ISSN 2278-8808
SJIF 2015: 5.403
An International Peer Reviewed & Referred
SCHOLARLY RESEARCH JOURNAL
FOR INTERDISCIPLINARY STUDIES

EFFICACY OF VITAMIN C FOR MITIGATING PRE TRANSPORTATION STRESS OF CATLA CATLA (HAMILTON, 1822) FINGERLING IN CONTEXT TO CORTISOL

Manoj M Ghughuskar[1], Neelam Saharan[2], P. P. Shrivastava[2], A. K. Reddy[2] and M. M. Shirdhankar[3]

[1]Freshwater Fish Research and Culture Project, College of Horticulture, Mulde, Tal.-Kudal, Dist.- Sindhudurg, Maharashtra-416520

[2] ICAR-Central Institute of Fisheries Education, Versova Mumbai-400061

[3]College of Fisheries, Shirgaon, Ratnagiri, Maharashtra-415612

Abstract

Catla catla fingerlings are given pre transportation stress i.e. handling, confinement and air exposure stress. These fingerlings were divided into two group one group (i.e. handling) and another group (i.e. without handling). Fishes of handling and without handling group was fed with and without vitamin –C for 4weeks duration and weekly sampling was done on 7th, 14th, 21st and 28th day .From each test group fishes are removed weekly and anesthetized for blood collection. Blood serum has been used for determining cortisol, .Here there is no significant interaction between condition (i.e. handling and without handling) and vitamin C concentration (i.e.T_0(0mg/kg), T_1(300mg/kg), T_2(600mg/kg), T_3(900mg/Kg), T_4(1200mg/Kg) and T_5(1500mg/Kg)) on cortisol, on 7th day, sampling but there is significant interaction($p<0.05$) between condition and vitamin C concentration on haemoglobin, Serum Protein ,albumin on 7th day sampling . But from 14th day up to 28th day sampling there is significant interaction ($p<0.05$) between condition and vitamin C concentration on Plasma cortisol, The level of cortisol, significantly differed ($p<0.05$)between T_0 and other treatments (i.e. T1,T2,T3,T4,T5) but not significantly different between the treatment T4(1200mg/Kg) and T5(1500mg/Kg). So economically 1200mg/kg vitamin C supplemented in feed for 14days or more can effectively ameliorate the pre transportation stress of catla fingerlings.

Keywords:,*Catla catla; fingerlings; pre transportation stress; Vitamin C; Cortisol*

Introduction

Carp dominate aquaculture production in freshwater pond, cages, pens and reticulating system and production in Inland fisheries. Indian major carps constitute 85% of the total fresh

water aquaculture production. Catla is the second most demanded after rohu as it is used as surface feeder component in polyculture. It is most preferred among the surface feeder, fast growing compared to other Indian major carps. Its low breeding response, low survival in nursery phase as compare to other Indian major carps (Rohu and Mrigal). Sometime leads to shortage in seed supply. (Dey etal,2005) In nursery stage rough handling ,crowding ,temperature fluctuation ,inadequate Dissolved oxygen are unfavorable environmental conditions which leads to the stress and lower the resistance of fishes (Wedemeyer,1999). Stress caused by environmental condition hampers normal growth and immune response of fish and makes susceptible to diseases. Ascorbic acid (vitamin C) has been known to improve the immune response. It is also an important molecule for normal growth and metabolic function of fish. Ascorbic acid is essential for producing collagen and bone minerals , assisting in metabolizing iron and helps in activation of Vitamin D. It also assist in reducing the harmful effect of hormones produced by the adrenal gland during prolonged period of stress(Lovell,1989;Navarve and Halver,1989). It provides protection of the living cells from oxidative damage as ascorbic acid neutralizing the reactive oxygen species. (Verlhac and Gabaudan, Undated)

In most animals ascorbic acid is synthesized from glucuronic acid, fish and crustaceans lack enzyme gluconalactone oxidise necessary for the last step in biosynthesis (Chatterjee,1973; Dabrowski,1990). Due to this fishes are dependent on constant supply of vitamin C in the form of ascorbic acid through feed. Lower dietary vitamin C shows deficiency syndrome of broken back syndrome in fish and black death disease in shrimp.

