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Effects of single or combination of dietary vitamin C and β-glucan supplementation on golden trevally, *Gnathanodon speciosus* (Carangidae)

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ABSTRACT

This study aims to test the single and combination effects of dietary vitamin C and β-glucan on golden trevally, *Gnathanodon speciosus.* The basal diet (D0) was added with vitamin C at 200 mg kg^{-1} diet (D1), β-glucan at 1.0 g kg^{-1} diet (D2), and a combination of vitamin C and β-glucan (200 mg vitamin C and 1 g β-glucan per kilogram diet) (D3). The diets were fed the fish for eight weeks. After eight weeks of diet feeding, the growth rate of the fish enhanced significantly with the presence of vitamin C, β-glucan, and the combination of Vitamin C and β-glucan ($p < 0.05$). The growth rate was significantly higher in a fish-fed diet combined with vitamin C and β-glucan. Survival rates were not significantly different among diet treatments. Muscle protein of fish ranged from 18.36–21.50% among diet treatments. Fish protein content in fish was not influenced by vitamin C, but the protein was higher in fish fed with the β-glucan-added diet and combination the β-glucan and vitamin C-added diet. The current results suggest that a supplemented combination of vitamin C and β-glucan could boost this golden trevally's growth and body composition at the juvenile stage.

Keywords: Growth, survival, body composition, vitamin C, β-glucan.

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INTRODUCTION

The golden trevally (*Gnathanodon speciosus*) are reef-dwelling marine species distributed in the tropical and subtropical waters of the Eastern Pacific, Western Indo-Pacific, and Eastern and Western Atlantic Ocean. The Golden Trevally is a popular food and ornamental fish native to the tropical Indo-Pacific region [1]. In Vietnam, this species has been induced to spawn successfully, becoming a new candidate for aquaculture. Recently, the golden trevally is more famous for being a cultured species in Viet Nam due to its fast growth and high market value. However, aquaculture was challenged by the environmental pressure such as water pollution, high stocking density, and low water quality [2, 3].

Vitamin C (ascorbic acid) is a water-soluble antioxidant and has been long considered to be a health-promoting agent when applied in aquaculture [4–8]. Vitamin C has a distinct role as a cofactor for enzymes engaged in the hydroxylation of proline and lysine and is required for synthesizing collagen and constructing bone matrix [9]. In several fish species, dietary vitamin C required for maximum body growth exceeds the basic requirement levels for growth, survival, and hydroxyproline concentrations [10]. Furthermore, immune response indicators are stimulated by vitamin C levels far above the physiological body necessities, protecting fish against stress [10, 11]. Our previous study also showed that the added vitamin C at 200 mg. kg^{-1} could boost the growth performance of golden trevally [12]. In addition, β-glucan application was reported to boost the growth performance of various aquaculture species such as large yellow croaker (*Pseudosciaena crocea*) [13], Atlantic salmon (*Salmo salar*) [14], koi carp (*Cyprinus carpio koi*) [15], rohu (*Labeo rohita*) [16] and snapper (*Pagrus auratus*) [17].

Although the efficacy of vitamin C and β-glucan has been demonstrated on different aquaculture species, to the authors' knowledge, the dietary combination of vitamin C and β-glucan on the growth performance of golden trevally is still not known. Therefore, the present study aimed to define the single and combination effects of dietary vitamin C and β-glucan on golden trevally, *Gnathanodon speciosus*.

MATERIALS AND METHODS

Experimental fish and culture systems

The golden trevally, *Gnathanodon speciosus*, was obtained from a hatchery in Nha Trang, Vietnam. 450 fish from the same bloodstock with a mean initial wet weight of 4.25 ± 0.11 g (SEM) were randomly stocked in 12 composite tanks.

Experimental design

The experiment tested the growth response and mortality of fish fed basal diet added with a single or combination of vitamin C and β-glucan and fed the fish for eight weeks. A design was used in which the four dietary treatments were randomly allocated to 3 replicate tanks, with twenty-five fish in each tank. A total of 18 tanks and 450 fingerlings golden trevally were stocked.

Experimental diets and feeding

The basal diet (content 39.3% crude protein, 8.71% lipid, and 19.72 GE MJ/kg) was used as control (D0), and basal was added with 200 mg kg^{-1} diet of vitamin C (D1), or 1.0 g β-glucan per 1-kilogram basal (D2), and the combination of 200 mg vitamin C and 1.0 g β-glucan per 1 kilogram basal (D3). The recommended concentration of supplementation was referenced from [12] for Vitamin C and Do-Huu et al., for β-glucan.

