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# EFFICACY OF VITAMIN C FOR MITIGATING PRE TRANSPORTATION STRESS OF CATLA CATLA FINGERLING IN CONTEXT TO SERUM GLUCOSE

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## Abstract

*Here pre transportation stress like handling, confinement and air exposure stress was given to the Catla catla fingerlings. These fingerlings are divided into two group one group (i.e. handling and 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 $7^{th}$,$14^{th}$ ,$21^{st}$ and $28^{th}$ day.From each test group fishes are removed weekly and anesthetized for blood collection. Blood serum has been used for determining glucose level .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 ,glucose on $7^{th}$ day, sampling .But from 14thday upto $28^{th}$ day sampling there is significant interaction ($p<0.05$) between condition and vitamin C concentration on glucose. The level of glucose, 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; Glucose*



## Introduction

High demand for aquaculture products due to daunting challenge to meet food security of expected global population of 9 billion by 2050.Aquaculture has grown impressive as compare to other agriculture and allied sector to keep the overall price of fish down and made fish and seafood more accessible to consumers all around the world (World Bank,2013).Carp dominate aquaculture production in freshwater pond,cages,pens and reticulating system and

production in Inland fisheries. Catla,rohu and mrigal constitute 85% of the total fresh water aquaculture production. Catla is thesecond 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). Some time 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 etal.,1999)

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 (Dabrowiski *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 in context to blood glucose level.

**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: $7^{th}$ day, 14th, $21^{st}$ and $28^{th}$ 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.*, Glucose.

**Estimation of Serum Glucose**

Glucose level in serum was determined with a commercial kit( Merkotest Glucose) based on GOD/POD. Glucose oxidase (GOD) converts the glucose into gluconate. The Hydrogenperoxide ($H_2O_2$) produced in the reaction is degraded by peroxidise (POD) and gives a coloured product phenol and 4-aminoantipyrine which is measurable using Trinder indicator reaction at 505nm. The increase in absorbance correlates with glucose concentration of sample.

$$\text{Glucose} + 0_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4H_20$$

10µl of serum /plasma of each treatment was taken in a labelled round bottom eppendoff tube of 1.5 ml capacity and then added 1000 µl reagent supplied with the help of pipette and labelled two eppendoff tubes as blank and standard. In blank tube, 10µl of distilled water was taken and 1000µl of Kit reagent (Phosphate Buffer, Glucose oxide, Peroxidase and 4 Amino Antipyrine with preservative and stabilizer) was added. In standard tube,10 µl of Standard (Glucose standard-100 mg/dl conc.) was added and then 1000 µl of Kit reagent was added. All the tubes with the reaction mixture was incubated for 10 minutes at 37$^{o}$C and reading was taken at 510nm wavelength on spectrophotometer.

**Conc. of unknown sample** =

Concentration of Standard x Abs. of unknown sample - Abs.of reagent blan
Abs. Standard-Abs. of Reagent Blank

**Physico-chemical parameters of Water**

Water quality parameters, *viz,*.temperature, pH ( pH meter having the temperature probe), dissolved oxygen by azide modification method (APHA-AWWA-WEF.,1998), free carbon dioxide titrimetric method (APHA-AWWA-WEF,1998), ammonia by spectrophotometerically at 640nm wavelength by phenate method (APHA-AWWA-WEF,1998), nitrite was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF,1998) and nitrate was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF.,1998) were recorded in this experiment.

**Statistical Analysis**

**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:-**

Glucose level differed significantly ($p<0.05$) between both condition (Handling and without handling) in case of $7^{th}$ ,$14^{th}$,$21^{st}$ and $28^{th}$ day sampling. Glucose level also differed significantly ($p<0.05$at various dietary vitamin C levels in case of $7^{th}$,$14^{th}$,$21^{st}$ and $28^{th}$ 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 glucose level.in case of $7^{th}$ day sampling period and significant interaction shows from $14^{th}$ ,$21^{st}$ and $28^{th}$ day sampling period as per **Table1.**

