E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2021; 9(3): 126-130

www.fisheriesjournal.com
Received: 16-03-2021
Accepted: 18-04-2021

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Effects of vitamin C supplementation on growth performance and immune responses of juvenile Waigieu seaperch (*Psammoperca waigiensis*)

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DOI: https://doi.org/10.22271/fish.2021.v9.i3b.2497

Abstract
This study was designed to investigate the effects of vitamin C supplement on growth performance and immune responses of Waigieu seaperch *(Psammoperca waigiensis*) at juvenile stage, 90 fish of an average initial weight of (33 ± 0.1 g and 14. ± 0.1 cm) were stocked in 10 tanks (20 L) during 30 days and fed with different level of supplemented feed with vitamin C 0, 150, and 400 mg/kg with three replicates for each and fed twice a day 8:00 and 16:00 hr. At the end of experiment both growth and hematological parameters were determined, results revealed that there was no significant difference in survival ratio (SR) was observed during among the experimental groups, fish fed with 400 mg/kg had significantly (P<0.05) higher specific growth rate, protein efficiency ratio; and significantly (P<0.05) better feed conversion ratios than non-supplemented fish. Hematological parameters showed a significant increase (P<0.05) with dietary AA level was increased as supplemented diets increase. Proximate compositions of the experimental such as average crude protein, crude fat, moisture, ash entire treatment diets utilized were no significant difference (P>0.05). Our data show that ascorbic acid is essential for Waigieu sea perch hematological and growth performance parameters.

Keywords: Waigieu seaperch, *Psammoperca waigiensis*, growth performance, immune responses of juvenile

Introduction
Vitamin C is a water-soluble vitamin required by most species, evidence form several study demonstrated that level of ascorbic acid (AA) varies form 30 mg/kg to 400 mg/kg below or higher can cause severe deformities (Dawood and Koshio 2018) [8] for instance locally made feed 92 mg/kg AA should be a minimum rage to supplement catfish (Okhionkpamwonyi and Edema 2017) [25] but for *O. karongae a maximum* supplementation of AA is 60 mg/kg (Nsonga *et al.* 2009) [24].
Some pathogen and strains can be treated by high level of AA at the same time increase immunity resistance of several fish (Patra 2008, Dawood and Koshio 2018) [8, 28]. Several published studies have demonstrated an enhanced immune response of fish through dietary supplementation with vitamin C (Landolt 1989, Dawood and Koshio 2018) [8, 17], feeding supplemented feed to Waigieu sea perch in its seasonality reproductive which are from April to September (Pham *et al.* 2012) [29] showed good performance based that estimation of gonadosomatic index (GSI) and histological shown preferred spawning season is from April to October (Shimose and Tachihara 2006) [33], but for induction spawning hormonal stimulation can be collected after 48 hours with limited dose in order to allow efficacy of milt properties (Le and Brown 2016, Le *et al.* 2014) [18, 19]. Waigieu seaperch brood stock can be well managed thought sperm cryopreservation by provision of hybrid breeding and its reproduction (Le and Pham 2017) [20]. Masengesho *et al.* (2021) [22] reported that fish size and feeding rate also influenced on growth performance and immune response of Waigieu seaperch at the juvenile stage.
Many studies showed that interplay between nutrition and immunity at the level of innate immune components (Barros *et al.* 2014, Barros *et al.* 2015, Biswas *et al.* 2010, Dawood and Koshio 2018, Dawood *et al.* 2018) [4-6, 8, 9]. The objective of this study was to determine effect of vitamin C on growth performances and immune response of Waigieu sea perch.

Materials and Methods

Location and time

Larval and fry rearing were conducted in Luong Son hatchery, Khanh Hoa province (20.8719° N, 105.5040° E). After 3 months, juveniles was transported in aerated plastic bags to wet laboratory in Nha Trang University, Khanh Hoa province (12'52' 35'' N and11 41 53''S) for experiment. All the samples were analyzed in biological laboratory in Nha Trang University, Vietnam.

