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EFFECT OF DIETARY *SPIRULINA* LEVEL ON GROWTH, FERTILITY, COLORATION AND LEUCOCYTE COUNT IN RED SWORDTAIL, *XIPHOPHORUS HELLERI*

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Abstract

Experiments were performed to investigate the influence of different levels of *Spirulina* (0, 1, 3, 5 and 8%) on feed consumption, growth, fertility, coloration, and leucocyte count in the ornamental red swordtail, *Xiphophorus helleri*. Feed intake, specific growth rate (SGR), and mean body length and weight increased as levels of *Spirulina* increased. Fish fed 8% *Spirulina* performed better than those fed lower levels. The gonad weight and gonadosomatic index (GSI) increased with the *Spirulina* level and rearing period. Fish fed 8% *Spirulina* had four times heavier gonads than fish fed 0-3% *Spirulina*. Female *X. helleri* fed 8% *Spirulina* released 89 young, significantly higher (p<0.01) than those fed 5, 3, 1, or 0% *Spirulina* (79, 64, 49, and 41 young, respectively). The total carotenoid content in the fins, skin, and muscle increased with the *Spirulina* level and the maximum carotenoid content was obtained in fish fed 8% *Spirulina*. The maximum coloration was in the fins, followed by the skin and muscle in all treatments. Fish fed 8% *Spirulina* had more monocytes, neutrophils, and lymphocytes and fewer basophils and thrombocytes while control fish showed the opposite trend. The necessity of incorporating an optimum level of *Spirulina* (8%) in the diet for maximum growth, reproduction, and coloration in *X. helleri* is discussed.

Introduction

Spirulina is one of the most concentrated natural sources of nutrition for all animals. Early interest in *Spirulina* focused mainly on its potential as a source of protein and vitamins. *Spirulina* contains 60-70% protein by weight, is the richest source of vitamins B_{12} and beta carotene (20 times more than carrots), and is loaded with essential fatty acids and minerals. Essential amino acids (62%) such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine are present in *Spirulina*. *Spirulina* improves

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the intestinal flora in fish by breaking down indigestible feed components to extract more nutrients. *Spirulina* stimulates the production of enzymes that transport fats within the fish for growth instead of storage. The cell wall of *Spirulina* is rich in muco-proteins that enhance the natural mucus layer of the skin, resulting in the shiny appearance of fins and skin and improving resistance to skin infections. More recently, new interests concern the therapeutic effects of *Spirulina* as a growth promoter and probiotic or booster of the immune system in all animals including fish.

Many factors influence fertility in female fish. Among them size, age, physical condition, reproductive history, and nutritional state are important (Woodhead, 1960). Previous authors have studied the effect of a *Spirulina*-rich diet or its extract on growth and immune responses in various animals (Hayashi et al., 1994; Qureshi et al., 1995; Scaria et al., 2000). However, no such study has been especially carried out on the effects of *Spirulina* on growth, coloration, fertility, and hematology in ornamental fishes. The present study was carried out to analyze the impact of different levels of dietary *Spirulina* on growth, coloration, fertility, and leucocyte count in the ornamental red swordtail, *Xiphophorus helleri*.

Materials and Methods

Fish and maintenance. Three hundred and seventy-five active 30-day-old juveniles of *X. helleri* (44±2.5 mg; 7.1±0.14 mm) were collected from laboratory bred brooders. They were divided into 15 groups corresponding to three replicates of five treatments. Each group consisted of 25 individuals of a similar body weight and was reared in a circular cement tank (0.5 x 0.5 m) containing 90 l of water. The water was clean, unchlorinated well water. Water quality, monitored biweekly, was 27.5±1.2°C, pH 7.8±0.01, salinity 0.15±0.11‰, water hardness 362±12 mg $CaCO_3$/l, and DO 3.98±0.07 ml/l. The tanks were completely drained twice a week and replenished with fresh water to remove accumulated feces from the bottom.

Feed and feeding. Five diets were prepared with five different *Spirulina* levels - 0, 1, 3, 5, and 8%. The diets contained 45% basal protein for maximum growth and reproductive potential in *X. helleri* (James and Sampath, 2004a). The feed was formulated according to Hardy (1980). Chemical compositions are given in Table 1. Protein and lipid contents were determined by spectrophotometer following Lowry et al. (1951) and Bragdon (1951), respectively. Mineral contents were estimated following the method of Paine (1964). Nitrogen free extract (NFE) was calculated by subtracting the protein, lipid, and mineral contents from the dry weight of the feed samples.

