Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 18, No. (3) : 2016 : 91-95

ISSN-0972-3005

EFFECT OF VITAMIN C DIETARY SUPPLEMENTATION ON GROWTH AND SURVIVAL OF GREY MULLET, *MUGIL CEPHALUS* (LINNAEUS, 1758) FRY

SAMBID SWAIN[A], N.K. CHADHA[A], J.K. SUNDARAY[B], P.B. SAWANT[A] AND E.M. CHHANDA PRAJNADARSINI[A]

[a] ICAR-Central Institute of Fisheries Education
[b] ICAR-Central Institute of Brackishwater Aquaculture

(Received 25 January, 2016; accepted 22 March, 2016)

Key words: *Vitamin C, Growth,* Mugil cephalus, *Growth performance, Survival*

Abstract–The present study was conducted to examine the effect of dietary supplementation of vitamin C on growth performance and survival of *Mugil cephalus* (Linnaeus, 1758) juveniles. Mullet juveniles of an average weight of 3.5 gm each were distributed into four treatment groups and were fed with one of the ten purified diets for 45 days. The growth performance and survival of the experimental animals were measured fortnightly. The experimental purified diets were formulated to contain with or without graded levels of vitamin C. The growth performance (absolute growth, percentage weight gain, SGR, PER, per day growth) were significantly higher in mullets fed with diet containing vitamin C supplementation than fed without vitamin supplementation. The vitamin had no significant effect on survival of the mullet juveniles and all the treatments were significantly similar to each other. These finding can be used for formulation of species specific feed for mullet fry for ensuring optimum growth

INTRODUCTION

Feed is crucial aspect as it provides essential nutrients for the better growth and survival of the species and accounts for 60 to 80% recurring cost. The major feed nutrients are the protein, carbohydrates, and lipids. Apart from these major nutrients, there are also some essential components that are required in minor quantities, and its deficiency could lead to several changes in body metabolism and can cause bodily deformities. These are the vitamins and minerals that are added to the diet as additives and have the potential of reducing the mortality and increase the growth of the fish. Vitamin C is found to have a significant role in fish nutrition.

Chatterjee, (1973) and Dabrowski, (1990) stated that most animals can synthesize Vitamin-C (ascorbic acid) from glucuronic acid, fish and crustaceans lack the enzyme gluconolactone oxidase necessary for the last step in this biosynthesis. Consequently, they are dependent on constant supplies of adequate quantities of vitamin C through the feed. Waagbo *et al.*, (1993) reported that smoltification is influenced by vitamin C as there is a significant effect of vitamin C on collagen formation, hormone synthesis (thyroid hormone, steroids), enzymatic activities (oxidase and brain neural activities and neurotransmitters). Thomas, (1984) suggested that ascorbic acid participates in the regulation of Na^+, K^+ - ATPase activities in mullets, *Mugil cephalus L*.

Grey mullet belong to the family mugilidae, having 13 species under genus *Mugil* (Jhingran and Gopalkrishanan, 1973), *Mugil cephalus* commonly known as striped grey mullet has a cosmopolitan distribution between latitude 40°N and 40°S covering all the oceans (Thomson, 1966; De Silva, 1980; Wells, 1984). It is an economically important euryhaline and eurythermal species contributing to sizable fisheries of estuarine and coastal regions in many countries. The protein and lipid requirements for the mullet juvenile were studied by many authors Ito and Barbosa (1997). However, the vitamin requirements for the mullets were not determined, this study was undertaken to estimate the optimal dietary ascorbic acid requirements for growth and survival for juvenile mullets.

***Corresponding author's email**: sambidcife@gmail.com

MATERIALS AND METHODS

Experimental design

The experiment was conducted for a period of 45days in completely randomized design with four treatment and three replications each. Each experimental tanks were stocked with 30 fishes (mean initial weight: 3.5 ±0.05 gand feeding was done at 8 % of the body weight. The daily ration was divided into three equal parts and fed during 09.00 h, 15.00 h and 21.00 h. The growth parameters and survival were measured fortnightly.