Experimental system

Juveniles were stocked in fiber-glass tanks (250 L/tank) arranged in series with 3 replicates per treatment. All tanks were connected to recirculating water system. The water of 35 ppt was exchanged 30% daily and 90% weekly. Each tank had one air-diffuser to maintain oxygen concentrations, which did not decrease below 5.5 mg/L. During the experiment, the oxygen was no less than 4.5 mg/L, total ammonia was less than 0.5 mg/L. The pH ranged from 7.5 – 8.0, while temperature was 28 ± 1 ℃.

Experimental design during

Fish was fed a commercial diet containing 44% protein and 7% lipid (C-5001, UP, Vietnam) with 3 levels (0, 150 and 400 mg/kg) of vitamin C supplementation. After 1 week of acclimatization, juveniles (33 ± 0.13g/fish) were randomly distributed into 9 fiber-glass tanks (250 L/tank) at the stocking density of 10 fish per tank. Each vitamin C supplemented treatment was randomly assigned into three positioned tanks. Fish were fed by hand at the feeing rate of 5% BW at 8.00 and 16.00 for four weeks. 10 minutes after feeding, uneaten feed was collected by siphoning.

Sample collection

At the end of the experiments, all fish were individually weighed. Before weighing fish were starved for 24 hours and anaesthetized with tricaine methanesulfonate (MS-222; Argent Chemical, Redmond, WA). Three fish in each tank were used to remove muscle tissues for proximate composition. Blood samples from three fish per tank were collected by puncturing their caudal vein using a syringe and transferred to BD vacutainer tubes (with K2E 5.4 mg, United Kingdom) for haematological analysis.

Proximate composition

Crude protein content was analyzed using Kjeltec Auto 1030 analyzer (Foss Tecator, Höganäs, Sweden), and crude lipid was analyzed by petroleum ether extraction in a Soxhlet extraction system. Moisture was determined by drying at 105 ℃ in an oven (Thermotec 2000, Contherm Scientific, Hutt, New Zealand). Ash was determined by combustion at 550 ℃ for 24 h in an electric furnace (Carbolite, Sheffield, UK).

Hematological analysis

Hematocrit (Hct) was determined using the microhematocrit method (Brown, 1980) and Sahli's haemoglobinometer was used to estimate hemoglobin (Hb) percentage (Hb %). The WBCs and RBCs were determined by diluting blood and enumeration in a haemocytometer or hemacytometer. MCV, MCH and MCHC were calculated indirectly with reference to RBC, Hct and Hgb; therefore, their changes are directly linked with these blood parameters. The mass spectrometry was used to determine blood protein; triglyceride was determined using VLDL test.

Calculation and statistical analysis

The following parameters in all tanks were calculated using the following equations:

Survival (%) = 100 × final fish number/initial fish number

Weight gain (WG g/fish) = final weight – initial weight

Specific growth rate (SGR %/day) = 100 × [(Ln FBW – Ln IBW)/feeding period (days)]

Final body weight ═ (FBW)

Feed conversion ratio (FCR) = feed intake in dry matter (g)/body weight gain (g)

Protein efficiency ratio (PER) = body weight gain (g)/protein fed (g)

Feed intake (FI) ═ Feed used - Uneaten feed

Mean corpuscular volume (MCV) = Hematocrit/Red blood cell

Mean corpuscular hemoglobin concentration (MCHC) = Hemoglobin/Hematocrit

All data were expressed as mean ± SD unless otherwise specified. All data were analyzed by one-way ANOVA. Duncan multiple comparison was used post hoc to rank mean values using SPSS for Windows version 22 (IBM, New York, USA). Significance level of $P< 0.05$ was used for all data.

