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Interaction of Spirulina with Different Levels of Vitamin E on Growth, Reproduction, and Coloration in Goldfish (*Carassius auratus*)

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Abstract

The interaction between spirulina (30 g/kg diet) and different levels of vitamin E (100, 200, 300, and 600 mg/kg diet) on growth, gonad weight, reproduction, and coloration were studied in goldfish, *Carassius auratus*, for 120 days. The mean body weight increased with time in all experimental diets. Growth, gonad weight, and fecundity in fish fed the diet containing spirulina+300 mg vitamin E were significantly (p<0.01) enhanced, compared to other diets. Supplementation of vitamin E beyond this level significantly (p<0.01) reduced gonad weight and, subsequently, fecundity. Control fish spawned only once, with fewer eggs per spawn, than other groups, which spawned twice with a greater number of eggs per spawn. Females fed spirulina without vitamin E laid 703 eggs in two spawnings compared to 1057 eggs in fish fed with the spirulina+300 mg vitamin E diet. Fish treated with other combinations laid fewer eggs. While all combinations of spirulina and vitamin E significantly enhanced coloration, the combination of spirulina+300 mg vitamin E was the most influential.

Introduction

Spirulina is used in aquaculture as a liquid feed for young fish and a dehydrated feed for adult fish. Spirulina contains 60-70% protein (by weight) and is a rich source of vitamins B-12 and β-carotene (20 times greater than carrots), minerals, essential amino acids (62%), and fatty acids. Spirulina improves the intestinal flora in fish by breaking down indigestible feed components to extract more nutrients from the feed. Spirulina stimulates the production of enzymes that transport fats within the fish for growth instead of storage (James et al., 2006). The cell wall of spirulina is rich in mucoproteins and enhances the natural mucous layer of the skin, resulting in a shiny appearance of the fins and skin and improved resistance to infection (Venkataraman, 1993).

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Vitamin E functions as a lipid-soluble antioxidant, protecting biological membranes, lipoproteins, and lipid stores against oxidation (Hamre and Lie, 1995). Several studies have demonstrated the individual effects of spirulina and vitamin E on growth, survival, skeletal deformities, reproduction, and immune responses in various organisms (Gupta et al., 1987; Braun, 1988; James et al., 2006). The present study investigates the interaction between spirulina and different levels of vitamin E on growth, gonad weight, reproduction, and coloration in goldfish, *Carassius auratus*.

Materials and Methods

Fish and maintenance. Three hundred and sixty healthy active 45-day-old *C. auratus* juveniles (0.27±0.05 g, 17.9±0.86 mm) were collected from laboratory bred brooders. They were sorted into six groups and offered one of six feeds. Each treatment was tested in triplicate, with 20 fish per circular cement tank (58.5 x 40 cm) containing 100 liters of water. Tanks were filled with dechlorinated well water (28.8±1°C, pH 7.7±0.6, salinity 0.13±0.01 ppt, water hardness 325 mg $CaCO_3$/l, dissolved oxygen 4.13±0.7 ml/l). The tanks were drained twice a week and replenished with fresh water to remove accumulated feces from the bottom.

Experimental diets and composition. The diets were prepared following the square method (Hardy, 1980). The control group was fed a 38% protein diet that contained neither spirulina nor vitamin E (Table 1). The optimum spirulina level for growth, coloration, and reproductive performance in goldfish is 30 g per kg diet (Vasudhevan, 2007). Thus, Group 2 was fed the control diet plus this amount of spirulina. Groups 3, 4, 5, and 6 were fed spirulina with 100, 200, 300, and 600 mg vitamin E per kg diet, respectively.

The dried ingredients were blended to make a homogenous mixture. The diets were mixed with an aliquot of boiled water and cooked by steam for 15-20 min. The source of vitamin E was α-tocopherol acetate. The α-tocopherol acetate (100, 200, 300, and 600 mg vitamin E/kg diet), dissolved in acetone, was sprayed on 1 kg of the diet ingredients and the diet was uniformly mixed. The required feeds (2 mm size) were prepared once in two weeks with a hand operated pelletizer, dried in sunlight, and stored separately in a refrigerator.

The protein and lipid contents of the experimental diets were determined in a spectrophotometer following Lowry et al. (1951) and Bragdon (1951), respectively. The moisture content was analyzed by drying in an electric hot air oven at 100°C. The mineral content was estimated following the method of Paine (1964). Nitrogen-free extract was calculated by subtracting the protein, lipid, and mineral contents from the dry weight of the feed samples.

