emergent
Life Sciences Research

Research Article

Effects of Optimum Vitamin E with Different levels of Vitamin C on Growth, Reproduction and Immune Response in Blue Gourami (*Trichogaster trichopterus*)

Vasudhevan I., Rama Devi P., Asokan K.

Received: 1 May 2017
Accepted: 28 June 2017
Online: 30 June 2017

Authors:
Vasudhevan I. ✉, Asokan K.
Department of Zoology, Vivekananda College, Agasteeswaram, Kanyakumari-629701,Tamil Nadu, India

Rama Devi P.
Department of PG Zoology, Adithanar College of Arts and Science, Tiruchendure, Thoothukudi-628216,Tamil Nadu, India

✉ gold_vasu@yahoo.com

Emer Life Sci Res (2017) 3(1): 57-62

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: http://dx.doi.org/10.7324/ELSR.2017.315762

Abstract

The effect of optimum level of vitamin E (300 mg kg^{-1} diet) and various levels vitamin C (50, 100, 200 and 300 mg kg^{-1} diet) on growth, reproduction and leucocytes count were studied in blue gourami (*Trichogaster trichopterus*) for 120 days. Test fish fed with the optimum vitamin E + 200 mg vitamin C kg^{-1} diet enhanced the chosen feeding and growth parameters, reproduction and disease resisting leucocytes count (lymphocyte and monocyte) than those fed with other diets. The control fish spawned only once with less number of eggs $spawn^{-1}$, while other groups spawned two times with more number of eggs $spawn^{-1}$ during the experiment. The female blue gourami fed with the optimum vitamin E laid 450 eggs in two spawning and it significantly ($P < 0.01$) enhanced to 570 eggs in two spawning fish fed with the optimum vitamin E and 200 mg vitamin C kg^{-1} diet respectively. However, the fish treated with other combinations laid less number of eggs than the above combinations. Lymphocyte and monocyte counts revealed that the fish fed with combination of optimum vitamin E and vitamin C diet produced more or less equal effects. The experiment revealed that vitamin C and vitamin E are the growth and reproduction promoting factors respectively and they interact with one another when these two nutrients are available simultaneously.

Keywords growth, leucocytes count, reproduction, *Trichogaster trichopterus*

Introduction

Vitamin E activity is present in a group of naturally occurring closely related tocopherols. Among those, α-tocopherol has the maximum vitamin E activity. DL α-tocopheryl acetate, a stable vitamin of α-tocopherol, is the most regularly utilized formula in animal feeds (1). Hydrolysis of this ester, α-tocopherol is absorbed from the intestine along with dietary fats (2).

As a fat soluble antioxidant, 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 symptoms witnessed in fish, such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte haemolysis, haemorrhages, depigmentation and lessening of fertility are related to the peroxidative damage to cellular membranes (1).

In aquaculture, Vitamin C (ascorbic acid) is an indispensable nutrient required to maintain the physiological process of different animals including fishes (3). Vitamin C is also directly involved in the maintenance of fish health and resistance to common infectious diseases (4). Vitamins E and C are functional as lipid and water-soluble chain breaking antioxidants respectively and they protect lipids, proteins and membranes from oxidative damage. The interaction between vitamins E and C can take place not only in homogenous solutions, but also in liposomal membrane systems in which vitamins E and C reside separately within and outside the membranes (5, 6). Several authors have investigated the individual effects of supplementation and deficiency of vitamin E or vitamin C on growth and immune response in fishes (7, 8, 9, 10). However, very little is known about the combined effect of dietary vitamins E and C on growth, reproduction and immune response (11,12) in fish. To the best of our knowledge, no studies are available on the interaction of the optimum level of vitamin E with various levels of vitamin C on growth, fecundity and immune response in ornamental fishes. Hence, the present study has been undertaken to investigate the interactions between the optimum level of vitamin E and various levels of vitamin C on growth, reproduction, and leucocytes count in blue gourami (*Trichogaster trichopterus*).

