On survival growth and some physiological parameters of *Oreochromis niloticus* as afftected by dietary ascorbic acid supplementation

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ABSTRACT

The present study was carried out to evaluate the effect of adding ascorbic acid to Tilapia fish diets on survival, growth, physiological parameters and total production. *O. niloticus* at average initial body weight $20.3^{+0.9}$g and length $13.5^{+0.6}$cm, were stocked in concrete ponds at a rate of 5 fish/ m^3, and offered a diet of 5% of body weight, six days weekly , twice daily . The basal diet contained 30% crude protein divided into five tested diets. Ascorbic acid was added to the basal diet at different levels, 0 , 300, 600, 900 and 1200 mg AA /kg diet.

The results show that tilapia growth performance indices (FBW, A.DG, SGR, GR and FCR) were increased with increasing ascorbic acid (AA) levels. Diet contained 900mg/kg recorded the best value, compared to the control diet. The best biological indices (hepato., gonado and gastrosomatic indices were recorded from diet (4) compared to the control diet. Survival and production were increased with the increase of ascorbic acid level until 900mg/kg diet which recorded the best results.

Similar results were obtained in blood properties (Erythrocyte, hematocrit and hemoglobin) as values showed the same pattern;, fish groups offered diet contained 900mg AA/kg had the best results comparing to the control diet; muscles, serum lipid values decreased with increasing A.A level. Protein content showed best values in fish fed A A up to 900mg/kg diet.

The observation on fish growth and health showed that Ascorbic Acid can be used as a commercial compound to reduce production costs and to increase total final profit.

Key words : *Oreochromis niloticus,* ascorbic acid , growth parameters, physiological properties.

INTRODUCTION

Vitamins are organic nutrients which arc called accessory factors required in small quantities for biochemical functions (Idris & Hasson ,2002). Metabolic reactions and normal growth in animals arise from functions of vitamins as cofactors for many growth enzymes in animal bodies that are indispensable nutrients required to maintain the physiological processes of

different animals including fishes (Tolbert, 1979). Fish depend upon an exogenous sources of vit. C. as they cannot sunthesize it due to the absence of the enzymes. Agylonolflon L. gulonolactone oxidase (Ec1.1.3.8) (Wilson, 1973).

Because of the unstable nature of vit C. , the use of non stable forms of ascorbic acid derivatives in aquatic feeds is necessary. vit. C. requirements for blue hybrid (Shiau & Jan , 1992) and hybrid tilapia (Shiau & Hsu , 2002) have been reported, L-Ascorbic acid (AA) is unstable and most of its activity in practical diets is lost during processing – storage. Shiau & Hsu (1993) found that 75% from vit. C. amount was lost at ambient temperature .

The influence of vit. C. on fish health was studied by several researchers . Halver (1992) and Shiau & Hsu (2007) cleared vit. C. role in fish health and tissue repair. Angrawal *et al.* (1978) showed the protective role of ascorbic acid in fish exposed to pesticide pollution and Navarre *et al*. (1989) reported that the diseases resistance and humoral antibodies production in fish can be offered different by levels of vit .C .

Vit. C. dietary levels influence on growth rate of tilapia fish was studied by Shiau & Jan (1992), while Lee *et al*. (1998) reported that growth performance of Korean roach fish increases with increased vit. C. levels in diet to 1500 mg/kg diet. Hung *et al*. (2007) observed that vit . C. can be used with minerals to obtain best results in fish health and growth.

The aim of this study was to evaluate the effect of adding vit. C. by various levels on *O. niloticus* growth , survival and physiological properties.

MATERIAL AND METHODS

1) Experimental site and used fish:

This study was carried out at El-Kanater fish research station in five concrete ponds with area of 8 m^3 (4x2xl for each pond) filled with fresh water from Darawa irrigation canal . *O. niloticus* fish were obtained from El-Abbasa fish research station of average initial body weight $20.3^{+o.90}$ g and 13.5 cm length. They were stocked at rate 5.0 fish/ m^3 and fed with a diet containing 30 % crude protein and the rearing period was 90 days.

2) Formulation of diets:

Supplemented diets chemical composition and proximate analysis is presented in Table (1). Basal diets contained 30% crude protein divided to five tested diets:

- Basal diet without adding vit. C. (control diet).
- diet 2 Basal diet contained 300 mg vit. C. /kg et .
- diet 3 Basal diet contained 600 mg vit. C. /kg et.
- diet 4 Basal diet contained 900 mg vit. C. /kg et
- diet 5 Basal diet contained 1200 mg vit. C./kg et.

The tested diets were offered to *O. niloticus* as dry pellets (3mm diameter and 5ml length)

Table (1) Feed intake, chemical composition, proximate analysis to *O. niloticaus* as affected by dietary supplemented vit. C.

