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*Full Length Article*

# Effects of Graded Level of Dietary L-Ascorbyl-2-Polyphosphate on Growth Performance and Some Hematological Indices of Juvenile Mahseer (*Tor putitora*)

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## Abstract

A 70 days feeding trial was conducted to evaluate the effects of graded level of L- ascorby-2- polyphosphate (APP) on growth performance and hematological indices of juvenile mahseer *Tor putitora*. Four different 40% protein experimental diets supplemented with APP at the rate of 0, 100, 200 and 300 mg APP $kg^{-1}$ diet were fed to triplicate group of fish. The APP supplementation showed their effect in a dose dependent manner. Significantly ($P < 0.05$) improved growth performance i.e. weight gain (WG%), feed conversion efficiency (FCE%), specific growth rate (SGR%) and hepatosomatic index (HSI%) was observed with $APP_{300}$, followed by $APP_{200}$ and then $APP_{100}$ enriched diet. The growth hormone (GH) level of fish also increased with increase in concentration of APP in the diet and showed a positive relationship with WG%. Moreover, the hematological parameters like red blood cell count, hemoglobin and hematocrit values also followed the same trend in response to graded levels of dietary APP supplementation, significantly ($P < 0.05$) higher values with $APP_{300}$ enriched diet followed by $APP_{200}$ diet while comparatively low, but significantly higher ($P < 0.05$) values as compared to control group of fish were obtained in response to $APP_{100}$ supplemented diets. The results of this study indicate the dose dependent beneficial effects of APP on growth performance and hematological indices of *T. putitora*. © 2015 Friends Science Publishers

**Keywords:** L-ascorbyl-2-Polyphosphate; Growth performance; Hematology; *Tor putitora*

## Introduction

Vitamins and minerals are the micronutrient, although require in small quantities but are essential for normal metabolism of all vertebrates including fish and their deficiency in the diet can cause diseases. Among them vitamin C is an important micronutrient that plays significant role in the growth (Ai *et al.*, 2006; Eo and Lee, 2008; Miar *et al.*, 2013), reproduction (Dabrowski and Ciereszko, 2001; Farahi, 2011), biosynthesis of various neuropeptides (Eipper *et al.*, 1993), maintenance of many enzymes in a reduced state (Aysun, 2009), healing of wounds (Tewary and Patra, 2008; Campos *et al.*, 2008) and immune reactions (Anbarasu and Chandran, 2001; Kumari and Sahoo, 2005; Ai *et al.*, 2006; Eo and Lee, 2008). Beside these also act as an antioxidant (Murmu and Shrivastava, 2011) and involve in the removal of hydrogen peroxide and deactivation of superoxide radicals. Thus improves the performance of cultured fish by decreasing mortality (Kumari and Sahoo, 2005; Ai *et al.*, 2006) physiological stress (Farahi, 2011) and improving hematological parameters (Affonso *et al.*, 2007; Nsonga *et al.*, 2009; Pimpimol *et al,.* 2012; Miar *et al.,* 2013) or by enhancing feed conversion efficiency, protein efficiency ratio and growth rate (Eo and Lee, 2008; Alam *et al.,* 2009; Dicu *et al.,* 2013).

However, deficiency of vitamin C cause spinal deformation, retarded growth, caudal fin erosion, lordosis and scoliosis (Lewis-McCrea and Lall, 2010), impaired wound healing (Campos *et al.,* 2008), anemia (Adham *et al.,* 2000), liver atrophy (Wang *et al.,* 2003), poor collagen formation (Aysun, 2009), increased susceptibility to bacterial diseases (Ai *et al.,* 2006), lower survival rates (Adham *et al.,* 2000; Wang *et al.,* 2003) in finfish, while black death disease in shrimp (Lightner *et al.,* 2009).

