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Effect of Dietary Vitamin E on Growth, Fecundity, and Leukocyte Count in Goldfish (*Carassius auratus*)

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Abstract

The effects of different levels of dietary vitamin E (0, 100, 200, 300, and 600 mg/kg diet) on growth, gonad weight, fecundity, and leukocyte count were studied in goldfish (*Carassius auratus*) for 120 days. Fish fed the 300 mg vitamin E/kg diet had the best feeding rate, weight gain, and specific growth rate. Fish fed the control diet lacking vitamin E began to develop gonads on day 60 and spawned only once, while those fed diets containing vitamin E began to develop gonads on day 40 and spawned twice. Females fed the 300 mg vitamin E/kg diet had significantly (p<0.01) heavier gonads and a higher number of eggs with better hatchability than those fed other diets. The second spawn was significantly (p<0.01) more prolific than the first in all groups. Egg weight and diameter and larvae weight and length were significantly (p<0.05) higher in fish fed 300 mg vitamin E than in fish fed the control diet but did not differ from other vitamin E treatments. Lymphocyte and monocyte populations were highest in fish fed 300 mg vitamin E. Thus, 300 mg vitamin E/ kg diet is the optimum level for improving reproduction and immune response in *C. auratus*. The probable mechanism for the action of vitamin E on growth and reproduction is discussed.

Introduction

Vitamin E activity is present in a group of naturally occurring closely related tocopherols. Among them, α-tocopherol has the highest vitamin E activity. DL-α-tocopherol acetate, a stable vitamin of α-tocopherol, is the most commonly used form in animal feeds (NRC, 1983). On hydrolysis of this ester, α-tocopherol is absorbed from the intestine along with dietary fats (Bjorneboe et al., 1990). As a fat-soluble antioxidant, the major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipids and cholesterol in cellular and subcellular membranes. Most of the deficiency signs observed in fish, such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte hemolysis, hemorrhage, depigmentation, and reduction of fertility, are related to peroxidative damage to cellular membranes (NRC, 1983). As a membrane-bound antioxidant, vit-

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amin E appears to scavenge free radicals at the site of their formation.

Many authors have studied the impact of vitamin E on growth and immune response in various organisms but studies related to effects of vitamin E on growth, reproduction, and immune response in ornamental fishes are scanty. Hence, the present study was undertaken to study the effect of different levels of dietary vitamin E on growth, gonad weight, reproduction, enzyme activity, and leukocyte count in the goldfish, *Carassius auratus*.

Materials and Methods

Fish and maintenance. Three hundred healthy 45-day-old juveniles of *C. auratus* (18±0.96 mm; 240±13 mg) from the same laboratory-bred brooders were divided into five groups, corresponding to five levels of vitamin E, i.e., 0 (control), 100, 200, 300, and 600 mg/kg diet, which were fixed in a pilot study. Each diet was tested in triplicate groups consisting of 20 individuals, reared in circular cement tanks (diameter 58.5 cm, height 40 cm) containing 100 l static water. Temperature was 28.3±1.1°C, hardness 325.13±13 mgCO_3/l, pH 7.8±0.05, dissolved oxygen 4.10±0.13 ml/l, and salinity 0.58±0.02 ppt. The tanks were drained twice a week and replenished with fresh water to remove accumulated feces from the bottom.

Feed and feeding. The diets were prepared using fishmeal, ground nut oil cake, tapioca, wheat flour, mineral mix, and cod liver oil (lipid source). The source of vitamin E was α-tocopherol acetate. The dried and powdered ingredients were blended to obtain a homogenous mixture, mixed with an aliquot of boiled water, and steam cooked for 15-20 min. The required level of vitamin E was prepared by dissolving an appropriate quantity of α-tocopherol acetate in 10 ml acetone and spraying it on the corresponding diets. Diets were prepared every fortnight and stored in a refrigerator to minimize nutrient loss.

Fish were fed ad libitum twice a day for 120 days. Feed was given in a feeding tray and left for one hour after which unconsumed feed was removed and dried in a hot air oven at 80°C. Feed consumption was estimated by subtracting the amount of unconsumed dry feed from the dry weight of the offered feed. The daily feeding rate was computed as the amount of feed consumed in mg/initial wet weight of fish in g/no. days.

