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Dietary ascorbic acid requirement for the optimum growth performances and normal skeletal development in juvenile hybrid grouper, *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*

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ABSTRACT

A 10-week feeding trial was conducted to determine the dietary ascorbic acid required by the juvenile hybrid grouper, *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* for its optimum growth, survival, and normal skeletal development. Eight experimental diets containing graded levels of ascorbic acid (4.8, 11.2, 24.1, 47.2, 75.6, 95.4, 156.2, and 303.0 mg/kg) were prepared and labeled as C5, C11, C24, C47, C76, C95, C156 and C303, respectively. Each diet was fed to triplicate groups of fish [initial weight 7.71 ± 0.06 g (mean ± SD)]. The fish were cultured in 150 L of fiber glass tank supplied with aeration and flow-through seawater system (3 L min^{-1}) with the stocking density of 15 individual per tank. During the feeding trial, fish were hand-fed with the experimental diets to apparent satiation twice a day (8:00 and 15:00). Bulk weight of each fish group was measured at 2 weeks interval. At the end of the experiment, fish were sacrificed and subjected to radiographic imaging to detect the presence of skeletal deformities. The body weight gain (BWG) of fish was in the range from 628.51 ± 39.61 to 880.18 ± 113.30%. Fish fed with the C156 diet gained the highest BWG and specific growth rate (SGR). In the present study, ascorbic acid level did not affect the survival of the hybrid grouper. The feed conversion ratio (FCR) value in all dietary treatments appeared to be less than 1, indicating hybrid grouper have high efficiency of converting feed into body mass. Multiple types of skeletal deformities (fusion, kyphosis, lordosis, and scoliosis) were observed in the fish fed with the diets containing less than 95 mg/kg of ascorbic acid. In conclusion, dietary ascorbic acid levels can affect the growth performance and normal skeletal development in the hybrid grouper. Although 95 mg/kg was sufficient for normal skeletal development, 156 mg/kg of dietary ascorbic acid is recommended for feed development to maintain the optimum growth and normal skeletal development in the fish.

1. Introduction

The hybrid grouper, *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* is a popular cultured fish in the Southeast Asia. It is first produced at the Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia in 2006. The hybrid grouper has several advantages than its maternal species (*E. fuscoguttatus*) as an aquaculture species especially in terms of faster growth and high tolerance to low salinity water (Othman et al., 2015). It has high demand and can fetch good market price especially in the live food fish trade in the Asia Pacific region (Senoo, 2010). Despite the emerging production and its popularity, deformities incidence is commonly observed. To the best of the authors' current knowledge, very limited literature has discussed the deformities of this species (if there is any). Vertebral deformities occurrences were widely discussed in marine fish species literatures (Witten et al., 2005; Boglione et al., 2009; Baeverfjord et al., 2009; Koumoundouros, 2010; Boglione et al., 2014). Deformed fish were usually discarded during the grow-out period or/and be sold at very low price than market value, which could lead to economic losses. Given its significance to the aquaculture industry, it is of importance to address the issue of deformities for this specific hybrid species.

There are several factors causes the deformities in fish such as rearing condition, genetic, and vitamin deficiency. One of many

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key vitamins needed by fishes is the ascorbic acid. Previous authors have discussed and published their works on the protein and lipid requirements, protein to energy ratio and carbohydrate utilization of this hybrid grouper (Jiang et al. 2015, 2016; Rahimnejad et al., 2015; Luo et al., 2016). However, there is still no information on its ascorbic acid requirement. Ascorbic acid is one of the essential micronutrients in the diets of fish, yet, most teleost fish are unable to synthesize ascorbic acid on its own due to the lack of L-gulonolactone oxidase (EC 1.1.3.8) enzyme (Wilson and Poe, 1973). It has many important roles to the fish health, including to promote growth and normal skeletal development (Lin and Shiau, 2005; Ai et al., 2006; NRC, 2011; Chen et al., 2015). Hence, supplementation of ascorbic acid in fish feeds is critical. Nonetheless, ascorbic acid requirement in fish varies among different fish species (e.g. Merchie et al., 1996; Alexis et al., 1997; Phromkunthong et al., 1997; Lee et al., 1998; Fournier et al., 2000; Wang et al., 2003a; Lin and Shiau, 2005; Xiao et al., 2010; Zhou et al., 2012; Kim and Kang, 2015). Information on the ascorbic acid requirement of the targeted fish species should be determined before dietary supplementation can be practiced. Therefore, the present study was conducted to determine the requirement of dietary ascorbic acid by the hybrid grouper for its optimum growth and skeletal development.