On $7^{th}$ day inhandling condition the maximum value of glucose level in $T_0$ (187.18 mg/dL) and the minimum value was found to be $T_5$ (152.76 mg/dL). Tukey HSD test revealed that in handling condition serum cortisol level is no significantly different ($p>0.05$) between the treatments.$T_0$,$T_1$,$T_2$,$T_3$;$T_2$,$T_3$,$T_4$; $T_3$,$T_4T_5$ and significantly different ($p<0.05$) between $T_0$ and $T_4$,$T_5$.

n $7^{th}$ day inwithout handling condition the maximum value of cortisol $T_0$ (134.29mg/dl) and the minimum value was found to be $T_5$ (116.36mg/dl).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 glucose level in $T_0$ (188.47mg/dl) and the minimum value was found to be $T_5$ (44.10mg/dl). 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 treatments$T_4$,$T_5$.

On 14th day inwithout handling condition the maximum value of glucose level in $T_0$ (135mg/dl) and the minimum value was found to be $T_5$ (42.69mg/dl).Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between $T_0$ and,$T_2$,$T_3$ $T_4$,$T_5$butnot significantly different ($p>0.05$) between the treatments$T_3$,$T_4$, $T_5$.

On 21st day inhandling condition the maximum value of glucose level in $T_0$ (189.65 mg/dL)and the minimum value was found to be $T_5$ (45.12 mg/dL). 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 treatments$T_4$,$T_5$.

On 21st day inwithout handling condition the maximum value of glucose level in $T_0$ (137.43 mg/dL) and the minimum value was found to be $T_5$ (39.80 mg/dL). Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between $T_0$ and$T_1$,$T_2$,$T_3$ $T_4$,$T_5$butnot significantly different ($p>0.05$) between the treatments$T_3$,$T_4$, $T_5$.

On 28th day inhandling condition the maximum value of glucose level in $T_0$ (187.95mg/dL) and the minimum value was found to be $T_5$ (46.52 mg/dL) 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 treatments$T_4$,$T_5$.

On 28th day inwithout handling condition the maximum value of glucose level in $T_0$ (137.78 mg/dL)and the minimum value was found to be $T_5$ (43.51mg/dL). Tukey HSD test revealed that in without handling condition serum cortisol level is significantly different ($p<0.05$)between $T_0$ and $T_2$,$T_3$ $T_4$,$T_5$butnot significantly different ($p>0.05$) between the treatments$T_3$,$T_4$, $T_5$.

**Table 1: Serum Glucose values of *Catlacatla* fingerlings when subjected to stress after $7^{th}$,$14^{th}$,$21^{st}$ and $28^{th}$ day**