Growth and gonad estimation. Fish were weighed at the beginning of the experiment and every 20 days. Specific growth rate was calculated from the difference between the wet weight at the beginning of the experiment and the weight on the day of calculation as $(\ln Wt_1 - \ln Wt_0)/t_1 \times 100$, 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 number of days between samplings.

Two females from each treatment were sacrificed at 20-day intervals from the time of gonad development until the commencement of spawning. Their ovaries were removed and weighed and the gonadosomatic index (GSI) was computed according to Dahlgren (1949) as (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.

Leukocyte count. Prior to sampling the fish for gonad estimation on day 100, the caudal peduncle of the sampled individuals was cut with a sharp sterilized knife to collect blood for counting leukocytes.

Spawning. Two males were chosen from each replicate and stocked with a female in a separate tank containing macrophytes of the *Hydrilla* species, until the end of the experiment. The *Hydrilla* plant was provided for adhesion of eggs during spawning. The remaining test animals were removed from the experimental tanks. After spawning, a sample of a few eggs was counted and weighed. Egg weight and diameter were recorded immediately after the eggs were laid. The mean wet weight was calculated by dividing the total wet weight of the eggs by the number of eggs. The diameter was measured using an ocular micrometer. After hatching, the length and weight of young ones were measured. Unhatched eggs were counted 65-72 h after fertilization.

Statistical analysis. Student's *t* test was applied to determine the significance of differ-

ences between group means. Two-way ANOVA was applied to find the significant effects of vitamin E level and rearing period on feeding and growth.

Results

Fish fed the diet enriched with 300 mg vitamin E/kg had a lower feeding rate, higher weight gain, and higher specific growth rate (SGR) than fish fed other levels (Table 1). Weight gains and SGR were relatively high at the start of the experiment but gradually decreased with time. Two-way ANOVA showed that differences in feeding rate and growth parameters were statistically significant ($p<0.05$) between levels of vitamin E and rearing period. The SGR before spawning was higher than after spawning for all groups but the difference was more pronounced in fish fed the 300 mg vitamin E diet. The difference in feed conversion ratio before and after spawning was opposite.

Females fed the 300 mg vitamin E diet had gonads of significantly higher weight and GSI ($p<0.01$) than fish fed other diets (Fig. 1). Gonad weight gradually increased with time in all treatments. Fish fed the control developed gonads on day 60 while those fed vitamin E supplemented diets began to develop gonads on day 40.

Fish fed the 300 mg vitamin E diet laid more eggs with greater hatchability than fish fed other diets (Fig. 2). Fish fed the control spawned only once while fish fed diets containing vitamin E spawned twice. The first spawns were less prolific than the second in all groups. The mean egg weight, egg diameter, larvae weight, and larvae length increased as the vitamin E level increased to 300 mg/kg but thereafter declined (Table 2).

Fish fed 300 mg vitamin E/kg diet had a higher level of lymphocytes and monocytes than those fed other diets (Fig. 3).

Discussion

The study showed that vitamin E significantly influences feeding, growth, fecundity, and leukocyte count in *C. auratus*. Fish fed 300 mg vitamin E/kg diet had the best growth, gonad weight, gonadosomatic index, egg production, egg size, and hatching rate. We suggest that vitamin E affects the development of gonads, leading to greater weight and GSI, and complete spawning in female *C. auratus*.

The reproductive cycle of most goldfish is very short, like in many other ornamental fishes (James and Sampath, 2003, 2004). In contrast, *C. auratus* is a continuous spawner with a short vitellogenic period. A low level of vitamin E probably reduces gonad development and fecundity. The significance of vitamin E in fish reproduction was confirmed in earlier studies. In a study of the effects of vitamin E and growth hormone on gonadal maturity in the common carp (*Cyprinus carpio*), dietary vitamin E resulted in a higher gonadosomatic index, larger ova, and more eggs with higher hatchability than the control (Gupta et al., 1987). Further, spawning was complete in fish fed a diet supplemented with vitamin E but partial in the majority of fish fed diets lacking vitamin E (Gupta et al., 1987). Red seabream (*Pagrus major*) fed diets supplemented with vitamin E, astaxanthin, and phosphatidylcholine shortly before spawning produced eggs with improved viability and hatchability (Watanabe et al., 1991).