Feeding. Fish were fed *ad libitum* twice a day for 120 days. Feed was weighed and given in a feeding tray for 1 h after which unconsumed feed was removed and dried in a hot air oven at 80°C. Feed consumption was estimated every 20 days by subtracting the amount of unconsumed dry feed from the total dry weight of the offered feed. Feeding and growth parameters were calculated for 20-day intervals (six throughout the 120-day period) and the means were combined to determine values before and after commencement of spawning. The feeding rate (mg/g live fish/day) was computed as: feeding rate = consumed feed/(initial wet wt of fish x no. of days).

Growth and gonad estimations. Fish were weighed at the beginning of the experiment and every 20 days thereafter. Growth (weight gain) was calculated as the difference between the wet weight at the beginning of the experiment and the wet weight on the day of calculation. The specific growth rate (%/day) was calculated as: $SGR = (\ln wt_1 - \ln wt_0)/t_1 \times 100$, where $\ln wt_0$ and $\ln wt_1$ 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. Feed conversion ratio (FCR) was calculated by relating feed consumption to weight gain.

The mean body weight (g) was calculated by dividing the total wet weight of the fish in the tank by the number of fish in the tank. Two females from each treatment were sacrificed at 20-day intervals from the time of gonad development till the commencement of spawning. Their

Table 1. Ingredients (per kg) and proximate composition (% dry matter basis) of experimental diets.

	Diet					
	Control	*Spirulina*	*Spirulina+100*	*Spirulina+200*	*Spirulina+300*	*Spirulina+600*
Ingredient						
Fishmeal	398	315	315	315	315	315
Ground nut oil cake (g)	368	320	320	320	320	320
Tapioca flour (g)	145	210	209.9	209.8	209.7	209.4
Wheat flour (g)	87	123	123	123	123	123
Mineral mix (g)	1	1	1	1	1	1
Spirulina (g)	0	30	30	30	30	30
Cod liver oil (ml)	1	1	1	1	1	1
Vitamin E (mg)	-	-	100	200	300	600
Proximate composition						
Crude protein	38.10±1.12	38.40±1.34	37.80±2.15	38.30±1.86	37.90±2.16	38.10±1.96
Crude fat	8.50±0.16	8.65±0.15	8.95±0.18	8.50±0.09	8.80±0.14	8.80±0.18
Ash	16.20±0.28	16.10±0.32	16.20±0.31	16.30±0.17	16.15±0.20	16.30±0.18
Nitrogen free extract	37.30 ±1.87	36.85±2.14	37.05±1.97	36.90±1.68	37.15±1.88	36.80±2.17

ovaries were removed and weighed and the gonadosomatic index (%) was computed according to Dahlgren (1979) as follows: GSI = wet wt of gonad/wet wt of fish x 100.

Fish, feed samples, unconsumed feed, and ovaries were weighed in an electric monopan balance to an accuracy of 1 mg.

Color estimation. After sampling the fish for gonad estimation, muscle, skin and fins and were collected for color estimation following Bjerkeng (1992).

Spawning. Two males were chosen from each replicate and reared with a female in a separate tank containing macrophytes of the *Hydrilla* species, until the end of the experiment. After spawning, eggs, adhered on leaves, were counted inside the water and a sample of a few eggs were taken and weighed. Egg weight and diameter were recorded immediately after the eggs were laid. The mean wet weight of the eggs was calculated by dividing the total wet weight of the eggs by the number of eggs. Diameter was measured using an ocular micrometer. After hatching, the length and weight of the young ones were measured. Unhatched eggs were counted only once, 65-72 h after fertilization.

Statistical analysis. Student's *t* test was applied to determine the significance of differences between group means. Two-way ANOVA was applied to find significant effects of nutrient levels and rearing periods on feeding and growth parameters (Zar, 1974). Tukey's multiple comparison test was applied to compare mean carotenoid contents of body parts as a function of the nutrients and rearing period.

Results

The mean body weight increased with time. Fish fed the spirulina+300 mg E diet had significantly higher mean body weight than other groups (Table 2). Vitamin E level and rearing period data were subjected to two-way ANOVA which revealed that both factors exerted significant effects on mean body weight (Fig 1). The mean feed consumption, weight gain, and specific growth rate were higher during the pre-spawning period than during the post-spawning period, however, the feeding rate and feed conversion ratio showed the opposite trend. Fish fed the spirulina+300 mg E diet had significantly ($p<0.01$) enhanced feeding and growth parameters.

The gonad weight and gonadosomatic index of female *C. auratus* generally increased with time (Fig. 2). The spirulina+300 mg diet produced five times more gonad weight than the control diet. However, vitamin E above this amount drastically reduced the gonad weight.

