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Increases resistance of convict cichlid (*Amatitlania nigrofasciata*) to environmental stress by spirulina powder

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Abstract

In recent decades, interest in using micro algae powders as growth promoters and immune stimulants in aquaculture has been increased. This study evaluated the effect of diets containing 0, 25, 50, 75 and 100 g kg^{-1} Spirulina powder on increasing resistance of Convict cichlid (*Amatitlania nigrofasciata*) to environmental stress. To order this, 375 fish by initial average weight of 1.20±0.025 g were distributed in a completely randomized design with five treatments and three replications for 8 weeks. The fish were fed four times a day. At the end of experiment, 7 fish from each tank were exposed with shortage of oxygen for 24 hours. Also, for assessment the survival of fish to challenge with density, the fish were hold in 500 cc container without aeration for 5 days. The results showed that spirulina powder increases survival rate of fish after encountering environmental stress. Therefore, to improve resistance of Convict cichlid, against stressful conditions, spirulina can be recommended at rate of 10 %.

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Introduction

Ornamental fish are reared as pets and are generally kept for home or business decoration, or as a hobby. They are both from fresh and marine water (FAO, 2011).

Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products (Craig and Helfrich, 2002). Feed additives such as dried algae improve growth, feed efficiency, carcass quality, and physiological response to stress and disease in several species of fish (Jaime-Ceballos *et al.*, 2005). Spirulina (Table 1) has been one of the most widely used microalgal species in aquafeeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007). Also, its immunomodulatory activity has been shown in animal experiments, which demonstrated its enhancement of phagocytic and natural killer activities (Qureshi and Ali, 1996). Several studies have been conducted to investigate the effects of spirulina on growth, nutrient utilization and immune responses of various fish species, including rainbow trout *Oncorhynchus mykiss* (Matty and Smith, 1978), red sea bream *Pagrus major* (Mustafa *et al.*, 1997), common carp *Cyprinus carpio* (Nandeesha *et al.*, 1998), tilapia *Oreochromis niloticus* (Takeuchi *et al.*, 2002), white sturgeon *Acipenser transmontanus* (Palmegiano *et al.*, 2008), Mekong giant catfish *Pangasianodon gigas* (Tongsiri *et al.*, 2010) and African sharptooth catfish *Clarias gariepinus* (Promya and Chitmanat, 2011) reported by Kim *et al.* (2013).

Convict cichlid Fish with scientific name *Amatitlania nigrofasciata* is one of the ornamental species of cichlidae family. These fish are also known as Zebra or Convict Cichlid in Iran. They are available in two colors yellowish and white to gray with black stripes exist. This fish reaches a length of 14 cm and can live in temperatures 23-27 degrees Celsius. The origin of the fish from Panama to Mexico, and tends to lower the water to swim. This fish is omnivorous and can be pretty wide variety of foods used in feeding fish consume.

There is no information in the literature on the effects of dietary spirulina levels on increasing the resistance of Convict cichlid to environmental stress. Therefore, the main objective of the present study is to investigate the effects of different levels of Spirulina powder on increasing the resistance of Convict cichlid to environmental stress (*Amatitlania nigrofasciata*).

Materials and methods

Diet preparation

Five iso-nitrogenous and iso-energetic diets containing different levels of microalgae Spirulina (Sina Rizjolbak Qeshm Co., Iran) containing 0, 25, 50, 75 and 100 gr·Kg^{-1} were formulated(Table 2). All ingredients were thoroughly mixed with 300 cc kg^{-1} distilled water, and pellets were prepared using a moist pelleting machine. The pellets were dried at room temperature for 24 h and ground into desirable particle sizes. The dried diet was packaged into plastic bag and stored frozen at -20°C until use.

Growth experiment

Convict cichlid (*Amatitlania nigrofasciata*) were obtained from a private commercial farm (Khorramshahr, Khuzestan, Iran). The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Fish (initial mean weight, 1.20±0.025 g) were allocated randomly into 60 L circular plastic tanks with 25 fish per each tank for the feeding trial after being collectively weighed. fish were hand-fed to apparent satiation 4 times a day (8:30, 11:30, 14:30 and 17:30) for 8 weeks. During the experimental period, mean water temperature was

26.22±0.93°C, dissolved oxygen was 7.16±0.41 mg L^{-1} and the pH was 7.33±0.21. The photoperiod was left under natural conditions during the feeding trail.

