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ESSENTIALILY OF VITAMIN C AND /OR VITAMIN E IN FEEDS FOR MONOSEX NILE TILAPIA *Oreochromis niloticus* REARED FOR ONE YEAR

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SUMMARY

The present study was carried out on monosex Nile tilapia *Oreochromis niloticus* for one year in Fish Research Station belonging to National Institute of Oceanography and Fisheries. The aim of work was to gain basic information about the effect of supplementation of 400-mg/ kg diet ± ascorbic acid (AA) and / or 2000 mg /kg diet α– tocopherol acetate (α– TOH).

The data showed that the highest average growth performance and protein utilization parameters were occurred when fish fed diet supplemented with (AA) and (α – TOH) together. Also, supplementation with (AA) and (α–TOH) had no adverse effect on the fish. The data cleared that the highest concentrations of (AA) and (α–TOH) were found in whole body, blood, liver and anterior kidney of fish fed diet supplemented with (AA) and (α-TOH) together. The highest values of haemoglobin, %; haematocrit, %; erythrocite count (10^6 / mm^3) and lowest values of leucocyte count (10^3 / mm^3) were occurred when fish fed diet supplemented with (AA) and (α– TOH) together. Also, the highest total serum protein (TSP) and their fractions (TSA) and (TSG) values were found when fish fed diet supplemented with (AA) and (α – TOH) together.

The present study suggested that the raise of vitamin C and vitamin E requirements to 400 mg /kg diet and 2000 mg/kg diet respectively, are adequate for high and rapid growth as well as no adverse effect on monosex Nile tilapia *O. niloticus*

INTRODUCTION

In nutrition research, good survival combined with continuous growth will often point to the

ability of a diet to meat the requirements of the species cultured. However, only these criteria do not guarantee an optimal physiological condition of the fish. High levels of Ascorbic Acid (AA) and (α-Tocopherol (α-TOH) on the other hand are reported to enhance tolerance to environmental stressors, e.g. aldrin toxicity (Agrawal *et al.*, 1978); Capture stress, salinity and temperature (Thomas, 1984) ; intermittent hypoxic stress (Ishibashi *et al.*, 1992) and to increase immunoresistance, e.g. in channel catfish (Li and Lovell, 1985); and Salmonids (Hardie *et al.*, 1991; Navarre and Halver, 1989). Ascorbic acid is an essential micronutrient in aqua-feeds since fish and crustaceans are not capable of vitamine C biosynthesis (Chatterjee , 1973). Several functions (Skeletal development, growth, resistance to toxicants and stress, immuno activity) are affected in aquaculture species by dietary ascorbate deficiency and result in increased fish mortality (Dabrowski, 1992). Ikeda and Sato (1964) found that carp were able to synthesize vitamin C but not in quantities sufficient to provid for rapid growth. Jauncey and Ross (1982); De Silva and Anderson (1995) recommended 100 mg/kg diet vitamin C (AA) as requirement of Nile tilapia Oreochromis niloticus. At present some controversies exist as to whether all teleost fish are in fact unable to ascorbic acid (Reviewed in Dabrowski *et al.*, 1994). The recommended amounts of vitamin C are adequate for normal growth and tissue development, but not in quantities sufficient to provide for rapid growth. Requirements for vitamin E and other cellular antioxidants have been shown to be dependent on the endogenous and exogenous generation of free radicals (Freeman and Crapo, 1984; Di-Giulio *et al.*, 1989) and the concentrations of oxidizable lipids in cellular membranes (Watanabe *et al.*, 1977; Cowey *et al.*, 1983). Jauncey and Ross (1982) recommended vitamin E (α-TOH) requirement of Nile tilapia *Oreochromis niloticus* to be 200 mg./kg. diet. Roem and Oines (1990) reported 25 mg./kg. diet of vitamin E requirement of blue tilapia

Oreochnomis aureus. NRC (1993) reported 50 mg./kg. diet of vitamine E requirement of Nile tilapia *Oreochromis niloticus*. De Silva and Anderson (1995) recommended 30 mg/kg for Nile tilapia *Oreochromis niloticus*. Diets with vitamin E above the recommended levels may prove beneficial in protecting channel catfish lipid membranes against oxidative damage when exposed to conditions that increase the demand for cellular antioxidants during the biotransformation of many contaminants for instrance (Reviewed in Di Giulio *et al.*, 1989). Poston and Livingston (1969) found that 5000 mg./kg. diet of vitamin E (α-TOH) caused reduced growth . In contrast, the highest dietary of (α– TOH), 2500 mg./kg. diet had no adverse effect on the fish. (Lovell, *et al.*; 1984).