Dabrowiski (1991a) suggested that metabolism rate is the primary factor regulating the AA requirement i.e larval fish displaying a relative faster growth and metabolism than juvenile and adult might need higher dietary ascorbic acid level to sustain optimal growth and physiological condition (Dabrowski*etal*., 1988; Dabrowiski,1990).Merchie *etal.,* 1995b revealed that European sea bass which when subjected to salinity stress shows positive effect on stress resistance when their diet is supplemented with high ascorbic acid.Effect of high dietary level of Ascorbic acid supplementation on stress and disease resistance important under suboptimal rearing condition like handling, transportation, crowding, poor water quality or disease outbreak. As it is in confirmation to the findings of Dabrowski,1992 that stress created increased ascorbate requirement.

Jaffa (1989) revealed that high dose of vitamin C supplementation has been suggestedto mitigate the effects of physiological stress . As per Kitabchi,1967 ascorbate in high

concentration inhibits steroid synthesis and therefore might reduce the severity of cortisol mediated stress response.

Therefore present study was carried out to investigate the effect of varying level of ascorbic acid (AA) on mitigation of pre transportation stress like confinement, handling and air exposure stress.

Materials and methods

Fingerlings of *Catla catla* (31.36±1.26g) were procured from Hans Aquarium, Roha,Dist.Raigad,Maharashtra,India and transported in polyethylene bags inflated with medical grade oxygento the wet lab of Aquaculture Division,Central Institute of Fisheries Education,Mumbai and were acclimatized to the experimental rearing condition for 30days.After acclimatizationfish were transferred to 36nos uniform size experimental tanks of 150 L and reared for 4weeks. Twenty fishes of uniform size per container were stocked in twelve distinct groups with three replicates for each treatment in plastic container (80x 57x42cm) of 150 L capacity each, following a completely randomized design.The fish were fed with experimental diet twice daily 2 to 3% of body weight of fishes stocked**.** The water is siphoned out daily 25 % of the total volume and volume makeup was done by fresh filtered conditioned water. Aeration was provided throughout the experimental period uninterruptedly through oil less aircompressor.

Hence, there were total twelve experimental groups *viz.*, without handling control T_{0WH}(basal feed +0mg/Kg Vitamin C);T_{1WH} (basal feed +300mg/Kg Vitamin C); T_{2WH} (basal feed +600mg/Kg Vitamin C); T_{3WH} (basal feed +900mg/Kg Vitamin C); T_{4WH} (basal feed +1200mg/Kg Vitamin C) and T_{5WH} (basal feed +1500mg/Kg Vitamin C). Handling control T_{0H}(basal feed +0mg/Kg Vitamin C);T_{1H} (basal feed +300mg/Kg Vitamin C); T_{2H} (basal feed +600mg/Kg Vitamin C); T_{3H} (basal feed +900mg/Kg Vitamin C); T_{4H} (basal feed +1200mg/Kg Vitamin C) and T_{5H} (basal feed +1500mg/Kg Vitamin C) were arranged in triplicates following a CRD design. The total volume of the water in each tub was maintained at 150 L throughout the experimental period. Round the clock aeration was provided. The aeration tube in each tub was provided with an air stone and a regulator to control the air pressure uniformly in all the tubs. Feed was given @ 3% of body weight for 30 days twice daily at 10:00 and 18:00 h under a normal light regime (light/dark 12/12 h). The fishes in handling stress was weekly chased with a hand net in order to capture all fish inside each tank and held captive in net for 3 minutes. After that, the net along with fishes was held in air for 3 minutes and put back to the respective tank. Fish in without handling groups were reared

without any disturbance except of daily siphoning and water exchange. Four sampling periods were established: 7th day, 14th, 21st and 28th day. Experimental sampling procedures were identified during four sampling period. Then fishes from each tank were sampled for determining the stress in both handling and without handling groups. For this, blood was drawn from fishes for determining the stress indicator parameters