Diets chemical analysis

Proximate analysis of diets was determined according to the method of AOAC (1995). Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System. Crude lipid was analyzed by ether extraction, moisture content by a dry oven drying at 105℃ for 24 h and ash by a furnace muffler (550℃ for 4 h).

Hypoxia stress

At the end trial period, seven fish from each tank were used randomly to induce hypoxia stress. The height water tanks are minimized and aeration was fully cut, fish were exposed to hypoxia for 24 hours. Mortality was recorded every 8 hours.

Density stress

Also, at end of the trial period, 7 fish per each tank were placed randomly in to container with approximate 500 cc of water with gentle aeration. Daily losses were record and dead fish exited. Record losses were carried out for 5 days.

Statistical analysis

In outline, this study was planned and executed entirely by accident. All data are collected normal distribution using the Shapiro-Wilk test was performed, and significant differences between treatments at different levels ($p \leq 0/05$) using ANOVA (One-way ANOVA) and post- Duncan test was examined. Analysis of all the data and the operations were performed by SPSS 19.0 software.

Results

The result of environmental stress on survival of fish fed diets containing different levels of spirulina powder were presented in Table 3, Figure 1 and 2. The result showed that, spirulina induces to increase significantly resistance of fish against hypoxia stress ($p<0.05$). So, higher survival rate was observed in treatment 3 with 7.5% spirulina.

Table 1. Biochemical composition of Spirulina (Ahmadzade-Nia *et al.*, 2011).

Componen	Percent (%)
Crude Protein	58-73
Carbohydrate	8-19
Fat	5-11
Moisture	3-7
Fiber	4-7
Ash	5-8
Digestible Energy(Kcal/kg)	3500

Also, the result of density stress showed, the survival rate of fish was increased with spirulina in diet of fish ($p<0.05$). So, higher survival rate was observed with 10 % spirulina. Also, control group had significantly lower survival.

Discussion

Among the microalgae used as foodstuffs for food supplements and animal feed in many parts of the world, Spirulina spp is the most popular due to high nutrient values and cost effectiveness at the farm scale (Meng-Umphan, 2009).

Research conducted by Duncan and Klesius (1996). found that spirulina alga was a good source of protein for animal feed, as well as containing high amounts of vitamins and minerals. Besides this, the cellular structure of spirulina alga is easily digestible and does not contain cellulose. Different levels of spirulina alga can be mixed with feeds according to the eating behaviors of the fish and differing abilities to digest the protein from plant sources (Promya and Chttmanat, 2011). The result of experiment showed that used spirulina in diet of fish induce to increase resistance of fish to environmental stress that it can be related to effective compounds attributed Spirulina powder.

Table 2. Formulation and proximate composition of experimental diets (%).

	Experimental Diets				
	0%	2.5%	5%	7.5%	10%
Dietary composition					
fish meal[a]	32.60	30.87	29.14	27.41	25.67
Spirulina powder	-	2.5	5	7.5	10
Beef heart powder	10	10	10	10	10
Wheat flour	27.53	26.49	25.45	24.41	23.39
Wheat bran	18	18	18	18	18
Fish Oil [b]	5.87	6.14	6.41	6.68	6.94
Vitamin premix[c]	2	2	2	2	2
Mineral premix[d]	2	2	2	2	2
Filler	2	2	2	2	2
Proximate Analyses (DM)					
Moisture	11.6	11.2	11.3	11.5	11.2
Crude protein (%)	35.6	35.6	35.8	35.7	35.5
Crude fat (%)	10.4	10.1	10.2	10.2	10.3
Energy(kcal/100gr)	300	300	300	300	300

[a]Clopeonella meal, Iran.

[b] Kilka oil, Mazandaran Co, Iran.

[c]Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, B1=6000 mg, B2=8000 mg, B3=12000 mg, B5=40000 mg, B6=4000 mg, B9=2000 mg, B12=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg.

[d]Mineral premix (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, $CoCl_2$:6000 mg.

[e] Antioxidant: Butylated hydroxytoluene (BHT).

DM, dry matter.

Resistance to stress is based on expose fish to a physical, chemical and biological unbalanced state (Correia *et al.*, 2003). In these studies, the effect of nutritional food and quality of fish can be determined by assessment the survival of fish against stress (Mohammadiazarm and Abedian-Kenari,2009). Spirulina has long been detected as a potential immunostimulant. Its aqueous extract was reported to effect the immune system by enhancement of phagocytic activity and stimulation of NK cells (Ravi *et al.*, 2010). The immunomodulatory activity of spirulina is related to its C-phycocyanin content (Vonshak, 1997). Recent studies showed that polyphenolic compounds have protective effects on immune system (Aquilano *et al.*, 2008; Franova *et al.*, 2010).

