Journal of Aquatic Biology & Fisheries | 2021 | **9** | pp. 48-54

ISSN 2321–340X

Combined Effects of Dietary β- Carotene and Vitamin C on the Growth Performance and Survival of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

Sumi, S.S.* and Prasad, G.

Department of Zoology, University of Kerala,
Thiruvananthapuram - 695 581, Kerala, India
**Email: sumiss376@gmail.com*

Abstract

The present study was conducted to examine the combined effect of two substances, Vitamin C and β- Carotene, on the growth and survival rate of Nile tilapia, *Oreochromis niloticus* fingerlings. Fish were divided into four diet groups, each with triplicates. Group 1 is regarded as a control group supplemented with a diet without Vitamin C and β- Carotene. The remaining groups, such as group 2, group 3, and group 4, were regarded as experimental groups supplemented with Vitamin C and β- Carotene in three different concentrations. Growth and feeding parameters and survival rate of fingerlings were evaluated. Group 3 and group 4 show significant ($P<0.05$) increasing variation in weight gain (WG), feed conversion efficiency (FCE), feed efficiency (FE), and protein efficiency ratio (PER) compared with group 1 and group2. Specific growth rate (SGR) shows significant ($P<0.05$) increasing variation in group 4 compared with group 1 and group 2. Feed conversion ratio (FCR) shows significant ($P<0.05$) decreasing variation in group 4 compared with group 1. Apparent digestibility co-efficiency for crude protein is significantly ($P<0.05$) increased in group 4, compared with group 1. Apparent digestibility co-efficiency for crude fat is significantly ($P<0.05$) increased in group 3 and group 4, compared with group 1 and group 2. Apparent digestibility co-efficiency of β–carotene shows the highest significant ($P<0.05$) value in all three groups compared with group 1. The digestibility of Vitamin C is optimum in all groups. There were no significant differences in survival rate observed between the treatments ($P>0.05$).

Keywords: Nile tilapia, β- Carotene, Vitamin C, Fish Feed, Growth, Survival, Nutrient Digestibility

1. Introduction

The global fish production is 177.8 million metric tons in 2019, up from 148.1 million metric tons in 2010, and fish is one of the most widely consumed foods globally, and it is only becoming more popular over time (Shahbandeh, 2020). India ranks third in fisheries production and second in aquaculture (NFDB, 2020). The Indian government recognizes tilapia farming as a critical sector in aquaculture, especially considering the success of other tilapia industries in tropical and subtropical regions worldwide, and the majority of tilapia currently produced in India are Nile tilapia strains (Menaga and Fitzsimmons, 2017). Tilapias are second only to carps as the most widely farmed freshwater fish in the world (FAO, 2012).

Tilapia is a group of freshwater omnivorous cichlids that are native to Africa and subsequently have been introduced, either deliberately or accidentally, throughout the world (Eknath, 2009). Towering fish costs will cause overfishing of unfarmed fishes and reduce food security for many customers that depend on fish as food (Workagegn *et al*., 2014). Besides, the fish supply can be increased through sustainable aquaculture production. The fish protein will become a scarce and costly commodity (FAO, 2012) since feed additives used as growth promoters and immunostimulants have a much important role in fish farming. Consequently, the expansion of research in aquafeed has much importance. Nowadays, various feed additives are included in fish feed as growth promoters and immune boosters. Nutraceuticals are foodstuffs, or parts of food, that supply medical or health benefits, such as enhanced growth rates and decreased disease susceptibility, which include fats, vitamins, and minerals that are essential for life and the proper growth and health of farmed fish (Mustafa *et al*., 2011).

Vitamin C (Ascorbic acid) is an essential molecule for the normal growth and metabolic functions of fish (Lim and Lovell, 1978). It can be broadly used as a dietary supplement as almost all fish lack the last enzyme of the Vitamin C biosynthetic pathway, L-gulonolactone oxidase (Chatterjee, 1973). The defence of living cells from oxidative damage is provided by ascorbic acid by neutralizing the reactive oxygen species (Verlhac and Gabaudan, 1994). Inadequate supply of dietary Vitamin C usually results in several deficiency signs such as spinal deformation, impaired collagen formation, internal haemorrhaging, and retarded growth (Coustans *et al*., 1998).