The purpose of the present study was to gain basic information about the effect of supplementation of 400 mg/kg Diet (AA) and or 2000 mg./kg. diet (α-TOH) of monosex Oreochromis niloti-

cus on :

1. The average growth performance of fish.
2. Protein utilization.
3. Gross deficiency sign.
4. Concentration of (AA) and (α-TOH) in whole fish body, blood, liver and anterior kidney.
5. Some haematological parameters .
6. Total serum protein (TSP), Total serum albumin (TSA) and Total serum globulin (TSG).

MATERIALS AND METHODS

Experimental fish :

Nile tilapia *O. niloticus* reared in this study was collected from the common populations of this species which cultured and propagated at the experimental concreet ponds (40 m^3 for each pond). The experimental fish were apparently healthy, negative for any parasites and had an average weight 31.12 gm. ± 0.61 at collection time. The collected fishes were taken randomly and transferred with special care after weight and randomly distributed into the experimental ponds. However, the first 14 days of the experimental period was considered as the adaptation period followed directly from 1 October 1997 to 30 November 1998 .

Experimental design :

Eight concreet fish ponds which were used in this study represented four nutritional treatments. Each of the four experimental diets was fed to duplicate groups of fish. The first group fed diet supplemented with 400 mg./kg. (AA) and 2000 mg./kg. (α-TOH) together, the second group fed diet supplemented 400 mg/kg (AA) Only, the third group fed diet supplemented with 2000 mg./kg. (α- TOH) only and the fourth group fed diet without supplementation of (AA) or (α TOH) (as a control group). The total area of each concreet pond were 40 m3. The depth of water was kept at about 1 m level throughout the period of study. Water inlet to experimental ponds was received by pumbing from a branch of the Nile (El-Monufy canal) and the outlet of the water was constructed to empty the ponds using the gravity. Each pond was filled with water and stocked by 80 fish (2 fish /m2) according to Viola *et al.* (1988) and Green (1992). Individual fish body weight and length were recorded at the beginning of experimental period to the nearest gram and centimeter fraction, respectively. The live fish weights and lengths were recorded individually every three weeks throughout the experimental period , which reached about one year. The experimental feeds were offered to fish as 1% of the live body weight per day through autumn and winter seasons, while, fish fed 3 % through summer and spring seasons. Feeds were offered twice daily

3. Preparation and Analysis of Experimental Fish diet:

All experimental diets as in table (1) contained 41%, wheat bran; 39%, soybean meal; 7%, fish meal; 7% meat meal; 4% cotton seed oil and 0.76% vitamin premix free of (AA) or (α-TOH) and 1% premix. However, the diets were differed in their content of (AA) or (α–TOH) as mentioned before. With the exception of (AA) or (a-TOH) supplementation, the four diets had the same nutritive values as shown in table (2), whereas the experimental diets were formulated according to the nutritional requirments of *O.niloticus* (NRC, 1993). The diet mixture was processed into California Pellet Meal (CPM) machine pekkets processed through a mincer 2-mm. diameter.

Analysis of Body Composition :

At the end of the experiment, fish in each treatment were netted, counted and weighed. Body composition analysis was performed using standard AOAC (1980) methods. Crude protein was determined as nitrogen content in the fish body as well as carcass energy content were estimated according to NRC (1993).

Determination of (AA) and (α TOH) in Whole body, Blood, Liver and Anterior Kidney of experimental fish :

Table (1) : The Composition Of Experimental Diets .

Ingredients	Diet Supplemented with (AA)+ (α-TOH)	Diet Supplemented with (AA)	Diet Supplemented with (α-TOH)	Diet Unsupplementeed with (AA) or (α-TOH)
Wheat bran , ...%	41	41	41	41
Soy bean meal.,.....%	39	39	39	39
Fish meal,.....%	7	7	7	7
Meat meal,.....%	7	7	7	7
Cotton seed meal,.....%	4	4	4	4
Ascorbic acid,.....%	0.04	0.04	-	-
α–Tocopherol acetate,..... %	0.20	-	0.20	-
Vit. premix (Free of Vit.C,E)%	0.76	0.96	0.80	1.00
Mineral Premix **.....%	1.00	1.00	1.00	1.00

* Vitamin premix (Each Kg. Contained):
Thiamine (B_1), 60 mg. : Riboflavin (B_2), 60 mg.; Phridoxine (B_6), 20 mg. ; Biotin, 10 mg.; Folic acid, 10 mg.; Para aminobenzoic acid, 2500 mg.; Choline, 2000 mg.; Niacin (Nicotinic acid, B_3), 150 mg.; Cyanocobalamin (B_{12}), 0.05 mg.; Retinol Palmitate (A), 2000 IU; Menadione (K), 40 mg.

** Mineral Premix (Each Kg. Contained) :
Calcium orthophosphate, 727.7775 gm.; Magnesium sulphate, 127.5 gm.; Naterium chloride, 60 gm.; Potassium chloride, 50 gm.; Iron sulphate, 25 gm.; Zink sulphate, 5.5 gm.; Manganese sulphate, 2.5375 gm.; Copper sulphate, 0.7850 gm.; Cabalt sulphate, 0.4775 gm.; Calcium iodate, 0.2950 gm.; Chromic chloride, 0.1275 gm.