Experimental diets

Experimental feed of crude protein 35% and lipid 8% were prepared by using Vitamin C (L-ascorbyl-2Triphospahate). Vitamin C was added in different concentration to the feed. *i.e.* T_0 - No supplementation of vitamin C in experimental diet, T_1-300mg/Kg Vitamin C in experimental diet, T_2- 600mg/kg VitaminC.in experimental diet T_3-900mg/kg VitaminC.in experimental diet,T_4-1200mg/Kg Vitamin C in experimental diet,T_5-1500mg/Kg Vitamin C in experimental diet.

Table: 1 Composition of experimental diet supplemented with varying amount of Vitamin C (g%) and proximate composition of feed.

Sl. No.	**Ingredient**	**Treatment**					
		Control	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
1.	Soybean Flour[a]	20.93	20.93	20.93	20.93	20.93	20.93
2.	Fish meal[a]	26.88	26.88	26.88	26.88	26.88	26.88
3.	Groundnut oil cake[a]	18.00	18.00	18.00	18.00	18.00	18.00
4	Wheat flour[a]	18.99	18.92	18.86	18.79	18.72	18.66
5.	Rice powder	5.00	4.97	4.95	4.93	4.91	4.89
6.	Corn flour[a]	3.10	3.10	3.10	3.10	3.10	3.10
7.	Codliver&sunflower oil[a]	5.0	5.0	5.0	5.0	5.0	5.0
8.	BHT[b]	0.6	0.6	0.6	0.6	0.6	0.6
9	Carboxymethylcellulose[b]	0.5	0.5	0.5	0.5	0.5	0.5
10.	Vitamin Premix	1.00	1.00	1.00	1.00	1.00	1.00
11.	Vitamin C[b] (L-ascorbate 2-triphosphate)	Nil	0.087	0.174	0.261	0.348	0.436
	Proximate Composition (% dry weight basis)						
12.	Crude protein	35.30 ±0.180	35.08 ±0.177	35.01 ±0.272	34.90 ±0.235	34.76 ±0.485	34.74 ±0.060
13.	Ether extract	7.64 ±0.029	7.35 ±0.086	7.55 ±0.057	7.62 ±0.040	7.12 ±0.070	6.80 ±0.623
14.	Ash	3.56 ±0.014	3.46 ±0.017	3.50 ±0.023	3.18 ±0.020	3.27 ±0.017	3.30 ±0.023
15.	Total carbohydrate	48.31 ±0.191	48.62 ±0.258	48.67 ±0.256	48.77 ±0210	49.30 ±0.514	49.55 ±0.613
16.	Digestible energy*	403.29 ±0.582	400.98 ±0.475	402.70 ±.0.303	403.27 ±0.178	400.33 ±0.335	398.45 ±3.16
17.	Moisture	7.12 ±0.015	7.13 ±0.026	6.61 ±0.0120	7.17 ±0.006	7.53 ±0.029	6.05 ±0.036

Digestible energy (Kcal100 per g)= (%CP.4)+(%EE.9)+(TC.4),DM%=100-moisture%

[a] Procured from local market

[b] HIMEDIA Laboratories, India.

Proximate analysis of feed

The proximate composition of the experimental diets was determined as per methods of AOAC (1995) and presented in Table 1. Sample were analyzed for crude protein (CP), ether extract(EE), ash and total carbohydrate(TC).

Serum Collection

For collection of serum, the blood was drawn from caudal vasculature of anesthetized fingerlings using 1 ml uncoated syringe. Collected blood was immediately transferred to dried eppendorff tube. These tubes were allowed to stand in tilted position at room temperature for clotting. After some time, due to clotting of blood, the yellow straw colour serum was carefully separated out and collected and transferred to another tube which was kept at -20 ^{o}C with proper labelling for further analysis, *i.e.,* Cortisol

Estimation of Cortisol

Cortisol level in serum was determined with a was quantified using Caymans Cortisol Enzyme Immunoassay kit (Cortisol EIA Kit Item No. 500360) .It is a competitive assay that has been used for estimating or quantifying of cortisol in serum.