| **eatment** | | **Glucose (mg/dl)** | | | |
|---|---|---|---|---|---|
| **Condition** | | 7 days | 14 days | 21 days | 28days |
| **H** | | $170.89^{a}$ | $102.17^{a}$ | $103.55^{a}$ | $104.04^{a}$ |
| **WH** | | $125.35^{b}$ | $82.20^{b}$ | $76.25^{b}$ | $78.63^{b}$ |
| **SEM** | | 1.644 | **1.252** | **1.192** | **1.27** |
| **P-value** | | **S(0.00)** | **S(0.00)** | **S(0.00)** | **S(0.00)** |
| **Vitamin C concentration** | | | | | |
| **$T_0$** | | $160.73^{d}$ | $161.87^{e}$ | $163.54^{e}$ | $162.86^{e}$ |
| **$T_1$** | | $156.63^{cd}$ | $130.25^{d}$ | $120.49^{d}$ | $121.57^{b}$ |
| **$T_2$** | | $151.03^{bcd}$ | $104.04^{c}$ | $98.99^{c}$ | $99.89^{c}$ |
| **$T_3$** | | $146.04^{abc}$ | $68.94^{b}$ | $70.71^{b}$ | $73.43^{d}$ |
| **$T_4$** | | $139.72^{ab}$ | $44.61^{a}$ | $43.21^{a}$ | $45.25^{e}$ |
| **$T_5$** | | $134.56^{a}$ | **$43.40^{a}$** | **$42.46^{a}$** | **45.01e** |
| **SEM** | | **2.84** | **2.16** | **2.064** | **2.21** |
| **P-value** | | **S(0.00)** | **S(0.00)** | **S(0.00)** | **S(0.00)** |
| **Vitamin C concentration * Condition** | | | | | |
| **H** | **$T_0$** | $188.47^{e}$ | $189.65^{e}$ | $103.55^{a}$ | $187.95^{e}$ |
| | **$T_1$** | $135.47^{d}$ | $137.67^{d}$ | $76.25^{b}$ | $138.43^{d}$ |
| | **$T_2$** | $112.69^{c}$ | $115.35^{c}$ | **1.192** | $116.39^{c}$ |
| | **$T_3$** | $86.16^{b}$ | $87.02^{b}$ | **S(0.00)** | $88.08^{d}$ |
| | **$T_4$** | $46.13^{a}$ | $46.49^{a}$ | | $46.91^{e}$ |
| | **$T_5$** | $44.10^{a}$ | $45.12^{a}$ | $163.54^{e}$ | $46.52^{e}$ |
| **WH** | **$T_0$** | $135.27^{d}$ | $137.43^{d}$ | $120.49^{d}$ | $137.78^{b}$ |
| | **$T_1$** | $125.04^{cd}$ | $103.32^{c}$ | $98.99^{c}$ | $104.70^{c}$ |
| | **$T_2$** | $95.4^{b}$ | $82.64^{b}$ | $70.71^{b}$ | $83.38^{d}$ |
| | **$T_3$** | $51.73^{a}$ | $54.39^{a}$ | $43.21^{a}$ | $58.79^{e}$ |
| | **$T_4$** | $43.10^{a}$ | $39.93^{a}$ | **$42.46^{a}$** | $43.60^{e}$ |
| | **$T_5$** | $42.69^{a}$ | $39.80^{a}$ | **2.064** | $43..51^{e}$ |
| **SEM** | | **4.027** | **3.06** | **2.91** | **3-12** |
| **P-value** | | **NS (0.220)** | **S(0.002)** | **S(0.00)** | S(0.00) |

**(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**

Vitamin C has been suggested to have positive role in amelioration of stress (Ortuno *et al.*, 2003; Chen *et al.*,2004). Under culture conditions, fish 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 enable 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 and glucose levels are often used as indicators of stress (Barton and Iwama, 1991a). The measurement of blood glucose level is still considered as an effective method to evaluate the stress effect of variety of stressors due to its simple methodology. The elevation of blood sugar levels in fish by both glucocorticoid, corticosteroids and catecholamines makes it the ideal parameter to study the secondary stress response, on activation of direct sympathetic (chromaffin tissue) as well as humoral (internal tissue) pathways (Wedemeyer and Mcleay, 1981). Ample literature exists on the rise of glucose level on application of various stressors like handling (Wedemeyer, 1972; Carey and McCormick, 1998), transportation (Barton and Schreck, 1987), claw ablation of prawn (Manush*et al.*, 2005) and packing density (Chatterjee *et al.*, 2006).

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 (*Oreochromisniloticus*)where the stressed conditions showed significantly higher level of plasma glucose indicating stress, both of which were reduced when fed with vitamin C supplemented diet. Orutuno*et al.*, 2003 found that in gilthead seabream (*Sparusaurata* L),

there was decrease in glucose level in stressed fish fed with vitamin C for 2 weeks and fish than the control group fed without vitamin C supplementation feed. But there was no ameliorating effect of Vitamin C on cortisol level of gilthead seabream. ,Basrur*et al.*2010 observed rise in in plasma glucose level during a repeated crowding stress investigation on Atlantic salmon (*Salmosalar*). 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 blood glucose level it can be proposed that vitamin C helps to reduce the stress response induced by handling.

**Conclusion**

It was also concluded that the doses 1200mg/Kg and 1500mg/Kg effectively mitigated the pre transportation stressas . But the dose 1200mg/Kg and 1500mg/Kg were not having significant difference to mitigate stress, so 1200mg/kg can be used for mitigating pre transportation stress of *Catla catla* fingerling. The nts like Vitamin C, which showed promising results in mitigating the pre-transportation stress like handling, crowding and confinement.

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