It is well documented that majority of fishes lack the L-gulonolactone oxidase (GLO) gene that is responsible for enzyme L-gulonolactone oxidase, necessary for the synthesis of ascorbic acid (AA) (Drouin *et al.,* 2011). Hence, they are unable to manufacture AA from D-glucose (Fracalossi *et al.,* 2001) and require it from external sources like in feed (Gouillou-Coustans and Kaushlic, 2000). Generally, vitamin C is heat labile degrades rapidly and loses its activity in the presence of oxygen, light and high temperature. Many researchers claimed about the loss of the preliminary quantity of AA from fish feedstuffs (Grant *et*

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*al.,* 1989; Zuberi *et al.,* 2011), and reported that sulfate and phosphate forms of AA are more resistant to oxidation and retain vitamin C activity for a longer period. Therefore, stable form of AA like ascorbyl-2- polyphosphate (APP) is getting popularity in the aquaculture industry (Zuberi *et al.,* 2011; EFSA panel, 2013).

Fish requires vitamin C for their proper development, growth and reproduction, but the amount varies according to fish species, size, feeding rates, environmental factors, nutrient interrelationships, health condition, water quality, feed formulation technique (Gouillou-Coustans and Kaushlic, 2000), culture conditions (NRC, 2011) and the form of the vitamin C that is supplied (Zuberi *et al.,* 2011: NRC, 2011). Therefore, the recommended values varied in different studies even for a single species.

In the last few years, extensive research has been published on the effect of dietary AA supplementation and reveals that the sufficient amount of vitamin C in fish feed significantly improves the growth performance, FCE%, condition factor and survival of different fish species by improving growth hormone concentration (Denny-Brown *et al.,* 2012), immunity (Anbarasu and Chandran, 2001; Ai *et al.,* 2006) and hematological parameters like plasma proteins, red blood cell (RBCs) count, hematocrit (Hct) value and white blood cell (WBCs) count of fish (Nsonga *et al.,* 2009; Pimpimol *et al.,* 2012). Moreover, also protect the body cells from oxidative damage (Sahoo and Mukherjee, 2003).

Golden mahseer or *Tor putitora* recognized as a king of mountain streams is a highly prized, delicious food fish of India, Pakistan, Bangladesh, Nepal, Afghanistan and Myanmar (Jha and Rayamajhi, 2010). For the last decade in most of the countries, including Pakistan, its population is continuously declining, due to multiple reasons, including overfishing, environmental pollution, slow growth rate and the loss of spawning grounds. In Pakistan, nowadays it is considered as a rare food fish. Because of its high economic importance as a game fish and high acceptability to the consumers, fisheries departments of Punjab and Khyber Pakhtunkhwa are using different techniques for its rearing, conservation and propagation and research is also in progress for increasing its growth rate in captivity. No work so far has been done on the dietary requirement of vitamin C of mahseer (*Tor putitora*). Thus, the present research was designed with aim to study if varying the amount of vitamin C supplementation of the diets regularly used in mahseer hatcheries could improve the growth performance and hematological parameter of fish.

## Materials and Methods

### Experimental Diets

Formulation and composition of 40% protein basal diet for *Tor putitora* is shown in Table 1. L-ascorbyl-2-polyphosphate (APP), a stable form of L. Ascorbic acid was used as a source of vitamin C in the experimental feed. Four experimental diets, APP0, $APP_{100}$, $APP_{200}$ and $APP_{300}$ containing 0, 100, 200 and 300 mg APP $kg^{-1}$ diet were formulated. All dried feed ingredients were grinded in a grinder, mixed with oil, water was added and then dough was made. It was passed through a meat grinder and then pellets were made which were dried at room temperature, saved in Ziploc bags and kept at low temperature.

### Experimental Fish and Feeding Trial

Juvenile mahseer (*Tor putitora*) were purchased and transferred from the Hattian Nursery unit, Attock, by adopting live hauling technique to the Fisheries and Aquaculture setup Quaid-i-Azam University, Islamabad. Prior to the start of the feeding experiment, the fish were weaned on a semi purified diet and acclimatized to laboratory condition for about 2 weeks. The feeding trial was conducted in a semi-static, flow-through system with 12 fiber tanks receiving aerated freshwater. The uniformed size fish, irrespective of sex, average body weight 2.27 ± 0.01 g were randomly allotted to each fiber tank. They were stocked at the rate of 30 fish/tank (Stocking density, 2.0 g $L^{-1}$) and the experiment was conducted in triplicate. Initially, the fish were fed their respective diets at a rate of 6% of wet body weight, twice a day and then feeding rate was adjusted fortnightly. Fish were fed to apparent satiation and daily feed intake was recorded by removing undigested feed and faces through siphoning. The fiber tanks were cleaned after every month in order to minimize algae and fungal growth that could provide a source of vitamin C. During feeding trial water temperature and pH ranged 21-23ºC and 7-8 respectively. The ammonia was less than 0.20 mg $L^{-1}$, while dissolve oxygen content was trying to keep at 6-6.5 mg $L^{-1}$ by providing compressed air via air stones.