Results

Growth and supplemented diet

It is evident from (Table 1) that, FBW, SGR and WG of Waigieu sea perch was significantly affected by feeding vitamin C. Fish fed 400 mg/kg had significantly higher FBW, SGR and WG compared with fish fed with 150 mg/kg and 0 mg/kg. Feed intake (FI) and protein efficiency ratio (PER) were significantly lower in fish fed 0 mg/kg ($P<0.05$); whereas, there was no significant effect on FI or PER of the fish fed 400 mg/kg and 150 mg/kg ($P<0.05$).

Fish fed with 400 mg/kg had significantly high FI and PER than with fish fed with 150 mg/kg and 0 mg/kg. There were no significant effects of supplemented diet of 0 mg/kg and 150 mg/kg on feed conversion ratio (FCR) ($p>0.05$) but there was significant effects on the fish fed with supplemented of 400 mg/kg ($P<0.05$). Fish fed with 400 mg/kg had significantly lower FCR than with fish fed with 150 mg/kg and 0 mg/kg.

Table 1: Vitamin C supplementary diets on growth performance of Waigieu sea perch after 4 weeks culture

Parameters	0 mg/kg	150 mg/kg	400 mg/kg
IBW (g/fish)	33 ± 0.01	33 ± 0.06	33 ± 0.13
FBW (g/fish)	39.33 ± 1.00a	45.00 ± 1.86b	51.57 ± 4.04c
SGR (%/day)	0.63 ± 0.09a	1.11 ± 0.15b	1.59 ± 0.28c
WG (g/fish)	6.33 ± 1.00a	12.00 ± 1.86b	18.67 ± 4.04c
FI (g/fish)	12.05 ± 2.54a	20.88 ± 3.10b	26.24 ± 6.19b
FCR	1.89 ± 0.10b	1.74 ± 0.09b	1.41 ± 0.13a
PER (%)	1.20 ± 0.06a	1.31 ± 0.07a	1.63 ± 0.16a
Survival (%)	100	100	100

Values are displayed as mean of triplicate group's ± SD. Means with different alphabets within a row are significantly different (P<0.05).

Proximate composition

Crude protein concentrations in fish fed 0 mg/kg vitamin C was significantly lower than fish fed the other two diets.

Crude lipid concentrations in fish fed 0 or 150 mg/kg vitamin C were significantly higher than in fish fed 400 mg/kg. Moisture concentrations in fish fed 400 mg/kg vitamin C were significantly higher than in fish fed lower concentrations of the vitamin. There were no significant differences in ash concentrations among dietary treatments.

Table 2: The results of the proximate composition (% of wet weight) for experimental diet

Parameters	0 mg/kg	150 mg/kg	400 mg/kg
Crude protein	17.89 ± 1.40a	18.58 ± 1.93b	18.75 ± 1.41b
Crude lipid	4.93 ± 0.28b	4.58 ± 0.41b	4.12 ± 0.27a
Moisture	72.96 ± 1.56a	72.65 ± 2.58a	73.78 ± 1.09b
Ash	1.85 ± 0.21a	1.70 ± 0.21a	1.75 ± 0.20a

Values are displayed as mean of triplicate groups ± SD. Means with different alphabets within a row are significantly different (P<0.05).

Hematological parameters

Fish fed 400 mg/kg had significantly higher WBC concentrations compared to fish fed 150 mg/kg and 0 mg/kg (Table 3). Hematocrit (Hct) and hemoglobin (Hb) concentrations were significantly higher than in fish fed lower concentrations of vitamin C.

There was significant increase in RBC concentrations across all three treatments, increasing as vitamin concentrations increased. MCV and MCH in increased as vitamin C concentrations increased, with significantly higher values observed in fish fed 400 mg/kg

Mean Corpuscular Hemoglobin Concentration (MCHC) was significantly higher in fish fed 400 mg/kg compared to fish fed lower concentrations. Platelet (PLT) concentrations were not significantly affected by dietary treatments. Triglyceride and protein /blood concentrations were significantly higher in fish fed 400 mg/kg compared to fish fed no supplemental vitamin C concentrations in the diet.