- (AA) Ascorbic Acid
- (α TOH) Alpha Tocopherol Acetate.

Table (2) :The Nutritive Value Of Experimental Diets

Nutritive Value	Diet Supplemented with (AA)+ (α-TOH)	Diet Supplemented with (AA)	Diet Supplemented with (α-TOH)	Control Diet
Ptotein,.....%	30.00	30.00	30.00	30.00
Lipid .....%	6.74	6.74	6.74	6.74
Nitrogren free extract...%	39.17	39.17	39.17	39.17
Metabolizable energy (kcal/kg)	3375.00	3375.00	3375.00	3375.00
Protein/energy ratio (P/E ratio)	88.89	88.89	88.89	88.89
Chemical Score of EAA.%	74.46	74.46	74.46	74.46
Index of A.A.....%	62.51	62.51	62.51	62.51
Ascorbic acid (mg/kg)	400.00	400.00	-	-
α-Tocopherol acetate (mg/kg.)	2000.00	-	2000.00	-
Vitamin premix free of Vit.C (mg/kg)	7600.00	9600.00	8000.00	10000.00
Mineral premix (mg/kg.)	10000.00	10000.00	10000.00	10000.00

EAA = Essential Amino Acids.

a) **Sampling Procedures :**

Fish samples were taken at the start and at the end of experimental period. The fish were fasted 24 hr. prior to sampling, and anaesthetized in water with the addition of saturated benzcaine ethanol solution. Four pooled samples per treatment (three fish per sample) were taken for analyses of (AA) and (α-TOH) in whole body and three different organs (blood, Liver and anterior kidney) were measured in the other two samples. These tissues were embedded in paraplast, sectioned, stained with hematoxylin and eosin (Humason, 1972) and examined for microscopic the lesions. Special stains were used when necessary to characterize lesions.

b) **Analytical Methods :**

The whole fish body, blood, liver and anterior kidney tissues of each sample were analyzed for (AA) according to methods of Polk *et al.*, (1960) and Wedemeyer (1969) , while, the whole body fish, blood , liver and anterior kidney tissues of each sample were analysed for (α- tocopherol by HPLC by the method of Lie *et al.*, (1994).

Haematological Examination :

At the end of the experimental, blood was collected from the caudal artery from 10 fish per treatment for measurement of Haemoglobin, Haematocrit, Erythrocyte count, total Leucocyte count, Sodium and Potassium. Haemoglobin content was measured using Sahli haemometer. Haematocrit was determined by the microcentrifugation method of Larson and Snieszko (1961). Erythrocite count was determined according to method of Hendrick (1952). Total leucocyte count determined according to the method of Hesser (1960).

Determination of Total serum protein (TSP) and Total serum albumin (TSA) and Total serum globulin (TSG) :

Serum was separated by centrifugation of uncoagulated (3000 r.p.m. for 10 min at 4RC). (TSP) and (TSA) and (TSG) were determind according to the method described by Reinhold (1953).

Statistical Analyses :

Statistical analyses was made by factorial design (3 x 2) according to the procedure reported by Steel and Torrie (1980). Duncans test was applied between treatments whenever, possible to test mean differences (Duncan, 1971).

RESULTS AND DISCUSSION

The Average Growth Performance :

The average growth performance of all male tilapia was shown in table (3). At one year, there are significant differences ($P < 0.05$) were found in final body weight, specific growth rate (SGR), among fish fed the various diets. The fish fed diet deficient in (AA) or (α-TOH) (Control group) had lower body weight than fish received other treatments. This may be due to the loss of appetite as reported for vitamin C or E deficiency. While the fish fed diet supplemented by (AA) and (α-TOH) together had higher body weight than fish received other treatments .The data also cleared that the fish fed diet supplemented by (AA) were larger than those fed diet supplemented by (α-TOH), Wise *et al* (1993) found that there is no differences in growth rate observed among dietary treatments group (0,60 and 2500 α- tocopherol acetate). Hamre and Lie (1995) indicated that the fish were growing normaly, but that there may have been differences in growth between (α–TOH) supplemented and unsupplemented fish. Merchie *et al.* (1996) showed that the (SGR) and calculated biomass of the control treatment were significantly lower than in the ascorbic acid supplemented group. Ikeda and Sato (1964) found that carp were able to synthesize vitamin C, but not in quantities sufficient to provide rapid growth. Lovell (1973) also revealed that the channel catfish probably synthesize limited quantities of the vitamin C which will prevent manifestation of clinical deficiency symptoms when they are not subjected to the stress of fast growth. It was apparent that the absence of supplemented vitamin C from the practical type diet used in this

