Braz. J. Aquat. Sci. Technol., 2009, 13(2):17-23.

EFFECT OF VARYING LEVELS OF DIETARY VITAMIN C (ASCORBIC ACID) ON GROWTH, SURVIVAL AND HEMATOLOGY OF JUVENILE TILAPIA, *Oreochromis karongae* (TREWAVAS 1941) REARED IN AQUARIA

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ABSTRACT

Nsonga, A.R.; Kang'ombe, J.; Mfitilodze, W.; Soko, C.K. & Mtethiwa, A.H. 2009. Effect of varying levels of dietary vitamin C (ascorbic acid) on growth, survival and hematology of juvenile tilapia, *Oreochromis karongae* (Trewavas 1941) reared in aquaria. Braz. J. Aquat. Sci. Technol. 13(2):17-23. ISSN 1808-7035. Vitamin C (ascorbic acid) requirement of juvenile *Oreochromis karongae* was studied by incorporating varying levels of ascorbyl-2-polyphosphate in a 40% crude protein diet to obtain 0, 20, 40, 60 and 80 mg ascorbic acid equivalent kg^{-1}diet. Juvenile fish of 3.7g ± 0.02g initial body weight were used for the study. After 84 days of the experiment, the fish fed AA-supplemented diets had significantly (P<0.05) higher specific growth rate protein conversion efficiency and protein efficiency ratio; and significantly (P<0.05) better feed conversion ratios than non supplemented fish. Fish fed non supplemented diet recorded a 33 % rate of mortality, where as those fed with a diet supplemented with 60 mg ascorbic acid kg^{-1} had mortality as low as 4.4 %. Hematological indices showed a significant increase (P<0.05) with dietary AA level. Diet containing 60 mg kg^{-1} of AA showed the maximum growth performance, while the broken line model gave 51 mg ascorbic acid kg^{-1} diet as the optimal level required by juvenile *O. karongae*. Our data show that ascorbic acid is essential for *O. karongae* growth performance.

Keywords: Clinical deficiency, Mortality, Conversion efficiency, Supplementation, Nutritional quality.

INTRODUCTION

Vitamin C (ascorbic acid) is an essential nutrient in aqua-feeds, and is an indispensable nutrient required to maintain physiological processes such as normal growth, immunity and reproduction of different animals including fishes (Tolbert, 1979). Ascorbic acid is water-soluble and is essential for several metabolic functions including the antioxidant system. Most fish, including tilapia, are not capable of vitamin C biosynthesis (Chatterjee, 1973) due to the absence of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of ascorbic acid (Wilson, 1973). L-ascorbic acid is extremely labile and the rate of degradation is a function of storage time, with the effect of temperature, oxygen, pH and light. Recent studies indicate that ascorbic acid derivatives that include sulfate and phosphates are more resistant to oxidation and retain ascorbic acid activity for fish (Abdelghany, 1996).

Ascorbic acid requirements of some tilapia species have been investigated. Stickney *et al*. (1984), reported the fortification of 50 mg of ascorbic acid equivalent kg^{-1} diet as the level that allows for maximum weight gain and absence of deficiency signs in blue tilapia (*Oreochromis aureus).* A diet of 79mg ascorbic acid kg^{-1} diet was found to be the requirement level for maximum weight gain of hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus)*(Shiau & Jan, 1992).

The ascorbic acid requirement varies among fish species, but intraspecies differences such as fish strain, size and age also affect the dietary requirement. The amount of ascorbic acid that must be added to the diet for normal function is also dependent on the form of the vitamin that is added to the diet (Lim & Webster, 2001).

In Malawi, nutritional studies are still in their infancy (Kang'ombe, 2004). This is the first attempt to determine the dietary ascorbic acid requirement of *O. karongae*. *Oreochromis karongae,* was recently isolated from the wild for aquaculture in Malawi and is farmed by both small holder and commercial farmers (Msiska & Costa-Pierce, 1999). Although there is general information on Vitamin C requirement for tilapia, specific data on the effects of vitamin C on growth, survival and hematology of *O. karongae* are scarce. The present study therefore, attempted to determine the quantitative dietary requirement of ascorbic acid and its effect on growth, survival and hematology of *O. karongae* by using L-Ascorbyl-2-polyphosphate which is a more stable form of ascorbic acid.