### Sample Collection and Analysis

At the end of the feeding trial, all fish from each tank were anesthetized immediately with MS222 (60 mg $L^{-1}$), weighed for calculating growth performance data, while blood was collected in eppendorf tubes by tail ablation. Subsequently, fish were decapitated on the ice box and liver of each fish was removed and weighed for the calculation of HIS%.

### Growth Performance

Percent weight gain (%WG), feed conversion ratio (FCR), feed conversion efficiency (FCE%), specific growth rate (SGR%), hepatosomatic index (HIS%) was calculated according to the method followed by Zhou *et al.* (2012).

### Growth Hormone Assay (GH)

Before centrifugation, blood was allowed to clot, then centrifuge at 3500 rpm for 10 min and blood serum was

collected in separate tubes. Serum growth hormone concentrations in all groups of *T. putitora* were estimated by using a Micro ELISA HGH kit (Amgenix MicroLISA$^{TM}$, USA). All samples were run in duplicate. The GH concentrations obtained from the kit were validated by verifying that the slope of the curve obtained by serial dilution of sample (0, 20, 40, 60 and 80%) matched the standard curve (P= 0.94). The intra- and inter assay coefficient of variation were found <12%.

### Hematology

Blood drawn by tail ablation was collected in VACUETTE® EDTA K3 tubes for the analysis of hematological indices. The red blood cells (RBCs) ($10^6$ $\mu L^{-1}$), hemoglobin (Hb) (g $dL^{-1}$) and hematocrit (%) values were determined by using a hematology analyzer (Sysmax KX-21 Japan).

### Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA) by using Statistic 8.1, Analytical Software. When a significant effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered with the significant level at $P < 0.05$.

## Results

### Growth Performance Indices

During feeding trial, no mortality was noted**.** The effect of graded level of APP on growth performance of *T. putitora* is shown in Table 2. The APP supplementation showed an effect in a concentration dependent manner, significant increase ($P < 0.05$) %WG was observed with $APP_{300}$ enriched diet (116.21 ± 1.04%) followed by $APP_{200}$ supplemented diet (Fig. 1). Moreover, $APP_{100}$ supplemented diet also indicated a positive effect ($P < 0.01$) on %WG as compared to basal diet, but the effect was less pronounced when compared with $APP_{200}$ and $APP_{300}$ enriched diets. Positive linear relationship was observed between the concentration of APP and %WG (Fig. 2).

The FCR, FCE% and SGR%, followed the same trend as % WG. Improved FCR, FCE% and SGR% were observed with $APP_{300}$ diet followed by $APP_{200}$ and then with $APP_{100}$ supplemented diets. APP supplemented diets also affected the HIS% value. The value increased with increase in concentration of APP in diet, therefore significantly ($P<0.05$) increased value was observed when fish reared on $APP_{300}$ enriched diet.

### Growth Hormone

The effect of graded level of dietary APP on serum GH levels of *T. putitora* is shown in Table 3. The growth hormone concentration in response to APP enriched diet showed the same trend as observed in %WG, significantly ($P < 0.05$) higher hormone level was observed with $APP_{300}$ supplemented diet followed by $APP_{200}$ and then with $APP_{100}$ enriched diets. The lowest level was observed in a group of fish reared on basal diet. The linear regression showed a positive relationship between dietary APP and GH level (Fig. 4), as well as between WG% and growth hormone level (Fig. 3).