Statistical Analysis

All data obtained was subjected to Two way ANOVA procedure of IBM SPSS(Ver.16.0) and significant means were separated by Tukey's HSD option of same software.

Result:-

Cortisol level differed significantly ($p<0.05$) between both condition (Handling and without handling) in case of 7th, 14th,21st and 28th day sampling. Cortisol level also differed significantly ($p<0.05$at various dietary vitamin C levels in case of 7th,14th,21st and 28th day sampling.

Two way ANOVA revealed that there is a no significant ($p>0.05$) interaction between various dietary vitamin C levels (T_0,T_1,T_2,T_3,T_4 and T_5) and conditions (handling and without handling) on cortisol levelin case of 7th day sampling period and significant interaction shows from 14th ,21st and 28th day sampling period as per Table 1 respectively.

On 7th day inhandling condition the maximum value of cortisol level in T_0 (225.66ng/ml) and the minimum value was found to be T_5 (187.29ng/ml). Tukey HSD test revealed that in handling condition serum cortisol level is not significantly different ($p>0.05$) between the treatments.T_0,T_1,T_2,T_3;T_1,T_2,T_3,$T_4$and significantly different ($p<0.05$) between T_0 and T_4,T_5.

On 7th day inwithout handling condition the maximum value of cortisol level in T_0 (161.03 ng/ml) and the minimum value was found to be T_4 (144.34ng/ml).Tukey HSD test revealed that in without handling condition serum cortisol level is no significantly different ($p>0.05$) between the treatments.T_0,T_1,T_2,T_3,T_4, T_5.

On 14th day inhandling condition the maximum value of cortisollevel in T_0 (224.08ng/ml) and the minimum value was found to be T_5 (75.38ng/ml). Tukey HSD test revealed that in handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_1,T_2,T_3 T_4,T_5 and no significant different ($p>0.05$) between the treatmentsT_4,T_5.

On 14th day inwithout handling condition the maximum value of cortisollevel in T_0 (159.3 ng/ml) and the minimum value was found to be T_5 (72.42ng/ml).Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_1,T_2,T_3 T_4,T_5butnot significantly different ($p>0.05$) between the treatmentsT_3,T_4, T_5.

On 21st day inhandling condition the maximum value of cortisollevel in T_0 (225.71ng/ml) and the minimum value was found to be T_5 (77.38ng/ml). Tukey HSD test revealed that in handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_1,T_2,T_3 T_4,T_5 and no significant different ($p>0.05$) between the treatmentsT_4,T_5.

On 21st day inwithout handling condition the maximum value of cortisol level in T_0 (159.99 ng/ml) and the minimum value was found to be T_5 (72.43ng/ml).Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_2,T_3 T_4,T_5butnot significantly different ($p>0.05$) between the treatmentsT_0, T_1; T_3,T_4, T_5

On 28th day in handling condition the maximum value of cortisol level in T_0 (226.20ng/ml) and the minimum value was found to be T_5 (76.85ng/ml). Tukey HSD test revealed that in handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_1,T_2,T_3 T_4,T_5 and no significant different ($p>0.05$) between the treatmentsT_4,T_5.

On 28th day in without handling condition the maximum value of cortisol level in T_0 (159.25ng/ml) and the minimum value was found to be T_5 (71.16ng/ml).Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between T_0 and T_2,T_3 T_4,T_5butnot significantly different ($p>0.05$) between the treatmentsT_0, T_1; T_1,$T_2$and T_3,T_4, T_5.

Table 1: Cortisol values of *Catlacatla* fingerlings when subjected to stress after 7th, 14th ,21st and 28th days.