Table (3): The Average Growth Performance Of Monosex Nile Tilapia Fed Diet Supplemented With (Aa) And / Or (α - TOH) Reared For One Year

ITEMS	Treatments				SE±
	Supplemented of (AA)+ (α-TOH)	Supplemented Of (AA)	Supplemented Of (α-TOH)	Without Supplemented Of (AA) or (α-TOH)	
Initial body weight (gm.)	32.90	30.69	30.14	30.75	0.61
Final body weight (gm.)	210.041^{a}	195.76^{b}	180.16^{c}	162.62^{d}	10.27
Gain in weight (gm.)	177.51^{a}	165.07^{b}	150.02^{c}	131.87^{d}	9.84
Specific growth rate* (% /day)	0.51^{a}	0.51^{a}	0.49^{c}	0.46^{d}	0.01
Feed consumption (gm.)	213.012^{a}	247.61^{b}	255.03^{bc}	262.00^{cd}	10.87
Feed converwsion ratio **	1.20^{a}	1.50^{b}	1.70^{c}	2.00^{d}	0.17

* Specific growth rate (% / day) = 100 (L n final weight L n initial weight) / days.
** Feed Conversion (gm./gm.) = dry feed intake (gm.)/ Wet weight- gain (gm.).
SE (, Standard error. Calculated from residual mean square in the analysis of variance.
a,b, and c. etc. means in same raw with different superscripts are different (P < 0.05).

study was responsible for the poor growth and spinal deformities in the fish. Soliman *et al.* (1994) found that the fingerling of *O. niloticus* fed diet devoid of ascorbic acid exhibited significantly poor growth and higher conditition factor. They added that the growth of fish was improved with increasing dietary ascorbic acid level up to 1250 mg /kg dry diet, of which is equivalent to a net requirement (after processing and storage) of 420 mg/kg diet It is worthly to note that the level of optimum vitamin C or vitamin E recommended were 100 and 200 mg/kg diet, respectively for *O. niloticus* Jauncey and Ross (1982). Roem and Oines (1990) reported that vitamin E- requirement of blue tilapia O. aureus was 25 mg/kg Diet. NRC (1993) recommended level of vitamin E-50 mg/kg diet for O. niloticus. De Silva and Anderson (1995) recommended 30 mg./kg. diet of vitamin E for Nile tilapia *O. niloticus.* While, this amount of vitamin C or vitamin E is adequate for normal growth and tissue development, but not in quantities sufficient to provide for rapid growth. In contrast, the highest dietary level of (tocopherol, 2500 mg/kg Had no adverse effect on the fish. (Lovell *et al.*, 1984). Also, Poston and Livingston (1969) found that 5000 mg/kg reduced growth rate in brook trout. The possible reason that explained the high average weights in fish fed

diet supplemented with (AA) and (α- TOH) together in the present study is that (AA) has been found to spare metabolism of (α– TOH) and proposed that (α– TOH) serves as the primary antioxidant and that (AA) reductively regenerates (tocopherol.

Protein Utilization :

Table, 4 summarized the protein utilization and energy utilization, the data cleared that the protein efficiency ratio (PER), protein productive value (PPV), Net protein utilization (NPU) and energy utilization (EU) values were higher in fish fed diet supplemented with (AA) and (α-TOH) together. The values of (PER), (PPV), (NPU) and (EU) were higher of fish fed diet supplemented with (AA) than fish fed diet supplemented with (α-TOH). Anadu *et al.*, (1990) revealed that the best growth, feed conversion (FC) and (PER) were obtained with feed containing ascorbic acid than those fed on the control diet. Soliman *et al.* (1994) showed that the food conversion, protein efficiency ratios and protein utilization of *O. niloticus* were improved with increasing dietary ascorbic acid level up to 1250 mg/kg dry diet, which is equivalent to a net requirement (after processing and storage) of 420 mg/kg. diet. Merchie *et al.* (1996) showed that the feed efficiency

Table (4): Protein Utilization And Energy Retention Of Monosex Nile Tilapia Fed Diet Supplemented With (AA) And / OR (α - TOH) .

ITEMS	Treatments				SE±
	Supplementation of (AA)+ (α-TOH)	Supplementation Of (AA)	Supplementation Of (α-TOH)	Without Supplementation Of (AA) or (α-TOH)	
Protein efficiency ratio*	2.78^{a}	2.22^{b}	1.96^{bc}	1.67^{d}	0.24
Protein productive value** %	39.64^{a}	29.84^{b}	26.66^{bc}	19.50^{d}	4.18
Net protein utilization***	8.97^{a}	8.43^{ab}	7.91^{bc}	6.34^{d}	0.57
Energy retention*** %	21.16^{a}	16.37^{b}	14.70^{bc}	10.42^{d}	2.22

* Protein Efficiency Ratio = Dry feed intake (gm.)/Wet weight gain (gm.).

