

Effect of Methods of Adding Vitamin C on Growth Performance and Feed Utilization and Survival of Fingerlings of Nile Tilapia Reared in Happa

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Abstract: A 56-days growth trial was conducted in happa under greenhouse culture system to examine the effects of different added vitamin C on growth, feed utilization and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings with average initial weight of 8.12 ± 0.02 g. Five treatments T1 control added dry vitamin C, T2 mix Vitamin C with water, T3 mix vitamin C with oil, T4 spray added vitamin with water and T5 spray added vitamin C with oil. The levels of vitamin C (70 mg/kg) and their combination were used to prepare diets used in nine experimental treatments with three replicates. Based on the results of this study, it can be concluded that addition of vitamin C with oil spray added was the best in terms of growth performance, feed utilization and survival rate.

Keywords: Growth performance, feed utilization, adds vitamin C, Nile Tilapia fingerlings

INTRODUCTION

Vitamin C (ascorbic acid) is an essential nutrient in aqua-feeds, and is an indispensable nutrient required to maintain physiological processes such as normal growth, immunity and reproduction of different animals including fishes (Teshima *et al.*, 1991). Ascorbic acid is water soluble and is essential for several metabolic functions including the antioxidant system. Most fish, including tilapia, are not capable of vitamin C biosynthesis (Chatterjee, 1973) due to the absence of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of ascorbic acid (Wilson, 1973). L-ascorbic acid is extremely labile and the rate of degradation is a function of storage time, with the effect of temperature, oxygen, pH and light. Recent studies indicate that ascorbic acid derivatives that include sulfate and phosphates are more resistant to oxidation and retain ascorbic acid activity for fish (Abdelghany, 1996). Ascorbic acid requirements of some tilapia species have been investigated Stickney *et al.* (1984), reported the fortification of 50 mg of ascorbic acid equivalent kg⁻¹ diet as the level that allows for maximum weight gain and absence of deficiency signs in blue tilapia (*Oreochromis aureus*). However, it

should be noted that this apparent interaction between method of adding and ascorbic acid stability. However, there is still a paucity of information on the interaction between method of adding vitamin C and stability. The objective of the present study was to assess the effects of different adding method on growth performance, feed utilization and survival of Nile tilapia fingerlings.

MATERIALS AND METHODS

The experiment was represented at Al-Amal private fish farm, El Kantara, Ismailia- Egypt. The experiment aim to study the Effect of different method of added Vitamins C to diets of Nile Tilapia fingerlings.

Water quality parameters

Were monitored during the study to follow water temperature and dissolved oxygen were measured by mettle Toledo, model 128.s/No1242. Other water quality including pH and ammonia were measured every two days by pH meter (Orion model 720A, s/no 13062) and ammonia meter by Hanna ammonia meter. The averages of water quality parameters are presented in Table (1).

Table (1): Water quality parameters

Experimental Parameters	Temperature	Dissolved Oxygen	Ammonia	pH
	28-25	5.7 -7 mg/l	0.07-0.04 mg/l	7.7-9

Experimental unit

Fish were stocked in 15 hapa (1.5 m x 1.00 m x 1. m) randomly divided into to equal experimental groups (250 fingerlings/three replicate hapas). The hapa were supplied all day with air blowers. Water temperature was maintained at (25-28°C) inside green house.

Experimental fish and Culture techniques

Two thousand and two hundred fifty fingerlings were obtained from Nile tilapia (*O. niloticus*) with an average initial body weight of $8 \pm$

0.1 gm from (Al Amal Fish Farm, Kantara, Ismailia) Governorate, Egypt. Fish were homogeneous in body weights and seemed to be healthy. Prior to the start of the experiment, fish was acclimatized to laboratory conditions for two weeks.