**Table 1:** Formulation of 40 % protein basal diet

| Ingredients | Amount (g $kg^{-1}$) |
|---|---|
| White Fish meal | 250 |
| Soybean meal | 130 |
| Sunflower meal | 50 |
| Gluten 60% | 500 |
| Wheat bran | 10 |
| Rice bran | 10 |
| Wheat flour | 10 |
| Vitamin premix* | 08 |
| Canola Oil | 20 |
| Vitamin C | 02 |
| Di-calcium phosphate | 10 |
| Total | 1000 g |

*(Vitamin premix contains vitamins, amino acid and minerals premix kg-1) Vitamin AB.P 40,000,000IU, Vitamin $D_3$ B.P 820,000 IU, Vitamin E B.P 6200 mg, vitamin $K_3$ B.P 800 mg, Vitamin $B_2$B.P 2500 mg, Vitamin B3 B.P 5100 mg, Vitamin B12 B.P 1000 mg, Vitamin PP B.P 10,500 mg, L. lysine B.P 10,500 mg, DL-Methionine B.P 50,500 mg, Choline chloride USP 125,500 mg, Manganese USP 30,000 mg, 15,100 mg, Zinc USP 17,555 mg, Copper B.P 1000 mg, Cobalt B.P 50 mg, Iodine B.P 300 mg

**Table 2:** Effect of graded level of dietary L-ascorbyl-2-Polyphosphate on growth performance of juvenile mahseer (*Tor putitora*)

| | Parameters | | | | |
|---|---|---|---|---|---|
| Diets | %WG | FCR | FCE (%) | SGR (%) | HSI (%) |
| $APP_0$ | $64.6\pm0.52^{g}$ | $3.56\pm0.06^{a}$ | $28.06\pm0.13^{g}$ | $0.40\pm0.02^{g}$ | $1.44 \pm 0.01^{e}$ |
| $APP_{100}$ | $90.04\pm0.63^{f}$ | $2.56\pm0.01^{b}$ | $39.01\pm0.05^{f}$ | $0.55\pm0.04^{f}$ | $1.52 \pm 0.02^{d}$ |
| $APP_{200}$ | $98.54\pm0.69^{d}$ | $2.42\pm0.02^{c}$ | $41.32\pm0.17^{e}$ | $0.59\pm0.03^{d}$ | $1.65 \pm 0.08^{c}$ |
| $APP_{300}$ | $116.21\pm1.04^{b}$ | $2.08\pm0.01^{f}$ | $48.15\pm0.27^{b}$ | $0.67\pm0.05^{b}$ | $1.81 \pm 0.06^{b}$ |

Data are represented as Mean ± SE (n=30). Means are followed by different letter within the column are significantly different ($P < 0.05$). (ANOVA followed by LSD test)

**Table 3:** Effect of diets supplemented with graded levels of L-ascorbyl-2-Polyphosphate on the status of GH of juvenile mahseer (*Tor putitora*)

| Parameters/Diets | Growth Hormone (ng $mL^{-1}$) |
|---|---|
| $APP_0$ | $0.09\pm0.06^{g}$ |
| $APP_{100}$ | $0.095\pm0.05^{f}$ |
| $APP_{200}$ | $0.112\pm0.07^{c}$ |
| $APP_{300}$ | $0.116\pm0.05^{b}$ |

Data are represented as Mean ± SE (n=30). Means are followed by different letter within the column are significantly different ($P < 0.05$). (ANOVA followed by LSD test)

### Hematological Indices

The APP enriched diet also significantly improved

hematological parameters like RBCs, Hb and Hct% of *T. putitora* in a dose dependent manner. Significantly highest RBCs, Hb and Hct% values were observed in a group of fish fed $APP_{300}$ enriched diets, while $APP_{100}$ supplemented diet considerably improved the RBCs, Hb and Hct% values in comparison to basal diet, but their effect was less pronounced as compared to $APP_{200}$ and $APPP_{300}$ enriched diets (Table 4).

