BIOLOGICAL PERFORMANCE OF ORANGE SPOTTED GROUPER, *Epinephelus coioides* SEED FED VITAMIN C AND CALCIUM FORTIFIED DIET

Titiek Aslianti and Agus Priyono

ABSTRACT

Further development of grouper culture facing the problems of unsucessful seed production, mostly due to high rate of larval deformity and mortality. Fortification of artificial feed with vitamin C and calcium is intended to increase vitality and improve vertebrae growth of orange spotted grouper, *Epinephelus coioides* seed. The experiment was conducted in a completely randomized design consisted of 4 treatments in terms of diets fortified with vitamin C + calcium (A), Vitamin C (B), Calcium (C), and control (D), with 3 replicates each. The treatments were applied to 20 days old seed which was reared for another 25 days. The growth rate of was checked at 5 days interval and survival rate was calculated based on mortality data when the experiment terminated. The results showed that the highest ($P<0.05$) survival rate (4.38%) and growth (TL 17.37 mm, BW 75.21 mg) were obtained from the seeds fed vitamin C + calcium fortified diet.

KEYWORDS: ***Epinephelus coioides*, feeds, larvae, fortified diet, vitamin C**

INTRODUCTION

The instability of marine-fish seed production in small scale hatcheries is often caused by mass mortality during rearing which lead to wide variation in survival rate (Sugama *et al.*, 1998) mostly due to malnutrition (Aslianti & Priyono, 2003) such as vitamin C deficiency. The optimal production of orange spotted grouper (*Epinephelus coioides*) seeds according to Indonesian Standard Quality has been unsuccessful due to problem during larval rearing. Some physiological aspects of orange spotted grouper larval rearing such-as live feed management, artificial diet substitutions, supplement of essential mineral and larval rearing environmental management had been conducted and the results showed that the abnormality of fry decreased to 9.26% (Aslianti & Priyono, 2004).

Vitamin C is one of essential nutrition elements for larval vitality (Lovell, 1973 *in* Suwirya *et al.*, 1999), but fish can not synthesize vitamin C (Ikeda & Sato, 1965 *in* Suwirya *et al.*, 1999), so vitamin C must be added into the feed (Kitamura *et al.*, 1965 *in* Suwirya *et al.*, 1999). However, for optimum growth rate, vitamin C requirement in fish depends on species, age and size of the fish.

Fish growth is related to backbone growth. Phosphorus and calcium is the essential mineral in bone formation for growth augmentation. In addition, calcium is an excellent larval feed ingredient and required in teeth and bone formation as well as in supporting vertebrate blood coagulation (Watanabe, 1988; Lall, 1989). Kompyang (1990) reported that calcium has a roll in neural-activity to transfer impulse, facilitating osmoregulation and as a cofactor in improve vertebrate enzyme-activity. Normal growth with zero deformity and low mortality can only be obtained if the diet sufficiently supplied with required nutrients. The experiment aims at improving biological performances of orange spotted grouper through the application of vitamin C and calcium fortified diet.

MATERIALS AND METHODS

Twelve 1.0 m^3 cylindrical fiberglass tanks filled with 500 *l* sea water (34 ± 1 ppt) and orange spotted grouper fertilized eggs were stocked in to the tanks with density of 20,000

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eggs/tank. Calculated based on the method developed by Effendi (1997), the larvae hatching rate was around 80%. SS type (60—110 µm) feed at density of 10—20 rotifers.ml^{-1} as initial feed were given from the second day after the grouper eggs hatched to D_3 and S type (120—180µm) with density of 20—30 rotifers.ml^{-1} were given to the larvae to D_{35}. Feed treatments were initiated to 20 days old larvae at density of 16,000 seeds.tank^{-1} and continued for another 25 days. The experiment was conducted in a completely randomized design consisting of four treatments i.e.: vitamin C + calcium (A), vitamin C (B), Calcium (C) and control (D). Vitamin C, 200 mg L (+) ascorbic acid.kg^{-1} feed, and calcium, 5 g calcium lactate.kg^{-1} feed, were blended into Suwirya *et al.* (1999) formulated diet.

The growth of the seeds was monitored every 5 days from 10—15 seed randomly sampled. A micrometer was used to measure total length under binocular microscope at 07.50—40.00 x magnification. The samples, after being dried with tissue paper, were individually weighted using digital scales at 0.1 mg precisions. Survival rates were counted when the experiment was terminated. Data were analyzed using analysis of variance at 95% confidence interval. Water quality such as water temperature, pH, DO, nitrite, nitrate, and ammonium was checked every three days. Larval vitality and deformity were also observed based on Potthof (1984) identification guide.

RESULT AND DISCUSSION

Growth rates in term of total length and body weight of the seed observed every 5 days are presented in Figures 1 and 2. The absolute growth rates are presented in Table 1; daily instantaneous growth rate (DGR) and survival rate counted at the end of the experiment are presented in Table 2.

The seed fed vitamin C+calcium fortified diet grew faster ($P<0.05$) than did the seed fed control diet, but not ($P>0.05$) from the seed fed vitamin C and Calcium fortified diets. Total length of the seed fed vitamin C+calcium fortified and control diets were 17.37 and 13.68 cm, respectively; respective to total length, final body weight were 75.21 and 32.03 mg.