2. Materials and methods

2.1. Experimental diets

Eight experimental diets with different supplementation levels of ascorbic acid were prepared in this study (Table 1). The basal diet was formulated to contain 50% crude protein and 16% crude lipid which are required for the optimum growth performance of juvenile grouper (Shapawi et al., 2014). L-ascorbic acid (Merck, Germany) was supplemented separately into each diet at the expense of alpha cellulose, and the corresponding levels of the dietary ascorbic acid were 4.8, 11.2, 24.1, 47.2, 75.6, 95.4, 156.2, and 303.0 mg/kg. These diets were labeled as the C5 (control – no ascorbic acid supplemented), C11, C24, C47, C76, C95, C156 and C303, respectively. The ascorbic acid content of the diets was determined by a reverse phase high performance liquid chromatography – HPLC (Shimadzu, Japan). Briefly, feed samples were homogenized in 10% cold metaphosphoric acid then the homogenates were centrifuged at 3000×*g* for 20 min. The supernatant were filtered through a 0.45 μm syringe filter then analyzed by a reversed phase HPLC with a Hypersil Gold C18 column (5 μm, 150 × 4.6 mm) with an ultraviolet detector at 254 nm. The mobile phase was 0.05 M KH_2PO_4 at pH 2.8 and the flow rate was 1.0 ml min^{-1}. For the diet preparation, all ingredients were ground into fine powder and thoroughly mixed with fish oil. Then, water was added to produce moist dough. The dough was then screw-passed through a 3-mm die and the strands of feeds were air dried at room temperature with the aid of air conditioner and an electrical fan. After drying, the strains were broken up, kept in plastic zip bag and stored in a freezer at −80 °C until use.

2.2. Feeding trial

Hybrid grouper, *E. fuscoguttatus* × *E. lanceolatus* juveniles were obtained from a local fish farmer in Tawau, Sabah. Prior to the feeding trial, all fish were acclimatized to tank culture condition for 2 weeks and fed with the control diet (C5 diet). The fish (average body weight of 7.71 ± 0.06 g) then were randomly distributed into 24 fiber glass tanks at a stocking density of 15 individual per tank. The fish were cultured in 150 L of tank supplied with aeration and flow-through seawater system (3 L min^{-1}). The water temperature and salinity were recorded at 28.5–31.0 °C and 30–33 gL^{-1}, respectively. Each dietary treatment was hand-fed to triplicate tanks of fish twice a day at 8:00 and 16:00. The fish were fed until apparent satiation in each feeding session. The amount of eaten feed and fish mortality were recorded daily for the calculation of feed utilization efficiency and survival. Bulk weight of the fish from all dietary treatments was measured once every 2 weeks.

2.3. Sample measurement and analysis

At the end of the feeding trial, all fish were fasted for 24 h and weighed individually. Consequently, the weight gain (WG), specific growth rate (SGR), survival, feed intake (FI), and feed conversion

Table 1
Diet formulation and proximate analysis of experimental diets (% dry matter basis).