Feed preparation

Purified ingredients such as casein, gelatin, dextrin, starch, cellulose, carboxymethyl cellulose, cod liver oil, vitamin mix, mineral mixture and vitamin C were used for feed formulation. Four different experimental diets were prepared to contain graded level of vitamin C as per formulation. The casein and gelatin were used as protein source whereas starch was used as a carbohydrate source, cellulose was used as filler, cod liver oil was used as a lipid source and carboxymethyl cellulose (CMC) was used as a binder. All the ingredients except vitamin-mineral mixture, betaine, cod liver oil were mixed properly in a mixture, and then the mixture was again mixed in a big mouth aluminium container. The dough was then transferred to one aluminum container and placed in pressure cooker for steaming for 15 minutes. Then the dough was taken out and is cooled to room temperature. The vitamin-mineral premix, vitamin C, and oil was added as per the formulation and were mixed after cooling. Pellets were prepared using the hand pelletizer having a diameter of 2 mm. Finally, they were air dried for one day and then sealed in polythene bags and kept inrefrigeratorat 5^0Cto avoid contamination till the completion of the experiment (Table 1).

Proximate composition of the feed

Rovimix stay C® (L-ascorbyl polyphosphate) containing 25% active ascorbic acid was supplemented separately to the basal diet at 0.0, 25.0, 50.0, 100.0 mgascorbic acid kg^{-1} diets, respectively which are denoted as C, T1,T2,T3. The corresponding levels of dietary ascorbic acid analyzed spectrophotometrically were 0.0, 23.25, 45.36, 82.30 mg kg^{-1} diet. The proximate compositions of the feeds were carried out following A.O.A.C method (1990) and represented in Table 2.

Table 1. Formulation and proximate composition of experimental diets

Ingredients (g/kg^{-1}feed)	Diet			
	T1	T2	T3	C
Casein, Vitamin free	256	256	256	256
Gelatin	50	50	50	50
Dextrin	300	300	300	300
Carboxymethyl cellulose	30	30	30	30
Cellulose	234	234	234	234
Mineral mix[a]	40	40	40	40
Vitaminmix[b] without Vitamin C	10	10	10	10
Betaine	10	10	10	10
Cod liver oil	40	40	40	40
Soya lecithin	30	30	30	30
Vitamin C (mg / kg of feed)[c]	23.25	45.36	82.30	0.00
NFE	96.40	96.60	96.58	96.80
Crude protein (% DM)	33.40	34.20	34.36	34.70
Crude lipid (% DM)	9.2	9.5	9.4	9.4
Ash (% DM)	3.60	3.40	3.42	3.20

[a]Mineral premix (mg/Kg) Calcium carbonate 300.0 Potassium di phosphate 319.0 Sodium phosphate (monobasic) 200.34, Magnesium sulphate (heptahydrate) 132.0, Zinc sulphate (monohydrate) 3.0, Sodium chloride 43.50, Cobalt chloride 1.0, Manganoussulphate (monohydrate) 0.80, Cuprous sulphate 0.20, Potassium iodite 0.15, Sodium selenite 0.0011.

[b]Vitamin premix (mg/Kg) Retinyl acetate, 1.20; Cholecalciferol, 0.17; Menadione, 3.33; inositole, 10; Choline choride, 150; Niacine, 9; Riboflavin, 2.0; Pyridoxine hydrochloride, 2.0; Thiamine hydrochloride, 2.0; D-calcium panththenate, 6.0;Biotin, 0.31; Folic acid, 0.18; Cyanocobalamin, 0.0027; α-tocoferol acetate, 20; Cellulose, 789.80.

[c] Supplemented as ascorbyl polyphosphate, Rovimix Stay C 15% mg AA equivalent kg dry diet .

Fish sampling procedure and Analysis of experimental data

Sampling was done at 15, 30 and 45 days of the experimental period. Prior to the experiment, the fishes were fed with the basal diet for ten days to utilize the body reserved Vitamin C by the fish. The data related to mortality, length and weight collected during experiment were used to calculate the Survival Rate (SR), Weight Gain (Wt Gain %), Specific Growth Rate (SGR %), Daily weight gain (DWG), Protein Efficiency Ratio (PER) (Table 3)

Water quality analysis

Water quality parameters of the experimental tanks were recorded throughout the study period (45 days). Physico-chemical parameters, such as water temperature (^{0}C), dissolved oxygen (mg/L), pH, ammonia-nitrogen (mg/L), nitrite-nitrogen (m/L), nitrate-nitrogen (mg/L), etc were closely monitored. Water temperature (^{0}C) was recorded every day. pH was determined immediately by a portable pH meter. Dissolved oxygen (DO), carbon dioxide (CO_2), hardness, chloride, alkalinity, nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and ammonia nitrogen (NH_3-N) were measured on each sampling date.