## Discussion

Among micronutrients, dietary ascorbic acid is essential for normal physiological functions, growth and immunity of fish (Ai *et al.*, 2006). No mortality was observed throughout the experiment, it seems that culture condition was favorable for the fish. In the present study, fish fed the basal diet had lower WG%, specific growth rate and FCE%, while APP enriched diet, increased the growth performance of *T. putitora* in a dose dependent manner. Therefore, fish fed $APP_{100}$, $APP_{200}$ and $APP_{300}$ enriched diet showed 25, 33.4 and 51%, respectively more WG as compared to fish fed basal diet. This finding indicated that AA could improve the growth performance and feed utilization of juvenile mahseer. Many other scientist also used the graded level of AA and reported variable amount for the optimum growth performance of different fish species like Kumari and Sahoo (2005) found positive relationship between dietary APP and weight gain and reported that Asian catfish (*Clarias batrachus*) showed best performance when they were fed 2000 mg APP kg-1 diet whereas parrot fish (*Oplegnathus fasciatus*) fed diet supplemented with 426 mg L-ascorbyl-2-monophosphate (AMP) kg-1 showed considerably comparable WG as observed when fish fed 1869 mg AMP enriched diet (Wang *et al.* 2003). Similarly, the growth performance of matrinxa (*Brycon amazonicus*) improved to increase concentration of AA in the diet and maximum WG was observed in response to 800 mg AA $kg^{-1}$ diet (Affonso *et al.,* 2007). However, in the Japanese sea bass (*Lateolabrax japonicus*) and Tilapia, *Oreochromis karongae*, optimum growth performance was observed with low doses of APP i.e., 53.5 and 60 mg kg−1 diet, respectively, while a further increase in AA level did not show any pronounced effect (Nsonga *et al.,* 2009). Moreover, Dicu *et al.* (2013) found an inverse relationship between dietary AA and specific growth rate of Stellate Sturgeon (*Acipenser stellatus*, Pallas, 1771). This inconsistency in dietary requirement of AA may arise due to variation of fish species, size, developmental stage, culture system, and environmental condition, interaction of nutrient and derivative of AA used in the study. In the present study, fry of *T. putitora* showed optimum growth performance when they were fed a diet containing higher amount of APP (300 mg $kg^{-1}$ diet) used in this study, thus there is a possibility that further increase in the amount of dietary further enhance the growth performance.

**Table 4**: Effect of graded level of dietary L-ascorbyl-2-Polyphosphate on hematological indices of juvenile mahseer (*Tor putitora*)

| | Parameters | | |
|---|---|---|---|
| Diets | RBCs ($10^6$ µL$^{-1}$) | Hb (g dL$^{-1}$) | Htc (%) |
| $APP_0$ | $2.26 \pm 0.08^{f}$ | $7.3 \pm 0.20^{e}$ | $32.92 \pm 0.01^{f}$ |
| $APP_{100}$ | $2.53 \pm 0.08^{e}$ | $7.33 \pm 0.03^{d}$ | $33.7 \pm 0.20^{e}$ |
| $APP_{200}$ | $2.86 \pm 0.04^{cd}$ | $8.04 \pm 0.14^{c}$ | $34.13 \pm 0.03^{d}$ |
| $APP_{300}$ | $3.26 \pm 0.08^{b}$ | $8.65 \pm 0.13^{b}$ | $35.1 \pm 0.10^{b}$ |

Data are represented as Mean ± SE (n=30). Means are followed by different letter within the column are significantly different ($P < 0.05$). (ANOVA followed by LSD test)

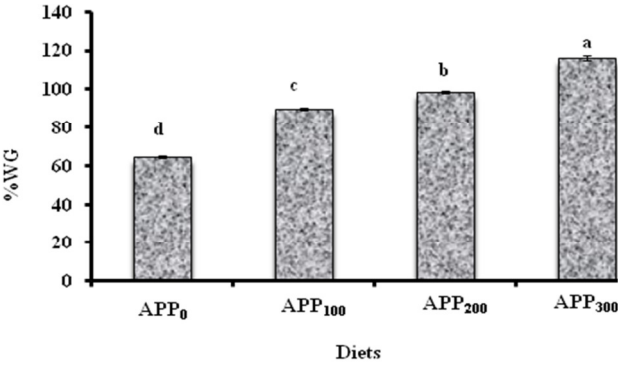


**Fig. 1:** The increase in %WG of juvenile *Tor putitora* after 70 days, in response to graded level of dietary L-ascorbyl-2-Polyphosphate. Each bar represents the values as Mean ± SE (n=30). Means are followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by LSD test)