Vitamin C and calcium added into feed significantly ($P<0.05$) affect seed total length and body weight growth as reflected by the absolute growth in each treatment (Table 1). Slow growth of the seed fed control diet was assumed due to nutrients, most probably vitamin C, insufficiency. According to Horning *et al.* (1984) vitamin C plays an important role in cartinine biosynthesis in fish body tissue since cartinine plays an important role in transferring lipid into mitochondria for further oxidation to produce energy. Insufficiency of vitamin C in the body tissue causes reduction of the rate of energy production in such a way that leads to weakening of the body and slow growth rate. Kosutarak *et al.* (1995) *in* Suwirya *et al.* (1999) reported that vitamin C is able to increase iron absorption in intestines and it has been known that iron is essential in circulation of oxygen in the body. Thus insufficiency of vitamin C would causes an impediment to oxygen circulation in such a way that makes the growth process runs abnormally. This was verified by daily growth rate of the seed fed control diet which was lower, 5.44%,

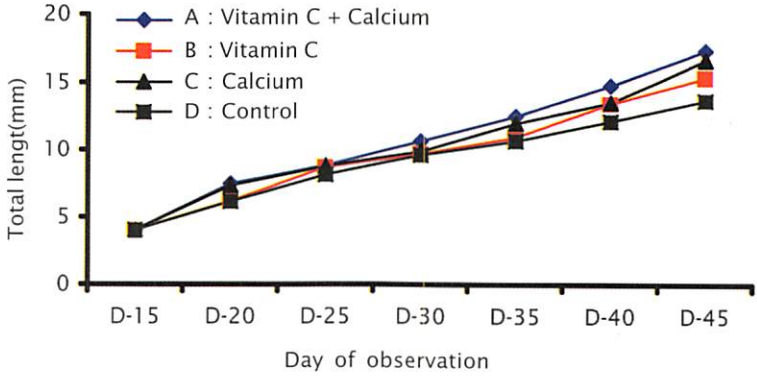

Figure 1. Total length growth of orange spotted grouper larvae fed different fortified diets

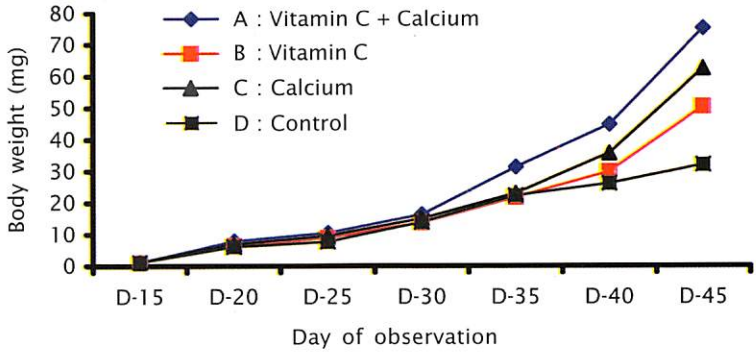

Figure 2. Body weight growth of orange spotted grouper larvae fed different fortified diets

Table 1. Initial and final total length (mm) and body weight (mg) of orange spotted grouper larvae fed fortified artificial diets

Treatments	Total Length		Body Weight	
	Initial (20-days old)	Final (45-days old)	Initial (20-days old)	Final (45-days old)
A : Vitamin C + Calcium	7.45	17.37 [a]	7.89	75.21 [a]
B : Vitamin C	6.18	15.39 [a]	6.53	50.44 [a]
C : Calcium	7.32	16.67 [a]	6.89	62.40 [a]
D : Control	6.13	13.68 [b]	6.1	32.03 [b]

*) Values followed by similar superscript are not significantly different (P>0.05)

than of the seed fed the other treatments (Table 2). According to Halver *et al.* (1969), vitamin C is essential in collagen synthesis. Lack of vitamin C causes an abnormal collagen synthesis and in turn causes cartilage distortion like what happened to coho and rainbow trout gill filaments, and also the low growing rate of red sea beam (Yano, 1975) and *Epinephelus malabarius* (Phromkunthong *et al.*, 1993 *in* Giri *et al.*, 1999). Lack of vitamin C in *Cromileptes altivelis* also causes lordosis and scoliosis, weakening of the body, hyperplasia in gills, and anemia; the number of hemoglobin, erythrocyte and leukocyte was much lower than those in the normal fish (Giri *et al.*, 1999). Slow growth rate of the seed fed control diet was observable in such a short rearing period, from day 25 to day 45, because it is closely related

Table 2. Survival rate and daily instantaneous growth rate (DGR) of orange spotted grouper larvae fed fortified artificial diets

Treatments	Survival rate	DGR (%)	
		Total length	Body weight
A : Vitamin C + Calcium	4.38 [a]	3.20 [a]	6.48 [a]
B : Vitamin C	2.80 [a]	3.42 [a]	6.17 [a]
C : Calcium	3.88 [a]	3.12 [a]	6.41 [a]
D : Control	1.54 [b]	3.05 [a]	5.44 [b]

*) Values followed by similar superscript are not significantly different (P>0.05)

to low availability of vitamin C in the body. Vitamin C is essential in the proline hydroxylation reaction to form lysine that is an essential compound in collagen formation and cartilage growth. The slow collagen formation leads to weaken tissues and cause abnormal bone growth (Horning *et al.*, 1984), which occurred in the seed fed control diet.