Treatment		Cortisol (ng/ml)			
		7th day	14th day	21st day	28th day
Condition					
H		209.28[a]	143.76[a]	145.43[a]	144.72[a]
WH		150.86[b]	108.53[b]	111.56[b]	111.16[b]
SEM		1.66	**0.990**	**1.359**	**1.40**
P-value		**S(0.00)**	**S(0.00)**	**S(0.00)**	**S(0.00)**
Vitamin C concentration					
T_0		193.34[d]	191.69[e]	192.85[a]	192.73[a]
T_1		188.32[cd]	169.99[d]	172.42[b]	172.18[b]
T_2		183.91[cd]	143.99[c]	154.67[c]	154.37[c]
T_3		177.28[abc]	101.08[b]	100.62[d]	99.98[d]
T_4		171.75[ab]	76.24[a]	75.94[e]	74.40[e]
T_5		165.82[a]	**73.90[a]**	**74.48[e]**	**74.00[e]**
SEM		**2.87**	**1.71**	**2.35**	**2.43**
P-value		**S(0.00)**	**S(0.00)**	**S(0.00)**	**S(0.00)**
Vitamin C concentration * Condition					
H	**T_0**	225.66[d]	224.08[g]	225.71[g]	226.20[a]
	T_1	220.69[cd]	194.15[f]	195.76[f]	194.95[b]
	T_2	213.19[cd]	173.18[e]	174.73[e]	173.93[cd]
	T_3	208.45[cd]	118.49[b]	120.16[b]	119.11[f]
	T_4	200.39[bc]	77.31[a]	79.15[a]	77.32[g]
	T_5	187.29[b]	75.38a	77.07[a]	76.85[g]
WH	**T_0**	161.03[a]	159.3d	159.99[de]	159.25[cd]
	T_1	155.96[a]	145.82c	149.08[cd]	149.42[de]
	T_2	154.63[a]	114.81[b]	114.81[b]	134.81[ef]
	T_3	146.10[a]	83.67[a]	134.617[a]	80.86[g]
	T_4	143.11[a]	75.18[a]	81.08[a]	71.47[g]
	T_5	144.34[a]	72.42[a]	72.43[a]	71.16[g]
SEM		**4.072**	**2.42**	**3.329**	**3.44**
P-value		**NS (0.112)**	**S(0.00)**	**S(0.00)**	**S(0.00)**

***Treatment means represent the average values of three plastic tubs per treatment. Tukey HSD range test was conducted for treatment means only if there was a significant interaction (ANOVA, p < 0.05). Means value in same column with different superscript differ significantly (p<0.05). S-Significant, NS-Non Significant, H-Handling, WH-Without Handling**

Discussion

(Ortuno *et al.,* 2003 and Chen *et al.,* 2004 revealed that Vitamin C has positive role in amelioration of stress). Fishes are subjected to procedures such as netting, handling, transportation, grading, overfeeding and over stocking all of which are considered stressful (Sandnes, 1991). These conditions are often associated with decreased resistance to pathogens, reduced capacity to maintain homeostasis and inability to withstand additional stressors.(Robertson *et al.*,1987). To face new stressful conditions, many morphological, biochemical and physiological changes take place (Halim*et al.,* 1987), which enables the organism to adapt to adverse conditions. Ascorbic acid demand has been shown to increase in fish suffering from diverse stress conditions (Hardie*et al.,* 1991, Henrique *et al.,* 1998).

Elevations of plasma cortisol level are often used as indicators of stress (Barton and Iwama, 1991a). Several studies have demonstrated the effects of stressors on concentrations of the corticosteroid cortisol in fish. Plasma concentration of cortisol is dependent on the duration and strength of the stressor (Barton and Iwama, 1991a) for which this parameter has been used as an indicator of stress. In the present study, the highest plasma cortisol was found in handling and without handling group control feed (without dietary vitamin C), indicated secretion of cortisol due to the stress caused by high density group.