Experimental diets

The diets were formulated from practical ingredients (Table 2). The experimental diets were formulated to contain almost 30% crude protein and gross energy 452.84 Kcal/100g. The experimental diets were prepared by individually weighing of each

component thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with powders. The methods used to add vitamins in the diet are as follows;

- Adddry (Control) T1
- Adding with water (T2)
- Adding with mix oil (T3)

- Adding with spray water (T4)
- Adding with spry oil (T5)

The produced diets were dried at room temperature then kept until experimental start. The composition and proximate analysis of the experimental diets are presented in Table (2). The fish were hand-fed to satiation 4 times /day (7, 10, 2, and 4 pm) throughout the experimental period 56 days.

Table (2): Formulation and chemical composition of the basal diet

Ingredients	30%
Fish meal (60%)	5
Soya bean meal	50
Yellow corn	17
rice bran	18
Soya oil	6
Vit. and Min. premix¹ free vitamin C	3
CMC (carboxy methyl cellulose)	1
Proximate Analysis	
Dry matter (%)	87.8
Protein (%)	30.08
Lipid %	10.49
Total carbohydrate (%)	44.71
Ash %	5.69
vitamin C mg/kg	70
Gross energy (Kcal /100g)	452.84

1. Each Kg vitamin & mineral mixture premix contained Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg,

2. Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 1993).

Experimental Methodology

Fish Samples

At the start and the end of the main experimental period (56 day), 5 fish were randomly taken from each experimental group. Fish were used for chemical analysis of the whole body. The tested diets and body were analyzed for crud protein (CP %) ether extract (EE %), crude fiber (CF %), ash (%) and moisture. The whole body composition of fish samples were analyzed except crud fiber (CF %) according to the procedures described by standard (AOAC, 2012). The nitrogen free-extract (NFE %) was calculated by differences.

Growth performance parameters

The growth performance and feed utilization parameters are calculated according to the follow.

Average Weight Gain (AWG)

(AWG) = Average final weight (g) – Average initial weight (g)

Average Daily Gain (ADG): -

(ADG) = [Average final weight (g) – Average initial weight (g)] / time (days)

Specific Growth Rate (SGR %/day): -

(SGR %/day) = 100 [Ln Wt1 - Ln Wt. 0 / t]

Where: - Ln: normal log Wt. 0: initial weight (g).

Wt. 1: final weight (g) T: time of days.

Feed and protein utilization parameters

Feed and protein utilization parameters are calculated according to the following equations:-

Conversion Ratio (FCR):-

FCR = Total feed consumption/ weight gain.

Feed Efficiency (FE) = weight gain/ Total feed consumption

Protein Efficiency Ratio (PER) = weight gain/protein consumed

Survival (%):

SR=Ni x 100/N0

Where: Nt = Total number of fish survived in tank at end of experiment.

N0 = Total number of fish survived in tank at beginning of experiment.

We analyzed the nutritional parameters of weight gain (WG), apparent feed intake (AFI), apparent feed conversion (AFC), carcass quality and yield (CY), according Equation 1: (1) where: DCW = dressed carcass weight. We also calculated the Viscerosomatic index (VSI), Gonadosomatic Index (GSI) and Hepatosomatic index (HSI), according Equations:

GSI = GW/BWX100

VSI = Viscera weight/BWX100

HIS = Liver Weight/BwX100

Carcass Yield = dressed carcass weight

Gonad weight = weight of gonad

Statistical analysis

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS Institute, 2005). Means were compared by Duncan's new multiple ranges test (Duncan, 1955).

Where: $Y_{ij} = \mu + D_i + e_{ij}$

Y_{ij} = The observation of the j th individual from D th Diet.

μ = the overall mean. D_i = The Fixed effect of the D th Diet.

e_{ij} = The random error associated with the individual j .