Carotenoids comprise a widespread group of plant synthesized polyene pigments, which vary in colour from yellow and orange to red (Tacon, 1981). These are lipid-soluble pigments divided into two groups: (red) capsanthin and (yellow) xanthophylls. Carotenoids have various beneficial effects on aquatic animals; they intensify larval growth and survival (Torrissen, 1984) and enhance the performance of broodstock (Watanabe *et al*., 1991; Verakunpiriya *et al*., 1997), as well as improve disease resistance (Tachibana *et al*., 1997). They also upgrade colouration in the flesh of salmonid fish (Yanar *et al*., 2007). As long as fish, like other animals, cannot synthesize carotenoids (Goodwin, 1984; Torrissen *et al*., 1989), they have to acquire carotenoids from dietary substances. Fishes can reform alimentary carotenoids and store them in the integument in addition to skin, flesh,

Received on: 02-01-2020; Revised on:16-04-2021; Accepted on: 03-05-2021

gonads, kidney, liver, intestines, and only in tiny quantity in the brain. Unfarmed carnivore fish obtain most of their carotenoids by feeding on small crustaceans and other vertebrates previously fed on algae. However, when fish are deprived of their natural food sources under rearing conditions, dietary supplementation of carotenoids is necessary. β- Carotene is one of the carotenoids (Kelestemur and Coban, 2016), which is the dimer of vitamin A and has been shown to have positive effects on various physiological and immunological parameters in humans and other vertebrates (Mustafa *et al*., 2013).

The mixed application of vitamins in feeds has also given better benefits to overcome growth retardation and disease problems. The present study was depicted to examine the combined effect of different dietary levels of Vitamin C (L-Ascorbic acid) and β- Carotene on the growth parameters and survival status of Nile tilapia *(Oreochromis niloticus*) fingerlings.

2. Materials and Methods

2.1. Experimental system and experimental fish

Oreochromis niloticus fingerlings were purchased from a fish hatchery situated in Alappuzha District, Kerala, and transported to the laboratory in aerated plastic bags. The fish were permitted to acclimatize for 2 weeks at laboratory conditions and then used for experiments. The weight of the animals ranged 5.61±0.109g. The experiments were carried out in glass tanks of 250 L capacity containing tap water. Each culture tank is maintained with 200 L of water throughout the experimental period, and the 80 L of water in each tank was changed on alternate days.

The study was conducted over 60 days to evaluate the efficacy of β- Carotene and Vitamin C in Nile tilapia (*O. niloticus)* fingerlings. One hundred and twenty *O. niloticus* fingerlings were divided into four equal groups. Ten fish were selected randomly into triplicate tanks for each dietary group with near-uniform biomass. All experimental fish were acclimated to the basal diet for 2 weeks before starting the growth trial. Four types of diets were prepared. The daily feed allowance was 3% of body weight per day. The experimental fishes were fed twice a day for 60 days. After seven days of the feeding trial, faecal matter was collected from week 2 to the experimental period. An excess amount of feed was collected one hour after each feeding, and then the faecal matter was collected by siphoning. To reduce the nutrient leaching in faecal matter, only fresh and intact faecal matter was collected. Faecal matter collected from a single tank was pooled each day and dried at 80 ^{0}C. Then it was used for proximate analysis. Fish were evaluated for growth performance and survival rate.

2.2. Diet formulation

Four types of diets were formulated for the experiment (Table 1). The group 1 diet was considered a basal diet devoid of Vitamin C and β-Carotene. The other three test diets were added with Vitamin C and β-Carotene in the following concentration. Group 2 diet contain β- Carotene 60mg/kg and Vitamin C 4000 mg/kg, group 3 diet contain β- Carotene 80mg/kg and Vitamin C 6000 mg/kg and group 4 diet contain β-Carotene 100mg/kg and Vitamin C 8000 mg/kg levels respectively. Fish meal, yellow corn, soybean meal, wheat bran, and cod liver oil were purchased from the local market. β- Carotene and Vitamin C (L ascorbic acid) were obtained from the Associated Scientific Company, Thiruvananthapuram. The diets were processed by blending the dry ingredients into a homogeneous mixture. All the powdered ingredients and vitamin-mineral premix were kneaded into a dough using the required amount of water. Vitamin C (L- Ascorbic acid) was added to the dough. β- Carotenewas dissolved in cod liver oil and mixed with dough very well. After that, the dough was pelleted using a noodle-making machine through a mesh at 2 mm diameter in size. The resulting pellet was dried at 60^{0}C for 12 hours and stored in an airtight container. The chemical compositions of the experimental and basal diets are presented in Table 1.

2.3. Water quality

The water quality parameters of the control and experimental tanks were measured individually. The temperature was recorded daily using a mercury thermometer. Dissolved oxygen (DO) was measured by the Winkler method (Winkler, 1888) and pH using a pH meter. Nitrite- N was measured at weekly intervals according to APHA, AWWA, and WPCF (1985).