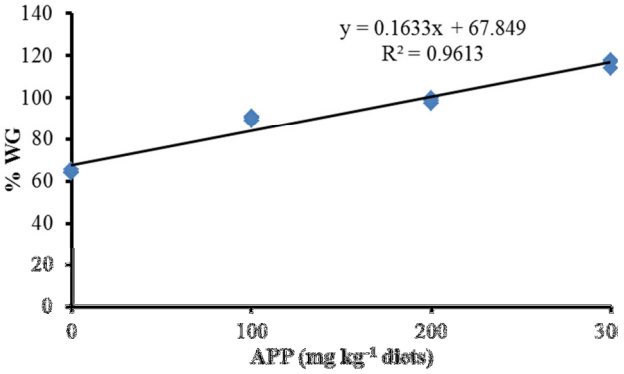


**Fig. 2:** Positive correlation between dietary APP and %WG of *Tor putitora*

The improved %WG, SGR, FCE% observed in the present study and reported previously in response to dietary AA supplementation may arise due to the positive effect of supplemented AA on protein metabolism and feed efficiency (Eo and Lee, 2008; Miar *et al.,* 2013). It is well recognized that vitamin C plays a significant role in the growth (Ai *et al.,* 2006; Zhou *et al.,* 2012) by enhancing

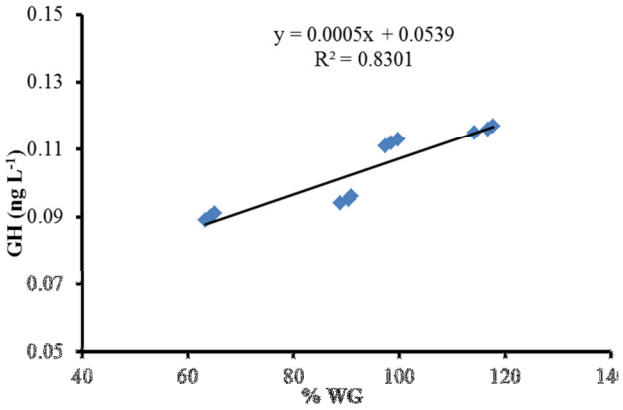


**Fig. 3:** Positive correlation between GH levels and % WG of *Tor putitora*

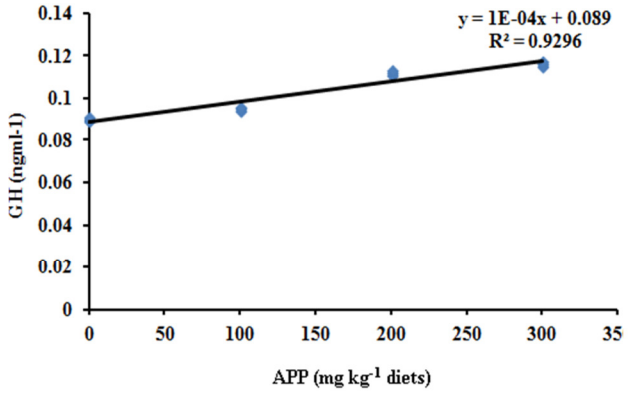


**Fig. 4:** Positive correlation between dietary APP and GH levels and % WG of *Tor putitora*

collagen and carnitine biosynthesis (Aysun, 2009), amidation of peptide hormones (Jimenez-Fernandez *et al.,* 2012), metabolism of iron (James, 2001) and immunity (Anbarasu and Chandran, 2001; Ai *et al.,* 2006) or by acting as a resistant modulator (Tewary and Patra, 2008; Eo and Lee, 2008).

Hepatosomatic index is the indicator of health and energy reserve of the organism. In the present study, APP supplementation has increased the HSI% of *Tor putitora* in a concentration dependent manner. This gain may be due to improve feed consumption. Similar results were obtained in other studies conducted on parrot fish (Wang *et al.*, 2003), tiger puffer (Eo and Lee, 2008), cobia (Zhou *et al.,* 2012). Moreover, Channel catfish (*Ictalurus punctatus*) fed ascorbic acid deficient diet showed a decrease hepatosomatic index (HIS %) than the fish fed ascorbic acid enriched diet (Gouillou-Coustans and Kaushlic, 2000).