Methodology

Fish and maintenance

Healthy and active juveniles of 40 days old *T. trichopterus* (180 Nos.) (MBL:14.6±0.60 mm; MBW:0.22±0.01 g) were collected from the local aquarium. They were sorted out into six groups. First group served as control and fed with diet containing 38% basal protein only and second group fed with 300 mg vitamin E kg^{-1} diet. James and Vasudhevan (10) found that 300 mg vitamin E kg^{-1} diet was the optimum level for gold fish to enhance the maximum growth, reproduction and leucocytes count. Pilot experiments were conducted to fix the range of vitamin C concentrations. The 3rd, 4th, 5th and 6th groups were fed with the optimum vitamin E diet (i.e. 300 mg vitamin E kg^{-1} diet) with 50, 100, 200 and 300 mg vitamin C kg^{-1} diet respectively. For convenience of presentation, hereafter the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups would be referred as C, C1, T1, T2, T3 and T4, respectively. Each group consisted of 10 individuals and they were reared in circular cement tank containing 100L of water (width: 58.5 cm; height: 40 cm, 120 L capacity). Triplicates were maintained for each test diets. The experimental tanks were filled with dechlorinated well water (Temperature: 28.5±0.80C; pH: 7.5±0.05; Salinity: 0.67±0.03 ppt; Water hardness: 333 mg CaCO3 L^{-1}; DO: 4.13±0.08 mLL^{-1}). The tanks were emptied twice a week and replenished with freshwater to eliminate collected surfaces from the bottom.

Feed preparation

Test diets were prepared using ingredients like fish meal, groundnut oil cake, tapioca and wheat flours and cod liver oil and mineral mix. α – tocopherol acetate was used as the source of vitamin E and L-ascorbic acid was used as vitamin C. Dried and powdered ingredients were blended at first to make a homogenous mixture, subsequently mixed with an aliquot of boiled water and then steam cooked for 15-20 minutes. Optimum vitamin E diet was prepared by dissolving 300 mg α-tocopherol in acetone and sprayed over 1 kg of 38% protein diet ingredients and then uniformly mixed. After moderate cooling, pellets (2mm size) were prepared with a hand operated pelletizer and dried in sunlight. The optimum vitamin E with chosen levels of vitamin C (50, 100, 200 and 300 mg kg^{-1}) diets were prepared by spraying the suitable quantity of L - ascorbic acid dissolved in distilled water and acetone dissolved α-tocopherol over the steam cooked diet. The control diet did not contain vitamins E and C. The sun dried diets were separately stored in refrigerator for experimental use. The experimental feeds were prepared once in two weeks to avoid the nutrient loss (13).

Feeding

Fish were fed *ad libitum* twice a day during the 120 day experiment. Feed was weighed and given in a feeding tray for 1 hr 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 feed offered. The feeding rate was computed as:

$$Feeding\ rate\ (mg\ g^{-1}\ live\ fish\ day^{-1}) = \frac{Amount\ of\ \ feed\ consumed\ (mg)}{Initial\ wet\ weight\ of\ \ fish\ (g)\ X\ \ No.\ of\ \ days}$$

Growth and gonad estimations

Fish were weighed at the beginning of the experiment and on every 20 days. Growth or gain in weight 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 the difference between the wet weight at the beginning of the experiment and on the day of calculation as:

$$SGR\ (\%) = \frac{l_n Wt_1 - l_n Wt_0}{t_1}\ x\ 100$$

where $l_n\ Wt_0$ and $l_n\ Wt1$ 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 the feed consumption to gain in weight of fish.

The mean body weight (g) was calculated by dividing 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 (mm) was measured. One female from each treatment were sacrificed at 20 days interval from the time of gonad development till the commencement of spawning. Their ovaries were removed and weighed and the gonadosomatic index (GSI) was computed according to (14).

$$Gonadosomatic\ index\ (\%) = \frac{Wet\ weight\ of\ \ gonad}{Wet\ weight\ of\ \ fish}\ x\ 100$$

The mean specific growth rate, gonad weight and GSI values were calculated as the respective 20 days sample data relating to number of samplings during the experiment.

Leucocytes count

Prior to sampling 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 (15). Fish, feed samples, unconsumed feed and ovaries were weighed in an electric monopan balance to an accuracy of 1 mg.

Spawning

One male were chosen from each replicate and reared with a female 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. After the spawning, eggs were counted and sample of few eggs were weighed without causing much disturbances. Unhatched eggs were also counted after 48-60 hours of fertilization.

Statistical analysis

Student's t test was applied to determine the significance of difference between group means. Two way – ANOVA test was applied to find the significant effects of chosen nutrients levels and rearing period on feeding and growth parameters(16).

Table 1. Effect of optimum level of vitamin E (mg kg^{-1} diet) with different levels of vitamin C (mg kg^{-1} diet)on feed consumption (g dry matter), feeding rate (mg g^{-1} live fish day^{-1}), gain in weight (g wet weight), specific growth rate (%), and feed conversion ratio in *T. trichopterus*. (mean±SD, n=3).