MATERIALS AND METHODS

Experimental set up

An indoor research trial was conducted in the wet laboratory of Bunda College of Agriculture, University of Malawi, over a period of 84 days (July to September 2006). Juvenile *O. karongae* (3.7g ± 0.02g) were collected from National Aquaculture Centre, Domasi. To eliminate possible external parasites, fish were treated in sodium chloride bath of 2 mg L^{-1} every two days during two weeks of acclimatization. During acclimatization the fish were offered a maintenance diet containing 25% protein, twice a day. The experimental tanks were filled to the brim with well water. Continuous aeration was provided with the use of pressure pumps that maintained oxygen levels above 5.0 mg L^{-1} (Lawson, 1994).

Experimental design

Fifteen (15) tanks of 200 L were stocked with 35 fish each. Fish were conditioned for one week and fed a basal diet containing 25% crude protein. Five experimental diets with 5 varying levels of L-ascorbic acid (0 mg, 20 mg, 40 mg, 60 mg and 80 mg kg^{-1} diet) with 99.9% purity (Kraft Chemical Company, Melrose Park, IL, US) containing 40% protein level (Table 1) were then assigned in triplicate to the experimental unit in a completely randomized design. The fish were hand fed to satiation twice daily at 6 % of live body weight in pellet form.

Sample Collection

Growth of fish was monitored fortnightly with fish body weight (g) and length (cm) measured individually to make adjustment to feeding. Feed intake was taken into account for later calculations of feed conversion ration, protein conversion efficiency and protein efficiency ratio. Prior to sampling, fish were kept without feeding for 24 hours. Precaution was taken to minimize stress during the weighing by anaesthetizing the fish with FA 100 (Yanabe Yakuhin Co. Palo Alto, USA)(1.2g L^{-1}). Every fortnight fish were euthanized for blood, liver and muscle analysis. The growth performance equations used are as indicated in the footnote of Table 2.

Analytical Methods

After measuring fish for growth, five fish per tank were then killed for liver and muscle ascorbate analysis using Indophenols Method (Seki 1990) and for hematological indices (differential, hematocrit and plasma protein) every fortnight. A hematocrit centrifugal machine at 12,000 rpm for 4 minutes and hematocrit reader were used to determine the packed cell volume of fish blood while the hand refractometer (Bellingham+Stanley, UK) was used for plasma protein

Table1 - Experimental diets with varying levels of vitamin C and their proximate composition used for feeding juveniles of *O. karongae*.

Ingredient	Varying levels of Vitamin C				
	0 mg	20 mg	40 mg	60 mg	80 mg
Fishmeal	38	38	38	38	38
Soybean(roasted)	38	38	38	38	38
Maize bran	8	8	8	8	8
Wheat bran	2	2	2	2	2
Vegetable oil	3	3	3	3	3
Rice bran	8	7.989	7.967	7.934	7.89
Vitamin premix(Vit C free)	1.5	1.5	1.5	1.5	1.5
Mineral premix	1,5	1.5	1.5	1.5	1.5
Vitamin C (mg/kg)	0	111	222	333	444
Total weight	100	99.99	99.97	99.93	99.89
Proximate composition (% Mean ± SD)					
Dry Matter	87.2±1.4	86.2±1.3	87.2±1.3	89.2±1.3	88.2±1.3
Crude protein	40.0±0.8	40.1±0.9	39.9±0.9	40.0±0.8	39.6±0.8
Crude fibre	2.8±0.4	2.8±0.4	2.7±0.4	2.8±0.4	2.8±0.4
Crude fat	13.9±0.3	13.8±0.3	13.9±0.3	13.8±0.3	13.8±0.3
Ash	9.8±0.9	10.2±0.9	10.2±0.9	9.9±0.9	10.1±0.9
Nitrogen free extract	21.9±1.6	22.1±1.5	21.8±1.7	22.0±1.5	21.8±1.6
Gross energy (kJ/g)	18.2±1.3	18.2±1.3	18.2±1.3	18.2±1.3	18.2±1.3
Ascorbic acid (mg/kg)	<0.01	19.5±1.2	38.9±1.2	58.9±1.2	79.0±1.2