Addition of calcium into feed apparently brought about a better growth rate to the seed. Hossain & Furuichi (1999) stated addition of calcium into feed increases the rate of bone growth and if calcium was available in a balanced amount, bone growth process would be normal; physically, the growth and survival will also be better. Furthermore, it ha been known that the role of calcium in animal body is in the formation of backbones, gills, exoskeleton, hemoglobin, delivery of impulses from central nervous system and also plays a role in osmoregulation. Absorption of calcium was influenced by parathyroid hormone that functioning in the speed of absorption. The more amount of calcium absorbed by the body, the more osteoblast bone formed and consequently speeds up bone formation process; physically, the seed growth will proceed normally. No doubt, addition of calcium into feed has a significant effect on the seed growth. However, according to Boyd (2000) marine fish, although capable of calcium absorption from their habitat, their calcium requirement for their growth and survival is much higher than fresh water fish.

Survival rate in each treatment counted at the end of the experiment (D_{45}) shown a significant different result ($P<0.05$); the survival rate of the seed fed vitamin C+calcium fortified diet, 4.38%, was the highest while the lowest was achieved in control diet, 1.54%, (Table 2). The difference is understandable, normal growth is a result sufficient availability of nutrients, in this case vitamin C and calcium, in feed which leads to normal bone formation growth process and maximum vitality. However, absorption of calcium into body for speeding up bone formation might cause abnormal larval growth. Abnormal (deformed) larvae are often having difficulty in finding feed and ended to death and low survival rate. The fact is reflected in the percentage of the number of deformed larvae in treatment A that was much lower (3.18%) than in treatment B (9.26%), C (8.3%), or D (18.58%). Addition of calcium into feed obviously reduced larval deformity rate (Figure 3).

Vitality test proven that the seed fed vitamin C+calcium fortified diet survived when it is exposed into air for 5 minutes while the seed fed control diet ended with death. According to Marzuqi *et al.* (1997), fish is not able to synthesize vitamin C in the body to maintain normal cell metabolism. Physically stressed fish suffer from increasing glucose metabolism and cortisol hormone content (Sandnes & Waagbo, 1991); cortisol is a hormone that keeps cell haemostatic functions. Horning, *et al.* (1984) stated that vitamin C contained in feed will accumulate in adrenal cortex tissue in the animal or in fish anterior gills, and plays a role as a cofactor in hydroxylation of biosynthesis of adrenals-corticosteroid hormone group that is essential in body metabolism regulation at the time of stress, so that vitamin C insufficiency reduces tolerance capacity of fish toward stress disturbance. Sufficient vitamin C content in body amplifies larvae capacity to cope with stress (Suwirya *et al.*, 1999). Lovell & Lim *in* Sandness (1991) reported that vitamin C requirement of catfish farmed in high density increases compared to that in normal density. This proves that in high density fish tend to be in stress condition.

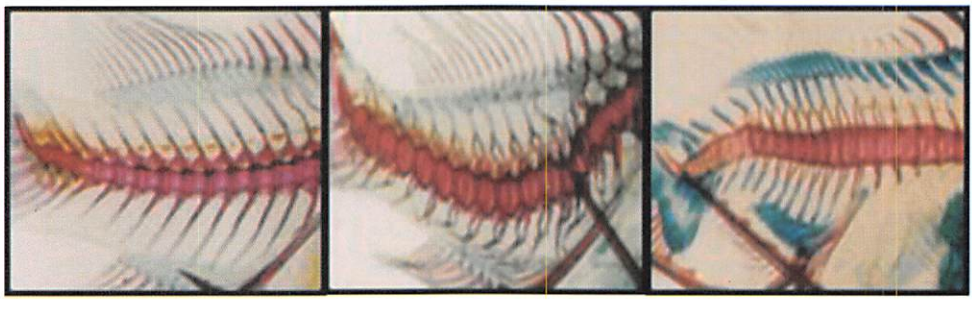

Figure 3. Seed samples showing normal (A) and deformed (B, C) backbone growth

CONCLUSION

Vitamin C and calcium are essentials ingredients of feed for reducing deformity and maintaining vitality in orange spotted grouper seed production. Vitamin C+calcium fortified diet generates the highest growth and survival rate in orange spotted grouper seed rearing

ACKNOWLEDGEMENT

I would like to thanks and show my appreciation to the author Dr. Haryanti, Dr. Gde Sumiarsa, and Dr. Taufik Ahmad for their help in reviewing manuscript. Special thanks for Wiwin Admadinata, Nyoman Restiada, Ayu Kenak, Ari Arsini, and Darsudi who technically support larval rearing, natural feed production, water quality and the proximate analysis of the diets.

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