Ingredients (per 100 g)	Diets							
	C0	C12	C24	C47	C76	C95	C156	C303
Fish meal[a]	70.8	70.8	70.8	70.8	70.8	70.8	70.8	70.8
Tapioca starch[b]	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
Alfa-Cellulose	0.1	0.098	0.095	0.092	0.085	0.075	0.06	0.02
CMC[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix[d]	3	3	3	3	3	3	3	3
Ascorbic acid	0	0.002	0.005	0.008	0.015	0.025	0.04	0.08
Mineral premix[e]	2	2	2	2	2	2	2	2
Dicalcium phosphate	1	1	1	1	1	1	1	1
Fish oil	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Proximate composition								
Moisture	11.84	11.28	12.00	11.11	11.36	11.77	11.32	12.23
Crude protein	50.13	50.79	50.47	50.18	50.74	50.95	49.77	50.37
Crude lipid	17.00	16.35	16.40	16.91	16.62	16.26	16.32	16.65
Ash	11.29	10.87	11.58	10.91	11.17	10.90	11.31	11.34

[a] Danish fish meal, TripleNine 999 Fish Protein, Denmark.
[b] Golden Fish brand. Bake with Me Sdn. Bhd., Malaysia.
[c] Carboxymethyl cellulose (CMC), Sigma.
[d] Vitamin mixture (g/kg mixture): Inositol, 5.0; choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl, 0.92; d-calcium panothenate, 3.0; retinyl acetate, 0.60; vitamin D3, 0.083; Menadione, 1.67; DL alpha tocopherol acetate, 8.0; d-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135. All ingredients were diluted with alpha cellulose to 1 kg.
[e] Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274.

ratio (FCR) of fish from all dietary treatments were calculated using the following formula:

$$\text{Body weight gain} \quad (\text{BWG})(\%) = [(\text{final weight},(\text{g}) - \text{initial weight}(\text{g}))/\text{initial weight}(\text{g})] \times 100$$

$$\text{Specific growth rate}(\text{SGR})(\%/\text{d}) = [ln(\text{final weight}(\text{g})) - \ln(\text{initial weight}(\text{g}))/\text{days}] \times 100$$

$$\text{Survival}(\%) = (\text{final fish number} - \text{initial fish number})/\text{initial fish number} \times 100$$

$$\text{Feed Intake}(\text{FI})(\text{g}) = \text{Total feed intake for10weeks}$$

$$\text{Feed conversion ratio}(\text{FCR}) = \text{dry feed consumed}(\text{kg})/\text{wet weight gain}(\text{kg})$$

2.4. Examination of skeletal deformities

Eight fish or half population of fish from each replicate (totally 24 samples from each dietary treatment) were randomly selected and sacrificed by immersing them into ice water. The samples were frozen in a freezer (−80 °C) then subjected to X-ray imaging to examine the fish skeletal morphology. Radiographs were taken using rotating anode X-ray tube, model DRX-1603B (Toshiba Electron Tubes & Devices Co., Ltd., Japan) with an exposure of 100 mAs at kV 65. The X-ray images were taken and used for the determination of skeletal deformities. Skeletal deformities were categorized into four types (fusion, lordosis, kyphosis, scoliosis) based on morphological features according to Boglione et al. (2009), and the ratio for each type of deformity in each dietary treatment was calculated based on the total number (24) of sample.

2.5. Determination of hepatic ascorbic acid concentration level in fish

Nine specimens were randomly sampled from each dietary treatment (three from each replicate) to determine the hepatic ascorbic acid concentration level. The fish were sacrificed by immersing them in ice water and subsequently dissected to obtain their livers. Liver samples were weighed and analyzed immediately or stored at −80 °C. The ascorbic acid concentration of liver samples was analyzed as mentioned before in Section 2.1.

2.6. Statistical analysis

The computer program IBM SPSS Statistics version 22 for Windows was used for the statistical analyses in the present study. All data (except the skeletal deformity data) were analyzed by one-way analysis of variance (ANOVA). Homogeneity of variance was tested using Levene's test, and multiple comparisons among treatments were performed using Duncan's multiple range test. Significant differences were assumed when $P < 0.05$.