RESULTS AND DISCUSSION

Effect of vitamin C on growth

The effect of different adding vitamin C on growth performance of Nile tilapia fingerlings are presented in Table 3. The group of fish on T5 had a significantly ($P < 0.05$) highest final body weight gain, weight gain percent and specific growth rate than the rest of other experimental treatment. The results of this study strongly indicate that different adding vitamin C significantly affects the growth, survival of Nile tilapia fingerlings. Growth is a function of both the nutritional quality and the rate of consumption, among other things (Stickney, 2000). These outcomes suggested that dietary vitamin C could improve the growth performance of tilapia fingerlings. The results of the present investigation are contrary to some past studies for different fish species (Ai *et al.*, 2004; Lin and Shiau, 2005). Nevertheless, Ai *et al.* (2006) stated that adding dietary vitamin C effect levels which didn't affect the growth parameters for juvenile large yellow croaker. There was stunted growth and haemorrhage in head of Japanese eels were observed when fed a vitamin C-deficient diet (Arai *et al.*, 1972). The reasons for this may be difference in individual size, development stage, cultivation environment, variation in experimental conditions including levels of nutrients interaction in the

treatment diets, other feed contents like other vitamins, for example vitamin E and the response

The group of fish on T5 had a significantly ($P < 0.05$) highest survival rate 98% than the rest of experimental groups. The lowest survival was reported around 90% in T1 which similar to reported by (Soliman *et al.*, 1986) in tilapia *O. mossambicus* fed a diet without supplemental ascorbic acid in 8 weeks. These results contradict Falcon *et al.* (2007), which stated that ascorbic acid directly influences the growth of fish.

Feed Utilization

The effect of different adding vitamin C on feed utilization of Nile tilapia fingerlings are presented in Table 4. The group of fish on T5 had a significantly ($P < 0.05$) lowest feed intake than the rest of experimental group. The group of fish on T1 had a significantly ($P < 0.05$) highest feed intake than the rest of experimental groups. The group of fish on T5 had a significantly ($P < 0.05$) lowest feed conversion ratio than the rest of experimental group. And the group on T1 had the highest FCR than the rest of experimental groups. The results of this study strongly indicate that different adding vitamin C significantly affects the growth, survival and hematology of Nile tilapia fingerlings. Feed utilization in this study was also affected by the dietary treatment of ascorbic acid adding Table 4. Total amount of feed consumed increased with ascorbic acid level. Both protein conversion efficiency and protein efficiency ratio were much lower in fish fed with diet without ascorbic acid. This indicates lower protein utilization by the fish. Fracalossi *et al.* (2001) observed a similar trend in juvenile Oscars (*Astronotus ocellatus*) cichlids. The diet used in the present experiment had high protein content (400 g kg⁻¹), which could have resulted in the fish consuming high levels of oxygen as consumption increases with protein in tilapia (Ross, 2000). The group of fish fed on T5 had a significantly ($P < 0.05$) highest Feed efficiency and Protein efficiency ratio. In agreement with Fracalossi *et al.* (2001). The relationship between spray added vitamin C with oil T5 showed a significant ($P < 0.05$) effect on the feed efficiency reaches the highest efficiency.

Table (3): Final weight (FW), total length (TL), standard length (SL), apparent feed intake (AFI), weight gain (WG) and apparent feed conversion (AFC) of Nile tilapia fingerlings fed with diets containing different levels of supplemental vitamin

Item	treatment				
	T1	T2	T3	T4	T5
Initial weight (g)	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10	8.0 ± 0.10	8.10 ± 0.10
Final Weight (g)	20.22 ± 0.30 ^c	21.00 ± 0.30 ^d	22.22 ± .30 ^c	23.22 ± 0.30 ^b	24.22 ± 0.30 ^a
Weight gain (g)	12.22 ± 0.20 ^e	13.0 ± 0.20 ^d	14.22 ± .10 ^c	15.22 ± .20 ^b	16.12 ± 0.10 ^a
Weight gain %	152.75 ± 0.10 ^e	162.5 ± 0.12 ^d	177.75 ± .14 ^c	190.25 ± 14 ^b	199.01 ± 0.14 ^a
SGR	1.65 ± 0.10 ^e	1.72 ± 0.10 ^d	1.82 ± 0.10	1.90 ± 0.10 ^b	1.95 ± 0.10 ^a
Survival %	90.00 ± 0.30 ^b	95.00 ± 0.30 ^a	97.00 ± 0.30 ^a	97.00 ± 0.30 ^a	98.00 ± 0.30 ^a

