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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.

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