3. Results

The initial body weight (IBW), final body weight (FBW), and survival of the hybrid grouper fed with different dietary supplementation levels of ascorbic acid are summarized in Table 2. Among all dietary treatments, fish fed with the C156 diet attained the highest FBW (880.18%) and SGR (3.24% d^{-1}) (Fig. 3). Although these results have no significant difference ($P > 0.05$) with those fed with the C76 (841.56%; 3.19% d^{-1}), C47 (835.31%; 3.19% d^{-1}), C95 (802.81%; 3.14% d^{-1}), C303 (778.90%; 3.10% d^{-1}) and C5 (723.16%; 2.99% d^{-1}), they were significantly higher than those fed with the C11 (628.51%; 2.83% d^{-1}) and C24 (634.23%; 2.85% d^{-1}) diets. Although the fish fed with the control (C5) diet showed the higher BWG (723.16%) and SGR (2.99% d^{-1}) than those fed with the C11 (628.51%; 2.83% d^{-1}) and C24 (634.23%; 2.85% d^{-1}) diets, no significant difference ($P > 0.05$) was detected among them (Figs. 2 and 3). At the end of the feeding trial, the survival of hybrid grouper in all treatments remained high (97.78–100%) with no significant difference ($P > 0.05$) among treatments.

Table 2
Growth performance and survival of hybrid grouper fed diets containing different levels of vitamin C for 10 weeks.

Diet	IBW (g)	FBW (g)	Survival (%)
C5	7.80 ± 0.09	64.16 ± 7.41	100 ± 0.0
C11	7.61 ± 0.11	55.44 ± 3.31	97.78 ± 2.22
C24	7.77 ± 0.12	57.08 ± 2.76	97.78 ± 2.22
C47	7.72 ± 0.10	72.27 ± 3.31	100 ± 0.0
C76	7.70 ± 0.01	72.55 ± 5.97	100 ± 0.0
C95	7.69 ± 0.07	69.49 ± 2.97	100 ± 0.0
C156	7.74 ± 0.11	75.95 ± 9.12	100 ± 0.0
C303	7.66 ± 0.06	67.31 ± 1.62	100 ± 0.0

Values are means of from three groups of fish (n = 3). Means in each row with different superscript are significantly different ($P < 0.05$).
IBW = Initial body weight.
FBW = Final body weight.

Table 3 show the feed utilization and hepatic ascorbic acid concentration level of the fish fed with the experimental diets. The highest feed intake (FI) was achieved by the fish fed with the C156 diet (57.16 g/fish), and this result was significantly higher than those fed with the C11 (42.09 g/fish) and C24 diet (42.01 g/fish). Although the fish fed with the C47 diet attained the best feed conversion ratio (FCR – 0.82) and feed efficiency (FE – 121.72), no significant difference was detected among treatments. The FCR and FE of diet C47 was also significantly higher than those from the C5 (0.9) and C11 (0.88), and C5 treatment (111.44 μg/g tissue), respectively. The hepatic ascorbic acid concentration level of the experimental fish increased [31.71 (control), 36.16–101.82 μg/g tissue] when the ascorbic acid dietary supplementation level increased. Although the fish fed with the C156 diet attained significantly higher ($P < 0.05$) hepatic ascorbic acid concentration level (86.11 μg/g tissue) than the other treatments, this result was significantly lower than that from the C303 treatment (101.82 μg/g tissue).

Table 4 shows the percentages of skeletal deformities in each dietary treatment. After 10 weeks of feeding trial, no skeletal deformity was observed in the fish fed with C95, C156 and C303 diets. Though, fish from the C5 dietary treatment suffered the highest percentage of kyphosis (54.17%) and this value was significantly higher than the other treatments. The radiographs of the sampled fish are shown in Fig. 1. Kyphosis was observed in the pre-hemal region of fish vertebral column which usually affected at the No. 2–6 vertebrae, where No. 3 and 4 vertebrae were broken. Lordosis occurred after kyphosis, and affected the No. 5–12 vertebrae (Fig. 1A and B). Those fish suffered from kyphosis and lordosis generally exhibited lethargic condition in the tank. Some of the fish only lay on the tank bottom and ingested when the feed was dropped right in front at its mouth. The occurrence of vertebral fusion and scoliosis was mostly found to occur in the hemal region at the No. 14–15 (Fig. 1C and D) and No. 8–16 vertebrae, respectively. The latter incidence showed the most curved vertebrae in No.13–16 (Fig. 1E and F). Those fish which suffered from scoliosis developed a permanent S-shaped body.