On 7^{th},14^{th} ,21^{st} and 28^{th} day in handling and without handling conditions, the maximum value of cortisol T_0(control *i.e.,* without supplement of vitamin C) and the minimum value was found to be T_5 (1500mg/kg).But there was no significant difference between T_4(1200mg/Kg) and T_5 (1500mg/kg)as on 14^{th},21^{st} and 28^{th} day sampling. These results are in conformation to the results obtained by Mustafa *et al.,* 2013 in fish Nile Tilapia (*Oreochromis niloticus*) where the stressed conditions showed significantly higher level of plasma cortisol, blood glucose indicating stress, both of which were reduced when fed with vitamin C supplemented diet.. Basrur *et al.*2010 observed rise in both plasma cortisol and glucose level during a repeated crowding stress investigation on Atlantic salmon (*Salmo salar*). Glucose metabolism is affected by cortisol concentration (Barton and Iwama, 1991;

Wedemeyer *et al*., 1990), and so a linear relationship is expected between these two chief stress parameters.

Therefore, the primary stress response by production of hormones was responded by concurrent rise of blood glucose, a secondary stress response to adapt with the stressed situations. From the results of plasma cortisol concentration it can be proposed that vitamin C helps to reduce the stress response induced by handling. Vitamin C supplementation @ 1000mg/kg as suggested by Verlhac and Gabaudan, 1993 reduced the cortisol concentration in the stressed group even lower than the unstressed group lacking the minimum required amount of dietary vitamin C.

Conclusion

It was also concluded that the dose 1200mg/Kg and 1500mg/Kg effectively mitigated the pre transportation stress as per the cortisol level is concerned. But the dose 1200mg/Kg and 1500mg/Kg were not having significant difference in cortisol level to mitigate stress, so 1200mg/kg can be used for mitigating pre transportation stress of *Catla catla* fingerling. The immunostimulants like Vitamin C, which showed promising results in mitigating the pre-transportation stress like handling, crowding and confinement. Cortisol is one of the parameter which indicate the stress level and helps in measuring the primary stress level.

Acknowledgement

Our thanks to, Director, Central Institute of Fisheries Education, Mumbai India for providing the facilities required for the present work .We acknowledge the support from Head Aquaculture Division of Central Institute of Fisheries Education ,Mumbai and Indian Council of Agriculture Education, New Delhi for their financial support.

References

Barton B. A. and Iwama G. K. (1991).Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids.Annual Review of Fish Diseases,1:3-26.

Barton, B. A. and C. B. Schreck. 1987. Influences of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (Oncorhynchustshawytscha). Aquaculture 62:299–310.

Basrur T. V., Longland R. and Wilkinson R. J. (2010). Effects of repeated crowding on the stress response and growth performance in Atlantic salmon (Salmosalar).Fish Physiology Biochemistry, 36:445-50.

Chatterjee, N., Pal, A. K., Das, T., Mohammed, M. S., Sarma, K., Venkateshwarlu, G., and Mukherjee, S. C. (2006). Secondary stress responses in Indian major carps Labeorohita (Hamilton), Catlacatla (Hamilton) and Cirrhinusmrigala (Hamilton) fry to increasing packing densities. Aquaculture Research,37(5), 472-476.

Chen R.Lochmann,R., Goodwin,A.,Parveen ,K.,Dobrowski ,K.andLee,K.J.(2004). Effect of dietary vitamin C and E on alternative complement activity, haematology tissue composition, vitamin concentration and response to heat stress in juvenile golden shiner (Notemigonuscrysoleucas) Aquaculture 242,553-569.

Dabrowski, K., 1990. Ascorbic acid status in the early life of whitefish (Coregonuslavaretus L.).Aquaculture 84, 61-70.

Dabrowski, K., 1991a. Administration of gulonolactone does not evoke ascorbic acid synthesis in teleost fish. Fish Physiol. Biochem. 9, 215-221.

Dabrowski, K., 1992. Ascorbate concentration in fish ontogeny.J. Fish'Biol. 40, 273-279.

Dabrowski, K., Hinterleitner, S., Sturmbauer, C., El-Fiky, N., W ieser, W., 1988. Do carp larvae require vitamin C. Aquaculture 72, 295-306.