In this study, we also observed positive correlation between GH level and APP supplementation (Fig. 4). Like our results Denny-Brown (2012) reported the similar close association between vitamin C intake and GH secretion in human and suggests a possible physiological connection between these two covariates. It is well known that vitamin C besides acting as a strong antioxidant also functions as a co-factor of an enzyme peptidylglycine α-amidating monooxygenase (PAM), which is responsible for amidation of various neuropeptides (Eipper *et al.,* 1993). Thus, many investigators including Eipper *et al.* (1993) observed the very high tissue concentrations of PAM activity in both the hypothalamus and pituitary and suggest its relation with the dose of vitamin C intake. Vitamin C actively transports across the membranes by using sodium-ascorbate cotransporter (SVCT) 1 and 2 (Savini *et al.,* 2008). The SVCT 2 are particularly expressed in the endocrine cells, neurons (Hediger, 2002) and hypothalamic tanycytes (García *et al.,* 2005), thus allowing local high concentration as compared to circulating vitamin C. Generally PAM activity has not been directly evaluated in regard to regulators of GH release instead it is related to growth hormone releasing hormone (GHRH), which is a C-terminal amidated peptide. Thus an increase in concentration of GH level in response to dietary vitamin C observed in the present study and reported previously may be due to increase affinity of SVCT2 with AA, which permit higher level of AA in the hypothalamus that leads to activation of PAM and higher efficiency of amidation of GHRH which in turn increase the concentration of GH (Denny-Brown *et al.,* 2012).

In several teleost, higher circulating GH level resulted in an increase in food intake that subsequently improve feed conversion efficiency and growth rate (Won and Borski, 2013). We also observed positive correlation between GH and APP supplementation (Fig. 4). The APP supplementation considerably ($P < 0.05$) increased the growth hormone levels and improved the FCR of *T. putitora*, in a dose dependent manner which in turn showed a positive relationship with the WG% (Fig. 3).

Ascorbic acid is a powerful antioxidant; provide protection to various tissues of fish including red blood cells against oxidative destruction (Sahoo and Mukherjee, 2003), while deficiency of AA caused anemia (NRC, 2011) because of poor absorption and redistribution of iron that lead to reduction in the synthesis of Hb. Among physiological markers, hematocrit is considered as an easy and immediate biomarker that could be used as a predictor of future growth performance. It represents the volume of RBCs, normally uses to diagnose anemia (Miar *et al.,* 2013). It is well documented that inadequate amount of AA, probably impair the membrane integrity of erythrocyte, cause hemolysis and eventually the mortality of erythrocyte due to peroxide production (Nayak *et al.,* 2007). There is a possibility that low levels of dietary AA reduce the antioxidative capacity of fish, thus causes tissue damage and make it susceptible to inflammation and infectious diseases (Chen *et al.,* 2003). In Catfish (*Pangasianodon gigas*) the best recorded values of Hct and RBCs count were attained, when fish fed AA at the rate of 500 and 750 mg $kg^{-1}$ diet (Pimpimol *et al.,* 2012), whereas in Juvenile matrinxa

(*Brycon amazonicus*) 600 and 800 mg AA $kg^{-1}$ diet showed the positive effect on RBCs, Hct and Hb values (Affonso *et al.,* 2007). Zhou *et al.* (2012) also reported the increased in Hct, Hb, and RBCs values of cobia (*Rachcentron canadum*) in response to dietary AA supplementation. Like these studies our results also showed significant increase in RBCs, Hb and Hct values of *T. putitora* in response to APP supplemented diets and support the view that AA involve in the removal of hydrogen peroxide and deactivation of superoxide radicals, singlet oxygen or superoxide, thus maintain the integrity of RBCs and protect them from oxidative stress.

## Conclusion

In conclusion, %WG, FCE%, serum GH level, RBCs count, Hct% and Hb level of *T. putitora* showed a close association with dietary vitamin C content. Therefore, feeding graded levels of APP increased the growth rate and hematological parameter of fish in a dose dependant manner. The results clearly indicated the positive effect of dietary AA and suggest that approximately 300 mg APP $kg^{-1}$ diet is adequate for better growth and health of juvenile *T. putitora.*

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