Fish were fed *ad libitum* twice a day during the 140-day experiment. Feed was given in a feeding tray for 2 h, after which unconsumed feed was removed and dried in a hot air oven at 80°C. Feed consumption was estimated by

Table 1. Proximate composition (%) of experimental diets.

Spirulina level (%)	*Protein*	*Lipid*	*Ash*	*Nitrogen free extract*
0	44.83±1.41	8.08±0.02	16.0	31.09
1	45.46±0.07	8.87±0.00	19.0	26.67
3	46.97±2.76	9.28±0.06	19.6	24.15
5	49.86±1.84	9.84±0.04	18.8	21.50
8	52.93±2.98	10.13±0.08	19.6	17.34

subtracting the amount of unconsumed dry feed from the dry weight of the feed offered.

Growth and gonad estimations. Fish were weighed at the beginning of the experiment and every 20 days. Growth was calculated as the difference between the wet weights at the beginning of the experiment and on the day of calculation. Specific growth rate (SGR) was calculated as $(Wt_1 - Wt_0)/t_1 \times 100$, where Wt_0 and Wt_1 are the weights of the fish at the beginning and end of each sampling period and t_1 is the period between samplings in days. The mean body weight (g) was calculated by dividing the total wet weight of the fish in the aquarium by the number of fish in the aquarium.

Every 20 days, three fish were chosen (one from each replicate in a treatment) and mean body length was measured. Two females from each treatment were sacrificed at 20 day intervals from the time of gonad development until the start of breeding. Their ovaries were removed and weighed and the gonadosomatic index (GSI) was computed according to Dahlgren (1979): (wet wt of gonad/wet wt of fish) x 100. Gonad maturity was determined according to ova development and the six stages of Meffe (1985) that indicate the readiness of the animal for breeding.

Prior to sacrificing the fish for gonad estimation, the caudal peduncle of the sample individuals was cut with a sharp sterilized knife to collect blood for counting leucocytes. Muscle, skin, and fins were collected for color estimation following Bjerkeng (1992). Fish, feed samples, unconsumed feed, and ovaries were weighed in an electric monopan balance to an accuracy of 1 mg.

Breeding. Two females were randomly chosen from each replicate and reared with a male in a separate tank containing a sufficient quantity of macrophytes of the *Hydrilla* species until the end of the experiment. The remaining test animals were removed from the experimental tanks. When the breeding females released their young, the young were isolated from the parents and counted.

Statistical analysis. Student's *t* test was applied to determine the significance of differences between group means. Two-way ANOVA was applied to find the significant effects of *Spirulina* level and rearing period on the mean body length and weight and the SGR. Tukey's multiple comparison test was used to compare mean carotenoid contents of body parts as a function of the *Spirulina* level and the rearing period (Zar, 1974).

Results

Feed consumption and SGR increased as the *Spirulina* level increased (Table 2). The body weight and length of fish fed the 8% diet were significantly greater ($p<0.01$) than in fish fed other diets (Fig. 1). The gonad weight and gonadosomatic index increased with time and *Spirulina* level and were significantly ($p<0.05$) higher in the 8% treatment. Fish fed the 8% diet had a significantly ($p<0.05$) higher number of young. The *Spirulina* diet significantly (one-way ANOVA: df 4, 10; F 20.94; $p<0.01$) influenced fertility in *X. helleri*.

The carotenoid content in the fins, skin, and muscle increased with the *Spirulina* level and the rearing period (Table 3). The highest carotenoid content in all three tissues was observed in fish fed 8% *Spirulina* and fins attained the maximum coloration in all treatments. Tukey's multiple comparison test showed that carotenoid content significantly differed ($p<0.05$).

The number of neutrophils, monocytes, and lymphocytes increased with the *Spirulina* level while basophils and thrombocytes decreased (Fig. 2). Fish fed 8% *Spirulina* had the highest number of monocytes, neutrophils, and lymphocytes and the lowest number of basophils and thrombocytes while the trend was opposite in control fish.