On the other hand, beneficial effects of dietary vitamin E on fish reproduction were not observed in some studies. There was no difference in egg hatchability in rainbow trout given diets with and without vitamin E (King et al., 1985) and high dietary α-tocopherol failed to increase the survival of eggs and fry in Atlantic salmon (*Salmo salar*; Eskelinen, 1989).

Vitamin E supplementation significantly influenced the leukocyte count. Lymphocytes and monocytes were the predominant cell types that varied in response to the vitamin E level. The increase of lymphocytes and monocytes in fish fed diets with vitamin E suggests that the immune mechanism in *C. auratus* is stimulated by vitamin E. Dietary levels of vitamin E enhanced immune responses in farmed fish (Hardie et al., 1990). Increased levels of vitamin E protected channel catfish (*Ictalurus punctatus*) from disease (Bai and Gatlin (1993). The impact of vitamin E on immune function is potentially great. Vitamin E in the

Table 1. Effect of different dietary levels of vitamin E on feeding rate, weight gain, specific growth rate, and feed conversion ratio in *Carassius auratus* (mean±SD for period since previous sampling; n = 3).

Day	*Vitamin E level (mg/kg diet)*				
	0	*100*	*200*	*300*	*600*
Feeding rate (mg/g live fish/day)					
20	92.18±7.84	120.21±8.69	120.73±10.15	116.20±8.14	115.30±8.14
40	128.89±8.97	149.20±9.14	138.19±7.19	124.45±7.65	143.55±6.15
60	131.78±8.14	151.11±7.65	141.06±9.17	127.29±6.13	148.67±7.93
80	89.31±2.81	127.90±6.16	121.15±4.81	111.11±5.79	125.31±8.16
100	47.36±2.81	42.92±2.85*	42.21±2.87*	38.44±1.85*	42.91±1.83*
120	176.80±6.79*	181.18±8.79	179.09±10.7	178.28±8.98	182.64±8.16
Wt gain (g dry matter)					
20	4.20±0.27	5.20±0.30	6.00±0.17	7.20±0.18	5.60±0.19
40	8.40±0.31	10.00±0.34	10.63±0.26	11.80±0.20	10.00±0.41
60	9.42±0.39	9.88±0.49	10.10±0.53	10.58±0.59	10.40±0.46
80	4.22±0.27	7.56±0.51	7.84±0.70	8.98±0.64	7.28±0.69
100	6.61±0.34	6.18±0.46*	6.89±0.39*	7.64±0.61*	6.47±0.38*
120	2.70±0.16*	3.75±0.21	3.87±0.17	4.59±0.26	3.87±0.15
Specific growth rate (%/day)					
20	3.10±0.04	3.70±0.08	4.10±0.10	4.60±0.11	3.90±0.08
40	3.35±0.05	3.45±0.09	3.40±0.09	3.45±0.09	3.35±0.07
60	2.20±0.07	2.00±0.07	1.95±0.07	1.80±0.07	2.05±0.08
80	0.70±0.03	1.15±0.06	1.05±0.08	1.20±0.07	1.10±0.11
100	1.72±0.04	1.86±0.04*	1.81±0.05*	1.96±0.03*	1.84±0.06*
120	1.10±0.01*	1.35±0.02	1.30±0.03	1.55±0.03	1.35±0.04
Pre-spawning	2.34±0.05	2.58±0.08	2.63±0.09	2.76±0.09	2.60±0.08
Post-spawning	1.41±0.03	1.60±0.03	1.56±0.04	1.76±0.03	1.59±0.05
Feed conversion ratio					
20	2.11±0.10	2.23±0.13	1.93±0.09	1.62±0.03	2.05±0.13
40	2.76±0.21	2.98±0.17	2.80±0.09	2.53±0.16	2.98±0.16
60	4.85±0.27	6.12±0.24	5.99±0.17	5.72±0.32	5.81±0.13
80	11.33±0.30	10.13±0.29	10.13±0.14	8.50±0.26	10.50±0.32
100	4.44±0.36	5.29±0.31*	4.78±0.31*	4.36±0.30*	5.05±0.19*
120	14.61±0.28*	11.94±0.51	11.58±0.47	9.76±0.53	11.58±0.47
Pre-spawning	5.26±0.22	5.36±0.21	5.21±0.12	4.59±0.19	5.33±0.18
Post-pawning	9.52±0.32	8.61±0.41	8.18±0.39	7.06±0.42	8.31±0.33