[1]Vitamin premix supplied the following (IU or mg kg)[1] diet): vitamin E (DL-a tocopherol), 250; vitamin A, 16060; vitamin D3, 4510 vitamin K (menadione sodium bisulphite), 30;;; thiamine, 32; riboflavin, 32; Ca-D-pantothenate, 80; niacin, 170; biotin, 10; folic acid, 10; cyanocobalamin, 0,032; pyridoxine, 32.
[2]Mineral premix supplied the following (mg kg)[1] diet): 0.7; MnO, 50; ZnO, 150; FeSO4, 150; CuSO4, 20; CoSO4, 0.5; I2Ca, 1.0. Na2SeO3,

Table 2 - Effect of Vitamin C (ascorbic acid, AA) on final mean body weights, weight gain, weight gain/day, percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein conversion efficiency (PCE), protein efficiency ratio (PER) of juveniles of *O. karongae* after 12 weeks of treatment (Means ± SE)[1].

Parameters	Treatments				
	0 mg AA /kg	20 mg AA /kg	40 mg AA /kg	60 mg AA /kg	80 mg AA /kg
Initial mean weight (g/fish)	3.75±0.01	3.76±0.01	3.76±0.01	3.76±0.01	3.76±0.06
Final mean weight (g/fish)	4.78±0.12^{a}	9.64±0.14^{b}	9.92±0.15^{b}	13.79±0.28^{c}	12.35±0.19^{c}
Weight gain (g/fish)[2]	1.03±0.35^{a}	5.88±0.30^{b}	6.16±0.32^{b}	10.03±0.33^{d}	8.59±0.31^{c}
Weight gain/day (g/fish)[3]	0.01±0.01^{a}	0.07±0.01^{b}	0.07±0.01^{b}	0.12±0.01^{d}	0.08±0.01^{c}
Weight gain (%)[4]	27 ±1.08^{a}	156 ±1.05^{b}	163±1.03^{b}	266±1.11^{c}	228±1.02^{c}
SGR (%/day)[5]	0.32±0.53^{a}	1.15±0.06^{b}	1.18±0.09^{b}	1.58±0.15c	1.45±0.65^{c}
FCR[6]	3.20±0.53^{c}	2.36±1.10^{b}	2.42±1.09^{b}	2.30±1.05^{a}	2.32±1.07^{a}
PCE[7]	2.32±1.07^{a}	22.28±1.06^{b}	22.65±1.08^{b}	28.18±1.07^{c}	22.9±1.09^{b}
PER[8]	0.03±0.03^{a}	0.15±0.06^{b}	0.15±0.03^{b}	0.25±0.04^{c}	0.21±0.10^{c}

[1]Means in the same column with different superscripts are significantly different (P<0.05). Data represent means of 30 fish/diet.

[2]Weight gain (g/fish) = final mean weight- initial mean weight.

[3]Weight gain/day = (final mean weight - initial mean weight)/(days)

[4]Weight gain (%) = (final mean weight - initial mean weight)/initial mean weight x100.

[5]SGR: specific growth rate (%/day) = 100 x [(In final weight (g) - In initial weight (g))/ (days)].

[6]FCR: feed conversion ratio = Total dry food offered (g)/weight wet gain (g). [7]PCE (%) = 100 (final body protein- initial body protein/amount of protein consumed).[8]PER = wet biomass gain (g)/ amount of protein consumed (g).

reading. Hemacytometer (Thoma's counting chamber) was used for total blood cell count which was delayed until week 12 to allow for enough blood to build up since the fish used in the experiment were small. Observations for clinical signs on fish continued throughout the experimental period. Water in tanks was changed 50% every two days and complete clean up of tanks was done every fortnight. Water quality was monitored using Horiba water checker (Horiba Ltd, Kyoto, Japan).