2.4. Evaluation of growth parameters and survival

At the end of the experimental period, the fishes were fasted for 24 h before harvest. The mean body weight was measured. Based on recording the weight of each fish, Weight Gain (WG g), Weight Gain in percentage (WG p), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), Feed Efficiency (FE), Protein Efficiency Ratio (PER) and survival (%) (SR) were calculated using the following equations:

Weight Gain (g) = final body weight (g) - initial body weight (g)

Weight Gain percentage = 100× (final body weight (g) - initial body weight (g)) ÷ initial body weight (g)

SGR (%) = 100× (ln final weight (g) -ln initial weight (g)) ÷ total duration of the experiment

FCR = feed given (g) ÷ weight gain (g)

FCE= weight gain (g) ÷ feed given (g)

FE=Wet body weight gain (g) ÷ total dry feed consumed (g)

PER =body weight gain (g) ÷ crude protein in the feed (g)

Survival (%) = 100 × [(Initial number of fish – Final number of fish)/initial number of fish]

2.5. Proximate composition analysis

Crude protein, crude fat, ash, and moisture contents in feed ingredients, diets, faecal matter, and whole-body fish samples were determined (AOAC, 1995). Crude protein content was measured by the Kjeldahl method (N×6.25). Fat content was measured by the Soxhlet method. Moisture content was estimated by the drying method, using an oven at 105^{0}C. Ash content was estimated by the method of combustion, using a muffle furnace at 550^{0}C. Acid-insoluble ash content was examined by using the muffle furnace at 550^{0}C after acid digestion. Acid-insoluble ash was used as an endogenous indicator, the Vitamin C content of diets and faecal matter were spectrophotometrically determined (Kapur, 2012), and β-

Table 1. Ingredients and composition of basal and test diet

Ingredients (Gram %)	Diets at different β-carotene and vitamin C concentration ratio			
	Group1	Group2	Group3	Group4
Fish meal	15	15	15	15
Soybean meal	32	32	32	32
Yellow corn	20	20	20	20
Wheat bran	28	27.594	27.392	27.19
Vitamin and mineral mix	2	2	2	2
Cod liver oil	3	3	3	3
β-carotene	-	0.006	0.008	0.01
vitamin C	-	0.4	0.6	0.8
Total	100	100	100	100
Proximate composition (%)				
Crude protein	32.75±0.856[a]	32.75 ±0.057 [a]	32.78±0.033 [a]	32.83±0.072 [a]
Crude fat	6.66±0.088 [a]	6.73±0.12 [a]	6.63±0.133 [a]	6.63±0.088 [a]
moisture	8.7±0.057 [a]	8.76±0.066 [a]	8.80±.00 [a]	8.80±0.057 [a]
Dry matter	91.30±0.057 [a]	91.23±0.066 [a]	91.2±0.00 [a]	91.20±0.057 [a]
ash	7.73±0.12 [a]	7.66±0.088 [a]	7.70±0.10 [a]	7.53±0.033 [a]
β-carotene	0.00±00 [a]	0.0059±00 [b]	0.0084±0 [c]	0.0098±00 [d]
Vitamin C	0.0037±0.0006 [a]	0.406±0.0024 [b]	0.606±0.0016 [c]	0.806±0.0021 [d]

Values are mean ± S.E. Values with different superscript letters at the same row are significant at p<0.05 (ANOVA). Mineral mixture (Agrimin[R] FORTE, virbac India) contains (nutritional value per kg): Vitamin A, 700000 I.U.; Vitamin D3, 70000 I.U., Vitamin E, 250 mg; Co, 150 mg; Cu, 1200 mg; I, 325 mg; Fe, 1500 mg; Mg, 6000 mg; K, 100 mg; Na, 5.9 mg; Mn, 1500 mg; S, 0.72%, Zn, 9600 mg; DL- Methionine, 1000 mg; Ca, 25.5 %; P, 12.75%.

Carotene content was spectrophometrically determined (Kelestemur and Coban, 2016; Metusalach *et al*., 1997; Amara *et al*., 2004).

The apparent digestibility co- efficients (ADCs) of the diets for dry matter, crude protein, crude fat, β carotene, and vitamin C were calculated according to the following equation (Spyridakis *et al*., 1989):

$$ADC\ (\%) = 100-100\times (Nf)/(Nd) \times (Md)/(Mf)$$

Where Nf is the percentage of a specific nutrient in faeces, Nd is the percentage of the same nutrient in the diet, Md is the percentage of acid-insoluble ash in the diet, and Mf is the percentage of acid-insoluble ash in faeces.

2.6. Statistical analysis

All the values were presented as mean ± SE. Differences between group means were assessed by one-way analysis of variance (ANOVA), and post hoc multiple comparisons with Duncan multiple range test was used by the SPSS 18 computer program. The result with $P < 0.05$ was considered statistically significant.

3. Results

3.1. Water quality

All water quality parameters were within the acceptable range at *O. niloticus* fingerlings. The water temperature ranged from 27.5^0 C to 29^{0}C, DO from 5.8 to 6.5mg L^{-1}, Nitrite- N from 0.05 to 0.2 mg $L^{-1,}$ and pH from 6.6 to 7.2.

3.2. Growth parameters and survival

Levels of Vitamin C and β- Carotene significantly influenced the weight gain of Nile tilapia fingerlings (Table 2). Weight gain significantly (P<0.05) increased in group 4, compared with group 1 and group 2. Group 3 shows significantly (P<0.05) increased variation in weight

Table 2. Combined effect of dietary β-carotene and vitamin C on the growth performance and survival status of *O. niloticus* fingerlings over a feeding period of 60 days.