4. Discussion

To our knowledge, this is the first report on the dietary ascorbic acid required by the hybrid grouper *E. fuscoguttatus* × *E. lanceolatus* for its optimum growth and normal skeletal development. In the

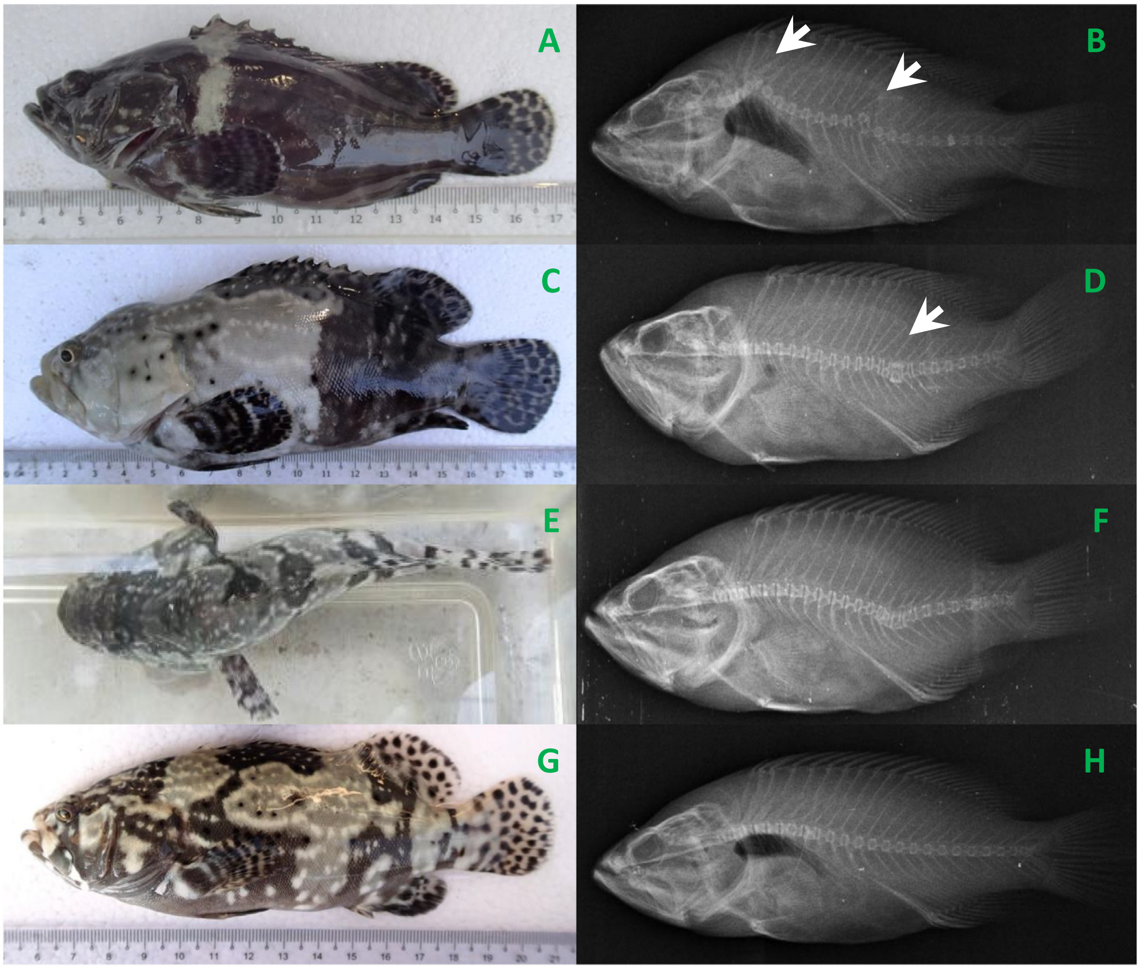

Fig. 1. Photos of deformed and healthy fish. A. Fish exhibited dark skin colour and suffered from kyphosis. B. X-ray image of the fish suffered from kyphosis. Arrow heads point the affected areas. C. Fish exhibited half-black skin colour and suffered from fused vertebrae. D. X-ray image of the fish suffered