* spawning commenced

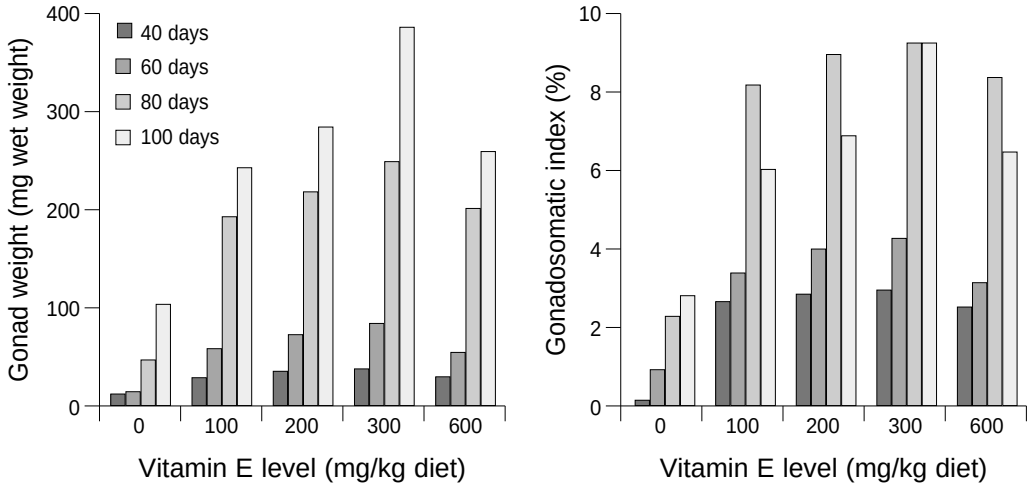

Fig. 1. Gonad weight (a) and gonadosomatic index (b) in *Carassius auratus* fed diets with different levels of vitamin E.

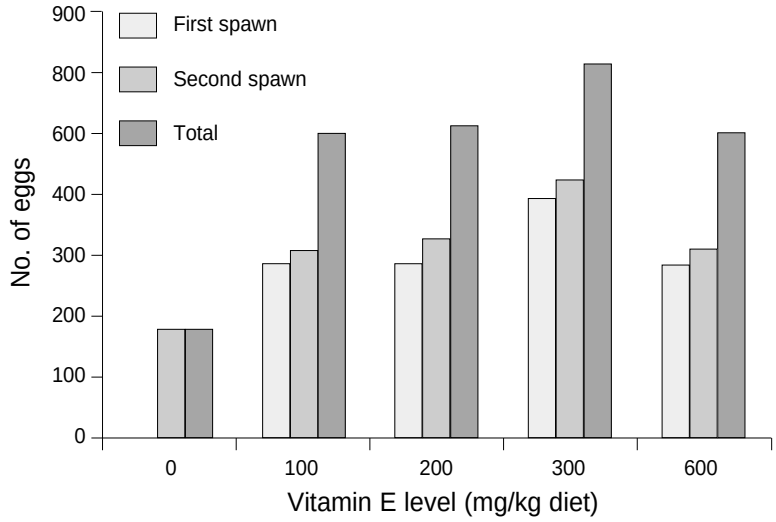

Fig. 2. Number of eggs/spawn and total number of eggs in *Carassius auratus* fed diets with different levels of vitamin E.

form of α-tocopherol is present in the lipid phase of animal tissue. By its ability to terminate lipid peroxidation, α-tocopherol protects biomembranes against oxidative damage. This is particularly important at sites of infection or in immunologically active tissues where phagocytic cells are activated (Lygren et al., 2001). It is likely that vitamin E influenced the immune response in *C. auratus* in this manner.