Growth parameters	Groups			
	Group1	Group2	Group3	Group4
IW	5.31±0.189 [a]	5.48±0.24 [a]	5.59±0.210 [a]	6.09±0.176 [a]
FW	6.92±0.55 [a]	7.30±0.601 [a]	8.75±0.726 [ab]	10.42±0.642 [b]
WG g	1.61±0.36 [a]	1.82±0.36 [a]	3.16±0.53 [b]	4.33±0.48 [b]
WG p	28.79±5.43 [a]	31.24±5.31 [ab]	54.18±8.54 [bc]	69.52±6.76 [c]
FI	9.55±0.34 [a]	9.90±0.42 [a]	9.77±0.39 [a]	10.96±0.316 [a]
FCE	0.159±0.03 [a]	0.173±0.029 [a]	0.327±0.045 [b]	0.388±0.036 [b]
FCR	9.678±2.65 [a]	8.811±2.149 [ab]	5.99±2.051 [ab]	2.81±0.293 [b]
SGR	0.458±0.085 [a]	0.459±0.069 [a]	0.695±0.101 [ab]	0.770±0.091 [b]
FE	0.159±0.030 [a]	0.172±0.029 [a]	0.358±0.046 [b]	0.388±0.036 [b]
PER	0.488±0.092 [a]	0.529±0.090 [ab]	0.918±0.145 [bc]	1.185±0.112 [c]
SR(p)	100	100	100	100

Values are mean ± S.E. Values with different superscript letters in the same row are significant at p<0.05 (ANOVA). IW(g)- initial weight in gram, FW (g)- final weight in gram, WGg- weight gain in gram, WGp- weight gain in percentage, FI(g)- feed intake in gram, FCE- feed conversion efficiency, FCR- feed conversion ratio, SGR (%)- specific growth rate in percentage, FE- feed efficiency, PER- protein efficiency ratio, SR (%)- survival rate in percentage

Table 3. Combined effect of dietary β-carotene and vitamin C on nutrient digestibility of *O. niloticus* fingerlings over a feeding period of 60 days

Groups	Apparent digestibility co- efficient (%)				
	Crude protein	Crude fat	Dry matter	β-carotene	Vitamin C
Group 1	82.96±0.07[a]	90.71±0.379 [a]	78.35±0.094 [a]	000 [a]	91.23±2.35 [a]
Group 2	83.03±0.34 [a]	91.076±0.234[a]	78.623±0.474[a]	39.58±2.98 [b]	94.00±0.15 [a]
Group 3	83.99±0.130[ab]	92.823±0.486[b]	79.523±0.546[a]	43.85±3.56 [b]	94.00±0.22 [a]
Group 4	84.27±0.370[b]	93.35±0.196 [b]	79.756±0.283[a]	46.21±1.33 [b]	94.42±0.28 [a]

Values are mean ± S.E. Values with different superscript letters in the same column are significant at p<0.05 (ANOVA).

gain compared with group 1, but group 2 did not show significant (P>0.05) variation from group 1. The highest feed intake (FI) was observed in group 4. Feed conversion efficiency (FCE) shows a significant (P<0.05) increasing rate in group 3 and group 4 compared with groups 1 and group 2 but group 2 did not show significant (P>0.05) variation from group 1. Feed conversion ratio (FCR) was significantly (P<0.05) lower in group 4 than in group 1 but group 2 and group 3 did not show significant (P>0.05) variation from group 1. The specific growth rate (SGR) of group 4 shows a significant (P<0.05) increasing variation compared with group 1 and group 2, but group 2 and group 3 did not show a significant (P>0.05) increase compared with group 1. The values of feed efficiency (FE) in group 3 and group 4 show the highest significant (P<0.05) increasing variation compared with group 1 and group 2 but group 2 did not show significant (P<0.05) variation than group 1. Protein efficiency ratio (PER) shows a significant (P<0.05) increasing trend in group 3 and group 4 compared with group 1, but group 2 did not show significant (P>0.05) variation from group1. Mortality was nil during the experimental period.

3.3. Nutrient digestibility

As shown in Table 3, the values of apparent digestibility co-efficiency for crude protein is significantly (P<0.05) increased in group 4 compared with group 1 and group 2. Group 2 and group 3 did not show significant (P>0.05) variation in apparent digestibility co-efficiency for crude protein from group 1. In apparent digestibility, co-efficiency for crude fat shows a significant (P<0.05) increasing variation in group 3 and group 4 compared with group 1 and group 2 (P<0.05), but group 2 did not show significant (P>0.05) variation from group 1. The digestibility value of dry matter did not significantly (P>0.05) differ among dietary treatments. However, it showed an increasing trend with increased concentration of dietary β carotene and vitamin C compared with the group1. Digestibility of β carotene is significantly (P<0.05) increased in all vitamins treated group compared with group 1 (P<0.05). The digestibility value of vitamin C shows an optimum range in all dietary treatments.