Table 3: Hematological parameters results in fish fed with different diets (With different vitamins C levels) throughout experimental period of 30 days

Parameters	0 mg/kg	150 mg/kg	400 mg/kg
WBC (×103 µl)	6.67 ± 0.37a	6.97 ± 0.49a	8.37 ± 0.41b
RBC (×106/µl)	1.93 ± 0.15a	2.3 ± 0.26b	3.17 ± 0.05c
Hb (mg%)	7.03 ± 0.20a	7.53 ± 0.49a	9.3 ± 0.10b
Hct (%)	22.63 ± 0.49a	23.6 ± 1.10a	26.7 ± 0.43b
MCV(fl)	117.58 ± 9.9b	103.22 ± 8.7b	84.35 ± 2.93a
MCH (pg)	36.49 ± 2.32b	33.15 ± 5.4ab	29.38 ± 0.62a
MCHC (g/dL)	31.08 ± 0.7a	31.97 ± 2.6ab	34.84 ± 0.64b
PLT (×109/L)	23.33 ± 1.52a	23.67 ± 1.52a	23.75 ± 1.73a
Triglycerides (mmol/L)	4.2 ± 0.52a	4.21 ± 0.31a	5.55 ± 0.88b
Protein/blood (%/ml)	37.4 ± 2.55a	38.1 ± 0.45ab	43.37 ± 4.23b

Values are displayed as mean of triplicate group's ± SD. Means with different lowercase alphabets within a row are significantly different (P< 0.05). WBC: White blood cell; Ht: Hematocrit, RBC: Red blood cell, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration.

Discussion

Present findings were in agreement with Nsonga *et al.* (2009) [24] indicated that vitamin C significantly affects the weight gain of juvenile Waigieu sea perch, in present study result shown that 400 mg/kg vitamin C in fish diet showed significantly better weight in terms of final body weight (FBW), specific growth rate (SGR) and weight gain (WG), also had significantly better feed efficiency indices such as feed conversion ratio (FCR), feed intake (FI) and protein efficiency ratio (PER), compared with others treatment, similar result was reported by Henrique *et al.* (1998) [14] and Ai *et al.* (2004) [2], contrary to prawn fed diet containing 0-100 vitamin C/kg were no difference for growth performance (Asaikkutti *et al.* 2018) [3].

Fish fed with 0 mg/kg had significantly lower final body weight (FBW) than 150 and 400 mg/kg, in contrast to the weight gain (WG) of fish fed supplemented feed with 400 mg/kg had significantly higher affect compared with fish fed at 150 and 0 mg/kg (Table 1), the proportion was the same as in specific growth rate (SGR) similarly findings were reported by Miar *et al.* (2013) [23] and Asaikkutti *et al.* (2018) [3], contrast to Caspian brown trout fed on 50, 100, 200, 400 mg/kg, where 200 mg/kg hard higher SGR compared to the other treatments (Islami and Arab 2015) [15]. Sufficient

vitamin C supplementation in fish feeds is necessary under difference aquatic culture system for better survival and growth (Lin and Shiau 2005) [21]. In our study, the lower value of feed conversion ratio (FCR) was observed in the fish fed with 400 mg/kg correspondence with better growth, compared to the higher FCR of 0 and 150 mg/kg, similar result was presented by Blom *et al.* (2000) [7] and Miar *et al.* (2013) [23] contrast to Caspian brown trout fed diet containing 50-400 vitamin C/kg were lower value of FCR was found in 200 mg/kg (Islami and Arab 2015) [15], addition to that vitamin C deficiencies read to the poor food conversion ration which will be poor growth and poor diseases resistance (Asaikkutti *et al.* 2018) [3].

The value of feed conversion ratio are indication of fish growth, quality and quantity of fed used as well as the water quality (Okumuş and Mazlum 2002) [26]. It is evident that fish fed with 400 mg/kg had higher figures of feed intake (FI) than others treatment; it is obvious that higher feed intake is proportional to the growth performance as result revealed in our study.