	Experimental diets					
	C	C1	T1	T2	T3	T4
			Feed consumption			
Pre-spawning	29.02±1.07	41.11±0.78	44.35±0.82	49.25±0.59	55.39±2.03	46.62±0.81
Post-spawning	-	47.50±1.09	53.67±0.17	58.19±1.45	63.73±0.79	52.14±0.63
			Feeding rate			
Pre-spawning	108.16±1.72	116.91±1.70	124.58±2.28	125.48±2.49	128.26±2.41	123.20±2.52
Post-spawning	-	149.90±2.75	166.10±1.74	168.04±2.16	173.75±2.19	163.39±2.81
			Gain in weight			
Pre-spawning	7.76±0.52	9.65±0.46	9.09±0.47	10.16±0.54	11.21±0.60	8.91±0.36
Post-spawning	-	3.18±0.25	3.36±0.24	3.60±0.22	3.78±0.33	3.51±0.28
			Specific growth rate			
Pre-spawning	2.23±0.21	2.57±0.20	2.46±0.17	2.76±0.22	2.98±0.16	2.40±0.18
Post-spawning	-	0.95±0.02	0.98±0.03	1.05±0.04	1.21±0.06	1.07±0.03
			Feed conversion ratio			
Pre-spawning	4.39±0.27	4.40±0.25	5.34±0.26	5.23±0.17	5.04±0.29	5.21±0.30
Post-spawning	-	14.82±0.42	15.57±0.63	15.41±0.49	15.70±0.47	14.48±0.39

Results and Discussion

The mean body weight was increased with increase in rearing period in *T. trichopterus* fed with all the experimental diets. Among the test diets, the fish fed with C1 followed by T3 diets showed significantly (t = P < 0.05) higher mean body weight than those fed on C1 and other diets. Two-way ANOVA revealed that the optimum vitamin E with different levels of vitamin C and rearing period hold significant effect on mean body weight in *T. trichopterus*. The mean feed consumption feeding rate and feed conversion ratio (FCR) of test animal were higher at the post-spawning as compared to the pre-spawning; however, gain in weight and specific growth rate showed the opposite trend. Among the experimental groups, the fish fed with C1 diet followed by T3 diet significantly (P<0.01) enhanced the high feeding and growth parameters than those fed on C and other diets (Table 1). The present study showed that dietary supplementation of vitamin C levels along with optimum vitamin E influenced the feeding and growth parameters in *T. trichopterus*. It might be due to the interactions of vitamin E with vitamin C supplementation. Working on juvenile hybrid striped bass (*Morone chrysops* female *X M. saxatilis* male), Sealey and Gatlin (17), found that dietary addition of vitamins E and C significantly (P<0.05) influenced the weight gain, feed efficiency and mortality. In addition, they also observed a significant interaction between vitamin C and vitamin E, which supports the present study. It is likely that effective interaction between vitamin E and vitamin C could have influenced the feeding and growth in *T. trichopterus*. The relatively high FCR value was probably as a result of a larger proportion of the feed allocated for the maintenance (18) and spawning of eggs (10) in *C. auratus*. The present study showed that combinations of the optimum vitamin E with various levels of vitamin C equally influenced the leucocytes (lymphocyte and monocyte) count in *T. trichopterus*. However, no synergistic effect was seen in leucocytes count. The fish fed with C1or T4 diets significantly induced the maximum response in lymphocyte production and indicated the triggering of immune mechanism of fish under stress to fight against the entry of foreign bodies into the blood stream. Bell et al. (21) found that there was a significant synergistic interaction between vitamin E and selenium on packed cell volume and NADPH dependent microsomal lipid peroxidation system in rainbow trout, *Salmo gairdneri*. Eicher -Pruiett et al. (22) observed a synergistic relationship of vitamins E and C on neutrophil and lymphocyte production in young calves. Montero et al. (23) reported that vitamins E and C are most important nutrients enhancing the immune system to ameliorate the stress and disease caused due to high stocking density in gilthead seabream, *Sparus aurata*. They also reported that vitamin E seems to have more protective role against

stress than vitamin C under crowded condition. Thus, from the present study, it can be concluded that utilization of two nutrients like vitamin E and vitamin C are very important while preparing diet for ornamental fish to enhance growth, fecundity and disease resistance.