3.4. Whole-body composition

The values of crude protein, crude fat, dry matter, ash, and moisture contents of the fish did not significantly (P>0.05) vary across all dietary treatments (Table 4).

4. Discussion

β- Carotene, a dimer of vitamin A, is another neutraceutical of interest that has been shown to increase the growth rate in Nile tilapia (Hu *et al*., 2006). Essential to the proper development and health of fish, vitamin A is a proven requirement for maintaining vital functions, including growth, normal vision, and epithelial cell differentiation (Olson 1991). Also, Nile tilapia has been shown to possess the ability to bio transform β- Carotene to vitamin A by a reduction reaction (Katsuyama and Matsuno, 1988). Dietary vitamin C is essential for normal growth and physiological functions in most fishes (Sakai, 1999). Dietary vitamin C can also improve fish growth performance (Al-Amoudi *et al*., 1992; Li *et al*., 1998). In the present study, our findings suggested *Oreochromis niloticus* fingerlings fed with a diet containing β- Carotene and vitamin C at the concentration rate of 80 mg/ kg- 6000 mg/ kg and 100 mg/ kg - 8000 mg/ kg shows highest significance (P<0.05) weight gain. Mustafa *et al*., 2013, observed similar results. Who studied the effect of phosphatidal choline and β- Carotene on juvenile Nile tilapia, *Oreochromis niloticus*. Similarly, vitamin A has been shown to increase growth rates in tilapia (Hu *et al*., 2006). That links carotenoids to growth enhancement in Atlantic salmon fry (*Salmo salar*) or the improvement of survival rate in kuruma prawn (*P. japonicus*) (Chien and Jeng, 1992). The highest values were obtained on FCE, FCR, SGR, PER, and FE in fish fed with a diet containing 100 mg/ kg β- Carotene - 8000 mg/ kg vitamins, Kelestemur and Coban, 2016., supported this result. Who studied the effect of the β- Carotene on the growth performance and skin pigmentation of rainbow trout and the result found that fish fed with β- Carotene carotene supplemented diet shows the highest value in weight gain, SGR, and survival. They also revealed that apparent digestibility co-efficiency of crude protein is significantly

Table 4. Combined effect of dietary β-carotene and vitamin C on whole body composition of *O. niloticus* fingerlings over a feeding period of 60 days

Groups	Whole body composition				
	Crude protein	Crude fat	Moisture	Dry matter	Ash
Group 1	13.07±0.07[a]	8.93±0.218 [a]	72.43±0.39[a]	27.56±0.300[a]	4.97±0.01[a]
Group 2	13.29±0.44[a]	8.10±0.20 [a]	72.62±0.34[a]	27.37±0.346[a]	4.77±0.12[a]
Group 3	13.36±0.46[a]	8.73±0.185 [a]	72.56±0.47 [a]	27.44±0.470[a]	4.56±0.29[a]
Group 4	13.28±0.26[a]	8.66±0.578 [a]	72.39±0.38[a]	27.61±0.383[a]	4.74±0.16[a]

Values are mean ± S.E. Values with different superscript letters in the same column are significant at p<0.05 (ANOVA).

higher in β- Carotene supplemented fish compared with fish fed without β- Carotene. However, the digestibility value of crude fat did not differ. But in our findings, the digestibility value of crude protein and crude fat higher in fish treated with β- Carotene and vitamin C; this groups also shows the highest nutrient digestibility in vitamin C and β- Carotene

Specific growth rate (SGR) is a reflection of the health status of fish. In this study, the fish group fed with β- Carotene 100mg/kg and vitamin C 8000 mg/kg showed a significant increase in SGR than the control group. This result is supported by Ibrahim *et al*. (2010), who studied the effect of dietary vitamin C and inulin in *O. niloticus*, which recorded the highest growth rate in the vitamin C supplemented group compared with the control group. These results are also supported by Kumari and Sahoo (2005). They determined the effects of high dietary ascorbic acid (vitamin C) on Asian catfish growth (*Clarias batrachus*). Their findings support the beneficial role of ascorbic acids as a growth promoter at the rate of 500mg/kg diet for a period of 4weeks in catfish farming rather than higher concentrations and more extended periods. Tewary and Patra (2015) found that Indian major carp, rohu (*Labeo rohita*), fed a Vitamin C-supplemented diet, showed a higher specific growth rate (SGR) β- Carotene upto1000 mg/kg compared with control fish. However, SGR data of group 4 showed significantly higher (P<0.05) than the control group. Feed intake was the highest in tilapia fed with β- Carotene 100mg/kg and vitamin C 8000 mg/kg supplemented diets, indicating that the nutrients were more efficiently used for growth performance. The WG and SGR of the tilapia increased, whereas the FCR decreased as the dietary β- Carotene and vitamin C levels increased. The results of vitamin C results were in accordance with (Kumari and Sahoo, 2005., Lovell, 1991), who determined the effects of high dietary ascorbic acid (Vitamin C) on Asian catfish growth (*Clarias batrachus*), their findings support the beneficial role of ascorbic acid as a growth.