Water quality monitoring

Water quality parameters remained in acceptable ranges during the twelve week experimental period and showed no significant differences (P>0.05) across treatment diets. Temperature ranged between 21.8°C ± 0.05 to 22.3 ± 0.01. Dissolved oxygen concentration averaged 6.5 mg L^{-1}. The pH values ranged from 7.53 ± 0.05 in treatment 0mg ascorbic acid kg^{-1} to 7.56 ± 0.04 in treatment 80mg ascorbic acid kg^{-1} diet. Ammonia averaged 0.16 ± 0.3 mg L^{-1} and means were not significantly different (P>0.05).

Statistical Analyses

All data were analysed using SPSS 12.0 software for Microsoft Windows (SPSS Inc., 2003). A One-way Analysis of variance was implemented and differences among the means were separated according to Duncan's multiple range tests at 5% (á) level. The Broken-line model analysis (Chou *et al*., 2001; Robbins *et al*. 2006) was used to estimate the optimum dietary ascorbic acid level requirement for juvenile *O. karongae.*

RESULTS

Effect of vitamin C on growth

Fish fed diets without ascorbic acid had significantly lower (P<0.05) weight gains than fish fed with ascorbic acid supplemented diets. Fish without ascorbic acid supplementation stopped growing at week 8 and began to lose weight. Fish fed ascorbic acid - supplemented diets did not stop growing during the 12-week feeding period and reached a significantly higher

(P<0.05) weight than did fish without dietary ascorbic acid. After 12 weeks of supplementation, 80 mg ascorbic acid kg^{-1} diet gave mean weight of 12.35g while 60 mg ascorbic acid kg^{-1} diet gave mean weight of 13.79g. The 80mg ascorbic acid kg^{-1} diet did not promote additional weight increase for juvenile *O. karongae*.

During the 84 day period, non-supplemented fed fish gained only 27% of their initial weight whereas fish fed 20, 40, 60 and 80 mg kg^{-1} diet gained 156, 163, 266, and 228% of their initial weight, respectively. The diet containing 60mg kg^{-1} produced highest weight gains (Table 2).

After 12 weeks, the specific growth rate, feed conversion ratio, protein conversion ratio and protein efficiency ratio were significantly lower (P<0.05) in fish without ascorbic acid supplementation than those fed ascorbic acid supplemented diets (Table 2).

Effect of vitamin C on hematology, ascorbate levels in liver and muscle

Hematological values increased significantly (P<0.05) with dietary ascorbic acid level. Diet without ascorbic acid supplementation showed the lowest values while diet 60mg ascorbic acid kg^{-1} indicated the highest hematological activity (Table 3). The packed cell volume also referred to as hematocrit, protein plasma, white blood cells, and red blood cell counts all showed significant (P<0.05) results with increase in values of ascorbic acid. The ascorbate levels in both liver and muscle increased in all treatments except in the 0 mg kg^{-1} treatment (Table 4).

Effect of vitamin C on disease condition

After week 12, fish without a supplemented diet showed clinical signs, such as hemorrhage in the eye and fin and also had eroded fins and appeared darker while fish with supplemented diet were all in good health condition.

Effect of vitamin C on condition factor and survival of fish

Condition factor indicates the stoutness of fish, and a well nourished fish has a condition factor of not less than 1. The initial condition factor varied between 1.55 and 1.56 while the final condition factors ranged from 1.46 to 1.72 (Table 5). Significant differences (P<0.05) were recorded in survival of juvenile *O. karongae* across diets with non-supplemented fed fish recording least figures. Survival significantly increased from 66.67 to 95.56 %, with ascorbic acid level. When supplementation of ascorbic acid was equal to or more than 60 mg kg^{-1}, the survival (93.34- 95.56%) was significantly (P< 0.05) higher to that of the control group (66.67%), and no significant differences in survival were observed among fish fed diets with 60 and 80 mg kg^{-1} of ascorbic acid (Table 5).