from fused vertebrae. Arrow head points the affected area. E. Fish develops a permanent S-shape even when at rest. F. X-ray image of fish suffered from scoliosis. Arrow head points the affected area. G. Healthy fish. H.X-ray image of normal fish skeletal.

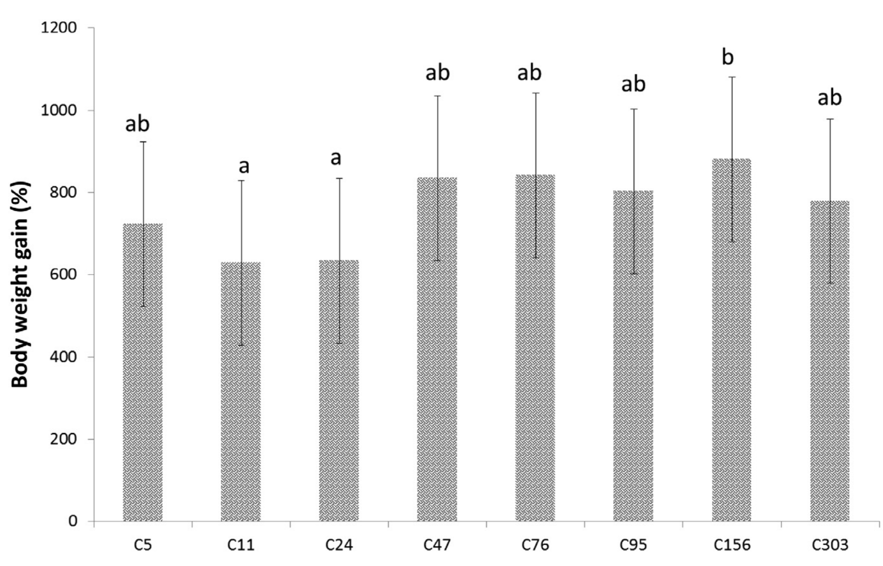

Fig. 2. Body weight gain (%) of hybrid grouper fed diets containing different levels of vitamin C for 10 weeks.

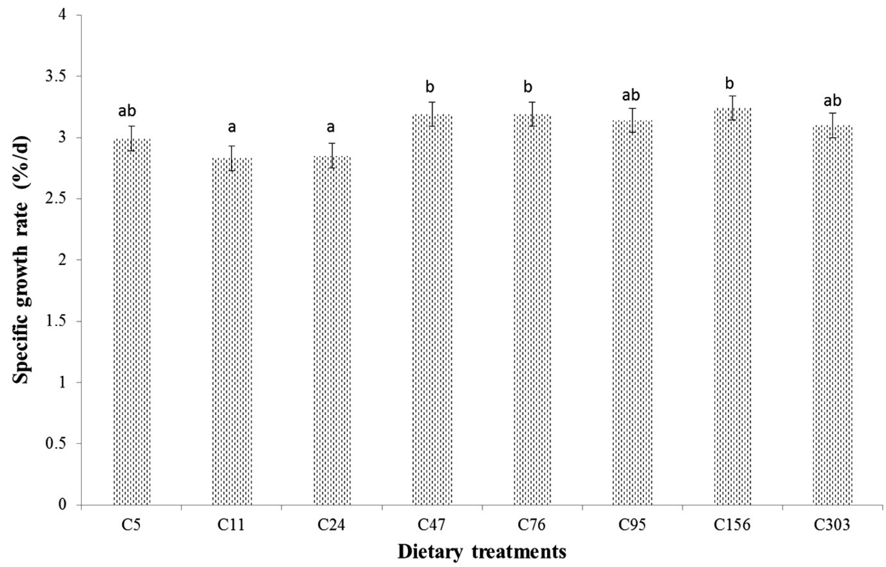

Fig. 3. Specific growth rate (%/d) of hybrid grouper fed diets containing different levels of vitamin C for 10 weeks.