** Protein Productive Value (%) = 100 {Carcass protein gain (gm.)/Dry protein intake (gm.) }.

*** Net Protein Utilization = Final body protein (1) (gm.) - Final body protein (2) / protein intake (gm.)
{(1): Protein diet group; (2) : None protein diet group }.

**** Energy utilization (retention) % = 100 {Carcass gross energy gain (kcal.) / Gross energy feed intake (kcal.)

of the control treatment were significantly lower than in the ascorbic acid supplemented groups. The same trend was observed by Roem and Oines (1990) concerning vitamin E requirement of the O. aureus. In the present study, it is worthly to mention that the values of (PER), (PPV), (NPU) and (EU) of fish fed diet supplemented with vitamin C and E together were higher than finding by Anadu *et al.*, (1990) or Roem and Oines (1990).

Gross Deficiency Signs :

After the summer season, the control fish had become anorexic and some appeared light in color. By the end of winter season, more fish on the control diet had the light skin pigmentation, and some were visibly thinner across the back, which indicated myopathy. At the end of the experiment (one-year) most of the control fish had varying degrees of skall lightness and over 90% of the fish showed visible indication of myophathy. The control fish, also were easily excitable and showed signs of fainting and loss of swimming coordination as reported for (tocopherol deficient (Lovell *et al.* 1984). None of these signs were found in fish fed diet supplemented with (AA) (α- TOH) together. Gross appearance of eyes and gills of all fish were normal. Internal examination did not reveal accumulation of fluids (ascites) in any fish or discoloration of visceral organs, as have been reported in other fish fed α-tocopherol deficient diets. The data reported herein showed that the vitamin C deficiency is suspected as being resposible for the deformity as developed spinal curvatures because none of the above symptoms have been observed in tilapia fed diet supplemented with vitamin C. Deformities were identified visually in fish fed vitamin C deficient diet. Most common was scoliosis, a lateral curvature of vertebral column, usually at approximately the mid-length of the fish. Many fish showed lordosis with or without scoliosis. Soliman *et al.* (1994) showed that *O. niloticus* fed ascorbic acid free diet exhibited deficiency signs including erratic and convulsive swimming, anorexia, lethargy, caudal fin erosion, skin haemorrhages and mortality. According to the nutritional and pathological parameters investigated, the recommended dietary inclusion level is 1250 mg /kg dry diet, which is equivalent to a net requirement (after processing and storage) of 420 mg /kg diet. With respect of vitamin E deficiency in diets caused lordasis as reported by Watanabe *et al.* (1970).Tengerdy (1990) reported that the supplemental levels of vitamin E in the diets of farm animals have been shown to enhance humoral and cellular immune responses and increase disease resistance. For this reason , increasing dietary levels of vitamin E to 2000 mg /kg may be an easy and effective means of increasing immune function and increasing disease resistance in monosex *O. niloticus* . Surface swimming or tetany, which have been observed in practical tilapia cultures where vitamin C deficiency was suspect (Lovell, 1973) were not found.

Gross examination of internal organs for all experimental group revealed no hemorrhagic areas that could be attributed to diet. Thus the data of gross deficiency signs cleared that the level of (tocopherol (2000 mg /kg) and ascorbic acid (400 mg/kg) supplemented together in the diet allowed for normal growth and prevention of muscular myopathy and anemia in *O. niloticus* and prevent incipient signs of muscle and liver pathology . De Silva and Anderson (1995) showed that ascorbic acid is required in the diet of some species of fish, the first recognized function of ascorbic acid is its role in hydroxylating the proline to hydroxyproline for use in cartilage synthesis.

Concentration of (AA) and (α-TOH) in whole body, blood, Liver and anterior kidney of experimental fish :

a) Ascorbic Acid :

Table; 5 showed the mean (AA) levels in whole body, blood, liver and anterior kidney of six fish from each treatment. All values were significantly higher ($P < 0.05$) for fish fed diet supplemented with (AA) and (α- TOH) together followed by those fed diet supplemented with (AA) only. Lovell (1973) and Merchie, (1996) found the same trend when compared between fish fed supplemental (AA) and others fed unsupplemental (AA). Blood concentration of (AA) in tilapia of control group have ranged 20 (1.00 mg. /kg. While, the blood concentration of ßascorbic acid in tilapia fed diet supplemented with vitamin C and E together was 57 (2.10 mg /kg Dabrowski *et al.* (1994) cited that the concentration of (AA)

Table (5): Concentration (mg/ kg)of ascorbic acid in whole body , blood , liver and anterior kidney of monosex Nile tilapia *O. niloticus* fed diets supplemented with (AA) and / or (α-TOH).