Experimental fish were fed 5% of the average biomass in each tank on the first day, and then every day, the amount fed was adjusted, based on the amount of uneaten or remaining pellets. Experimental diets were fed to the fish twice daily (1/2 at 08:00 and 1/2 at 17:00). Uneaten feed and feces were siphoned daily before morning feed. These procedures were followed for 8 weeks.

Sampling and data collection

All fish were weighed, and the total length of the experiment's beginning and end. At the end of the experiment, two fish from each tank (6 fish in each treatment) was randomly sampled and sacrificed for measurement of muscle composition. Fish were starved for 24 h prior to measuring or sampling.

Chemical analysis of culture fish

Crude protein and lipid were measured following the Kjeldahl standard method, with the procedure described by [18].

Data calculation

Growth rate, daily growth coefficient (*DGC*, % d^{-1}), and survival were computed by the following equations [19]: *DGC* (% d^{-1}) = 100 × ($W_t^{1/3}$ – $W_o^{1/3}$)/days; Survival rate = 100 × (N_t)/N_o, where W_t and W_o were weight of fish at the end (week 8) and initial weight (g), respectively. N_o was the initial number of fish and N_t was the number of fish at the end of the experiment. Final biomass was calculated by sum-up the total weight of all fish in each tank at the end of the experiment.

Statistical analysis

Data are presented as means ± standard error (SEM). To compare growth performance and body composition data of fish among diet treatments, ANOVA tests and the least significant difference (LSD) were used. Differences were significant when $p < 0.05$. Non-parametric Kruskal-Wallis test was used to compare survival rates. Statistical analysis were performed in SPSS 18 (IBM, Chicago, IL).

Animal research

Based on the National Regulations for the Use of Animals in Research in Vietnam, golden trevally (Gnathanodon speciosus) is not listed in two groups IB (endangered and critically endangered species) and IIB (threatened and rare species) (Decree32/, 2006/ND-CP, 2006). Therefore, this study does not require a permit or ethical approval. However, the authors have implemented their best practice of using animals in research.

RESULTS

Growth performance of golden trevally fed different diets

The weight of fish at the experiment start was uniform between the experimental treatments, 4.25 ± 0.11 g (SEM). There was no significant difference in the weight of fish between treatments at commencement (ANOVA, $p \geq 0.581$). However, significant differences in fish weight among diets were observed at the end of the experiment (week 8).

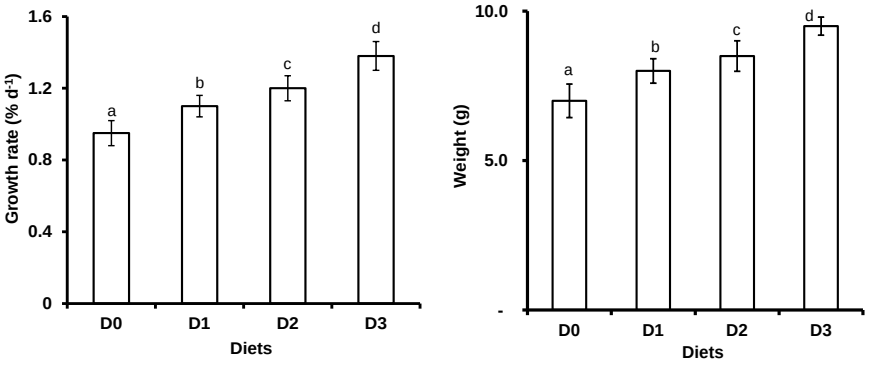

Figure 1. Average daily growth rate (% d^{-1}) and mean weight (± SEM) of golden trevally fed single or combination of vitamin C and β-glucan. D0: Control; D1: Vitamin C, D2: β-glucan; D3: Combination Vitamin C and β-glucan. Different letters indicate significant differences between treatments (week 8)

After eight weeks of diet feeding, the mean weight ranged from 7.01 g to 9.52 g among treatments. All fish fed the diet with supplementation (Vitamin C or β-glucan) showed a higher growth rate than fish fed control diet. The most increase in weight was observed in fish fed a combination vitamin C and β-glucan supplemented diet. The weight of fish fed combination vitamin C and β-glucan diets was significantly higher than those fish fed the control and fish single supplementation (vitamin C or β-glucan) ($p \leq 0.001$). (Figure 1).