Vitamin C requirement for juvenile O. karongae

Vitamin C requirement for juvenile *O. karongae* was estimated by fitting a broken line model to the weight data and was found to be 51 mg ascorbic acid kg^{-1} diet (Figure 1).

Table 3 - Initial and final haematological parameters of *O. karongae* fed observed under varying levels of ascorbic acid diets during 12 weeks of treatments (Mean ± SE)[1].

	Haematological parameters							
Diet AA mg/kg	Packed Cell Volume (g /100L)		Plasma Proteins Counts/mm^3		White Blood Cells Count /mm^3		Red Blood Cells (%)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	12±1.04	15±1.04^a	1.5±1.14	1.0±1.09^a	5.55 x 10^4±1.03	6.66 x 10^4±1.43^a	1.00 x 10^6±1.04	1.99x10^6± 1.11^a
20	12±1.09	29±1.07^b	1.5±1.02	2.8±1.11^b	5.55 x 10^4±1.43	6.96 x 10^4±0.85^b	1.00 x 10^6±1.23	2.05x 10^6±1.34^b
40	12±1.04	30±1.03^b	1.5±1.02	2.8±1.11^b	5.55 x 10^4±1.19	7.00 x 10^4±0.49^b	1.00 x 10^6±1.13	2.61x10^6± 1.20^b
60	12±1.05	30±1.12^b	1.5±1.01	3.0±1.12^b	5.55 x 10^4±1.07	7.30 x 10^4±1.49^b	1.00 x 10^6±1.20	3.05x 10^6±1.19^c
80	12±1.12	30±1.09^b	1.5±1.09	3.0±1.22^b	5.55 x 10^4±1.06	7.20 x 10^4±1.65^b	1.00 x 10^6±1.01	3.34x 10^6±1.41^c

[1]Means in the same column sharing different superscripts are significantly different (P<0.05).

Table 4 - Initial and final ascorbate levels in liver and muscle of *O. karongae* fed with varying levels of ascorbic acid diets during 12 weeks of treatments (n=3) (Mean ± SE).

Diet AA (mg k g^{-1})	Ascorbate levels (µg g^{-1} tissue)			
	Liver		Muscle	
	Initial	Final	Initial	Final
0	23.1±1.2	5.1±0.9	7.1±1.3.	1.8±0.1
20	23.2±1.4	33.2±1.7	7.1±1.7	18.2±1.7
40	23.2±1.5	45.1±1.3	7.3±1.6	18.6±1.6
60	23.4±1.2	59.3±1.6	7.1±1.8	24.3±1.3
80	23.6±1..5	75.4±1.2	7.2±1.3	27.1±1.5

Table 5 - Initial and final condition factor (K), and Survival of *O. karongae* cultured in indoor tanks after 12 weeks of treatment (Mean ± SE)[1].

AA mg/diet	Parameters		
	Initial Condition (K)	Final Condition (K)	Survival (%)
0	1.56 ± 0.02	1.46 ± 0.09[a]	66.67 ± 0.32[a]
20	1.55 ± 0.03	1.60 ± 0.08[b]	86.67 ± 0.16[b]
40	1.55 ± 0.04	1.68 ± 0.11[b]	87.67 ± 0.18[b]
60	1.55 ± 0.01	1.72 ± 0.12[b]	95.56 ± 0.01[c]
80	1.56 ± 0.10	1.70 ± 0.07[b]	93.34 ± 0.21[c]

[1]Values of different superscripts in a column are significantly different at $P<0.05$.