ITEMS	Treatments			
	Supplemented of (AA)+ (α-TOH)	Supplemented Of (AA)	Supplemented Of (α-TOH)	Without Supplemented Of (AA) or (o-TOH)
Whole body, Vit.C.Content (mg.)	120 ± 18.0	92 ± 11.0	0.76 ± 0.30	0.61 ± 0.40
Ratio of Vit.C in whole body/feed.	1.40 ± 0.01	0.92 ± 0.03	0.81 ± 0.02	0.78 ± 0.02
Blood, Vit. C. content.....(mg.)	57 ± 2.10	49 ± 1.70	23 ± 0.80	20 ±1.00
Ratio of Vit.C in blood/feed	0.67 ± 0.01	0.49^{7} ± 0.03	31.8 ± 3.71	26.32 ± 2.21
Liver, Vit.C. content....(mg.)	88 ± 2.70	83 ± 3.10	26 ± 1.10	22 ± 0.7
Ratio of Vit.C in liver/ feed	1.03 ± 0.03	0.83 ± 0.01	0.35 ± 0.01	0.28 ± 0.02
Anterior kidney Vit.C content (mg.)	166 ± 4.00	160 ± 3.2	73 ± 1.30	69 ± 1.30
Ratio of Vit.C in interior kidney/feed	1.93 ± 0.02	1.61 ± 0.03	98.65 ± 7.8	90.79 ± 6.11

in blood plasma of the fish fed 500 ppm was 34.8 (g/ml. Indicating that (AA) level in blood of fish probably vary with dietary levels as has been found to occur with salmonids (Halver, *et al* 1969), channel catfish (Lovell, 1973; 1975) and sea bass (Merchie, 1996) who reported that the liver, anterior kidney and blood analysis all reflected the differences in content of (AA) in the diet. Also, the liver concentration of (AA) in tilapia fed diet supplemented with vitamin C and E together was 88 (2.7 mg. /kg. higher than the unsupplemented fish (22 ± 0.7 mg/kg.) Dabrowski *et al.*, (1994) found that rainbow trout fed diets supplemented with 40 ppm ascorbic acid had a liver concentration 38.59 ppm (AA). Dabrowski *et al* (1994) reported that the concentration of (AA) in the liver of common carp and rainbow trout was significantly different after they had been fed a diet supplemented with 500 ppm (AA) for 5-6 weeks. While, Dabrowski *et al.*, (1994) found that channel catfish fed diets supplemented with 132 ppm of ascorbic acid had either no detectable or only no amounts of (AA) in the liver and kidney. Soliman *et al.*, (1994) found that the tissue ascorbate concentrations of *O. niloticus* were depressed in fish fed the ascorbic acid free diet While, they found that the tissue ascorbate concentrations, especially for liver, were highly correlated with dietary ascorbic acid level. The anterior kidney concentration of (AA) in tilapia fed diet supplemented with (AA) and (α– TOH) together was 166 ±4.00 mg /kg and higher than those fed (AA) and (α-TOH) deficient diet (69 (1.30 mg. /kg.) as reported by Lovell (1973).

Concerning, supplementation of (AA) only increased whole body levels 121 fold compared by fish fed diet supplemented with (α–TOH) only, 151 fold of those fed unsupplemented diet. While, the supplementation of (AA) and (α– TOH) together increased the whole body level of (AA) to 1.3 fold compared by those fish supplemented with (AA) only. The ratios of (AA) in whole body / feed of the (AA) supplemented fish were 0.92 ±0.03 of those found for the supplemented fish with (α– TOH) only 0.81 ± 0.07 and of the unsupplemented fish 0.78 ± 0.02. While, this ratio was 1.4 ±0 0.01 when fish supplemented by (AA) and (α– TOH) together.

b) α- Tocopherol :

Table, 6 shows mean (α– TOH) levels in whole body, blood, liver and anterior kindey of six fish from each treatment. All values were significantly ($P < 0.05$) higher for the fish fed supplemental (α–TOH) and (AA) together. Also, the concentrations of (α– TOH) in blood, liver and anterior kidney were higher of fish suplemented with (α–TOH) only than those fed diet supplemented with (AA) only or those unsupplemented (control group) . Hamre and Lie (1995) found that the (α–TOH) concentration increased in blood, liver and anterior kidney of fish fed diet supplemented with

