Evaluation of growth and survival of Zebra fish, *Danio rerio* by flake food that formulated with spirulina

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Abstract

Danio rerio is one of the most beautiful ornamental fish living in fresh water and tropical water having a lot of fans in our country. This study aimed at investigating the effect of flaked diet with spirulina microalga feeding on growth and survival rates of *Danio rerio* in Mahshahr, south of Iran in 2014. Three hundred and sixty zebra fish have been purchased and were randomly distributed among 12 aquariums, which the first group or control have been received commercial concentrated food (Energy co.), the 2nd group were fed basal diet enriched with 4% Spirulina. The 3rd group received enriched food with 12% of the same supplement diet and and finally, the 4th group was designed for 25% of Spirulina.

The highest levels of condition factor and specific growth ratio were calculated for 4.394± 0.35 and 0.92 ±0.004, respectively. In addition, the lowest levels of daily feeding consumption and feed conversion ratio of 0. 35 ±0.035 and 0.037±0.01 were respectively obtained. In general, the inclusion of spirulina powder in feeding level of this fish would lead to gaining weight. Furthermore, the increase of spirulina microalga powder would affect the feed conversion ratio in its feeding level within a raising period of 60 days. Since there is no significant difference between 12 and 25% of the basal diet, it is concluded that the best feeding level is the basal diet enriched with4% spirulina.

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Keywords: spirulina, flake, *Danio rerio*, feeding level, growth, survival

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Introduction

The zebra fish, *Danio rerio,* is a small fish from the minnow family (Cyprinidae) with vibrantly colored horizontal stripes and was first described by the British surgeon Francis Hamilton (Hamilton 1822). Conveniently for developmental biology and geneticis, zebra fish have transparent and externally developing embryos and an essentially complete vertebrate body by 24 hours of development (though the embryonic/early larval pigment pattern is not fully formed for several more days). It has crucial model organism in genetics and developmental biology (Grunwald & Eisen 2002). Its reproduction stage is short and shows a single spawning with hundreds of offspring (Detrich, Westerfield & Zon 1999). Subsequently, the larvae metamorphose quickly into juveniles, and reproductive maturity can be attained in as little as six weeks. Adult zebra fish are easy to care for and breed and their reproduction is essentially a seasonal in the laboratory. Moreover, large numbers of embryos (50–300) can be obtained from females at intervals of as little as two weeks (Parichy 2006; Quigley and Parichy 2002; Clark, Hennig, Herwig, Cifton, Marra, Lehrach, Johnson & Group 2001; Vogel 2000).The Zebra danio is small-bodied, elongate, and without defensive morphology such as fin spines, making it vulnerable to a wide array of predators (Hill and Kapuscinski & Pavlowich 2011).

Spirulina is a cyanobacterium that has been commercially cultivated for many years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and b- carotene (Vonshak 1997). It can be considered as a nutritional supplement that has various health benefits for humans, and a feed supplement for animals having economic benefits. As an example, it can be a suitable food supplement when fed to trout, sea bass, fancy carp, red tilapia, shrimp and mollusk. It has been found that the alga can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat. Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp (Watanuki, Ota, Malin, Tassakka, Kato & Sakai 2006; Tongsiri, Mang-Amphan & Yuwadee 2010).

This study was designed to evaluate the use of enriched food by spirulina powder in practical diets for zebra Fish In Terms of Growth and Survival.

Materials and Methods

Three hundred and sixty zebra fish have been perched and were randomly divided in 12 aquariums and dedicated in the laboratory in Mahshahr: the first group or control have been received concentrated food called Energy in the market, the 2nd group received enriched food with 4% Spirulina. The 3rd group that received enriched food with 12% of the same supplement and the 4th group received enriched food with 25% of Spirulina, A feeding regime of 3% body weight per day was employed throughout the

trail. The amount of food was calculated and readjusted weekly according to change in the body weight and distributed in three equal portions for 60 days.

Data calculation

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

Specific Growth Rate (SGR) was calculated as the difference between the wet weight at the beginning of the experiment and on the day of sampling as

$$\text{SGR } (\%\text{day}^{-1}) = \frac{\ln W t_1 - \ln W t_0}{t_1} \times 100$$

Where lnWto and lnWt1 are the natural logarithm of weights of the fish at the beginning and end of each sampling period and t1 is the period between samplings in days.