In conclusion, 300 mg vitamin E/kg diet elicited the best response on gonad develop-

Table 2. Effect of different dietary levels of vitamin E on egg weight, egg diameter, larvae weight, and larvae length in *Carassius auratus* (mean±SD; n = 3).

Vitamin E (mg/kg diet)	*Egg wt (mg wet wt)*	*Egg diameter (mm)*	*Larvae wt (mg wet wt)*	*Larvae length (mm)*
0	1.34±0.01	0.71±0.01	1.51±0.03	3.90±0.03
100	1.38±0.02	0.74±0.01	1.54±0.01	4.11±0.01
200	1.39±0.03	0.74±0.01	1.54±0.02	4.28±0.02
300	1.41±0.02	0.77±0.02	1.57±0.01	4.30±0.01
600	1.37±0.02	0.73±0.02	1.54±0.02	4.27±0.02

Student's *t* test for 0 vs 300 mg vitamin E/kg diet
Mean egg weight: $t = 4.43$; $p<0.01$
Mean egg diameter: $t = 3.80$; $p<0.05$
Mean larval weight: $t = 2.68$; $p<0.05$
Mean larval length: $t = 17.89$; $p<0.01$

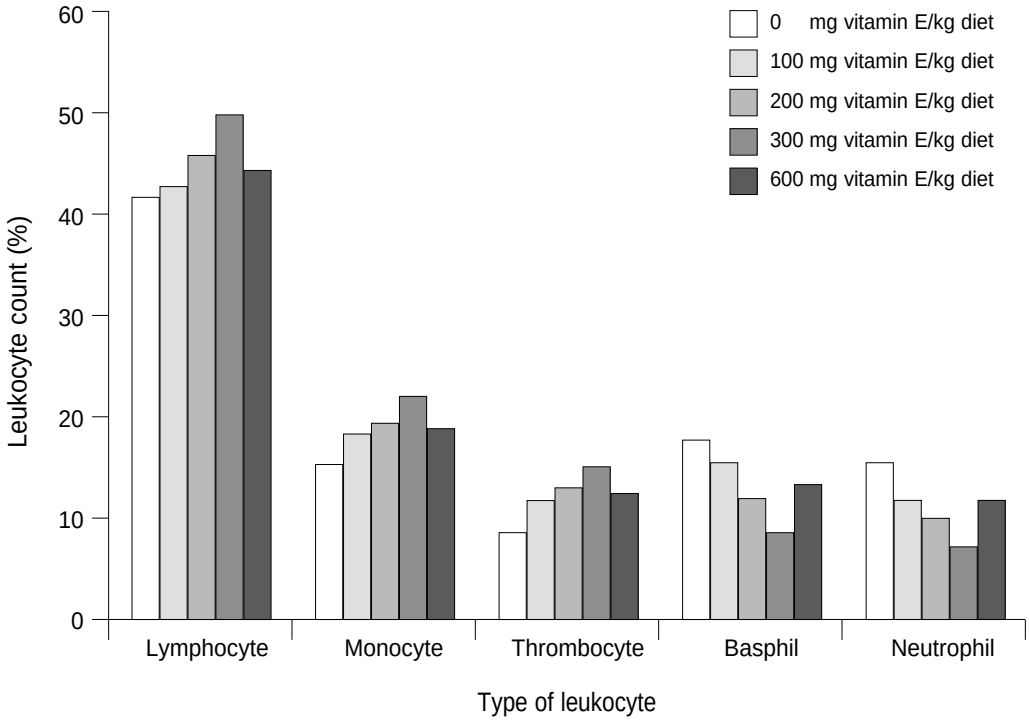

Fig. 3. Effect of different levels of dietary vitamin E on leukocyte count in *Carassius auratus* on day 100.

ment, reproduction, and immunity. Thus, this level of vitamin E can effectively be used as a feed additive to enhance reproduction and immunity of the goldfish, *C. auratus*.

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