Control fish spawned only once with fewer eggs per spawn while the other groups spawned twice with more eggs per spawn. *C. auratus* fed spirulina+300 mg E produced better quality eggs (weight and diameter) and larvae (weight and length) (Table 3). Similarly, this diet elicited significantly ($p<0.05$) higher egg hatchability (Fig. 3).

The total carotenoid content in the skin and fins increased with time, while the content in the muscle showed the opposite trend (Table 4). Skin contained the most carotenoid, followed by fins and muscle. Tukey's multiple comparison test revealed that combinations of spirulina and vitamin E levels significantly ($p<0.05$) enhanced the coloration in muscle, skin, and fins of *C. auratus*. The spirulina+300 mg E produced the maximum coloration in tested tissues as compared to other diets.

Discussion

Dietary supplementation of vitamin E levels and spirulina influenced feeding and growth parameters in *C. auratus*. Spirulina enhanced feed intake and growth in red swordtail, *Xiphophorus helleri* (James et al., 2006) and has been identified as a potential protein source for animal feed (Braun, 1988; James et al., 2006). There is a synergistic interaction between spirulina and vitamin C that increased body weight gain, feed efficiency, and digestibility in red seabream, *Pagrus major* (Mustafa et al., 1994). It is likely that an interaction between spirulina and vitamin E influenced the feeding and growth in *C. auratus*. The relatively high feeding rate and FCR values

Table 2. Effect of spirulina with different levels of vitamin E on feed consumption, feeding rate, weight gain, specific growth rate, and feed conversion ratio in *Carassius auratus* (means±SD; n = 3).

	Diet					
	Control	*Spirulina*	*Spirulina+100*	*Spirulina+200*	*Spirulina+300*	*Spirulina+600*
Feed consumption (g dry matter)						
Pre-spawn	31.83±1.73	46.94±1.83	49.55±3.10	54.01±3.01	64.72±3.45	49.64±2.63
Post-pawn	-	43.57±1.17	48.01±2.55	52.60±1.36	64.75±1.27	47.71±1.62
Feeding rate (mg/g live fish/day)						
Pre-spawn	110.22±1.37	107.05±1.30	112.16±1.38	110.14±1.09	108.85±1.15	108.97±1.05
Post-pawn	-	123.71±2.76	135.85±1.36	140.94±1.24	151.78±1.36	132.09±0.97
Weight gain (g wet wt)						
Pre-spawn	7.91±0.25	10.30±0.30	10.12±0.51	11.14±0.50	13.97±0.26	10.16±0.36
Post-pawn	-	5.37±0.19	4.86±0.32	5.25±0.19	5.19±0.23	4.20±0.26
Specific growth rate (%/day)						
Pre-spawn	2.14±0.21	2.55±0.23	2.51±0.16	2.65±0.20	2.82±0.24	2.50±0.23
Post-pawn	-	1.35±0.13	1.20±0.02	1.25±0.02	1.25±0.01	1.05±0.04
Feed conversion ratio						
Pre-spawn	4.34±0.31	4.49±0.34	4.87±0.54	5.05±0.29	5.20±0.25	5.05±0.29
Post-pawn	-	8.11±0.36	9.88±0.27	10.02±0.45	12.54±0.37	11.36±0.43

The control group spawned on day 120, the last day of the experiment.
Results of Student's *t* test comparing weight gain of spirulina+300 mg vitamin E diet with:
Spirulina+200 diet, $t = 15.82$, $p<0.01$
Spirulina+400 diet, $t = 19.51$, $p<0.01$