In this study, apparent digestibility co-efficiency of crude fat shows a significant (P<0.05) increasing variation in the fish group fed with diet contain β Carotene 80mg/kg - Vitamin C 6000 mg/kg and diet β Carotene 100mg/kg - Vitamin C 8000 mg/kg, which means that increasing concentration of dietary β- Carotene and vitamin C led to increasing ADC of fat levels. Apparent digestibility co-efficiency of the crude protein shows only an increasing trend in the fish group fed with diet contain β Carotene 80mg/kg - Vitamin C 6000 mg/kg compared with the control group but shows significant (P<0.05) increase in the group fed with diet containing β Carotene 100mg/kg - Vitamin C 8000 mg/kg compared with the control group. These results conform with Lara, 2003. His study revealed that the better digestibility obtained with the addition of probiotics is improved diet and protein digestibility, which may, in turn, explain the better growth and feed efficiency noticed with the supplemented diet. Digestibility of β- Carotene shows an increasing trend in increasing the concentration of β- Carotene in feed and digestibility value of vitamin C shows optimum range all dietary treatment indicate that maximum absorption of vitamin C. Dietary vitamin C is essential for normal growth and physiological functions in most fishes (Sakai, 1999). The fact that teleost fish lack the enzyme responsible for the endogenous synthesis of ascorbic acid made it of premium importance (Roy and Guma, 1958; NRC, 1993). However, fish growth is the most flexible and is one of the complex activities. It denotes the net outcome of a series of biological factors that begin with food

The present results revealed that the combination of β- Carotene and vitamin C as a feed additive for Nile tilapia is recommended to stimulate productive growth performance and nutrient utilization. Further research is still needed to detect the mode of action of these vitamins on tilapia digestibility. Another possible explanation for increased growth performance with added vitamins is the improvement in digestibility, which may, in turn, explain the better growth and feed efficiency observed with the supplemented diets. Proximate composition analysis of whole body of fish in all dietary treatments did not show a significant difference.

As the demand for fish increases, heterogeneity of species in aquaculture by including more species for increasing production levels has become necessary. The introduction of tilapia in our culture systems is preferable because it represents a lower level in the food chain and, thus, its culture will be economical and eco-friendly. Several authors suggest that the addition of supplementary feeds may lead to increased size variation by increasing competition for feed, especially if the feed is given at a central feeding point (Grant, 1993; Alanara, 1996; Doupe and Lymbery, 2003; Rutten *et al*., 2005). Meanwhile, Vitamin C is cheap and showed promising results regarding growth and survival and enhanced disease resistance in Nile tilapia (Ibrahim *et al*., 2010). These data collectively suggest that the addition of Vitamin C and β-carotene to the commercially available basal diet used in this study can be used in tilapia aquaculture to enhance fish growth. This information can also help producers of commercial feed in improving feed composition.

5. Conclusion

In conclusion, our findings suggest that a combination of vitamins at the concentration of 80 mg/kg β carotene- 6000 mg/kg vitamin C and 100 mg/kg β carotene- 8000 mg/kg vitamin C can be used as an effective growth promoter in *Oreochromis niloticus* fingerling's fish feed. Increasing the concentration of β carotene and vitamin C in the diet will also increase the nutrient digestibility of crude fat and crude protein that will also increase the fish's growth. However, only supplementation levels of 100 mg/kg β carotene-8000 mg/kg Vitamin C shows optimum growth for *O.niloticus* fingerlings. In addition, these growth promoters are locally available at much lower prices in the local market. So the use of these vitamins could be the possible strategy for better growth of fishes in the aquaculture industry. Further research will be needed to scrutinize the effects of these vitamins in other fishes and apply different combinations of vitamins in diets on large sizes of fish under field conditions.

Acknowledgements

The financial support produced by the Government of Kerala, India, for our experimental research in the laboratory is greatly acknowledged. The authors wish to thank the Department of Zoology, University of Kerala, Thiruvananthapuram, for providing their technical facilities.

6. References

Al-Amoudi, M.M., El-Nakkadi, A.M.N. and El-Nouman, B.M. 1992. Evaluation of optimum dietary requirement of vitamin C for the growth of *Oreochromis spilurus* fingerlings in water from the Red Sea. *Aquaculture.*, 105, 165–173.

Alanara, A., 1996. The use of self-feeders in rainbow trout Oncorhynchus mykiss production. *Aquaculture.*, 145, 1–20.

Amara, E.C, Kiron, V., Satoha, S. and Watanabe, T. 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) associated with dietary intake of carotenoids from natural products. *Fish Shellfish Immunol.*, 16: 527-537.