Items / Ingredients	Total weight %					Proximat analys	
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5		
- Cottonseed cake	20.0	20.0	20.0	20.0	20.0	crude protein%	29.8
- Soybean meal	20.0	20.0	20.0	20.0	20.0	crude fiber %	6.9
- Fish meal	10.0	10.0	10.0	10.0	10.0	Elher extract%	8.9
- Rice bran	10.0	10.0	10.0	10.0	10.0	ASH %	8.9
- Wheat bran	39.0	39.0	39.0	39.0	39.0	Nitrogen free extract %	10.5
- Oil	1.0	1.0	1.0	1.0	1.0	Gross-energy k Cal/kg diet	4250
- Vitamin C mg /100g diet	-	300	600	900	1200		

3) Blood analysis and sexual maturity:

The blood samples (15 fish examined from each pond) were taken over heparinized vials by severing the caudal peduncle of the fish (Dabroweska *el al.*, 1989) The erythrocytes count was determined using a double hemocytometer (Wintrobe 1934). The hematocrit value was measured by drawing blood directly from fish into heparinized hemopipette, then centrifuged at 3000 rpm (Hesser, 1960). Hemoglobin content was estimated by Van Kampen & Zijfstra (1961). Serum glucose was measured by using Bohringer Mannheim kits as described by Tinder (1969) and serum lipid by Zoliner & Kirsch (1962) methods. Protein in serum and muscles was measured according to Gornall, *et al*., (1949), Glycogen in muscles was determined by using anthrone reagent (Handel, 1965), total lipid in muscles was extracted with mixture of chloroform and methanol (2:1) and determined using Bligh & Dyer (1959) method.

4) Growth rate and feed utilization:

During fish rearing period every 15 days fish from each pond were measured, (Length – weight), and physico-chemical parameters were measured to determine pH . dissolved oxygen, total alkalinity and water temperature to study the water quality for the reared fish. At the end of study: growth performance of reared fish were measured according to the following :

TWG = final body weight - initial body weight.

SGR = Ln final weight - Ln initial weight / period x 100

GR = final weight - initial weight / initial weight x l00

TFC. = (Average weight x fish number x 3 / 100

F.C.R = Total food consumption / weight gain g x fish number.

5) Statistical analysis :

The experimental results were statistically analyzed using T test and F test of significance according to Snedecor & Cochran (1967) .

RESULTS

Table (2) shows fish growth performance, final body weight (FBW), Average daily gain (AD.G) , specific growth rate (SGR), Growth ratio ('GR), and feed conversion ratio (FCR) as affected by adding vit. C. (Ascorbic acid) at different levels (0 , 300, 600 , 900, and 1200 g /kg diet).

Table (2) shows that maximum differences were observed in growth performance with significantly higher values (97.2 ،0.88 ، 1.23 and 380.0 respect.) were obtained from fish offered diet (4) that contained vit. C. at level 900 mg/kg diet, compared to fish group fed control diet which recorded the lowest values (70.5 ، 0.55 ، 0.91 and 244.8 % respect.). Nutrient , efficiency as shown from table (2), revealed that *O. niloticus* nutrient ratio (Protein effeciency ratio: values increased with increasing Ascorbic acid levels until 900 mg/kg diet, the highest value (1.89) was recorded from fish fed diet (4) containing vit. C. at level 900 mg compared with control diet which recorded lowest value (1.3).

Feed conversion ratio showed the same pattern. Differences were significant while the lowest and be value was recorded from fish groups offered diet (4), at A.A. level 900mg compared to diet (1) without adding vit. C .

Table (2) Average growth performance, P.E.R and F.C.R. for *O. niloticaus* as affected by adding vit. C with different levels .

Treatments **Vit .C. concentration mg/kg diet Items**	**T_1** **0.0**	**T_2** **300**	**T_3** **600**	**T_4** **900**	**T_5** **1200**	**MSE$^+$**
- aver. initial body weight (g)	20.3	20.3	20.3	20.3	20.3	0.90
- aver. final body weight (g)	705^{d}	75.4^{c}	90.7^{ab}	97.2^{a}	92.3^{b}	3.5
- aver. daily Iain (g/fish/day)	0.55^{d}	0.61^{c}	0.78^{ab}	0.85^{a}	0.80^{b}	0.01
- S.G.R%	0.91^{d}	0.97^{c}	$0.16a^{b}$	1.23^{a}	1.17^{b}	0.07
- Growth rate%	244.8^{d}	271.4^{c}	346.7^{ab}	380.6^{a}	354^{b}	16.5
- P.E.R%	1.3^{d}	1.59^{c}	1.66^{ab}	1.89^{a}	1.86^{b}	
- Feed consumed.	150.5	153.4	1.37	142.0	138.3	0.11
- F.C.R%	2.44^{d}	2.79^{c}	1.95	1.85^{a}	1.92	0.06

a,b,c. etc mean in the same row with different superscript are different ($p<O.05$).
MSE= mean standard error calculated from residual mean square in the analysis of variance.