Ascorbic acid analysis

Quantification of vitamin C in the experimental diet and fish was carried out by calorimetric method. The feed and tissue samples were weighed 1-5 g separately and 5mL of TCA was added in each sample. It was then homogenate at a low rpm and 200-300mg of activated charcoal was added to it. Then, the homogenate solution was filtered by using Whatman N filter paper (No.2) for 2 to 3 times. 5ml of supernatant was collected and one drop of thiourea and 1ml of 2,4, dinitrophenylhydrazine was added. The supernatant was then incubated at 36^{0}C for 3 hours and was transferred to ice bath. 5ml of 85% sulphuric acid was added drop by drop. The solution was then allowed to cool for 30 minutes to room temperature and then measured by spectrophotometerat 540nm.

Calculations

The following variables were calculated:

Weight Gain percentage = (Wt-W0/W0) x 100

Specific growth rate % (SGR)={(Ln Wt -Ln W0)/t} x 100

Daily weight gain = Wt-W0/t

Protein efficiency ratio (PER) = Wet Weight Gain/ Protein Fed

Survivalrate % =Nt/ N0x 100

Where,

Wt and W0 were final and initial fish weights, respectively;

Nt and N0 were final and initial numbers of fish in each replicate, respectively;

t is the experimental duration in day.

Statistical Analysis

After confirming normality and homogeneity of variance, data were analyzed by one-way analysis of variance (ANOVA) (SPSS software, version 19, Chicago, IL, USA) to test for the effects of the dietary treatments. Differences between the means were tested by Duncan multiple range test. The level of significance was chosen at 0.05.

RESULTS AND DISSCUTION

The highest percentage weight gain was recorded for T2(68.45 ± 0.96 %) treatment group followed by T3 (52.07 ± 0.96 %T1 (25.91 ± 0.96%)and control group treatment group. All the treatments were different from each other and were significantly (p, 0.05%) higher than the Control diet (17.05 ± 0.96%).

Table 2. Growth performance of *Mugil cephalus* juvenile

Variable	TI	T2	T3	Control
Initial weight (g)	3.50^a ± 0.05 g	3.50^a ± 0.05 g	3.50^a ± 0.05 g	3.50^a ± 0.05 g
Final weight (g)/45 days	4.40^a ± 0.96 g	5.80^c ± 0.96 g	5.32^b ± 0.96 g	4.09^a ± 0.96 g
PercentWeight gain	25.91^a ± 0.96	68.45^c ± 0.96	52.07^b ± 0.96	17.05^a ± 0.96
SGR %/day	0.51^a ± 0.02	1.16^c ± 0.02	0.93^b ± 0.02	0.35^a ± 0.02
Daily weight gain (mg)	18.64^a ±0.53	48.92^c ±0.53	34.04^b ±0.53	12.00^a±0.53
PER	25.11^a ±0.7	64.37^c ±0.7	47.20^b ±0.7	15.72^a±0.7
Survival (%)	93.20^a ± 1.2	93.38^a ± 1.2	93.40^a ± 1.2	93.00^a ± 1.2

Means within the same row not sharing a common superscript letter are significantly different (P< 0.05)

The enhanced weight gain is due to the beneficial effect of Vitamin-C as co-factor in the synthesis of collagen which ultimately increased the weight gain (Padh and Aleo, 1987), and can promote the transformation of lysine and proline to hydroxylysine and hydroxyproline, respectively. Similar finding was observed in different fish species such as common carp *Cyprinus carpio* (Gouillou-coustans *et al.,* 1998); Chinese white shrimp *Fenneropenaeus chinensis* (Wang and Li, 1996); African catfish *Clarias gariepinus* (Merchie *et al.,* 1997); channel catfish *Ictalurus punctatus* (Mustin and Lovell, 1992); Asian seabass *Lates calcarifer* (Boonyaratpalin *et al.,*1994); red sea bream *Pagus major* (Kousutarak *et al.,* 1994); rainbow trout *Oncohrynchus mykiss* (Ashley *et al.,* 1975) but several authors have reported that vitamin C has no effect on growth in different fish species such as gilthead sea bream *Sparus aurata* (Henrique *et al.,* 1998); Atlantic salmon (Hardie *et al.,* 1991).