300 mg /kg DL-α- toco pherol acetate. Cowey *et al.* (1981, 1983) were successful in utilizing the (AA) stimulated lipid peroxidation assay in liver microsomes as a sensitive index of vitamin E status in rainbow trout. Therefore , this assay was perfected for monosex *O. niloticus* and was performed on tissue samples of fish that had been fed their respective diets for one year. Supplementation of (α– TOH) only increased the whole body (α- TOH) concentration 7.8 fold compared by fish fed diet supplemented with (AA) only, 7 fold of those fed unsupplemented diet {free of supplemental (α -TOH) and (AA) }. While, the supplementation of (α– TOH) and (AA) together increased the whole body level of (α– TOH) to 0.05 fold compared by those fish fed supplemented with (α– TOH) only. Hamre and Lie (1995) reported that the supplementation of vitamin E increased the whole body (α– TOH) concentration 5-7 fold affecting all analysed organs. The liver (α– TOH) concentration increased approximately 10.6 fold when compared between fish supplemented with (α-TOH) only and those supplemented with (AA) only , 0.12 fold when compared between fish supplemented with (AA) and (α TOH) together and those supplemented with (α– TOH) only. While, Hamre and Lie (1995) found the liver (α–TOH) concentration increased approximately 10 fold in case of fish supplemented with 300 mg/kg. Apparently, the liver is capable of storng excess (α– TOH) . In the fish supplemented with (α– TOH) only, the ratio of whole body / feed were 1.17 ± 0.03 of those found for the supplemented fish with (AA) only

Table (6): Concentration (mg/ kg) of α- Tocopherol in Whole body, Blood, liver and Anterior kidney of monosex Nile tilapia O. niloticus fed diets supplemented with (AA) and / or (α - tTOH) .

ITEMS	Supplemented of (AA)+ (α-TOH)	Supplemented Of (AA)	Supplemented Of (α-TOH)	Without Supplemented Of (AA) or (α-TOH)
Whole body, (α–TOH) Content (mg.)	630 ± 91.0	68 ± 2.30	600 ± 11.0	75 ± 2.10
Ratio of (α–TOH) in whole body/ feed.	1.41 ± 0.02	34.52 ± 2.25	1.17 ± 0.03	35.89 ± 3.17
Blood, (α–TOH) content.....(mg.)	561 ± 24.0	61 ± 08.00	528 ± 18.0	59.00 ± 0.7
Ratio of (α–TOH) in blood / feed	1.31 ± 0.03	30.96 ± 3.55	1.03 ± 0.02	28.23 ± 2.57
Liver, (α–TOH) content....(mg.)	9990 ± 109	840 ± 26.00	8881 ± 118	811 ±29.00
Ratio of (α–TOH) in liver / feed	22.46 ± 1.09	447.21 ± 23.7	16.41 ± 1.7	388.04 ± 29.23
Anterior kidney (α–TOH) content (mg.)	947 ± 31.0	101 ± 18.00	882 ± 25.0	105 ± 13.00
Ratio of (α–TOH in interior kidney/feed	2.21 ± 0.11	51.27 ± 8.34	1.72 ± 0.01	50.24 ± 6.91

34.52 ±2.25 and of the unsupplemented fish 35.89 ± 3.17. While, this ratio was 1.47 (0.02 when fish supplemented with (α– TOH) and (AA) together. Also, Hamre and Lie (1995) found in the fish supplemented with DL (tocopherol acetate, the ratio of [α– TOH] tissue : [α–TOH] feed was generally lower than in the unsupplemented fish. Hamre and Lie (1995) also found that in Atlantic salmon Juveniles, the ratio of (α– TOH) concentration in whole body / feed was unchanged at dietary DL (tocopheryl acetate levels between 30 and 300 mg /kg Concentration dependent rates of absorption or turnover of (α– TOH) are therefore unlikely to have caused the different relative body concentrations. In mammals, a hepatic tocopherol binding protein discriminates between different stereoisomers of (α– TOH), binding the D-form with a higher affinity. Consequently, D and DL α–tocopherol have different biological activities (Kayden and Traber, 1993). A similar discrimination mechanism is probably present in fish, although this remains to be shown. The relative incorporation of (α–TOH) in the fish body in supplemented and unsupplemented fish was similar to the relative activities of D- and DL- (α–TOH in mammals. All organs in the present study showed a reduction of the tissue / feed ratio of (α– TOH) upon supplementation .Thus , (α–TOH) appears to be retained in the tissues according to a distribution key , independent of supplementation and body level.

Haematological Changes :

The effect of (AA) and / or (α– TOH) supplementation on some haematological parameters were shown in table (7). The most prominents feature was a significant (P < 0.05) increase in Haemoglobin (%) from 48.47% {Without supplementation of (AA) (α– TOH)}to 58.91 % {with supplementation of (AA) and (α– TOH) together}. In contrest, insignificant differences were found between fish supplemented with (AA) (52.35 %) and those supplemented with (α-TOH) only (51.88 %). It means that the supplementation of (AA) and (α– TOH) together increased Haemoglobin content to (58.91 %) and was significantly higher (P < 0.05) than unsupplennented fish. While, Wilson *et al.* (1984) showed no differences were observed in haemoglobin concentration among fish fed the, O,20, 20, 40 and 60 mg /kg DI (α–TOH levels for 20 weeks. While, the present data agreed with finding of Soliman *et al.*, (1994) who found that the haemoglobin level was depressed in Nile tilapia *O. niloticus* fed the ascorbic acid free diet. Haematocrit values were lower (P < 0.05) for fish fed the control diet. Soliman *et al.*, (1994) showed that the haematocrit level was depressed in Nile tilapia *O. niloticus* fed the ascorbic acid free diet. Klar *et al* (1986) showed that the increase in haematocrit of anemic fish that were placed on a control diet indicated that recovery from the disease by individual fish was possible. No individual haematocrits were near the extremely low range (0--5 %) observed

Table (7): Changes in some haematological parameters of monosex O. niloticus fed diets supplemented with (AA) / or (α- - TOH).