AOAC. 1995. Association of Official Analytical Chemists. In: Cunniff, P.A. (Eds), Official Methods of Analysis 16th edn. AOAC International, Arlington, VA, USA.

APHA and AWWA, 1985. Standard Methods for Examination of Water and Wastewater. 16th American Public, Health Association, Washigton, DC.

Chatterjee, I. B. 1973. Vitamin-C synthesis in animals: evolutionary trend. *Sci. Cult.*, 210–211.

Chien, Y.H. and Jeng, S.C. 1992. Pigmentation of kuruma prawn, Penaeus japonicus Bate, by various pigment sources and levels and feeding regimes. *Aquaculture.*,102: 333-346.

Doupe, R.G.and Lymbery, A.J. 2003. Toward the genetic improvement of feed conversion efficiency in fish. *J. World Aquac. Soc*. 245–254.

Eknath, A.E. and Hulata, G. 2009. Use and exchange of genetic resources of Nile tilapia (*Oreochromis niloticus*). *Rev Aquac.*, 1: 197-213.

Fitzsimmons, K. 2010. Potential to increase global tilapia production. Presented at the global outlook for aquaculture leadership, Kuala Lumpur. Aquaculture Leadership, 2010 August 28, Kuala Lumpur.

Food and Agriculture Organization of the United Nations (FAO). 2012. The State of World Fisheries and Aquaculture. FAO, Rome.

Goodwin, T.W. 1984. *The biochemistry of the carotenoids*. Volume II Animals;Chapman and Hall (eds). New York, NY, USA.

Gouillou-Coustans, M.F., Bergot, P. and Kaushik, S.J. 1998. Dietary ascorbic acid needs of common carp (*Cyprinus carpio*) larvae. *Aquaculture*, 161: 453-461.

Grant, J.W.A., 1993. Whether or not to defend? The influence of resource distribution. *Mar. Behav. Physiol*. 23: 137–153.

Hu, C.J., Chen, S.M., Pan, C.H. and Huang, H.C. 2006. Effects of dietary vitamin A or β-carotene concentrations on growth of juvenile Nile hybrid tilapia, *Oreochromis niloticus x O. aureus. Aquacult.*, 253: 602–607.

Ibrahem, M.D, Fathi, M., Mesalhy, S. and Abd El-Aty, A.M. 2010. Effect of dietary supplementation of inulin and vitamin C on the growth, hematology,innate immunity, and resistance of Nile tilapia (*Oreochromi niloticus*). *Fish Shellfish Immunol.*, 29: 241e 246.

Kapur, A., Haskovic, A., Copra-Janicijevic, A., Klepo, L., Topcagic, A., Tahirovic, I. and Sofic, E. 2012. Spectrophotometric analysis of total ascorbic acid contetnt in various fruits and vegetables. *Bulletin of the Chemists and Technologistsof Bosnia and Herzegovina*, 38: 39-42.

Katsuyama, M. and Matsuno, T. 1988. Carotenoid and Vitamin A, and metabolism of carotenoids, β-carotene, canthaxanthin, zeaxanthin, lutein, and tunaxanthin in tilapia (*Tilapia nilotica*). *Comp. Biochem. Physiol.*, 90B: 131–139.

Kelestemur and Coban. 2016. Effects of The β-Carotene on the Growth Performance and Skin Pigmentation of Rainbow Trout (*Oncorhynchus mykiss*, W. 1792). *J Fisheries Live st Prod*, 4: 1.

Kumari. J. and Sahoo P.K. 2005. High dietary vitamin C affects growth, non-specific immune responses and disease resistance in Asian catfish, *Clarias batrachus. Mol Cell Bio chem.*, 280 (1e2): 25e33.

Li, M.H., Wise, D.J. and Robinson, E.H. 1998. Effect of dietary vitamin C on weight gain, tissue ascorbate concentration, stress response, and disease resistance of channel catfish, Ictalurus punctatus. *J. World Aquacult. Soc.*, 29: 1–8.

Lim, C. and Lovell, R.T. 1978. Pathology of vitamin C deficiency syndrome in channel catfish. *JNutr.*, 108: 1137e41.

Lovell, R.T. 1991. Nutrition of aquaculture species. *J Anim Sci.*, 69:4193e200.

Menaga, M. and Fitzsimmons, K. 2017. Growth of the Tilapia Industry in India. world aquaculture, 48(3): 49-52.

Metusalach, J., Brown, A. and Shahidi, F. 1997. Effects of stocking density on colour characteristics and deposition of carotenoids in cultured arctic charr (Salvelinus alpinus). *Food Chem.*, 59: 107-114.

Mohan, C.V. and Sorgeloos, P. 2010. (eds). Farming the Waters for People and Food. Proceedings of the Global Conference on Aquaculture Phuket, Thailand. FAO, Rome and NACA, Bangkok. 22–25 September 2010. pp: 705-717.