Table 3. survival rate after induction of environmental stress.

	Diets				
	Con	2.5%	5%	7.5%	10%
anoxia	47.62±4.76[a]	52.38±4.76[ab]	66.66±4.76[ab]	71.42±8.24[b]	66.66±9.52[ab]
density	42.86±8.24[a]	52.38±4.76[ab]	47.62±4.76[ab]	57.14[ab]	61.90±4.76[b]

Values (means ± SE of three replication) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

Algae Spirulina contains various immune stimulator such as vitamin B, vitamin E and as well as antioxidant pigments including chlorophyll a, xanthophyll, betacarotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin. (Habib *et al.*, 2008).

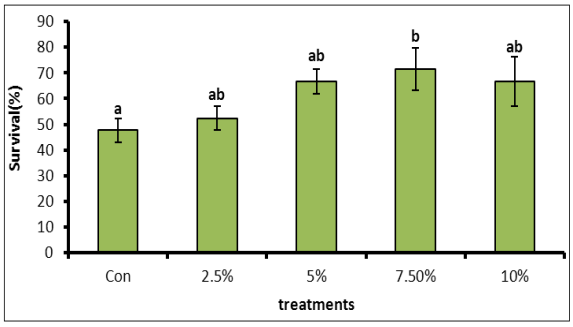

Fig. 1. survival rate of fish fed experimental diet against hypoxia stress.

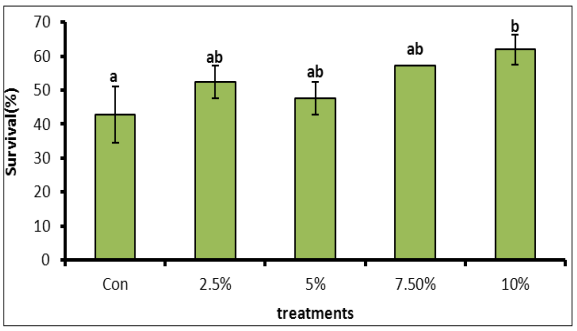

Fig. 2. survival rate of fish fed experimental diet after induction of density stress.

Vitamin E has an essential role in protecting the body against the destructive effects of active oxygen species which are formed during the metabolism or in the environment. Vitamin E is an important antioxidant, soluble in fat cells and cell membranes which it protects the phospholipids of membrane against active oxygen species and free radicals. Now vitamin E comes to the account as a membrane cleaner of free radicals, and important component of the cellular defense system that contains the other enzymes such as superoxide dismutase (SODs), glutathione peroxidase (GSH-PX), glutathione reductase (GR), catalase, thioredoxin reductase (TR) and non-enzymatic factors such as uric acid, glutathione, that many of which are need to other essential nutrients. Expression of SOD depends on the status of copper, zinc and manganese and activities of GR are related to the appropriate situation of riboflavin. These antioxidant performances show that vitamin E and related nutrients can have important may be side by side to protect the body and to treat conditions associated with oxidative stress (Kathleen and Sylvia, 2008). Also, the increased lysozyme activity in fish fed spirulina algae probably due to phycocyanin-C of it (Vonshak, 1997). The lysozyme is a strong parser enzyme which is available in the blood and lymphoid tissues of fish. This enzyme has a big role in the safety of the fish and is one of the most important natural resistances in fish. It isolates the link between molecules of N-Acetyl-D-Glucosamine and N-acetylmuramic acid in the cell wall of gram positive bacteria. In the case of gram negative bacteria, they are not destroyed directly by lysozyme, but destroyed indirectly by activated lysozyme after activity of complements and other enzymes of cells which induce to destroy the external walls (Magnadottir, 2006). The use of immune stimulator in fish feed through the activity of lysozyme in the blood of fish, increases the resistance of fish to stressful conditions. (Sakai, 1999). Also, the existence of carotenoid pigments in spirulina algae, increases fish resistance against pathogens through reducing the amount of stress (Promya and Chitmanat, 2011).

Conclusion

The result showed that 10% spirulina in diet of Convict cichlid can be resulted to increase environmental stress resistance of fish.

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