DISCUSSION

The results of this study strongly indicate that vitamin C significantly affects the growth, survival and hematology of juvenile *O. karongae*. Growth is a function of both the nutritional quality and the rate of consumption, among other things (Stickney, 2000). In this research trial, a diet containing 51mg of ascorbic acid kg^{-1} diet was found to be the optimal dietary requirement for juvenile *O. karongae* while 60mg ascorbic acid kg^{-1} diet was found to be the requirement level for maximum growth and performance of *O. karongae.* The ascorbic acid requirement value by *O. karongae* attained in this experiment was higher than those reported for *Oreochromis aureus* between 10 and 25 mg ascorbic acid who also examined juvenile hybrid tilapia, *Oreochromis niloticus x O. aure*us (Shiau & Hsu, 1999).

However, the requirement based on growth performance in this study was lower than that for *Oreochromis spilurus* (100-200 mg ascorbic acid kg^{-1} diet) (Al-Amoudi *et al.,* 1992). The difference is probably related to fish species, size, the form of vitamin C and experimental conditions in different studies (Lovell, 1989). The 60 mg ascorbic acid kg^{-1} diet found as requirement level for maximum growth agrees with Li & Lovell (1985) who demonstrated that fish raised from 3 to 19g required 60 mg ascorbic acid kg^{-1} diet for maximum weight gain. Weight gain increase with dietary level is considered by many nutritionists to be the most important and meaningful response in nutritional requirement studies (Stickney, 2000).

The diet without ascorbic acid supplementation decreased the specific growth rate (0.32 % day^{-1}) of juvenile *O. karongae* and this is in accordance with studies conducted by Ai *et al.* (2004) who also observed declining specific growth rate with ascorbic acid deficient diet for seabass (*Scophthalmus maximus*). Stickney (1994) reported that feed conversion factor values can actually be less than 1 in water systems where there is natural food. Goddard (1996) observed that poor or fluctuating feed conversion ratios may reflect problems with diets or feeding methods. In the present study, fish fed with non supplemented diet had poor food conversion ratio (4.2) while fish fed with the supplemented diet recorded 2.0-2.42 as feed conversion ratios.

Feed utilization in this study was also affected by the dietary level of ascorbic acid. Total amount of feed consumed increased with ascorbic acid level. Both protein conversion efficiency and protein efficiency ratio were much lower in fish fed with diet without ascorbic acid. This indicate lower protein utilization by the fish. Fracalossi *et al.* (1998) observed a similar trend in juvenile Oscars (*Astronotus ocellatus)* cichlids. The diet used in the present experiment had high protein content (400 g kg^{-1}), which could have resulted in the fish consuming high levels of oxygen as consumption increases with protein in tilapia (Ross, 2000).

In this study, a number of other healthy related conditions were uncovered due to ascorbic acid deficiency. For instance, hematology of the fish was significantly affected by ascorbic acid. Fish without ascorbic acid supplementation showed lower values for hematocrit (15%), total white blood cell (6.66 x $10^4/mm^3$), red blood cells (1.99 x $10^6/mm^3$) and plasma protein (1.0g $100L^{-1}$). These negative trends compromised the healthy status of the fish that showed clinical signs such as fin erosion and broken back disease. Shiau & Jan (1992) found similar results in hybrid tilapia without dietary ascorbic acid that showed lower hematocrit than fish receiving ascorbic acid supplementation. Shiau & Jan (1992) reported that anemia is common in animals with ascorbic acid deficiency because there is reduction in the absorption and redistribution of iron and consequently a reduction in the synthesis of hemoglobin. Fracalossi *et al.* (1998) also reported signs of scurvy on Oscars (*Astronotus ocellatus)* such as reduced growth, impaired collagen formation and lordosis due to non supplementation of ascorbic acid. In this study, juvenile

O. karongae not supplemented with ascorbic acid showed elevated levels of neutrophils. According to Roberts (1989), excess release of neutrophils into the blood of mammals and fish is a response to stress. Absence of ascorbic acid therefore may have induced a physiological stress on juvenile *O. karongae*.