Dey, M. M., Paraguas, F. J., Bhatta, R., Alam, F., Weimin, M., Piumsombun, S., ...& Sang, N. V. (2005). Carp Production in Asia: Past Trends and Present Status1. Carp Genetic Resources for Aquaculture in Asia, 6.

Halim, Y., Faisal, M., Ahmed, I., 1987. Fish diseases, an index of water pollution: a review. FAO/UNEP Meeting on the Effects of Pollution on Marine Ecosystems, Blanes, Spain, 7–11 October 1985. FAO Fish. Rep. Suppl., 352, pp. 97–104.

Hardie, L. J., Fletcher, T. C. and Secombes, C. J. (1991). The effect of dietary vitamin C on the immune response of the Atlantic salmon (Salmo salar L.).Aquaculture 95,201–214.

Henrique, M. M. F., Gomes, E. F., Gouillou-Coustans, M. F., Oliva-Teles, A., and Davies, S. J. (1998). Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of seabream, Sparusaurata Aquaculture, 161(1), 415-426.

Jaffa, M. 1989.Vitamin C can curb those stress associated losses.Fish Farming International,12:18–19,

Kitabchi. A. E1967.Ascorbic acid in steroidogenesis.Nature,215:1385–1386,

Lehninger, A. L. 1974. Role of phosphate and other proton-donating anions in respiration-coupled transport of Ca^{2+} by mitochondria. Proceedings of the National Academy of Sciences, 71(4), 1520-1524.

Lovell, R.T. 1989. Vitamin C (ascorbic acid). pp. 54-60. In: Nutrition and Feeding of Fish. An AVI Book, Van Nostrand Reinhold Publication.

Merchie,G.,Lavens,P.,Dhert,P.Pector,R.,MaiSoni,A,F.,Nelis,H.,Oliver,F,De Leenheer and Sorgeloos,P.1995b. Live food medicated vitamin C transfer to Decentrarchuslabraxand Clariasgariepinus. Journal of Applied Ichthyology,11:336-341.

Mustafa, A., Hayat, A. S. and Quarrar, P., 2013. Stress modulated responses in Nile Tilapia Oreochromis niloticus treated with Non ascorbic acid supplemented feed. Advances in Zoology and Botany, 1(2): 39-45.

Navarre, O. and Halver, J.E. 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture, 79: 207-221.

Ortuno,,J.,Esteban,M.A. and Meseguer,J.(2003) The effect of dietary intake of Vitamin C and E on the stress response of gilthead seabream (Sparusauratus L.) Fish and Shell Fish Immunology: 14,145-156

*SandnesK .1991, **Vitamin C in fish nutrition—a review** Fisk. Dir. Skr., Ser. Ernaering, 5 pp. 3–32*

Shalaby, A. M., and Abbassa, A. H. (2004).The opposing effect of ascorbic acid (vitamin C) on ochratoxin toxicity in Nile tilapia (Oreochromisniloticus).InProceedings of the 6th International Symposium on Tilapia in Aquaculture (RB Remedios, GC Mair and K. Fitzsimmons, eds) (pp. 209-221).

Verlhac V., Doye A. N', Gabaudan J., Troutaud D. and Deschaux P.(1993). Vitamin nutrition and fish immunity: influence of antioxidant vitamins (C and E) on immune response of rainbow trout. Fish Nutrition in Practice Les Colloques (INRA eds,) 61: 167-177.

Wedemeyer G. A., Barton B., Mcleay,D., Schreck, C. and Moyle P.B. (1990). Stress and acclimation.Pages 451-477 in C.B. Schreck and P. B. Moyle, editors.Methods for fish biology.American Fisheries Society, Bethesda, Maryland,

Wedemeyer G.A. and McLeay D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. In: Pickering A (ed) Stress and fish. Academic Press, New York, pp 247–268

Wedemeyer, W. J., and Scheraga, H. A. (1999). Exact analytical loop closure in proteins using polynomial equations. Journal of Computational Chemistry,20(8), 819-844.