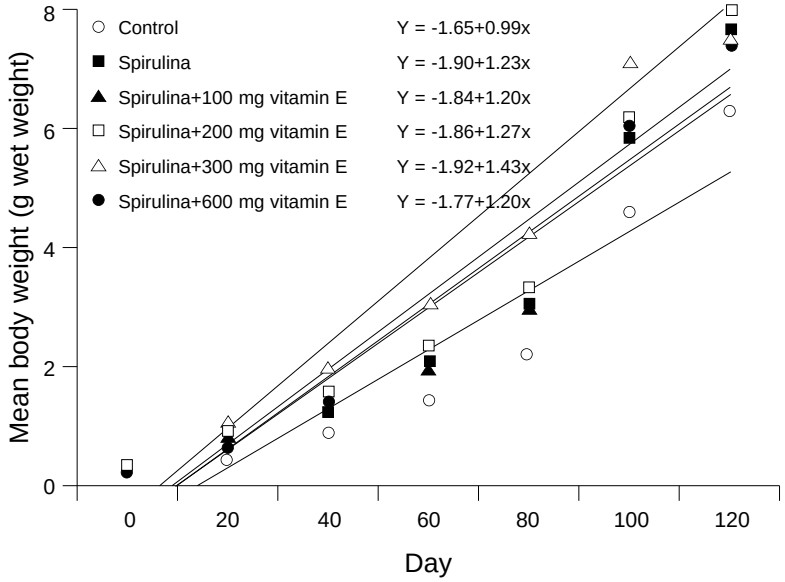

Fig. 1. Regression lines for mean body weight in goldfish, *Carassius auratus*, fed spirulina with different levels of vitamin E as a function of time.

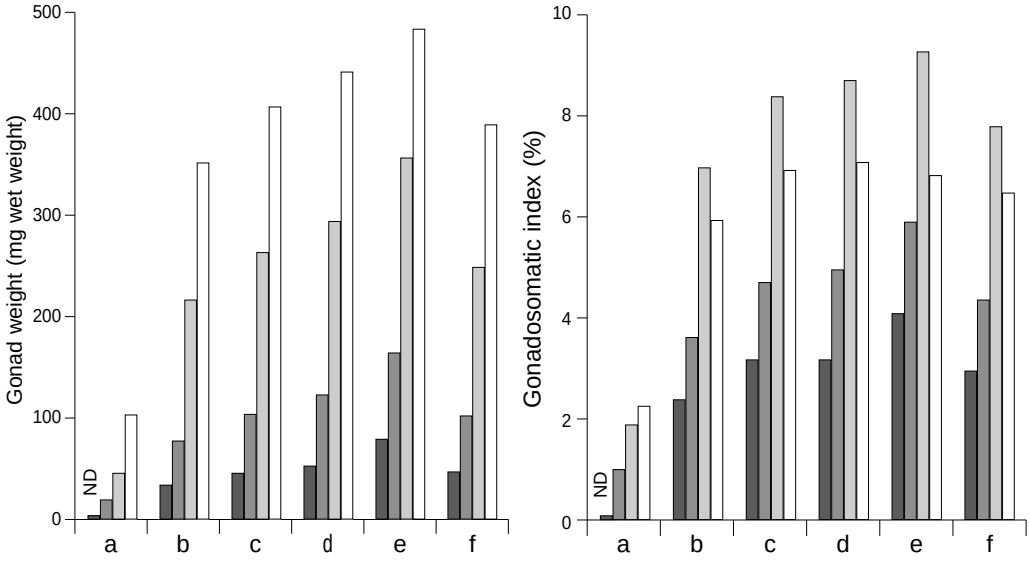

Fig. 2. Gonad weight and gonadosomatic index in goldfish, *Carassius auratus*, fed (a) control diet with neither spirulina nor viamin E, (b) control diet enriched with spirulina, diets containing spirulina plus (c) 100 mg, (d) 200 mg, (e) 300 mg, or (f) 600 mg vitamin E per kg diet. Measurements were taken on days 40 (■), 60 (■), 80 (■), and 100 (□). ND indicates undeveloped gonad.

Table 3. Effects of supplementary spirulina and vitamin E on mean egg weight and diameter, and mean larvae weight and length in goldfish, *Carassius auratus* (means±SD; n = 3).

	Diet					
	Control	*Spirulina*	*Spirulina+100*	*Spirulina+200*	*Spirulina+300*	*Spirulina+600*
Egg wt (mg wet wt)	1.33±0.01	1.36±0.03	1.36±0.02	1.38±0.03	1.46±0.02	1.36±0.01
Egg diameter (mm)	0.71±0.03	0.75±0.02	0.76±0.05	0.78±0.04	0.80±0.02	0.77±0.01
Larva wt (mg wet wt)	1.50±0.02	1.53±0.02	1.54±0.04	1.56±0.02	1.58±0.03	1.54±0.02
Larva length (mm)	3.7±0.07	3.8±0.04	4.0±0.02	4.1±0.03	4.3±0.04	3.9±0.02

Results of Student's *t* test comparing spirulina diet with spirulina+300 mg vitamin E diet:
For mean egg weight, $t = 3.92$, $p<0.01$
For mean larva weight, $t = 2.50$, $p<0.05$
For mean egg diameter, $t = 2.50$, $p<0.05$
For mean larva length, $t = 12.50$, $p<0.01$

Fig. 3. Reproductive performance in goldfish, *Carassius auratus*, fed (a) control diet with neither spirulina nor viamin E, (b) control diet enriched with spirulina, diets containing spirulina plus (c) 100 mg, (d) 200 mg, (e) 300 mg, or (f) 600 mg vitamin E per kg diet.

were probably due to allocation of a larger proportion of the feed to maintenance (Brett and Groves, 1979) and spawning (James and Sampath, 2003).