Feed conversion ratio (FCR) was calculated by related feed consumption to gain in weight of fish. $FeedConversionRatio$=Feed intake (g)/Weight Gain (g)

The mean body weight (g) was calculated by dividing total wet weight of the fish in the aquarium by the number of fish in the aquarium.

Statistical analysis of data

Statistical analysis was performed using the Analysis of variance (ANOVA) two-way classification and Duncan's multiple Range Test, to determine differences between treatments means at significance rate of $P < 0.05$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis System (SAS) program.

Results

Body weight

Body weight indices were increased and between treatments were significantly different in zebra fish ($P<0.05$).There was no significant difference between treatments 12% and 25 % ($P> 0.05$).The highest and the lowest of this indices obtained in 25% (683.673±59.96) and control (310.713±41.55), respectively (Table 1).

Table 1 The average percentage of body weight gain in zebra fish (*Danio rerio*) during the study period of 60 days fed a formulated food Spirulina

During 60 days	The average weight (%)
Control	310.713±41.55[a]
T1: 4% spirulina	683.673±59.96[c]
T2: 12% spirulina	680.101±68.14[c]
T3: 25% spirulina	548.979±41.55[b]

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Specific growth rate

The highest specific growth rate was shown in treatment 12% (0.092±0.004) and the lowest indicator was in control on 60th day (0.022 ± 0.001), respectively. Between treatments were no significant differences on 20th, 40th and 60th day (p>0.05) (table 2).

Table 2 Average specific growth rate in zebra fish (*Danio rerio*) during the period of 60 days fed a formulated food Spirulina

Average specific growth rate	20th day	40th day	60th day
Control	0.045 ±0.003[a]	0.029± 0.0002[a]	0.022± 0.0001[a]
4%	0.090±0.004[c]	0.048± 0.0001[b]	0.033± 0.0001[a]
12%	0.092± 0.004[c]	0.049± 0.0001[b]	0.034± 0.0001[a]
25%	0.079± 0.003[a]	0.044± 0.0003[b]	0.033± 0.0001[a]

Different letters in each column indicate significant differences (p<0.05).

Conditional factor

The highest conditional factor was in 4% spirulina on 20th day (4.394±0.035) and the lowest was in 12% spirulina on 60thday (1.077% ±0.018). The average of conditional factor was no significant difference on 20th, 40th and 60th day (p>0.05) (table 3).

Table 3 The average of conditional factor in zebra fish (*Danio rerio*) during the period of 60 days fed a formulated food Spirulina

Conditional factor	20th day	40th day	60th day
Control	3.805 ±0.028[a]	2.342± 0.019[a]	3.093± 0.015[a]
4%	2.520±0.018[a]	1.951± 0.014[b]	1.077± 0.018[b]
12%	3.275± 0.012[a]	2.135± 0.022[ab]	1.241± 0.014[a]
25%	4.394± 0.035[a]	3.332± 0.011[a]	2.259± 0.011[a]

Different letters in each column indicate significant differences (p<0.05)

Food Conservation Ratio (FCR)

The highest food conservation ratio (FCR) was in control group on 20th day (0.162±0.01) and the lowest was in 4% Spirulina on this day (0.037±0.01). Between treatments were significant differences on 20th, 40th and 60th day (p<0.05) but between treatment 12% spirulina and control on 40th day and 60th day were no

significant differences (table 4). The results of this study were shown, using of different percent of Spirulina had no effect on survival rate ($p>0.05$).

Table 4 The food conservation ratio in zebra fish (*Danio rerio*) during the period of 60 days fed a formulated food Spirulina

Conditional factor	20th day	40th day	60th day
Control	0.162 ±0.01 [a]	0.086± 0.05[a]	0.086± 0.02[a]
4%	0.095±0.03[d]	0.132± 0.04[c]	0.136± 0.01[c]
12%	0.056± 0.02[c]	0.081± 0.05[a]	0.082± 0.05[a]
25%	0.037± 0.01[b]	0.038± 0.02[b]	0.040± 0.05[b]

Different letters in each column indicate significant differences ($p<0.05$).