Data given in Table (3) Show that *O. niloticus* as affected by adding A.A. at different levels had biological indices (hepato - gonado and gastro somatic indices at a significant differences ($p<0.05$), Maximum values (5.9، 10.85 and 12.8 respect.) , for the male and (5.9 ، 6.28 and 12.8 respect.) for female were obtained from diet (4) with (900) mg/Kg diet. while the lowest values (4.45 , 7.9 and 11.5 respect.) for males and (4.45 ، 4.92 and 11.51 respect.) for females were recorded from fish groups fed the control diet.

Table (3) shows the survival and total production which revealed the same better value. Differences were significantly higher (100%) in fish groups offered diet (4), with contrast to the control fish which recorded the lowest survival value (90%).Table (3) shows the total production (Kg / pond) and the net production (kg / m^3) and the net production kg/ fedd. for *O. niloticus* as affected by adding AA . Maximum values (3.08 ، 0.285 and 1190.0 respect.) were obtained from fish fed diet contained 900mg AA/kg diet, while fish groups offered the control diet recorded the lowest values (1.8 ، 0.125 and 5250.00 respect .)

Table (3) Biological indices, survival performance and net return of *O. niloticaus* as affected by adding vit. C .

Treatments **Vit .C. concentration mg/kg diet Items**	**T_1** **0.0**	**T_2** **300**	**T_3** **600**	**T_4** **900**	**T_5** **1200**	**MSE$^+$**
Biological indices:						
- hepato soma index	4.45^{d}	4.9^{c}	5.88^{ab}	5.9^{a}	5.5	0.9
- gastro somatic index	11.51^{d}	12.53^{c}	12.7^{ab}	12.8^{a}	12.66	1.02
- Goanado somatic index:						
1) : Male fish	7.9^{d}	8.52^{c}	987^{ab}	10.95^{a}	9.55^{b}	1.03
2) : female fish.	4.92^{d}	5.90^{c}	6.11	6.28^{a}	16.10^{b}	1.90
Survival performance:						
- fish number at starting.	40.0	40.0	40.0	40.0	40.0	
- fish number at harvesting.	36.0	37.0	38.0	40.0	38.0	
- losses number.	4.0^{d}	3.0	2.0	0.0	2.0	
- survival rate.	90^{d}	92.5^{c}	195^{ab}	100^{a}	95	
Total production I pond:	1.80^{d}	2.042^{c}	2.865^{ab}	3.080^{a}	2.73^{b}	
- net production (kg) / pond	1.00^{d}	1.23^{c}	2.065^{ab}	2.268^{a}	1.9^{b}	
- net production (kg)/ m3	0.125^{d}	0.154^{c}	0.258^{ab}	0.283^{a}	0.32^{b}	
- net production.(kg)1 fed.	525.0^{d}	645.0^{c}	1084^{ab}	1190^{a}	982.2^{b}	

a,b,c. etc mean in the same row with different superscript are different ($p<O.05$).
MSE = mean standard error calculated from residual mean square in the analysis of variance.

Table (4) illustrates blood parameters (Erythrocyte count, (million)/mm^3 Hematocrit values and Hemoglobin content for *O. niloticaus* as affected by adding vit. C. at five levels. Differences were obtained in blood parameters with significantly higher values, (2.59 ، 34.70 and 13.57 respect.) from fish groups offered diet contained 900 g /kg diet . Compared to fish groups fed the control diet which recorded the lowest values (1.85 ، 31.58 and 8.57 respect.).

Data in Table (4) show that muscles composition (total protein g/l00g. fresh tissues. Glycogen mg/100g fresh tissues and total lipid g/100g fresh tissues, had a significant differences ($p<0.05$) Differences obtained in lipid content with significant higher values(7.4) shown in fish offered control diet without AA, while fish group fed the diet contained 900mg AA/Kg diet had the best value (5.95).

In order to clear the differences in protein and glycogen results, maximum values (18.2 and .1.18) were obtained from fish fed diet (4) compared to fish offered control diet with the lowest value (15.61 , 1.35 respect.).

From Table (4) , serum analysis (glucose mg /100 ml serum, lipid g /100 ml serum and protein g/100ml serum .The same pattern of highest value (5.55) in lipid content was recorded from fish fed the control diet, in contrast to diet (4) which recorded the lowest and better valeu . Differences were shown in protein and glucose with significantly higher values (14.7and 60.33) from fish offered diet contained vit . C . At 900 mg /kg diet , while fish fed diet without add. vit . C. had the lowest value (11.3 and 54.5 respect.).