Mustafa, A. Randolph, L. and Dhawale, S. 2011. Effect of Phosphatidylcholine and Beta-Carotene Supplementation on Growth and Immune Response of Nile Tilapia, *Oreochromisniloticus,* in Cool Water.*J Appl Aquaculture.*, 23:136–146.

Mustafa, A., Randolph, L. Dhawale, S. 2013.Effects of Nutritional Supplements Phosphatidylcholine and Beta-Carotene on Growth and Selected Stress and Immune Parameters in Nile Tilapia, *Oreochromis Niloticus* (L.*). Advances in Zoology and Botany*, 1(3): 57-61.

National Fisheries Development Board (NFDB). 2020. India's Blue Economy net getting bigger! Country ranks third in fisheries and second in aquaculture. India.

National Research Council (NRC). 1993. *Nutrient requirements of fish*. National polyphosphate (AsPP) as a dietary ascorbicacid source for channel catfish. Washington, DC: Academy Press.

Olson, J.A. 1991. Vitamin A. In *The handbook of vitamins*, Zempleni, J., Rucker, R.B., Suttie, J.W. and McCormick, D.B.(eds). New York: Marcel Dekker, 1–59.

Roy, R.N. and Guma B.C. 1958. Species difference in regard to the biosynthesis of ascorbic acid. Nature (Lond.);182: 319- 20.

Rutten, M.J.M., Bovenhuis, H., Komen, J., Bijma P., 2005c. Is competitive behavior in Nile tilapia (Oreochromis niloticus L.) heritable? In: Rutten, M.J.M., 2005. Breeding for improved production of Nile tilapia (*Oreochromis niloticus* L.), Doctoral Thesis, Wageningen University, pp. 74–85.

Sakai, M. 1999. Current research status of fish immunostimulants. *Aquaculture.,*172: 63- 92.

Shahbandeh, M. 2020. Fish production worldwide 2002-2019. https://www.statista.com/.

Spyridakis, P., Metailler, R., Gabaudan, J. and Riaza, A. 1989. Studies on nutrient digestibility in European sea bass (*Dicentrarchus labrax*): 1. Methodological aspects concerning faeces collection. *Aquaculture.*, 77:61-70.

Subasinghe, R., Ahmad, I., Kassam, L., Krishnan, S., Nyandat, B., Padiyar, A., Phillips, M., Reantaso, M., Miao, W. and Yamamoto, K. 2012. Protecting small-scale farmers: a reality within a globalized economy? In Subasinghe, R.P., Arthur, J.R., Bartley, D.M., De Silva, S.S., Halwart, M. and Hishamunda, N., Mohan, C.V. and Sorgeloos, P. eds. Farming the Waters for People and Food. Proceedings of the Global Conference on Aquaculture 2010, Phuket, Thailand. 22–25 September 2010. pp. 705–717. FAO, Rome and NACA, Bangkok.

Tacon, A.G.J. 1981. Speculative review of possible carotenoid function in fish. *Prog. fish- cult*., 43(4): 205–208.

Tewary A, Patra, B.C. 2008. Use of vitamin C as an immunostimulant .Effect on growth, nutritional quality,and immune response of *Labeo rohita* (Ham.). *Fish Physiol Biochem.,* 34(3): 251e9.

Torrissen O.J. 1984. Pigmentation of salmonids. Effects of carotenoids in eggs and start feeding diet on survival and growth rate.*Aquaculture*43: 185-193.

Torrissen, O.J., Hardy, R.W and Shearer, K.D. 1989. Pigmentation of salmonids: Carotenoid deposition and metabolism. *Crit Rev Aquat Sci*, 1: 209-225.

Verakunpiriya, V., Watanabe, K., Mushiake, K., Kawano, K., Kobayashi, T and Hasegawa, I. 1997. Effect of krill meal supplementation in soft-dry pellets on spawning and quality of egg of yellowtail. *Fish Sci.* 63: 433–439.

Verlhac, V. and Gabaudan, J. 1994. Inûuence of vitamin C on the immune system of salmonids. *Aquacult. Fish. Manage.*, 25: 21–36.

Watanabe, T., Lee, M.J., Mizutani, J., Yamada, T., Satoh, S. and Takeuchi, T. 1991. Effective component of cuttlefish meal and raw krill for improvement of quality of red sea bream Pagrus major eggs. *Nippon Suisan Gakkaishi*, 57: 681–694.

Winkler, L. W. 1888. Die Bestimmung des in Wasser gelösten Sauerstoffen. *Berichte der Deutschen Chemischen Gesellschaft,* 21: 2843–2855.

Workagegn, K.B., Ababbo, E.D., Yimer, G.T and Amare, T.A., Growth Performance of the Nile Tilapia (*Oreochromis niloticus* L.). 2014. Fed Different Types of Diets Formulated from Varieties of Feed Ingredients, *J Aquac Res Development*., 5: 3.

Yanar, Y., Buyukçapar, H., Yanar, M. and Gocer, M. 2007. Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout. *Food Chem,* 100: 326-330.