Different letters in the row indicate significant differences ($p < 0.05$) by Duncan's test; Mean \pm standard error. There was a significant difference ($P < 0.05$) between experimental groups (Table 5) in HSI values. The group of Nile tilapia fingerlings on T5 had the highest HSI and the group on T1 had the lowest value of HSI. In agreement with Furuita *et al.* (2009). There was a significant difference in GSI values between the treatments, with higher values for the treatments T5 and lowest on T1. Similar results was obtained by (Navarro *et al.*, 2009). There was a significant difference ($P < 0.05$) between experimental groups (Table 4) in GSI values in this way, other

studies registered a positive effect on fish reproduction. In agreement with Navarro *et al.* (2009). There was a significant difference ($P < 0.05$) between experimental groups (Table 5) in GW values among experimental groups. In agreement with Soliman *et al.* (1986). There was a significant difference ($P < 0.05$) between experimental groups (Table) in GW. The highest GW was recorded in T5, T4, and T3 and the lowest value of GW in T1, In agreement with Martins *et al.* (2016).

There was a significant difference ($P < 0.05$) in carcass yield (CY) among experimental groups. In agreement with Martins *et al.* (2016).

Table (4): Effect of adding methods of vitamin C on Feed utilization of Nile tilapia fingerling

Item	Treatment				
	T1	T2	T3	T4	T5
Feed intake (g)	26.88 \pm .20 ^b	21.58 \pm 0.20 ^e	29.01 \pm 0.20 ^a	25.87 \pm 0.20 ^c	22.75 \pm 0.20 ^d
FCR	2.2 \pm 0.20 ^a	1.66 \pm 0.20 ^b	1.80 \pm 0.10 ^d	1.70 \pm 0.20 ^c	1.60 \pm 0.20 ^b
FE	0.45 \pm 0.10 ^e	0.60 \pm 0.10 ^b	0.55 \pm 0.10 ^c	0.58 \pm 0.20 ^e	0.62 \pm 0.20 ^a
PER	1.52 \pm 0.20 ^e	2.00 \pm 0.20 ^b	1.85 \pm 0.20 ^d	1.96 \pm 0.20 ^c	2.09 \pm 0.20 ^a

Different letters in the row indicate significant differences ($p < 0.05$) by Duncan's test; Mean \pm standard error.

The 60 mg ascorbic acid kg-1 diet found as requirement level for maximum growth agrees with Li and Lovell (1985) who demonstrated that fish rose from 3 to 19g required 60 mg ascorbic acid kg-1 diet for maximum weight gain. Weight gain increase with dietary level is considered by many nutritionists to be the most important and meaningful response in nutritional requirement studies (Stickney, 2009). Body proximate composition did not affect with adding methods of vitamin C.

No significant difference was found for viscerossomatic index, hepatosomatic index and

gonad weight. The results of chemical analysis of the carcass (dry matter, protein, ether extract) and carcass yield showed no significant difference (Table 5). Probably, the amount and period of experiment were not enough to stimulate collagen synthesis. There was a significant difference ($P < 0.05$) in GSI values between the treatments, with higher values for the treatments T5 . This result demonstrates the importance of the spray adding vitamin C to diets (Navarro *et al.*, 2009).