ITEMS	Treatments				SE±
	Supplemented of (AA)+ (α-TOH)	Supplemented Of (AA)	Supplemented Of (α-TOH)	Without Supplemented Of (AA) or (α-TOH)	
Haemoglobin, %	58.91^{a}	52.35^{b}	51.88^{b}	48.47^{c}	2.09
Haematocrit, %	38.64^{a}	31.91^{b}	38.72^{a}	25.38^{c}	2.51
Erythrocite count (million/mm^3)	1.98^{a} 1	1.32^{b}	1.38^{b}	0.88^{c}	0.45
Total Leucocyte count ($10^3/mm^3$)	11.60^{c}	13.08^{c}	13.10^{b}	15.66^{a}	1.30

SE ± , Stand error. Calculated from residual mean square in the analysis of variance.
a, b, ... etc. means in same raw with different superscripts are different (P < 0.05).

in the anemic fish as reported by Klar *et al* (1986). Haematocrits were not differed (P < 0.05) among groups fed (AA) and (α– TOH) together or those supplemented with (α– TOH) only. Lovell *et al.* (1984) found the haematocrit values were not difered among catfish fed 25, 75, 250 and 2500 mg. /kg. They also, found the lower value were observed among fish fed the control diet. Poston and Livingston (1969) found that 5000 mg /kg caused reducing in haematocrit values in brook trout. The present study suggests that the level of (α-TOH) (2000 mg. /kg.) and the level of (AA) (400 mg. /kg.) together allowed for normal growth and erythrocyte production.

Total serum protein, Total serum albumin and Total serum globulin :

As shown in table (8), the values of Total serum protein (TSP), Total serum albumin (TSA) and Total serum globulin (TSG) values were significantly affected (P < 0.05) by supplementation of (AA) and / or (α– TOH). The suppulementation of (AA) and (α–TOH) together showed higher values of (TSP), (TSA) and (TSG) and followed by those fed diet supplemented with (AA) only, supplemented with (α– TOH) only and unsupplemented fish. The values of (TSP), (TSA) and (TSG) which obtained by those supplemented with (AA) and (α–TOH) together were higher than the values found by Blazer and Wolk (1984) who reported these values reflects the higher level of immunoglobulin. This data may explain the possible reason, that the (AA) and (α–TOH) would quickly reflect changes in the catabolic anabolic relationship. Helmy *et al.* (1974) reported that, the increases in serum protein would result

Table (8):Total serum protein, Albumin, Globulin and Albumin / globulin ratio of monsex Nile tilapia *O. niloticus* fed diets supplemented with (AA) and / or (α- TOH)

ITEMS	Treatments				SE±
	Supplemented of (AA)+ (α-TOH)	Supplemented Of (AA)	Supplemented Of (α-TOH)	Without Supplemented Of (AA) or (α-TOH)	
Total serum protein (g./dl).	3.87^{a}	3.18^{b}	2.80^{bc}	2.11^{d}	0.37
Total serum albumin (g.dl).	2.82^{a}	2.00^{b}	1.49^{c}	1.01^{d}	0.39
Total serum globulin (g.dl).	1.33^{a}	1.23^{b}	1.19^{c}	1.03^{c}	0.06
Albumin / Globulin ratio.	2.11^{a}	1.63^{b}	1.23^{c}	0.97^{d}	0.25

SE ±, standard error. Calculated from residual mean square in the analysis of variance.
a, b, ... etc. means in same raw with different superscripts are different ($P < 0.05$).

when anabolic processes exceeded catabolic ones, and reserve proteins are being produced in greater quantity to meat increased metabolic requirements of the fish . They added that , an increase catabolic rate would explain the decreases in serum protein level. Thus, the fish fed (AA) or (α– TOH) deficient diet can cuse reduced growth rate, myopathy, anemia and possibly other pathologies as found by (Merchie, *et al.* 1996).

CONCLUSION :

It was apparent that the absence of vitamin C or E from the practical - type diet used in this study was responsible for the poor growth and spinal deformities in the fish. The present study suggested that 400 mg/kg (AA) and 2000 mg /kg (α–TOH) together were adequate for high growth rate of monsex O. niloticus, while many authers recommended 100 mg. /kg. (AA) and 25 200 mg. /kg. (α– TOH) for normal growth and tissue development, but not in quantities sufficient to provide rapid growth.

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