Discussion

Fish fed the diet with 8% *Spirulina* elicited the maximum feeding and growth parameters, possibly due to the high amount of protein (53%) and the growth stimulatory effects of *Spirulina*. Scaria et al. (2000) found that the ornamental guppy (*Poecilia reticulata*) and platy (*Xiphophorus maculatus*) consumed more feed containing *Spirulina* than feed containing mushrooms or azolla. A higher growth

Table 2. Feed consumption (g dry matter), specific growth rate (%), gonad weight (mg wet wt), gonadosomatic index (%), and fertility (no. young) in red swordtail, *Xiphophorus helleri*, fed diets with different levels of *Spirulina*. Values are means±SD of three observations.

Rearing period (days)	*Spirulina level (%)*				
	0	*1*	*3*	*5*	*8*
Feed consumption					
20	3.17±0.67	3.93±0.67	4.05±0.86	5.14±0.56	5.11±0.16
40	6.47±0.32	8.22±0.67	9.34±0.86	9.53±0.56	10.50±0.16
60	8.05±1.01	10.68±1.09	11.44±1.01	12.22±0.35	15.03±1.06
80	10.04±0.77	11.66±0.69	12.94±0.07	15.38±0.07	16.80±0.24
100	12.05±0.09	14.53±0.02	16.73±0.03	23.06±0.04	16.92±0.01
120 *	3.95±0.02	4.78±0.01	5.35±0.00	5.64±0.01	6.48±0.05
140	3.65±0.01	4.15±0.01	4.76±0.01	4.96±0.01	5.89±0.01
Specific growth rate					
20	2.056±0.09	1.913±0.03	2.521±0.18	2.241±0.09	2.176±0.15
40	4.734±0.09	4.007±0.01	3.481±0.13	5.926±0.08	4.854±0.24
60	6.528±0.33	6.214±0.01	5.070±0.33	7.363±0.22	6.676±0.71
80	9.046±0.56	8.054±0.15	6.282±0.19	9.436±0.14	9.413±0.93
100	10.062±0.03	8.943±0.52	7.060±0.04	9.490±0.37	11.408±0.60
120 *	12.880±0.71	10.535±1.30	7.687±0.19	11.406±0.05	15.795±0.68
140	5.961±0.14	6.363±0.20	5.768±0.22	4.470±0.01	6.967±0.01
Gonad weight					
80	9.00±0.41	29.25±0.96	27.00±1.65	38.00±2.00	88.33±2.08
100	29.00±1.41	42.5±3.53	57.50±3.53	93.00±4.24	165.00±7.07
120 *	78.00±2.83	92.00±3.82	112.01±4.24	167.50±10.60	410.00±14.14
Gonadosomatic index					
80	1.918±0.30	6.937±0.30	8.256±0.81	7.754±0.41	18.100±0.42
100	4.809±0.22	8.235±0.68	14.092±0.86	16.877±0.77	25.075±1.07
120 *	9.363±0.34	13.256±0.41	22.400±0.85	22.634±1.43	123.123±4.24
Fertility					
No. young	41.25±4.10	48.5±3.75	64.2±4.69	78.75±7.55	89.25±8.26

* Breeding commenced

rate was found in fish consuming feed containing *Spirulina* than in fish consuming feed with no *Spirulina* (Daniel and Kumuthakalavalli, 1991; Okada et al., 1991). Dietary beta carotene (10-30 mg/100 g) increased the specific growth rate (in terms of mean body length and weight) in the gold fish, *Carassius auratus*, with no significant differences between diets containing different levels of beta carotene (Aravindan et al., 2001). Feed consumption and growth rate in *X. helleri* were significantly enhanced when fish were fed 45% animal protein or plant protein (James and Sampath, 2004a), supporting findings of the present study.

