Int. J. Environ. Res., 5(3):763-768, Summer 2011
ISSN: 1735-6865

Effects of Daphnia magna fed with B group vitamins-enriched Chlorella sp. and Scenedesmus obliquus on the growth rate of Rutilus frisii kutum fry

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Received 12 Sep. 2010; Revised 4 March 2011; Accepted 14 March 2011

ABSTRACT: Currently, white fish or Kutum *Rutilus frisii* is considered to be a good candidate for commercial aquaculture. However, little information is available regarding the nutritional requirement of this fish fry. Experiments were conducted to examine the effects *Daphnia magna* as live food cultured on two freshwater green algae, *Chlorella sp*. and *Scenedesmus obliquus* enriched with different B group vitamin dosages (0.00 as control, 0.50, 1.00 and 2.00 mL of enriching solution per liter of algae culture medium) on the growth and survival rates of *Rutilus frisii kutum* fry. Results demonstrated that increasing in B group vitamin dosages are caused an increasing in nutritional value (Kcal) of two freshwater green algae species *Chlorella sp.* and *Scenedesmus obliquus* significantly. Significant differences were observed in BWG (%) and SGR between different vitamins treatments both the fed *Daphnia magna* cultured on *Chlorella sp*. and *Scenedesmus obliquus* enriched ($P<0.05$) but not in survival percentage ($P>0.05$).

Key words: *Rutilus frisii kutum fry* growth rate**,** Live food enrichment**,** Bgroup vitamins

INTRODUCTION

A wide variety of live organisms has been utilized in aquaculture, mainly because of their nutritional value, which is higher than that of prepared diets (Sipauba-Tavares, 2001). Live prey organisms, especially zooplankton, are generally used as initial food for certain species of fish fry (Legar *et al*., 1986). *Daphnia* is a frequently used food source in the freshwater larviculture (i.e. for different carp species) and in the ornamental fish industry (i.e. guppies, sword tails, black mollies and plattys etc.). However, since *Daphnia* is a freshwater species, it is not a suitable prey organism for marine organisms, because of its low content of essential fatty acids (Lavens & Sorgeloos, 1996). Live prey organisms like *Daphnia* can be bio-encapsulated with a variety of enrichment diets to manipulate their content in certain nutrients (Coutteau & Sorgeloos, 1997). Although the main vitamins of B group in nature are synthesized by unicellular organisms and plant cells, results of several investigations indicate that when algae are produced in mass culture for use as live food in aquaculture, enrichment of these algae with vitamins and other minerals more than the amount required to support their culture is essential. Vitamin B1, B7 and B12 are among the B group vitamins which are absorbed from the culture media are essential for the growth of algae. It has been estimated that about 70% of all planktonic algae require vitamin B_{12} as a growth factor in their culture (Azari Takami & Amini Charmehini, 2009).

Maruyama et al. (1989) estimated the intake of vitamin B12 by different strains of freshwater *Chlorella vulgaris in vitro* and found that all strains of the green algae *C. vulgaris* were capable of absorbing vitamin B12 from the culture medium although the amount of vitamin B12 absorbed differed in different strains. Evidence also showed that some of the strains were capable of absorbing 81% of the vitamins added to the culture medium, more than the required amount to support their culture. Some methods have significantly enhanced nutritional value of *Daphnia* as an important live food in the aquaculture (Ravet *et al*., 2003; Von Elert, 2002). This research presents a specific approach to enrich *D. magna* with a viamin mix (Goulden *et al*., 1982) for the

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monospecific culture of *Daphnia* on *Chlorella sp.* and *Scenedesmus obliquus*. This study aims at evaluating the role of the *Daphnia* cultured on freshwater green algae enriched with different dosages of B group vitamins on the growth performance of *kutum* fry.

MATERIALS & METHODS

Mass culture of green algae *Chlorella sp.* and *Scenedesmus obliquus*: The Zander (Z-8+N) medium was used for mass culture of the two green algae species. This is a general medium for culturing green and blue-green algae. Since green algae do not have heterocyst for nitrification, nitrogen should be added to their medium, therefore their medium is in the form of Z-8+N. The culture of algae was carried out in the laboratory by adding pure algal stock of the respected species to the medium. The amount of inseminated algae samples was equivalent to 1mg dried substance per liter of the new medium. The algae were culture at optimal conditions using mono white color (3500±350 Lux) with photoperiod of 14L:10D, at 25±2 ºC and pH=7.5 – 8 (Ordog, 1981). The algae were collected from the medium after 96 hours when they were at the end of their logarithmic growth phase and when they were at their maximum nutritional value and density. Three subsamples were used to determine the dry weight of the cultured algae while the rest were determined and before using as food for *Daphnia*. Algae at the density of 10 mg/L of *D. magna* culture medium was used for *Daphnia* feeding (Ordog, 1981). Preparation of enriching solution: Appropriate vitamin mixture containing all B group vitamins is applied for mono culture of *D. magna* with green algae (Table 1) was offered by Goulden *et al.* (1982). One mL of this vitamin mixture should be added to one liter of the enrichment medium (Lavens & sorgeloos, 1996). This vitamin mixture can be preserved in dark, cold and dry place of a freezer at -18 ºc for three weeks (Goulden *et al*., 1982).