In the present study, the fish fed with 400 mg/kg had a high protein efficiency ratio (PER) compared with fish fed with 0 and 150 mg/kg, this is the indication of higher protein utilization by fish fed with supplemented diet, our study is in agreement with Fracalossi *et al.* (1998) [13] reported similar trend in juvenile oscars (*Astronauts ocelots)* cichlids. And Miar *et al.* (2013) [23] presented that PER was lower in the trout fed with lower AA compared to trout fed with higher AA. In our study, the numbers of PER increased with increasing vitamin C supplementation, contrary to Fauconneau *et al.* (1986) [11] reported that the relationship between dietary protein and PER differs from species to species and fed contents.

Hematological parameters are reliable indicators for health status in several animal fish included, but they can vary with numerous factors like: season, temperature and nutritional status (Fazio 2019) [12]. It is clearly depicted in Table 3 that the fish fed with ascorbic acid diet had higher figures of WBC, RBC, Hb, Hct, MCHC, and triglyceride and protein blood; Similar to present findings of hematological parameters have also reported by Shahkar *et al.* (2015) [32]. Contrary to the higher figures of MCV and MCH was noticed in none -supplemented dies and exception were observed in of the figures of platelet which was nearly the same in all feeding trials. Our finding indicates a higher white blood cell (WBC) in the fish feed with 400 mg/kg compared with 150 and 0 mg/kg; similarly finding were reported by Miar *et al.* (2013) [23]. A similar proportion was observed in the flowing blood parameters, hemoglobin (Hb), red blood cell (RBC) and hematocrit (Hct). A higher number of RBC in the fish fed with 400m/kg in our study is the indication of enhancement of immune responses and the ability of O_2 carrying capacity similar result were observed in catfish fed on supplemented diet of 750 mg vitamin C/kg compared with other treatment of 0, 250, 500 and 750 mg vitamin C/kg (Pimpimol *et al.* 2012) [30]. Most immune response and the best growth are enhanced by supplemented diets with vitamin C (Henrique *et al*., 1998) [14].

For erythrocyte content index a higher number of MCV and MCH were noticed in the fish fed without ascorbic acid In agreement with our results (Adel and Khara 2016) [1] whereas higher MCHC was observed in fish fed with 400 mg/kg. A high number of erythrocyte content indexes were implication of good physiological status of fish. In the present study, the higher value of protein in blood was observed in the fish fed with 400 mg similarly result was reported by de Andrade *et al.* (2007) [10]. A high vitamins C in fish feed could enhance protein synthesis and total serum protein. Moreover, innate immune response could be implication of total protein (Sahoo and Mukherjee 2003, Ortuno *et al.* 1999, Ai *et al*. 2004) [2, 27, 31]. The result from our study showed that a high number of triglyceride was recorded in the fish fed with 400 mg/kg compared with others feeding trials. So high vitamin C concentrations are able to enhance growth performance and immune responses of many species as well as we presented in our study of Waigieu sea perch juveniles (Table 2). Proximate composition of crude protein in the fish fed with 400 mg/kg had high numbers compared with 150 and 0 mg/kg. Contrary to the high body lipid content of the fish fed with none-supplemented diet another wards, this experiment whole-body crude lipid content increased with the decreasing supplementation dietary levels which agreed with the results found by Kim and Kang (2015) [16]. The proportion was the same in the ash but for protein and moisture increases as supplementation increases. In conclusion growth performance and hematological of fish under supplemented diet should be further investigated.

In conclusion, the results from the present study indicate that the estimated optimum dietary AA at 400 mg/kg may improve the growth performance and enhance the hematological parameters as well as increase proximate compositions in juvenile Waigieu seaperch (*P. waigiensis*). We recommend that Waigieu seaperch farmers may supplement the diets of fish with 400 mg/kg AA for normal fish growth, physiology and disease protection in aquaculture.

Acknowledgement

This research was supported by Vietnam National Foundation for science and technology Development (NAFOSTED) under grant number 106.05-2017.343 is greatly acknowledged.

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