The survival rate of golden trevally fed different diets

At the end of the experiment (week 8), fish survival rates ranged from 87.08–90.60% (Figure 2). There were higher survival rates in fish in the supplemented diet. However, no significance was found between fish survival rates among treatments ($p = 0.471$).

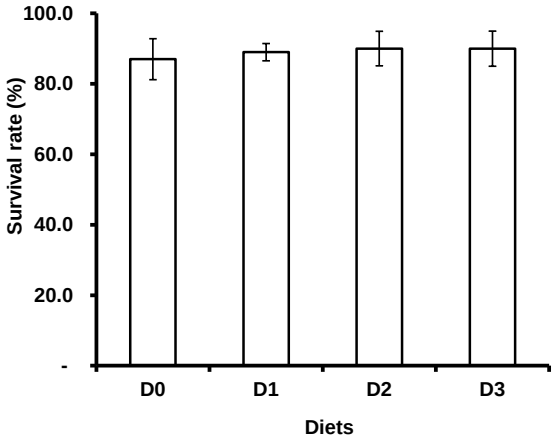

Figure 2. Survival rate of golden trevally fed single or combination of vitamin C and β-glucan. D0: Control; D1: Vitamin C; D2: β-glucan; D3: Combination Vitamin C and β-glucan. Different letters indicate significant differences between treatments

Size variation of golden trevally fed different diets

Our results revealed that the variation coefficient (CV, %) of body weight of golden trevally was the highest in the fish-fed control diet (10.15%), followed by the CV of fish-fed diets D1, D2, and D3. The CV had the lowest value of 7.00% in the group of fish-fed diet D3 (a combination of 200 mg C and 1 g β-glucan kg^{-1}diet). The CV values of fish-fed diets D2 (added β-glucan) and D3 (a combination of 200 mg vitamin C and β-glucan kg^{-1}diet) were significantly lower compared to the CV of fish-fed control diet ($p = 0.004$). However, the CV value in fish fed only level of vitamin C did not differ significantly compared to CV in the control group ($p = 0.112$).

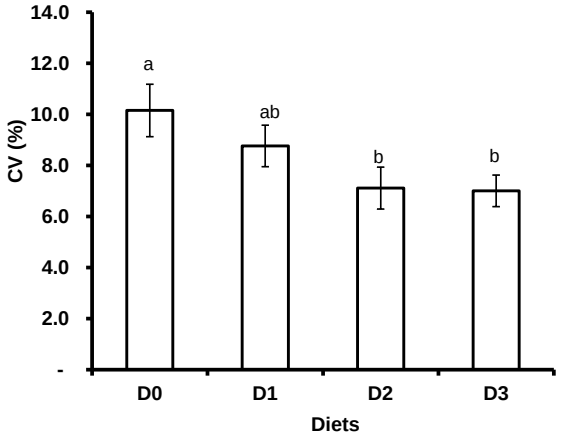

Figure 3. Coefficient of variations in weight (CV, %) of golden trevally fed single or combination of vitamin C and β-glucan. D0: Control; D1: Vitamin C; D2: β-glucan, D3: Combination Vitamin C and β-glucan. Different letters indicate significant differences between treatments

Protein and lipid content in flesh of golden trevally fed different diets

Our results revealed that protein content in the muscle of golden trevally ranged from 18.36% in the group fed the basal to 20.01% in the group fed diet combined with vitamin C and β-glucan. There was a significantly higher protein content in the fish-fed diet added β-glucan (D2), and the diet added both vitamin C and β-glucan (D3) compared to the protein in fish-fed control ($p = 0.031$). However, there was no significant difference in protein content in the fish-fed diet with only vitamin C (D1) compared to the fish-fed control diet. Lipids in

the flesh of fish fed diet content both vitamin C and β-glucan (D3) was significantly lower than fish in control (p = 0.032), but there was no difference between fish fed vitamin C or β-glucan compared to fish fed basal diet ($p \geq 0.142$) (Figure 4).