Gonad weight reflects fecundity. Fish receiving the spirulina+300 mg E diet laid 50% more eggs than fish fed the control diet, indicating that an additive interaction exists between spirulina and vitamin E. Vitamin E enhanced gonad weight, gonadosomatic index, ova size, and egg production in *Cyprinus carpio* when combined with a growth hormone (Gupta et al., 1987). Spirulina contains fat soluble vitamins and β-carotene that might have interacted with the vitamin E, exerting an impact on gonad weight and fecundity in *C. auratus*. Total egg production, mean number of eggs per spawning, number of spawns, and mean egg diameter were not affected by individual supplementation of vitamin C or E in milkfish, *Chanos chanos* (Emata et al., 2000). However, the dietary supplementation of both resulted in a higher percentage of spawn with a higher (>90%) percentage of egg viability, hatching, and cumulative survival than those of the control (Emata, 2000). Similarly, egg viability and hatchability of red seabream

Table 4. Effects of supplementary spirulina and vitamin E on carotenoid content (mg/100 mg wet tissue) in muscle, skin, and fins of goldfish, *Carassius auratus*, as a function of time (means±SD; n = 3).

	Diet					
Day	*Control*	*Spirulina*	*Spirulina+100*	*Spirulina+200*	*Spirulina+300*	*Spirulina+600*
Muscle						
20	0.056±0.003	0.109±0.002	0.114±0.003	0.119±0.004	0.126±0.005	0.107±0.002
40	0.037±0.001	0.092±0.005	0.098±0.002	0.102±0.002	0.106±0.004	0.094±0.003
60	0.024±0.002	0.076±0.001	0.081±0.004	0.087±0.001	0.089±0.002	0.076±0.001
80	0.015±0.001	0.044±0.003	0.049±0.001	0.052±0.003	0.056±0.001	0.046±0.002
Skin						
20	0.126±0.013	0.249±0.012	0.252±0.009	0.259±0.006	0.265±0.006	0.249±0.007
40	0.149±0.011	0.289±0.010	0.294±0.005	0.301±0.006	0.313±0.005	0.289±0.006
60	0.169±0.009	0.336±0.017	0.341±0.003	0.346±0.008	0.357±0.002	0.337±0.008
80	0.193±0.016	0.379±0.014	0.384±0.002	0.389±0.003	0.396±0.005	0.379±0.002
Fins						
20	0.109±0.007	0.174±0.005	0.178±0.008	0.182±0.004	0.191±0.005	0.174±0.003
40	0.138±0.008	0.211±0.014	0.206±0.003	0.209±0.002	0.215±0.004	0.202±0.002
60	0.165±0.004	0.276±0.019	0.269±0.009	0.273±0.006	0.281±0.003	0.264±0.008
80	0.183±0.006	0.308±0.015	0.297±0.005	0.304±0.007	0.309±0.004	0.292±0.004

Tukey's test :	*Control*		*Spirulina*		*Spirulina+100*		*Spirulina+200*		*Spirulina+300*		*Spirulina+600*
Muscle	μ_1	≠	μ_2	≠	μ_3	≠	μ_4	≠	μ_5	≠	μ_6
Skin	μ_1	≠	μ_2	≠	μ_3	≠	μ_4	≠	μ_5	≠	μ_6
Fins	μ_1	≠	μ_2	≠	μ_3	≠	μ_4	≠	μ_5	≠	μ_6

≠ significant over other treatments at $p<0.05$ level.

improved when the fish were fed diets supplemented with vitamin E, astaxanthin, and phosphatidylcholine shortly before spawning (Watanabe et al., 1991).

The spirulina+300 mg diet produced the maximum coloration in tested tissues as compared to other diets. The combination of spirulina and vitamin C improved coloration in red seabream (Mustafa et al., 1994). However, no comparable studies are available on the effects of the combination of spirulina and vitamin E on coloration in fish.

In conclusion, spirulina and vitamin E are growth and reproduction promoting factors, respectively, and effectively interact with each other by triggering the activities of one another when these two nutrients are available simultaneously. Therefore, utilization of two nutrients such as spirulina and vitamin E (instead of including only one nutrient) are very important when preparing diets to enhance growth, fecundity, and coloration in ornamental fish.

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