Enrichment of algae with B group vitamins: Zero, 0.5, 1 and 2 mL of prepared B vitamin mixture were added to each liter of the algae culture medium separately, each with three replicates. The nutritional value of enriched algae was measured by the methods described by Parvaneh (2006).

Mass culture of *Daphnia magna*: Fifteen 50 liters fiberglass tanks containing 45 liters dichloride tap water were used for mass production of *D. magna*. The density of *Daphnia* considered in the culture was 50 individual per liter of the aquarium water. Those cultured in 12:12 h L:D light condition at the temperature of 22±1ºC were daily fed with green algae *Chlorella sp.* or *Scenedesmus obliquus* (Fallahi *et al*., 2005).

Table 1. A vitamin mixture for the monospecific culture of *Daphnia magna* on *Chlorella sp.* and *Scenedesmus obliquus* (Lavens & Sorgeloos, 1996)

Nutrient	Concentration of stock solution (µg/L)
Biotin	5
Thiamine	100
Pyridoxine	100
Pyridoxamine	3
Calcium Panthothenate	250
B12 (as mannitol)	100
Nicotinic acid	50
Nicotinomide	50
Folic acid	20
Riboflavin	30
Inositol	90

Rearing of *Rutilus frisii kutum* fry: *kutum* fry were obtained from Shahid Beheshti center in Rasht, Iran on July 2008. They were kept for 24 hours in two 500 liters fiberglass tanks to adapt to the new condition. The fish fry with initial wet weight of 260.87±20 mg were randomly distributed in groups of 10 individuals per tank in to 24 elliptic fiberglass tanks of 10 liter each (1 fish fry/L). Water was continuously aerated to keep oxygen levels close to 6.3±0.3 mg/L. Water quality was checked periodically; pH was 7.8±0.04, temperature was 21±2 ºC and photoperiod was 12L: 12D (OECD, 1980). *Daphnia magna* of the average weight 0.97± 0.1 mg that was cultured purely and massively was collected from fiberglass tanks. They were randomly distributed in to 24 incubators of 1 liter each. They were nourished by *Chlorella sp.* and *Scenedesmus obliquus* that already enriched with different dosages of B group vitamins (0, 0.5, 1 and 2 mL of B group vitamins per liter of algal culture medium each with three replicates) for 24 hours prior to be consumed by *kutum* fry. *Rutilus* fry were fed three times per day with *Daphnia magna* (Stickiney, 1991). After 10 days of fish fry rearing, growth index of fish fry were calculated based on following equations (Omergie *et al*., 2009):

$$BWG\% = W1 - W0 / W0 \times 100 \quad (1)$$

$$SGR = \ln(W1) - \ln(W0) / t \times 100 \quad (2)$$

BWG%=Body Weight Gain%

SGR=Specific Growth Rate

W0=Initial weight, W1=Final weight, t= time (10 days)

Shapiro wilk was used for examining the normality test of data distribution and parametric test was used for analyzing any of the measured factors with normal distribution. One-way analysis of variance (ANOVA) in 95% confidence interval was used for factors independently and tested by Duncan for comparing the average of different replicates of each treatment.

RESULTS & DISCUSSION

The nutritional value of enriched and non-enriched *Chlorella sp.* is shown in Table 2. The lowest nutritional value was observed in non-enriched algae (256.87 kcal). The nutritional value of algae enriched with 0.5, 1 and 2 dosages of enriching solution showed respectively 42%, 39% and 27% higher than non-enriched algae.

The nutritional value of enriched and non-enriched *Scenedesmus obliquus* is shown in Table 3. The lowest nutritional value was observed in non-enriched algae (337.63 kcal). The nutritional value of algae enriched with 0.5, 1 and 2 dosages of enriching solution obtained respectively 25%, 11% and 4% higher than non-enriched-algae.