References

[1] National Research Council **(1983)**. Nutrient Requirement of Warm-water Fishes and Shell fishes. National Academy Press. Washington, DC, .pp. 102.
[2] A. Bjorneboe., G. E. Bjorneboe and C.A. Drevon **(1990)**. Absorption, transport and distribution of vitamin E. J. Nutr., **120:** 233-242.
[3] B. M. Tolbert **(1979)**. Ascorbic acid metabolism and physiological function. Int. J. Vit. Nutr. Res., **19:** 127–142.
[4] O. Navarre and J. E. Halver **(1989)**. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture, **79:** 207-221.
[5] P. B. McCay **(1985)**. Vitamin E interactions with free radicals and ascorbate. Annu. Rev. Nutr., **5:** 323-340.
[6] E. Niki **(1987)**. Antioxidants in relation to lipid peroxidation. Chem. Phy. Lipids, **44:** 227-253.
[7] N. Ruff., P. Lavens., J.-Z. Huo., P. Sorgeloos., H. J. Nelis and A. De Leenheer **(2001)**. Antioxidant effect of dietary tocopherol and ascorbic acid on growth and survival of *Litopenaeus vannamei* post larvae. Aquacult. Int., **9:** 115–126.
[8] M. M. Harlioglu., K. Koprucu and Y. Ozdemir **(2002)**. The effects of dietary vitamin E on the pleopodal egg number of *Astacus leptodactylus* (Eschscholtz, 1823). Aquacult. Int., **10:** 391-397.
[9] R. James., K. Sampath., R. Thangarathinam and I. Vasudhevan **(2006)**. Effects of dietary *Spirulina* on growth, fertility, coloration and leucocytes count in red swordtail, Xiphophorus helleri. Isr. J. Aquac., **58:** 97–104.
[10] R. James and I. Vasudhevan **(2011)**. Effect of dietary vitamin C on growth, reprodution and leucocytes count in the gold fish, *Carassius auratus* (Linnaeus,1758). Indian J. Fish., **58:** 65-71.
[11] R. Frischknecht., T.Wahli and W. Meier **(1994)**. Comparison of pathological changes due to deficiency of vitamin C, vitamin E and combinations of vitamin C and E in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., **17:** 31-45.
[12] T.Wahli., V.Verlhac., J.Gabaudan., W.Schuep and W. Meier **(1998)**. Influence of combined vitamin C and E on non-specific immunity and disease resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., **21:** 127–137.
[13] V. S. Blazer and R. E. Wolke **(1984)**. The effect of α-tocopherol on the immune responses and non-specific resistance factors of rainbow trout (*Salmo gairdneri Richardson*). Aquaculture, **37:** 1-9.
[14] B.T.Dahlgren **(1979)**. The effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata* (Peters). J. Fish Biol., **15:** 71-91.
[15] I.Vasudhevan **(2008)**. Effect of selected growth promoting immunostimulants on growth, reproduction, coloration and leucocytes count in gold fish, *Carassius auratus*. Ph.D. Thesis submitted to Manonmanian Sundaranar University, Tirunelveli, TN, India.
[16] J. H. Zar **(1999)**. Biostatical Analysis. 4th edn. New Jersey.
[17] M.Sealey and M. Gatlin **(2002)**. Dietary vitamin C and vitamin E interest influence growth and tissue composition of juvenile hybrid striped bass (*Morone chrysops* (female) *x M. saxatilis* (male)) but have limited effects on immune responses. J. Nutr, **132:** 748-755.
[18] Fange, R., and D. Grove **(1979)**. Fish Physiology, Vol. 8 Bioenergetics and Growth.
[19] A. C. Emata., I. G. Borlongan and J. P. Damaso **(2000)**. Dietary vitamin C and E supplementation and reproduction of milkfish *Chanos chanos*. Aquacul. Res., **3:** 557-564.
[20] T.Watanabe., M. J. Lee., J .Mizutani., T. Yamada., S. Satoh and T. Takeuchi **(1991)**. Nutritional studies in the seed production of fish. 20. Effective components in cuttlefish meal and raw krill for improvement of quality of red seabream, Pagrus major eggs. Nippon Suisan Gakkaishi, **57:** 681-694.

[21] J. G. Bell., C. B. Cowey., J. W. Adron and A. M. Shanks **(1985)**. Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdneri*). Br. J. Nutr., **53:** 149–157.
[22] S. D. Eicher -Pruiett., J. L. Morrill., F. Blecha., J. J. Higgins., N. V. Anderson and P. G. Reddy. **(1992)**. Neutrophil and Lymphocyte response to supplementation with vitamins C and E in young calves. J. Dairy Sci., **75:** 1635–1642.
[23] D. Montero., M. Marrero., M. S. Izquierdo., L. Robaina.,J. M. Vergara and L. Tort **(1999)**. Effect of vitamin E and dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. Aquaculture, **171:** 269-278.