Highest SGR (Specific growth rate) was recorded in T2 (1.16 ± 0.02%) group followed by T3 (0.93 ± 0.02%) and T1 (0.51 ± 0.02%) groups. The groups were significantly different from each other and were higher from Control diet (0.35 ± 0.02 %) and daily weight gain was recorded highest in T2 (48.92 ± 0.53 mg) group followed by T3 (34.04 ± 0.53 mg) and T1 (18.64 ± 0.53 mg). These groups were significantly different to each other and were higher than the C (12.00± 0.53mg). This increase in SGR as well as DWG which may be due to the effect of vitamin C on the physiological function of the species and this is similar to studies conducted by Ai *et al.,* (2004) who also observed declining specific growth rate with ascorbic acid deficient diet for turbot(*Scophthalmus maximus*; large yellow croaker *Pseudosciaena crocea* (Ai *et al.,* 2006) but several authors have reported that vitamin C has no effect on SGR or DWG in different fish species such as atlantic salmon *Salmo salar* (Waagbo *et al.,* 1993).

PER (Protein efficiency ratio) was recorded highest in T2 (64.37 ± 0.7) group followed by T3 (47.2 ± 0.7) and T1 (25.1 ± 0.7) group. All the groups were significantly different to each other and also higher than the C (15.72± 0.7). It may be due to vitamins C in fish feed as it could enhance protein synthesis (Andrade *et al.,* 2007) similar results were found in juvenile cobia (Zhou *et al.,* 2012); *Heterobranchus longifilis* fingerlings (Ibiyo *et al.,* 2007); grouper *Epinephelus malbaricus* (Lin and Shiau, 2005). Japanese seabass *Lateolabrax japonicus* (Ai *et al.,* 2004).

Highest survival rate was recorded in T3 (93.4 ± 1.2 %) treatment group followed by T2 (93.38 ± 1.2 %) and T1 (93.2 ± 1.2 %) treatment group but all the treatment groups were similar to each other and also with C (93.00 ± 1.2 %).Vitamin C had no significant effects on fish mortality, might be due to high resistance of *Mugilcephalus.* Similar finding was also reported in other fishes such as gilthead seabream *Sparus aurata* (Henrique *et al.,* 1998), atlantic salmon *Salmo salar* (Lall *et al.,* 1989), but differs from the studies asreported in other fish species such as channel catfish *Ictalurus punctatus* (Duncan and Lovell, 1994), atlantic salmon (Hardie *et al.,*1991).

CONCLUSION

It can be concluded that relatively low vitamin C has a negative effect on the growth performance of *Mugil cephalus* fry whereas the level of 45.36mg ascorbic acid kg^{-1} feed significantly increases the growth performance while vitamin C dietary supplementation has no effect on the survival of *Mugil cephalus* juveniles.

Acknowledgements
The authors would like to thank the Director, ICAR-CIFE and Director, ICAR-CIBA for providing necessary facility to carry out this research.

REFERENCES

Ai, Q., K. Mai, C. Zhang, W. Xu, Q. Duan, B. Tan and Z. Liufu. 2004. Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus. Aquaculture*. 242: 489– 500

Ai, Q.H., Mai, K.S., Tan, B.P., Xu, W., Zhang, W., Ma, H. and Liufu, Z. 2006. Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker. *Pseudosciaenacrocea. Aquaculture.* 261: 327-336.

Andrade, J.I.A., Ono, E.A., Menezes, G.C., Brasil, E.M., Roubach, R., Urbinati, E.M., Tavares-Dias, M., Marcon, J.L. and Affonso, E.G. 2007. Influence of diets supplemented with vitamins C and E on pirarucu (*Arapaima gigas*) blood parameters. *Comp. Biochem. Physiol. Physiol.* 146: 576–580

AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists International. Arlington, VA, USA.

Ashley, L.M., Halver, J.E. and Smith, R.R. 1975. Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing. In: Ribelin, W.E., Migaki, G. (Eds.), The Pathology of Fishes. *The University of Wisconsin Press*, Wisconsin: 769–786.

Boonyaratpalin, M. and Supamataya, S K. 1994. Ascorbyl phosphate-Mg as a dietary vitamin C source for sea

bass Latescalcarifer. In: Chou, L.M., Munri, A., Lam, T.J., Chen, T.W. Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., Phang, V.P.E., Shim, K.F., Tan, C.H. (Eds.), *Proceedings Third Asian Fisheries Forum*, Asian Fisheries Society, Manilla, Philippines: pp. 725-728.

Chatterjee, I B. 1973. Evolution and the biosynthesis of ascorbic acid; *Science.* 182: pp.1271–1272

Dabrowski, K. 1990. Gulonolacone oxidase is missing in teleost fish. The direct spectrophotometric assay. *Biol. Chem. Hope-Seyler*. 371 : 207-214

De Silva, S.S. and Silva, E.I.L. 1980. Biology of young grey mullet *Mugil cephalus L.*, populations of a coastal lagoon in Sri Lanka. *Journal of Fish Biology.* 15: 9–20.