Initial weight, Final weight, Body Weight Gain percentage (BWG%) and Specific Growth Rate (SGR) of *Rutilus* fry fed with *Daphnia magna* nourished with *Chlorella sp.* enriched with different dosages of B group vitamins are shown in Table 4. The highest BWG% and SGR were obtained from the treatment that *kutum* fry fed with *D. magna* nourished with *Chlorella sp.* enriched with dosages of 1 of B group vitamins. Table 4 shows significant differences between growth of the fish fry in this treatment and other treatments ($P<0.05$).

Table 2. Average nutritional value of *Chlorella sp.* enriched with different dosages of B group vitamins

Enriching solution dosages (mL/L)	0.0	0.5	1.0	2.0
Protein (g per 100g dry weight)	28.00[a]	52.85[b]	49.58[b]	46.37[b]
Lipid (g per 100g dry weight)	7.70[a]	8.46[b]	9.37[b]	6.58[a]
Carbohydrate (g per 100g dry weight)	16.66[a]	16.32[a]	15.45[a]	17.60[a]
Chlorella sp. Nutritional value (kcal)	256.87[a]	366.65[b]	357.54[b]	327.89[b]

Values are the mean (n=3). Numbers within the same row with different letters are significantly different ($P<0.05$).

Table 3. Average nutritional value of *Scenedesmus obliquus* enriched with different dosages of B group vitamins

Enriching solution dosages (mL/L)	0.0	0.5	1.0	2.0
Protein (g per 100g dry weight)	50.05[a]	51.62[a]	56.33[b]	50.05[a]
Lipid (g per 100g dry weight)	8.02[a]	8.38[a]	9.52[b]	10.44[b]
Carbohydrate (g per 100g dry weight)	12.58[b]	12.08[b]	12.34[b]	10.92[a]
Scenedesmus Nutritional value (kcal)	337.63[a]	345.47[ab]	376.95[b]	353.16[b]

Values are the mean (n=3). Numbers within the same row with different letters are significantly different ($P<0.05$).

Table 4. Average growth index of the *kutum* fry fed with *Daphnia magna* were nourished by *Chlorella sp.* enriched with different dosages of B group vitamins

Enriching solution dosages (mL/L)	0.0	0.5	1.0	2.0
Initial weight(mg)	254.93±12.29	218.93±30.51	207.50±12.8	237.70± 14.65
Final weight(mg)	290.76±15.43	253.53±28.46	251.27±9.81	273.50± 18.62
BWG (%)	14.06± 3.05[a]	16.1±3.22[a]	21.19±2.86[b]	15.03±1.53[a]
SGR	1.31 ±0.27[a]	1.49±0.28[a]	1.92 ±0.23[b]	1.4 ±0.13[a]
Survival rate (%)	100±0.0	100±0.0	100±0.0	100±0.0

Values are the mean ± SD (n=3). Numbers within the same row with different letters are significantly different (P<0.05)

Initial weight, Final weight, Body Weight Gain percentage (BWG%) and Specific Growth Rate (SGR) of *kutum* fry fed with *Daphnia magna* nourished with *Scenedesmus obliquus* enriched with different dosages of B group vitamins are shown in table 5. The highest BWG% and SGR were obtained from the treatment that *kutum* fry fed with *D. magna* nourished with *Scenedesmus obliquus* enriched with dosages of 2 of B group vitamins, and the lowest were obtained were obtained from non-enriched algae. Table 5 shows significant differences between growth of the fish fry in this treatment and treatments which algae enriched with dosages of 1.0 and 2.0 of enriching solution (P<0.05).

Enrichment of *Scenedesmus obliquus* and *Chlorella sp*. with a suitable mix of vitamin B group (Goulden *et al*., 1982) improved their nutritive value (Tables 2 and 3). Highest values in the two green algal species enriched with 0.5 and 1 mL/L culture medium were 42% and 11%, respectively which were significantly higher than control group (Tables 2 and 3). In the past few decades various techniques have been used to improve nutritive values of green algae as food for *Daphnia* among them enrichment with vitamins B_1 and B_{12} (D Agostino & Provasoli, 1970), vitamins B_1, B_7 and B_{12} (Provasoli & Carlucci, 1974) vitamins B12 (Keating, 1985) and with a suitable mixed culture medium of vitamin B group (Goulden *et al*., 1982). Although it appears that being autotrophic in nature green algae only require light and inorganic nutrients, investigations carried out by (Lwoff & Dusi, 1937 in Croft *et al*., 2006) found that some of the members of the phylum chlorophyta require vitamin B as a growth factor in their culture. Vitamins are not sources of energy, but they have important functions in relation to metabolism (metabolism of carbohydrates,

Table 5. Average growth index of the *kutum* fry fed with *Daphnia magna* were nourished by *Scenedesmus obliquus* enriched with different dosages of B group vitamins