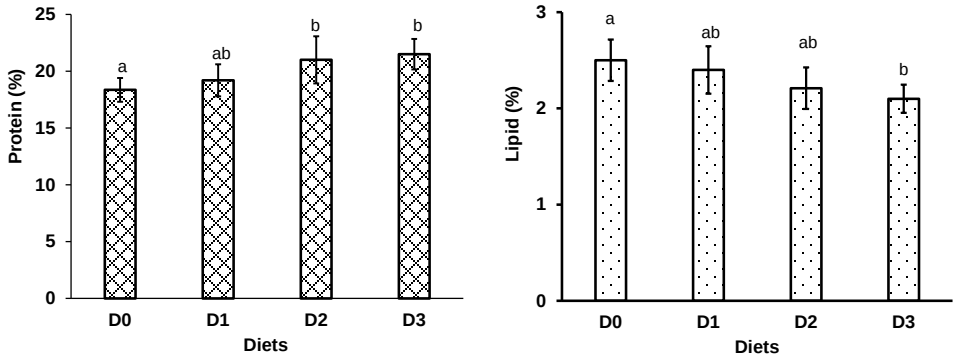

Figure 4. Protein content in the flesh of golden trevally fed single or combination of vitamin C and β-glucan. D0: Control; D1: Vitamin C; D2: β-glucan; D3: Combination Vitamin C and β-glucan. Different letters indicate significant differences between treatments

DISCUSSION

The current study reveals the role of vitamin C in the growth of golden trevally, *Gnathanodon speciosus*. Supplement of 0.02% vitamin C in the diet enhances the growth of the fish. This result was in agreement with other studies that reported the critical role of vitamin C in boosting growth and survival of in juvenile rainbow trout (*Oncorhynchus mykiss*) [9]; red sea bream, *Pagrus major* [20] *Heterobranchus longifilis* fingerlings [8]. The recent study on the effect of Vitamin C on Coho Salmon *Oncorhynchus kisutch* revealed that VC markedly improved the growth performance indexes and liver VC concentration, enhanced the hepatic and serum antioxidant activities, and increased the contents of serum alkaline phosphatase (AKP) activity, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) whereas decreased the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, and triglyceride (TG) level [21].

However, vitamin C supplements in the diet did not improve growth in juvenile cobia (*Rachycentron canadum* [7]. An explanation was that the benefit of vitamin C added to the diet may be species-specific. In the present study, there is no significant difference in the protein content of golden trevally fed different concentrations of vitamin C. Our results are similar to Zhou et al., (2012) [7], who reported that protein content in juvenile cobia (*Rachycentron canadum*) was not influenced by the presence of vitamin C added to the diet. In another finding, Ibiyo et al., (2006) [8] stated that the protein content in *Heterobranchus longifilis* fingerlings fed vitamin C did not differ from the fish-fed control diet. However, the feed utilization in the case of protein efficiency ratio was significantly improved in fish-fed vitamin C.

The current study also reveals the importance of dietary β-glucan requirement to the growth of the juvenile golden trevally, *Gnathanodon speciosus.* The results show that growth, coefficient of variations in weight, and survival of juvenile golden trevally were significantly enhanced when supplemented with 0.1% β-glucan in their diet. In addition, this study also indicates that dietary β-glucan increases the protein level in the fish muscle. The current result is consistent with other publications reported on various cultured fish species such as pompano fish, *Trachinotus ovatus* [22], koi carp (*Cyprinus carpio koi*) [15], large yellow croaker (*Pseudosciaena crocea*) [13], rohu (*Labeo rohita*) fingerlings [16], snapper (*Pagrus auratus*) [17] and white fish (*Rutilus frisii kutum*) [23]. By contrast, β-glucan also shows no improvement in the growth of sea bass (*Dicentrarchus labrax*) [24]. The effect

of β-glucan on the growth of fish is not fully understood. Possible factors affecting the growth and survival of fish fed dietary β-glucan diet should be the concentration of β-glucan, species-specific response, and experimental conditions [25].

This study emphasized the benefit of a combination of vitamin C and β-glucan supplement in the diet to the growth and survival of golden trevally, *Gnathanodon speciosus.* Fish growth was boosted when they were fed diets with 0.02% vitamin C and 0.1% β-glucan inclusion in the diet of golden trevally. The mechanism for that still needs to be clarified in further research. However, the combination effect of Vitamin C and β-glucan has improved the immune response [26–28] and reduced the harmful effects of heavy metals [29] in the fish, resulting in higher growth and survival in the culture fishes.

CONCLUSION

A dietary combination of vitamin C and β-glucan could enhance juvenile golden trevally's growth, survival, and body composition. However, the combination of the two additives inclusion in the diet should be applied to gain better performance. This study found the benefits of a dietary combination of vitamin C and β-glucan on golden trevally juveniles. However, further study should focus on this combination's effects on this species' whole culture cycle.

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