Table (5): Viscerossomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI), gonad weight (GW), carcass yield (CY) and carcass composition in dry matter (DM), crude protein (CP) and ether extract (EE) of Nile tilapia fingerlings fed different levels of supplemental vitamin C

Item	Treatment				
	T1	T2	T3	T4	T5
HSI	11.28 \pm 4.07	11.04 \pm 3.04	12.04 \pm 3.04	12.94 \pm 3.90	13.22 \pm 3.90
VSI	3.10 \pm 1.13	3.16 \pm 1.05	3.36 \pm 1.13	3.86 \pm 1.70	3.96 \pm 1.80
GSI	0.49 \pm 0.09 ^c	0.53 \pm 0.13 ^b	0.53 \pm 0.13 ^b	0.58 \pm 0.15 ^b	0.70 \pm 0.12 ^a
GW	0.11 \pm 0.07 ^b	0.10 \pm 0.05 ^b	0.17 \pm 0.22 ^a	0.17 \pm 0.22 ^a	0.17 \pm 0.22 ^a
CY	85.53 \pm 1.57	86.10 \pm 1.36	86.10 \pm 1.36	86.66 \pm 2.34	86.10 \pm 1.36
Carcass composition in dry matter (DM)					
DM	25.69 \pm 2.67	26.13 \pm 1.45	26.68 \pm 5.5	26.68 \pm 5.5	26.31 \pm 1.6
CP	48.86 \pm 3.56	48.87 \pm 2.35	48.73 \pm 7.8	48.73 \pm 7.8	48.90 \pm 3.8
EE	27.57 \pm 2.56	27.40 \pm 4.5	29.59 \pm 4.8	29.59 \pm 4.8	28.59 \pm 8.9

Different letters in the row indicate significant differences ($p < 0.05$) by Duncan's test; Mean \pm standard error.

CONCLUSION

It could be concluded that T5 (spry added vitamin c with oil) was the best of growth performance and feed utilization of fingerlings Nile tilapia (*Oreochromis niloticus*) under these experimental conditions.

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تأثير طرق إضافة فيتامين سي على أداء النمو والاستفادة الغذائية لاصبغيات سمك البلطي النيلي المربي في هابيات

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أجريت تجربة نمو لمدة ٥٦ يومًا في لدراسة طرق إضافة فيتامين سي على أداء النمو والاستفادة الغذائية ونسبة الإعاشة لاصبغيات سمك البلطي النيلي التي متوسط وزنها 8.12 ± 0.02 جرام المربي في هابيات تحت صوبة زراعية. يبلغ 8.12 ± 0.02 جم وتم إضافة فيتامين سي إلى العليقة بعدة طرق كالاتي: المعاملة الأولى الكنترول تم إضافة فيتامين سي جاف إلى باقي المكونات، المعاملة الثانية إضافة فيتامين سي بالعجن بالماء، المعاملة الثالثة تم إضافة فيتامين سي بالعجن بالزيت، المعاملة الرابعة تم إضافة فيتامين سي بالخلط بالماء ورشة على العليقة، المعاملة الخامسة تم خلط فيتامين سي بالزيت ورشه على العليقة، وتم تغذية المجموعات التجريبية على العلائق المختبرة بنسبة ٥% من وزن الجسم أربع مرات في اليوم وكانت تغذى الأسماك لدرجة الشبع لمدة ٥٦ يوم وكان يتم وزن الأسماك كل ١٤ يوم. ويتم حساب كميته العلاف المستهلكة خلال هذه الفترة. وتم حساب مقاييس أداء النمو والاستفادة الغذائية وكانت النتائج كالاتي: أعطت المعاملة أفضل النتائج من حيث الزيادة في الوزن ومعدل النمو النوعي والنسبة المئوية للزيادة في الوزن ومعامل التحويل الغذائي والكفاءة الغذائية ومعامل الاستفادة من البروتين وكانت اقل معاملة هي الكنترول التي أتم إضافة فيتامين سي جاف إلى العليقة. كذلك وجد أن طرق إضافة فيتامين سي لم تؤثر على مكونات الجسم (المادة الجافة - البروتين الخام - والدهن). ويستنتج من هذه الدراسة أن إضافة فيتامين سي بالرش بالزيت على مكونات العليقة كانت الأفضل من حيث أداء النمو والاستفادة الغذائية تحت هذه الظروف التجريبية.

الكلمات الدالة: سمك البلطي النيلي- طرق إضافة فيتامين سي - أداء النمو - الاستفادة الغذائية - الإعاشة