Enriching solution dosages (mL/L)	0.0	0.5	1.0	2.0
Initial weight(mg)	304.99±9.46	296.66±8.12	281.56± 24.70	284.70±8.87
Final weight(mg)	378.66±12.07	377.20±3.39	364.06± 30.75	375.66±2.31
BWG (%)	21.16 ± 1.08[a]	27.2±3.59[a]	29.32±0.25[b]	32.02±3.44[b]
SGR	2.16±0.09[a]	2.4± 0.28[a]	2.57±0.57[b]	2.78±0.26[b]
Survival rate (%)	100±0.0	100± 0.0	100±0.0	100±0.0

Values are the mean ± SD (n=3). Numbers within the same row with different letters are significantly different (P<0.05)

lipids and protein) as well as in normal growth of body cells and the lack or deficiency of each one of them can cause serious deformities in the animal body (Shahbazi & Maleknia, 2003). It is known that vitamins of the B group and their analogues act as co-enzymes (Co-enzyme A, steel co-enzyme A, NAD^+, $NADP^+$, FMN, FAD, B_6PO_4, THF and Cobamide) to catalyze cell metabolism. Animal cells are not able to synthesize this group of co-enzymes and it is therefore supplied to them through food (Shahbazi & Maleknia, 2003).

Hirauyama et al. (1989) found that enrichment of freshwater *Chlorella vulgaris* K-22 by adding a suspension of vitamin B12 or by culturing the algae in a medium containing vitamin B12 resulted in a significant increase the nutritive value of chlorella, sometimes even reaching the nutritive value of marine chlorella. Watanabe et al. (1983) found a significant increase in nutritive value of rotifers, *Artemia* nauplii and *Moina* fed to fish larvae when they were enriched with fat soluble vitamins A & E.

Measurements of protein, lipid and carbohydrate content in each of the treatments indicate that highest nutritive value belonged to the treatment with highest protein content (Tables 2 and 3). Guisando & Serrano (1989) and McKee & Knowles (1987) determined protein, lipid and carbohydrate levels in *Daphnia magna* and *Brachionus calciflorus* and found that biomass and protein content in these two species was directly related to the dietary protein source, whereby increase in dietary protein levels resulted in an increase in the protein content and in turn in the biomass of these two species.

In the present study significant increase ($P<0.05$) in mean specific growth rates (SGR) and percentage body weight gain (BWG%) were observed in *kutum* fry fed *Daphnia magna* cultured on *Chlorella* and *Scenedesmus* enriched with different doses of vitamin B group as compared to the control group. the highest mean BWG% and SGR recorded in fish fry fed *Daphnia* cultured on *Chlorella* enriched with 1 mL/L vitamin B group and on *Scenedesmus* enriched with 2 mL/L vitamin B group (Table 4 and 5). However, since *Daphnia* is a freshwater organism it is not suitable as a prey organism for marine organisms because of its low content of essential fatty acid (Von Elert, 2002) and hence various methods are used to increase its nutritive value. To date no investigation has documented the effects of feeding *Daphnia* cultured on algae enriched with vitamin B group to marine fish and therefore the results of the present study are compared with other investigations using live food enriched with other vitamins. Azari Takami et al. (2004) showed the positive effects of vitamin C intake on growth factors, survival and resistance to environmental stress in rainbow trout larvae. Abediyan et al. (2006) studied the dietary effects of *D. magna* enriched with cod liver oil on growth and survival rates in *Acipenser persicus* larvae in two separate experiments. In the first experiment significant differences ($P<0.05$) were observed in growth in trials fed *Daphnia* enriched with cod liver oil while no significant differences ($P>0.05$) were detected in growth among the different trials studied in the second experiment. Jawaheri Baboli (2007) reported highest growth and survival rates in Caspian *salmon* (*Salmo truta caspius*) larvae fed *Artemia urmiana* nauplii enriched with long chain highly unsaturated fatty acids (HUFA) and vitamin C (20% ascorbyl palmitate) and lowest growth and survival in those fed formulated diets. *Huso huso* larvae fed *Artemia urmiana* enriched with vitamin E and long chain HUFA showed improved growth and resistance to environmental stress (Jalali *et al*., 2008), but Hafezieh et al. (2010) reported no better growth rate in Persian sturgeon larvae were fed *Artemia urmiana* enriched with HUFA and Vitamin C.

CONCLUSION

Based on the results of this study it may be concluded that feeding *kutum* fingerlings with *D. magna* cultured in freshwater algae enriched with vitamin B group resulted in improved SGR and BWG% in *kutum* fingerlings by increasing nutritive value of *D. magna.*

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