Table 3
Feed utilization and hepatic concentration of vitamin C in hybrid grouper fed diets containing different levels of vitamin C for 10 weeks.

Diet	Total FI (g/fish)	FCR	FE	Hepatic vitamin C(μg/g wet tissue)
C5	50.30 ± 5.18^{ab}	0.90 ± 0.02^{b}	111.44 ± 3.09^{a}	31.71 ± 3.50^{a}
C11	42.09 ± 3.43^{a}	0.88 ± 0.02^{b}	113.90 ± 2.58^{ab}	36.16 ± 4.17^{a}
C24	42.01 ± 2.56^{a}	0.85 ± 0.02^{ab}	117.55 ± 3.06^{ab}	52.76 ± 7.33^{b}
C47	53.03 ± 2.42^{ab}	0.82 ± 0.01^{a}	121.72 ± 1.38^{b}	54.23 ± 3.90^{b}
C76	54.46 ± 3.36^{ab}	0.84 ± 0.03^{ab}	118.63 ± 3.63^{ab}	54.54 ± 1.60^{b}
C95	52.27 ± 2.58^{ab}	0.85 ± 0.00^{ab}	118.25 ± 0.29^{ab}	58.05 ± 2.85^{b}
C156	57.16 ± 6.41^{b}	0.85 ± 0.02^{ab}	117.85 ± 2.41^{ab}	86.11 ± 8.79^{c}
C303	52.16 ± 1.95a^{b}	0.87 ± 0.01^{ab}	114.47 ± 1.19^{ab}	101.82 ± 9.81^{d}

Values are means of from three groups of fish (n = 3). Means in each row with different superscript are significantly different ($P < 0.05$).
FI = Feed intake.
FCR = Feed conversion ratio.
FE = Feed efficiency.

Table 4
Vertebral deformities occurrence in juvenile hybrid grouper fed diets containing different levels of vitamin C for 10 weeks.

Diet	Fusion (%)	Scoliosis (%)	Kyphosis (%)	Lordosis (%)
C5	4.17 ± 7.22	12.50 ± 0.00	54.17 ± 5.18^{b}	8.33 ± 7.22
C11	8.33 ± 7.22	ND	8.33 ± 7.22^{a}	8.33 ± 7.22
C24	4.17 ± 7.22	4.17 ± 7.22	4.17 ± 7.22^{a}	4.17 ± 7.22
C47	8.33 ± 7.22	ND	ND	ND
C76	ND	ND	4.17 ± 7.22^{a}	ND

Values are means of from three groups of fish (n = 3) with 8 fish per group. Means in each row with different superscript are significantly different ($P < 0.05$).
ND = Not detected.

present study, the BWG (628.51–880.18%) and SGR (2.83–3.24%/d) of the hybrid grouper were very high compared to its maternal species tiger grouper, *Epinephelus fuscoguttatus*. According to our previous study, the highest BWG and SGR of *E. fuscoguttatus* with 8.8 ± 1.0 g initial weight were only 298.9% and 2.4%/day after the fish was cultured for 8 weeks under the similar environment (Shapawi et al., 2014). In addition, the hybrid grouper in the present study attained about 0.8–0.9 in FCR. These results indicated that the hybrid grouper indeed is a fast-growth species (Rahimnejad et al., 2015; Jiang et al., 2015, 2016).