Fish fed the 8% *Spirulina* diet had four-

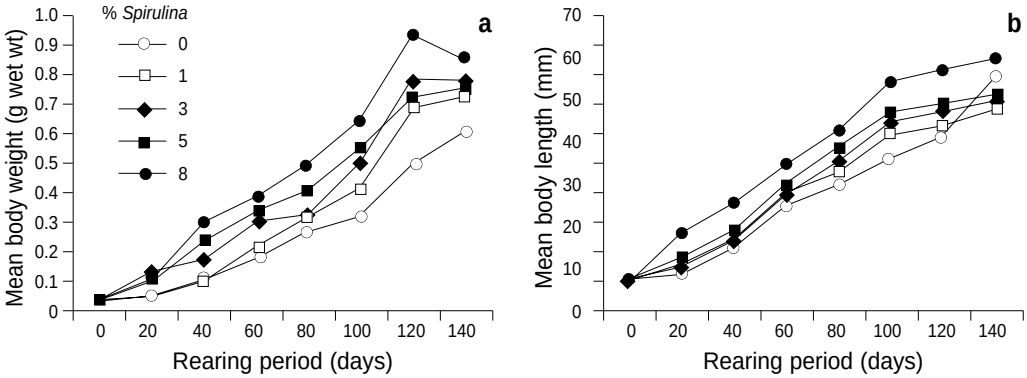

Fig. 1. Mean body weight (a) and length (b) in red swordtail, *Xiphophorus helleri*, fed diets with different levels of *Spirulina* (%).

times heavier gonads than fish fed the 1-3% *Spirulina* diets, possibly due to greater availability of protein and gonad stimulatory substances. Female guppies, *P. reticulata*, fed a 47% protein diet had greater ovarian lengths, widths, gonad weights, and GSI (Dahlgren, 1979). This level was close to our findings (50-53%) on *X. helleri*. Female *X. helleri* fed high protein diets (45%) had a greater ovary weight and GSI than those fed low protein diets (10-35%; James and Sampath, 2004a).

The present study shows that diet composition and palatability significantly affect reproduction in *X. helleri*. The palatability of the *Spirulina* diet enhanced feed consumption which directly increased the growth rate, gonad development, and fertility. *Spirulina* contains a high level of protein (53%) and all the essential amino acids, vitamins, fatty acids, etc., that might have stimulated gonad development and thereby fertility compared to fish fed the *Spirulina*-free diet. As in the present study, a greater number of young were produced by *X. helleri* fed a diet containing *Artemia*, possibly due to the high level of protein (57%) and complete essential amino acids in that diet (James and Sampath, 2004b).

Even though the red swordtail is brightly colored, dietary supplementation of *Spirulina* significantly enhanced coloration in the fins and skin. The increase in carotenoid content in the fins, skin, and muscle was proportionate to the increase in dietary carotenoid, demonstrating that *X. helleri* has the capacity to efficiently utilize carotenoids. Similar observations in trout and salmon muscle were made by earlier authors (Storebakken et al., 1987; Bjerkeng et al., 1990). A dose-dependent carotenoid content was reported in the muscle of Arctic char and salmon (Bijerkeng et al. 1990; Ando et al., 1994; Hatlen et al. 1997; Wathne et al., 1998). In ornamental fishes (unlike salmon and Arctic char), pigmentation was high only in the fins and skin, possibly because dietary carotenoids are acquired, digested, utilized, transported, and stored more directly in the skin and fins than in the muscle (Aravindan et al., 2001). The low carotenoid content in the muscle indicates that assimilated carotene is directly transported to the skin and fins to provide necessary pigmentation. According to Schiedt et al. (1985), this is achieved by establishing reductive metabolic pathways from the muscle to the fins and skin. In salmon, Arctic char, and trout, the pigmentation of the integument and fins occurs only during sexual maturation and a reduction of muscle carotenoid indicates that carotenoids are mobilized directly to the integument and fins from the muscle during that season.

Table 3. Carotenoid contents (mg/100 mg wet tissue) in fins, skin, and muscle of red swordtail fed different levels of *Spirulina*. Values are means±SD of three observations.

Rearing period	*Spirulina level (%)*				
(days)	*0*	*1*	*3*	*5*	*8*
Fins					
80	0.067±0.008	0.129±0.004	0.143±0.002	0.184±0.106	0.281±0.004
100	0.097±0.015	0.173±0.009	0.187±0.006	0.218±0.002	0.332±0.028
120	0.129±0.005	0.216±0.475	0.242±0.014	0.293±0.009	0.378±0.026
Skin					
80	0.036±0.005	0.053±0.004	0.128±0.019	0.146±0.006	0.154±0.005
100	0.061±0.009	0.101±0.002	0.136±0.152	0.152±0.006	0.162±0.014
120	0.092±0.041	0.121±0.005	0.158±0.009	0.166±0.002	0.189±0.002
Muscle					
80	0.006±0.003	0.007±0.002	0.019±0.004	0.040±0.012	0.043±0.006
100	0.020±0.013	0.032±0.002	0.036±0.002	0.048±0.003	0.058±0.028
120	0.025±0.001	0.036±0.002	0.051±0.009	0.061±0.001	0.082±0.005