Table (4) Blood parameters . serum analysis and biochemical composition of muscles in *O. niloticaus* as affected by adding dietary vit. C .

Treatments **Vit .C. concentration mg/kg diet Items**	**T_1** **0.0**	**T_2** **300**	**T_3** **600**	**T_4** **900**	**T_5** **1200**	**MSE$^+$**
Blood parameters:						
- Erythrocyte count (milion / mm^3).	1.86^d	1.85^c	2.01^{ab}	2.59^a	2.95^b	0.156
- Hematocrit value %.	31.75^d	34.06^c	39.55^{ab}	43.78^a	42.9^b	2.55
- Hemoglobin content (g /100)	8.56^d	8.95c	12.3^{ab}	13.57^a	12.7^b	1.7
Mnscle compositions						
- Protein g /100g fresh tissue	15.61^d	15.95c	16.59^{ab}	18.2^a	17.3^b	1.9
-Glycogen (mg /100g fresh tissue).	11.35^d	1.56c	1.71^{ab}	1.18	1.69^b	0.02
- Lipid (g /100 g fresh tissue).	7.41^d	6.31^c	6.1	5.95^a	6.0^b	0.90
Serum analysis						
- Glucose (mg / 100ml serum.	54.5^d	70.33^c	55.73^{ab}	60.33^a	58.82^b	5.33
- lipid (g) / 100 ml serum.	5.55^d	4.84^c	4.5^{ab}	4.00^a	4.45^b	0.08
- proteien (g) / 100 ml serum.	11.3^d	12.9c	13.55^{ab}	17.7	14.5^b	1.9

a,b,c. etc mean in the same row with different superscript are different($p<O.05$).
MSE = mean standard error calculated from residual mean square in the analysis of variance.

DISCUSSION

O. niloticaus as affected by adding dietary supplemented Ascorbic acid (AA), growth performance increas with the increase in (AA) level until 900 mg/kg diet. This result is in agreement with the observation of Sweilum (2005), who found that growth performance of such fish increased with increasing vit. C. level until 750 mg /kg diet, also with previous studies of Mohajon & Agrawal (1980) on the nutritional requirement of Ascorbic Acid for Indian major carp that showed that growth rate was increased by increasing the levels of (A A) in the diets up to 600.0 mg /kg diet. Similar observation was obtained by Shiau & Jan (1992), who mentioned that vit. C. required for maximum growth in Tilapia fish is 790 mg /kg diet. While Lee *et al*. (1998) reported that the weight gain, feed conversion factor showed the highest value for Korean-rock fish with feeding dietary ascorbic acid level at 1500 mg /kg diet.

The requirement levels of dietary (AA) for growing fish were illustrated from different fish as following: 100mg/kg diet for rainbow trout (Hilton *et al.,* 1978), 50mg/kg diet for blue Tilapia (Stickney *et al.*,1990), 60 mg/kg for

channel cat fish (El- Naggar & Lovel, 1991) and 400 mg/ kg diet EI-Hammady *et al.* (2002).

Data of survival rate had better results shown in fish offered diet contained 900 mg /kg, diet, in accordance with that postulated by EI-Shandaweily (1999) and sweilum (2005) in Tilapia fish. Total production recorded better values for fish fed diet contained 900 mg /kg diet. This agrees with the observation recorded by El-Hammady *et al.* (2002), that ascorbic acid at level of 600 mg/kg diet, recorded the highest value in fish production for *O. niloticaus*.

The present results of Red-blood cells count, hematocrit and hemoglobin had the maximum values in fish fed diet contained 900 mg/kg diet with contrast to the control diet. Similar observation was shown by Shiau & Jan (1992) which revealed that macrocytic anemia has been shown in Tilapia sp., when fed diet deficient of (AA), EL Hammady & EL said (2002) cited that the best hematocril and hemoglobin of Tilapia sp. were recorded with increasing dietary (A.A) level up to 600 mg /kg diet .

Serum glucose and lipid were decreased with increasing (A.A) levels up to 900 mg /kg diet. while serum protein values were increased directly with the increasing in vitamin C levels. Similar results were observed by Kibatchi (1967) and Sweilum (2005) who reported that adding vit .C. (optimum), level to fish showed the conversion of unsaturated fatty acid to lipid.

The present results show that biochemical composition of fish muscles were also affected by adding ascorbic acid in the diets, where total protein and glycogen were increased with the increasing of (AA) requirement, while total lipids decreased with the increase in vit. C. level until 900 mg/kg diet. Simitar record were observed by Mahanjan *et al*. (1980). when fed carp fishes on diets contained 650-750 mg /kg diet of (AA).

CONCLUSION

At the end of the present research, the observation enhancing fish growth and health , reported that Ascorbic acid can be used as a commercial product to reduce the production costs and to increase the final net production and profit in aquaculture.

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