There is a relationship between tissue ascorbate and the fish health (Halver, 1985). In the present study fish with non supplemented diets showed declining ascorbate levels in both the liver and the muscle. On the other hand , fish with supplemented diet showed increased levels of ascorbate. Ai *et al.* (2004) got similar results on Japanese seabass (*Lateolabrax japonicus*) and according to Lim & Lovell (1978), requirement for tissue saturation is much higher than that for normal fish growth that could prevent deficiency signs. Therefore increased tissue ascorbate in *O. karongae* fed with supplemented diet of ascorbic acid had a positive effect on the health of the fish (Halver, 1985). A relatively low rate of survival (60%) was observed in juvenile *O. karongae* fed with a non-supplemented diet of ascorbic acid. The significantly lower survival in fish fed with non-supplemented diet can only be attributed to the physiological stress caused by dietary ascorbic acid deficiency and not due to water quality since all low water quality parameters were within the ranges known to be required by tilapia. The level of dissolved oxygen was above 5 mg L^{-1} in all treatments, which is ideal for warm water fishes (Buttner *et al.,* 1993). The oxygen level was above 2.5 and 1 mg L^{-1} where *O. niloticus* showed signs of stress (decrease in activity accompanied by body coloration and erection of dorsal fin) in similar systems (Ross, 2000). The water pH of 7.5 across the treatments was within the limits for the growth of several species of tilapias (Boyd & Tucker, 1998; Ross, 2000).

In conclusion, supplementation of 60mg ascorbic acid kg^{-1} in the diet significantly yielded the maximum fish performance in terms of weight gain and overall health condition in this nutrient dose-response experiment. We also conclude that 51 mg ascorbic acid kg^{-1} in the diet is the optimal dietary requirement for *O. karongae* and must be fortified to pelleted diets in order to maximize ascorbic acid bioavailability to juvenile *O. karongae.*

ACKNOWLEDGEMENTS

The authors thank the Icelandic International Development Agency (ICEIDA) for financial support of experiments during the Master's studies of the first author. Special thanks should also go to the former Aquaculture Farm Manager, Late Mr. A.G. Matambo for tireless effort in the logistical arrangements of this research. We thank Mr. B. Lusangasi for daily fish care and Mr. E. Nyali for laboratory analyses. Thanks go to Bunda College of Agriculture for making facilities available for the research. Mr Iain Elliot (Institute of Aquaculture, University of Stirling, UK) provided insightful comments to the manuscript.

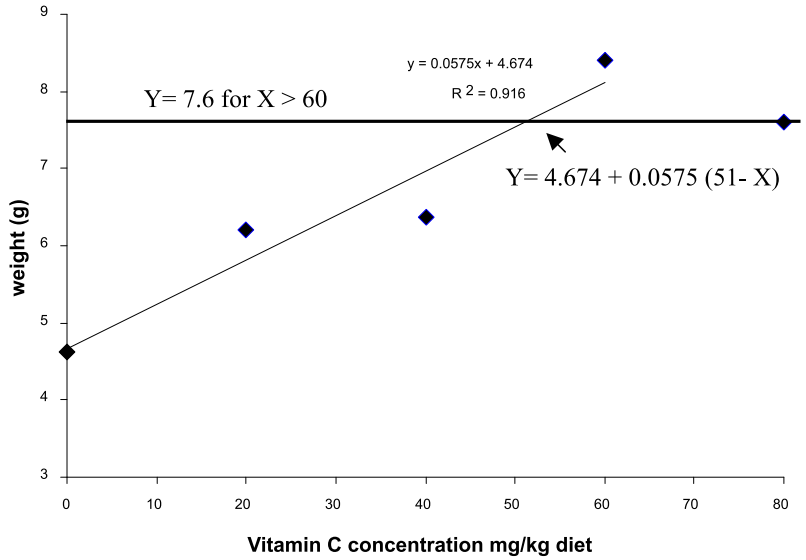

Figure 1 - Broken-Line model for the ascorbic acid requirement of juvenile *O. karongae.* Weight breakpoint is at 51 mg ascorbic acid kg^{-1} diet (n=3 tanks).

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Received: October 13, 2008
Accepted: November 11, 2009