Discussion

The present study showed that dietary supplementation with flake food that formulated with spirulina affected the feeding and growth parameters in *Danio rerio*. These results may possibly due to the improved feed intake and nutrient digestibility. Moreover, Spirulina contains several nutrients especially vitamins and minerals that may promote fish growth. According to growth performance in the present study considered to be T1 (4% Spirulina diet) was the best feed for the *Danio rerio* as the weight gain (683.673±59.96) and specific growth rate 0.034± 0.0001 were increased with the concentration of *Spirulina.* FCR decreases up to 0.040± 0.05 in T3 (25% Spirulina).

The highest levels of condition factor and specific growth ratio of 4.394± 0.35 and 0.92 ±0.004 were respectively obtained. In addition, the lowest levels of daily feeding consumption and feed conversion ratio of 0. 35 ±0.035 and 0.037±0.01 were respectively obtained. In general, the inclusion of spirulina powder in feeding level of this fish would lead to this fish gaining weight. Smith (1981)found that the effect of *Spirulina i*n the diet enhanced feed intake and growth in Red swordtail, *Xiphophorus helleri*. Ezhil, Jeyanthi, & Narayanan (2008) mentioned that the feed supplemented with *Spirulina* powder improved the feed conversion ratio and growth of rainbow trout. Ungsethaphand, Peerapornpisal, Whangehai and Sardsud (2010) reported that maximum survivability (91.11%) in hybrid red tilapia fed on 20% *Spirulina* . Scaria, Kumuthakalavalli and Xavier (2000) notified that the ornamental guppy and platy (*X. maculates*) consumed more feed inclusing *Spirulina* than other mushrooms and Azolla. Because, it is rich in proteins, vitamins, essential

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amino acids and fatty acids, *Spirulina* has been identified as a potential protein source for fish feed. In addition, this study found that the optimum rate of *Spirulina* in the fish practical diet is 30% or 300g/kg additives in feeds.

The present study suggests, up to 12% of *Spirulina* diet can be substituted in a formulated flake diet for zebra fish will not produce any adverse effects on fish growth and survival rate. In addition Spirulina promotes the growth and survival rate of the fish. Further the incorporated fishmeal can be reduced by the substitution of Spirulina in diet for culture of most ornamental fish species.

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بررسی رشد و بقاء ماهی زبرا *Danio rerio* در تغذیه با غذای پولکی غنی شده با اسپیرولینا

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چکیده

ماهی زبرا یکی از زیباترین ماهیان تزئینی آبهای شیرین و گرمسیری است که در کشور ما طرفداران زیادی دارد. این مطالعه بررسی اثر رژیم غذایی غذای پولکی غنی شده با ریزجلبک اسپیرولینا را روی میزان رشد و بقای ماهی زبرا در ماهشهر در جنوب ایران در سال ۱۳۹۴بود. برای این مطالعه ۳۶۰ ماهی زبرا خریداری شد و بطور تصادفی در ۱۲ آکواریوم قرار داده شد. گروه اول به عنوان شاهد از غذای کنسانتره تجاری (شرکت انرژی)،گروه دوم با غذای پایه غنی شده با ۴درصد اسپیرولینا، گروه سوم با ۱۲ درصد اسپیرولینا و گروه چهارم با ۲۵ درصد اسپیرولینا تغذیه شدند. بیشترین سطح فاکتور وضعیت و رشد ویژه به ترتیب ۰/۳۵ ± ۴/۳۹۴ و ۰/۰۰۴ ± ۰/۹۲ بود. همچنین پائین ترین سطح مصرف روزانه FCR به ترتیب ۰/۰۳۵ ±۰/۳۵ و ۰/۰۱± ۰/۰۳۷ بود. بطور کلی پودر اسپیرولینا سبب افزایش وزن شد. افزایش میزان پودر اسپیرولینا روی FCR در مدت ۶۰ روز تاثیر گذار بود. اختلاف معنی داری بین تیمار ۱۲ و ۲۵ درصدی اسپیرولینا در جیره پایه مشاهده نشده است. در مجموع بهترین سطح اسپیرولینا تیمار ۴ درصد بود.

کلمات کلیدی: اسپیرولینا، غذای پولکی، ماهی زبرا، سطوح تغذیه، رشد، بقاء

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