Gouillou-Coustans, M.F., Bergot, P. and Kaushik, S.J. 1998. Dietary ascorbic acid needs of common carp (*Cyprinuscarpio*) larvae. *Aquaculture*. 161 : 453–461.

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. and Gouillou-Coustans, M.F. 1998. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of sea bream, *Sparusaurata*. *Aquaculture.* 161 : 415–426.

Ibiyo, L.M.O., Atteh, J.O., Omotosho, J.S. and Madu, C.T. 2007. Vitamin C (ascorbic acid) requirements of *Heterobranchuslongifilis* fingerlings. *Afr. J. Biotech*. 6: 1559-1567.

Ito, K. and Barbosa, J.C. 1997. N. velprote.Ico e proporc_a_ o de prote|.Na de origemanimalemdietasarticiais para a tainha, Mugilplatanus.*Boletim do Instituto de Pesca* 24 : 111-117.

Jhingran, V. G. and Gopalakrishnan, V. 1973. Estuarine fishery resources of India in relation to adjacent seas. *Journal of the Marine Biological Association of India*. 15: 323–334.

Kousutarak, P., Kanazawa, A., Teshima, S., Koshio, S., Itoh, S. 1994. L-ascorbyl-2- phosphate Mg as a vitamin C source for red sea bream juveniles In Chou, L.M., Munri, A., Lam, T.J., Chen, T.W. Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., Phang, V.P.E., Shim, K.F., Tan, C.H. (Eds.), *Proceedings Third Asian Fisheries Forum, Asian Fisheries Society*, Manilla, Philippines: 729-732.

Lall, S.P., Olivier, G., Weerakoon, D.E.M. and Hines, J.A. 1990. The effect of vitamin C deficiency and excess on immune response in Atlantic salmon (*Salmo salar* L.). In: Takeda, M., Watanabe, T. (Eds.), 3. Int. Symp. On Feeding and Nutrition in Fish, 28 Aug–1 Sep 1989, Toba, Japan. Tokyo University of Fisheries, Tokyo, Japan, 427– 441.

Merchie, G., Lavens, P., Verreth, J., Ollevier, F., Nelis, H., De Leenheer, A., Storch, V. and Sorgeloos, P. 1997. The effect of supplemental ascorbic acid in enriched live food for *Clariasgariepinus* larvae at start feeding. *Aquaculture.* 151: 245–258.

Mustin, W.G., Lovell, R.T. 1992. Na-L-ascorbyl-2-monophosphate as a source of vitamin C for channel catfish. *Aquaculture*. 105 : 95-100.

Duncan, R.T. Lovell. 1994. Influence of vitamin C on the folate channel catfish, *Ictalurus punctatus*, for growth, hematopoiesis, and resistance to *Edwardsiellaictaluri* infection. *Aquaculture* 127 : 233-244.

Padh, H. and Aleo, JJ. 1987. Characterization of the ascorbic acid transport by 3T6 fibroblasts. *BiochimBiophysActa*. 23; 901(2) : 283–290.

Thomas, P. and Wofford, H. W. 1984. Effects of metals and organic compounds on hepatic glutathione, cysteine, and acid-soluble thiol levels in mullet (*Mugilcephalus L.*). *Toxicology and Applied Pharmacology.* 76 : 172-182.

Thomson, J.M. 1966. The grey mullet. In: Barnes, H. (Ed.), Oceanography and Marine Biology.An Annual Review, vol. 4. Allen and Unwin, London, pp. 301–355.

Waagbo, R., Glette, J., Raa-Nilsen, E. and Sandnes, K. 1993. Dietary vitamin C, immunity and disease resistance in Atlantic salmon, *Salmo salar. Fish PhysiolBiochem*. 12 : 61–73.

Wang, W. and Li, A. 1996. Influences of LAPP on the growth, resistance to low oxygen content and immunoresistance of *Penaeuschinensis*.*Trans. Oceanol. Limnol.* 1 : 42–49.

Wells, R.D.S. 1984. The food of the grey mullet (*Mugil cephalus L.*) in Lake Waahi and the Waikato River at Huntly. New Zealand Journal of Marine and Freshwater Research. 18: 13–19.

Zhou, Q., Wang, L., Wang, H., Xie, F. and Wang, T. 2012. Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (*Rachycentron canadum*). *Fish Shellfish Immunol.* 32(6): 969-75.