Results of the present study also strongly evidenced that ascorbic acid dietary supplementation can improve the growth performance of the hybrid grouper. The BWG (628.51–880.18%) and SGR (2.83–3.24%/day) of the hybrid grouper generally increased when the ascorbic acid dietary supplementation level increased (from 11.2 mg/kg to 156 mg/kg). Besides that, the hepatic ascorbic acid concentration level in the hybrid grouper increased when the ascorbic acid dietary supplementation level increased. Such results were similar with those reported on other marine and freshwater fish species (e.g. Ishibashi et al., 1992; Ai et al., 2004; Lin and Shiau, 2004; Xie and Niu, 2006; Chen et al., 2015). Nevertheless, the hepatic ascorbic acid concentration level of the hybrid grouper in the present study did not reach a plateau when the ascorbic acid dietary supplementation level increased from 156 to 303 mg/kg. Fish fed with the C303 diet contained significantly higher ($P < 0.05$) ascorbic acid level in their liver (101.82 μg/g tissue) than those fed with the C156 diet (86.11 μg/g tissue). Apparently, the hybrid grouper required higher dietary ascorbic acid to saturate its hepatic ascorbic acid concentration although 156 mg/kg of dietary ascorbic acid was enough to fulfill its requirement for optimum growth. In fact, similar results were also reported in other fish species. For instances in the Japanese parrot fish, *Oplegnathus fasciatus* as reported by Ishibashi et al. (1992) (dietary ascorbic acid requirement for optimum growth was 250 mg/kg while to saturate the ascorbic acid concentration in liver was 500 mg/kg), *L. japonicus* by Ai et al. (2004) (47.6 and 89.7 mg/kg, respectively), ayu, *Plecoglossus altivelis* by Xie and Niu (2006) (160 and 226 mg/kg, respectively), and the *M. salmoides* by Chen et al. (2015) (109.5 and 147.8 mg/kg, respectively).

Survival of the hybrid grouper in the present study was relatively high in all dietary treatments (97.8–100%), seemingly that it was not affected by the ascorbic acid dietary supplementation levels. The hybrid grouper fed with the low ascorbic acid content diets (C5, C11, C24, and C47) first shown the visible ascorbic acid deficiency signs (such as the darker skin coloration and loss of equilibrium) at the end of the 9th week during the feeding trial. In the previous study on *E. malabaricus*, the fish fed with the low ascorbic acid diets exhibited the first occurrence of the ascorbic acid deficiency signs after 8 weeks and the fish survival was in the range of 83–100% (Lin and Shiau, 2004). Although comparison was hard to be made, it seemed that the hybrid grouper has slightly better toleration than the original grouper species to the ascorbic acid deficiency which may be contributed by its hybrid vigor.

The present study is the first attempt to report the skeletal deformities associated with ascorbic acid deficiency in the juvenile hybrid grouper. Multiple types of skeletal deformities such as fusion, kyphosis, lordosis, and scoliosis were observed in fish fed with the diets containing less than 95 mg/kg of ascorbic acid. Indeed, skeletal deformity is one of the classical sign of ascorbic acid deficiency in fish, including both marine and freshwater species (Lovell, 1989; Aguirre and Gatlin, 1999; Wang et al., 2002, 2003b; Ai et al., 2004; Ibiyo et al., 2007). It has been reported that, the imbalance of other vitamins as well might induce deformities in fish. Vertebral deformity were found in Japanese flounder (*Paralichthys olivaceus*) and zebra fish (*Danio rerio*) due to excessive dose of vitamin A (Miki et al.,1990; Haga et al., 2009) and vitamin D3 (Haga et al., 2004).

Among all types of skeletal deformity, kyphosis scored the highest in the present study. Although some fish suffered from kyphosis with fusion, all fusion cases in the present study involved only 2 consecutive vertebrae; fusion will be considered severe only when it involved more than 3 consecutive vertebrae (Boglione et al., 2014). Apparently, the developed skeletal deformities were not up to the lethal level for the fish. Such results probably explain the high fish survival in the present study.

5. Conclusion

In conclusion, ascorbic acid dietary supplementation can affect the growth performance and normal skeletal development in the juvenile hybrid grouper. Although the minimum dietary ascorbic acid required by the hybrid grouper for normal skeletal development was determined at 95 mg/kg; 156 mg/kg of dietary ascorbic acid recommended for feed development to maintain the optimum growth and normal skeletal development in the fish.

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