}$	$25.37±1.10^{ab}$	$24.70±1.48^{ab}$
A:G ratio	$1.44±0.04^{a}$	$1.36±0.04^{a}$	$1.39±0.09^{a}$	$1.41±0.03^{a}$	$1.47±0.09^{a}$	$1.45±0.09^{a}$
Glucose (GLU)	$3.26±0.10^{a}$	$3.55±0.26^{a}$	$4.11±0.77^{a}$	$4.37±0.59^{a}$	$3.44±0.24^{a}$	$4.01±0.58^{a}$
Cholesterol	$7.19±0.08^{a}$	$6.78±0.08^{abc}$	$7.042±0.35^{ab}$	$7.07±0.04^{ab}$	$6.44±0.13^{c}$	$6.59±0.12^{bc}$
POD	$37.18±0.85^{b}$	$40.56±1.08^{b}$	$41.48±0.58^{b}$	$49.11±2.36^{a}$	$50.07±1.90^{a}$	$47.78±0.89^{a}$
SOD	$22.83±0.84^{d}$	$24.42±1.64^{d}$	$30.54±3.31^{c}$	$39.99±0.21^{b}$	$49.62±0.68^{a}$	$44.35±0.75^{b}$
Lysozyme	$14.30±0.51^{d}$	$18.41±0.42^{c}$	$18.99±0.75^{bc}$	$20.47±0.4^{b}$	$23.21±0.84^{a}$	$23.61±0.42^{a}$

improve the blood parameters of gibel carp. Firstly, the *Chlorella* could increase the total protein (TP), albumin and globulins in the serum of gibel carp. Serum TP includes two groups: albumins and globulins. The albumins are synthesized by liver and served as protein transporters (Anderson *et al*., 1979). The globulins are synthesized by the mononuclear phagocyte system and play important roles in the immune system (Kumar *et al*., 2012). Our findings suggested that the dietary adding *Chlorella* could increase the immune response of gibel carp. In mammals, albumin-globulin ratio is used to evaluate different diseases in liver and kidney. The albumin-globulin ratio remained unchanged after 60 days feeding test suggesting that fish fed with *Chlorella* maintain healthy status. Secondly, the *Chlorella* could decrease the level of blood cholesterol, not the glucose of gibel carp, suggesting that the *Chlorella* might involve in the metabolism of lipid. The same case also found by Güroy *et al.* (2011). Thirdly, the *Chlorella* could increase the superoxide dismutase (SOD) and lysozyme of gibel carp. The superoxide dismutase (SOD) is one of the first lines of antioxidant defense which can protect cell and tissues against oxidative stress, and also play some roles in the protective immunity against bacterial (Muñoz-Montesino *et al*., 2004; Pham *et al*., 2009). Lysozyme is also an important defence molecule of the innate immune system, which is important in mediating protection against microbial invasion (Saurabh and Sahoo, 2008). Increasing of SOD and lysozyme by dietary *Chlorella* suggested that the chlorella might contain some bioactive substances involving in the regulating of fish immune response.

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 al*., 2012). In the current study, we found that the dietary *Chlorella* could significantly increase the digestive enzyme in the hepatopancreas and intestine of gibel carp, suggesting the *Chlorella* could enhance the diet utilization rate by increasing the activity of digestive enzyme.

In conclusion, *Chlorella* could be used as a feed additive in gibel carp farming. The optimal adding level of dietary *Chlorella* was 0.8-1.2%. Dietary *Chlorella* could promote the growth performance, immune response and the activity of digestive enzyme.

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