Tukey's multiple comparison test

Spirulina concentration (%):	8 vs		0		1		3		5
Fins	μ1	≠	μ2	≠	μ3	≠	μ4	=	μ5
Skin	μ1	≠	μ2	≠	μ3	≠	μ4	=	μ5
Muscle	μ1	≠	μ2	=	μ3	=	μ4	=	μ5
Spirulina concentration (%):	5 vs		0		1		3		
Fins	μ1	≠	μ2	=	μ3	=	μ4		
Skin	μ1	≠	μ2	≠	μ3	=	μ4		
Muscle	μ1	=	μ2	=	μ3	=	μ4		

≠ significant over other treatment at $p<0.05$.

High density lipoproteins are responsible for transport of carotenoids from the muscle to the integument in salmon (Ando and Hatano, 1988). Further, several abiotic and biotic factors are expected to influence the ingestion, mobilization, and metabolism of carotenoids, as in other feed constituents (Hatlen et al., 1996). It is likely that a similar mechanism also operates in *X. helleri*.

Neutrophils, monocytes, and lymphocytes were the leucocytes with the most positive response to the addition of *Spirulina* to the diet. Feeding *Spirulina platensis* in processed form enhanced specific and non-specific immunity and resistance against *Edwardsiella ictaluri* infection in channel catfish, *Ictaluras punctatus*, (Duncan and Kiesius, 1996). Monocytes and neutrophils, involved in the first line of the defense mechanism and phagocytosis, had the maximum increase among leucocyte types, indicating that *Spirulina* in the diet may benefit the immune system in *X. helleri* as well.

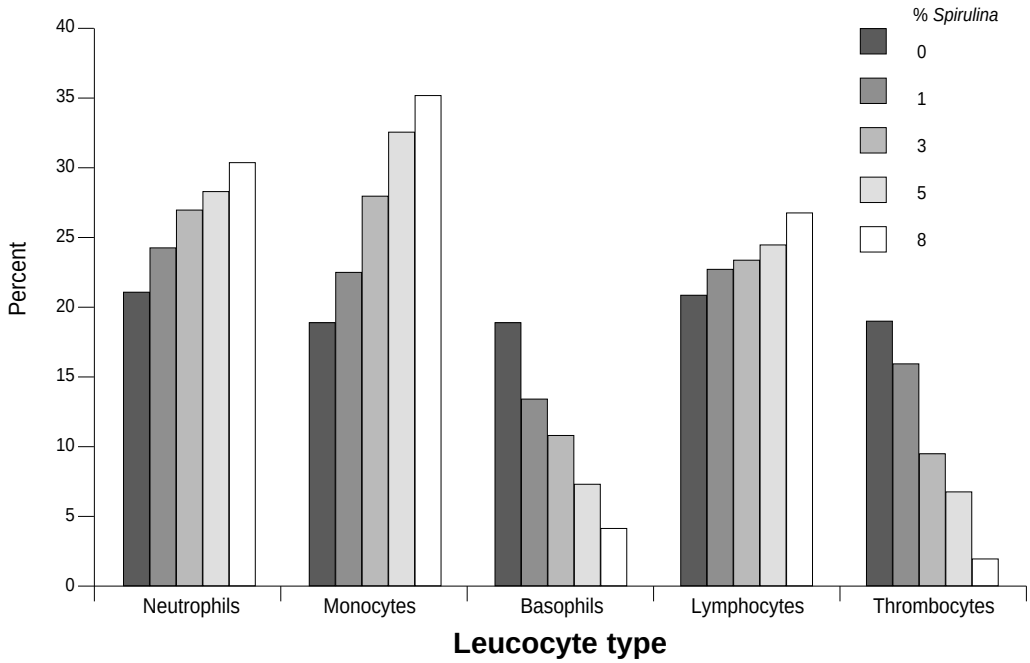

Fig. 2. Leucocyte counts in red swordtail, *Xiphophorus helleri*, fed diets with different levels of *Spirulina*.

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